

World Journal of *Diabetes*

World J Diabetes 2018 January 15; 9(1): 1-52



**REVIEW**

- 1 Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment
Serhiyenko VA, Serhiyenko AA

ORIGINAL ARTICLE**Basic Study**

- 25 Short-term effects of obestatin on hexose uptake and triacylglycerol breakdown in human subcutaneous adipocytes
Carpéné C, Les F, Estève D, Galitzky J

Observational Study

- 33 Heart rate is an independent predictor of all-cause mortality in individuals with type 2 diabetes: The diabetes heart study
Prasada S, Oswalt C, Yeboah P, Saylor G, Bowden D, Yeboah J

META-ANALYSIS

- 40 Association of obesity with hypertension and type 2 diabetes mellitus in India: A meta-analysis of observational studies
Babu GR, Murthy GVS, Ana Y, Patel P, Deepa R, Benjamin-Neelon SE, Kinra S, Reddy KS

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Dr. Adejuwon A Adeneye, MD, PhD, Department of Pharmacology, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, Ikeja 100001, Nigeria

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, and Scopus.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Ya-Jing Lu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Li-Jun Cui*
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL

World Journal of Diabetes

ISSN

ISSN 1948-9358 (online)

LAUNCH DATE

June 15, 2010

FREQUENCY

Monthly

EDITORS-IN-CHIEF

Lu Qi, MD, PhD, Assistant Professor, Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, United States

Jingbo Zhao, PhD, Associate Professor, Aalborg Hospital Science and Innovation Centre, Aalborg Hospital, Aarhus University Hospital, Aalborg 9000, Denmark

EDITORIAL BOARD MEMBERS

All editorial board members resources online at <http://www.wjgnet.com/1948-9358/editorialboard.htm>

EDITORIAL OFFICE

Xiu-Xia Song, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER

Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE

January 15, 2018

COPYRIGHT

© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS

<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION

<http://www.f6publishing.com>

Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment

Victoria A Serhiyenko, Alexandr A Serhiyenko

Victoria A Serhiyenko, Alexandr A Serhiyenko, Department of Endocrinology, Lviv National Medical University Named by Danylo Halitsky, Lviv 79010, Ukraine

ORCID number: Victoria A Serhiyenko (0000-0002-6414-0956); Alexandr A Serhiyenko (0000-0002-3905-0326).

Author contributions: Serhiyenko VA and Serhiyenko AA contributed equally to this work, they have conceptualized, designed, performed and wrote the review.

Conflict-of-interest statement: All authors declare no conflict of interest.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Victoria A Serhiyenko, MD, PhD, Department of Endocrinology, Lviv National Medical University Named by Danylo Halitsky, Pekarska 69 Str., Lviv 79010, Ukraine. serhiyenko@inbox.ru
Telephone: +380-322-769496
Fax: +380-322-769496

Received: October 28, 2017
Peer-review started: October 29, 2017
First decision: November 23, 2017
Revised: December 9, 2017
Accepted: December 29, 2017
Article in press: December 29, 2017
Published online: January 15, 2018

Abstract

Cardiac autonomic neuropathy (CAN) is a serious com-

plication of diabetes mellitus (DM) that is strongly associated with approximately five-fold increased risk of cardiovascular mortality. CAN manifests in a spectrum of things, ranging from resting tachycardia and fixed heart rate (HR) to development of "silent" myocardial infarction. Clinical correlates or risk markers for CAN are age, DM duration, glycemic control, hypertension, and dyslipidemia (DLP), development of other microvascular complications. Established risk factors for CAN are poor glycemic control in type 1 DM and a combination of hypertension, DLP, obesity, and unsatisfactory glycemic control in type 2 DM. Symptomatic manifestations of CAN include sinus tachycardia, exercise intolerance, orthostatic hypotension (OH), abnormal blood pressure (BP) regulation, dizziness, presyncope and syncope, intraoperative cardiovascular instability, asymptomatic myocardial ischemia and infarction. Methods of CAN assessment in clinical practice include assessment of symptoms and signs, cardiovascular reflex tests based on HR and BP, short-term electrocardiography (ECG), QT interval prolongation, HR variability (24 h, classic 24 h Holter ECG), ambulatory BP monitoring, HR turbulence, baroreflex sensitivity, muscle sympathetic nerve activity, catecholamine assessment and cardiovascular sympathetic tests, heart sympathetic imaging. Although it is common complication, the significance of CAN has not been fully appreciated and there are no unified treatment algorithms for today. Treatment is based on early diagnosis, life style changes, optimization of glycemic control and management of cardiovascular risk factors. Pathogenetic treatment of CAN includes: Balanced diet and physical activity; optimization of glycemic control; treatment of DLP; antioxidants, first of all α -lipoic acid (ALA), aldose reductase inhibitors, acetyl-L-carnitine; vitamins, first of all fat-soluble vitamin B1; correction of vascular endothelial dysfunction; prevention and treatment of thrombosis; in severe cases-treatment of OH. The promising methods include prescription of prostacyclin analogues, thromboxane A2 blockers and drugs that contribute into strengthening and/or normalization of Na^+ , K^+ -ATPase (phosphodiesterase inhibitor), ALA, dihomogamma-linolenic acid (DGLA), ω -3 polyunsaturated fatty acids (ω -3 PUFAs), and the simultaneous prescription of ALA, ω -3 PUFAs and DGLA, but the future investigations

are needed. Development of OH is associated with severe or advanced CAN and prescription of nonpharmacological and pharmacological, in the foreground midodrine and fludrocortisone acetate, treatment methods are necessary.

Key words: Diabetes mellitus; Risk factors; Cardiac autonomic neuropathy; Screening for cardiac autonomic neuropathy; Cardiovascular reflex tests; Orthostatic hypotension; Heart rate variability; Prophylaxis; Treatment

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Cardiac autonomic neuropathy (CAN) is a serious complication of diabetes mellitus, which is strongly associated with increased risk of cardiovascular mortality. Although it is common complication, the significance of CAN has not been fully appreciated and there are no unified treatment algorithms for today. In this review we have analyzed the existing data about the known risk factors, screening and diagnostic algorithm, staging of CAN and possible treatment, including effectiveness of lifestyle modification, intensive glycemic control; treatment of diabetic dyslipidemia; antioxidants; vitamins; correction of vascular endothelial dysfunction; prevention and treatment of thrombosis; treatment of orthostatic hypotension.

Serhiyenko VA, Serhiyenko AA. Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment. *World J Diabetes* 2018; 9(1): 1-24 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i1/1.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i1.1>

INTRODUCTION

It was estimated that there were 415 million people with diabetes mellitus (DM) aged 20-79 years in 2015, and the number was predicted to rise to 642 million by 2040^[1]. The development of cardiac autonomic neuropathy (CAN) is associated with the lesion of the autonomic nervous system (ANS), and may be accompanied by coronary vessels ischemia, arrhythmias, "silent" myocardial infarction (MI), severe orthostatic hypotension (OH) and sudden death syndrome^[2-6]. At the early stages CAN can be subclinical and it becomes clinically evident as the disease progresses^[7-9].

Based on the CAN Subcommittee of the Toronto Consensus Panel on Diabetic Neuropathy^[5] and the American Diabetes Association (ADA)^[3], CAN is defined as the impairment of cardiovascular autonomic control in patients with DM following the exclusion of other causes. Cardiovascular autonomic reflex tests (CARTs) are usually used for CAN diagnosis and staging^[5,10].

CAN treatment is a complex process, that includes: Lifestyle modification; reducing insulin resistance (IR); tight glucose control; management of diabetic dyslipidemia (DLP); antioxidants; vitamins; treatment of myocardial metabolic abnormalities; thrombosis;

management of OH; symptomatic treatment of concomitant diseases and others^[11-18]. This study was aimed to review the existing data about the risk factors, prophylaxis, early diagnosis, treatment, and treatment perspectives of patients with DM and CAN.

The PubMed and MEDLINE, Scopus, BIOSIS, EMBASE, Google Scholar and Springer Online Archives Collection were used to conduct a search of the literature. Keywords used were "cardiac autonomic neuropathy", "silent myocardial infarction", "sudden death syndrome", "heart rate variability", "orthostatic hypotension", "cardiovascular autonomic reflex tests" in combination with the term "diabetes" for the years from 1990 until today. In addition, a manual search of some reference lists of relevant reviews and trials was performed.

RISK FACTORS FOR CAN

The risk of developing autonomic dysfunction in DM depends on several factors. However, two of them are common to both type 1 DM (T1DM) and type 2 DM (T2DM): Degree of glycemic control and disease duration. Inadequate glucose control plays an important role in the initial pathophysiology [microcirculation dysfunction due to nitric oxide (NO) loss, oxidative stress (OS) and accumulation of free radicals with lesion of Schwann cell] as well as in its progression (neuronal apoptosis and axonal degeneration)^[19-21].

The pathophysiological mechanism of diabetic neuropathies development is multifactorial, and there is enough evidence that small-fiber diabetic polyneuropathy (DPN) and even CAN may precede DM^[22].

Several studies reported the important role of cardiovascular risk factors, such as systolic blood pressure (BP), triglycerides (TGs) level, body mass index (BMI) and smoking, in the development of CAN^[21].

Even more important, however, were the results of the Intensified Multifactorial Intervention in Patients With Type 2 Diabetes and Microalbuminuria (Steno 2) study, in which the intensified multifactorial intervention (hyperglycemia, DLP, hypertension, and microalbuminuria) in patients with T2DM reduced the risk of CAN progression by 68%^[23,24]. The role of intensive control in preventing and slowing the progression of CAN in patients with T1DM is also well-known: In the Diabetes Control and Complications Trial (DCCT), its prevalence was reduced by 53%^[21,25].

The main predictors for the development of CAN in patients with T2DM are age, gender, ethnicity and presence of microvascular complications [nephropathy, retinopathy, and peripheral neuropathy (PNP)]^[6]. In a cohort of 1000 T2DM people, the development of CAN 7.5 years of follow-up was correlated with older age and the presence of microvascular disease^[26]. In terms of gender, in a multicenter study of 3250 patients with DM, there was no difference in the prevalence of CAN between men and women (men 35% and women 37%)^[27]. However, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study which involved more than 8000 T2DM patients, CAN was more prevalent in

Table 1 Cardiac autonomic neuropathy in type 1 and type 2 diabetes mellitus: Differences in relation to risk factors and natural history^[21]

Risk factors	Type 1 DM	Type 2 DM
Age	+	+
Gender (female)	+	-
Obesity	-	+
Hyperinsulinemia	NA	+
Duration of DM	++	++
Smoking	+	+
HbA1c	++	++
Hypertension	++	+
Retinopathy	++	+
Hypertriglyceridemia	+	+
Classical DPN	++	++
Microalbuminuria	++	++
Dyslipoproteinemia (> LDL and < HDL)	+	(+)
Prevalence at diagnosis of DM	7.70%	5%
Prevalence after 10 yr	38%	65%
Prevalence (random)	25%	34%

++: Strong association; +: Moderate association; -: Not found; (+): Controversial; NA: Not applicable; DM: Diabetes mellitus; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

women (2.2% in women and 1.4% in men for severe; 4.7% in women and 2.6% in men for moderate to severe)^[28].

According to data obtained from cross-sectional or longitudinal studies clinical predictors or correlates of CAN were age, diabetes duration, glycemic control, the presence of other chronic DM complications, such as diabetic retinopathy, DPN, diabetic nephropathy, and renal failure^[5,19,22,29,30]. The value of several cardiovascular risk factors in development of CAN has also been reported: Hypertension, smoking (only in cross-sectional studies), decreased high-density lipoprotein cholesterol (HDL-C), increased low-density lipoprotein cholesterol (LDL-C), TGs levels, obesity in T2DM (with some controversy), insulin levels in T2DM, waist circumference, cardiovascular disease, and use of anti-hypertensive drugs^[5,19,29-31]. Current data that differentiate CAN in T1DM and in T2DM in terms of risk factors and natural history are summarized in Table 1^[21].

Possible factors associated with high mortality and sudden death due to autonomic neuropathy are^[27]: Cardiorespiratory arrest/increased perioperative and peri-intubation risk; silent myocardial ischaemia (SMI)/infarction; hypertension; ventricular arrhythmias/prolongation of the QT interval (QTi); resting tachycardia; orthostatic hypotension (OH); exaggerated BP responses with supine position and exercise; flattening of the nocturnal reduction of BP and heart rate (HR); abnormal diastolic/systolic left ventricular function; impaired cardiovascular responsiveness; poor exercise tolerance; heat intolerance due to defective sympathetic thermoregulation; hypoglycemia unawareness; increased risk of severe hypoglycemia; obstructive sleep apnoea syndrome; susceptibility to foot ulcers and amputations

due to arteriovenous shunting and sudomotor dysfunction.

MORBIDITY AND MORTALITY IN CAN

Reduced heart rate variability (HRV) has been shown to have direct independent consequences in terms of morbidity and mortality in patients with prediabetes and DM^[32]. Development of autonomic dysfunction in T1DM is accompanied by the four time higher risk of mortality^[33,34].

CAN is strongly associated with increased mortality^[5,35,36], and in some studies with morbidity, such as stroke, coronary artery disease (CAD) and SMI. A diminished Valsalva heart rate (HR) ratio was significantly associated with development of SMI^[5,37]. According to the European Epidemiology and Prevention of Diabetes (EURODIAB) study autonomic dysfunction was associated with coexisting cardiovascular disease (CVD), glycated hemoglobin (HbA_{1c}) level, duration of T1DM and was diagnosed in one-third of patients^[32]. Results from the ACCORD trial again confirmed the association of CAN and mortality. These investigations showed that the individuals in this trial with baseline CAN were 1.55-2.14 times as likely to die as individuals without CAN^[5,28]. Furthermore, CAN in the presence of DPN was the highest predictor of CVD mortality. There is also strong evidence, based on studies in patients with T1DM and patients with T2DM that prolongation of QTi is an independent predictor of cardiovascular deaths and all-cause mortality^[5,8,34,35,38].

There is definitive evidence for a predictive value of CAN on overall mortality (class I) and some evidence on morbidity (class II). Prolongation of QTi (class II), tachycardia (class II) and non-dipping status (class III) are associated with increased mortality rate. Poor glycemic control in T1DM (class I), and a combination of obesity, DLP, hypertension and poor glycemic control in T2DM (class II) are established risk factors for CAN^[5].

CLASSIFICATION OF DIABETIC AUTONOMIC NEUROPATHIES^[39]

CAN, that is associated with reduction in HRV, resting tachycardia, OH and sudden death syndrome; Gastro-intestinal, that includes diabetic gastropathy, enteropathy and colonic hypomotility; Urogenital, that includes erectile dysfunction, diabetic cystopathy and female sexual dysfunction; Sudomotor dysfunction with development of gustatory sweating and distal hyperhidrosis; Abnormal pupillary function; Hypoglycemia unawareness.

Classification of diabetic CAN^[5]

Subclinical phase: Decreased HRs variability.

Early phase: Resting tachycardia.

Advanced stage: Exercise intolerance; Cardiomyopathy with left ventricular dysfunction; OH; Silent myocardial

Table 2 Abnormalities associated with cardiovascular autonomic neuropathy at the level of cardiovascular system and peripheral vascular function^[5,45,46]

Cardiovascular system	Peripheral vascular function
Perioperative instability	↑ Peripheral blood flow and warm skin
Resting tachycardia	↑ Arteriovenous shunting and swollen veins
Loss of reflex heart rate variations	↑ Venous pressure
Hypertension	Leg and foot oedema
Exercise intolerance	Loss of protective cutaneous vasomotor reflexes
Orthostatic hypotension	Loss of venoarteriolar reflex with microvascular damage
Postprandial hypotension	↑ Transcapillary leakage of macromolecules
Silent myocardial ischaemia	↑ Medial arterial calcification
Left ventricular dysfunction and hypertrophy	-
QT interval prolongation	-
Impaired baroreflex sensitivity	-
Non-dipping, reverse dipping	-
Sympathovagal imbalance	-
Dysregulation of cerebral circulation	-
↓ Sympathetically mediated vasodilation of coronary vessels	-
↑ Arterial stiffness	-

ischaemia.

SCREENING AND DIAGNOSIS

Cardiovascular autonomic neuropathy is by far one of the most studied forms among the various forms of diabetic autonomic neuropathies^[40,41]. Screening for CAN should be performed in T2DM patients at diagnosis and T1DM patients after 5 years of disease, in particular those at greater risk for CAN due to a history of poor glycemic control ($HbA_{1c} > 7\%$), or the presence of one major CVD risk factor, or other chronic complications of DM (level B). CAN screening may be also required in asymptomatic patients for pre-operative risk assessment before major surgical procedures (level C)^[5]. Assessment of symptoms and signs, associated with CAN should be considered in patients with hypoglycemia unawareness (level C). Patients with chronic complications of DM should be screened for CAN symptoms and signs and in case of the presence tests excluding other drug effects/interactions or comorbidities that could mimic CAN should be performed (level E)^[2,5,39]. CAN assessment can be used for cardiovascular risk stratification and as a marker for increased risk of intraoperative cardiovascular lability.

CLINICAL IMPACT OF CAN

Clinical manifestations of CAN

Symptomatic manifestations of CAN include sinus tachycardia, exercise intolerance and OH. Depending on studied diabetic populations OH was present in 6%-32% of patients with DM^[5,21,42]. The symptoms of OH, such as dizziness, light-headedness, fainting, blurred vision were found out in 4%-18% of diabetic patients^[5,22]. Orthostatic intolerance symptoms may be worse in the early morning, during prolonged standing, after meals, or physical activity^[5,43,44], that may contribute to the associated with CAN burden (Table 2).

Light-headedness, palpitations, weakness, faintness,

and syncope are the most common symptoms of CAN, that occurs upon standing^[5,45,46] (Table 2). It may be considered to perform screening among patients with unawareness of hypoglycemia, as this condition may be associated with CAN^[30,39,45,47-50].

Development of OH is associated with advanced disease stage and is easy to recognize in the office. There is no compensatory increase in the HR, despite hypotension in most cases of CAN^[5,39,46,51]. CAN diagnosis includes evaluation of symptoms (Table 3) and signs of CAN (higher resting HR, presence of OH and impaired HRV). In patients with microvascular and neuropathic complications should be performed evaluation for symptoms and signs of autonomic neuropathy (level E)^[39,49,52].

CAN ASSESSMENT

Assessment of CAN symptoms

According to the Rochester Diabetic Neuropathy Study the correlation between the autonomic deficits and symptoms was weak in patients with T1DM and absent in T2DM patients^[5,43,44].

Assessment of CAN signs

Resting tachycardia: A fixed HR that is unresponsive to moderate exercise, stress or sleep indicates almost complete cardiac denervation^[8,32,53]. Higher resting HR (> 78 bpm) compared with lower resting HR (< 58 bpm) and a rise in HR with time have been shown to be independent risk predictors for all-cause and CVD mortality^[5,32,36].

Exercise intolerance: Autonomic dysfunction impairs exercise tolerance, reduces response in HR and BP, and blunts increases in cardiac output in response to exercise. To avoid hazardous levels of intensity of exercise patients with CAN need to rely on their perceived exertion, not HR. Presently, there is inadequate evidence to

Table 3 Symptoms and signs associated with diabetic cardiovascular autonomic neuropathy^[39]

Cardiovascular autonomic neuropathy	
Resting tachycardia	
Abnormal blood pressure regulation	Nondipping Reverse dipping
Orthostatic hypotension (all with standing)	Light-headedness Weakness Faintness Visual impairment Syncope
Orthostatic tachycardia or bradycardia and chronotropic incompetence (all with standing)	Light-headedness Weakness Faintness Dizziness Visual impairment Syncope
Exercise intolerance	

recommend routine screening of asymptomatic diabetic patients with an exercise ECG test^[5,8,32].

OH: OH is an excessive fall in BP level (is a drop of > 20 mmHg systolic or/and > 10 mmHg diastolic BP) within 3 min of standing and a fall of 30 mmHg systolic BP when a person assumes a standing position. OH is characterized by symptoms that occur after standing: Lightheadedness, weakness, faintness, dizziness, palpitations, blurred vision, and even nausea and syncope^[5,8,32,43,51].

Orthostatic tachycardia syndrome: Symptoms compatible with orthostasis, such as feeling faint or dizzy, circumoral paresthesia may be caused by postural tachycardia syndrome (POTS), neurocardiogenic syncope, inappropriate sinus tachycardia, or abnormalities in baroreceptor function^[5,8,32].

QTi prolongation: Prolongation of QTi has been defined as a QTc (corrected QT for HR) ≥ 450 ms in men and ≥ 460 ms in women^[54]. Hyperinsulinemia can induce reversible prolongation of QTi in healthy subjects, hyperglycemia and acute hypoglycemia can induce the prolongation of QTi in both healthy and diabetic patients^[38,55,56]. In patients with T1DM prolongation of QTc was found out during overnight hypoglycemia and support an arrhythmic basis for the "dead in bed" syndrome^[5,57].

Impaired HRV: Decrease in HRV is the earliest clinical indicator of CAN. In health people the HR has a high degree of beat-to-beat variability and HRV fluctuates increasing with inspiration and decreasing with expiration. Impaired HRV is a strong, independent predictor of increased mortality after acute MI^[8,46].

Reverse dipping and non-dipping pattern: At night, normal individuals exhibit reduction in nocturnal BP, associated with predominance of vagal tone and decreased sympathetic activity. In diabetic patients with CAN this pattern is altered, resulting in predominance of sympathetic tone during night and development

of nocturnal hypertension. This is associated with a development of left ventricular (LV) hypertrophy and increased cardiovascular morbidity and mortality rate in patients with DM and CAN^[5,46]. In research and management of arterial hypertension ambulatory blood pressure monitoring (ABPM) is a standard tool with regard to diagnostic, prognostic, and therapeutic issues^[58]. CAN was associated with both violations of the circadian variation in BP, namely non-dipping or reverse dipping condition. So, ABPM may be useful in detecting of the circadian variation in BP violations, orthostatic and postprandial hypotension, and in achieving BP goals. The presence of non-dipping or reverse dipping in ABPM requires CAN testing and may suggest the presence of CAN^[5].

"Silent" myocardial ischemia/cardiac denervation syndrome: "Silent" ischemia in diabetic patients can either result from CAN, from autonomic dysfunction attributable to CAD itself, or from both. Altered pain thresholds, subthreshold by ischemia not sufficient to induce pain and dysfunction of the afferent cardiac autonomic nerve fibers have all been suggested as possible mechanisms^[32,59]. Development of nausea, vomiting, cough, dyspnea, tiredness and electrocardiography (ECG) changes are the features of an MI in patients with CAN^[8].

CAN and "dead in bed" syndrome

Sudden, unexpected deaths occur among subjects with CAN^[60]. Imaging of myocardial sympathetic innervation has shown that predisposition to arrhythmias may also be related to intracardiac sympathetic imbalance^[61,62]. In the Rochester Diabetic Neuropathy Study^[61,63], the investigators found that all cases of sudden death in individuals with and without DM had severe CAD or LV dysfunction.

Intraoperative cardiovascular liability

Development of DM is accompanied by the two-three times higher risk of perioperative cardiovascular morbidity and mortality^[32,64]. Preoperative screening

Table 4 Cardiovascular autonomic reflex tests^[29,42]

Test	Technique	Normal response and values
Beat-to-beat HRV	With the patient at rest and supine, heart rate is monitored by ECG while the patient breathes in and out at 6 breaths per minute, paced by a metronome or similar device	A difference in HR of > 15 beats per minute is normal and < 10 beats per minute is abnormal. The lowest normal value for the expiration-to inspiration ratio of the R-R interval decreases with age: age 20-24 yr, 1.17; 25-29, 1.15; 30-34, 1.13; 35-39, 1.12; 40-44, 1.10; 45-49, 1.08; 50-54, 1.07; 55-59, 1.06; 60-64, 1.04; 65-69, 1.03; and 70-75, 1.02
Heart rate response to standing	During continuous ECG monitoring, the R-R interval is measured at beats 15 and 30 after standing	Normally, a tachycardia is followed by reflex bradycardia. The 30:15 ratio should be > 1.03, borderline 1.01-1.03
Heart rate response to the Valsalva maneuver	The subject forcibly exhales into the mouthpiece of a manometer to 40 mmHg for 15 s during ECG monitoring	Healthy subjects develop tachycardia and peripheral vasoconstriction during strain and an overshoot bradycardia and rise in BP with release. The normal ratio of longest R-R to shortest R-R is > 1.2, borderline 1.11-1.2
Systolic blood pressure response to standing	Systolic BP is measured in the supine subject. The patient stands and the systolic BP is measured after 2 min	Normal response is a fall of < 10 mmHg, borderline fall is a fall of 10-29 mmHg and abnormal fall is a decrease of > 30 mmHg
Diastolic blood pressure response to isometric exercise	The subject squeezes a handgrip dynamometer to establish a maximum. Grip is then squeezed at 30% maximum for 5 min	The normal response for diastolic BP is a rise of > 16 mmHg in the other arm, borderline 11-15 mmHg

HRV: Heart rate variability; ECG: Electrocardiography; BP: Blood pressure; HR: Heart rate.

for CAN should be performed in patients with reduced hypoxic-induced ventilatory drive^[32] and identify patients with greater intraoperative complications risk^[8,32]. Thus, resting HR is not a specific sign of CAN (class IV). After exclusion of other causes OH suggests an advanced CAN that should be confirmed by cardiovascular autonomic reflex tests (CARTs) (class I). Specific but insensitive CAN indices are QT_i prolongation (class II), OH (class III) and reverse dipping (class III)^[32].

DIAGNOSTIC TESTING FOR CAN

Cardiovascular autonomic reflex tests

CARTs are considered as gold-standard measures of autonomic function^[32]. Postural change of BP (OH) and sustained isometric muscular strain provide indices of sympathetic function, whereas the HR variations during deep breathing, lying-to-standing (HR tests) and Valsalva maneuver are indices mainly of parasympathetic function. Diagnostic tests of CAN are summarized in Table 4. The normal, borderline and abnormal values in tests of cardiovascular autonomic function are summarized in Table 5.

According to CAN subcommittee in the Toronto Diabetic Neuropathy Consensus Panel, CAN diagnostic criteria are divided as follows: A positive one test is early diagnosis of CAN; the presence of two or three positive tests is required for definitive diagnosis of CAN; the presence of OH combined with one of the previous criteria is defined as severe CAN^[5].

The main clinical indications of the autonomic reflex tests^[5,52,65]: Diagnosis and staging of CAN in T2DM patients (at diagnosis and annually thereafter); diagnosis and staging of CAN in T1DM patients (5 years after diagnosis and annually thereafter); stratification of cardiovascular risk: In pre-operative testing, pre-physical activity, indication of selective beta-blocker, and suspected silent

ischemia; differential diagnosis of other manifestations of CAN (regardless of DM duration): Assess whether gastroparesis, erectile dysfunction, OH, dizziness, syncope, or tachycardia in diabetic persons are due to dysautonomia; evaluate the progression of autonomic failure and monitor response to therapy (e.g., continuous infusion of insulin, post-transplants, and use of antioxidants); differential diagnosis of other causes of neuropathy such as autoimmune autonomic neuropathy (chronic inflammatory demyelinating polyneuropathy, celiac disease, amyotrophy) or toxic-infectious neuropathy (alcohol, primary neuritic Hansen's disease, human immunodeficiency virus) as well as in cases where the presence of autonomic neuropathy is disproportionate to the sensory-motor neuropathy.

To the most sensitive and specific diagnostic tests available for CAN evaluation belongs HRV, muscle sympathetic nerve activity (MSNA), baroreflex sensitivity (BRS), plasma catecholamines, and heart sympathetic imaging^[50,66].

Short-term ECG recording

The short-term ECG recordings can be analyzed by dedicated software in the frequency domain. This method usually uses the Fourier method, which transform R-R intervals into waves with three basis components: Very low frequency ≤ 0.04 Hz (VLF); low frequency 0.04-0.15 Hz (LF) and high frequency 0.15-0.4 Hz (HF). LF represents combined effects of sympathetic and parasympathetic influence, whereas HF represents vagal activity. A decrease in HF is a sign of parasympathetic dysfunction, in the early stages of autonomic dysfunction in DM, when sympathetic predominance is observed it leads to an increase in LF/HF^[67]. It is not clear if classical Ewing's tests or time-domain methods are better for diagnosis of CAN. However, Ewing's tests are simpler and can be more easily implemented during routine clinical use.

Table 5 Normal, borderline and abnormal values in tests of cardiovascular autonomic function^[27]

	Normal	Borderline	Abnormal
Tests reflecting mainly parasympathetic function			
Heart rate response to Valsalva Manoeuvre (Valsalva ratio)	≥ 1.21	1.11–1.20	≤ 1.10
Heart rate (R-R interval) variation	≥ 15 beats/min	11–14 beats/min	≤ 10 beats/min
During deep breathing (maximum-minimum heart rate) immediate heart rate response to standing (30:15 ratio)	≥ 1.04	1.01–1.03	≤ 1.00
Tests reflecting mainly sympathetic function			
Blood pressure response to standing (fall in systolic blood mmHg mmHg mmHg pressure)	≤ 10	11–29	≥ 30
Blood pressure response to sustained handgrip (increase in diastolic blood pressure)	≥ 16 mmHg	11–15 mmHg	≤ 10 mmHg

HR variability

Possible mechanisms, which can affect HR are: Efferences of sympathetic and parasympathetic nervous system to the sinus node, ionic changes in the sinus node, neurohumoral influences, local temperature changes. The short-term HRV is essentially determined by the sympathetic and parasympathetic efferences and stretch of the sinus nod under resting conditions.

The state of sympathetic and parasympathetic is responsible for a physiologic variation in the HR and HRV. The evaluation of HRV can be performed in the time and frequency domains^[5,50,66].

Time domain measures include the standard deviation of 5-min average of normal R-R intervals (SDANN), the difference between the longest and shortest R-R intervals and the root-mean square of the difference of successive R-R intervals (RMSSD). The number of instances per hour in which two consecutive R-R intervals differ by more than 50 ms over 24 h (pNN50) can be calculated by longer recordings. All these indices explore the parasympathetic activity^[67].

It is obvious that reduction in HRV is associated with CAN, but this method has no standard values for diagnosis CAN^[68,69]. Also during 24 h recording many factors can have an influence on HRV parameters, such as concomitant illness, use of medication, and lifestyle factors (exercise, stress, smoking, etc.). The analysis of ECG recordings in conjunction with respiration and beat-to-beat BP recordings is the best approach to HRV testing (level C).

HR turbulence

HR turbulence (HRT) is a method for CAN detection by Holter-based technique^[70,71].

Baroreflex sensitivity

The interesting approach that combines information derived from BP and HR is BRS that can be done with several methods: Spontaneous BP variations can be measured and drugs or physical manoeuvres can be applied to modify BP. None of the BRS tests available today shown a clinically relevant difference or definite advantage over the others^[72]. Although the results of some studies in diabetic patients suggest an early impairment of BRS, the diagnostic accuracy of BRS measures was evaluated in very few studies^[50,73]. Cardiac vagal BRS is a independent

prognostic index for cardiovascular mortality in the general (class II). The presence of early abnormalities with respect to CARTs warrant the clinical use of BRS in identifying subjects at risk for CAN (classes II–III).

Muscle sympathetic nerve activity

Blunted responsiveness to physiological hyperinsulinemia or glucose ingestion and increased resting MSNA have been described among T2DM with neuroadrenergic autonomic dysfunction and obesity. MSNA abnormalities reverse with weight loss^[50,66], but in contrast, T1DM is associated with a by about half decrease in the number of bursts^[74]. MSNA allows direct and continuous measurement of sympathetic nerve traffic (class I). Resting MSNA might be increased in early T2DM, possibly due to hyperinsulinemia and type 1 diabetes is associated with a MSNA reduction (class IV). This technique requires specialized personal, is difficult, time-consuming, invasive, and cannot be repeated often (class II)^[50].

Cardiovascular sympathetic tests and catecholamine assessment

The determination of norepinephrine in plasma is in principle the biochemical equivalent of MSNA. While norepinephrine clearance is low in idiopathic autonomic neuropathy, this was not in the case of CAN^[50,75]. The plasma catecholamine measurements can not be mandatory recommended for routine CAN diagnosis in clinical practice (level C)^[50].

Heart sympathetic imaging

Cardiac sympathetic innervation is possible to assess by using radiolabelled sympathomimetic amines or catecholamines(^[123]I)-meta-iodobenzylguanidine (MIBG), [¹¹C]-meta-hydroxyephedrine (HED), 6-^{[18}F] dopamine, and [¹¹C]-epinephrine^[50,76–78]). Regional differences in vesicular uptake or retention was determined in subjects with T1DM and CAN by analysing the washout rates of [¹¹C]-epinephrine parallels those of [¹¹C]-HED^[50,79,80].

Scintigraphic tracers directly assess the structural integrity of the sympathetic nervous system supply to the heart (class III). Heart sympathetic imaging has greater sensitivity to detect changes in sympathetic neuronal function and/or structure^[50,81]. The indices of myocardial perfusion and LV dysfunction in T1DM correlate with scintigraphic data (class III).

Table 6 Diagnostic algorithm for diabetic cardiac autonomic neuropathy^[3,39]

Symptoms		Signs/diagnostic tests	Differential workup
Resting tachycardia	Palpitations could be asymptomatic	Clinical exam: Resting heart rate > 100 bpm	Anemia hyperthyroidism fever CVD (atrial fibrillation, flutter, other) Dehydration Adrenal insufficiency Some medications Smoking, alcohol, caffeine Recreational drugs (cocaine, amphetamines, methamphetamine, mephedrone) Adrenal insufficiency
Orthostatic hypotension	Light-headedness Weakness Faintness Visual impairment Syncope	Clinical exam: A reduction of > 20 mmHg in the systolic blood pressure or > 10 mmHg in diastolic blood pressure	Intravascular volume depletion Blood loss/acute anemia Dehydration Pregnancy/postpartum CVD Alcohol Medication Antiadrenergics Antianginals Antiarrhythmics Anticholinergics Diuretics ACE inhibitors/angiotensin receptor blocker Narcotics Neuroleptics Sedatives

CAD: Coronary artery disease; CVD: Cardiovascular disease.

Diagnostic testing for orthostatic symptoms

A standard test for establishing the cause of postural symptoms is the head-up tilt-table study. Other functional syndromes may also be revealed, such as paradoxical orthostatic bradycardia syndrome and the vasoconstrictor syndrome (paradoxical orthostatic hypertensive syndrome, also known as OH)^[8].

Diagnostic algorithm for diabetic CAN (Table 6)**Differential diagnosis of diabetic neuropathies:**

Differential diagnosis of diabetic neuropathies should be performed by excluding other causes of neuropathy (Table 7), by undertaking a medication history and family history and performing relevant testing (*e.g.*, blood count, folic acid, serum B₁₂, metabolic panel, thyroid hormones)^[49].

Neuropathy end points for research and clinical practice^[3,39]:

For clinical trials the recommended CAN measures include: standardized CARTs that are specific, sensitive, simple^[5,39,49,82,83]; HRV indices^[39,45,50,84]; resting QTc and HR^[28,34,39,85]; other methods are expensive and time-consuming, require trained personnel (baro-reflex sensitivity, cardiac sympathetic imaging, and microneurography)^[5,39,50,86].

Diagnostic criteria for CAN

The CAN Subcommittee of the Toronto Consensus Panel on Diabetic Neuropathy established four reasons why

the diagnosis of CAN is relevant to clinical practice^[5]: For diagnosing and staging the different clinical forms of CAN: Initial, definite, and advanced or severe; for stratifying the degree of cardiovascular risk and the risk of other diabetic complications; for the differential diagnosis of clinical manifestations and their respective treatment; to adapt the goal of HbA_{1c} in each patient: For example, those with initial stages of CAN should have a more intensive glycemic control while patients with severe CAN should have a less aggressive glycemic control due to the risk of asymptomatic hypoglycemia. CARTs are the “gold” standard clinical tests for cardiovascular autonomic neuropathy^[5]. In the CAN Subcommittee of the Toronto Consensus Panel statement are defined criteria for CAN definition and severity^[5,6]. For the early CAN diagnosis only one abnormal CART result (among the 7 tests: 5 CARTs and HRV tests in time- and frequency-domains) is sufficient; definite CAN should be confirmed by 2 or 3 abnormal tests and severe CAN can be indicated by development of OH^[5,71,87].

Staging of CAN

Ewing *et al.*^[42] (1985) proposed a classification based on “early involvement” (two borderline test results or one abnormal result on HR test), “definite involvement” (two or more abnormal results on HR tests), and “severe involvement” (development of OH).

The following CARTs are the “gold” standard for clinical autonomic testing: HR response to deep breathing,

Table 7 Differential diagnosis of diabetic neuropathies^[39]

Metabolic disease	Thyroid disease (common)
	Renal disease
Systemic disease	Systemic vasculitis
	Nonsystemic vasculitis
	Paraproteinemia (common)
	Amyloidosis
Infectious	Human immunodeficiency virus
	Hepatitis B
	Lyme
Inflammatory	Chronic inflammatory demyelinating polyradiculoneuropathy
Nutritional	B12
	Postgastroplasty
	Pyridoxine
	Thiamine
	Tocopherol
	Industrial agents, drugs, and metals
	Industrial agents
	Acrylamide
	Organophosphorous agents
Drugs	Alcohol
	Amiodarone
	Colchicine
	Dapsone
	Vinka alkaloids
Metals	Platinum
	Taxol
	Arsenic
	Mercury
Hereditary	Hereditary motor, sensory, and autonomic neuropathies

standing, and Valsalva manoeuvre, and BP response to standing (class II); these CARTs are sensitive, specific, reproducible, easy to perform, safe and standardized (classes II and III); the Valsalva manoeuvre is not advisable in the presence of increased risk of retinal haemorrhage and proliferative retinopathy (class IV). Age is the most relevant factor affecting HR tests (class I); a definite diagnosis of CAN and CAN staging requires more than one HR test and the OH test (class III)^[5].

PREVENTION OF THE CAN

Prevention of diabetic neuropathies focuses on lifestyle modifications and tight glucose control. Early optimization of glucose control in patients with T1DM (class A) and a multifactorial approach targeting glycaemia among other cardiovascular risk factors in patients with T2DM (class C) were considered for prevention or delay of CAN development^[39].

TREATMENT OF THE CAN

Implementation of tight glucose control as early as possible to prevent or delay the development of CAN in the course of T1DM (class A); consider a multifactorial approach in the course of T2DM (class C).

CAN treatment is a complex process, that includes: Lifestyle modification; reducing IR; intensive glycemic control; treatment of DLP; antioxidants, first of all

α -lipoic acid (ALA), aldose reductase inhibitors, acetyl-L-carnitine; vitamins, first of all fat-soluble vitamin B₁; correction of vascular endothelial dysfunction; prevention and treatment of thrombosis; in severe cases-treatment of OH^[88].

Glucose control

In the DCCT intensive glucose control reduced the risk of CAN development by 45% and in the Epidemiology of Diabetes Interventions and Complications (EDIC) study, this risk was reduced by 31%^[39,48].

The large sample size in DCCT/EDIC, the robust definitions used for CAN and the highly reproducible and sensitive testing protocol support tight glycemic control for prevention or delay of CAN development in the course of T1DM. In contrast, intensive glucose control has not consistently lowered the risk of CAN development in T2DM^[39,47]. Lifestyle modification, tight glycemic control and targeting cardiovascular disease risk factors reduced the risk of CAN development by 60% in patients with T2DM^[24,39].

Lifestyle modifications

Lifestyle modifications include rational nutrition and optimal level of physical activity and correction of obesity. Active lifestyle is accompanied by the three times less risk of increased mortality rate than sedentary lifestyle (less than 1000 kcal/wk)^[89].

The ADA does not recommend a specific diet over another for the diabetic patients and lists three different diets for individuals who have or are at risk of having DM (low-carbohydrate, low-fat calorie-restricted or Mediterranean diet)^[90]. Although there are no studies looking at the cardiovascular outcome in diabetic patients only there is some cardiovascular benefit of adhering to a Mediterranean diet in diabetic patients.

Although the DPP^[39,91] and the Impaired Glucose Tolerance Neuropathy (IGTN) study^[39,92] reported benefits of lifestyle modification on diabetic symmetrical sensory neuropathy (DSPN) and CAN measures, respectively, these trials did not include DM patients. The best models to date regarding effectiveness of intensive lifestyle intervention come from the DPP^[24], the Steno-2 Study, the Italian supervised treadmill study^[93], and the University of Utah T2DM study^[94]. The risk of adverse events or exercise-induced injury through decreased cardiac responsiveness to exercise, impaired thermoregulation, OH, impaired night vision due to impaired papillary reaction, and greater susceptibility to hypoglycemia can be increased in patients with autonomic neuropathy^[5]. CAN is considered also as an independent risk factor for development of SMI and cardiovascular death^[28]. Therefore, individuals with diabetic CAN should undergo cardiac investigation before beginning physical activity more intense than that to which they are accustomed^[3].

Most peripheral neuropathy affects the extremities, particularly the lower legs and the feet, but also the hands, whereas damage to the ANS may lead to imbalances

between the sympathetic and parasympathetic nerve fibers that innervate the heart and blood vessels, as well as abnormalities in HR control and vascular dynamics. To prescribe or engage in exercise that is both safe and effective, health care providers and patients with DM need to increase their understanding of the pathophysiological nature of neuropathies and the physical activity hurdles that may arise from the presence of a neuropathy. With proper care and preventative measures, patients with DM that experience either type of neuropathy can benefit from regular participation in mild to moderate aerobic, resistance, and balance activities, assuming they take any potential alterations into account to ensure that exercise is safe and effective^[95,96]. Individuals with CAN should be screened and receive physician approval and possibly an exercise stress test before exercise initiation. Exercise intensity is best prescribed using the HR reserve method with direct measurement of maximal HR^[95,96].

Individuals with autonomic neuropathy (particularly CAN) should avoid high-intensity physical activities unless they have been cleared by a physician to participate: They should also avoid physical exertion in hot or cold environments since dehydration may be a risk for those who have difficulty with thermoregulation; individuals must be made aware that hypotension may occur after vigorous activities; recumbent cycling or water aerobics may be safer activities for individuals with OH; for better accuracy, individuals should monitor exercise intensity using the HR reserve method using a measured maximal HR, if possible, or use perceived exertion. The results indicate that 6-mo aerobic exercise training improves the cardiac ANS function in T2DM patients. However, more favourable effects are found in T2DM patients with definite CAN^[97].

Glucose control

The DCCT and the follow-up observational EDIC study (DCCT/EDIC) stands as the pivotal trial demonstrating clear and persistent benefits of tight glucose control for both DSPN and CAN in patients with T1DM^[47,48,94,98-100]. DCCT enrolled patients with T1DM who were randomly assigned to intensive or conventional insulin therapy^[47,48,101-103]. The risk reduction in incident CAN with intensive therapy during DCCT was 45%^[47,48,101,103]. The DCCT/EDIC has furthered the understanding of the role of glucose control in the development and progression of neuropathy^[47,48,103,104]. The Kumamoto trial, the first randomized controlled trial to report beneficial effects of tight glucose control, reported no differences on CAN measures^[47,105]. The UKPDS trial enrolled 3,867 relatively young patients with newly diagnosed T2DM. By the end of the trial, intensive glucose control had no effect on DSPN or CAN^[47,106,107]. The VADT trial randomized 1,791 veterans with T2DM to either intensive or standard glucose control. After approximately 5.6 years of follow-up, there were no differences in the rates of new DSPN in the intensive vs standard arm, despite significant differences in the mean HbA_{1c} between groups^[47,108,109]. The ADDITION trial did not obtain baseline evaluations

for DSPN or CAN, preventing objective evaluations of change in DSPN or CAN with intervention^[110-112].

Drugs for treatment of hypercholesterolemia

The 3-hydroxy-3-methylglutaryl-coenzyme reductase inhibitors: The 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase inhibitors (lovastatin, pravastatin, fluvastatin, simvastatin, atorvastatin, rosuvastatin, pitavastatin). By inhibiting HMG-CoA reductase, statins block the pathway for synthesizing cholesterol in liver. The reduction in cholesterol level induces an increased expression of the low density lipoprotein receptor (LDLR), which results in decreased concentration of LDL-C and other apolipoprotein B (apoB)-containing lipoproteins^[113].

Secondary prevention statin studies such as MRC/BHF Heart Protection Study (HPS) showed significant risk reduction among individuals with DM. Based on this, the primary prevention of CVD with atorvastatin in T2DM in the Collaborative Atorvastatin Diabetes Study (CARDS) was designed to assess the effects of aggressive lipid lowering on the primary prevention of atherosclerotic CVD in individuals with T2DM. In individuals with average or mildly elevated LDL-C at baseline (mean 117 mg/dL), an LDL-C reduction to a mean of 82 mg/dL was accompanied by a 37% reduction in major cardiovascular events compared with placebo. CARDS, which originally planned a mean follow-up of 4 years, was terminated 2 years early because of the significant benefit achieved in the statin group^[114,115].

Cholesterol absorption (ezetimibe): In summary, cholesterol absorption inhibitors^[113,115]: ↓ LDL-C 10%-18% by inhibiting intestinal absorption of cholesterol and decreasing delivery to the liver (primarily); ↓ ApoB 11%-16%; ↓ LDL-C 25%, total LDL-C 34%-61% (in combination with statins); ↓ LDL-C 20%-22% and apo B 25%-26% without reducing increasing HDL-C (in combination with fenofibrate). Ezetimibe: Usual recommended starting daily dosage 10 mg; dosage range 10 mg^[115].

PCSK9 inhibitors (proprotein convertase subtilisin/kexin type 9) inhibitors (alirocumab, evolocumab):

Two monoclonal antibody inhibitors of PCSK9, a protein that regulates the recycling of LDLR, have recently been approved by the Food and Drug Administration (FDA)^[116,117]. Alirocumab and evolocumab are subcutaneously injectable LDL-lowering agents capable of further reducing LDL approximately 60% when added to maximum statin therapy^[118-122]. Alirocumab: Usual recommended starting daily dosage 75 mg every 2 wk; dosage range 75-150 mg every 2 wk. Evolocumab. Usual recommended starting daily dosage 140 mg every 2 wk or 420 mg once mo; dosage range not applicable^[115].

Fibric acid derivatives (gemfibrozil, fenofibrate, fenofibric acid): Fibrates are agonists of peroxisome

proliferator-activated receptor- α (PPAR- α), acting *via* transcription factors regulating various steps in lipid and lipoprotein metabolism^[113,123]. Fenofibrate: Usual recommended starting daily dosage 48-145 mg; dosage range 48-145 mg; Gemfibrozil: Usual recommended starting daily dosage 1.200 mg; dosage range 1.200 mg; Fenofibric acid: Usual recommended starting daily dosage 45-135 mg; dosage range 45-135 mg^[115].

Niacin (nicotinic acid): Nicotinic acid has been reported to decrease fatty acid influx to the liver and the secretion of VLDL by the liver; this effect appears to be mediated in part by the effects on hormone-sensitive lipase in the adipose tissue. Nicotinic acid has key action sites in both liver and adipose tissue. In the liver nicotinic acid is reported to inhibit diacylglycerol acyltransferase-2 (DGAT-2) that results in the decreased secretion of VLDL particles from the liver, which is also reflected in reductions of both IDL and LDL particles. Nicotinic acid raises HDL-C and apolipoprotein A1 (apoA1) primarily by stimulating apoA1 production in the liver^[124]. The effects of nicotinic acid on lipolysis and fatty acid mobilization in adipocytes are well established^[125,126]. Nicotinic acid (immediate-release): Usual recommended starting daily dosage 250 mg; dosage range 250-3000 mg; Nicotinic acid (extended-release): Usual recommended starting daily dosage 500 mg; dosage range 500-2000 mg^[115].

Bile acid sequestrants: In summary, bile acid sequestrants^[115,127]: ↓ LDL-C (primarily) 15%-25% by binding bile acids and preventing their reabsorption in the ileum; ↓ glucose and HbA_{1c} (approximately 0.5%) (colesevelam); is FDA approved to treat T2DM. Cholestyramine: Usual recommended starting daily dosage 8-16 g; dosage range 4-24 g; Colestipol: Usual recommended starting daily dosage 2 g; dosage range 2-16 g; Colesevelam: Usual recommended starting daily dosage 3.8 g; dosage range 3.8-4.5 g; Ezetimibe/simvastatin: Usual recommended starting daily dosage 10/20 mg; dosage range 10/10-10/80 mg; Extended-release niacin/simvastatin: Usual recommended starting daily dosage 500/20 mg; dosage range 500/20-1.000/20 mg^[115].

Inhibitors of microsomal TG transfer protein

Within the lumen of the endoplasmic reticulum, lomitapide inhibits microsomal TG transfer protein (MTP), which prevents the formation of apoB, and, thus, the formation of VLDL and chylomicrons as well. Altogether, this leads to a reduction of LDL-C. Lomitapide, the MTP inhibitor, and mipomersen, the antisense oligonucleotides against apo B, have shown their efficacy in lowering LDL-C in recent phase III trials and they were already approved for treating patients with homozygous familial hypercholesterolemia^[128]. Lomitapide: Usual recommended starting daily dosage 5 mg, with subsequent titration; dosage range 5-60 mg^[115].

Antisense apolipoprotein B oligonucleotide (mi-

pomersen *via* subQ injection): Mipomersen is a second-generation antisense oligonucleotide targeted to human apoB-100, large protein synthesized by the liver that plays a fundamental role in human lipoprotein metabolism. Mipomersen predominantly distributes to the liver and decreases the production of apoB-100, the primary structural protein of the atherogenic lipoproteins including LDL, thereby reducing plasma LDL-C and apoB-100 concentrations^[129]. Mipomersen (SubQ injection): Usual recommended starting daily dosage 200 mg once weekly, with subsequent titration; dosage range 200 mg once weekly^[115].

Omega-3 fatty acids: Omega-3 polyunsaturated fatty acids (PUFAs) (eicosapentaenoic acid and docosahexaenoic acid) are used at pharmacological doses to lower TGs. Prescription of omega-3 fatty acids (2-4 g/d) results in decreased plasma concentration of TGs and VLDL concentration^[113].

In summary, omega-3 fatty acids^[115]: ↓ TG 27%-45%, TC 7%-10%, VLDL-C 20%-42%, apoB 4%, and non-HDL-C 8%-14% in individuals with severe hypertriglyceridemia, most likely by reducing hepatic VLDL-TG synthesis and/or secretion and enhancing TG clearance from circulating VLDL particles. Other potential mechanisms of action include^[115]: ↑ β -oxidation; ↓ inhibition of acyl-CoA; 1,2-diacylglycerol acyltransferase; ↓ decreased hepatic lipogenesis; ↑ increased plasma lipoprotein activity; ↓ LDL-C 5% (Icosapent ethyl); ↑ LDL-C 45% (omega-3-acid ethyl esters). Omega-3-acid ethyl esters (Lovaza): Usual recommended starting daily dosage 4 g per day; dosage range 4 g per day. Icosapent ethyl (Vascepa®) Usual recommended starting daily dosage 4 g per day; dosage range 4 g per day^[115].

Specific features of DLP in insulin resistance and type 2 diabetes

Diabetic DLP is a cluster of plasma lipid and lipoprotein abnormalities that are metabolically interrelated. The increase in large VLDL particles in T2DM initiates a sequence of events that generates atherogenic remnants, small TG-rich dense HDL particles and small dense LDL^[113,130-132].

Evidence for low-density lipoprotein-lowering therapy

The Cholesterol Treatment Trialists' meta-analysis further indicates that subjects with T2DM will have a relative risk reduction that is comparable to that seen in non-diabetic patients, but being at higher absolute risk, the absolute benefit will be greater, resulting in a lower number needed to treat^[113,133,134].

Triglycerides and high-density lipoprotein cholesterol

Clinical benefits achieved by the treatment of atherogenic DLP (high TGs and low HDL-C) are still a matter of discussion. Although the Helsinki Heart Study reported a significant reduction in CVD outcomes with gemfibrozil, neither the Fenofibrate Intervention and Event Lowering

in Diabetes (FIELD) nor the ACCORD study showed a reduction in total CVD outcomes^[113,135-137].

Treatment strategies for patients with T2DM and metabolic syndrome

Recommendations for the treatment of DLP in DM^[113]: In all patients with T1DM and in the presence of microalbuminuria and/or renal disease, LDL-C lowering (at least 50%) with statins as the first choice is recommended irrespective of the baseline LDL-C concentration (class I, level C)^[113,138,139]; in patients with T2DM and CVD, and in patients without CVD who are > 40 years of age with one or more other CVD risk factors, the recommended goal for LDL-C is < 1.8 mmol/L (< 70 mg/dL), for non-HDL-C is < 2.6 mmol/L (< 100 mg/dL) and for apoB is < 80 mg/dL (class I, level B)^[133,139].

In all patients with T2DM and no additional risk factors and/or evidence of target organ damage, LDL-C < 2.6 mmol/L (< 100 mg/dL) is the primary goal. Non-HDL-C < 3.4 mmol/L (< 130 mg/dL) and apoB < 100 mg/dL are the secondary goals (class I, level B)^[133,139].

Fatty acids metabolism disorders

Vasoactive prostanoids, metabolites and dihomono- γ -linolenic acid (DGLA) are necessary for the normal nerve conductivity and blood flow. According to the data from double-blind, placebo-controlled studies prescription of DGLA to patients with DPN was accompanied by the increase in the speed of nerve conductivity. Prescription of L-carnitine can be recommended as one of the lipid-lowering therapy components to T2DM patients^[140,141].

Antioxidant therapy

Hyperglycemia-induced OS and nitrosative stress has been singled out as one of the major links between DM and diabetic complications; leads to generation of free radicals due to autooxidation of glucose and glycosylation of proteins^[142,143]. The persistent increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) accompanied by a decrease in antioxidant (AO) activity leads to the occurrence of OS and nitrosative stress which can cause endothelial dysfunction, IR, and eventually leads to diabetic microvascular and macrovascular complications^[144]. Reactive species can be eliminated by a number of enzymatic and nonenzymatic antioxidant mechanisms. Superoxide dismutase (SOD) immediately converts O_2^- to hydrogen peroxide (H_2O_2), which is then detoxified to water either by catalase in the lysosomes or by glutathione peroxidase (GPx) in the mitochondria. Another enzyme that is important is glutathione reductase (GSR), which regenerates glutathione that is used as a hydrogen donor by GPx during the elimination of H_2O_2 ^[142,143].

Hyperlipidemia in the presence of hyperglycemia generates additional ROS that are also implicated in cell dysfunction^[143,145]. OS has been implicated in causing nerve damage in several animal, human, and experimental models of diabetes^[143,146]. The mechanisms involved in OS-induced nerve dysfunctions include

generation of ROS, increased RNS, lipid peroxidation (LPO), deoxyribonucleic acid (DNA) damage, and reduction in cellular antioxidants^[143,147]. Increased ROS and RNS together with reductions in the AO defense mechanisms within the neurons contribute to the manifestations of DPN which include nerve blood flow impairment, endoneurial hypoxia, nerve degeneration, axonal atrophy. Recent findings implicate free radicals in the development of DN in addition to the impairment of AO defense system in T2DM^[142].

Also, induction of aldose reductase enzyme depletes the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), a requirement for the regeneration of the cellular AO, reduced glutathione (GSH), contributing to OS^[7,143,148,149]. Intra- and inter-molecular cross-linking reactions with proteins, lipids, or DNA lead to the formation of stable, covalent, and irreversible adducts collectively referred to as advanced glucose end-products (AGEs) that accumulate within cells with age^[143,148]. Increased formation of AGEs leads to the elevation of OS and subsequently damage to cells and tissues, an occurrence that has been found in experimental animals and in humans^[150]. AGEs have also been shown to decrease axonal transport within neurons leading to their degeneration^[143,151].

Antioxidants are available endogenously as a physiological defense mechanism of the cell or obtained exogenously from diet. The enzymatic AO systems, such as copper, zinc, manganese and selenium, SOD, GPx, GSR, and catalase may remove the ROS directly or sequentially, preventing their excessive accumulation and consequent adverse effects. Non-enzymatic AO systems consist of scavenging molecules that are endogenously produced such as GSH, ubiquinol, and uric acid or derivatives of the diet such as vitamins A, C and E, carotenoids, lipoic acid (LA); coenzyme Q₁₀ (CoQ₁₀); and cofactors like albumin, vitamins B₁, B₃, folic and uric acids^[152,153]. Vitamins C, E and LA are involved in the termination of the LPO process^[152]. The abilities of flavonoids to scavenge free radicals have also been reported^[143]. However, in the case of macrovascular/microvascular complications, the antioxidant therapy is beneficial together with BP control, management of atherogenic DLP, and optimal glucose control^[143,153,154].

Strategies targeted directly against reactive oxygen species and reactive nitrogen species^[143]

Diabetes-induced nerve dysfunction is established to be caused by an increase in the overproduction of ROS and RNS. It was therefore hypothesized that antioxidants or agents that directly scavenge free radicals can reduce the formation or progression of ROS reactions which in turn decreases OS thereby improving DPN conditions^[143]. Some of the most important antioxidants include ALA, vitamins A, C, and E, acetyl L-carnitine, taurine, and melatonin.

ALA: ALA can be biosynthesized in plants and animals where it is metabolized to dihydrolipoic acid (DHLA)

upon uptake into cells. Both ALA and DHLA are potent free radical scavengers that are also involved in the regeneration of vitamins C and E and oxidized GSH within the cell^[155]. ALA is also a cofactor for a number of mitochondrial enzymes^[143]. ALA is known to reduce OS by inhibiting hexosamine and AGEs pathways^[143].

ALA, a critical co-factor for mitochondrial dehydrogenase reactions, is another compound with free radical-scavenging activity^[156,157]. ALA was found to increase glucose transport in muscle cells in culture by stimulating translocation of glucose transporter type 4 (GLUT4) from internal pools to the plasma membrane^[153].

Treatment with ALA protected the insulin receptor from oxidative damage, maintaining its functional integrity in cultured adipocytes. Oral administration of ALA significantly increased insulin-mediated glucose uptake, presumably by modulating insulin sensitivity in patients with T2DM^[153]. ALA600SOD (an oral formulation of ALA and SOD) improved symptoms and electroneurographic parameters among subjects with DPN^[158].

Vitamins A, B₁, B₃, C and E

Dietary antioxidant vitamins such as vitamins A, C, and E detoxify free radicals directly and also interact with recycling processes to create reduced forms of the vitamins. Antioxidant vitamins have a number of biological activities such as immune stimulation and prevention of genetic changes by inhibiting DNA damage induced by the ROS metabolites^[159].

Vitamin A: Vitamin A has a plethora of cellular actions. Besides modulating gene expression, cell growth and differentiation, this vitamin may also act as AO, although the mechanisms of action in this role are not fully deciphered^[159]. The AO potential of carotenoids (vitamin A) depends on their distinct membrane-lipid interactions, while some carotenoids can decrease LPO, others can stimulate it^[159].

Vitamin B₁: Thiamine derivatives are cofactor for enzymes involved in the production of chemical energy from carbohydrates and fat. Thiamine deficiency (TD) may be associated with specific and selective neuronal cell death and damage of the blood-brain barrier. DM might be considered as TD state, if not in absolute terms at least relative to the increased requirements deriving from accelerated and amplified glucose metabolism in non-insulin dependent tissues that, like the vessel wall, are prone to complications. The TD in clinical diabetes may increase the fragility of vascular cells to the adverse effects of hyperglycemia and there by the increase of the risk of developing microvascular complications^[160].

Nicotinamide (vitamin B₃): The vitamin plays an important role in mitochondrial energy generation and DNA repair. Deficiency of nicotinamide is associated with dermatological, gastrointestinal, hematological and

nervous system dysfunction. Sensory neuropathy due to vitamin B₃ deficiency is characterized by decreased sensation to touch and vibration^[154].

Vitamin C: Ascorbic acid serves as a cofactor for hydroxylation and function of monooxygenase enzymes in the synthesis of sub-tissues (collagen), neurotransmitters and carnitine. Ascorbic acid is an antioxidant acting as an enzymatic cofactor in maintaining tissue integrity and plays an important role in formation of epithelial and endothelial barriers and aids in regeneration of oxidized vitamin E^[154].

Vitamin C has a role in scavenging ROS and RNS by becoming oxidated itself. The oxidized products of vitamin C, ascorbic radical and dehydroascorbic radical are regenerated by GSH, the reduced form of nicotinamide adenine dinucleotide (NADH) or NADPH. In addition, vitamin C can reduce the oxidized forms of vitamin E and GSH. There is paucity of information on the role of vitamin C in DPN despite evidence that it normalizes sorbitol concentration in the blood, scavenges LPO, and regenerates GSH in diabetes^[143]. In a prospective cohort study, vitamin C intake was found to be significantly lower among incident cases of T2DM^[153].

Vitamin E: Vitamin E is a group of fat-soluble compounds that includes the AO compound alpha-tocopherol, which is a lipid-soluble AO that increases resistance of LDL-C to oxidation, reduces smooth muscle cell proliferation, and reduces adhesiveness of platelets to collagen^[154]. It inhibits LPO by scavenging reactive oxygen species and preserving cell membranes. Neurological conditions associated with vitamin E deficiency includes: Posterior spinal columns disease, spinocerebellar ataxia, peripheral neuropathy, and optic neuropathy^[154].

Vitamin E has been reported to alleviate symptoms of DM and diabetes-induced complications in animals through reduction in OS biomarkers. In clinical trials, vitamin E did not however show a significant relief of the symptoms of microvascular and macrovascular complications despite reducing OS biomarkers in the subjects^[143].

The lack of performance of vitamin E may not however be unconnected to the fact that the design of each study was not targeted directly at diabetes end-points such as HbA_{1c} < 7% levels, BP < 130/180 mmHg, avoiding hypoglycemic events, and maintaining weights but rather at complications that may have multiple causal factors^[143]. Vitamin E supplementation reduced blood glucose and HbA_{1c} levels significantly and had a neuroprotective effect on the total myenteric population, without affecting intestinal area or thickness of the intestinal wall or muscular tunic^[143,161].

Vitamin doses may also be part of the problem, as the effect of vitamins depends on dietary concentrations and/or supplement intake. The wide variety of doses reached with diet and supplements, and the lack of an established "pharmacological" dose of vitamins, makes

it difficult to ascertain the true net effect of vitamin status or supplementation needed to generate beneficial effects^[161-163]. Other AOs are taurine, acetyl L-carnitine, and N-acetylcysteine which have been demonstrated to reduce the progression of DPN^[15].

Strategies targeted against individual OS pathways^[143]

The pathways of hyperglycemia-induced OS discussed earlier are potential therapeutic targets in DPN. Some of the interventions have resulted in specific therapies, for example, aldose reductase inhibitors (ARIs), protein kinase C (PKC) inhibitors, and anti-AGE agents.

Aldose reductase inhibitors: Therefore, ARIs are agents that reduce the flux of glucose into the polyol pathway thereby preventing the harmful effects of excess sorbitol and fructose in neurons. Results from *in vivo* and *in vitro* animal studies highlighted the positive effect of inhibiting ARI on DPN^[143]. These studies have been the foundation for embarking on several clinical trials with ARIs with AO activities such as Fidarestat (SNK-860), Epalrestat, and Ranirestat (AS-3201)^[143]. Among the ARIs that have made it to clinical trials, Epalrestat was licensed in Japan while others [e.g., Tolrestat (AY-2773), Zenarestat (FK-366; FR-74366), and Ponalrestat] were withdrawn due to inefficacy or safety concerns^[143]. ARIs prevent the progression of DPN, enhance sural motor and sensory nerve conduction velocities (NCV), and improve wrist and ankle F-wave latency together with alleviating neuropathic pain^[143,164]. In addition, it is reported that the prescription of eparestat may improve subjective neuropathy symptoms, sensory and motor nerve conduction velocity^[143].

Protein kinase C inhibitors: PKC is involved in the activation of key regulatory proteins responsible for nerve function and synthesis of neurotransmitters. Inhibiting PKC was reported to suppress neuropathic pain. Ruboxistaurin, a specific inhibitor of neuronal protein kinase C (PKC1B) that possesses antioxidant effects, improves NCV and endoneurial blood flow in diabetic rats. In clinical trials, Ruboxistaurin reduces the progression of DPN^[143] but fails to achieve its primary end-points, vibration detection threshold and symptoms reduction. Ruboxistaurin had effects on diabetic DPN in some studies, but the evidence is not enough for meta-analysis and firm conclusion.

Anti-advanced glucose end-products agents: Anti-AGE agents prevent the formation and accumulation of AGEs. They also counteract the AGE-receptor for AGE interactions that might aggravate the OS damage in DPN. Examples are benfotiamine, aminoguanidine, and aspirin which are known for their AO properties through the inhibition of AGEs formation^[7,143].

Benfotiamine

Benfotiamine (BFT) has been reported to increase

transketolase enzyme activity which directs AGE substrates to the pentose phosphate pathway resulting in the reduction of hyperglycemic damage. It also inhibits the increase in UDP-N-acetylglucosamine that induces the hexosamine pathway activity ultimately reducing tissue AGEs^[143,165-167]. In combination with pyridoxamine and cyanocobalamin, BFT improves the vibration perception threshold, motor function, and symptom score^[143,168].

Aminoguanidine

Aminoguanidine has been reported to react with 3-deoxyglucosone, a precursor of AGE, thereby trapping the reactive carbonyls and preventing the formation of AGEs although it has been withdrawn from clinical trial as a result of toxicity^[143,169].

Aspirin

Aspirin has been reported to inhibit the production of pentosidine, a cross-linking AGE, by scavenging free radicals and chelating metal ions in collagen incubated with glucose *in vitro*^[170].

Strategies targeted at mitochondria^[143]

It has been demonstrated that excess superoxide anion radicals, hydroxyl radicals, and H₂O₂ are produced during the generation of adenosine triphosphate (ATP) in mitochondria under hyperglycemic conditions contributing to increased oxidative damage^[143].

Coenzyme Q: Coenzyme Q (a mitochondrial antioxidant) or ubiquinone may decrease OS not only by quenching reactive oxidant species but also by "recoupling" mitochondrial oxidative phosphorylation, thereby reducing superoxide production^[153,156]. CoQ₁₀ supplements can be either the oxidized form (ubiquinone) or reduced form (ubiquinol) as both forms seem pretty equally potent in increasing circulating levels of total CoQ₁₀ in the body. "Total CoQ₁₀" refers to the sum of both forms, since CoQ₁₀ can readily swap between forms as it acts in the body^[171]. Ubiquinone and ubiquinol form a pair of molecules known as a REDOX couplet (reduction/oxidation) which is a property that is crucial for the functioning of CoQ₁₀ within the electron transport chain, where it transports electrons from complex I and II to complex III. CoQ₁₀ is an important micronutrient acting on the electron transport chain of the mitochondria with two major functions: (1) synthesis of ATP; and (2) a potent antioxidant. Deficiency in CoQ₁₀ is often seen in patients with T2DM^[171]. CoQ₁₀ also has the ability to prevent LPO from either inhibiting lipid peroxyl radicals and has been noted to restore α-tocopherol from its radical state back to its AO state^[171]. Protein carbonylation has also been noted to be reduced with CoQ₁₀ (direct inhibition of protein oxidation) but has been noted to not influence the conversion of NO into peroxynitrite. *Via* its AO potential, ubiquinone can protect DNA from excess oxidation from H₂O₂ and potentially act as an anticarcinogen (as noted in human lymphocytes at least)^[171].

Deficiency in CoQ₁₀ is often present among pati-

ents with T2DM due to various reasons. As a potent antioxidant, CoQ₁₀ is assumed to scavenge excessive ROS and provide protection to cells, especially mitochondria from oxidative damage. Therefore, restoration of CoQ₁₀ level among patients with T2DM by supplementation of exogenous CoQ₁₀ could potentially alleviate OS, preserve mitochondrial function, and eventually lead to improvement of glycemic control^[171]. In DM, CoQ₁₀ has been reported to show promising therapeutic potential^[171]. The standard dose for CoQ₁₀ is generally 90 mg for a low dose and 200 mg for the higher dose, taken once daily with a meal due to its reliance on food for absorption^[171].

Telmisartan

Telmisartan is a well-known unique angiotensin II (Ang II) type 1 receptor blocker (ARB) that exerts a powerful AO effect. Furthermore, a number of properties like the best binding affinity to Ang II type 1 receptors, the maximum plasma half life and the highest lipophilicity among the presently available ARBs make this molecule a long lasting antioxidant^[172]. Telmisartan has a potential neuro-protective effect on PNP; this is mediated through its anti-inflammatory effects and its dual properties as an ARB, and a partial PPAR- γ ligand^[172]. Usual adult dose for hypertension: Initial dose: 40 mg orally once a day. Maintenance dose: 40 to 80 mg orally once a day. Usual adult dose for cardiovascular risk reduction: 80 mg orally once a day.

Metformin

Both American and European guidelines recommend metformin as the first-line agent for the pharmacological management of T2DM and preventing its complications^[3]. It possesses AO property and causes reduction of albumin excretion rate in the urine of diabetic patients. In addition, it decreases the production of AGEs, improves free radical defense system by its ability to directly scavenge oxygenated free radicals and thereby reduces intracellular ROS levels. The glycemic control-independent neuroprotective and antineuropathic effects of metformin recently reported in animal studies^[173]. Usual adult dose for T2DM: Initial dose: 500 mg PO bid or 850 mg PO qd. Dose titration: Increase in 500 mg weekly increments or 850 mg every 2 wk as tolerated. Maintenance dose: 2.000 mg daily in divided doses. Maximum dose: 2500 mg/d.

Pioglitazone

Thiazolidinedione (TZD) drugs such as pioglitazone are approved by the FDA for the treatment of T2DM. TZDs also reduce the molecular and behavioral sequelae of neurological disease. Positive and protective effects of TZD group of drugs, like pioglitazone, in the amelioration of AO enzyme levels in renal histopathology and renal tissue associated with diabetic nephropathy has recently been investigated by many researchers. Increased expression of nuclear transcription factor p65 in renal tubules and glomeruli during diabetic nephropathy has

been reduced by pioglitazone therapy thereby showing protection from renal pathophysiology. But TZDs has limited clinical uses due to the occurrence of fluid retention, hemodilution, and heart failure in about 15% of patients. Usual adult dose for T2DM: Initial dose: 15-30 mg PO with meal qDay initial; may increase dose by 15 mg with careful monitoring to 45 mg qDay maximum. Some drugs with AO properties which have antioxidant effect in patients with DM are shown in Table 1^[163].

Triple antioxidant therapy

Participants with T1DM with early complications were randomly assigned to a combination AO regimen or to placebo. Allopurinol (300 mg qd), ALA (600 mg bid) and nicotinamide (750 mg bid), or matched PO placebos were administered for 24 mo. The administration of each individual active drug or placebo component was titrated in consecutive weeks (first ALA, then nicotinamide, finally allopurinol) such that the participant began receiving full therapeutic doses of all the medications 3 wk postrandomisation. In cohort of T1DM patients with mild-to-moderate CAN, a combination AO treatment regimen did not prevent progression of CAN, had no beneficial effects on myocardial perfusion or DPN, and may have been detrimental. However, a larger study is necessary to assess the underlying causes of these findings^[83].

Correction of vascular endothelial dysfunction^[174,175]

Trimetazidine: Prescription of this medication is accompanied by glucose metabolism improvement, endothelin-1 reduction in patients with diabetic cardiomyopathy, significantly contributes to the improvement of ejection fraction (EF) in patients with heart failure^[174,175].

Perhexiline: Prescription of this pharmacological agent to patients with HF significantly improve the EF and VO₂max, but unfortunately, the clinical use is limited because of the increased risk of PNP development and hepatotoxicity^[175,176].

Ranolazine: Unfortunately the prescription of this drug with possible metabolism modification properties is associated with the increased possibility of QTc prolongation^[175,177].

Beta blockers: Prescription of beta blockers, particularly the β_1 -selective, is associated with endothelial protective effects. In patients with essential hypertension prescription of nebivolol was accompanied by endothelium-dependent vasodilator function improvement^[178-183]. Endothelium-dependent responses in patients with essential hypertension were improved after prescription of carvedilol (non-selective $\beta_{1,2}$ antagonist with α -antagonist property), but this can be due its antioxidant capacity^[182,183]. The combined prescription of angiotensin-converting enzyme inhibitor and carvedilol was accompanied with more pronounced endothelium-dependent vasodilator

responses^[184].

Calcium channel blockers: Prescription of dihydropyridine calcium channel blockers is accompanied by endothelial protective effect, mainly mediated by reduction in LPO and associated ROS generation^[183,185,186]. Prescription of isradipine to cholesterol-fed rabbit was associated with endothelial function improvement^[183,187].

Prescription of some dihydropyridines (amlodipine, nifedipine and azelnidipine) was associated with decrease of leucocyte activation and interleukin-6 and C-reactive protein levels^[183,188], also improvement of endothelial function by treatment with amlodipine was found^[183,189,190].

The combination of statins with amlodipine produces more beneficial effect on endothelial function in rats with DM^[191,192]. Thus, prescription of dihydropyridine calcium channel blockers is suitable for treatment of endothelial dysfunction.

Phosphodiesterase-5 inhibitors: Phosphodiesterase-5 (PDE5) is highly specific for hydrolysis of cyclic nucleotides monophosphate, such as cyclic guanosine monophosphate (cGMP), which is a molecular messenger involved in regulation of vascular function, axon guidance, the modulation of DPN and pain perception^[193-195]. PDE5 inhibitors including sildenafil, tadalafil, and vardenafil, are primarily used as pharmacological agents for the treatment of erectile dysfunction, but they also have a potential therapeutic application for the treatment of neurovascular dysfunction, neuroinflammatory and neurodegenerative diseases by inducing accumulation of cGMP and activation of cGMP dependent protein kinase, e.g., PKG, signaling pathways^[195,196]. Clinical study demonstrates that PDE5 inhibitors are safe and generally well tolerated with no serious side effects in patients. Sildenafil improves vascular function and blood supply to the vasa nervorum while ameliorating neurological function of neuropathy in diabetic patients^[197].

The considerably longer duration of action for tadalafil may permit less frequent dosing and could potentially reduce adverse effects associated with treatment. Moreover, the absorption and activity of tadalafil is unaffected by food ingestion, age, diabetes, or mild to moderate hepatic insufficiency. Also, tadalafil did not lower systemic BP in clinical trials^[198].

The angiopoietin-Tie (ANG/Tie) signaling system was identified as a vascular-specific receptor tyrosine kinase pathway that is essential for vessel development. PDE5 inhibitor-induced activation of the cGMP/PKG and ANG/Tie2 signaling pathways promotes neurovascular remodeling both directly through these signaling pathways to ameliorate neurovascular function, and indirectly *via* endothelial cells and Schwann cells, which produce neurotrophic factors and provide a permissive restorative microenvironment in the sciatic nerve. Both direct and indirect approaches, in concern, improve neurological function of diabetic neuropathy^[199].

Ivabradine, the cardiac pacemaker “funny” [I_f] inhibitor:

Ivabradine is a heart-rate-lowering agent that acts by selectively and specifically inhibiting the I_f , a mixed Na^+ - K^+ inward current that controls the spontaneous diastolic depolarization in the sinoatrial node and hence regulates the HR^[200,201]. Ivabradine slows down HR and exerts cardioprotective effects^[183,202,203].

According to data obtained from clinical studies the influence of ivabradine on flow-mediated vasodilation is nonsignificant, so the effects of this drug are controversial^[183,204,205]. In patients with stable CAD without heart failure, the additional prescription of the cardiac pacemaker “funny” [I_f] inhibitor was associated with increased frequency of atrial fibrillation^[183,206].

Prevention and treatment of thrombosis

Administration of antiplatelet agents (acetylsalicylic acid, clopidogrel and others) can lead to prevention of blood clots, stenocardia and development of MI. Clopidogrel is more effective medication for the reduction of cardiovascular risk factors^[207,208].

Treatment of OH

Treatment of OH should involve both non pharmacological and pharmacological interventions. Non-pharmacologic treatment should be the initial approach. OH should be treated by volume repletion with fluids and salt. Patients should be advised to avoid hot baths, to get out of bed slowly and if their diabetes is being treated with insulin, patients should administer this medication while lying down^[8,209,210]. Although there are concerns on risk of supine hypertension by administration of fludrocortisones, rescription of low-dose may be beneficial in supplementing volume repletion^[8,209,210].

Pharmacological intervention includes prescription of mineralocorticoids and/or adrenergic agonists. Supplementary salt intake together with mineralocorticoid (fludrocortisones) increases plasma volume. In generally it is ineffective until edema develops, which carries a risk of causing hypertension and congestive HF^[81]. Prescription of adrenergic agonist (ephedrine, midodrine, clonidine) is effective in some patients, but titration of this medications should be performed gradually^[81]. The somatostatin analog (octreotide) can also be prescribed to patients with refractory OH after eating^[7].

OH can be aggravated by different forms of therapy [e.g. tricyclic antidepressant (amitriptyline)] used for the treatment of other complications (e.g., painful sensory neuropathy). Therefore, careful attention to other medications that may aggravate OH in these patients is mandatory^[8,211]. Similarly, the use of β -adrenergic blockers may benefit the tachycardia and anticholinergics, the orthostatic bradycardia. Pyridostigmine (inhibitor of acetylcholinesterase) has also been shown to improve symptoms and orthostatic BP for patients with POTS and HRV in healthy young adults^[8,212]. Treatment with somatostatin (Octreotide) can be recommended for patients with pooling of blood in the splanchnic bed, and

prescription of erythropoietin for patients with contracted plasma volume^[8]. Sympathomimetic drugs (midodrine) are the first-line medicines in the treatment of patients with OH^[3,39,81]. The titration of midodrin should be performed gradually to efficacy.

CONCLUSION

CAN is common and often underdiagnosed complication of DM which is strongly associated with increased rate of cardiovascular morbidity and mortality. As the development and progression of cardiovascular denervation can be slowed down and is partly reversible in the early disease stages, it is recommended to perform screening for that complication among DM patients. A variety of methods can be used for CAN assessment, but the "gold" standard clinical tests are CARTs. The basic CAN prevention and treatment tools are intensive glycemic control, lifestyle modification and management of CVD risk, but the unified algorithm and known disease modifying treatment is lacking.

CAN treatment is a complex process, that includes: Lifestyle modification; reducing IR; intensive glycemic control; treatment of DLP, antioxidants, vitamins, correction of vascular endothelial dysfunction, prevention and treatment of thrombosis and OH. The new possible perspective areas of CAN treatment are administration of thromboxane A₂ blockers and prostacyclin analogues, PDE5 inhibitors, ALA, ω -3 PUFAs, DGLA and the combined prescription of ALA, DGLA and ω -3 PUFAs. In addition the combined administration of ALA, ω -3 PUFAs and benfotiamine promotes reduction of chronic inflammation markers and increase of HRV parameters, that might be useful in preventing the development and progression of CAN. Development of OH is associated with severe or advanced CAN and prescription of nonpharmacological and pharmacological, in the foreground midodrine and fludrocortisone acetate, treatment methods are necessary.

REFERENCES

- 1 **Ogurtsova K**, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 2017; **128**: 40-50 [PMID: 28437734 DOI: 10.1016/j.diabres.2017.03.024]
- 2 **Marazzi G**, Volterrani M, Rosano GM. Metabolic agents in the management of diabetic coronary patients: a new era. *Int J Cardiol* 2008; **127**: 124-125 [PMID: 18199501 DOI: 10.1016/j.ijcard.2007.10.042]
- 3 Standards of Medical Care in Diabetes-2017: Summary of Revisions. *Diabetes Care* 2017; **40**: S4-S5 [PMID: 27979887 DOI: 10.2337/dc17-S013]
- 4 **Tesfaye S**, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P; Toronto Diabetic Neuropathy Expert Group. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care* 2010; **33**: 2285-2293 [PMID: 20876709 DOI: 10.2337/dc10-1303]
- 5 **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]
- 6 **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]
- 7 **Edwards JL**, Vincent AM, Cheng HT, Feldman EL. Diabetic neuropathy: mechanisms to management. *Pharmacol Ther* 2008; **120**: 1-34 [PMID: 18616962 DOI: 10.1016/j.pharmthera.2008.05.005]
- 8 **Vinik AI**, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation* 2007; **115**: 387-397 [PMID: 17242296 DOI: 10.1161/CIRCULATIONAHA.106.634949]
- 9 **Vinik AI**, Erbas T. Diabetic autonomic neuropathy. *Handb Clin Neurol* 2013; **117**: 279-294 [PMID: 24095132 DOI: 10.1016/B978-0-444-53491-0.00022-5]
- 10 **Callaghan BC**, Cheng HT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol* 2012; **11**: 521-534 [PMID: 22608666 DOI: 10.1016/S1474-4422(12)70065-0]
- 11 **Cannon CP**. Combination therapy in the management of mixed dyslipidaemia. *J Intern Med* 2008; **263**: 353-365 [PMID: 18324928 DOI: 10.1111/j.1365-2796.2008.01933.x]
- 12 **Bril V**. Treatments for diabetic neuropathy. *J Peripher Nerv Syst* 2012; **17** Suppl 2: 22-27 [PMID: 22548619 DOI: 10.1111/j.1529-8027.2012.00391.x]
- 13 **Tandon N**, Ali MK, Narayan KM. Pharmacologic prevention of microvascular and macrovascular complications in diabetes mellitus: implications of the results of recent clinical trials in type 2 diabetes. *Am J Cardiovasc Drugs* 2012; **12**: 7-22 [PMID: 22217193 DOI: 10.2165/11594650-000000000-00000]
- 14 **Soares-Miranda L**, Sandercock G, Vale S, Santos R, Abreu S, Moreira C, Mota J. Metabolic syndrome, physical activity and cardiac autonomic function. *Diabetes Metab Res Rev* 2012; **28**: 363-369 [PMID: 22238216 DOI: 10.1002/dmrr.2281]
- 15 **Hosseini A**, Abdollahi M. Diabetic neuropathy and oxidative stress: therapeutic perspectives. *Oxid Med Cell Longev* 2013; **2013**: 168039 [PMID: 23738033 DOI: 10.1155/2013/168039]
- 16 **Vinik AI**, Nevoret ML, Casellini C, Parson H. Diabetic neuropathy. *Endocrinol Metab Clin North Am* 2013; **42**: 747-787 [PMID: 24286949 DOI: 10.1016/j.ecl.2013.06.001]
- 17 **Isik A**, Firat D. Bilateral intra-areolar polythelia. *Breast J* 2017; Epub ahead of print [PMID: 28590581 DOI: 10.1111/tbj.12838]
- 18 **Isik A**, Soyuturk M, Süleyman S, Firat D, Peker K, Yilmaz İ, Celebi F. Correlation of Bowel Wall Thickening Seen Using Computerized Tomography With Colonoscopies: A Preliminary Study. *Surg Laparosc Endosc Percutan Tech* 2017; **27**: 154-157 [PMID: 28291060 DOI: 10.1097/SLE.0000000000000389]
- 19 **Ziegler D**. Cardiovascular autonomic neuropathy: clinical manifestations and measurement. *Diabetes Rev* 1999; **7**: 300-315
- 20 **Vinik AI**, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. *Diabetes Care* 2003; **26**: 1553-1579 [PMID: 12716821 DOI: 10.2337/diacare.26.5.1553]
- 21 **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]
- 22 **Valensi P**, Pariès J, Attali JR; French Group for Research and Study of Diabetic Neuropathy. Cardiac autonomic neuropathy in diabetic patients: influence of diabetes duration, obesity, and microangiopathic complications--the French multicenter study. *Metabolism* 2003; **52**: 815-820 [PMID: 12870154 DOI: 10.1016/S0026-0495(03)00095-7]
- 23 **Gaede P**, Vedel P, Parving HH, Pedersen O. Intensified multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: the Steno type 2 randomised study. *Lancet* 1999; **353**: 617-622 [PMID: 10030326 DOI: 10.1016/S0140-6736(98)07368-1]
- 24 **Gaede P**, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 2003; **348**: 383-393 [PMID: 12556541 DOI: 10.1056/NEJMoa021778]

- 25 The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). *Diabetologia* 1998; **41**: 416-423 [PMID: 9562345 DOI: 10.1007/s001250050924]
- 26 Gaede P, Lund-Andersen H, Parving HH, Pedersen O. Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Engl J Med* 2008; **358**: 580-591 [PMID: 18256393 DOI: 10.1056/NEJMoa0706245]
- 27 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]
- 28 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]
- 29 Boulton AJ, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, Malik RA, Maser RE, Soslenko JM, Ziegler D; American Diabetes Association. Diabetic neuropathies: a statement by the American Diabetes Association. *Diabetes Care* 2005; **28**: 956-962 [PMID: 15793206 DOI: 10.2337/diacare.28.4.956]
- 30 Low PA, Benrud-Larson LM, Sletten DM, Opfer-Gehrking TL, Weigand SD, O'Brien PC, Suarez GA, Dyck PJ. Autonomic symptoms and diabetic neuropathy: a population-based study. *Diabetes Care* 2004; **27**: 2942-2947 [PMID: 15562211 DOI: 10.2337/diacare.27.12.2942]
- 31 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]
- 32 Vinik AI, Erbas T, Casellini CM. Diabetic cardiac autonomic neuropathy, inflammation and cardiovascular disease. *J Diabetes Invest* 2013; **4**: 4-18 [PMID: 23550085 DOI: 10.1111/jdi.12042]
- 33 Orchard TJ, LLOYD CE, Maser RE, Kuller LH. Why does diabetic autonomic neuropathy predict IDDM mortality? An analysis from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes Res Clin Pract* 1996; **34** Suppl: S165-S171 [PMID: 9015687 DOI: 10.1016/S0168-8227(96)90025-X]
- 34 Ziegler D, Zentgraf 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]
- 35 Maser RE, Lenhard MJ. Cardiovascular autonomic neuropathy due to diabetes mellitus: clinical manifestations, consequences, and treatment. *J Clin Endocrinol Metab* 2005; **90**: 5896-5903 [PMID: 16014401 DOI: 10.1210/jc.2005-0754]
- 36 Vinik AI, Maser RE, Ziegler D. Autonomic imbalance: prophet of doom or scope for hope? *Diabet Med* 2011; **28**: 643-651 [PMID: 21569084 DOI: 10.1111/j.1464-5491.2010.03184.x]
- 37 Wackers FJ, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE; Detection of Ischemia in Asymptomatic Diabetics Investigators. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. *Diabetes Care* 2004; **27**: 1954-1961 [PMID: 15277423 DOI: 10.2337/diacare.27.8.1954]
- 38 Veglio M, Chinaglia A, Cavallo-Perin P. QT interval, cardiovascular risk factors and risk of death in diabetes. *J Endocrinol Invest* 2004; **27**: 175-181 [PMID: 15129815 DOI: 10.1007/BF03346265]
- 39 Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, Soslenko 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]
- 40 Dyck PJ, Albers JW, Andersen H, Arezzo JC, Biessels GJ, Bril V, Feldman EL, Litchy WJ, O'Brien PC, Russell JW; Toronto Expert Panel on Diabetic Neuropathy. Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity. *Diabetes Metab Res Rev* 2011; **27**: 620-628 [PMID: 21695763 DOI: 10.1002/dmrr.1226]
- 41 Albers JW, Pop-Busui R. Diabetic neuropathy: mechanisms, emerging treatments, and subtypes. *Curr Neurol Neurosci Rep* 2014; **14**: 473 [PMID: 24954624 DOI: 10.1007/s11910-014-0473-5]
- 42 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]
- 43 Low PA. Prevalence of orthostatic hypotension. *Clin Auton Res* 2008; **18** Suppl 1: 8-13 [PMID: 18368301 DOI: 10.1007/s10286-007-1001-3]
- 44 Low PA, Walsh JC, Huang CY, McLeod JG. The sympathetic nervous system in diabetic neuropathy. A clinical and pathological study. *Brain* 1975; **98**: 341-356 [PMID: 810214 DOI: 10.1093/brain/98.3.341]
- 45 Heart 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]
- 46 Pop-Busui R. Cardiac autonomic neuropathy in diabetes: a clinical perspective. *Diabetes Care* 2010; **33**: 434-441 [PMID: 20103559 DOI: 10.2337/dc09-1294]
- 47 Ang L, Jaiswal M, Martin C, Pop-Busui R. Glucose control and diabetic neuropathy: lessons from recent large clinical trials. *Curr Diab Rep* 2014; **14**: 528 [PMID: 25139473 DOI: 10.1007/s11892-014-0528-7]
- 48 Martin CL, Albers JW, Pop-Busui R; DCCT/EDIC Research Group. Neuropathy and related findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes Care* 2014; **37**: 31-38 [PMID: 24356595 DOI: 10.2337/dc13-2114]
- 49 Ziegler D, Keller J, Maier C, Pannek J; German Diabetes Association. Diabetic neuropathy. *Exp Clin Endocrinol Diabetes* 2014; **122**: 406-415 [PMID: 25014092 DOI: 10.1055/s-0034-1366435]
- 50 Bernardi L, Spallone V, Stevens M, Hilsted J, Frontoni S, Pop-Busui R, Ziegler D, Kempler P, Freeman R, Low P, Tesfaye S, Valensi P; Toronto Consensus Panel on Diabetic Neuropathy. Methods of investigation for cardiac autonomic dysfunction in human research studies. *Diabetes Metab Res Rev* 2011; **27**: 654-664 [PMID: 21695761 DOI: 10.1002/dmrr.1224]
- 51 Consensus statement on the definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy. The Consensus Committee of the American Autonomic Society and the American Academy of Neurology. *Neurology* 1996; **46**: 1470 [PMID: 8628505 DOI: 10.1212/WNL.46.5.1470]
- 52 Spallone V, Bellavere F, Scionti L, Maule S, Quadri R, Bax G, Melga P, Viviani GL, Esposito K, Morganti R, Cortelli P; Diabetic Neuropathy Study Group of the Italian Society of Diabetology. Recommendations for the use of cardiovascular tests in diagnosing diabetic autonomic neuropathy. *Nutr Metab Cardiovasc Dis* 2011; **21**: 69-78 [PMID: 21247746 DOI: 10.1016/j.numecd.2010.07.005]
- 53 Hage FG, Iskandrian AE. Cardiovascular imaging in diabetes mellitus. *J Nucl Cardiol* 2011; **18**: 959-965 [PMID: 21785921 DOI: 10.1007/s12350-011-9431-7]
- 54 Valensi PE, Johnson NB, Maison-Blanche P, Extramania F, Motte G, Coumel P. Influence of cardiac autonomic neuropathy on heart rate dependence of ventricular repolarization in diabetic patients. *Diabetes Care* 2002; **25**: 918-923 [PMID: 11978691 DOI: 10.2337/diacare.25.5.918]
- 55 Santini V, Ciampittiello G, Gigli F, Bracaglia D, Baroni A, Cicconetti E, Verri C, Gambardella S, Frontoni S. QTc and autonomic neuropathy in diabetes: effects of acute hyperglycaemia and n-3 PUFA. *Nutr Metab Cardiovasc Dis* 2007; **17**: 712-718 [PMID: 17324562 DOI: 10.1016/j.numecd.2006.09.006]
- 56 Prince CT, Secrest AM, Mackey RH, Arena VC, Kingsley LA, Orchard TJ. Cardiovascular autonomic neuropathy, HDL cholesterol,

- and smoking correlate with arterial stiffness markers determined 18 years later in type 1 diabetes. *Diabetes Care* 2010; **33**: 652-657 [PMID: 20040653 DOI: 10.2337/dc09-1936]
- 57 **Desouza CV**, Bolli GB, Fonseca V. Hypoglycemia, diabetes, and cardiovascular events. *Diabetes Care* 2010; **33**: 1389-1394 [PMID: 20508232 DOI: 10.2337/dc09-2082]
 - 58 **Mancia G**, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Boudier HA, Zanchetti A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Erdine S, Kiowski W, Agabiti-Rosei E, Ambrosioni E, Lindholm LH, Viigimaa M, Adamopoulos S, Agabiti-Rosei E, Ambrosioni E, Bertomeu V, Clement D, Erdine S, Farsang C, Gaita D, Lip G, Mallion JM, Manolis AJ, Nilsson PM, O'Brien E, Ponikowski P, Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Waeber B, Williams B; Management of Arterial Hypertension of the European Society of Hypertension; European Society of Cardiology. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007; **25**: 1105-1187 [PMID: 17563527 DOI: 10.1097/HJH.0b013e3281fc975a]
 - 59 **Shakespeare CF**, Katritsis D, Crowther A, Cooper IC, Coltart JD, Webb-Peploe MW. Differences in autonomic nerve function in patients with silent and symptomatic myocardial ischaemia. *Br Heart J* 1994; **71**: 22-29 [PMID: 8297687 DOI: 10.1136/hrt.71.1.22]
 - 60 **Veglio M**, Borra M, Stevens LK, Fuller JH, Perin PC. The relation between QTc interval prolongation and diabetic complications. The EURODIAB IDDM Complication Study Group. *Diabetologia* 1999; **42**: 68-75 [PMID: 10027581 DOI: 10.1007/s001250051115]
 - 61 **Kahn JK**, Sisson JC, Vinik AI. QT interval prolongation and sudden cardiac death in diabetic autonomic neuropathy. *J Clin Endocrinol Metab* 1987; **64**: 751-754 [PMID: 3818902 DOI: 10.1210/jcem-64-4-751]
 - 62 **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]
 - 63 **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]
 - 64 **Burgos LG**, Ebert TJ, Asiddao C, Turner LA, Pattison CZ, Wang-Cheng R, Kampine JP. Increased intraoperative cardiovascular morbidity in diabetics with autonomic neuropathy. *Anesthesiology* 1989; **70**: 591-597 [PMID: 2929996 DOI: 10.1097/0000542-198904000-00006]
 - 65 **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]
 - 66 **Bernardi L**. Clinical evaluation of arterial baroreflex activity in diabetes. *Diabetes Nutr Metab* 2000; **13**: 331-340 [PMID: 11232758]
 - 67 **Freeman R**, Saul JP, Roberts MS, Berger RD, Broadbridge C, Cohen RJ. Spectral analysis of heart rate in diabetic autonomic neuropathy. A comparison with standard tests of autonomic function. *Arch Neurol* 1991; **48**: 185-190 [PMID: 1993010 DOI: 10.1001/archneur.1991.00530140079020]
 - 68 **Mogensén UM**, Jensen T, Køber L, Kelbæk H, Mathiesen AS, Diken U, Rossing P, Hilsted J, Kofoed KF. Cardiovascular autonomic neuropathy and subclinical cardiovascular disease in normoalbuminuric type 1 diabetic patients. *Diabetes* 2012; **61**: 1822-1830 [PMID: 22498696 DOI: 10.2337/db11-1235]
 - 69 **Sammito S**, Böckelmann I. Reference values for time- and frequency-domain heart rate variability measures. *Heart Rhythm* 2016; **13**: 1309-1316 [PMID: 26883166 DOI: 10.1016/j.hrthm.2016.02.006]
 - 70 **Bauer A**, Malik M, Schmidt G, Barthel P, Bonnemeier H, Cygankiewicz I, Guzik P, Lombardi F, Müller A, Oto A, Schneider R, Watanabe M, Wichterle D, Zareba W. Heart rate turbulence: standards of measurement, physiological interpretation, and clinical use: International Society for Holter and Noninvasive Electrophysiology Consensus. *J Am Coll Cardiol* 2008; **52**: 1353-1365 [PMID: 18940523 DOI: 10.1016/j.jacc.2008.07.041]
 - 71 **Balcioğlu AS**, Müderrisoğlu H. Diabetes and cardiac autonomic neuropathy: Clinical manifestations, cardiovascular consequences, diagnosis and treatment. *World J Diabetes* 2015; **6**: 80-91 [PMID: 25685280 DOI: 10.4239/wjd.v6.i1.80]
 - 72 **La Rovere MT**, Pinna GD, Maestri R, Robbi E, Caporotondi A, Guazzotti G, Sleight P, Febo O. Prognostic implications of baroreflex sensitivity in heart failure patients in the beta-blocking era. *J Am Coll Cardiol* 2009; **53**: 193-199 [PMID: 19130988 DOI: 10.1016/j.jacc.2008.09.034]
 - 73 **Rosengård-Bärlund M**, Bernardi L, Fagerudd J, Mäntysaari M, Af Björkstén CG, Lindholm H, Forsblom C, Wadén J, Groop PH; FinnDiane Study Group. Early autonomic dysfunction in type 1 diabetes: a reversible disorder? *Diabetologia* 2009; **52**: 1164-1172 [PMID: 19340407 DOI: 10.1007/s00125-009-1340-9]
 - 74 **Hoffman RP**, Sinkey CA, Anderson EA. Microneurographically determined muscle sympathetic nerve activity levels are reproducible in insulin-dependent diabetes mellitus. *J Diabetes Complications* 1998; **12**: 307-310 [PMID: 9877463 DOI: 10.1016/S1056-8727(98)00010-5]
 - 75 **Hilsted J**. Catecholamines and diabetic autonomic neuropathy. *Diabet Med* 1995; **12**: 296-297 [PMID: 7600741 DOI: 10.1111/j.1464-5491.1995.tb00479.x]
 - 76 **Freeman MR**, Newman D, Dorian P, Barr A, Langer A. Relation of direct assessment of cardiac autonomic function with metaiodobenzylguanidine imaging to heart rate variability in diabetes mellitus. *Am J Cardiol* 1997; **80**: 247-250 [PMID: 9230179 DOI: 10.1016/S0002-9149(97)00337-8]
 - 77 **Schnell O**, Muhr D, Weiss M, Dresel S, Haslbeck M, Standl E. Reduced myocardial 123I-metaiodobenzylguanidine uptake in newly diagnosed IDDM patients. *Diabetes* 1996; **45**: 801-805 [PMID: 8635656 DOI: 10.2337/diab.45.6.801]
 - 78 **Nagamachi S**, Jinnouchi S, Kurose T, Ohnishi T, Flores LG 2nd, Nakahara H, Futami S, Tamura S, Matsukura S. 123I-MIBG myocardial scintigraphy in diabetic patients: relationship with 201Tl uptake and cardiac autonomic function. *Ann Nucl Med* 1998; **12**: 323-331 [PMID: 9972369 DOI: 10.1007/BF03164921]
 - 79 **DeGrado TR**, Hutchins GD, Toorongian SA, Wieland DM, Schwaiger M. Myocardial kinetics of carbon-11-meta-hydroxyephedrine: retention mechanisms and effects of norepinephrine. *J Nucl Med* 1993; **34**: 1287-1293 [PMID: 8326386]
 - 80 **Allman KC**, Stevens MJ, Wieland DM, Hutchins GD, Wolfe ER Jr, Greene DA, Schwaiger M. Noninvasive assessment of cardiac diabetic neuropathy by carbon-11 hydroxyephedrine and positron emission tomography. *J Am Coll Cardiol* 1993; **22**: 1425-1432 [PMID: 8227801 DOI: 10.1016/0735-1097(93)90553-D]
 - 81 **Freeman R**. Clinical practice. Neurogenic orthostatic hypotension. *N Engl J Med* 2008; **358**: 615-624 [PMID: 18256396 DOI: 10.1056/NEJMc074189]
 - 82 **Low PA**, Denq JC, Opfer-Gehrking TL, Dyck PJ, O'Brien PC, Slezak JM. Effect of age and gender on sudomotor and cardiovascular function and blood pressure response to tilt in normal subjects. *Muscle Nerve* 1997; **20**: 1561-1568 [PMID: 9390669 DOI: 10.1002/(SICI)1097-4598(199712)20:123.0.CO;2-3]
 - 83 **Pop-Busui R**, Stevens M. Autonomic neuropathy in diabetes. In: *Therapy for Diabetes Mellitus and Related Disorders*. 6th ed. Umpierrez GE, Ed. Alexandria, VA: American Diabetes Association, 2014: 834-863
 - 84 **Ziegler D**, Voss A, Rathmann W, Strom A, Perz S, Roden M, Peters A, Meisinger C; KORA Study Group. Increased prevalence of cardiac autonomic dysfunction at different degrees of glucose intolerance in the general population: the KORA S4 survey. *Diabetologia* 2015; **58**: 1118-1128 [PMID: 25724570 DOI: 10.1007/s00125-015-3534-7]
 - 85 **Lykke JA**, Tamow L, Parving HH, Hilsted J. A combined abnormality

- in heart rate variation and QT corrected interval is a strong predictor of cardiovascular death in type 1 diabetes. *Scand J Clin Lab Invest* 2008; **68**: 654-659 [PMID: 19378439 DOI: 10.1080/00365510802018330]
- 86 **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]
- 87 **Ziegler D,** Gries FA, Mühlen H, Rathmann W, Spüler M, Lessmann F. Prevalence and clinical correlates of cardiovascular autonomic and peripheral diabetic neuropathy in patients attending diabetes centers. The Diacan Multicenter Study Group. *Diabete Metab* 1993; **19**: 143-151 [PMID: 8314418]
- 88 **Serhiyenko VA,** Serhiyenko AA. Diabetic cardiac autonomic neuropathy: Do we have any treatment perspectives? *World J Diabetes* 2015; **6**: 245-258 [PMID: 25789106 DOI: 10.4239/wjd.v6.i2.245]
- 89 **Esposito K,** Giugliano D. Mediterranean diet and type 2 diabetes. *Diabetes Metab Res Rev* 2014; **30** Suppl 1: 34-40 [PMID: 24357346 DOI: 10.1002/dmrr.2516]
- 90 **Sleiman D,** Al-Badri MR, Azar ST. Effect of mediterranean diet in diabetes control and cardiovascular risk modification: a systematic review. *Front Public Health* 2015; **3**: 69 [PMID: 25973415 DOI: 10.3389/fpubh.2015.00069]
- 91 **Carnethon MR,** Prineas RJ, Temprosa M, Zhang ZM, Uwaifo G, Molitch ME; Diabetes Prevention Program Research Group. The association among autonomic nervous system function, incident diabetes, and intervention arm in the Diabetes Prevention Program. *Diabetes Care* 2006; **29**: 914-919 [PMID: 16567837 DOI: 10.2337/diacare.29.04.06.dc05-1729]
- 92 **Smith AG,** Russell J, Feldman EL, Goldstein J, Peltier A, Smith S, Hamwi J, Pollari D, Bixby B, Howard J, Singleton JR. Lifestyle intervention for pre-diabetic neuropathy. *Diabetes Care* 2006; **29**: 1294-1299 [PMID: 16732011 DOI: 10.2337/dc06-0224]
- 93 **Balducci S,** Iacobellis G, Parisi L, Di Biase N, Calandriello E, Leonetti F, Fallucca F. Exercise training can modify the natural history of diabetic peripheral neuropathy. *J Diabetes Complications* 2006; **20**: 216-223 [PMID: 16798472 DOI: 10.1016/j.jdiacomp.2005.07.005]
- 94 **Singleton JR,** Marcus RL, Jackson JE, K Lessard M, Graham TE, Smith AG. Exercise increases cutaneous nerve density in diabetic patients without neuropathy. *Ann Clin Transl Neurol* 2014; **1**: 844-849 [PMID: 25493275 DOI: 10.1002/actn.125]
- 95 **Colberg SR,** Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, Braun B; American College of Sports Medicine; American Diabetes Association. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement executive summary. *Diabetes Care* 2010; **33**: 2692-2696 [PMID: 21115771 DOI: 10.2337/dc10-1548]
- 96 **Colberg SR,** Vinik AI. Exercising with peripheral or autonomic neuropathy: what health care providers and diabetic patients need to know. *Phys Sportsmed* 2014; **42**: 15-23 [PMID: 24565817 DOI: 10.3810/psm.2014.02.2043]
- 97 **Pagkalos M,** Koutlianos N, Kouidi E, Pagkalos E, Mandroukas K, Deligiannis A. Heart rate variability modifications following exercise training in type 2 diabetic patients with definite cardiac autonomic neuropathy. *Br J Sports Med* 2008; **42**: 47-54 [PMID: 17526623 DOI: 10.1136/bjism.2007.035303]
- 98 **Epidemiology of Diabetes Interventions and Complications (EDIC).** Design, implementation, and preliminary results of a long-term follow-up of the Diabetes Control and Complications Trial cohort. *Diabetes Care* 1999; **22**: 99-111 [PMID: 10333910 DOI: 10.2337/diacare.22.1.99]
- 99 **Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group.** Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *JAMA* 2003; **290**: 2159-2167 [PMID: 14570951 DOI: 10.1001/jama.290.16.2159]
- 100 **Cefalu WT,** Ratner RE. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: the “gift” that keeps on giving! *Diabetes Care* 2014; **37**: 5-7 [PMID: 24356590 DOI: 10.2337/dc13-2369]
- 101 **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]
- 102 **Effect of intensive diabetes treatment on nerve conduction in the Diabetes Control and Complications Trial.** *Ann Neurol* 1995; **38**: 869-880 [PMID: 8526459 DOI: 10.1002/ana.410380607]
- 103 **Pop-Busui R,** Herman WH, Feldman EL, Low PA, Martin CL, Cleary PA, Waberski BH, Lachin JM, Albers JW; DCCT/EDIC Research Group. DCCT and EDIC studies in type 1 diabetes: lessons for diabetic neuropathy regarding metabolic memory and natural history. *Curr Diab Rep* 2010; **10**: 276-282 [PMID: 20464532 DOI: 10.1007/s11892-010-0120-8]
- 104 **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]
- 105 **Ohkubo Y,** Kishikawa H, Araki E, Miyata T, Isami S, Motoyoshi S, Kojima Y, Furuyoshi N, Shichiri M. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract* 1995; **28**: 103-117 [PMID: 7587918 DOI: 10.1016/0168-8227(95)01064-K]
- 106 **Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33).** UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837-853 [PMID: 9742976 DOI: 10.1016/S0140-6736(98)07019-6]
- 107 **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]
- 108 **Duckworth W,** Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R, Warren SR, Goldman S, McCarren M, Vitek ME, Henderson WG, Huang GD; VADT Investigators. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 2009; **360**: 129-139 [PMID: 19092145 DOI: 10.1056/NEJMoa0808431]
- 109 **Albers JW,** Herman WH, Pop-Busui R, Feldman EL, Martin CL, Cleary PA, Waberski BH, Lachin JM; Diabetes Control and Complications Trial /Epidemiology of Diabetes Interventions and Complications Research Group. Effect of prior intensive insulin treatment during the Diabetes Control and Complications Trial (DCCT) on peripheral neuropathy in type 1 diabetes during the Epidemiology of Diabetes Interventions and Complications (EDIC) Study. *Diabetes Care* 2010; **33**: 1090-1096 [PMID: 20150297 DOI: 10.2337/dc09-1941]
- 110 **Ismail-Beigi F,** Craven T, Banerji MA, Basile J, Calles J, Cohen RM, Cuddihy R, Cushman WC, Genuth S, Grimm RH Jr, Hamilton BP, Hoogwerf B, Karl D, Katz L, Krikorian A, O'Connor P, Pop-Busui R, Schubart U, Simmons D, Taylor H, Thomas A, Weiss D, Hramiak I; ACCORD trial group. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. *Lancet* 2010; **376**: 419-430 [PMID: 20594588 DOI: 10.1016/S0140-6736(10)60576-4]
- 111 **Charles M,** Fleischer J, Witte DR, Ejksjaer N, Borch-Johnsen K, Lauritzen T, Sandbaek A. Impact of early detection and treatment of diabetes on the 6-year prevalence of cardiac autonomic neuropathy in people with screen-detected diabetes: ADDITION-Denmark, a cluster-randomised study. *Diabetologia* 2013; **56**: 101-108 [PMID: 23064291 DOI: 10.1007/s00125-012-2744-5]
- 112 **Charles M,** Ejksjaer N, Witte DR, Borch-Johnsen K, Lauritzen T, Sandbaek A. Prevalence of neuropathy and peripheral arterial disease

- and the impact of treatment in people with screen-detected type 2 diabetes: the ADDITION-Denmark study. *Diabetes Care* 2011; **34**: 2244-2249 [PMID: 21816977 DOI: 10.2337/dc11-0903]
- 113 **Catapano AL**, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Ž, Riccardi G, Taskinen MR, Tokgozoglu L, Verschuren WM, Vlachopoulos C, Wood DA, Zamorano JL; Authors/Task Force Members; Additional Contributor. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. *Eur Heart J* 2016; **37**: 2999-3058 [PMID: 27567407 DOI: 10.1093/eurheartj/ehw272]
 - 114 **Colhoun HM**, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ, Thomason MJ, Mackness MI, Charlton-Menys V, Fuller JH; CARDS investigators. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet* 2004; **364**: 685-696 [PMID: 15325833 DOI: 10.1016/S0140-6736(04)16895-5]
 - 115 **Jellinger PS**, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, Grunberger G, Guerin CK, Bell DSH, Mechanick JI, Pessah-Pollack R, Wyne K, Smith D, Brinton EA, Fazio S, Davidson M. American association of clinical endocrinologists and american college of endocrinology guidelines for management of dyslipidemia and prevention of cardiovascular disease. *Endocr Pract* 2017; **23**: 1-87 [PMID: 28437620 DOI: 10.4158/EP171764.APPGL]
 - 116 **Abifadel M**, Varret M, Rabès JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, Derré A, Villéger L, Farnier M, Beucler I, Bruckert E, Chambaz J, Chanu B, Lecerf JM, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003; **34**: 154-156 [PMID: 12730697 DOI: 10.1038/ng1161]
 - 117 Praluent (alirocumab) prescribing information. Bridgewater, NJ: Sanofi-Aventis US, 2015
 - 118 **Raal FJ**, Stein EA, Dufour R, Turner T, Civeira F, Burgess L, Langslet G, Scott R, Olsson AG, Sullivan D, Hovingh GK, Cariou B, Gouni-Berthold I, Somaratne R, Bridges I, Scott R, Wasserman SM, Gaudet D; RUTHERFORD-2 Investigators. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015; **385**: 331-340 [PMID: 25282519 DOI: 10.1016/S0140-6736(14)61399-4]
 - 119 **Robinson JG**, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, Stroes ES, Langslet G, Raal FJ, El Shahawy M, Koren MJ, Lepor NE, Lorenzato C, Pordy R, Chaudhari U, Kastelein JJ; ODYSSEY LONG TERM Investigators. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015; **372**: 1489-1499 [PMID: 25773378 DOI: 10.1056/NEJMoa1501031]
 - 120 **Stoekenbroek RM**, Kastelein JJ, Huijgen R. PCSK9 inhibition: the way forward in the treatment of dyslipidemia. *BMC Med* 2015; **13**: 258 [PMID: 26456772 DOI: 10.1186/s12916-015-0503-4]
 - 121 **Schwartz GG**, Bessac L, Berdan LG, Bhatt DL, Bittner V, Diaz R, Goodman SG, Hanotin C, Harrington RA, Jukema JW, Mahaffey KW, Moryusef A, Pordy R, Roe MT, Rorick T, Sasiela WJ, Shirodaria C, Szarek M, Tamby JF, Tricoci P, White H, Zeiher A, Steg PG. Effect of alirocumab, a monoclonal antibody to PCSK9, on long-term cardiovascular outcomes following acute coronary syndromes: rationale and design of the ODYSSEY outcomes trial. *Am Heart J* 2014; **168**: 682-689 [PMID: 25440796 DOI: 10.1016/j.ahj.2014.07.028]
 - 122 **Norata GD**, Tibolla G, Catapano AL. Targeting PCSK9 for hypercholesterolemia. *Annu Rev Pharmacol Toxicol* 2014; **54**: 273-293 [PMID: 24160703 DOI: 10.1146/annurev-pharmtox-011613-140025]
 - 123 **Chapman MJ**, Redfern JS, McGovern ME, Giral P. Niacin and fibrates in atherogenic dyslipidemia: pharmacotherapy to reduce cardiovascular risk. *Pharmacol Ther* 2010; **126**: 314-345 [PMID: 20153365 DOI: 10.1016/j.pharmthera.2010.01.008]
 - 124 **Thompson A**, Danesh J. Associations between apolipoprotein B, apolipoprotein AI, the apolipoprotein B/AI ratio and coronary heart disease: a literature-based meta-analysis of prospective studies. *J Intern Med* 2006; **259**: 481-492 [PMID: 16629854 DOI: 10.1111/j.1365-2796.2006.01644.x]
 - 125 **AIM-HIGH Investigators**, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med* 2011; **365**: 2255-2267 [PMID: 22085343 DOI: 10.1056/NEJMoa1107579]
 - 126 **HPS2-THRIVE Collaborative Group**, Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, Wallendszus K, Craig M, Jiang L, Collins R, Armitage J. Effects of extended-release niacin with laropiprant in high-risk patients. *N Engl J Med* 2014; **371**: 203-212 [PMID: 25014686 DOI: 10.1056/NEJMoa1300955]
 - 127 **Ooi CP**, Loke SC. Colesevelam for Type 2 diabetes mellitus: an abridged Cochrane review. *Diabet Med* 2014; **31**: 2-14 [PMID: 24024701 DOI: 10.1111/dme.12295]
 - 128 **Ahn CH**, Choi SH. New drugs for treating dyslipidemia: beyond statins. *Diabetes Metab J* 2015; **39**: 87-94 [PMID: 25922802 DOI: 10.4093/dmj.2015.39.2.87]
 - 129 **Bell DA**, Hooper AJ, Burnett JR. Mipomersen, an antisense apolipoprotein B synthesis inhibitor. *Expert Opin Investig Drugs* 2011; **20**: 265-272 [PMID: 21210756 DOI: 10.1517/13543784.2011.547471]
 - 130 **Adiels M**, Olofsson SO, Taskinen MR, Borén J. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2008; **28**: 1225-1236 [PMID: 18565848 DOI: 10.1161/ATVBAHA.107.160192]
 - 131 **Scott R**, O'Brien R, Fulcher G, Pardy C, D'Emden M, Tse D, Taskinen MR, Ehnholm C, Keech A; Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study Investigators. Effects of fenofibrate treatment on cardiovascular disease risk in 9,795 individuals with type 2 diabetes and various components of the metabolic syndrome: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. *Diabetes Care* 2009; **32**: 493-498 [PMID: 18984774 DOI: 10.2337/dc08-1543]
 - 132 **Taskinen MR**, Borén J. New insights into the pathophysiology of dyslipidemia in type 2 diabetes. *Atherosclerosis* 2015; **239**: 483-495 [PMID: 25706066 DOI: 10.1016/j.atherosclerosis.2015.01.039]
 - 133 **Cholesterol Treatment Trialists' (CTT) Collaboration**, Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhalra N, Peto R, Barnes EH, Keech A, Simes J, Collins R. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010; **376**: 1670-1681 [PMID: 21067804 DOI: 10.1016/S0140-6736(10)61350-5]
 - 134 **Sattar N**, Preiss D, Murray HM, Welsh P, Buckley BM, de Craen AJ, Seshasai SR, McMurray JJ, Freeman DJ, Jukema JW, Macfarlane PW, Packard CJ, Stott DJ, Westendorp RG, Shepherd J, Davis BR, Pressel SL, Marchioni R, Marfisi RM, Maggioni AP, Tavazzi L, Tognoni G, Kjekshus J, Pedersen TR, Cook TJ, Gotto AM, Clearfield MB, Downs JR, Nakamura H, Ohashi Y, Mizuno K, Ray KK, Ford I. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *Lancet* 2010; **375**: 735-742 [PMID: 20167359 DOI: 10.1016/S0140-6736(09)61965-6]
 - 135 **Keech A**, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, Forder P, Pillai A, Davis T, Glasziou P, Drury P, Kesäniemi YA, Sullivan D, Hunt D, Colman P, d'Emden M, Whiting M, Ehnholm C, Laakso M; FIELD study investigators. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005; **366**: 1849-1861 [PMID: 16310551 DOI: 10.1016/S0140-6736(05)67667-2]
 - 136 **ACCORD Study Group**, Ginsberg HN, Elam MB, Lovato LC, Crouse JR 3rd, Leiter LA, Linz P, Friedewald WT, Buse JB, Gerstein HC, Probstfield J, Grimm RH, Ismail-Beigi F, Biggar JT, Goff DC Jr, Cushman WC, Simons-Morton DG, Byington RP. Effects of combination lipid therapy in type 2 diabetes mellitus. *N Engl J Med* 2010; **362**: 1563-1574 [PMID: 20228404 DOI: 10.1056/NEJMoa1001282]
 - 137 **Saha SA**, Arora RR. Fibrates in the prevention of cardiovascular disease in patients with type 2 diabetes mellitus--a pooled meta-analysis of randomized placebo-controlled clinical trials. *Int J Cardiol* 2010; **141**: 157-166 [PMID: 19232762 DOI: 10.1016/j.ijcard.2008.11.211]

- 138 **Collins R**, Armitage J, Parish S, Sleight P, Peto R; Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet* 2003; **361**: 2005-2016 [PMID: 12814710 DOI: 10.1016/S0140-6736(03)13636-7]
- 139 **Brugts JJ**, Yetgin T, Hoeks SE, Gotto AM, Shepherd J, Westendorp RG, de Craen AJ, Knopp RH, Nakamura H, Ridker P, van Domburg R, Deckers JW. The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: meta-analysis of randomised controlled trials. *BMJ* 2009; **338**: b2376 [PMID: 19567909 DOI: 10.1136/bmj.b2376]
- 140 **Power RA**, Hulver MW, Zhang JY, Dubois J, Marchand RM, Ilkayeva O, Muoio DM, Mynatt RL. Carnitine revisited: potential use as adjunctive treatment in diabetes. *Diabetologia* 2007; **50**: 824-832 [PMID: 17310372 DOI: 10.1007/s00125-007-0605-4]
- 141 **Solfrizzi V**, Capurso C, Colacicco AM, D'Introno A, Fontana C, Capurso SA, Torres F, Gadaleta AM, Koverech A, Capurso A, Panza F. Efficacy and tolerability of combined treatment with L-carnitine and simvastatin in lowering lipoprotein(a) serum levels in patients with type 2 diabetes mellitus. *Atherosclerosis* 2006; **188**: 455-461 [PMID: 16384561 DOI: 10.1016/j.atherosclerosis.2005.11.024]
- 142 **Kasznicki J**, Kosmowski M, Sliwinski A, Mrowicka M, Stanczyk M, Majsterek I, Drzewoski J. Evaluation of oxidative stress markers in pathogenesis of diabetic neuropathy. *Mol Biol Rep* 2012; **39**: 8669-8678 [PMID: 22718504 DOI: 10.1007/s11033-012-1722-9]
- 143 **Oyenih A**, Ayeleso AO, Mukwevho E, Masola B. Antioxidant strategies in the management of diabetic neuropathy. *Biomed Res Int* 2015; **2015**: 515042 [PMID: 25821809 DOI: 10.1155/2015/515042]
- 144 **de M Bandeira S**, da Fonseca LJ, da S Guedes G, Rabelo LA, Goulart MO, Vasconcelos SM. Oxidative stress as an underlying contributor in the development of chronic complications in diabetes mellitus. *Int J Mol Sci* 2013; **14**: 3265-3284 [PMID: 23385234 DOI: 10.3390/ijms14023265]
- 145 **Furukawa S**, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; **114**: 1752-1761 [PMID: 15599400 DOI: 10.1172/JCI200421625]
- 146 **Vincent AM**, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr Rev* 2004; **25**: 612-628 [PMID: 15294884 DOI: 10.1210/er.2003-0019]
- 147 **Obrosova IG**, Ilnytska O, Lyzogubov VV, Pavlov IA, Mashtalir N, Nadler JL, Drel VR. High-fat diet induced neuropathy of pre-diabetes and obesity: effects of "healthy" diet and aldose reductase inhibition. *Diabetes* 2007; **56**: 2598-2608 [PMID: 17626889 DOI: 10.2337/db06-1176]
- 148 **Negre-Salvayre A**, Coatrieux C, Ingueneau C, Salvayre R. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. *Br J Pharmacol* 2008; **153**: 6-20 [PMID: 17643134 DOI: 10.1038/sj.bjp.0707395]
- 149 **Mahmood D**, Singh BK, Akhtar M. Diabetic neuropathy: therapies on the horizon. *J Pharm Pharmacol* 2009; **61**: 1137-1145 [PMID: 19703362 DOI: 10.1211/jpp/61.09.0002]
- 150 **Kalousova M**, Skrha J, Zima T. Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. *Physiol Res* 2002; **51**: 597-604 [PMID: 12511184]
- 151 **King RH**. The role of glycation in the pathogenesis of diabetic polyneuropathy. *Mol Pathol* 2001; **54**: 400-408 [PMID: 11724915]
- 152 **Valko M**, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; **39**: 44-84 [PMID: 16978905 DOI: 10.1016/j.biocel.2006.07.001]
- 153 **Bajaj S**, Khan A. Antioxidants and diabetes. *Indian J Endocrinol Metab* 2012; **16**: S267-S271 [PMID: 23565396 DOI: 10.4103/2230-8210.104057]
- 154 **Balarabe SA**, Adamu MD, Watila MM, Jiya N. Neuromyelitis optica and myasthenia gravis in a young Nigerian girl. *BMJ Case Rep* 2015; **2015**: pii: bcr2014207362 [PMID: 26338241 DOI: 10.1136/bcr-2014-207362]
- 155 **Hamilton SJ**, Chew GT, Watts GF. Therapeutic regulation of endothelial dysfunction in type 2 diabetes mellitus. *Diab Vasc Dis Res* 2007; **4**: 89-102 [PMID: 17654442 DOI: 10.3132]
- 156 **Packer L**, Kraemer K, Rimbach G. Molecular aspects of lipoic acid in the prevention of diabetes complications. *Nutrition* 2001; **17**: 888-895 [PMID: 11684397 DOI: 10.1016/S0899-9007(01)00658-X]
- 157 **Ziegler D**, Schatz H, Conrad F, Gries FA, Ulrich H, Reichel G. Effects of treatment with the antioxidant alpha-lipoic acid on cardiac autonomic neuropathy in NIDDM patients. A 4-month randomized controlled multicenter trial (DEKAN Study). *Deutsche Kardiale Autonome Neuropathie. Diabetes Care* 1997; **20**: 369-373 [PMID: 9051389 DOI: 10.2337/diacare.20.3.369]
- 158 **Bertolotto F**, Massone A. Combination of alpha lipoic acid and superoxide dismutase leads to physiological and symptomatic improvements in diabetic neuropathy. *Drugs R D* 2012; **12**: 29-34 [PMID: 22329607 DOI: 10.2165/11599200-000000000-00000]
- 159 **McNulty H**, Jacob RF, Mason RP. Biologic activity of carotenoids related to distinct membrane physicochemical interactions. *Am J Cardiol* 2008; **101**: 20D-29D [PMID: 18474269 DOI: 10.1016/j.amjcard.2008.02.004]
- 160 **Berrone E**, Beltramo E, Solimine C, Ape AU, Porta M. Regulation of intracellular glucose and polyol pathway by thiamine and benfotiamine in vascular cells cultured in high glucose. *J Biol Chem* 2006; **281**: 9307-9313 [PMID: 16452468 DOI: 10.1074/jbc.M600418200]
- 161 **Lazo de la Vega-Monroy ML**, Larrieta E, German MS, Baez-Saldana A, Fernandez-Mejia C. Effects of biotin supplementation in the diet on insulin secretion, islet gene expression, glucose homeostasis and beta-cell proportion. *J Nutr Biochem* 2013; **24**: 169-177 [PMID: 22841397 DOI: 10.1016/j.jnutbio.2012.03.020]
- 162 **Sheikh-Ali M**, Chehade JM, Mooradian AD. The antioxidant paradox in diabetes mellitus. *Am J Ther* 2011; **18**: 266-278 [PMID: 19797943 DOI: 10.1097/MJT.0b013e3181b7badf]
- 163 **Rahimi R**, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother* 2005; **59**: 365-373 [PMID: 16081237 DOI: 10.1016/j.biopha.2005.07.002]
- 164 **Hotta T**, Akanuma Y, Kawamori R, Matsuoka K, Oka Y, Shichiri M, Toyota T, Nakashima M, Yoshimura I, Sakamoto N, Shigeta Y. Long-term clinical effects of epalrestat, an aldose reductase inhibitor, on diabetic peripheral neuropathy: the 3-year, multicenter, comparative Aldose Reductase Inhibitor-Diabetes Complications Trial. *Diabetes Care* 2006; **29**: 1538-1544 [PMID: 16801576 DOI: 10.2337/dc05-2370]
- 165 **Haupt E**, Ledermann H, Köpcke W. Benfotiamine in the treatment of diabetic polyneuropathy--a three-week randomized, controlled pilot study (BEDIP study). *Int J Clin Pharmacol Ther* 2005; **43**: 71-77 [PMID: 15726875 DOI: 10.5414/CP43071]
- 166 **Stracke H**, Gaus W, Achenbach U, Federlin K, Bretzel RG. Benfotiamine in diabetic polyneuropathy (BENDIP): results of a randomised, double blind, placebo-controlled clinical study. *Exp Clin Endocrinol Diabetes* 2008; **116**: 600-605 [PMID: 18473286 DOI: 10.1055/s-2008-1065351]
- 167 **Balakumar P**, Rohilla A, Krishan P, Solairaj P, Thangathirupathi A. The multifaceted therapeutic potential of benfotiamine. *Pharmacol Res* 2010; **61**: 482-488 [PMID: 20188835 DOI: 10.1016/j.phrs.2010.02.008]
- 168 **Stracke H**, Lindemann A, Federlin K. A benfotiamine-vitamin B combination in treatment of diabetic polyneuropathy. *Exp Clin Endocrinol Diabetes* 1996; **104**: 311-316 [PMID: 8886748 DOI: 10.1055/s-0029-1211460]
- 169 **Yan H**, Guo Y, Zhang J, Ding Z, Ha W, Harding JJ. Effect of carnitine, aminoguanidine, and aspirin drops on the prevention of cataracts in diabetic rats. *Mol Vis* 2008; **14**: 2282-2291 [PMID: 19081783]
- 170 **Urios P**, Grigorova-Borsos AM, Sternberg M. Aspirin inhibits the formation of pentosidine, a cross-linking advanced glycation end product, in collagen. *Diabetes Res Clin Pract* 2007; **77**: 337-340 [PMID: 17383766 DOI: 10.1016/j.diabres.2006.12.024]
- 171 **Shen Q**, Pierce JD. Supplementation of Coenzyme Q10 among Patients with Type 2 Diabetes Mellitus. *Healthcare (Basel)* 2015; **3**:

- 296-309 [PMID: 27417763 DOI: 10.3390/healthcare3020296]
- 172 **Burnier M.** Telmisartan: a different angiotensin II receptor blocker protecting a different population? *J Int Med Res* 2009; **37**: 1662-1679 [PMID: 20146864 DOI: 10.1177/147323000903700602]
 - 173 **Ahmed MA,** Muntingh GL, Rheeder P. Perspectives on Peripheral Neuropathy as a Consequence of Metformin-Induced Vitamin B12 Deficiency in T2DM. *Int J Endocrinol* 2017; **2017**: 2452853 [PMID: 28932240 DOI: 10.1155/2017/2452853]
 - 174 **Fragasso G,** Pallosi A, Puccetti P, Silipigni C, Rossodivita A, Pala M, Calori G, Alfieri O, Margonato A. A randomized clinical trial of trimetazidine, a partial free fatty acid oxidation inhibitor, in patients with heart failure. *J Am Coll Cardiol* 2006; **48**: 992-998 [PMID: 16949492 DOI: 10.1016/j.jacc.2006.03.060]
 - 175 **Lee WS,** Kim J. Diabetic cardiomyopathy: where we are and where we are going. *Korean J Intern Med* 2017; **32**: 404-421 [PMID: 28415836 DOI: 10.3904/kjim.2016.208]
 - 176 **Lee L,** Campbell R, Scheuermann-Freestone M, Taylor R, Gunaruwan P, Williams L, Ashrafian H, Horowitz J, Fraser AG, Clarke K, Frenneaux M. Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment. *Circulation* 2005; **112**: 3280-3288 [PMID: 16301359 DOI: 10.1161/CIRCULATIONAHA.105.5514]
 - 177 **Morrow DA,** Scirica BM, Karwatowska-Prokopczuk E, Murphy SA, Budaj A, Varshavsky S, Wolff AA, Skene A, McCabe CH, Braunwald E; MERLIN-TIMI 36 Trial Investigators. Effects of ranolazine on recurrent cardiovascular events in patients with non-ST-elevation acute coronary syndromes: the MERLIN-TIMI 36 randomized trial. *JAMA* 2007; **297**: 1775-1783 [PMID: 17456819 DOI: 10.1001/jama.297.16.1775]
 - 178 **Witteles RM,** Fowler MB. Insulin-resistant cardiomyopathy clinical evidence, mechanisms, and treatment options. *J Am Coll Cardiol* 2008; **51**: 93-102 [PMID: 18191731 DOI: 10.1016/j.jacc.2007.10]
 - 179 **Nikolaidis LA,** Poornima I, Parikh P, Magovern M, Shen YT, Shannon RP. The effects of combined versus selective adrenergic blockade on left ventricular and systemic hemodynamics, myocardial substrate preference, and regional perfusion in conscious dogs with dilated cardiomyopathy. *J Am Coll Cardiol* 2006; **47**: 1871-1881 [PMID: 16682315 DOI: 10.1016/j.jacc.2005.11.082]
 - 180 **Sytze Van Dam P,** Cotter MA, Bravenboer B, Cameron NE. Pathogenesis of diabetic neuropathy: focus on neurovascular mechanisms. *Eur J Pharmacol* 2013; **719**: 180-186 [PMID: 23872412 DOI: 10.1016/j.ejphar.2013.07.017]
 - 181 **Tzemos N,** Lim PO, MacDonald TM. Nebivolol reverses endothelial dysfunction in essential hypertension: a randomized, double-blind, crossover study. *Circulation* 2001; **104**: 511-514 [PMID: 11479245 DOI: 10.1161/hc3001.094207]
 - 182 **Zepeda RJ,** Castillo R, Rodrigo R, Prieto JC, Aramburu I, Brugere S, Galdames K, Noriega V, Miranda HF. Effect of carvedilol and nebivolol on oxidative stress-related parameters and endothelial function in patients with essential hypertension. *Basic Clin Pharmacol Toxicol* 2012; **111**: 309-316 [PMID: 22703478 DOI: 10.1111/j.1742-7843.2012.00911.x]
 - 183 **Su JB.** Vascular endothelial dysfunction and pharmacological treatment. *World J Cardiol* 2015; **7**: 719-741 [PMID: 26635921 DOI: 10.4330/wjc.v7.i11.719]
 - 184 **Kelly AS,** Gonzalez-Campoy JM, Rudser KD, Katz H, Metzger AM, Thalín M, Bank AJ. Carvedilol-lisinopril combination therapy and endothelial function in obese individuals with hypertension. *J Clin Hypertens* (Greenwich) 2012; **14**: 85-91 [PMID: 22277140 DOI: 10.1111/j.1751-7176.2011.00569.x]
 - 185 **Mak IT,** Boehme P, Weglicki WB. Antioxidant effects of calcium channel blockers against free radical injury in endothelial cells. Correlation of protection with preservation of glutathione levels. *Circ Res* 1992; **70**: 1099-1103 [PMID: 1576732 DOI: 10.1161/01.RES.70.6.1099]
 - 186 **Matsubara M,** Hasegawa K. Benidipine, a dihydropyridine-calcium channel blocker, prevents lysophosphatidylcholine-induced injury and reactive oxygen species production in human aortic endothelial cells. *Atherosclerosis* 2005; **178**: 57-66 [PMID: 15585201 DOI: 10.1016/j.atherosclerosis.2004.08.020]
 - 187 **Habib JB,** Bossaller C, Wells S, Williams C, Morrisett JD, Henry PD. Preservation of endothelium-dependent vascular relaxation in cholesterol-fed rabbit by treatment with the calcium blocker PN 200110. *Circ Res* 1986; **58**: 305-309 [PMID: 2936528 DOI: 10.1161/01.RES.58.2.305]
 - 188 **Fukao K,** Shimada K, Hiki M, Kiyonagi T, Hirose K, Kume A, Ohsaka H, Matsumori R, Kurata T, Miyazaki T, Daida H. Effects of calcium channel blockers on glucose tolerance, inflammatory state, and circulating progenitor cells in non-diabetic patients with essential hypertension: a comparative study between azelnidipine and amlodipine on glucose tolerance and endothelial function—a crossover trial (AGENT). *Cardiovasc Diabetol* 2011; **10**: 79 [PMID: 21906391 DOI: 10.1186/1475-2840-10-79]
 - 189 **Celik T,** Balta S, Karaman M, Ahmet Ay S, Demirkol S, Ozturk C, Dinc M, Unal HU, Yilmaz MI, Kılıc S, Kurt G, Tas A, Iyisoy A, Quarti-Trevano F, Fıçı F, Grassi G. Endocan, a novel marker of endothelial dysfunction in patients with essential hypertension: comparative effects of amlodipine and valsartan. *Blood Press* 2015; **24**: 55-60 [PMID: 25390761 DOI: 10.3109/08037051.2014.972816]
 - 190 **Fukutomi M,** Hoshida S, Mizuno H, Kario K. Differential effects of aliskiren/amlodipine combination and high-dose amlodipine monotherapy on endothelial function in elderly hypertensive patients. *Am J Hypertens* 2014; **27**: 14-20 [PMID: 24008122 DOI: 10.1093/ajh/hpt158]
 - 191 **Okamura T,** Tawa M, Geddawy A, Shimosato T, Iwasaki H, Shintaku H, Yoshida Y, Masada M, Shinozaki K, Imamura T. Effects of atorvastatin, amlodipine, and their combination on vascular dysfunction in insulin-resistant rats. *J Pharmacol Sci* 2014; **124**: 76-85 [PMID: 24389820 DOI: 10.1254/jphs.13178FP]
 - 192 **Zhou MS,** Tian R, Jaimes EA, Raij L. Combination therapy of amlodipine and atorvastatin has more beneficial vascular effects than monotherapy in salt-sensitive hypertension. *Am J Hypertens* 2014; **27**: 873-880 [PMID: 24413709 DOI: 10.1093/ajh/hpt272]
 - 193 **Jain NK,** Patil CS, Singh A, Kulkarni SK. Sildenafil-induced peripheral analgesia and activation of the nitric oxide-cyclic GMP pathway. *Brain Res* 2001; **909**: 170-178 [PMID: 11478933 DOI: 10.1016/S0006-8993(01)02673-7]
 - 194 **Patil CS,** Singh VP, Singh S, Kulkarni SK. Modulatory effect of the PDE-5 inhibitor sildenafil in diabetic neuropathy. *Pharmacology* 2004; **72**: 190-195 [PMID: 15452368 DOI: 10.1159/000080104]
 - 195 **Wang L,** Chopp M, Szalad A, Liu Z, Bolz M, Alvarez FM, Lu M, Zhang L, Cui Y, Zhang RL, Zhang ZG. Phosphodiesterase-5 is a therapeutic target for peripheral neuropathy in diabetic mice. *Neuroscience* 2011; **193**: 399-410 [PMID: 21820491 DOI: 10.1016/j.neuroscience.2011.07.039]
 - 196 **Peixoto CA,** Nunes AK, Garcia-Osta A. Phosphodiesterase-5 Inhibitors: Action on the Signaling Pathways of Neuroinflammation, Neurodegeneration, and Cognition. *Mediators Inflamm* 2015; **2015**: 940207 [PMID: 26770022 DOI: 10.1155/2015/940207]
 - 197 **Schäfer A,** Fraccarollo D, Pförtsch S, Flierl U, Vogt C, Pfrang J, Kobsar A, Renné T, Eigenthaler M, Ertl G, Bauersachs J. Improvement of vascular function by acute and chronic treatment with the PDE-5 inhibitor sildenafil in experimental diabetes mellitus. *Br J Pharmacol* 2008; **153**: 886-893 [PMID: 17891166 DOI: 10.1038/sj.bjp.0707459]
 - 198 **Wang L,** Chopp M, Szalad A, Lu X, Jia L, Lu M, Zhang RL, Zhang ZG. Tadalafil Promotes the Recovery of Peripheral Neuropathy in Type II Diabetic Mice. *PLoS One* 2016; **11**: e0159665 [PMID: 27438594 DOI: 10.1371/journal.pone.0159665]
 - 199 **Wang L,** Chopp M, Zhang ZG. PDE5 inhibitors promote recovery of peripheral neuropathy in diabetic mice. *Neural Regen Res* 2017; **12**: 218-219 [PMID: 28400802 DOI: 10.4103/1673-5374.200804]
 - 200 **Tse S,** Mazzola N. Ivabradine (Corlanor) for Heart Failure: The First Selective and Specific I f Inhibitor. *PT* 2015; **40**: 810-814 [PMID: 26681903]
 - 201 **DiFrancesco D.** The role of the funny current in pacemaker activity. *Circ Res* 2010; **106**: 434-446 [PMID: 20167941 DOI: 10.1161/CIRCRESAHA.109.208041]
 - 202 **Reil JC,** Tardif JC, Ford I, Lloyd SM, O'Meara E, Komajda M, Borer JS, Tavazzi L, Swedberg K, Böhm M. Selective heart rate reduction with ivabradine unloads the left ventricle in heart failure patients. *J Am*

- Coll Cardiol* 2013; **62**: 1977-1985 [PMID: 23933545 DOI: 10.1016/j.jacc.2013.07.027]
- 203 **Rienzo M**, Melka J, Bizé A, Sambin L, Jozwiak M, Su JB, Hittinger L, Berdeaux A, Ghaleh B. Ivabradine improves left ventricular function during chronic hypertension in conscious pigs. *Hypertension* 2015; **65**: 122-129 [PMID: 25350985 DOI: 10.1161/HYPERTENSIONAHA.114.04323]
- 204 **Jochmann N**, Schröter F, Knebel F, Hättasch R, Gericke C, Stangl K, Baumann G, Stangl V. Effect of ivabradine-induced heart rate reduction on flow-mediated dilation measured with high-sensitivity ultrasound in patients with stable coronary heart disease. *Cardiovasc Ultrasound* 2014; **12**: 5 [PMID: 24479706 DOI: 10.1186/1476-7120-12-5]
- 205 **Nerla R**, Di Franco A, Milo M, Pitocco D, Zaccardi F, Tarzia P, Sarullo FM, Villano A, Russo G, Stazi A, Ghirlanda G, Lanza GA, Crea F. Differential effects of heart rate reduction by atenolol or ivabradine on peripheral endothelial function in type 2 diabetic patients. *Heart* 2012; **98**: 1812-1816 [PMID: 23086971 DOI: 10.1136/heartjnl-2012-302795]
- 206 **Fox K**, Ford I, Ferrari R. Ivabradine in stable coronary artery disease. *N Engl J Med* 2014; **371**: 2435 [PMID: 25517716 DOI: 10.1056/NEJMc1413158]
- 207 **Dhule SS**, Gawali SR. Platelet aggregation and clotting time in type II diabetic males. *Natl J Physiol Pharm Pharmacol* 2014; **4**: 121-123 [DOI: 10.5455/njppp.2014.4.290920131]
- 208 **Güven F**, Yilmaz A, Aydin H, Korkmaz I. Platelet aggregation responses in type 2 diabetic patients. *Health* 2010; **2**: 708-712 [DOI: 10.4236/health.2010.27108]
- 209 **Vinik AI**. Diabetic neuropathy: pathogenesis and therapy. *Am J Med* 1999; **107**: 17S-26S [PMID: 10484041 DOI: 10.1016/S0002-9343(99)00009-1]
- 210 **Jordan J**, Shannon JR, Black BK, Ali Y, Farley M, Costa F, Diedrich A, Robertson RM, Biaggioni I, Robertson D. The pressor response to water drinking in humans : a sympathetic reflex? *Circulation* 2000; **101**: 504-509 [PMID: 10662747 DOI: 10.1161/01.CIR.101.5.504]
- 211 **Singer W**, Opfer-Gehrking TL, McPhee BR, Hilz MJ, Bharucha AE, Low PA. Acetylcholinesterase inhibition: a novel approach in the treatment of neurogenic orthostatic hypotension. *J Neurol Neurosurg Psychiatry* 2003; **74**: 1294-1298 [PMID: 12933939 DOI: 10.1136/jnnp.74.9.1294]
- 212 **Nóbrega AC**, dos Reis AF, Moraes RS, Bastos BG, Ferlin EL, Ribeiro JP. Enhancement of heart rate variability by cholinergic stimulation with pyridostigmine in healthy subjects. *Clin Auton Res* 2001; **11**: 11-17 [PMID: 11503945 DOI: 10.1007/BF02317797]

P- Reviewer: Isik A, McMillin MA, Radenovic L, Xavier-Elsas P

S- Editor: Ji FF **L- Editor:** A **E- Editor:** Lu YJ



Basic Study

Short-term effects of obestatin on hexose uptake and triacylglycerol breakdown in human subcutaneous adipocytes

Christian Carpéné, Francisco Les, David Estève, Jean Galitzky

Christian Carpéné, David Estève, Jean Galitzky, Institut des Maladies Métaboliques et Cardiovasculaires, Institut National de la Santé et de la Recherche Médicale, Université Paul Sabatier, Toulouse 31432, France

Francisco Les, Department of Pharmacy, Faculty of Health Sciences, Universidad San Jorge, Villanueva de Gállego 50830, Spain

Author contributions: Carpéné C and Galitzky J substantially contributed to the conception, design of the study and drafted the manuscript; Carpéné C, Les F and Estève D worked in data acquisition and orientated their analysis after bibliographical research; and all authors approved the submitted version of the paper after careful perusal.

Supported by Erasmus plus Traineeship program.

Institutional review board statement: The study was reviewed by the Institutional Review Board of Institut des Maladies Métaboliques et Cardiovasculaires, Toulouse, France. The study was approved by the local Ethics Committee: "Comité de Protection des Personnes Sud Ouest et Outre mer II".

Conflict-of-interest statement: All authors declare that they do not have any potential conflict of interest in relation to this article.

Data sharing statement: No supplementary data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Dr. Christian Carpéné, Institut des

Maladies Métaboliques et Cardiovasculaires, Institut National de la Santé et de la Recherche Médicale, Université de Toulouse, CHU Rangueil, UPS, Toulouse 31432, France. christian.carpene@inserm.fr
Telephone: +33-5-61325640

Received: February 7, 2017

Peer-review started: February 12, 2017

First decision: May 17, 2017

Revised: November 17, 2017

Accepted: December 4, 2017

Article in press: December 4, 2017

Published online: January 15, 2018

Abstract

AIM

To study complete dose-dependent effects of obestatin on lipolytic and glucose transport activities in human adipocyte preparations highly responsive to insulin.

METHODS

Adipocytes were prepared by liberase digestion from subcutaneous abdominal adipose tissue obtained from overweight subjects undergoing plastic surgery. The index of lipolytic activity was the glycerol released in the incubation medium, while glucose transport was assessed by [³H]-2-deoxyglucose uptake assay.

RESULTS

When tested from 0.1 nmol/L to 1 μmol/L, obestatin did not stimulate glycerol release; it did not inhibit the lipolytic effect of isoprenaline and did not alter the insulin antilipolytic effect. Obestatin hardly activated glucose transport at 1 μmol/L only. Moreover, the obestatin stimulation effect was clearly lower than the threefold increase induced by insulin 100 nmol/L.

CONCLUSION

Low doses of obestatin cannot directly influence lipolysis and glucose uptake in human fat cells.

Key words: Insulin; Lipolysis; Adipokines; Glucose uptake; Obestatin; Human adipocytes

© **The Author(s)** 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: We have compared in adipocytes the well-known glucose uptake stimulation and lipolysis inhibition induced by insulin to the effects of obestatin, a gut peptide derived from ghrelin gene recently proposed to act on fat cells. Obestatin was much less efficient than insulin in adipocytes from human abdominal subcutaneous adipose tissue. Indeed, obestatin weakly activated hexose transport while it could not reproduce the antilipolytic effect of insulin at any tested concentration. We therefore propose that obestatin does not rapidly modulate lipogenesis and lipolysis and that its contribution to energy homeostasis depends on actions other than a direct control of adipocyte metabolism.

Carpéné C, Les F, Estève D, Galitzky J. Short-term effects of obestatin on hexose uptake and triacylglycerol breakdown in human subcutaneous adipocytes. *World J Diabetes* 2018; 9(1): 25-32 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i1/25.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i1.25>

INTRODUCTION

Obestatin is a 23-amino acid peptide with highly conserved sequence among mammalian species that corresponds to the 76-98 segment of pre-proghrelin, a polypeptide of 117 residues, also generating by cleavage of its 24-51 segment the multifunctional hormone ghrelin. The receptor initially proposed for obestatin was the orphan G protein-coupled receptor GPR39^[1]. However, this assumption has never been confirmed^[2], and to our knowledge it remains unclear to what receptor can selectively bind obestatin.

Hardly clearer is the overall physiological action of obestatin, which suppresses food intake and decreases body-weight gain, and counteracts the appetite-stimulating properties of ghrelin. At the first glance, the anorectic and catabolic properties attributed to obestatin appear to be opposite to the insulin panel of actions. In fact, obestatin has been reported to limit food intake in rodents under special conditions only, such as fasting-refeeding challenges^[1]. Then, it has been evidenced that obestatin failed to affect food intake and gut motility in ghrelin-deficient mice, and in further studies, obestatin administration did not exert clear-cut influence on food intake and body weight^[3]. It is therefore currently suggested that obestatin is not a major regulator of satiety signalling^[4] while it is still admitted that ghrelin and obestatin may have opposite effects on digestive

physiology.

Similarly, the *in vitro* effects of obestatin directly measured on one of its targets, namely the adipose cell, are far from being univocally demonstrated. Several reports have evidenced that obestatin activates glucose uptake in 3T3-L1 cultured preadipocytes and in mature fat cells^[5,6]. Accordingly, obestatin inhibited isoproterenol-induced lipolysis, promoted AMP-activated protein kinase phosphorylation, enhanced adiponectin secretion in both mice and human mature adipocytes. Obestatin also enhanced glucose uptake either in the absence or in the presence of insulin, promoted GLUT4 translocation and increased Akt phosphorylation, according to the studies of Granata and coworkers^[6,7]. Also like insulin, obestatin promoted adipogenesis in rat^[8] or murine^[5] preadipocytes. However, other studies that described an antilipolytic action of obestatin on non-esterified fatty acid and glycerol release, failed to detect any influence on glucose transport^[9]. Even a lack of obestatin effect was observed regarding glycerol release or adipogenesis in 3T3-L1 preadipocytes^[10], while a pro-lipolytic action was evidenced in other models^[11]. Such ability of obestatin to trigger lipid catabolism^[12] was therefore hardly conceivable together with the above-reported insulin-like actions. Anyhow, such controversy was dealing with previous observations indicating that obestatin inhibits proliferation and differentiation of 3T3-L1 preadipocytes^[3].

In this context, the putative ability of obestatin to modulate glucose uptake deserved to be verified in human native fat cells rather than in any additional engineered insulin-sensitive model. To this aim, and in order to also verify whether obestatin was able to acutely influence adipocyte lipolytic activity, we decided to study its acute effects on human subcutaneous adipocytes. Our approach was further justified by the fact that obestatin is proposed to belong to the large family of adipokines^[13] secreted by adipose tissue^[7]. A special attention was paid to use insulin-responsive fat cells, thereby to include human insulin as a positive control in our comparative study. Similarly, lipolytic agents such as isoprenaline (a β -adrenoceptor agonist also known as isoproterenol), atrial natriuretic peptide (ANP)^[14] and antilipolytic factors such as UK14304 (α_2 -adrenoceptor agonist) were used as references for the fine regulation of lipolytic activity. Lastly, hydrogen peroxide (H₂O₂) was also used in our tests since it is known to activate glucose transport independently from insulin^[15]. In the following results, we have therefore tested increasing doses of obestatin (0.1 nmol/L - 1 μ mol/L) on human fat cells preparations highly responsive to insulin under conditions already validated to investigate the properties of other adipokines^[16,17], drugs^[18] or dietary components^[19].

MATERIALS AND METHODS

Chemicals

Recombinant human obestatin was purchased from Phoenix Pharmaceuticals Inc. (Belmont, CA, United

Table 1 Clinical parameters of the study group and characteristics of adipocyte preparations

Clinical characteristics of SCAT donors	
BMI of subjects, kg/m ²	26.1 ± 0.7
Age, yr	40 ± 3
Biochemical features of adipocyte preparations	
Cell lipid content/lipolysis assay, mg (<i>n</i>)	14.1 ± 1.3 (7)
Cell lipid content/glucose uptake assay, mg (<i>n</i>)	15.9 ± 1.3 (10)
Lipolytic responsiveness (fold increase over basal glycerol release, <i>n</i> = 7)	
Basal	1.00 ± 0.17
Isoprenaline 10 µmol/L	5.14 ± 0.67 ^b
Human atrial natriuretic peptide 1 µmol/L	5.16 ± 0.44 ^b
Glucose transport capacity (fold increase over basal 2DG uptake, <i>n</i> = 10)	
Basal	1.00 ± 0.13
Insulin 100 nmol/L	3.14 ± 0.28 ^b
Hydrogen peroxide 1 mmol/L	1.72 ± 0.27 ^a

Adipocytes were isolated by liberase digestion from pieces of SCAT obtained from a total of 13 women then incubated for lipolysis and/or glucose uptake assays for the number of individual preparations indicated in parenthesis. Different from respective basal values at: ^a*P* < 0.05; ^b*P* < 0.001. SCAT: Subcutaneous adipose tissue.

States). Human insulin, bovine serum albumin, and other reagents were obtained from Sigma-Aldrich (Saint Quentin Fallavier, F). Liberase TM was from Roche Diagnostic (Indianapolis, IN, United States). [³H]-2-deoxyglucose was from Perkin Elmer (Boston, MA, United States). UK 14304 (bromoxidine) was a generous gift from late Dr Hervé Paris (INSERM, Toulouse, France).

Subjects and preparation of adipose cells

Samples of subcutaneous adipose tissue (SCAT), were obtained from non-obese premenopausal women (age range 29-53 year) undergoing abdominal lipectomy at the plastic surgery department of Rangueil hospital (Toulouse, France) under the agreement of INSERM guidelines and the ethic committee for the protection of individuals under the reference DC-2008-452. The clinical characteristics of the donors and the biochemical profiles of the corresponding adipocyte preparations are described in Table 1. The removed pieces of fat depot were transferred in less than 30 min to the laboratory. SCAT was immediately treated by liberase digestion (15 µg/mL) in the presence of 3.5% of bovine serum albumin in the digestion buffer (Krebs-Ringer containing 15 mmol/L sodium bicarbonate, 10 mmol/L HEPES, 2 mmol/L pyruvate). Separation, washing and dilution of the buoyant adipocytes were performed in the same buffer without liberase as previously described^[19], immediately prior biological assays.

Lipolysis and deoxyglucose transport measurements in isolated adipocytes

Fat cells were diluted in around 10-fold their volume of buffer, and cell suspension was distributed into plastic vials. Lipolytic activity was assessed by the glycerol released by fat cells medium after a 90-min incubation in 400 µL final volume with the tested agents, as previously described^[19]. Results were expressed as µmoles of glycerol released/100 mg cellular lipids/90 min, or as percentage of isoprenaline-induced stimu-

lation.

For hexose uptake assays, incubations of the tested agents with fat cell suspensions lasted 45 min at 37 °C before 10 min exposure to 0.1 mmol/L [³H]-2-deoxyglucose (2-DG) as previously described^[19]. Separation of internalized hexose was performed on 200 µL aliquots by centrifugation through dynonyl-phtalate silicon oil to separate buoyant intact fat cells from medium^[17]. Lipid content was determined as previously reported^[20,21]. Uptake was expressed as fold increase over basal uptake, which accounted for 0.30 ± 0.05 nmol 2-DG internalized/100 mg cellular lipids/10 min.

3T3 F442A cultured preadipocytes

3T3 F442A cells were grown at 37 °C under 5% CO₂ in DMEM supplemented with 10% foetal calf serum and antibiotic mixture (100 U/mL penicillin + 100 µg/mL streptomycin) until confluence. Contrarily to their parent cell line 3T3-L1, 3T3-F442A cells do not need isobutylmethylxanthine and dexamethasone to trigger adipogenic process and are in this regard only insulin-dependent^[22]. Cells were therefore induced to differentiate by 50 nmol/L insulin for 8 d before being tested for 2-DG uptake.

Statistical analysis

Results are given as means ± SEM. Statistical significance was assessed by use of Student's *t*-test or one-way ANOVA followed by Bonferroni test using Prism 5 for Mac OS X.

RESULTS

Preliminary verification of obestatin biologic activity in 3T3-F442A adipocytes

Since obestatin has been reported to activate glucose uptake in 3T3-L1 cultured preadipocytes, it was first verified whether our preparation could reproduce such

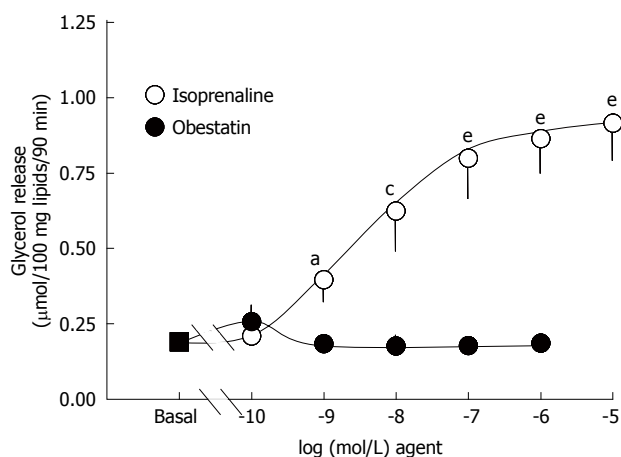


Figure 1 Effects of isoprenaline and of obestatin on lipolysis activation in human adipocytes. Fat cells were incubated for 90 min with isoprenaline (open circles) or obestatin (closed circles) at the indicated concentrations. Mean \pm SEM of 7 experiments. In several occurrences, SEM bar lies within the symbol. Different from basal lipolysis (black square) at: ^a $P < 0.05$; ^c $P < 0.01$; ^e $P < 0.001$.

insulin-like activity. However, our preliminary tests were performed on 3T3-F442A lineage, which is slightly distinct from 3T3-L1 cells since only requiring insulin to promote adipocyte differentiation. Eight days after confluence, cells were serum starved overnight and their basal [³H]-2-deoxyglucose uptake was activated by 1.79 ± 0.03 fold and by 1.21 ± 0.04 by 10 nmol/L insulin and 10 nmol/L obestatin, respectively ($n = 3$; $P < 0.001$ and $P < 0.02$). These preliminary observations indicated that obestatin preparation reproduced almost two-third of the insulin effect on glucose uptake and prompted us to treat human fat cells with obestatin.

Preparations of highly responsive human adipocytes

As shown in Table 1, human adipocytes were isolated from subjects belonging to the normal-to-mild overweight class, according to the body mass index-based classification of obesity. From this group, constituted by a total 13 non-obese premenopausal women undergoing abdominal plastic surgery, there was sufficient SCAT material to test the influence of obestatin on triacylglycerol breakdown in seven cases while glucose uptake assays could be performed on 10 individual adipocyte preparations. When measuring glycerol release, one of the end-products of complete hydrolysis of triacylglycerols, the β -adrenergic agonist isoprenaline maximally stimulated fivefold the baseline, qualifying our test conditions as discriminative enough for studying the effects of any agent supposed to alter lipolytic activity. Other control conditions included atrial natriuretic peptide, which stimulated glycerol release as well as isoprenaline. Regarding glucose transport, human insulin induced a threefold increase of basal uptake (Table 1), which can be considered as a substantial stimulation for insulin-responsive cells. Hydrogen peroxide also significantly activated glucose transport.

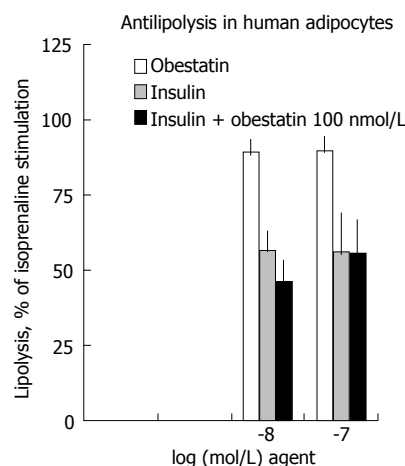


Figure 2 Effects of obestatin and of insulin on isoprenaline-induced lipolysis in human adipocytes. The submaximal stimulation of lipolysis by 5 nmol/L isoprenaline was set at 100 % (control) and determined in the presence of indicated doses of obestatin (open columns), insulin (shaded columns), or the combination of insulin/+ 100 nmol/L obestatin (dark columns). Mean \pm SEM of 7 experiments.

Influence of obestatin on lipolytic responses of human adipocytes

While isoprenaline dose-dependently stimulated the lipolytic activity, obestatin did not modify basal lipolysis, when tested from 10^{-10} to 10^{-6} mol/L (Figure 1). In the same conditions, another peptide tested in parallel was able to maximally stimulate lipolysis to the same level than isoprenaline: Atrial natriuretic peptide 1 μ mol/L (Table 1), indicating that diverse lipolytic agents other than isoprenaline could activate triglyceride breakdown in the tested preparations.

To check whether obestatin needed a pre-activated state of triglyceride breakdown to regulate lipolysis, we co-incubated obestatin with 5 nmol/L isoprenaline. The glycerol release provoked by such threshold dose of isoprenaline also enabled to observe antilipolytic actions. Lipolysis was not altered by obestatin at 10 or 100 nmol/L, indicating that the adipokine was not potentiating or inhibiting a moderate lipolytic activation (Figure 2). On the opposite, insulin, at 10-100 nmol/L, provoked a partial inhibition of the β -adrenergic-induced triglyceride breakdown. Obestatin did not significantly hamper or improve such antilipolytic action, clearly indicating that the adipokine was devoid of antilipolytic effect on its own, or unable to acutely enhance that of insulin. Further tests were performed in the presence of a higher, submaximal dose of isoprenaline. Again no clear-cut antilipolysis was found with obestatin while the α_2 -adrenergic agonist (UK 14304, also known as bromoxidine) impaired the lipolytic response to isoprenaline (Table 2).

Glucose transport response to obestatin or insulin in human adipocytes

Insulin dose-dependently stimulated the 2-DG uptake of human adipocytes, with a detectable effect at 10

Table 2 Influence of obestatin on antilipolytic and glucose transport activities of human subcutaneous adipocytes

	<i>n</i>	Treatment	Control	Obestatin 1 nmol/L	Obestatin 10 nmol/L	Obestatin 100 nmol/L	UK14304 1 μ mol/L
Lipolysis, μ mol glycerol/ 100 mg lipid/90 min	3	Isoprenaline	0.64 \pm 0.10	0.61 \pm 0.09	0.62 \pm 0.10	0.62 \pm 0.10	0.29 \pm 0.04 ^a
Glucose transport, nmol 2-DG/100 mg lipids/10 min	10	Insulin	0.46 \pm 0.14	0.45 \pm 0.09	0.45 \pm 0.09	0.43 \pm 0.09	ND

Fat cells were incubated with a submaximal dose of the reference activator of lipolysis (isoprenaline 100 nmol/L), or glucose transport (insulin 5 nmol/L) alone (control) or with the indicated agents. Mean \pm SEM. Different from corresponding control at: ^a $P < 0.05$. ND: Not determined.

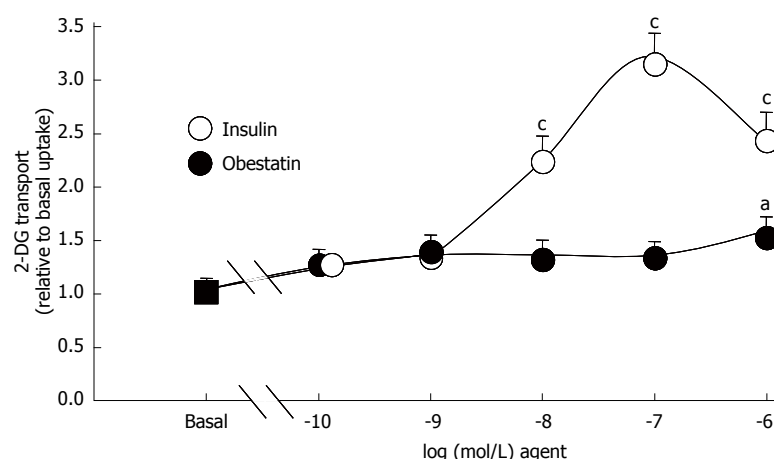


Figure 3 Effects of obestatin and insulin on glucose transport in human adipocytes. 2-deoxyglucose uptake (2-DG) was measured after 45-min incubation without (basal) and with the indicated doses of human insulin (open circles), or obestatin (closed circles). Mean \pm SEM of 10 experiments. Statistically different from basal uptake (black square) at: ^a $P < 0.05$; ^c $P < 0.001$.

nmol/L and a maximum at 100 nmol/L. A decline relative to the maximal insulin stimulation was observed at the high concentration of 1 μ mol/L. By contrast, no clear-cut change in glucose uptake was observed in response to obestatin, save at this high micromolar dose (Figure 3). The significant stimulation of hexose uptake observed with 1 μ mol/L obestatin was increasing baseline by 1.52 ± 0.19 fold. However such increase of hexose uptake by obestatin accounted for only 29% of the maximal response to insulin. During these tests, 1 mmol/L hydrogen peroxide also partially reproduced the insulin stimulation of hexose uptake (Table 1).

Since obestatin on its own was not able to fully mimic the insulin activation of glucose transport, it was further tested whether it was favouring the action of a threshold dose of the pancreatic hormone. However, obestatin, from 1 to 100 nmol/L, did not modify the 5 nmol/L insulin action (Table 2).

DISCUSSION

Taken together, our results indicate that obestatin does not act as a fast-acting antilipolytic agent or as a strong activator of glucose transport in human subcutaneous adipocytes.

Our observations are therefore in apparent contradiction with those of Granata and coworkers, who previously reported that obestatin inhibits lipolysis and activates glucose transport in 3T3-L1 murine

preadipocytes, and in human omental and subcutaneous adipocytes^[6]. However, in our study, the stimulation of glucose transport by insulin was equivalent to a threefold increase over basal uptake in adipocytes from overweight subjects, *i.e.*, reaching a magnitude greater than the insulin responsiveness found in the human fat cell preparations used by Granata *et al.*^[6] or other research teams^[23], which hardly reached a doubling of baseline. Indeed, when looking into details of glucose transport, the human fat cell preparations studied by Granata *et al.*^[6] were not overtly insulin-responsive: Insulin 100 nmol/L was activating basal hexose uptake by approximately a 1.3 fold factor. Consequently, it was feasible, for Granata *et al.*^[6], to conclude that obestatin largely reproduced the feeble action of insulin, while we observed here that 1 μ mol/L obestatin concentration of peptide hardly induced one-third of the maximal response to insulin. Therefore, with a similar feeble activation of hexose uptake by obestatin, two distinct interpretations could be drawn since the difference lies mainly in the maximal activation by insulin, the “golden reference” for stimulation of glucose utilization. In fact, insulin responsiveness can dramatically decline until complete resistance when obesity is complicated with type 2 diabetes, making that the use of insulin-resistant fat cells is not a good tool to underscore insulin-mimicking factors. In this view, hydrogen peroxide, known as a partial insulin mimicker^[15] regarding glucose uptake^[24], was effective in human adipocytes under our conditions.

At this time, it is important to note that the lack of clear-cut stimulation of glucose uptake into human adipocytes reported here for 0.1-100 nmol/L obestatin totally agrees with a previous observation made on 3T3-L1 differentiated preadipocytes^[9] and with its antiadipogenic properties found in the same cell lineage^[3]. All these findings are therefore contrasting with the reported obestatin ability to improve insulin effect on glucose carrier translocation in several fat cell models^[6]. Although the equipment in GPR 39, the controversial obestatin receptor^[25], is less abundant in adipocytes from obese and diabetic subjects^[26], it is difficult to support that such putative insulin-like effect of the adipokine on hexose uptake was improved in the insulin-resistant preparations and lowered in the insulin-sensitive ones.

Another amazing observation was that 1 μ mol/L obestatin was able to stimulate glucose uptake weakly but significantly, while at lower doses it was unable to improve the submaximal action of insulin at 5 nmol/L. One could ask about the purity of our used preparation, but unfortunately we did not verify by chemical analyses the composition given by the furnisher. It could also be argued that the peptide was degraded before/during incubation with fat cells. Though we did not perform a before/after comparison of the incubation medium containing obestatin and fat cells, it can be assessed that the peptide preparation was correctly efficient on its own since it activated glucose uptake in 3T3-F442A preadipocytes. Moreover, in our hands, another peptide preparation, that of ANP, fully exhibited its recognized lipolytic action in human adipocytes^[14]. Lastly, it is barely conceivable that a putative contaminant inhibited obestatin action and not that of insulin, since there was no impairment when obestatin preparation was tested in combination with insulin. Therefore, despite all the precautions that may be taken for the interpretation of our data, we propose that the only detected effect of obestatin on human adipocytes, occurring at 1 μ mol/L, has to be considered as extraphysiological. This should also apply to the same micromolar dose of insulin, which also behaved strikingly, since less efficient than 100 nmol/L of the pancreatic hormone, the recognized reference for maximal activation of glucose uptake. Such assessment against the specificity of relatively high dosages does not mean that the maximal insulin action cannot be overpassed in adipocyte preparations. On the opposite, we confirmed in human fat cells, that the antilipolytic effect of the α -adrenergic agonist (UK 14304) largely overpassed that of insulin. In contrast, no clear-cut antilipolytic action of obestatin could be detected when tested alone or even when combined with insulin. Again, our observations were not so different from those previous studies^[6] in which only a modest antilipolytic effect of obestatin was observed, but without exhibiting a classical sigmoidal dose-dependent curve. Taken together, the data reported so far do not support that obestatin is directly regulating triglyceride breakdown in human adipocytes, at least during short-term incubations.

Our observations do not definitely close the chara-

cterization of the short-term insulin-like effects of obestatin, but prompt to recall the history of the insulin-like properties attributed transiently to visfatin by Shimomura and coworkers before a retraction of their original findings^[27] and a lack of confirmation of such properties by various verification studies^[16]. Thus, the capacity of obestatin to fully mimic short-term insulin-like actions (such as glucose transport activation or triglyceride breakdown inhibition) remains questionable owing to the small magnitude of the responses, if any. Obviously, it cannot be definitively ruled out that obestatin can promote some modulation of other lipolytic and lipogenic regulators, or act after longer exposure *via* other cells present in adipose tissue, therefore operating by mechanisms different from direct activation of fat cell receptors.

Anyhow, no insulin-like property is necessary for obestatin to exert a physiological adipokine role, together with other members of the ghrelin family. The concern is to clarify whether obestatin can be considered as a "fair" adipokine, like adiponectin, increasing insulin responsiveness and decreasing with obesity, or as a deleterious one, like many other pro-inflammatory cytokines linked to obesity-related insulin resistance.

In conclusion, our results did not confirm a direct biological regulatory effect of obestatin on glucose transport and triglyceride breakdown in fat cells from human subcutaneous adipose tissue, rendering questionable the occurrence of an obestatin-dependent modulation of lipogenic and lipolytic activities that might relay or help the defective responsiveness to insulin in pre-diabetic and diabetic states.

ARTICLE HIGHLIGHTS

Background

Obestatin is a gut hormone, derived from the same gene as ghrelin and involved in food intake regulation. This peptide, initially proposed to bind to the G protein-coupled receptor GPR39 is active in the digestive tract, pituitary and adipose tissues. Initially, obestatin was reported to inhibit triacylglycerol hydrolysis in cultured murine 3T3-L1 adipocytes and in human adipocytes. Another insulin-like property was added to the panel of obestatin actions: The stimulation of glucose transport into fat cells. However, several recent reports have indicated that obestatin may activate lipolysis and raised confusion about its role in the modulation of triacylglycerol storage/mobilization. Thus, it was of interest to verify whether processes that are exquisitely regulated by insulin (glucose utilisation and lipid mobilisation by adipocytes) were also modulated by obestatin in human adipocytes.

Research frontiers

The study aimed at determining complete dose-dependent effects of human obestatin in human subcutaneous fat cells. Such approach brings additional evidence that obestatin cannot readily and rapidly reproduce the antilipolytic action of insulin, while it confirms that the α -adrenergic agonist bromoxidine surpasses the insulin-induced inhibition of lipolysis in human fat cells. At 1 μ mol/L, obestatin induces a moderate activation of hexose uptake in fat cells, the magnitude of which is too modest to assess definitively that the peptide acts as an insulin mimicker.

Innovations and breakthroughs

Although a direct regulatory action on adipocyte lipolysis/lipogenesis does not seem to contribute to the multifunctional *in vivo* actions of obestatin, our

observations do not exclude a long-term influence of the peptide on adipocyte biology in healthy, obese or diabetic subjects. Whether such long-term actions might be beneficial to combat obesity and diabetes linked complications remains to be clarified.

Applications

Obestatin is primarily a gut hormone, derived from the same gene as ghrelin and should belong to the multiple steps linking digestive tract function and food intake regulation. Nevertheless, its apparent lack of direct action on target cells such as the adipocytes, which are involved in the regulation of energy balance and glucose handling, does not allow proposing novel obestatin-based therapeutic approaches in combating obesity and diabetes.

Terminology

ANP: Atrial natriuretic peptide; SCAT: Subcutaneous adipose tissue; BMI: Body mass index SEM: Standard error of the mean; 2-DG: 2-deoxyglucose.

ACKNOWLEDGMENTS

We thank the staff of plastic surgery of Rangueil Hospital (Toulouse, France) for providing us with surgical samples from abdominal lipectomy and Estelle Wanecq for technical assistance. The authors also thank Anne Bouloumié (I2MC, Toulouse, France) for helpful discussions and Anaïs Briot for improving the manuscript. In memoriam to Michel Berlan and to Jean Claude Murat.

REFERENCES

- 1 **Zhang JV**, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C, Hsueh AJ. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* 2005; **310**: 996-999 [PMID: 16284174 DOI: 10.1126/science.1117255]
- 2 **Chartrel N**, Alvear-Perez R, Leprince J, Iturrioz X, Reaux-Le Goazigo A, Audinot V, Chomarat P, Coge F, Nosjean O, Rodriguez M, Galizzi JP, Boutin JA, Vaudry H, Llorens-Cortes C. Comment on "Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake". *Science* 2007; **315**: 766; author reply 766 [PMID: 17289961 DOI: 10.1126/science.1135047]
- 3 **Tang SQ**, Jiang QY, Zhang YL, Zhu XT, Shu G, Gao P, Feng DY, Wang XQ, Dong XY. Obestatin: its physicochemical characteristics and physiological functions. *Peptides* 2008; **29**: 639-645 [PMID: 18325633 DOI: 10.1016/j.peptides.2008.01.012]
- 4 **Depoortere I**, Thijs T, Moechars D, De Smet B, Ver Donck L, Peeters TL. Effect of peripheral obestatin on food intake and gastric emptying in ghrelin-knockout mice. *Br J Pharmacol* 2008; **153**: 1550-1557 [PMID: 18204478 DOI: 10.1038/sj.bjp.0707683]
- 5 **Gurriarán-Rodríguez U**, Al-Massadi O, Roca-Rivada A, Crujeiras AB, Gallego R, Pardo M, Seoane LM, Pazos Y, Casanueva FF, Camiña JP. Obestatin as a regulator of adipocyte metabolism and adipogenesis. *J Cell Mol Med* 2011; **15**: 1927-1940 [PMID: 21029370 DOI: 10.1111/j.1582-4934.2010.01192.x]
- 6 **Granata R**, Gallo D, Luque RM, Baragli A, Scarlatti F, Grande C, Gesmundo I, Córdoba-Chacón J, Bergandi L, Settanni F, Togliatto G, Volante M, Garetto S, Annunziata M, Chancón B, Gargantini E, Rocchietto S, Matera L, Datta G, Morino M, Brizzi MF, Ong H, Camussi G, Castaño JP, Papotti M, Ghigo E. Obestatin regulates adipocyte function and protects against diet-induced insulin resistance and inflammation. *FASEB J* 2012; **26**: 3393-3411 [PMID: 22601779 DOI: 10.1096/fj.11-201343]
- 7 **Granata R**, Ghigo E. Products of the ghrelin gene, the pancreatic β -cell and the adipocyte. *Endocr Dev* 2013; **25**: 144-156 [PMID: 23652400 DOI: 10.1159/000346306]
- 8 **Wojciechowicz T**, Skrzypski M, Kołodziejewski PA, Szczepankiewicz D, Pruszyńska-Oszmolek E, Kaczmarek P, Strowski MZ, Nowak KW. Obestatin stimulates differentiation and regulates lipolysis and leptin secretion in rat preadipocytes. *Mol Med Rep* 2015; **12**: 8169-8175 [PMID: 26498652 DOI: 10.3892/mmr.2015.4470]
- 9 **Miegueu P**, St Pierre D, Broglio F, Cianflone K. Effect of desacyl ghrelin, obestatin and related peptides on triglyceride storage, metabolism and GHSR signaling in 3T3-L1 adipocytes. *J Cell Biochem* 2011; **112**: 704-714 [PMID: 21268092 DOI: 10.1002/jcb.22983]
- 10 **Ren G**, He Z, Cong P, Yu J, Qin Y, Chen Y, Liu X. Effect of TAT-obestatin on proliferation, differentiation, apoptosis and lipolysis in 3T3-L1 preadipocytes. *J Pept Sci* 2013; **19**: 684-691 [PMID: 24106000 DOI: 10.1002/psc.2550]
- 11 **Pruszyńska-Oszmolek E**, Szczepankiewicz D, Hertig I, Skrzypski M, Sassek M, Kaczmarek P, Kołodziejewski PA, Mackowiak P, Nowak KW, Strowski MZ, Wojciechowicz T. Obestatin inhibits lipogenesis and glucose uptake in isolated primary rat adipocytes. *J Biol Regul Homeost Agents* 2013; **27**: 23-33 [PMID: 23489684]
- 12 **Nagaraj S**, Raghavan AV, Rao SN, Manjappa UV. Obestatin and Nt8U influence glycerolipid metabolism and PPAR gamma signaling in mice. *Int J Biochem Cell Biol* 2014; **53**: 414-422 [PMID: 24937751 DOI: 10.1016/j.biocel.2014.06.006]
- 13 **Aktas B**, Yilmaz Y, Eren F, Yonal O, Kurt R, Alahdab YO, Celikel CA, Ozdogan O, Imeryuz N, Kalayci C, Avsar E. Serum levels of vaspin, obestatin, and apelin-36 in patients with nonalcoholic fatty liver disease. *Metabolism* 2011; **60**: 544-549 [PMID: 20580037 DOI: 10.1016/j.metabol.2010.05.008]
- 14 **Sengenès C**, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. *FASEB J* 2000; **14**: 1345-1351 [PMID: 10877827]
- 15 **Ludvigsen C**, Jarett L. Similarities between insulin, hydrogen peroxide, concanavalin A, and anti-insulin receptor antibody stimulated glucose transport: increase in the number of transport sites. *Metabolism* 1982; **31**: 284-287 [PMID: 7043172]
- 16 **Wanecq E**, Prévot D, Carpené C. Lack of direct insulin-like action of visfatin/Nampt/PBEF1 in human adipocytes. *J Physiol Biochem* 2009; **65**: 351-359 [PMID: 20358348 DOI: 10.1007/bf03185930]
- 17 **Carpéné C**, Galitzky J, Saulnier-Blache JS. Short-term and rapid effects of lysophosphatidic acid on human adipose cell lipolytic and glucose uptake activities. *AIMS Molec Sci* 2016; **3**: 222-237 DOI: 10.3934/molsci.2016.2.222]
- 18 **Mercader J**, Wanecq E, Chen J, Carpené C. Isopropyl norsynephrine is a stronger lipolytic agent in human adipocytes than synephrine and other amines present in Citrus aurantium. *J Physiol Biochem* 2011; **67**: 443-452 [PMID: 21336650 DOI: 10.1007/s13105-011-0078-2]
- 19 **Gomez-Zorita S**, Tréguer K, Mercader J, Carpené C. Resveratrol directly affects in vitro lipolysis and glucose transport in human fat cells. *J Physiol Biochem* 2013; **69**: 585-593 [PMID: 23315205 DOI: 10.1007/s13105-012-0229-0]
- 20 **Bour S**, Daviaud D, Gres S, Lefort C, Prévot D, Zorzano A, Wabitsch M, Saulnier-Blache JS, Valet P, Carpené C. Adipogenesis-related increase of semicarbazide-sensitive amine oxidase and monoamine oxidase in human adipocytes. *Biochimie* 2007; **89**: 916-925 [PMID: 17400359 DOI: 10.1016/j.biochi.2007.02.013]
- 21 **Atgié C**, Sauvant P, Ambid L, Carpené C. Possible mechanisms of weight loss of Siberian hamsters (*Phodopus sungorus sungorus*) exposed to short photoperiod. *J Physiol Biochem* 2009; **65**: 377-386 [PMID: 20358351 DOI: 10.1007/bf03185933]
- 22 **Pairault J**, Lasnier F. Control of the adipogenic differentiation of 3T3-F442A cells by retinoic acid, dexamethasone, and insulin: a topographic analysis. *J Cell Physiol* 1987; **132**: 279-286 [PMID: 2442179 DOI: 10.1002/jcp.1041320212]
- 23 **Sancho V**, Nuche B, Arnés L, Cancelas J, González N, Díaz-Miguel M, Martín-Duce A, Valverde I, Villanueva-Peñacarrillo ML. The action of GLP-1 and exendins upon glucose transport in normal human adipocytes, and on kinase activity as compared to morbidly obese patients. *Int J Mol Med* 2007; **19**: 961-966 [PMID: 17487430]
- 24 **May JM**, de Haën C. The insulin-like effect of hydrogen peroxide on pathways of lipid synthesis in rat adipocytes. *J Biol Chem* 1979; **254**: 9017-9021 [PMID: 479177]
- 25 **Dong XY**, He JM, Tang SQ, Li HY, Jiang QY, Zou XT. Is GPR39 the natural receptor of obestatin? *Peptides* 2009; **30**: 431-438 [PMID: 18977259 DOI: 10.1016/j.peptides.2008.09.022]

- 26 **Catalán V**, Gómez-Ambrosi J, Rotellar F, Silva C, Gil MJ, Rodríguez A, Cienfuegos JA, Salvador J, Frühbeck G. The obestatin receptor (GPR39) is expressed in human adipose tissue and is down-regulated in obesity-associated type 2 diabetes mellitus. *Clin Endocrinol (Oxf)* 2007; **66**: 598-601 [PMID: 17371481 DOI: 10.1111/j.1365-2265.2007.02777.x]
- 27 **Fukuhara A**, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I. Retraction. *Science* 2007; **318**: 565 [PMID: 17962537 DOI: 10.1126/science.318.5850.565b]

P- Reviewer: Hussain SAR, Mitra A **S- Editor:** Kong JX

L- Editor: A **E- Editor:** Lu YJ



Observational Study

Heart rate is an independent predictor of all-cause mortality in individuals with type 2 diabetes: The diabetes heart study

Sameer Prasada, Cameron Oswalt, Phyllis Yeboah, Georgia Saylor, Donald Bowden, Joseph Yeboah

Sameer Prasada, Cameron Oswalt, Department of Medical School (Medical students), Wake Forest University, Winston Salem, NC 27157, United States

Phyllis Yeboah, Department of Internal Medicine, Wake Forest Baptist Health, Winston Salem, NC 27157, United States

Georgia Saylor, Joseph Yeboah, Department of Heart and Vascular Center of Excellence, Wake Forest Baptist Health, Winston Salem, NC 27157, United States

Donald Bowden, Department of Biochemistry, Genomics and Personalized Medicine Research, Wake Forest University, Winston Salem, NC 27157, United States

Author contributions: Bowden D enrolled subjects and collected data for the Diabetes Heart Study; Prasada S and Yeboah J designed the study and performed statistical analysis using Statistical Analysis System JMP; Prasada S and Oswalt C wrote the manuscript; Yeboah P and Yeboah J helped write and edit the manuscript; all authors contributed to this article.

Institutional review board statement: The Diabetes Heart Study was approved by the Wake Forest University Institutional Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment in the Diabetes Heart Study (DHS).

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

[licenses/by-nc/4.0/](http://creativecommons.org/licenses/by-nc/4.0/)

Manuscript source: Unsolicited manuscript

Correspondence to: Joseph Yeboah, MD, MS, Department of Heart and Vascular Center of Excellence, Wake Forest Baptist Health, Medical Center Blvd, Winston Salem, NC 27157, United States. jyeboah@wakehealth.edu
Telephone: +1-336-7167015
Fax: +1-336-7169188

Received: May 4, 2017

Peer-review started: May 5, 2017

First decision: July 20, 2017

Revised: July 25, 2017

Accepted: November 25, 2017

Article in press: November 25, 2017

Published online: January 15, 2018

Abstract

AIM

To assess the association of resting heart rate with all-cause and cardiovascular disease (CVD) mortality in the Diabetes Heart Study (DHS).

METHODS

Out of a total of 1443 participants recruited into the DHS, 1315 participants with type 2 diabetes who were free of atrial fibrillation and supraventricular tachycardia during the baseline exam were included in this analysis. Heart rate was collected from baseline resting electrocardiogram and mortality (all-cause and CVD) was obtained from state and national death registry. Kaplan-Meier (K-M) and Cox proportional hazard analyses were used to assess the association.

RESULTS

The mean age, body mass index (BMI) and systolic blood

pressure (SBP) of the cohort were 61.4 ± 9.2 years, 32.0 ± 6.6 kg/m², and 139.4 ± 19.4 mmHg respectively. Fifty-six percent were females, 85% were whites, 15% were blacks, 18% were smokers. The mean \pm SD heart rate was 69.8 (11.9) beats per minute (bpm). After a median follow-up time of 8.5 years (maximum follow-up time is 14.0 years), 258 participants were deceased. In K-M analysis, participants with heart rate above the median had a significantly higher event rate compared with those below the median (log-rank $P = 0.0223$). A one standard deviation increase in heart rate was associated with all-cause mortality in unadjusted (hazard ratio 1.16, 95%CI: 1.03-1.31) and adjusted (hazard ratio 1.20, 95%CI: 1.05-1.37) models. Similar results were obtained with CVD mortality as the outcome of interest.

CONCLUSION

Heart rate is an independent predictor of all-cause mortality in this population with type 2 diabetes. In this study, a 1-SD increase in heart rate was associated with a 20% increase in risk suggesting that additional prognostic information may be gleaned from this ubiquitously collected vital sign.

Key words: Diabetes mellitus; Mortality; Resting heart rate; Prevention

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Persons with type 2 diabetes mellitus (T2DM) have a higher rate of morbidity and mortality compared with those without diabetes. Prevention is the best way of reducing the risk in this population. Unlike the general population, the predictive value of resting heart rate for mortality in persons with T2DM is not well established. We used baseline data and a median of 8.5 years of follow up from the Diabetes Heart Study to show that resting heart rate is an independent predictor of mortality in individuals with T2DM. Our data suggests that efforts that reduce heart rate in T2DM may be useful.

Prasada S, Oswalt C, Yeboah P, Saylor G, Bowden D, Yeboah J. Heart rate is an independent predictor of all-cause mortality in individuals with type 2 diabetes: The diabetes heart study. *World J Diabetes* 2018; 9(1): 33-39 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i1/33.htm> DOI: <http://dx.doi.org/10.4239/wjcd.v9.i1.33>

INTRODUCTION

Diabetes mellitus is a major health problem affecting 29.1 million (9.3%) Americans^[1-3]. Type 2 diabetes mellitus comprises 90-95% of these diagnosed cases^[1,2]. The Center for Disease Control (CDC) estimates that one-third of Americans will develop type 2 diabetes at some point in their lifetime. Cardiovascular disease (CVD) death rates are 1.7 times higher for adults with

diabetes than those without diabetes^[1]. Understanding which specific factors and findings are associated with increased risk of mortality may help us prognosticate patients as well as provide specific, earlier therapies for those at highest risk.

Resting heart rate (RHR) is an easily and ubiquitously collected vital sign at every clinical patient encounter. RHR is a function of many factors including recent activity, tobacco use, medications, emotional stability, air temperature, and position^[4-7]. Resting heart rate is associated with increased cardiovascular risk in the general population^[4-18]. Zhang *et al*^[4] in meta-analysis of 46 studies including 1246203 patients showed that higher resting heart rate is associated with increased risk of all-cause and cardiovascular mortality, independent of traditional cardiovascular risk factors. Zhang *et al*^[4] hypothesized that association is due to higher resting heart rate signaling an imbalance between vagal and sympathetic tone and thus dysfunctional autonomic nervous system activity. The prevalence of autonomic dysfunction is very high in individuals with diabetes mellitus raising the possibility that resting heart rate may not be as informative as a risk marker in diabetes as in the general population. It remains unclear if the association between resting heart rate and CVD risk exist in higher risk populations such as those with type-2 diabetes mellitus^[19-25]. We sought to examine the association between resting heart rate, all-cause and CVD mortality in individuals with type 2 diabetes in the Diabetes Heart Study (DHS).

MATERIALS AND METHODS

Study population

The details of the National Institutes of Health -funded Diabetes Heart Study have been published^[26-30]. There were 1443 type 2 diabetic concordant siblings from 564 different families included in the study. Type 2 Diabetes mellitus (DM) was defined as diagnosed diabetes after 35 years of age managed with oral agents and/or insulin without any history of diabetic ketoacidosis. Of these participants, 85% are European Americans and 15% are African Americans. From 1998 to 2005, participants were recruited primarily from western North Carolina from outpatient medicine clinics, health fairs, community outreach programs, and referrals by physicians without any inclusions or exclusions based on prior cardiovascular disease history. Potential participants were recruited by letters which included a telephone number to call if interested. Interviews were performed by telephone and then by an examination visit. Potential participants were sent the informed consent forms and questionnaires before their examination visits for them to review. Written informed consent was obtained at these visits for all participants. The Wake Forest School of Medicine Institutional Review Board approved all study protocols. The study sample represents a cross-section of the diabetic community

living in western North Carolina.

Participant examination visits were performed in the General Clinical Research Center at Wake Forest Baptist Medical Center. Exams included medical history and health behavior interviews. In addition, anthropometric measures, blood pressure, fasting blood draw, and a spot urine collection were measured. Laboratory analyses included total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, fasting glucose, glycated hemoglobin, blood chemistries, and urine albumin and creatinine. Prior CVD history was based on each participant's history including events (heart attack, stroke) and/or interventions (coronary artery bypass grafting, carotid endarterectomy, coronary angiography). Hypertension was defined as blood pressure measurements over 140 mmHg systolic/90 mmHg diastolic or prescription of anti-hypertensive medication. The four-variable Modification of Diet in Renal Disease equation was used to calculate estimated glomerular filtration rate (eGFR). In DHS patients' medication list was not rigorously collected during the baseline exam and therefore is not complete.

Resting heart rate measurement

All DHS participants had a resting electrocardiogram (ECG) during the baseline examination. The resting 12-lead electrocardiogram was performed using Marquette MAC 500 ECG instrument (Marquette Electronics, Milwaukee, WI, United States) after a uniform resting period (after 5 min of rest). The electrocardiogram was read at the Wake Forest Epidemiologic Cardiology Research Center using analytical software. Resting heart rate used in this analysis were those reported from the participants resting ECG. For this study, we included type 2 diabetic participants ($n = 1315$) without atrial fibrillation and supraventricular tachycardia.

Ascertainment of outcomes ascertainment

Ascertainment has been described in detail previously^[25,27]. For all participants in this study, the National Social Security Death Index maintained by the United States Social Security Administration was used to determine vital status. Length of follow-up was measured from the date of the initial study visit to the end of 2012, unless the participant was confirmed as deceased. In those cases, length of follow-up was measured from the date of the initial examination visit to the date of death.

Statistical analysis

Summary statistics were described for continuous variables as mean \pm SD and for categorical variables as frequency (percentage). Summary statistics of participants above and below the median heart rate [heart rate (HR) = 69] was compared using chi-square test for categorical variables and students *t*-test for continuous variables. Kaplan-Meier analysis was used to assess the events-free survivals of DHS participants with resting heart rate above and below the median

heart rate and the curves compared using log-rank test.

Cox proportional hazards regression analysis was subsequently used to assess the association between resting heart rate, all-cause and cardiovascular disease mortality adjusting for confounders *via* 4 models; Model 1- unadjusted; Model 2- adjusted for age, sex, and ethnicity; Model 3- Model 2+ body mass index (BMI), hemoglobin A1c, diabetes duration, systolic blood pressure, hypertension, total cholesterol level, triglyceride level, current smoking status, and eGFR and Model 4- Model 3+ comorbidities. A two sided *P* value of < 0.05 was accepted as statistically significant. All analyses were performed using Statistical Analysis System (SAS) JMP Pro software, version 12.0.1 (SAS Institute, Cary, NC, United States).

RESULTS

Baseline characteristics

At baseline, mean age, diabetes duration, HbA1c, RHR, BMI, and systolic blood pressure of the cohort were 61.4 years, 10.4 years, 7.4%, 69.8 bpm, 32.0 kg/m², and 139.4 mmHg respectively (Table 1). The majority of participants were European Americans (84.6%) and there were more women (55.9%) in the study. Of the 1315 participants, 652 (49.6%) had below median RHR and 663 (50.4%) had above median RHR (Table 1). Participants with resting heart rate below the median were older and had higher prevalence of prior CVD. Those with resting heart rate greater than the median had higher BMI, diastolic blood pressure, HbA1c, glucose, triglyceride and total cholesterol levels.

Resting heart rate and all-cause mortality

After a median follow-up time of 8.5 years (maximum follow-up time of 14.0 years), 258 participants (19.6%) were deceased. As shown in Figure 1A, participants with resting heart rate \geq median had significantly less mortality event-free survival compared with those with resting HR $<$ median (Log rank $P = 0.022$). Table 2 shows the CVD mortality risk associated with 1 standard deviation increase in resting heart rate in the 4 models. In the full Cox regression model, each 1-SD increase in RHR was associated with a 20% increase in risk for all-cause mortality [HR 1.20 (95%CI: 1.05-1.37), $P = 0.01$; Table 2] after controlling for age, sex, ethnicity, BMI, hemoglobin A1c, diabetes duration, systolic blood pressure, hypertension, total cholesterol level, triglyceride level, current smoking status, eGFR, and baseline CVD history. An interaction term of resting heart rate and either sex or race was not significant in our full model.

Resting heart rate and cvd mortality

After the same follow-up period (median follow-up 8.5 years; maximum follow-up 14.0 years), 111 participants (8.4%) died from CVD causes. Participants with resting heart rate $>$ median had a lower CVD mortality event-free survival compared with those $<$ median (Log rank

Table 1 Baseline characteristics of participants in the diabetes heart study

Characteristics	All (n = 1315)	< Median RHR (n = 652)	≥ Median HR (n = 663)	P value
Age (yr)	61.4 (9.2)	62.2 (9.3)	60.6 (9.1)	0.0015
Caucasian (%)	1113 (84.6)	557 (85.4)	556 (83.9)	
African American (%)	202 (15.4)	95 (14.6)	107 (16.1)	
Women (%)	735 (55.9)	323 (49.5)	412 (62.1)	
BMI (kg/m ²)	32.0 (6.6)	31.0 (6.5)	33.0 (6.5)	< 0.0001
Current smoker (%)	234 (17.9)	98 (15.1)	136 (20.6)	0.1336
Ex-smoker (%)	541 (41.3)	296 (45.7)	245 (37.2)	0.0223
Diabetes duration (yr)	10.4 (7.04)	10.0 (7.0)	10.7 (7.1)	0.0953
Systolic BP (mmHg)	139.4 (19.4)	139.1 (19.1)	139.7 (19.7)	0.5661
Diastolic BP (mmHg)	73.4 (10.4)	72.6 (10.1)	74.2 (10.6)	0.0044
Hypertension (%)	1116 (84.9)	543 (83.3)	573 (86.4)	0.1118
Prior CVD (%)	397 (30.7)	218 (33.7)	179 (27.6)	0.0161
HbA1c (%)	7.4 (1.9)	7.1 (1.67)	7.7 (2.1)	< 0.0001
Glucose (g/L)	1.4 (0.6)	1.3 (0.5)	1.5 (0.7)	< 0.0001
Total cholesterol (g/L)	1.8 (0.5)	1.8 (0.4)	1.9 (0.5)	0.0006
HDL (g/L)	0.44 (0.1)	0.4 (0.1)	0.4 (0.1)	0.7662
LDL (g/L)	1.0 (0.4)	1.0 (0.3)	1.05 (0.4)	0.2616
Triglycerides (g/L)	1.8 (1.2)	1.7 (1.1)	2.0 (1.3)	< 0.0001
eGFR (mL/min × 1.73 m ²)	67.9 (20.5)	68.2 (20.0)	67.7 (20.9)	0.6865
RHR (bpm)	69.8 (11.9)	60.2 (5.6)	79.3 (8.4)	< 0.0001

RHR: Resting heart rate; BMI: Body mass index; CVD: Cardiovascular diseases; HDL: High density lipoprotein; LDL: Low density lipoprotein; eGFR: Estimated glomerular filtration rate.

Table 2 Association between 1- standard deviation of resting heart rate with mortality in the diabetes heart study in cox proportional hazard models after a median follow-up of 8.5 years

Models	Hazard ratio	95%CI	P value
All-cause mortality (model)			
1	1.16	1.03-1.31	0.0151
2	1.26	1.12-1.42	0.0020
3	1.15	1.01-1.32	0.0355
4	1.20	1.05-1.37	0.0079
Cardiovascular mortality (model)			
1	1.19	0.98-1.43	0.0688
2	1.29	1.07-1.54	0.0073
3	1.14	0.93-1.40	0.2164
4	1.19	0.97-1.47	0.0975

Model 1: Unadjusted; Model 2: Adjusted for age, sex, ethnicity; Model 3: Model 2 + body mass index, hemoglobin A1c, diabetes duration, systolic blood pressure, hypertension, total cholesterol level, triglyceride level, current smoking status, estimated glomerular filtration rate; Model 4: Model 3 + baseline cardiovascular diseases history.

$P = 0.045$) (Figure 1B). Resting heart rate showed trends similar to that if all-cause mortality but some of the models did not attain statistical significance likely because of the lower number of CVD mortality that occurred during the follow up (Table 2).

DISCUSSION

The goal of this study was to assess the association between resting heart rate and mortality in type-2 diabetics, a high risk group with very high prevalence of cardiac autonomic dysfunction^[29,30]. Our study showed that despite the high prevalence of cardiac autonomic

dysfunction in type-2 diabetics, resting heart rate predicts mortality similar to that found in the general population.

Current data is consistent with an association between resting heart rate and mortality in the general population^[4-18]. In the absence of medication use and cardiac arrhythmias, resting heart rate variability is controlled by a balance between sympathetic and parasympathetic systems. Persistently high resting heart rates are seen in stressful situations, chronic illness, physical inactivity, etc., all of which have been associated with higher mortality and morbidity in the general population. In diabetes mellitus, however, complex cascades of pathways are activated by hyperglycemia resulting in neuronal ischemic and cellular death^[21,22]. This neuronal death leads to conditions such as poly-neuropathies and cardiac autonomic neuropathy. Symptoms of cardiac autonomic neuropathy include resting tachycardia, exercise intolerance, postural hypotension and diabetes cardiomyopathy. Thus resting tachycardia may represent a stressful state in both diabetic and non-diabetic individuals but the pathophysiology may be different. Hillis *et al.*^[24,25] used data from the Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation study of about 11140 patients with type-2 diabetes mellitus, recruited from 215 centers in 20 countries, to show that resting heart rate was associated with all-cause mortality, macrovascular and microvascular complications. However, Bartáková *et al.*^[20] used a smaller cohort of 421 type 2 diabetes mellitus (T2DM) patients to show that resting heart rate was not associated with advanced cardiovascular events and all-cause mortality. The present study findings are consistent with the findings by Hillis *et al.*^[24,25]. In our study a 1 standard deviation increase in resting heart rate

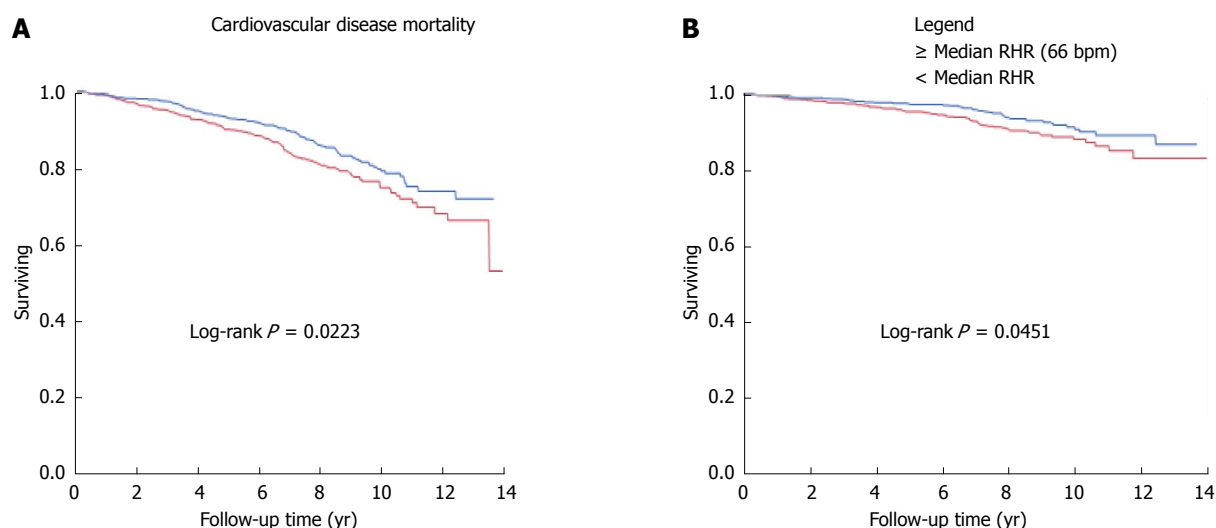


Figure 1 Result of Kaplan Meier curves. A: Kaplan Meier curves showing the Mortality free- survival of Type-Diabetics with resting heart rate (RHR) above and below the median (Median RHR = 69 bpm) in the Diabetes Heart Study; B: Kaplan Meier curves showing the Cardiovascular disease Mortality free- survival of Type-Diabetics with RHR above and below the median (Median RHR = 69 bpm) in the Diabetes Heart Study; Red line: \geq Median RHR (66 bpm); Blue line: $<$ Median RHR.

was associated with a 20% increase in CVD/ all-cause mortality.

In our study, the mean BMI of participants with resting heart rate greater than the median was higher than those with resting heart rate less than the median suggesting that factors such as obesity, physical inactivity/deconditioning, and endocrine abnormalities such as thyroid function may play a significant role in the increased risk observed. We adjusted for BMI in our final models but data on physical activity and thyroid function were not collected in the DHS so it is unclear if adequate adjustment for these variables will change our estimates in this analysis. Nonetheless, this suggests that targeting factors such as obesity, physical activity and other factors that leads to reduced resting heart rate may help reduced the high mortality risk seen in persons' with diabetes mellitus. Additionally Aggressive control of hyperglycemia to minimize the prevalence of cardiac autonomic dysfunction^[22] which may manifest as resting tachycardia and reduction of stress among others, all of which leads to reduce resting heart rate in the general population may all be beneficial targets for reducing mortality in patients with type-2 diabetes mellitus.

Limitations

This study is an observational study and therefore despite the effort to adjust for all possible confounders available to us, our results may still be due to residual confounding. We did not have adequate documentation of medications that influence resting heart rate in the Diabetes Heart Study and therefore could not eliminate nor adjust for them in our full model. This may have affected our results and findings. Our study results and findings should therefore be interpreted with this limitation in mind. The DHS only included whites and blacks and therefore our results may not be extended to other race/ethnicities. The number of events especially CVD mortality that occurred during the follow up was

small hence the non-significant p values seen in Table 2.

In conclusion, heart rate is an independent predictor of all-cause and CVD mortality in this population with type 2 diabetes. In this study, a 1-SD increase in HR was associated with a 20% increase in risk suggesting that additional prognostic information may be available from this ubiquitously collected vital sign.

ARTICLE HIGHLIGHTS

Research background

Individuals with type 2 diabetes mellitus have a significantly higher risk of morbidity and mortality compared with those without diabetes mellitus. Cardiovascular diseases still remains the number one cause of death in persons with diabetes mellitus. Current efforts at reducing this risk include tight glycemic control, control of cardiovascular risk factors and weight reduction among others. Despite these measures, morbidity and mortality in diabetes mellitus still remains high. There is therefore the need for identifying other non-traditional risk factors to further reduce this risk. Resting heart rate has been associated with mortality in the general population. However the association of resting heart rate and mortality risk in diabetes mellitus is unclear.

Research motivation

There are several ways (pharmacological and non-pharmacological) that resting heart rate can be reduced. Establishing an association between resting heart rate and mortality in individuals with diabetes mellitus provides a whole new avenue and pathway for further reducing the high mortality risk associated with the disease.

Research objectives

This study used a large population of individuals with diabetes mellitus.

Research methods

Heart rate was collected from baseline resting electrocardiogram and mortality (all-cause and CVD) was obtained from state and national death registry. Kaplan-Meier (K-M) and Cox proportional hazard analyses were used to assess the association.

Research results

The results show that a 1 standard deviation increase in resting heart rate is associated with a 20% increase in the risk mortality.

Research conclusions

Resting heart rate is a risk factor for all-cause and cardiovascular disease mortality in individuals with diabetes mellitus and may provide additional prognostic information.

Research perspectives

Resting heart rate is a cheap ubiquitous vital sign that is obtained during every doctor's visit. The information gleaned from this vital sign maybe be useful to guide therapy choices which will ultimately reduce mortality in this population.

REFERENCES

- Centers for Disease Control and Prevention. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. Atlanta, GA: US Department of Health and Human Services
- Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR, Jiménez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Mackey RH, Matsushita K, Mozaffarian D, Mussolino ME, Nasir K, Neumar RW, Palaniappan L, Pandey DK, Thiagarajan RR, Reeves MJ, Ritchey M, Rodriguez CJ, Roth GA, Rosamond WD, Sasson C, Towfighi A, Tsao CW, Turner MB, Virani SS, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation* 2017; **135**: e146-e603 [PMID: 28122885 DOI: 10.1161/CIR.0000000000000485]
- World Health Organization. Global report on diabetes. USA: Geneva, 2016. Available from: http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf
- Zhang D, Shen X, Qi X. Resting heart rate and all-cause and cardiovascular mortality in the general population: a meta-analysis. *CMAJ* 2016; **188**: E53-E63 [PMID: 26598376 DOI: 10.1503/cmaj.150535]
- Hansen TW, Thijs L, Boggia J, Li Y, Kikuya M, Björklund-Bodegård K, Richart T, Ohkubo T, Jeppesen J, Torp-Pedersen C, Lind L, Sandoya E, Imai Y, Wang J, Ibsen H, O'Brien E, Staessen JA; International Database on Ambulatory Blood Pressure in Relation to Cardiovascular Outcomes Investigators. Prognostic value of ambulatory heart rate revisited in 6928 subjects from 6 populations. *Hypertension* 2008; **52**: 229-235 [PMID: 18574073 DOI: 10.1161/HYPERTENSIONAHA.108.113191]
- Legeai C, Jouven X, Tafflet M, Dartigues JF, Helmer C, Ritchie K, Amouyel P, Tzourio C, Ducimetière P, Empana JP. Resting heart rate, mortality and future coronary heart disease in the elderly: the 3C Study. *Eur J Cardiovasc Prev Rehabil* 2011; **18**: 488-497 [PMID: 21450655 DOI: 10.1177/1741826710389365]
- Inoue R, Ohkubo T, Kikuya M, Metoki H, Asayama K, Kanno A, Obara T, Hirose T, Hara A, Hoshi H, Totsune K, Satoh H, Kondo Y, Imai Y. Predictive value for mortality of the double product at rest obtained by home blood pressure measurement: the Ohasama study. *Am J Hypertens* 2012; **25**: 568-575 [PMID: 22318510 DOI: 10.1038/ajh.2012.3.]
- Wannamethee G, Shaper AG, Macfarlane PW. Heart rate, physical activity, and mortality from cancer and other noncardiovascular diseases. *Am J Epidemiol* 1993; **137**: 735-748 [PMID: 8484365 DOI: 10.1093/oxfordjournals.aje.a116734]
- Hartaigh Bó, Allore HG, Trentalange M, McAvay G, Pilz S, Dodson JA, Gill TM. Elevations in time-varying resting heart rate predict subsequent all-cause mortality in older adults. *Eur J Prev Cardiol* 2015; **22**: 527-534 [PMID: 24445263 DOI: 10.1177/2047487313519932.]
- Custodis F, Roggenbuck U, Lehmann N, Moebus S, Laufs U, Mahabadi AA, Heusch G, Mann K, Jöckel KH, Erbel R, Böhm M, Möhlenkamp S. Resting heart rate is an independent predictor of all-cause mortality in the middle aged general population. *Clin Res Cardiol* 2016; **105**: 601-612 [PMID: 26803646 DOI: 10.1007/s00392-015-0956-7]
- Palatini P, Julius S. Elevated heart rate: a major risk factor for cardiovascular disease. *Clin Exp Hypertens* 2004; **26**: 637-644 [PMID: 15702618 DOI: 10.1081/CEH-200031959]
- Ohira T, Diez Roux AV, Prineas RJ, Kizilbash MA, Camethon MR, Folsom AR. Associations of psychosocial factors with heart rate and its short-term variability: multi-ethnic study of atherosclerosis. *Psychosom Med* 2008; **70**: 141-146 [PMID: 18256350 DOI: 10.1097/PSY.0b013e318160686a]
- American Heart Association. All about Heart Rate (Pulse); 2015. Available from: http://www.heart.org/HEARTORG/Conditions/More/MyHeartandStrokeNews/All-About-Heart-Rate-Pulse_UCM_438850_Article.jsp#.WPQFZfnyuM8
- Seccareccia F, Pannozzo F, Dima F, Minoprio A, Menditto A, Lo Noce C, Giampaoli S; Malattie Cardiovascolari Aterosclerotiche Istituto Superiore di Sanita Project. Heart rate as a predictor of mortality: the MATISS project. *Am J Public Health* 2001; **91**: 1258-1263 [PMID: 11499115 DOI: 10.2105/AJPH.91.8.1258]
- Fox K, Borer JS, Camm AJ, Danchin N, Ferrari R, Lopez Sendon JL, Steg PG, Tardif JC, Tavazzi L, Tendera M; Heart Rate Working Group. Resting heart rate in cardiovascular disease. *J Am Coll Cardiol* 2007; **50**: 823-830 [PMID: 17719466 DOI: 10.1016/j.jacc.2007.04.079]
- Boudoulas KD, Borer JS, Boudoulas H. Heart Rate, Life Expectancy and the Cardiovascular System: Therapeutic Considerations. *Cardiology* 2015; **132**: 199-212 [PMID: 26305771 DOI: 10.1159/000435947]
- Lang CC, Gupta S, Kalra P, Keavney B, Menown I, Morley C, Padmanabhan S. Elevated heart rate and cardiovascular outcomes in patients with coronary artery disease: clinical evidence and pathophysiological mechanisms. *Atherosclerosis* 2010; **212**: 1-8 [PMID: 20152981 DOI: 10.1016/j.atherosclerosis.2010.01.029]
- Johansen CD, Olsen RH, Pedersen LR, Kumarathurai P, Mouridsen MR, Binici Z, Intzilakis T, Køber L, Sajadieh A. Resting, night-time, and 24 h heart rate as markers of cardiovascular risk in middle-aged and elderly men and women with no apparent heart disease. *Eur Heart J* 2013; **34**: 1732-1739 [PMID: 23306958 DOI: 10.1093/eurheartj/ehs449]
- Anselmino M, Ohrvik J, Rydén L; Euro Heart Survey Investigators. Resting heart rate in patients with stable coronary artery disease and diabetes: a report from the euro heart survey on diabetes and the heart. *Eur Heart J* 2010; **31**: 3040-3045 [PMID: 20935002 DOI: 10.1093/eurheartj/ehq368]
- Bartáková V, Klimešová L, Kianičková K, Dvořáková V, Malúšková D, Řehořová J, Svojanovský J, Olšovský J, Bělobrádková J, Kaňková K. Resting Heart Rate Does Not Predict Cardiovascular and Renal Outcomes in Type 2 Diabetic Patients. *J Diabetes Res* 2016; **2016**: 6726492 [PMID: 26824046 DOI: 10.1155/2016/6726492]
- Pop-Busui R. Cardiac autonomic neuropathy in diabetes: a clinical perspective. *Diabetes Care* 2010; **33**: 434-441 [PMID: 20103559 DOI: 10.2337/dc09-1294]
- Manzella D, Paolisso G. Cardiac autonomic activity and Type II diabetes mellitus. *Clin Sci (Lond)* 2005; **108**: 93-99 [PMID: 15476437 DOI: 10.1042/CS20040223]
- Stettler C, Bearth A, Allemann S, Zwahlen M, Zanchin L, Deplazes M, Christ ER, Teuscher A, Diem P. QTc interval and resting heart rate as long-term predictors of mortality in type 1 and type 2 diabetes mellitus: a 23-year follow-up. *Diabetologia* 2007; **50**: 186-194 [PMID: 17096116 DOI: 10.1007/s00125-006-0483-1]
- Hillis GS, Hata J, Woodward M, Perkovic V, Arima H, Chow CK, Zoungas S, Patel A, Poulter NR, Mancia G, Williams B, Chalmers J. Resting heart rate and the risk of microvascular complications in patients with type 2 diabetes mellitus. *J Am Heart Assoc* 2012; **1**: e002832 [PMID: 23316296 DOI: 10.1161/JAHA.112.002832]
- Hillis GS, Woodward M, Rodgers A, Chow CK, Li Q, Zoungas S, Patel A, Webster R, Batty GD, Ninomiya T, Mancia G, Poulter NR, Chalmers J. Resting heart rate and the risk of death and cardiovascular complications in patients with type 2 diabetes mellitus. *Diabetologia* 2012; **55**: 1283-1290 [PMID: 22286552 DOI: 10.1007/s00125-012-2471-y]
- Bowden DW, Cox AJ, Freedman BI, Hugenschmidt CE, Wagenknecht LE, Herrington D, Agarwal S, Register TC, Maljdian

- JA, Ng MC, Hsu FC, Langefeld CD, Williamson JD, Carr JJ. Review of the Diabetes Heart Study (DHS) family of studies: a comprehensively examined sample for genetic and epidemiological studies of type 2 diabetes and its complications. *Rev Diabet Stud* 2010; **7**: 188-201 [PMID: 21409311 DOI: 10.1900/RDS.2010.7.188]
- 27 **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]
- 28 **Bowden DW**, Lehtinen AB, Ziegler JT, Rudock ME, Xu J, Wagenknecht LE, Herrington DM, Rich SS, Freedman BI, Carr JJ, Langefeld CD. Genetic epidemiology of subclinical cardiovascular disease in the diabetes heart study. *Ann Hum Genet* 2008; **72**: 598-610 [PMID: 18460048 DOI: 10.1111/j.1469-1809.2008.00446.x]
- 29 **Cox AJ**, Hsu FC, Freedman BI, Herrington DM, Criqui MH, Carr JJ, Bowden DW. Contributors to mortality in high-risk diabetic patients in the Diabetes Heart Study. *Diabetes Care* 2014; **37**: 2798-2803 [PMID: 24989706 DOI: 10.2337/dc14-0081]
- 30 **Cox AJ**, Hugenschmidt CE, Wang PT, Hsu FC, Kenchaiah S, Daniel K, Langefeld CD, Freedman BI, Herrington DM, Carr JJ, Stacey B, Bowden DW. Usefulness of biventricular volume as a predictor of mortality in patients with diabetes mellitus (from the Diabetes Heart Study). *Am J Cardiol* 2013; **111**: 1152-1158 [PMID: 23351459 DOI: 10.1016/j.amjcard.2012.12.044]
- P- Reviewer:** Gómez-Sáez JM, Huang J, Koch TR, Tamemoto H, Zhao J
S- Editor: Cui LJ **L- Editor:** A **E- Editor:** Lu YJ



Association of obesity with hypertension and type 2 diabetes mellitus in India: A meta-analysis of observational studies

Giridhara R Babu, GVS Murthy, Yamuna Ana, Prital Patel, R Deepa, Sara E Benjamin-Neelon, Sanjay Kinra, K Srinath Reddy

Giridhara R Babu, Yamuna Ana, R Deepa, Public Health Foundation of India, IIPH-H, Bangalore Campus, SIHFW Premises, Beside Leprosy Hospital, Bangalore 560023, India

GVS Murthy, Indian Institute of Public Health-Hyderabad, Plot # 1, A.N.V.Arcade, Amar Co-op Society, Kavuri Hills, Madhapur, Hyderabad 500033, India

GVS Murthy, London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom

Prital Patel, Indian School of Business, Hyderabad 500111, India

Sara E Benjamin-Neelon, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, United States

Sanjay Kinra, Department of Non-Communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine and University College London Hospital, London WC1E 7HT, United Kingdom

K Srinath Reddy, Public Health Foundation of India, ISID Campus, 4 Institutional Area Vasant Kunj, New Delhi 110070, India

ORCID number: Giridhara R Babu (0000-0003-4370-8933); GVS Murthy (0000-0002-5695-866X); Yamuna Ana (0000-0002-6795-6846); Prital Patel (0000-0003-2922-8204); R Deepa (0000-0002-3781-496X); Sara E Benjamin Neelon (0000-0003-4643-2397); Sanjay Kinra (0000-0001-6690-4625); K Srinath Reddy (0000-0003-3416-3548).

Author contributions: Babu GR conceived the study aims and design, contributed to the data extraction, planned the analysis, interpreted the results and drafted the final version of the paper; Murthy GVS has contributed to manuscript development and critical review; Ana Y and Deepa R evaluated the study articles and made decisions on inclusion and exclusion of the articles; Patel P was involved in manuscript development, provided inputs for estimations and critical review; Neelon SEB has reviewed the manuscript critically; Kinra S has contributed to

the article critically for important intellectual content; Reddy KS has contributed to the article critically for important intellectual content and final approval of the version to be published.

Supported by Wellcome Trust DBT India Alliance Intermediate Fellowship (Clinical and Public Health) to Giridhara R Babu.

Conflict-of-interest statement: The authors deny any conflict of interest.

Data sharing statement: All data generated or analysed during this study are included in this published article. No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Giridhara R Babu, MBBS, MBA, MPH, PhD, Wellcome Trust-DBT India alliance Research Fellow in Public Health, Additional Professor, Public Health Foundation of India, IIPH-H, Bangalore Campus, SIHFW Premises, Beside Leprosy Hospital, 1st Cross, Magadi Road, Bangalore 560023, India. giridhar@iiphh.org
Telephone: +91-080-23206124

Received: August 3, 2017

Peer-review started: August 7, 2017

First decision: September 7, 2017

Revised: November 5, 2017

Accepted: November 19, 2017

Article in press: November 19, 2017

Published online: January 15, 2018

Abstract

AIM

To perform a meta-analysis of the association of obesity with hypertension and type 2 diabetes mellitus (T2DM) in India among adults.

METHODS

To conduct meta-analysis, we performed comprehensive, electronic literature search in the PubMed, CINAHL Plus, and Google Scholar. We restricted the analysis to studies with documentation of some measure of obesity namely; body mass index, waist-hip ratio, waist circumference and diagnosis of hypertension or diagnosis of T2DM. By obtaining summary estimates of all included studies, the meta-analysis was performed using both RevMan version 5 and "metan" command STATA version 11. Heterogeneity was measured by I^2 statistic. Funnel plot analysis has been done to assess the study publication bias.

RESULTS

Of the 956 studies screened, 18 met the eligibility criteria. The pooled odds ratio between obesity and hypertension was 3.82 (95%CI: 3.39 to 4.25). The heterogeneity around this estimate (I^2 statistic) was 0%, indicating low variability. The pooled odds ratio from the included studies showed a statistically significant association between obesity and T2DM (OR = 1.14, 95%CI: 1.04 to 1.24) with a high degree of variability.

CONCLUSION

Despite methodological differences, obesity showed significant, potentially plausible association with hypertension and T2DM in studies conducted in India. Being a modifiable risk factor, our study informs setting policy priority and intervention efforts to prevent debilitating complications.

Key words: Obesity; Meta-analysis; Hypertension; Type 2 diabetes mellitus

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: India with population explosion and high burden of non-communicable diseases (NCDs) poses a great challenge for the public health specialists to find the route cause for it. Meta-analysis to find the association of obesity with hypertension and type 2 diabetes mellitus in India proved the statistical significance association of obesity with major NCD's with high degree of variability. Results provided with the possible risk factors for the NCD's and what need to be done for the preventive aspect of such diseases. As obesity being a risk factor, setting up a priority policy decisions related to interventions for the prevention of obesity can result in a huge dynamic change in the trend of NCD's in the country like India.

Neelon SE, Kinra S, Reddy KS. Association of obesity with hypertension and type 2 diabetes mellitus in India: A meta-analysis of observational studies. *World J Diabetes* 2018; 9(1): 40-52 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i1/40.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i1.40>

INTRODUCTION

Indians have a higher burden of obesity and have relatively lower muscle mass compared to the whites^[1]. Indians develop metabolic syndrome, hypertension, and type 2 diabetes mellitus (T2DM) earlier compared to whites, which is independent of BMI^[2,3]. The available evidence suggests the age-adjusted prevalence of obesity has doubled in men and has increased three folds in women over two decades (1970s-1990s) in India^[4]. Subsequent economic reforms in India (1991) have initiated overpowering changes in the quality and quantity in a number of lifestyle factors in Indians^[5]. For example, increased consumption of unhealthy food and lower levels of physical activity might likely have contributed to an increase in the prevalence of obesity and its comorbidities^[6].

In India, hypertension and T2DM are the major non-communicable diseases (NCDs) leading to catastrophic complications including death. It is important to investigate the role of modifiable risk factors resulting in NCDs such as obesity, physical inactivity, tobacco use, and alcohol consumption^[7]. Among these shared risk factors of NCDs, limiting the use of tobacco has fittingly received the greater attention of policy makers compared to other risk factors. However, the risk factors seldom act in isolation and it is important to alleviate the impact of their confluence. It is, therefore, important to determine the quantum of the risk contribution by individual risk factor like obesity. Available evidence suggests strong associations between obesity and NCDs^[8,9]. However, none of the earlier reviews have specifically evaluated the role of obesity in the etiology of hypertension and T2DM in India.

The prevalence of obesity has increased significantly in India over the last few decades. About a third of the adult population in urban India is currently estimated to be overweight or obese. As a result, the number of persons with hypertension and T2DM could increase exponentially^[10]. Apart from contributing to T2DM and hypertension, obesity is a major risk factor for pulmonary diseases, metabolic diseases, osteoarthritis, several cancers and serious psychiatric illness^[9,11]. We limit our investigation to T2DM and hypertension. Specifically, we plan to systematically review studies exploring the plausible role of obesity in the etiology of hypertension and T2DM, synthesize the evidence, and perform a meta-analysis if appropriate. Understanding the putative role of obesity and its impact on NCDs will inform future interventions to reduce the burden of

Babu GR, Murthy GVS, Ana Y, Patel P, Deepa R, Benjamin-

these diseases.

MATERIALS AND METHODS

The objective of our study is to estimate the association of obesity with hypertension and T2DM in Indian settings in adults. We developed a protocol for conducting the meta-analysis; with the searching strategy encompassing key MeSH terms, selection of article based on inclusion and exclusion criteria, data extraction, quality assessment of the study, the summary of evidence and analysis.

Literature search and article selection

We included only studies published in English and are conducted in India. We included both the original and review articles restricting the analysis to studies having: (1) documentation of some measure of obesity; AND (2) diagnosis of hypertension was reported; OR (3) T2DM was reported and diagnosed using World Health Organization (WHO) and American Diabetes Association (ADA) criteria. In addition, case-control studies must have compared participants with the disease (T2DM or hypertension) with controls without the disease. We excluded intervention studies, as this was beyond the scope of our review. We defined the exposure variable (obesity as adults with BMI ≥ 30 (studies have considered obesity as BMI with ≥ 25 and ≥ 30), waist circumference (WC) (≥ 80 cm for females and ≥ 90 cm for males), and waist to hip ratio (≥ 0.80 for females and ≥ 0.90 for males). We followed the Joint National Committee VII (JNC VII) criteria for the diagnosis of hypertension; with readings of Systolic Blood Pressure (SBP) ≥ 140 mmHg or Diastolic Blood Pressure (DBP) ≥ 90 mmHg. T2DM was diagnosed as per WHO and ADA classification, when Fasting Blood Sugar (FBS) is ≥ 126 mg/dL (≥ 7.0 mmol/L) or 2-h Post Prandial Blood Sugar (2 h-PPBS) is ≥ 200 mg/dL (≥ 11.1 mmol/L)^[12] (Table 1).

We conducted a comprehensive search of all papers published between January 1980 and January 2016 using MeSH terms for articles in PubMed (Table 2). We also screened other databases, including CINAHL Plus and Google Scholar for additional papers from January to October 2016. We contacted individual authors as necessary to clarify information and assess other relevant papers. We also reviewed cross-referenced papers cited in the assessed articles.

Data extraction and analysis

Stage 1: Identification of studies for inclusion: As a preliminary step two authors (Yamuna Ana and R Deepa) independently assessed the study abstracts retrieved from electronic databases.

Stage 2: Choice of valid studies: Studies selected in stage 1 with necessary information were independently assessed against the inclusion criteria. We included only those studies which aided in the calculation of the relative risk or odds ratio of exposure (obesity) and

outcome (T2DM or hypertension).

Stage 3: Quality assessment: The primary author (Giridhara R Babu) developed the protocol for the review and monitored the overall quality of the review at each step. Criteria for defining obesity, T2DM, and hypertension were noted and crosschecked by primary and secondary authors (Giridhara R Babu, GVS Murthy). Two authors (Yamuna Ana and R Deepa) independently reviewed each article in its entirety for inclusion. The primary author (Giridhara R Babu) conducted random checks before data were extracted and tabulated.

We employed the following set of criteria to evaluate the papers: (1) suitability of the study design; (2) appropriate sample size; (3) evidence regarding obesity and attributes of participants; and (4) accuracy of the tools used for quantifying obesity, diabetes and blood pressure. We also reviewed controlling for confounding, selection bias, reduction of reporting errors and strategies employed to minimize measurement bias.

For assessing eligibility, 2 authors (Yamuna Ana and R Deepa) individually reviewed the full-text papers. Discrepancies were resolved by agreement among both authors which arose during the selection of articles based on study inclusion criteria. Disagreements regarding the inclusion of article were resolved by consulting Giridhara R Babu. If there were multiple reports related to a single study, we included the report with the details relevant to obesity and the outcome of interest.

Stage 4: Extraction of the data and synthesis of results: We did a preliminary search of the electronic databases, after which we selected papers with a title and abstract that matched our criteria. We obtained additional articles from the references provided in the reviewed articles, downloaded the full texts of the article for review. We noted the following details; first author of the paper, year of publication, study design deployed, cut-off values for defining obesity, the prevalence of exposure (obesity), relative risk and odds ratio for T2DM and hypertension. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines were used as the reference for assessing the quality of each study^[13].

We derived the summary estimate by combining estimates from all the selected studies^[14-24]. We did statistical analysis using RevMan version 5 and STATA version 11^[25]. We used double data entry procedure and analysed in the Cochrane Collaboration's Review Manager Software version 5 for Windows (Cochrane Collaboration, Oxford, England). Further, the data in the spreadsheet was analysed using the "metan" command of STATA 11 version for Mac (STATA Corporation, College Station, Texas, United States)^[25]. Crosschecking of outputs for internal consistency has been done and we obtained the pooled odds ratios reported in selected studies using Generic Inverse variance for overall estimates. We strictly conformed to the guidelines for meta-analysis of observational studies used in epidemiology^[26]. We used RevMan for developing flowcharts and for examining

Table 1 Criteria for obesity, hypertension, and type 2 diabetes mellitus

Criteria for obesity, hypertension and T2DM		
Obesity	Hypertension (JNC VII criteria)	T2DM
BMI (≥ 30)	SBP greater than or equal to 140 mmHg or	WHO and ADA classification: Fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL) or 2 h plasma glucose ≥ 11.1 mmol/L (200 mg/dL)
Waist-hip ratio (> 0.80 for females and > 0.90 for males)	DBP greater than or equal to 90 mmHg respectively	
Waist circumference (≥ 90 cm, > 88 cm for female and > 102 cm for male)		

DBP: Diastolic blood pressure; SBP: Systolic blood pressure; ADA: American Diabetes Association; JNC: Joint National Committee; WHO: World Health Organization; T2DM: Type 2 diabetes mellitus; BMI: Body mass index.

Table 2 Search terms used for literature review

Search terms for obesity and hypertension	Search Terms for Obesity and type 2 diabetes
((obesity[MeSH Terms]) AND hypertension[MeSH Terms]) AND prevalence[MeSH Terms] AND India [MeSH Terms]	((obesity[MeSH Terms]) AND type 2 diabetes[MeSH Terms]) AND incidence[MeSH Terms] AND India[MeSH Terms]
((obesity[MeSH Terms]) AND hypertension[MeSH Terms]) AND incidence[MeSH Terms] AND India[MeSH Terms]	((obesity[MeSH Terms]) AND type 2 diabetes[MeSH Terms]) AND prevalence[MeSH Terms] AND India[MeSH Terms]
((obesity[MeSH Terms]) AND hypertension[MeSH Terms]) AND relative risk[MeSH Terms] AND India[MeSH Terms]	((obesity[MeSH Terms]) AND type 2 diabetes [MeSH Terms]) AND risk ratio[MeSH Terms] AND India[MeSH Terms]
((obesity[MeSH Terms]) AND hypertension[MeSH Terms]) AND risk ratio[MeSH Terms] AND India[MeSH Terms]	((obesity[MeSH Terms]) AND type 2 diabetes[MeSH Terms]) AND relative risk[MeSH Terms] AND India [MeSH Terms]
((obesity[MeSH Terms]) AND hypertension[MeSH Terms]) AND attributable risk[MeSH Terms] AND India[MeSH Terms]	((obesity[MeSH Terms]) AND type 2 diabetes [MeSH Terms]) AND attributable risk[MeSH Terms] AND India[MeSH Terms]
((obesity[MeSH Terms]) AND hypertension[MeSH Terms]) AND prevalence[MeSH Terms] OR incidence[MeSH Terms] AND India [MeSH Terms]	((obesity[MeSH Terms]) AND type 2 diabetes [MeSH Terms]) AND prevalence[MeSH Terms] OR incidence[MeSH Terms] AND India [MeSH Terms]

the quality of study methodology. We calculated the unadjusted odds ratios with 95%CI using random-effects model for all analyses^[27]. We used funnel-plot analysis to assess small-study and publication bias. We calculated odds ratio for individual study from the data cell values. We calculated the pooled odds ratio using the individual unadjusted odds ratios of each study within each subgroup of case-control and cohort studies. Hence the pooled odds ratio was also unadjusted. We measured heterogeneity using I^2 statistic. This describes the percentage of total variation across studies that is due to heterogeneity rather than mere chance alone producing this^[28]. I^2 can be readily calculated from basic results obtained from a typical meta-analysis as $I^2 = 100\% \times (Q - df)/Q$, where Q is Cochran's heterogeneity statistic and df being the degrees of freedom. An advantage of I^2 is that it does not depend on the number of studies included in the meta-analysis^[29].

Risk of bias

To assess the risk of publication bias we constructed funnel plots for all the association between exposure and outcome variables.

RESULTS

Study selection

The initial search identified 6907 studies. After checking

for duplicates, we screened 956 studies and excluded 774 that were not relevant. Hence we included 182 studies for full article review and among those we excluded 164 studies from the meta-analysis. Of these, 131 articles were not eligible due to non-availability of exposure or outcome criteria (Figure 1). The ineligible studies were rejected for the following reasons: Exposure criteria were not defined (46), obesity or overweight was not used as an exposure (26), studies were conducted outside India (21), T2DM or hypertension was not included in study (23) and data provided was insufficient to calculate odds ratio or relative risk (15). Finally, 6 studies satisfying the review criteria for hypertension and 12 for T2DM were involved in the meta-analysis.

A descriptive overview of studies included in meta-analysis

One cohort study was included and rest were cross-sectional studies. The age groups of the participants ranged from 20 to 55.5 years. In studies with T2DM as the outcome, the exposure was assessed using BMI in 5 studies, WC in 3 studies and WHR in 4 studies. For the studies involving hypertension as an outcome of interest, five studies used BMI and one used WHR (Tables 3 and 4).

Methodological quality

Information regarding confounding factors is reported in all the studies and in 2 studies, the selection bias is

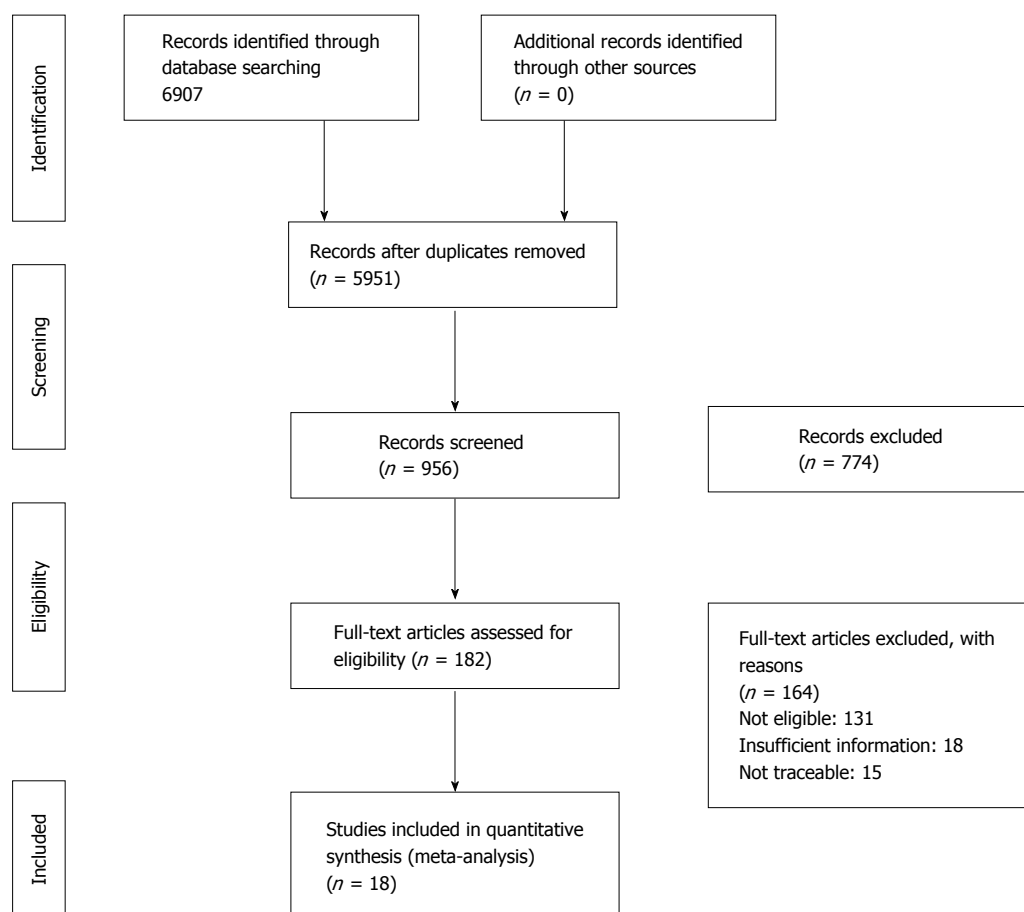


Figure 1 Preferred reporting items for systematic reviews and meta-analysis study flow diagram.

discussed. In studies with hypertension as an outcome, all studies discussed measurement error vs 6 studies with T2DM as the outcome (Tables 3 and 4).

Publication bias

The funnel plot that depicts the publication bias showed an inverted funnel shape with studies of higher precision relatively closer to the pooled odds ratio. This corroborates minimal publication bias (Figures 2 and 3).

Combined effect of obesity and type 2 diabetes mellitus

Odds ratio pooled from all the included studies in meta-analysis exhibited statistically significant association between obesity and T2DM (OR = 1.14, 95%CI: 1.043 to 1.237). We noticed substantial heterogeneity among these study estimates, with the I^2 statistic being 83.9% and $P = 0.0001$. Similarly, the pooled odds ratio of obesity and hypertension was 3.820 (95%CI: 3.392 to 4.248). The heterogeneity around this estimate (I^2 statistic) was 0%, and $P = 0.435$ indicating low variability among the included studies.

DISCUSSION

Our results show that the association between obesity and hypertension is strongly positive and T2DM is

moderately positive compared with healthy non-obese adults in India. Through the synthesis of available evidence using random effects meta-analysis, we show that obesity in India is a formidable independent risk factor to mitigate; albeit the risk appears to be relatively less for T2DM. With industrialization and urbanization, the prevalence of obesity has increased gradually in India, heightening the need to focus on the prevention of these NCDs.

Our analysis suggests that after adjustment for covariates, obesity is significantly associated with hypertension. These estimates were stable, suggested by low variability in the heterogeneity (I^2 statistic, 0%)^[30]. The findings concur with other studies linking body mass as an important risk factor to hypertension^[31-33]. This also coincides with the observed trend of increasing prevalence of hypertension in India across different risk groups for obesity^[34-37]. More specifically, the estimates of meta-analysis are analogous to the estimates from (odds ratio, 3.7; 95%CI: 2.1-6.8) synthesis of evidence covering 6 middle-income countries by Sanjay Basu *et al.*^[34], indicating increased correlation of obesity prevalence with hypertension across dissimilar cultures. The pathophysiology of developing hypertension in obese individuals is explained by elevated cardiac output, perhaps due to excess intravascular volume and reduced

Table 3 Characteristics of included obesity and hypertension studies

Ref.	Year	Participants characteristics	Study characteristics				Measurements		Methodological quality of study			
		Age M (sd) in yr	Setting	Study design	Sample size	Inclusion criteria	Exposure	Outcome	Adjusting confounders	Selection bias	Measurement error	Response rate
Reddy <i>et al</i> ^[14]	2003	20-30	Urban slums	Cross-sectional	1000 (500 male and 500 female)	Adults of 20-60 yr age	BMI > 25	Mean blood pressure levels	Important Confounders ¹	Not mentioned	Mentioned	100%
Mandal <i>et al</i> ^[15]	2008	40-49	Kolkata Municipal Corporation	Cross-sectional	887	Aged 20 yr or more	BMI ≥ 25	JNC VII guideline	Important confounders ¹ + religion, marital status, nature of work, family type, animal protein intake	Not mentioned	Mentioned and discussed	98.30%
Bhadoria <i>et al</i> ^[16]	2014	38-50	Urban wards	Cross-sectional	939	Individuals aged 20 yr and above	BMI ≥ 27.5	JNC VII guideline	Important confounders ¹	Not mentioned	Mentioned	97.02%
Bhadoria <i>et al</i> ^[16]	2014	Males: 25-52 Female: 24-53	48 villages and 15 urban wards of Jabalpur District	Cross-sectional	939	Aged 20 yr and above	W/H ratio > 0.85 for females and > 0.90 for males	JNC VII guideline	Important confounders ¹	Not mentioned	Mentioned	97.02%
Bhadoria <i>et al</i> ^[16]	2014	Males: 25-52 Female: 24-53	Villages of Jabalpur district	Cross-sectional	939	Aged 20 yr and above	BMI ≥ 27.5	JNC VII guideline	Important confounders ¹	Not mentioned	Mentioned	97.02%
Adhikari <i>et al</i> ^[17]	2015	53.9 ± 12.7	Semi-urban in Mangalore city	cross-sectional	800	≥ 20 yr	BMI ≥ 25	JNC VII criteria	Important confounders ¹ + serum cholesterol, serum triglycerides	Mentioned and discussed	Mentioned and discussed	68.80%

¹Important confounder: Age, sex, family history, history of previous events, DM, diet, smoking, alcohol, no regular exercise, saturated fat intake, excess salt intake, sedentary physical activity. JNC VII criteria for diagnosis: Considering JNC 7 category guideline, normal blood pressure is defined as < 120/80 mmHg, prehypertension state is detected when systolic blood pressure (SBP) and diastolic blood pressure (DBP) is 120-139 mmHg and 80-89 mmHg respectively. If the blood pressure is > 140/90 mmHg it is diagnosed as hypertension with Stage 1 hypertension (when SBP and DBP are 140-159 mmHg and 90-99 mmHg respectively) and Stage 2 hypertension (when SBP and DBP are ≥ 160 mmHg and ≥ 100 mmHg respectively). BMI: Body mass index; JNC: Joint National Committee.

cardiac contractility^[38]. Recent evidence suggests that among obese, alteration in nutritional status, gut microbiota, sunlight exposure and increased physical activity have an important role in the presence or absence of hypertension^[39]. Future studies may provide more details on these variables, including possible mediation.

Our results indicate that obesity is only moderately associated with T2DM. Also, we observed considerable heterogeneity in studies involving T2DM. The results also indicate that this is not explained by differences in participant age, baseline characteristics, or study quality. Such heterogeneity might be seen for several reasons. First, the "Asian Indian Phenotype" refers to unique abnormalities characterized by higher chances of adverse effects of obesity despite lower BMI, higher WHR, comparatively low WC and thin stature as compared to other ethnic groups^[40]. The lean T2DM

is a distinct clinical entity in India. Due to temporal ambiguity in cross-sectional studies, it is possible that loss of weight might have ensued after the diagnosis of T2DM. In a recent survey covering eleven cities of India, 45% patients with diabetic retinopathy reported already had the visual loss when they first detected to have T2DM^[41]. This indicates that nearly half of the persons with T2DM in India are undiagnosed, and therefore, apart from other complications would have lost considerable weight by the time of diagnosis. It is reported that nearly 53% of patients may have weight loss as the presenting symptom of T2DM^[42]. Given this evidence, we estimate that nearly one-fourth of the undiagnosed persons with T2DM will have weight loss and therefore will spuriously indicate that obesity may not be a significant risk factor. Using cut-off points of BMI, WC and WHR as surrogates for percentage body fat in Indians, and thereby making classifications

Table 4 Characteristics of included obesity and type 2 diabetes mellitus studies

Ref.	Year	Participants characteristics			Study characteristics		Measurements		Methodological quality of study			
		Age M (sd) in yr	Setting	Study design	Sample size	Inclusion criteria	Exposure	Outcome	Adjusting confounders	Selection bias	Measurement error	Response rate
Mohan <i>et al</i> ^[19]	1996	55.5 ± 11.9	Tamilnadu	Cross-sectional	1399	Individuals aged ≥ 20 yr	BMI ≥ 30 kg/m ²	Diabetes (WHO criteria)	Important confounders ¹ +, SBP, DBP	Not mentioned	Mentioned and discussed	90.20%
Mohan <i>et al</i> ^[19]	1996	55.5 ± 11.9	Tamilnadu	Cross-sectional	1399	individuals aged ≥ 20 yr	WC ≥ 90 cm	Diabetes (WHO criteria)	Important confounders ¹ +, SBP, DBP	Not mentioned	Mentioned and discussed	90.20%
Kumar <i>et al</i> ^[20]	Published year 2008	36.4	Kolkata	Cross-sectional	2200	Policemen with (monthly income: Rs.6000-15000), age (20 and 60 yr)	BMI	T2DM	Important confounders ¹ +, SBP, DBP,	Not mentioned	Mentioned and discussed	98.18%
Kumar <i>et al</i> ^[20]	Published year 2008	36.4	Kolkata	Cross-sectional	2200	policemen with (monthly income: Rs.6000-15000), age (20 and 60 yr)	WHR	T2DM	Important confounders ¹ + SBP, DBP	Not mentioned	Mentioned and discussed	98.18%
Kumar <i>et al</i> ^[20]	Published year 2008	36.4	Kolkata	Cross-sectional	2200	Policemen with (monthly income: Rs.6000-15000), age: 20 and 60 yr	WC	T2DM	Important confounders ¹ SBP, DBP	Not mentioned	Mentioned and discussed	98.18%
Bharati <i>et al</i> ^[21]	2007	20-49	Rural and urban field practice area.	Cross-sectional	1370	Adults: ≥ 20 yr	BMI > 30	T2DM (ADA classification)	Important confounders ¹ + blood cholesterol, hypertension	Not mentioned	Not mentioned	100%
Bharati <i>et al</i> ^[21]	2007	20-49	Rural and urban field practice area	Cross-sectional	1370	Adults: ≥ 20 yr	WHR	T2DM (ADA classification)	Important confounders ¹ + blood cholesterol, hypertension	Not mentioned	Not mentioned	100%
Ravindra Singh <i>et al</i> ^[24]	2012-13	30-39	Agra City	Cross-sectional	633	Adults: ≥ 30 yr residing in Agra City	BMI	T2DM (WHO criteria)	Important confounders ¹	Not mentioned	Not mentioned	100%
Ravindra Singh <i>et al</i> ^[24]	2012-13	30-39	Agra City	Cross-sectional	633	Adults: ≥ 30 yr residing in Agra City	WHR	T2DM (WHO criteria)	Important confounders ¹	Not mentioned	Not mentioned	100%
Ravindra Singh <i>et al</i> ^[24]	2012-13	30-39	Agra City	Cross-sectional	633	Adults: ≥ 30 yr residing in Agra City	WC (> 88 cm for female and > 102 cm for male)	T2DM (WHO criteria)	Important confounders ¹	Not mentioned	Not mentioned	100%
Ghorpade <i>et al</i> ^[22]	2007	35-50	Rural Tamilnadu	Cohort	1403	Adults > 25 yr of age from selected population	BMI ≥ 23	T2DM	Important confounders ¹ + n work status, Alcohol intake	Mentioned	Mentioned and discussed	85%
Vijaya-kumar <i>et al</i> ^[23]	2007	30-44	Urban Kerala	Cross-sectional	1990	≥ 18 yr, residing since t 6 mo	WHR (< 0.80 in women, 0.90 in men)	T2DM (Those with diabetes, and ADA classification)	Important confounders ¹ + hypercholesterolemia, elevated BP	Not mentioned	Not mentioned	82.70%

¹Important confounders: Age, family history, sex, dietary habit, social economic status. As per WHO and ADA classification, diagnosis of diabetes is confirmed when fasting plasma glucose is ≥ 7 mmol/L (126 mg/dL) or 2 h plasma glucose is ≥ 11.1 mmol/L (200 mg/dL). Impaired glucose tolerance (IGT) test and impaired fasting glucose (IFG) test is considered as positive when the fasting plasma glucose is < 7 mmol/L (126 mg/dL) and 6.1 to 6.9 mmol/L (110 mg/dL to 125 mg/dL) respectively, 2 h plasma glucose is ≥ 7.8 and < 11.1 mmol/L (140 mg/dL and 200 mg/dL) and < 7.8 mmol/L (140 mg/dL) respectively. Both: Males and females; NA: Not available; ADA classification of diabetes: Fasting: ≥ 126 mg/dL; DBP: Diastolic blood pressure; SBP: Systolic blood pressure; ADA: American Diabetes Association; JNC: Joint National Committee; WHO: World Health Organization; T2DM: Type 2 diabetes mellitus; WC: Waist circumference; WHR: Waist to hip ratio; BMI: Body mass index.

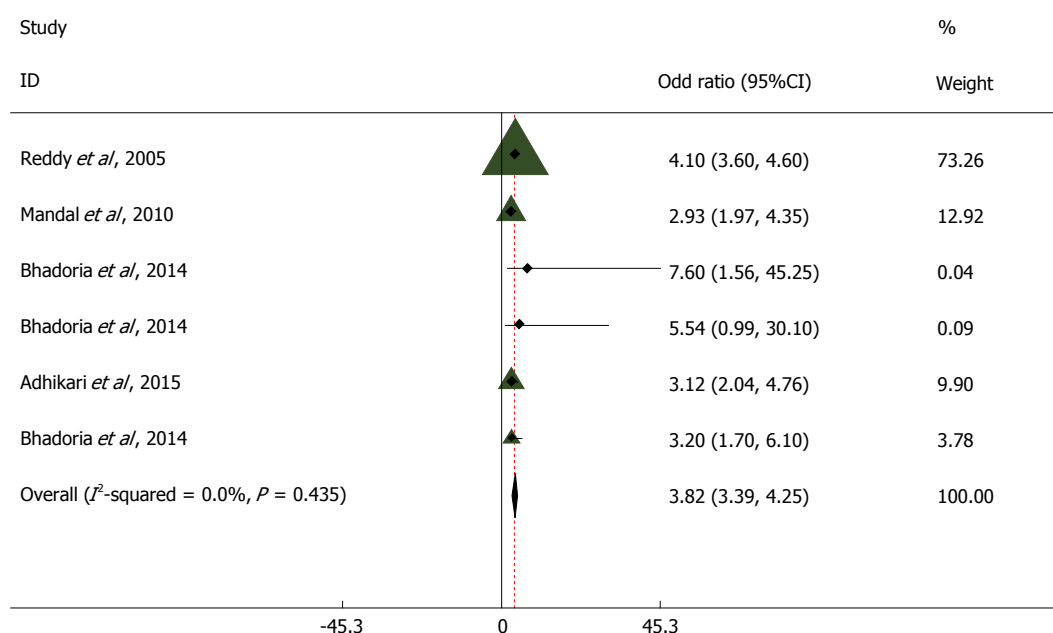


Figure 2 Meta-analysis of studies exploring association between obesity and hypertension in India.

of obesity might have underestimated the overall measures^[43]. The validity of universal cut-off points for Indians is uncertain; it would be better only to treat it continuous variable^[8]. Future examinations should include analysis of the data sets from these studies for a continuous association. The association of obesity with T2DM and hypertension is highly probable at lower levels than the cut-off points used in this paper. Therefore, we might have grossly underestimated the association between obesity and T2DM. Further, Survival bias might have resulted in underestimation; since, people with T2DM, who are dead, debilitated, disabled or have severe illness might not have captured by the cross-sectional studies^[44]. The available evidence concurs with our finding; while the majority of persons with T2DM are obese in the west, 27% of people with diabetes in India are lean^[45-47]. These individuals may have different clinical and biochemical profiles, including predisposition to microvascular complications^[46-49].

Such variations in phenotype used in different studies might include inconsistencies in specific cut-points employed. It is also possible that most of the evidence from cross-sectional studies is derived from hospital-based populations and is, therefore, subject to considerable survivor bias^[50]. Hence, the included participants in the final sample represent only survivors who might have had better glucose control compared to individuals with poor glucose control confounded by obesity^[50]. Finally, those with T2DM may lose substantial amounts of weight from the disease and as a function of treatment^[51]. Due to the cross-sectional nature of these studies, the temporality of obesity prior to the onset of T2DM cannot be established. Despite the heterogeneity, most estimates are in the same direction with only 2 studies reporting less than a null association for T2DM.

The association of obesity with NCDs in India has several challenges. First, despite posing a major public health challenge, the rising prevalence of childhood obesity has received very little attention from policy makers in India. Second, compared to whites, Indians are more prone for obesity and decreased muscle mass for any proposed value of BMI^[1]. With 46%^[52] in the south and 50%^[53] in the north, recent estimates suggest that obesity affects the unvaryingly high proportion of urban Indians, predisposing them to future NCDs. This complicates the issue since Indians within normal BMI can develop insulin resistance, metabolic syndrome, and T2DM^[1]. Therefore, the severity and consequences of obesity might be grossly underestimated, including the challenge of finding an appropriate definition of obesity in Indians. The implications of obesity on the growth of the nation and future expenditures are undervalued. Given that India is projected to have 135 million individuals with generalized obesity^[54], around 44 million might develop insulin resistance^[55-57]. If we were to apply similar methodology employed by Popkin *et al.*^[57] in previous estimates, the annual costs attributable to overweight and obesity in India will surpass approximately \$100 billion in 2025.

To our estimate, this is the first meta-analysis to summarize association of obesity with hypertension and T2DM in India. Our results indicate that it is important to consider further explorations of obesity and NCD associations. Intervention and policy efforts to alleviate the adverse effects of obesity in India, including hypertension and T2DM are also needed. However, there are number of limitations to our review. First, the possibility of conclusive evidence is limited due to the availability of evidence from cohort studies. Second, there can be considerable measurement issues due to heterogeneous definitions

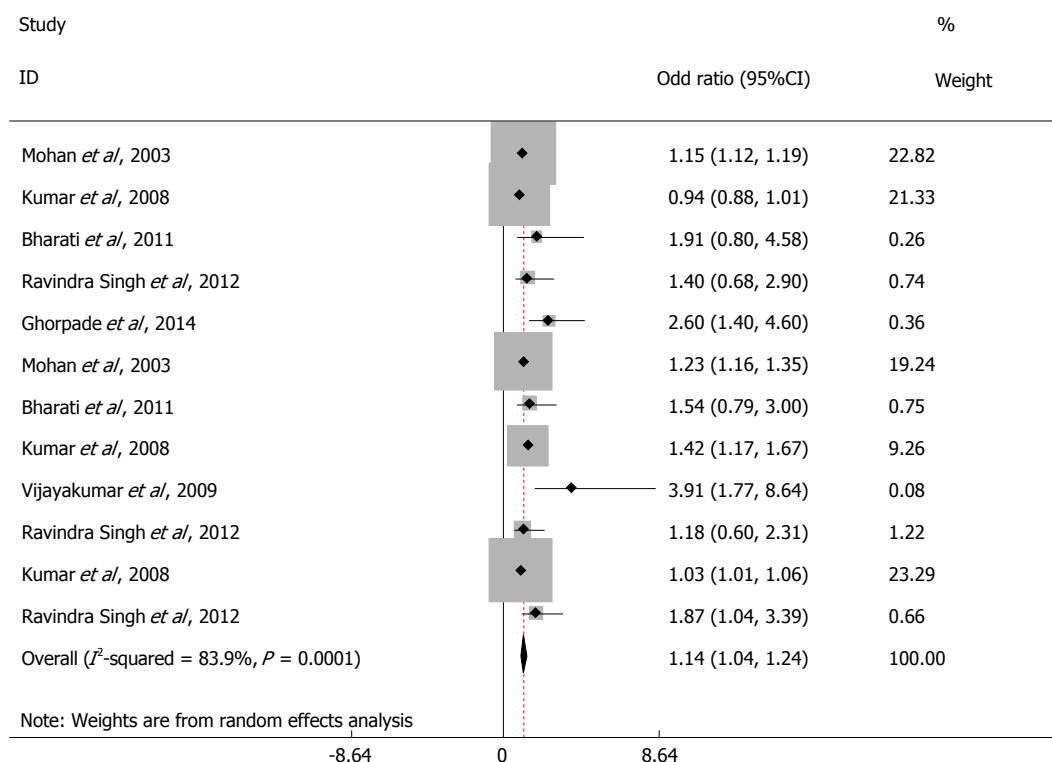


Figure 3 Meta-analysis of studies exploring association between obesity and type 2 diabetes mellitus in India.

in different population subgroups. Third, a standard definition of what constitutes "obesity" in Indians remains elusive and therefore, combining different measures of obesity might have led to misclassifications in this study. Also, in the absence of India specific cut-off points, inability to treat obesity as a continuous variable might have underestimated the association between obesity and T2DM. Finally, the reliance on cross-sectional studies may be particularly susceptible to biases, including survivor bias and therefore restricts causal inference.

Obesity is an important driver of NCDs in India. The current stage of the obesity epidemic presents an opportunity for policy and intervention efforts related to prevention. This opportunity necessitates developing a clear strategy for the control of NCDs through rigorous screening and management. The adverse effects of obesity cannot be assessed without robust documentation of obesity indicators throughout the life course. The increasing prevalence of obesity, hypertension, and diabetes in India has enormous implications for the healthcare system. Policymakers, Government officials, and public health professionals can focus policy and intervention efforts on obesity as an important risk factor to prevent NCDs like diabetes and hypertension.

ARTICLE HIGHLIGHTS

Research background

It is well known that hypertension and type 2 diabetes mellitus (T2DM) are the major non-communicable diseases (NCDs) leading to catastrophic complications and death in India. It is important to investigate the role of modifiable risk factors such as obesity resulting in NCDs. The authors are

aware that the risk factors seldom act in isolation and it is important to alleviate the impact of their confluence. It is therefore important to determine the significance of risk contribution by individual risk factor like obesity. Available evidence suggests strong associations between obesity and NCDs. However, none of the earlier reviews have specifically evaluated the role of obesity in the etiology of hypertension and T2DM in India.

Research motivation

As obesity is one of the key NCD's and risk factor for the majority of other NCD's in India, the authors need to provide evidence to show its association with other major diseases like hypertension and T2DM. By exhibiting the evidence and its association, preventive measures can be taken for root cause of disease.

Research objectives

To perform a meta-analysis of the association of obesity with hypertension and T2DM in India among adults to assess potential causal factors and improve prevention and control measures for these NCDs.

Research methods

The authors have followed rigorous methodology in doing comprehensive meta-analysis with a predefined protocol. The authors entered and analysed data using the Cochrane Collaboration's Review Manager software version 5 for Windows (Cochrane Collaboration, Oxford, England), and subsequently entered into a spreadsheet and re-analysed data using the "metan" command of STATA 11 version for Mac. The authors have used the RevMan for developing flow chart according to PRISMA guidelines, and also assessed the methodological quality of studies. The authors found that the pooled estimate between obesity and hypertension and the heterogeneity around this estimate which indicating low variability among the included studies. The pooled estimate from all studies showed a statistically significant association between obesity and T2DM. The authors observed considerable heterogeneity among these estimates of studies.

Research results

The results shows that the association of obesity and hypertension is strongly positive and T2DM moderately positive compared with healthy non-obese

adults in India. This study provides evidence regarding the putative role of obesity and its impact on NCDs. This also coincides with the observed trend of increasing prevalence of hypertension in India across different risk groups for obesity.

Research conclusions

The current stage of the obesity epidemic presents an opportunity for policy and intervention efforts related to prevention. This opportunity necessitates developing a clear strategy for the control of NCDs through rigorous program management at national and state levels. The increasing prevalence of obesity, hypertension, and diabetes in India has enormous implications for the healthcare system. Policy makers, government officials, and public health professionals can focus policy and intervention efforts on obesity as an important risk factor to prevent NCDs like diabetes and hypertension.

Research perspectives

Study provides with experience of route cause associated with major NCD's like hypertension and T2DM. As the evidence suggested obesity is associated with these NCD's, it is the time to think regarding preventive aspect of obesity to prevent future outcome. With limited earlier statistically proved evidence, the current meta-analysis the association of obesity with hypertension and T2DM in India proved the statistical significance association of obesity with major NCD's such as T2DM and hypertension with high degree of variability and substantial heterogeneity. Results provided the possible common risk factors for the NCD's and made a way for the researchers to think of the research on interventional measures to prevent obesity in coming future. Research involving Randomized Controlled Trials nested within cohort for the prevention of obesity will provide affirmation of fruitful interventions which can be included in future evidence based policy formulation.

ACKNOWLEDGMENTS

We thank Dr. Jotheeswaran A Thiyagarajan for his guidance in performing the statistical analysis..

REFERENCES

- 1 **Bhardwaj S**, Misra A, Khurana L, Gulati S, Shah P, Vikram NK. Childhood obesity in Asian Indians: a burgeoning cause of insulin resistance, diabetes and sub-clinical inflammation. *Asia Pac J Clin Nutr* 2008; **17** Suppl 1: 172-175 [PMID: 18296330]
- 2 **Enas EA**, Mohan V, Deepa M, Farooq S, Pazhoor S, Chennikkara H. The metabolic syndrome and dyslipidemia among Asian Indians: a population with high rates of diabetes and premature coronary artery disease. *J Cardiometaab Syndr* 2007; **2**: 267-275 [PMID: 18059210 DOI: 10.1111/j.1559-4564.2007.07392.x]
- 3 **Yajnik CS**, Ganpule-Rao AV. The obesity-diabetes association: what is different in indians? *Int J Low Extrem Wounds* 2010; **9**: 113-115 [PMID: 20705620 DOI: 10.1177/1534734610380028]
- 4 **Gupta R**, Gupta VP, Bhagat N, Rastogi P, Sarna M, Prakash H, Deedwania PC. Obesity is major determinant of coronary risk factors in India: Jaipur Heart Watch studies. *Indian Heart J* 2008; **60**: 26-33 [PMID: 19212018]
- 5 **Murty S**. Multinational Corporations: One Dimension of Economic Reforms. In Singh BN, Shrivastava MP, Prasad N, editors. *Economic Reforms in India*, New Delhi: APH Publishing Corporation, 2003: 261-280
- 6 **Hu FB**. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes Care* 2011; **34**: 1249-1257 [PMID: 21617109 DOI: 10.2337/dc11-0442]
- 7 **GBD 2013 Risk Factors Collaborators**. Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S, Brauer M, Burnett R, Casey D, Coates MM, Cohen A, Delwiche K, Estep K, Frostad JJ, Astha KC, Kyu HH, Moradi-Lakeh M, Ng M, Slepak EL, Thomas BA, Wagner J, Aasvang GM, Abbafati C, Abbasoglu Ozgoren A, Abd-Allah F, Abera SF, Aboyans V, Abraham B, Abraham JP, Abubakar I, Abu-Rmeileh NM, Aburto TC, Achoki T, Adelekan A, Adofo K, Adou AK, Adsuar JC, Afshin A, Agardh EE,

Al Khabouri MJ, Al Lami FH, Alam SS, Alasfoor D, Albittar MI, Alegretti MA, Aleman AV, Alemu ZA, Alfonso-Cristancho R, Alhabib S, Ali R, Ali MK, Alla F, Allebeck P, Allen PJ, Alsharif U, Alvarez E, Alvis-Guzman N, Amankwaa AA, Amare AT, Ameh EA, Ameli O, Amini H, Ammar W, Anderson BO, Antonio CA, Anwari P, Argeseanu Cunningham S, Arnlöv J, Arsenijevic VS, Artaman A, Asghar RJ, Assadi R, Atkins LS, Atkinson C, Avila MA, Awuah B, Badawi A, Bahit MC, Bakfalouni T, Balakrishnan K, Balalla S, Balu RK, Banerjee A, Barber RM, Barker-Collo SL, Barquera S, Barregard L, Barrero LH, Barrientos-Gutierrez T, Basto-Abreu AC, Basu A, Basu S, Basulaiman MO, Batis Ruvalcaba C, Beardsley J, Bedi N, Bekele T, Bell ML, Benjet C, Bennett DA, Benzian H, Bernabé E, Beyene TJ, Bhalla N, Bhalla A, Bhutta ZA, Bikbov B, Bin Abdulhak AA, Blore JD, Blyth FM, Bohensky MA, Bora Başara B, Borges G, Bornstein NM, Bose D, Boufous S, Bourne RR, Brainin M, Brazinova A, Breitborde NJ, Brenner H, Briggs AD, Broday DM, Brooks PM, Bruce NG, Brugha TS, Brunekreef B, Buchbinder R, Bui LN, Bukhman G, Bulloch AG, Burch M, Burney PG, Campos-Nonato IR, Campuzano JC, Cantoral AJ, Caravanos J, Cárdenas R, Cardis E, Carpenter DO, Caso V, Castañeda-Orjuela CA, Castro RE, Catalá-López F, Cavalleri F, Çavlin A, Chadha VK, Chang JC, Charlson FJ, Chen H, Chen W, Chen Z, Chiang PP, Chimed-Ochir O, Chowdhury R, Christophi CA, Chuang TW, Chugh SS, Cirillo M, Claßen TK, Colistro V, Colomar M, Colquhoun SM, Contreras AG, Cooper C, Cooperrider K, Cooper LT, Coresh J, Courville KJ, Criqui MH, Cuevas-Nasu L, Damsere-Derry J, Danawi H, Dandona L, Dandona R, Dargan PI, Davis A, Davitoiu DV, Dayama A, de Castro EF, De la Cruz-Góngora V, De Leo D, de Lima G, Degenhardt L, del Pozo-Cruz B, Dellavalle RP, Deribe K, Derrett S, Des Jarlais DC, Dessalegn M, deVeber GA, Devries KM, Dharmaratne SD, Dherani MK, Dicker D, Ding EL, Dokova K, Dorsey ER, Driscoll TR, Duan L, Durrani AM, Ebel BE, Ellenbogen RG, Elshrek YM, Endres M, Ermakov SP, Erskine HE, Eshrati B, Esteghamati A, Fahimi S, Faraon EJ, Farzadfar F, Fay DF, Feigin VL, Feigl AB, Fereshtehnejad SM, Ferrari AJ, Ferri CP, Flaxman AD, Fleming TD, Foigt N, Foreman KJ, Paleo UF, Franklin RC, Gabbe B, Gaffikin L, Gakidou E, Gamkrelidze A, Gankpé FG, Gansevoort RT, García-Guerra FA, Gasana E, Geleijnse JM, Gessner BD, Gething P, Gibney KB, Gillum RF, Ginawi IA, Giroud M, Giussani G, Goenka S, Goginashvili K, Gomez Dantes H, Gona P, Gonzalez de Cosio T, González-Castell D, Gotay CC, Goto A, Gouda HN, Guerrant RL, Gugrani HC, Guillemin F, Gunnell D, Gupta R, Gupta R, Gutiérrez RA, Hafezi-Nejad N, Hagan H, Hagstromer M, Halasa YA, Hamadeh RR, Hammami M, Hankey GJ, Hao Y, Harb HL, Haregu TN, Haro JM, Havmoeller R, Hay SI, Hedayati MT, Heredia-Pi IB, Hernandez L, Heuton KR, Heydarpour P, Hijar M, Hoek HW, Hoffman HJ, Hornberger JC, Hosgood HD, Hoy DG, Hsairi M, Hu G, Hu H, Huang C, Huang JJ, Hubbell BJ, Huiart L, Hussein A, Iannarone ML, Iburg KM, Idrisov BT, Ikeda N, Innos K, Inoue M, Islami F, Ismayilova S, Jacobsen KH, Jansen HA, Jarvis DL, Jassal SK, Jauregui A, Jayaraman S, Jeemon P, Jensen PN, Jha V, Jiang F, Jiang G, Jiang Y, Jonas JB, Juel K, Kan H, Kany Roseline SS, Karam NE, Karch A, Karema CK, Karthikeyan G, Kaul A, Kawakami N, Kazi DS, Kemp AH, Kengne AP, Keren A, Khader YS, Khalifa SE, Khan EA, Khang YH, Khatibzadeh S, Khonelidze I, Kieling C, Kim D, Kim S, Kim Y, Kimokoti RW, Kinfu Y, Kinge JM, Kissela BM, Kivipelto M, Knibbs LD, Knudsen AK, Kokubo Y, Kose MR, Kosen S, Kraemer A, Kravchenko M, Krishnaswami S, Kromhout H, Ku T, Kuate Defo B, Kucuk Bicer B, Kuipers EJ, Kulkarni C, Kulkarni VS, Kumar GA, Kwan GF, Lai T, Lakshmana Balaji A, Lalloo R, Lallukka T, Lam H, Lan Q, Lansingh VC, Larson HJ, Larsson M, Laryea DO, Lavados PM, Lawrynowicz AE, Leasher JL, Lee JT, Leigh J, Leung R, Levi M, Li Y, Li Y, Liang J, Liang X, Lim SS, Lindsay MP, Lipshultz SE, Liu S, Liu Y, Lloyd BK, Logroscino G, London SJ, Lopez N, Lortet-Tieulent J, Lotufo PA, Lozano R, Lunevicius R, Ma J, Ma S, Machado VM, MacIntyre MF, Magis-Rodriguez C, Mahdi AA, Majdan M, Malekzadeh R, Mangalam S, Mapoma CC, Marape M, Marcenes W, Margolis DJ, Margono C, Marks GB, Martin RV, Marzan MB,

- Mashal MT, Masiye F, Mason-Jones AJ, Matsushita K, Matzopoulos R, Mayosi BM, Mazorodze TT, McKay AC, McKee M, McLain A, Meaney PA, Medina C, Mehndiratta MM, Mejia-Rodriguez F, Mekonnen W, Melaku YA, Meltzer M, Memish ZA, Mendoza W, Mensah GA, Meretoja A, Mhimbira FA, Micha R, Miller TR, Mills EJ, Misganaw A, Mishra S, Mohamed Ibrahim N, Mohammad KA, Mokdad AH, Mola GL, Monasta L, Montañez Hernandez JC, Montico M, Moore AR, Morawska L, Mori R, Moschandreas J, Moturi WN, Mozaffarian D, Mueller UO, Mukaigawara M, Mullany EC, Murthy KS, Naghavi M, Nahas Z, Naheed A, Naidoo KS, Naldi L, Nand D, Nangia V, Narayan KM, Nash D, Neal B, Nejjari C, Neupane SP, Newton CR, Ngalesoni FN, Ngirabega Jde D, Nguyen G, Nguyen NT, Nieuwenhuijsen MJ, Nisar MI, Nogueira JR, Nolla JM, Nolte S, Norheim OF, Norman RE, Norrving B, Nyakarahuka L, Oh IH, Ohkubo T, Olusanya BO, Omer SB, Opio JN, Orozco R, Pagcatipunan RS Jr, Pain AW, Pandian JD, Panelo CI, Papachristou C, Park EK, Parry CD, Paternina Caicedo AJ, Patten SB, Paul VK, Pavlin BI, Pearce N, Pedraza LS, Pedroza A, Pejin Stokic L, Pekerici A, Pereira DM, Perez-Padilla R, Perez-Ruiz F, Perico N, Perry SA, Pervaiz A, Pesudovs K, Peterson CB, Petzold M, Phillips MR, Phua HP, Plass D, Poenaru D, Polanczyk GV, Polinder S, Pond CD, Pope CA, Pope D, Popova S, Pourmalek F, Powles J, Prabhakaran D, Prasad NM, Qato DM, Quezada AD, Quistberg DA, Racapé L, Rafay A, Rahimi K, Rahimi-Movaghar V, Rahman SU, Raju M, Rakovac I, Rana SM, Rao M, Razavi H, Reddy KS, Refaat AH, Rehm J, Remuzzi G, Ribeiro AL, Riccio PM, Richardson L, Riederer A, Robinson M, Roca A, Rodriguez A, Rojas-Rueda D, Romieu I, Ronfani L, Room R, Roy N, Ruhago GM, Rushton L, Sabin N, Sacco RL, Saha S, Sahathevan R, Sahraian MA, Salomon JA, Salvo D, Sampson UK, Sanabria JR, Sanchez LM, Sánchez-Pimienta TG, Sanchez-Riera L, Sandar L, Santos IS, Sapkota A, Satpathy M, Saunders JE, Sawhney M, Saylan MI, Scarborough P, Schmidt JC, Schneider JJ, Schöttker B, Schwebel DC, Scott JG, Seedat S, Sepanlou SG, Serdar B, Servan-Mori EE, Shaddick G, Shahraz S, Levy TS, Shangquan S, She J, Sheikhbahaei S, Shibuya K, Shin HH, Shinohara Y, Shiri R, Shishani K, Shieue I, Sigfusdottir ID, Silberberg DH, Simard EP, Sindi S, Singh A, Singh GM, Singh JA, Skirbekk V, Sliwa K, Soljak M, Soneji S, Søreide K, Soshnikov S, Sposato LA, Sreeramareddy CT, Stapelberg NJ, Stathopoulou V, Steckling N, Stein DJ, Stein MB, Stephens N, Stöckl H, Straif K, Stroumpoulis K, Sturua L, Sunguya BF, Swaminathan S, Swaroop M, Sykes BL, Tabb KM, Takahashi K, Talongwa RT, Tandon N, Tanne D, Tanner M, Tavakkoli M, Te Ao BJ, Teixeira CM, Téllez Rojo MM, Terkawi AS, Texcalac-Sangrador JL, Thackway SV, Thomson B, Thorne-Lyman AL, Thrift AG, Thurston GD, Tillmann T, Tobollik M, Tonelli M, Topouzis F, Towbin JA, Toyoshima H, Traebert J, Tran BX, Trasande L, Trillini M, Trujillo U, Dimbuene ZT, Tsilimbaris M, Tuzcu EM, Uchendu US, Ukwaja KN, Uzum SB, van de Vijver S, Van Dingenen R, van Gool CH, van Os J, Varakin YY, Vasankari TJ, Vasconcelos AM, Vavilala MS, Veerman LJ, Velasquez-Melendez G, Venketasubramanian N, Vijayakumar L, Villalpando S, Violante FS, Vlassov VV, Vollset SE, Wagner GR, Waller SG, Wallin MT, Wan X, Wang H, Wang J, Wang L, Wang W, Wang Y, Warouw TS, Watts CH, Weichenthal S, Weiderpass E, Weintraub RG, Werdecker A, Wessells KR, Westerman R, Whiteford HA, Wilkinson JD, Williams HC, Williams TN, Woldeyohannes SM, Wolfe CD, Wong JQ, Woolf AD, Wright JL, Wurtz B, Xu G, Yan LL, Yang G, Yano Y, Ye P, Yenewew M, Yentür GK, Yip P, Yonemoto N, Yoon SJ, Younis MZ, Younoussi Z, Yu C, Zaki ME, Zhao Y, Zheng Y, Zhou M, Zhu J, Zhu S, Zou X, Zunt JR, Lopez AD, Vos T, Murray CJ. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015; **386**: 2287-2323 [PMID: 26364544 DOI: 10.1016/S0140-6736(15)00128-2]
- 8 **Deurenberg P**, Yap M, van Staveren WA. Body mass index and percent body fat: a meta analysis among different ethnic groups. *Int J Obes Relat Metab Disord* 1998; **22**: 1164-1171 [PMID: 9877251 DOI: 10.1038/sj.ijo.0800741]
- 9 **de Onis M**, Blössner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. *Am J Clin Nutr* 2010; **92**: 1257-1264 [PMID: 20861173 DOI: 10.3945/ajcn.2010.29786]
- 10 **Zimmet P**. Globalization, coca-colonization and the chronic disease epidemic: can the Doomsday scenario be averted? *J Intern Med* 2000; **247**: 301-310 [PMID: 10762445 DOI: 10.1046/j.1365-2796.2000.00625.x]
- 11 **Samanic C**, Chow WH, Gridley G, Jarvholm B, Fraumeni JF Jr. Relation of body mass index to cancer risk in 362,552 Swedish men. *Cancer Causes Control* 2006; **17**: 901-909 [PMID: 16841257 DOI: 10.1007/s10552-006-0023-9]
- 12 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; **33** Suppl 1: S62-S69 [PMID: 20042775 DOI: 10.2337/dc10-S062]
- 13 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009; **151**: 264-269, W64 [PMID: 19622511 DOI: 10.7326/0003-4819-151-4-200908180-00135]
- 14 **Reddy S**, Prabhu G. Prevalence and risk factors of hypertension in adults in an Urban Slum, Tirupati, AP. *IJCM* 2005; **30**: 84 [DOI: 10.4103/0970-0218.42855]
- 15 **Mandal PK**, Roy AS, Chatterjee C, Mallik S, Manna N. Burden of hypertension and its risk factors in an urban community of India: are we aware and concerned? *SJPH* 2010; **5**: 130-135
- 16 **Bhadoria AS**, Kasar PK, Toppo NA, Bhadoria P, Pradhan S, Kabirpanthi V. Prevalence of hypertension and associated cardiovascular risk factors in Central India. *J Family Community Med* 2014; **21**: 29-38 [PMID: 24695988 DOI: 10.4103/2230-8229.128775]
- 17 **Adhikari P**, Pemminati S, Pathak R, Kotian MS, Ullal S. Prevalence of Hypertension in Bolloor Diabetes Study (BDS-II) and its Risk Factors. *J Clin Diagn Res* 2015; **9**: IC01-IC04 [PMID: 26674015 DOI: 10.7860/JCDR/2015/16509.6781]
- 18 **Dowse GK**, Zimmet PZ, Gareeboo H, George K, Alberti MM, Tuomilehto J, Finch CF, Chitson P, Tulsidas H. Abdominal obesity and physical inactivity as risk factors for NIDDM and impaired glucose tolerance in Indian, Creole, and Chinese Mauritians. *Diabetes Care* 1991; **14**: 271-282 [PMID: 2060430 DOI: 10.2337/diacare.14.4.271]
- 19 **Mohan V**, Shanthirani CS, Deepa R. Glucose intolerance (diabetes and IGT) in a selected South Indian population with special reference to family history, obesity and lifestyle factors--the Chennai Urban Population Study (CUPS 14). *J Assoc Physicians India* 2003; **51**: 771-777 [PMID: 14651136]
- 20 **Kumar S**, Mukherjee S, Mukhopadhyay P, Pandit K, Raychaudhuri M, Sengupta N, Ghosh S, Sarkar S, Mukherjee S, Chowdhury S. Prevalence of diabetes and impaired fasting glucose in a selected population with special reference to influence of family history and anthropometric measurements--the Kolkata policeman study. *J Assoc Physicians India* 2008; **56**: 841-844 [PMID: 19263680]
- 21 **Bharati DR**, Pal R, Kar S, Rekha R, Yamuna TV, Basu M. Prevalence and determinants of diabetes mellitus in Puducherry, South India. *J Pharm Bioallied Sci* 2011; **3**: 513-518 [PMID: 22219584 DOI: 10.4103/0975-7406.90104]
- 22 **Ghorpade AG**, Majgi SM, Sarkar S, Kar SS, Roy G, Ananthanarayanan PH, Das AK. Diabetes in rural Pondicherry, India: a population-based study of the incidence and risk factors. *WHO South East Asia J Public Health* 2013; **2**: 149-155 [PMID: 28615590 DOI: 10.4103/2224-3151.206761]
- 23 **Vijayakumar G**, Arun R, Kutty VR. High prevalence of type 2 diabetes mellitus and other metabolic disorders in rural Central Kerala. *J Assoc Physicians India* 2009; **57**: 563-567 [PMID: 20209716]
- 24 **Singh R**, Kaushal M, Agarwal V. Prevalence of Diabetes Mellitus and its Risk Factors in Urban Population of Agra District: A Community Based Study. *Medical Science* 2015; **5**: 439-439

- 25 **Stata S.** Stata Statistical Software: Release 11.2009. Available from: URL: https://www.researchgate.net/publication/256294412_Stata_Statistical_Software_Release_MP_101
- 26 **Greenland S.** Quantitative methods in the review of epidemiologic literature. *Epidemiol Rev* 1987; **9**: 1-30 [PMID: 3678409 DOI: 10.1093/oxfordjournals.epirev.a036298]
- 27 **Clarke M, Oxman AD.** Review Manager (RevMan) Version 5.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration [Computer programme] 2008. Available from: URL: http://xueshu.baidu.com/s?wd=paperuri%3A%286c1387eadc2781b4d929be1db00d0638%29filter=sc_long_sigtntn=SE_xueshu_source_2kduw22vsc_vurl=http%3A%2F%2Fwww.scienceopen.com%2Fdocument%3Fvid%3Da73ee5f0-8d2f-4bea-9e24-d1e8cd7cfef8ie=utf-8sc_us=1249986841050500996
- 28 **Higgins JP, Thompson SG.** Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539-1558 [PMID: 12111919 DOI: 10.1002/sim.1186]
- 29 **Harris R, Bradburn M, Deeks J, Sterne JAC.** metan: fixed- and random-effects meta-analysis. *Stata J* 2008; **8**: 3-28 [DOI: 10.1201/9781420064759.ch1]
- 30 **Higgins JP, Thompson SG, Deeks JJ, Altman DG.** Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]
- 31 **Kannel WB.** Risk stratification in hypertension: new insights from the Framingham Study. *Am J Hypertens* 2000; **13**: 3S-10S [PMID: 10678282 DOI: 10.1016/S0895-7061(99)00252-6]
- 32 **Manicardi V, Camellini L, Bellodi G, Coscelli C, Ferrannini E.** Evidence for an association of high blood pressure and hyperinsulinemia in obese man. *J Clin Endocrinol Metab* 1986; **62**: 1302-1304 [PMID: 3517032 DOI: 10.1210/jcem-62-6-1302]
- 33 **Stevens VJ, Corrigan SA, Obarzanek E, Bernauer E, Cook NR, Hebert P, Mattfeldt-Beman M, Oberman A, Sugars C, Dalcin AT.** Weight loss intervention in phase 1 of the Trials of Hypertension Prevention. The TOHP Collaborative Research Group. *Arch Intern Med* 1993; **153**: 849-858 [PMID: 8466377 DOI: 10.1001/archinte.1993.00410070039006]
- 34 **Basu S, Millett C.** Social epidemiology of hypertension in middle-income countries: Determinants of prevalence, diagnosis, treatment, and control in the WHO SAGE study. *Hypertension* 2013; **62**: 18-26 [PMID: 23670299 DOI: 10.1161/HYPERTENSIONAHA.113.01374]
- 35 **James WP.** The epidemiology of obesity: the size of the problem. *J Intern Med* 2008; **263**: 336-352 [PMID: 18312311 DOI: 10.1111/j.1365-2796.2008.01922.x]
- 36 **Ghosh JR, Bandyopadhyay AR.** Comparative evaluation of obesity measures: relationship with blood pressures and hypertension. *Singapore Med J* 2007; **48**: 232-235 [PMID: 17342293]
- 37 **Gupta R, Gupta V.** Hypertension epidemiology in India: lessons from Jaipur heart watch. *Curr Sci* 2009; **97**: 349-355
- 38 **Diaz ME.** Hypertension and obesity. *J Hum Hypertens* 2002; **16** Suppl 1: S18-S22 [PMID: 11986887 DOI: 10.1038/sj.jhh.1001335]
- 39 **Kotsis V, Nilsson P, Grassi G, Mancia G, Redon J, Luft F, Schmieder R, Engeli S, Stabouli S, Antza C, Pall D, Schlaich M, Jordan J; WG on Obesity, Diabetes, the High Risk Patient, European Society of Hypertension.** New developments in the pathogenesis of obesity-induced hypertension. *J Hypertens* 2015; **33**: 1499-1508 [PMID: 26103132 DOI: 10.1097/HJH.0000000000000645]
- 40 **Mohan V, Deepa R.** Obesity and abdominal obesity in Asian Indians. *Indian J Med Res* 2006; **123**: 593-596 [PMID: 16873902]
- 41 **Gilbert CE, Babu RG, Gudlavalleti AS, Anchala R, Shukla R, Ballabh PH, Vashist P, Ramachandra SS, Allagh K, Sagar J, Bandyopadhyay S, Murthy GV.** Eye care infrastructure and human resources for managing diabetic retinopathy in India: The India 11-city 9-state study. *Indian J Endocrinol Metab* 2016; **20**: S3-S10 [PMID: 27144134 DOI: 10.4103/2230-8210.179768]
- 42 **Maisey A.** A Practical Approach to Gastrointestinal Complications of Diabetes. *Diabetes Ther* 2016; **7**: 379-386 [PMID: 27431262 DOI: 10.1007/s13300-016-0182-y]
- 43 **Bodicoat DH, Gray LJ, Henson J, Webb D, Guru A, Misra A, Gupta R, Vikram N, Sattar N, Davies MJ, Khunti K.** Body mass index and waist circumference cut-points in multi-ethnic populations from the UK and India: the ADDITION-Leicester, Jaipur heart watch and New Delhi cross-sectional studies. *PLoS One* 2014; **9**: e90813 [PMID: 24599391 DOI: 10.1371/journal.pone.0090813]
- 44 **Tobias DK, Pan A, Jackson CL, O'Reilly EJ, Ding EL, Willett WC, Manson JE, Hu FB.** Body-mass index and mortality among adults with incident type 2 diabetes. *N Engl J Med* 2014; **370**: 233-244 [PMID: 24428469 DOI: 10.1056/NEJMoa1304501]
- 45 **Das S.** Nutritional status and profile of NIDDM of recent onset. *J Diab Assoc India* 1998; **28**: 99-101
- 46 **Prabhu M, Sudha V, Shashikiran U.** Clinical Profile of Type 2 Diabetes Mellitus And Body Mass Index-Is There Any Correlation? *Calicut Medical Journal* 2004; **2**: 4
- 47 **Barma PD, Ranabir S, Prasad L, Singh TP.** Clinical and biochemical profile of lean type 2 diabetes mellitus. *Indian J Endocrinol Metab* 2011; **15**: S40-S43 [PMID: 21847453 DOI: 10.4103/2230-8210.83061]
- 48 **Sinharoy K, Mandal L, Chakrabarti S, Paul UK, Bandyopadhyay R, Basu AK.** A study on clinical and biochemical profile of low body weight type 2 diabetes mellitus. *J Indian Med Assoc* 2008; **106**: 747-750 [PMID: 19368101]
- 49 **Unnikrishnan AG, Singh SK, Sanjeevi CB.** Prevalence of GAD65 antibodies in lean subjects with type 2 diabetes. *Ann N Y Acad Sci* 2004; **1037**: 118-121 [PMID: 15699503 DOI: 10.1196/annals.1337.018]
- 50 **Barr EL, Zimmet PZ, Welborn TA, Jolley D, Magliano DJ, Dunstan DW, Cameron AJ, Dwyer T, Taylor HR, Tonkin AM, Wong TY, McNeil J, Shaw JE.** Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation* 2007; **116**: 151-157 [PMID: 17576864 DOI: 10.1161/CIRCULATIONAHA.106.685628]
- 51 **Doehner W, Erdmann E, Cairns R, Clark AL, Dormandy JA, Ferrannini E, Anker SD.** Inverse relation of body weight and weight change with mortality and morbidity in patients with type 2 diabetes and cardiovascular co-morbidity: an analysis of the PROactive study population. *Int J Cardiol* 2012; **162**: 20-26 [PMID: 22037349 DOI: 10.1016/j.ijcard.2011.09.039]
- 52 **Deepa M, Farooq S, Deepa R, Manjula D, Mohan V.** Prevalence and significance of generalized and central body obesity in an urban Asian Indian population in Chennai, India (CURES: 47). *Eur J Clin Nutr* 2009; **63**: 259-267 [PMID: 17928807 DOI: 10.1038/sj.ejcn.1602920]
- 53 **Bhardwaj S, Misra A, Misra R, Goel K, Bhatt SP, Rastogi K, Vikram NK, Gulati S.** High prevalence of abdominal, intra-abdominal and subcutaneous adiposity and clustering of risk factors among urban Asian Indians in North India. *PLoS One* 2011; **6**: e24362 [PMID: 21949711 DOI: 10.1371/journal.pone.0024362]
- 54 **Pradeepa R, Anjana RM, Joshi SR, Bhansali A, Deepa M, Joshi PP, Dhandania VK, Madhu SV, Rao PV, Geetha L, Subashini R, Unnikrishnan R, Shukla DK, Kaur T, Mohan V, Das AK; ICMR-INDIAB Collaborative Study Group.** Prevalence of generalized & abdominal obesity in urban & rural India--the ICMR-INDIAB Study (Phase-I) [ICMR- NDIAB-3]. *Indian J Med Res* 2015; **142**: 139-150 [PMID: 26354211 DOI: 10.4103/0971-5916.164234]
- 55 **Misra A, Vikram NK, Arya S, Pandey RM, Dhingra V, Chatterjee A, Dwivedi M, Sharma R, Luthra K, Guleria R, Talwar KK.** High prevalence of insulin resistance in postpubertal Asian Indian children is associated with adverse truncal body fat patterning, abdominal adiposity and excess body fat. *Int J Obes Relat Metab Disord* 2004; **28**: 1217-1226 [PMID: 15314636 DOI: 10.1038/sj.ijo.0802704]
- 56 **Kelly T, Yang W, Chen CS, Reynolds K, He J.** Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)* 2008; **32**: 1431-1437 [PMID: 18607383 DOI: 10.1038/ijo.2008.102]
- 57 **Popkin BM, Kim S, Rusev ER, Du S, Zizza C.** Measuring the full economic costs of diet, physical activity and obesity-related





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 February 15; 9(2): 53-58



**ORIGINAL ARTICLE****Retrospective Study**

- 53 Blood glucose changes surrounding initiation of tumor-necrosis factor inhibitors and conventional disease-modifying anti-rheumatic drugs in veterans with rheumatoid arthritis

Wood PR, Manning E, Baker JF, England B, Davis L, Cannon GW, Mikuls TR, Caplan L

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Rajesh Kumar, MD, Associate Professor, Division of Molecular Cardiology, College of Medicine, Texas A and M Health Science Center, Temple, TX 76504, United States

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, and Scopus.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Rui-Fang Li*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Li-Jun Cui*
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

Lu Qi, MD, PhD, Assistant Professor, Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, United States

Jingbo Zhao, PhD, Associate Professor, Aalborg Hospital Science and Innovation Centre, Aalborg

Hospital, Aarhus University Hospital, Aalborg 9000, Denmark

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com/1948-9358/editorialboard.htm>

EDITORIAL OFFICE
Xiu-Xia Song, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE
February 15, 2018

COPYRIGHT
© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
<http://www.wjgnet.com/bpg/ggerinfo/204>

ONLINE SUBMISSION
<http://www.f6publishing.com>

Retrospective Study

Blood glucose changes surrounding initiation of tumor-necrosis factor inhibitors and conventional disease-modifying anti-rheumatic drugs in veterans with rheumatoid arthritis

Patrick R Wood, Evan Manning, Joshua F Baker, Bryant England, Lisa Davis, Grant W Cannon, Ted R Mikuls, Liron Caplan

Patrick R Wood, Evan Manning, Liron Caplan, Denver Veterans Affairs Medical Center, Division of Rheumatology, University of Colorado, Aurora, CO 80045, United States

Joshua F Baker, Philadelphia Veterans Affairs Medical Center, Division of Rheumatology, University of Pennsylvania, Philadelphia, PA 19104, United States

Bryant England, Ted R Mikuls, Omaha Veterans Affairs Medical Center, Division of Rheumatology, University of Nebraska, Omaha, NE 68198, United States

Lisa Davis, Denver Health and Hospital, Division of Rheumatology, University of Colorado, Aurora, CO 80045, United States

Grant W Cannon, George Wahlen Veterans Affairs Medical Center, Division of Rheumatology, University of Utah, Salt Lake City, UT 84148, United States

ORCID number: Patrick R Wood (0000-0002-6386-4805); Evan Manning (0000-0002-4971-2610); Joshua F Baker (0000-0003-0799-7563); Bryant England (0000-0002-9649-3588); Lisa Davis (0000-0002-1801-174X); Grant W Cannon (0000-0001-6640-9173); Ted R Mikuls (0000-0002-0897-2272); Liron Caplan (0000-0002-2799-3036).

Author contributions: All authors helped to perform the research and reviewed and edited the final manuscript; Wood PR and Caplan L contributed to writing the manuscript, conception and design; Wood PR, Manning E and Caplan L contributed to data analysis.

Supported by VA HSR&D MERIT Award IIR, No. 14-048-3 for Dr Caplan; and Dr. Wood is supported by a VA GME Enhancement Award.

Institutional review board statement: This study was approved by local IRBs (Colorado Multiple Institutional Review

Board #06-0956) and the Scientific and Ethical Advisory Board of the VARA registry for analysis of VARA and VA administrative data.

Informed consent statement: All patients provided written consent prior to enrollment in the VARA registry.

Conflict-of-interest statement: All authors declare no conflicts-of-interest related to this article.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Liron Caplan, MD, PhD, Associate Professor, Denver Veterans Affairs Medical Center, Division of Rheumatology, University of Colorado, POB 6511, B115, Aurora, CO 80045, United States. liron.caplan@ucdenver.edu
Telephone: +1-720-8575103
Fax: +1-720-8575992

Received: January 8, 2018

Peer-review started: January 8, 2018

First decision: February 9, 2018

Revised: February 13, 2018

Accepted: February 14, 2018

Article in press: February 14, 2018

Published online: February 15, 2018

Abstract

AIM

To determine the scope of acute hypoglycemic effects for certain anti-rheumatic medications in a large retrospective observational study.

METHODS

Patients enrolled in the Veterans Affairs Rheumatoid Arthritis (VARA) registry were selected who, during follow-up, initiated treatment with tumor necrosis factor inhibitors (TNFi's, including etanercept, adalimumab, infliximab, golimumab, or certolizumab), prednisone, or conventional disease-modifying anti-rheumatic drugs (DMARDs), and for whom proximate random blood glucose (RBG) measurements were available within a window 2-wk prior to, and 6 mo following, medication initiation. Similar data were obtained for patients with proximate values available for glycosylated hemoglobin A1C values within a window 2 mo preceding, and 12 mo following, medication initiation. RBG and A1C measurements were compared before and after initiation events using paired *t*-tests, and multivariate regression analysis was performed including established comorbidities and demographics.

RESULTS

Two thousands one hundred and eleven patients contributed at least one proximate measurement surrounding the initiation of any examined medication. A significant decrease in RBG was noted surrounding 653 individual hydroxychloroquine-initiation events (-3.68 mg/dL, $P = 0.04$), while an increase was noted for RBG surrounding 665 prednisone-initiation events ($+5.85$ mg/dL, $P < 0.01$). A statistically significant decrease in A1C was noted for sulfasalazine initiation, as measured by 49 individual initiation events (-0.70% , $P < 0.01$). Multivariate regression analyses, using methotrexate as the referent, suggest sulfasalazine ($\beta = -0.58$, $P = 0.01$) and hydroxychloroquine ($\beta = -5.78$, $P = 0.01$) use as predictors of lower post-medication-initiation RBG and A1C values, respectively. Analysis by drug class suggested prednisone (or glucocorticoids) as predictive of higher medication-initiation event RBG among all start events as compared to DMARDs, while this analysis did not show any drug class-level effect for TNFi. A diagnosis of congestive heart failure ($\beta = 4.69$, $P = 0.03$) was predictive for higher post-initiation RBG values among all medication-initiation events.

CONCLUSION

No statistically significant hypoglycemic effects surrounding TNFi initiation were observed in this large cohort. Sulfasalazine and hydroxychloroquine may have epidemiologically significant acute hypoglycemic effects.

Key words: Disease modifying anti-rheumatic drugs; Drug toxicity; Glucocorticoids; Rheumatoid arthritis; Tumor necrosis factor inhibitors

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Clinicians should be cognizant of the potential for rare hypoglycemic effects of the conventional disease-modifying anti-rheumatic drugs hydroxychloroquine and sulfasalazine, in addition to the well-known hyperglycemic effects of glucocorticoids. Although case reports describe dramatic sporadic hypoglycemic events with the initiation of tumor necrosis factor inhibitors, these effects were not confirmed in our large retrospective study.

Wood PR, Manning E, Baker JF, England B, Davis L, Cannon GW, Mikuls TR, Caplan L. Blood glucose changes surrounding initiation of tumor-necrosis factor inhibitors and conventional disease-modifying anti-rheumatic drugs in veterans with rheumatoid arthritis. *World J Diabetes* 2018; 9(2): 53-58 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i2/53.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i2.53>

INTRODUCTION

Current disease models suggest important links between activation of the innate immune system and obesity, the metabolic syndrome, and diabetes^[1]. In particular, obesity has been associated with activation of the tumor necrosis factor (TNF)-alpha pathway, a cytokine system important in the treatment of many autoimmune diseases.

Tumor necrosis factor TNF inhibitors have revolutionized the care of patients with rheumatoid arthritis (RA), proving highly effective in controlling signs and symptoms of disease, and reducing erosive progression in patients with moderate-to-severe disease. Etanercept, a soluble fusion protein inhibitor of TNF, has an extensive safety record that is generally highly favorable, but sporadic and anecdotal events have been reported indicating potential side effects on glucose homeostasis.

These reports include descriptions of radical and immediate hypoglycemia and improved glucose tolerance with drug initiation among diabetics^[2]. These have occurred in patients with established diagnoses of type II diabetes and psoriasis^[3,4] or psoriatic arthritis^[5]. Events have also occurred in RA patients taking etanercept^[6]. Hypoglycemic episodes have also been observed in non-diabetic patients^[7]. Furthermore, prospective study of obese subjects has shown improved fasting glucose, adiponectin ratios, and other glucose tolerance markers in patients treated prospectively with etanercept^[8].

In addition to anecdotal effects of TNF inhibitors (TNFi) on blood glucose homeostasis, literature exists which suggests hypoglycemic effects for other immunosuppressive medications used in the treatment of RA. These include the conventional disease modifying anti-rheumatic drug (DMARD) sulfasalazine^[9]. Hydroxychloroquine, another DMARD and anti-malarial, has also been noted to have effects on glucose and lipid metabolism beyond its anti-inflammatory role^[10], and prospective studies have shown a decreased risk of incident diabetes among RA patients who use hydroxy-

chloroquine^[11].

Effects on blood glucose and hemoglobin A1C surrounding medication initiation were therefore examined in a cohort of veterans with established RA who initiated treatment on TNFi, prednisone, and DMARDs.

MATERIALS AND METHODS

VARA registry

This retrospective analysis used data obtained from US veterans with RA who were enrolled in the Veterans Affairs Rheumatoid Arthritis (VARA) registry. VARA is an ongoing, longitudinal, multicenter registry that included patients from 12 VA medical centers (Birmingham AL, Brooklyn NY, Dallas TX, Denver CO, Jackson MS, Iowa City IA, Little Rock AR, Omaha NE, Portland OR, Philadelphia PA, Salt Lake City UT, and Washington, DC, United States). The study was approved by local IRBs (Colorado Multiple Institutional Review Board #06-0956) and the Scientific and Ethical Advisory Board of the VARA registry for analysis of VARA and VA administrative data; all patients provided written consent prior to enrollment in the VARA registry.

Patients

All patients reported disease onset after 18 years of age and fulfilled the 1987 American College of Rheumatology (ACR) classification criteria for RA^[12]. Registry members were selected for analysis if they began DMARD, prednisone, or TNFi therapy for the first time between the periods of March 2003 (the first date after which multiple TNFi agents were available within VA) and December 2014. Initial therapy with any of the following agents qualified a subject for inclusion: etanercept, adalimumab, infliximab, golimumab, certolizumab, methotrexate, leflunomide, hydroxychloroquine, sulfasalazine, or prednisone. Patients with or without established diagnoses of diabetes were included for analysis.

Data sources

In addition to VARA registry data, administrative VA databases were utilized in the analysis, including the Corporate Data Warehouse (CDW), National Pharmacy Extract, and Pharmacy Benefits Management (PBM) database^[13]. Descriptions of these databases in the context of this registry have been published elsewhere^[14].

Statistical analysis

The primary outcomes in this study were the differences in RBG and A1C between measurements prior to, and subsequent to, medication exposure. Pre-exposure RBG and A1C values were selected as the most proximal preceding value available before medication initiation within a 6 mo window. For RBG, post-exposure values were selected as a most proximal value post-initiation within a 2 wk to 6 mo window. For A1C, post-values were selected as the most proximal value post-initiation within a 2- to 12- mo window.

Paired *T*-tests were performed comparing pre- and post- values, with an alpha of 0.05 set as a significance threshold. Baseline demographic characteristics were obtained, as well as baseline characteristics for validated comorbidities^[15]: Age, sex, diabetes diagnosis, cancer diagnosis, chronic kidney disease, chronic lung disease, and congestive heart failure. Finally, multivariate linear regression analysis was performed, examining specific agent and medication class initiated, age, sex, and comorbidities as predictors for change in blood glucose and A1C at the time of medication initiation. Statistical analysis was performed using Stata v. 11.2 (College Station TX).

RESULTS

Baseline characteristics

A total of 2111 patients contributed 4,028 medication-initiation events with glucose measurements available for analysis. Individual patients could contribute more than one initiation event for analysis when applicable, but only one, first-start, event was included per medication-patient pairing. The mean age of examined patients was 63.9 years, and 90% were male, reflecting demographics of the Veterans Affairs health care system patient population at-large, which is disproportionately male and middle-aged. Large numbers were initiated on methotrexate, etanercept, sulfasalazine, leflunomide, hydroxychloroquine, adalimumab, and prednisone during follow-up. Initiation of certolizumab and golimumab were far less represented in these data.

Change in A1C and RBG

Paired *t*-tests (Table 1) revealed significant RBG decreases in 653 initiation-events of hydroxychloroquine (-3.68 mg/dL, $P = 0.04$), and higher RBG values following 665 prednisone-initiation events (+5.85 mg/dL, $P < 0.01$). A1c values were significantly lower following 49 sulfasalazine starts (-0.70%, $P < 0.01$).

A larger, unpaired analysis of all available pre- and post- initiation glucose values surrounding drug initiation events confirmed lower post-A1C values following sulfasalazine initiation and higher RBG values following prednisone. In addition, a trend towards lower RBG values following sulfasalazine initiation was seen (-2.73 mg/dL, $P = 0.09$). In addition, a trend was seen towards lower A1C values following initiation of etanercept (-0.30%, $P = 0.10$) and higher A1C values following certolizumab initiation (0.48%, $P = 0.09$).

Multivariate linear regression analyses

In multivariate analyses that accounted for comorbidities and demographic characteristics, we evaluated the effect of individual DMARDs and TNFi's on individual changes in A1C and RBG using methotrexate as the referent. In these analyses, hydroxychloroquine initiation predicted a decrease in RBG (Table 2) compared to methotrexate initiation when accounting for other variables (coefficient

Table 1 Changes in paired A1C and random blood glucose surrounding medication-initiation events

Medication	<i>n</i>	Difference (mg/dL or % glycosylation)	Confidence interval		<i>P</i> value
Random blood glucose					
Etanercept	311	-0.06	-4.91	4.79	0.98
Adalimumab	302	1.03	-3.41	5.46	0.65
Certolizumab	5	-5.20	-26.60	16.19	0.54
Golimumab	13	-17.61	-37.54	2.31	0.08
Infliximab	147	-1.90	-9.45	5.65	0.62
Methotrexate	831	2.08	-7.48	4.90	0.15
Leflunomide	290	-0.48	-6.84	5.88	0.88
Hydroxychloroquine	653	-3.68	-7.15	0.20	0.04
Sulfasalazine	335	-0.38	-4.92	4.17	0.87
Prednisone	665	5.85	2.20	9.51	< 0.01
Hemoglobin A1C					
Etanercept	35	-0.17	-0.50	0.17	0.33
Adalimumab	43	0.04	-0.34	0.42	0.83
Certolizumab	1	0.20	n/a	n/a	n/a
Golimumab	4	-0.65	-3.47	2.17	0.52
Infliximab	20	-0.36	-0.95	0.23	0.22
Methotrexate	107	-0.11	-0.35	0.14	0.40
Leflunomide	51	-0.02	-0.40	0.35	0.91
Hydroxychloroquine	98	-0.15	-0.40	0.11	0.25
Sulfasalazine	49	-0.70	-1.08	0.31	< 0.01
Prednisone	68	-0.01	-0.25	0.24	0.96

n/a: Insufficient numbers to calculate.

Table 2 Multivariate regression analysis of glucose changes surrounding medication-initiation events

Predictor variable	Hemoglobin A1C				Random blood glucose			
	Coefficient	Confidence interval		<i>P</i> value	Coefficient	Confidence interval		<i>P</i> value
Sex	-0.12	-0.60	0.36	0.63	1.75	-3.80	7.30	0.54
Age	0.00	-0.01	0.02	0.71	0.00	-0.16	0.15	0.98
Diabetes	0.10	-0.20	0.40	0.50	0.02	-3.33	3.36	0.99
Malignancy	-0.20	-0.50	0.09	0.17	0.54	-3.31	4.39	0.78
Coronary artery disease	0.07	-0.30	0.43	0.72	-1.48	-6.54	3.58	0.57
Congestive heart failure	-0.07	-0.37	0.22	0.62	4.69	0.45	8.92	0.03
Chronic lung disease	0.14	-0.11	0.38	0.27	-0.95	-4.16	2.27	0.56
Chronic kidney disease	-0.35	-0.78	0.07	0.11	-5.57	-12.67	1.52	0.12
Hypertension	0.01	-0.35	0.38	0.95	-0.81	-4.50	2.88	0.67
Medication (comparator is methotrexate)								
Adalimumab	0.17	-0.28	0.62	0.46	-0.87	-6.83	5.09	0.78
Golimumab	-0.56	-1.81	0.70	0.39	-19.88	-44.57	4.82	0.12
Certolizumab	0.41	-2.06	2.87	0.75	-7.02	-46.55	32.51	0.73
Infliximab	-0.30	-0.90	0.30	0.32	-3.85	-11.76	4.06	0.34
Etanercept	-0.08	-0.56	0.40	0.75	-2.07	-7.97	3.84	0.49
Leflunomide	0.05	-0.37	0.47	0.81	-2.66	-8.68	3.36	0.39
Hydroxychloroquine	-0.01	-0.36	0.33	0.94	-5.78	-10.38	-1.17	0.01
Sulfasalazine	-0.58	-1.00	-0.16	0.01	-2.56	-8.26	3.15	0.38
Prednisone	0.11	-0.26	0.49	0.55	3.76	-0.82	8.34	0.11
Medication class (comparator is conventional DMARDs)								
TNFi	0.06	-0.23	0.35	0.68	0.30	-3.47	4.07	0.88
Glucocorticoid	0.20	-0.13	0.53	0.23	6.32	2.40	10.24	< 0.01

DMARDs: Disease-modifying anti-rheumatic drugs.

= -5.77, CI = -10.4- -1.2, *P* = 0.01). Additionally, sulfasalazine initiation predicted decreased A1C values compared to methotrexate-initiators (β = -0.58, *P* = 0.01). Of the various comorbidities examined, only congestive heart failure (ever) was found to predict changes in RBG surrounding medication initiation (β = 4.57, *P* = 0.03). A separate regression analysis by

medication class rather than individual agent revealed corticosteroid (prednisone) use as a predictor for positive change in RBG as compared to DMARD initiators as the referent (β = 6.32, *P* < 0.01); medications as analyzed by class did not, however, predict A1C change, and CHF was the only demographic or comorbidity predictor for higher RBG.

DISCUSSION

Our data are unable to firmly demonstrate an effect on blood glucose for etanercept or other TNFi in a cohort of rheumatoid arthritis patients, although trends in these data are somewhat supportive of hypoglycemic effects previously suggested in case reports and series for etanercept, in particular. As these events are rare, risk factors and biologic processes underlying TNF-associated hypoglycemic events might be better clarified through the use of case-control studies comparing specific patients with these events with selected controls. In addition, a similar investigation of glycemic effects of both DMARD and TNFi in spondyloarthritis patients is warranted, as soon as adequate registry data are available in these diseases.

The detection of blood glucose increases following the initiation of prednisone—a well-established phenomenon and pharmacologic effect—lends internal validity to our study. Utilizing the described techniques, modest but statistically significant medication-initiation effects towards lower blood glucose also appear to be present for sulfasalazine and hydroxychloroquine.

The strong signal for lower A1C following sulfasalazine initiation (but not in RBG) may be consistent with the latency of action for some traditional DMARDs, which may also include their hypoglycemic effects. The lack of predictive value for change in glucose when these data are analyzed by drug class (other than glucocorticoids) also suggest that at least some of these effects are medication-specific. These may be separate from, or in addition to, global anti-inflammatory effects on glucose tolerance. These effects may combine with favorable effects for these agents on lipid profiles^[16] and to partially explain the previously described cardioprotective profile of hydroxychloroquine. The effects of sulfasalazine and hydroxychloroquine seen in our cohort may also support further investigation of these medications as therapeutic hypoglycemic agents.

It is notable that a diagnosis of diabetes did not strongly influence change in glucose values relative to DMARD medications by our regression analyses. This result suggests that hypoglycemic effects for hydroxychloroquine and sulfasalazine are possibly independent of an insulin-resistant state.

Limitations of our study include the wide variation in RBG values at any given time. In addition, we did not investigate effects of glucocorticoids besides prednisone, nor non-TNFi biologic agents, as we did not expect adequate data for analysis in this registry for these agents. Our results may be confounded by variations in body mass index and other unmeasured patient characteristics using these data sources. These include our failure to include disease activity measures, which may be only partially accounted for by our inclusion of prednisone as a surrogate for disease activity and severity. Finally, as with other VARA publications, our data are somewhat limited by the atypical demographics of the Veterans Affairs population (dis-

proportionately male) relative to the general population with RA, and it is theoretically possible that these results could be somewhat different in a younger, more female population.

Clinicians should be cognizant of the potential for rare hypoglycemic effects of the conventional DMARDs hydroxychloroquine and sulfasalazine, in addition to the well-known hyperglycemic effects of prednisone.

ARTICLE HIGHLIGHTS

Research background

Several case reports and series have described dramatic acute hypoglycemic effects for the immunosuppressive agents collectively called tumor necrosis factor inhibitors (TNFi's). In addition, studies have shown various cardioprotective and metabolic effects for conventional anti-rheumatic therapies; however, few studies have examined acute blood glucose effects for these agents in large population studies.

Research motivation

A better estimate of frequency and scale hypoglycemia associated with the initiation of medicines for users of TNFi and other anti-inflammatory medications may give insight into the role of cytokines and the inflammatory cascade in glucose tolerance as well as better estimate risks and benefits for these medications to the clinician.

Research objectives

We wished to determine whether initiation of tumor necrosis factor inhibitors in rheumatoid arthritis patients leads to reductions in blood glucose as measured by random blood glucose or glycosylated hemoglobin A1c. Simultaneously, we wished to investigate for similar effects in conventional antirheumatic drugs, given the established lipid homeostasis and cardioprotective effects for these agents.

Research methods

An observational registry linking pharmacy, clinical laboratory, and other data was utilized to retrospectively identify the time of prescription of the agents in question. This registry and linked data was used to retrospectively identify glucose measures proximate to these medication start events, so that changes in blood glucose surrounding the medication start could be inferred.

Research results

Cohort-level glucose effects were not identified in this registry surrounding the start of tumor-necrosis factor inhibitors, although glucose-lowering changes were identified surrounding the initiation of the conventional antirheumatic treatments sulfasalazine and hydroxychloroquine. Hyperglycemic changes surrounding prednisone were identified, lending further internal validity to these results.

Research conclusions

This study adds to the literature supporting the potentially beneficial metabolic effects of conventional anti-rheumatic therapies sulfasalazine and hydroxychloroquine beyond their general anti-inflammatory effects. These data also lend reassurance against large-scale prevalence for previously reported adverse hypoglycemic effects for tumor-necrosis factor inhibitors. The results point to the need for additional, similar studies in other populations, particularly those with spondyloarthritis syndromes such as psoriatic arthritis.

Research perspectives

This study, while a null result regarding tumor necrosis factor-inhibitor-associated hypoglycemic effects, points to the need for additional clarification on the physiology and causes for hypoglycemic events with these medications. We suggest the need for similar studies in psoriasis and psoriatic arthritis patients, as well as the potential utility of a case-control approach for the future study of dramatic hypoglycemic effects and events

with immunosuppressive medications.

REFERENCES

- 1 **Lumeng CN**. Innate immune activation in obesity. *Mol Aspects Med* 2013; **34**: 12-29 [PMID: 23068074 DOI: 10.1016/j.mam.2012.10.002]
- 2 **Farrokhi F**, Taylor HC, McBride NM. Etanercept-induced hypoglycemia and improved glycemic control in a patient with type 2 diabetes. *Endocr Pract* 2011; **17**: 306-307 [PMID: 21474422]
- 3 **Wambier CG**, Foss-Freitas MC, Paschoal RS, Tomazini MV, Simao JC, Foss MC, Foss NT. Severe hypoglycemia after initiation of anti-tumor necrosis factor therapy with etanercept in a patient with generalized pustular psoriasis and type 2 diabetes mellitus. *J Am Acad Dermatol* 2009; **60**: 883-885 [PMID: 19389539 DOI: 10.1016/j.jaad.2008.10.009]
- 4 **Cheung D**, Bryer-Ash M. Persistent hypoglycemia in a patient with diabetes taking etanercept for the treatment of psoriasis. *J Am Acad Dermatol* 2009; **60**: 1032-1036 [PMID: 19217693 DOI: 10.1016/j.jaad.2008.12.012]
- 5 **Pfeifer EC**, Saxon DR, anson RW. Etanercept-Induced Hypoglycemia in a Patient With Psoriatic Arthritis and Diabetes. *J Investig Med High Impact Case Rep* 2017; **5**: 1-4 [DOI: 10.1177/2324709617727760]
- 6 **Boulton JG**, Bourne JT. Unstable diabetes in a patient receiving anti-TNF-alpha for rheumatoid arthritis. *Rheumatology (Oxford)* 2007; **46**: 178-179 [PMID: 16998233 DOI: 10.1093/rheumatology/ kel322]
- 7 **Czajkowska JB**, Shutty B, Zito S. Development of low blood glucose readings in nine non-diabetic patients treated with tumor necrosis factor-alpha inhibitors: a case series. *J Med Case Rep* 2012; **6**: 5 [PMID: 22234148 DOI: 10.1186/1752-1947-6-5]
- 8 **Stanley TL**, Zanni MV, Johnsen S, Rasheed S, Makimura H, Lee H, Khor VK, Ahima RS, Grinspoon SK. TNF-alpha antagonism with etanercept decreases glucose and increases the proportion of high molecular weight adiponectin in obese subjects with features of the metabolic syndrome. *J Clin Endocrinol Metab* 2011; **96**: E146-E150 [PMID: 21047923 DOI: 10.1210/jc.2010-1170]
- 9 **Haas RM**, Li P, Chu JW. Glucose-lowering effects of sulfasalazine in type 2 diabetes. *Diabetes Care* 2005; **28**: 2238-2239 [PMID: 16123497 DOI: 10.2337/diacare.28.9.2238]
- 10 **Hage MP**, Al-Badri MR, Azar ST. A favorable effect of hydroxychloroquine on glucose and lipid metabolism beyond its anti-inflammatory role. *Ther Adv Endocrinol Metab* 2014; **5**: 77-85 [PMID: 25343023 DOI: 10.1177/2042018814547204]
- 11 **Bili A**, Sartorius JA, Kirchner HL, Morris SJ, Ledwich LJ, Antohe JL, Dancea S, Newman ED, Wasko MC. Hydroxychloroquine use and decreased risk of diabetes in rheumatoid arthritis patients. *J Clin Rheumatol* 2011; **17**: 115-120 [PMID: 21441823 DOI: 10.1097/RHU.0b013e318214b6b5]
- 12 **Arnett FC**, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31**: 315-324 [PMID: 3358796 DOI: 10.1002/art.1780310302]
- 13 **Smith MW**, Joseph GJ. Pharmacy data in the VA health care system. *Med Care Res Rev* 2003; **60**: 92S-123S [PMID: 15095548 DOI: 10.1177/1077558703256726]
- 14 **Cannon GW**, DuVall SL, Haroldsen CL, Caplan L, Curtis JR, Michaud K, Mikuls TR, Reimold A, Collier DH, Harrison DJ, Joseph GJ, Sauer BC. Persistence and dose escalation of tumor necrosis factor inhibitors in US veterans with rheumatoid arthritis. *J Rheumatol* 2014; **41**: 1935-1943 [PMID: 25128516 DOI: 10.3899/jrheum.140164]
- 15 **England BR**, Sayles H, Mikuls TR, Johnson DS, Michaud K. Validation of the rheumatic disease comorbidity index. *Arthritis Care Res (Hoboken)* 2015; **67**: 865-872 [PMID: 25186344 DOI: 10.1002/acr.22456]
- 16 **Kerr G**, Aujero M, Richards J, Sayles H, Davis L, Cannon G, Caplan L, Michaud K, Mikuls T. Associations of hydroxychloroquine use with lipid profiles in rheumatoid arthritis: pharmacologic implications. *Arthritis Care Res (Hoboken)* 2014; **66**: 1619-1626 [PMID: 24692402 DOI: 10.1002/acr.22341]

P- Reviewer: Gheita TAA, Sakkas LI **S- Editor:** Cui LJ
L- Editor: A **E- Editor:** Li RF





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 March 15; 9(3): 59-65



**ORIGINAL ARTICLE****Basic Study**

- 59 Patients with type 2 diabetes demonstrate proprioceptive deficit in the knee

Ettinger LR, Boucher A, Simonovich E

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Timothy R Koch, MD, Doctor, Professor, Department of Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, and Scopus.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Xiu-Xia Song*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Li-Jun Cui*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

Lu Qi, MD, PhD, Assistant Professor, Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, United States

Jingbo Zhao, PhD, Associate Professor, Aalborg Hospital Science and Innovation Centre, Aalborg

Hospital, Aarhus University Hospital, Aalborg 9000, Denmark

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com/1948-9358/editorialboard.htm>

EDITORIAL OFFICE
Xiu-Xia Song, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.fjopublishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.fjopublishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE
March 15, 2018

COPYRIGHT
© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.fjopublishing.com>

Basic Study

Patients with type 2 diabetes demonstrate proprioceptive deficit in the knee

Lucas Richard Ettinger, Ami Boucher, Elisabeth Simonovich

Lucas Richard Ettinger, Ami Boucher, Elisabeth Simonovich,
Department of Exercise Science, Willamette University, Salem,
OR 97302, United States

ORCID number: Lucas Richard Ettinger (0000-0002-4770-2128);
Ami Boucher (0000-0003-2556-3654); Elisabeth Simonovich
(0000-0001-9259-9388).

Author contributions: Boucher A and Simonovich E performed the majority of experiments and were also involved in editing the manuscript; all authors were involved with data analysis and interpretation; Ettinger LR designed the study and wrote the manuscript.

Supported by The Willamette University Mary Stuart Rogers Science Collaborative Research Program Endowment.

Institutional review board statement: This study was reviewed and approved by the Willamette University Institutional Review Board.

Informed consent statement: Written and verbal consent to enrollment in the study was obtained by all study participants prior to all other aspects of this study.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Lucas Richard Ettinger, PhD, Professor, Department of Exercise Science, Willamette University, 900 State

Street, Salem, OR 97302, United States. lettinge@willamette.edu
Telephone: +1-503-3706240
Fax: +1-503-3706773

Received: January 25, 2018
Peer-review started: January 26, 2018
First decision: February 27, 2018
Revised: March 5, 2018
Accepted: March 14, 2018
Article in press: March 14, 2018
Published online: March 15, 2018

Abstract

AIM

To investigate proprioceptive discrepancies in the lower extremity in persons with type 2 diabetes mellitus (T2DM).

METHODS

In this cross-sectional study, a total of 46 older persons were divided into a T2DM group ($n = 23$) and a control group who did not have T2DM ($n = 23$). Participants were given a brief warm up with stretching exercises. Diabetic neuropathy scores were collected prior to proprioceptive testing. For proprioceptive testing, participants performed leg extensions to randomized target positions of 15°, 30°, 45, 60° degrees of elevation in the sagittal plane, each target was repeated a total of four times. Subjects were guided to target positions in the absence of visual feedback *via* auditory cues from a custom JPS application. When the participant entered the target position, they memorized the location of their limb in space and subsequently attempted to re-locate this position in space. Proprioceptive errors were measured from the target positioned, target remembered, target repositioned protocol.

RESULTS

Proprioceptive accuracy was lower in the diabetic group

at all levels of target angle than the control group ($P < 0.05$). The diabetic group had 46% greater inaccuracy than the control group at all levels of target position. Diabetics also reported greater neuropathy scores than controls in the past 12 mo $P < 0.01$.

CONCLUSION

Deficits in lower limb localization and greater diabetic neuropathy scores were identified in this study. Our findings may be associated with deafferentation as peripheral neuropathy is a common complication with the disease. These findings may help to explain the declining balance function in the older persons with T2DM which is also commonly reported.

Key words: Proprioception; Diabetic neuropathy; Joint position sense; Knee position in space; Deafferentation in diabetic limb

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Diabetic peripheral neuropathy is often associated with small diameter afferent nerve damage. Here, we demonstrate participants with type-2 diabetes performing proprioceptive tasks as measured by a joint position sense activity of the lower extremity, result in greater errors with limb localization than matched controls. Findings from this study indicate that both large and small diameter afferent nerves are likely involved in diabetic neuropathy. These findings warrant future studies involving joint position sense as a measurement tool for disease progression and treatment.

Ettinger LR, Boucher A, Simonovich E. Patients with type 2 diabetes demonstrate proprioceptive deficit in the knee. *World J Diabetes* 2018; 9(3): 59-65 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i3/59.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i3.59>

INTRODUCTION

According to recent data provided by the International Diabetes Federation and the World Health Organization, type 2 diabetes mellitus (T2DM) is one of the largest, expensive and most pervasive diseases with an estimated prevalence of approximately 350-400 million cases worldwide^[1,2]. The epidemiologic evidence with respect to diabetic peripheral neuropathy (DPN) has not been as well established in the literature, where reports estimate the prevalence to be near 15%-30% but as high as 50%^[3,4]. Regardless, DPN is the most frequent condition, secondary to diabetes mellitus^[4]. DPN is characterized by various levels of nerve damage and has been linked to patient economic burdens, quality of life and productivity^[5]. Predominantly, DPN is

attributed to loss of nociceptive feedback from the lower extremities due to small fiber degeneration^[6]. However, from a recent review, discrepancies in the literature are reported with respect to the extent of pain perception threshold^[4]. These discrepancies raise questions as to the range of tissue disruption, where previously DPN was thought to include superficial anatomy such as the skin, but recent data suggests deeper structures, including muscle may be affected^[4].

Recent evidence suggests T2DM patients have greater risk of falls requiring a visit to the ER^[7]. Furthermore, T2DM generally exhibit worse balance during activity than non-diabetics, which may account for the high frequency of ER visits due to falls^[8]. Patients with DPN have worse balance during quiet stance, and are more off balance during perturbations than non-diabetics. Further, balance during quiet stance is exacerbated when the eyes are closed^[9]. These patients also demonstrate reduced toe-obstacle clearance during gait, which may additionally account for the high degree of falls^[10]. These data suggest that patients with T2DM may experience proprioceptive disruption with respect to the general population as proprioception plays a major role in the stabilizing body equilibrium^[11]. In a prospective cohort study, the number of falls was measured over a calendar year. Both ankle and hip strength and proprioceptive thresholds were successfully used as predictor variables to determine the number of falls in this population^[12]. DPN has historically been characterized as a superficial loss of nociception, which involves small diameter afferent nerve fibers (A-delta and C-fibers)^[4]. However, outside of DPN, factors such as age have been found to disrupt larger diameter afferents such as A-alpha proprioceptive nerve fibers which are found in the deeper tissues such as muscle^[13]. To the author's knowledge, no studies have investigated the effect of DPN on large diameter afferent nerve fibers.

Knee proprioception has been commonly measured using joint position sense tasks for a number of populations of patients including Anterior Cruciate Ligament injury, knee arthroplasty and osteoarthritis, to name a few^[14,15]. However, no recent studies have examined joint position sense of the lower extremity for patients with T2DM or for DPN. Recently, studies examining joint position sense tasks in the upper and lower extremity have found linear improvements in proprioception to increasing elevation angle of the target position^[16-20]. However, we recently demonstrated that with disease, the linear behavior between joint position sense and target angle of elevation is disrupted in the upper extremity independently of pain^[21]. It is unknown whether or not patients with T2DM will demonstrate proprioceptive imbalances when compared to healthy controls during a joint position sense test. Furthermore, it is unknown if T2DM will respond normally with respect to target angle of elevation as compared to matched controls. It is the goal of the study to measure knee

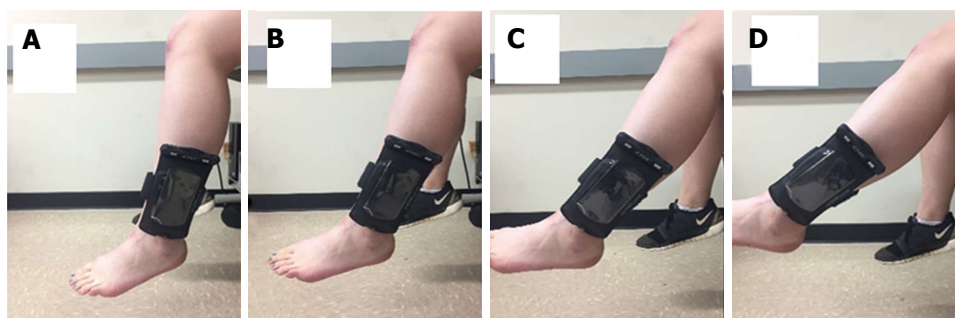


Figure 1 iPod orientation and experimental setup. Target angles A: 15°; B: 30°; C: 45°; D: 60° of leg elevation in the sagittal plane.

proprioception as measured by joint position sense in this population. We aim to address the magnitude of the differences between T2DM and healthy matched controls. We hypothesize that patients with T2DM will have a greater magnitude of proprioceptive errors than healthy controls. Our secondary hypothesis predicts that patients with T2DM will not have linear improvements to proprioceptive acuity by target angle of elevation.

MATERIALS AND METHODS

Subjects and demographics

Twenty-three healthy adults (16 females, 7 males) with a mean age of 65 ± 8 years and twenty-three individuals with T2DM (16 females, 7 males) with a mean age of 63 ± 10 years participated in this study. Diabetics had an average of 11.3 years of exposure to diabetic symptoms. Prior to testing, all subjects signed an informed consent form approved by the Institutional Review Board of Willamette University. Only two participants in both diabetic and control group were left leg dominant, and dominance was determined by the leg used to kick a ball. Exclusion criteria included a history of injury to the hip, knee or ankle; patients with osteoarthritis were also excluded from the study. For scoring peripheral neuropathy within the diabetic group, the diabetic neuropathy symptom score (DNS) was used. The DNS included an assessment of pain, numbness, tingling and ataxia. The maximum DNS score was four points, where one point or more indicates neurological abnormalities^[22]. The DNS scores were compared between diabetics and healthy controls using an independent samples *t*-test. Diabetics had significantly higher DNS than controls ($P < 0.01$) where the average DNS was 1.8 for diabetics and, was 0.2 for controls.

Instrumentation

Joint position sense was measured with an iPod touch (Apple®), using a custom made JPS application^[23]. The JPS application utilizes data from the tri-axial accelerometers which estimate humeral elevation angle using methods previously validated^[23,24]. For calibration of the iPod, the longitudinal axis of the device was aligned to the long axis of the fibula with the bottom of

the device mounted to the leg at the level of the lateral malleolus. The participant sat on an adjustable chair (Biodex) so that the height of the participant could be modified so that the foot lost contact with the ground (Figure 1). With the leg relaxed and hanging so that the long axis of the leg was aligned to the vector of gravity in a position referred to as zero-G, which coincides with the absolute angular reference position^[23]. Throughout the protocol, motion was constrained to the knee joint. All data were collected at 40 Hz. An external Bluetooth speaker was used to minimize external noise distraction as well as to administer targeting cues. The participant was instructed to keep their eyes closed throughout the entire joint testing protocol.

Protocol

All testing was performed in a single session. Participants completed a standardized warmup on their dominant leg, which consisted of 5 min of treadmill walking at a self-selected pace, and several hamstrings and quadriceps stretching exercises. The iPod was secured to the leg at the level of the lateral malleolus using Velcro straps and a flexible iPod case (Overboard™). Joint position data were collected at four absolute angular target positions 15°, 30°, 45, 60° with respect to the axis of gravity (Figure 1). Target angles were repeated on the ipsilateral side four times and were presented in a randomized order. All participants completed practice trials using a dummy target position of 25 degrees of elevation until competency in the protocol was demonstrated.

Subjects were guided to target positions (15°, 30°, 45, 60° degrees of elevation in the sagittal plane) *via* auditory feedback from the JPS application^[23]. A low frequency tone was heard through the speaker, indicating to the subject to elevate their leg in the sagittal plane with their ankle dorsiflexed. When the tone stopped, this indicated to the subject that they were in the “target range” ($\pm 1^\circ$ boundary with respect to the target) and should hold their leg in that position. Once in the target position, the subject was instructed to focus on the position of their leg in space for 3 s until an automated voice instructed them to go to the relaxed position. After 2 s in the relaxed position, the same automated voice instructed the subject to “find target”, upon which the subject tried to find the

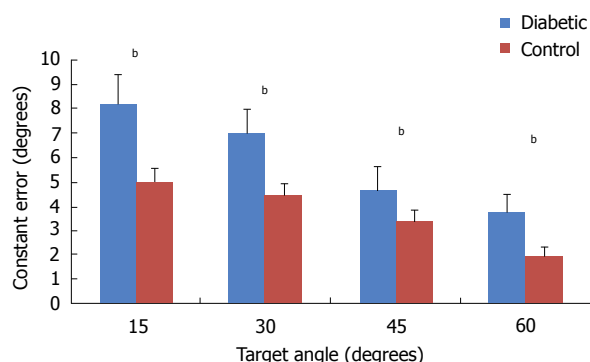


Figure 2 Joint sense constant errors for diabetic (blue) and control (red) participants by target angle of elevation at 15, 30, 45 and 60 degrees of elevation. ^bDenote significant differences.

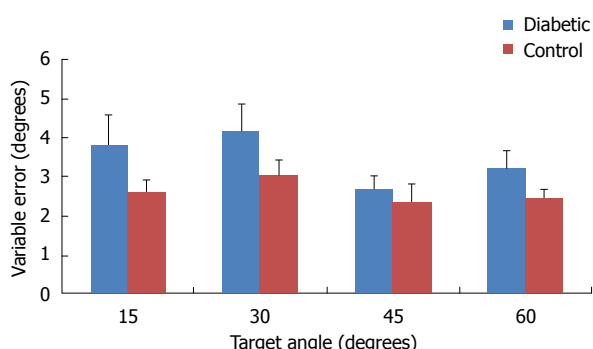


Figure 3 Joint sense variable errors for diabetic (blue) and control (red) participants by target angle of elevation at 15, 30, 45 and 60 degrees of elevation.

previous target position without any auditory or visual feedback. The subject was then instructed to go back to the relaxed position until the next trial began. Figure 1 demonstrates the experimental setup and depicts the target angles described above.

Data reduction and error score calculations

All JPS data were downloaded from the iPod using iTunes software. Three-dimensional accelerometer data were converted into knee angular data in a custom Labview program using the second integral function and Pythagorean theorem, which has been validated and described in the literature for accelerometers when estimating humeral elevation angles^[24]. Data from each condition were averaged by target angle and represent the constant error. Constant error represents the angular accuracy and directional bias during the angle matching task^[25]. Further, the deviation of repositioned data with respect to positioned data were quantified and reported as the variable error, which represents the precision.

Statistical analysis

Proprioceptive differences between our two populations of participants (type II diabetics and healthy controls) were analyzed using a two-way Mixed effects ANOVA (Table 1), where elevation angle (15, 30, 45, 60

Table 1 Two-way ANOVA table for the effects of group and target angle on proprioceptive constant errors

ANOVA factor	df	F-ratio	P-value
Group × target angle	3	0.527	0.664
Group	1	5.735	0.021 ^b
Target	3	7.342	0.001 ^b

^bDenote significant findings.

Table 2 Two-way ANOVA table for the effects of group and target angle on proprioceptive variable errors

ANOVA factor	df	F-ratio	P-value
Group × target angle	3	0.327	0.806
Group	1	2.61	0.114
Target	3	0.825	0.467

degrees) was the within subject-measure and groups (type II diabetics and controls) was the between-subjects measure. Two separate ANOVA analyses were used to investigate constant error (accuracy) and variable error (precision), further information pertaining to these results are reported in Tables 1 and 2 respectively. All statistics were computed using SYSTAT version 13, from Systat Software, Inc., San Jose California, United States.

RESULTS

Constant error

For constant errors, results of our two-way ANOVA analysis (Table 1) do not indicate a significant interaction between group and target angle ($P = 0.66$), however, for main effects we found significant differences between groups ($P = 0.021$) and significant differences between levels of target angle were also observed ($P = 0.001$). On average the diabetic group had 2° greater constant errors for all levels of target angle. Both diabetic and controls groups demonstrated a linear reduction in error by target angle (Figure 2).

Variable error

For variable errors, results of our two-way ANOVA analysis (Table 2) do not indicate a significant interaction between group and target angle ($P = 0.806$). No significant main effects of group were detected ($P = 0.114$) and no significant differences were found by target angle ($P = 0.467$) (Figure 3).

DISCUSSION

We had two hypotheses in the current study. First, we hypothesized that diabetics with DPN would have worse accuracy and precision than matched controls on joint position sense matching tests with respect to proprioceptive constant errors and variable errors

respectively. Second, we hypothesized that diabetics would not be sensitive to linear position changes with respect to target angle of elevation as would be expected in healthy controls. For each of our hypothesis and corresponding results will be discussed below.

Our data suggest that our first hypothesis was supported for accuracy but not for precision (Figures 2 and 3). Our results indicated that the magnitude of the constant errors were significantly greater in the diabetic group than the control group by 46% (Figure 2) which represented an average of 1.6 degree angular difference in accuracy at each level of target position. The greater inaccuracy in the diabetic group could be the result of diminished A-alpha nerve fibers which contain the primary receptors for proprioceptive acuity; however, previous reports indicate that disruption of smaller afferent nerve fibers such as A-delta and C-fibers are most common with DPN^[4,26]. A secondary explanation for the disruption in proprioceptive accuracy could be related to the loss of nociception in some areas, but the heightened nociceptive response in non-nociceptive receptors through a process known as allodynia^[27]. We previously reported that patients with pain syndromes have large proprioceptive deficits in both accuracy and precision of similar magnitudes that are consistent with the present study. However, it remains unclear if pain disrupts proprioceptive information or can be used as additional sensory input to improve limb in space awareness^[21]. Further, our previous findings demonstrate that when patients receive a local nerve block, accuracy but not precision is affected^[21]. Questionnaire data from the present study indicate that 60% of the diabetic participants reported some discomfort in the lower extremity during the proprioceptive task; however, we did not separate participants into groups based on pain. Due to the similarities between our previous study results and the present study, it is possible that our results are confounded by pain associated with diabetic symptoms^[21]. Future studies could examine proprioceptive acuity in DPN with and without symptomatic pain to help resolve this question.

With respect to our second hypothesis, diabetics appeared to have reduced angular errors by target angle, which indicates that as the limb approached higher elevation angles, proprioceptive awareness was augmented and thus joint errors were reduced (Figure 1 and Table 1). Joint proprioceptive studies of healthy individuals have indicated a strong linear trend in proprioceptive acuity by target angle of elevation of limbs in the sagittal plane^[16-21,28]. Our data indicate that diabetics behave normally with respect to angle of elevation; however, the overall magnitudes of the errors are greater, with an average error of 1.6 degrees greater error at all angles of elevation. The mechanisms responsible for enhanced proprioceptive awareness at greater elevation errors are somewhat unknown; however, it is possible that receptors in the muscle and

joints are most active in these positions^[18,29]. Thus it is possible that the mechanoreceptors that are responsible for sensation of degree of elevation are unaffected in diabetic populations, whilst the mechanoreceptors that are responsible for accuracy and precision are affected. Together these findings help to explain heightened instability and fall risk in this population. Furthermore, the larger errors we observed at 15-30 degree target angles could help to explain why patients with T2DM demonstrate poorer obstacle clearance during gait as obstacle clearance is likely to occur within a similar degree of knee angles to our target angle^[9,10].

DPN is well documented with respect to afferent nerves in distal limbs^[3,6,8,12,22,30,31]. However, the extent of the peripheral disruption has previously been limited to specific aspects of the afferent nerve which predominantly involves smaller nociceptive A-delta and C-fibers^[4]. Here, we present data which suggests that other afferent nerve fibers are likely affected by DPN as larger A-alpha nerve fibers carry the principal sensory component of proprioception to the CNS. Neurovascular ischemia has long been attributed to be the culprit of DPN and was recently shown to be greater throughout various levels of DPN as measured by ischemia-modified albumin biomarkers^[32]. In studies examining physiologic responses during laboratory induced ischemia; afferent nerves are more commonly affected than efferent nerves due to the greater size and oxidative demands of the afferent nerve^[33-36]. With the ischemic nerve block, proprioception is one sensory system shown to be disrupted^[33]. Therefore, it is likely that proprioception is also disrupted with the loss of the afferent nerve in cases of DPN. It is further possible that joint position sense could be used as a test for the symptoms of DPN. Future studies could examine the extent of which joint position sense is disrupted in diabetic populations. Examining JPS errors by severity of DPN symptoms could help clinicians and researchers diagnose DPN, and could be used as a measurement tool for effectiveness of treatment.

We acknowledge that proprioception as measured by a joint position sense task does involve a memory component (remembering where your arm was moments ago), further our findings could be influenced by alterations of the central nervous system and small sample size.

ARTICLE HIGHLIGHTS

Research background

Type 2 diabetics are at greater risk for falls and balance disruption than the general population. This phenomenon may be explained by the interruption of afferent nerves associated with peripheral neuropathy, which is common within this population. The link between afferent nerve disturbance and falls may involve diminished proprioceptive awareness in the distal limb.

Research motivation

The main objective of this study was to investigate limb localization and

proprioceptive acuity in the distal limb of persons with type 2 diabetes.

Research objectives

Identification of proprioceptive disturbance in the diabetic population informs clinicians towards alternative forms of diabetic instrumentation for disease progression, identification and efficacy of treatment.

Research methods

We collected knee proprioceptive acuity using a joint position sense (JPS) task. We collected data on 23 diabetics and 23 age and gender matched controls. Instrumentation of JPS was conducted using an Apple iPod touch and a custom JPS application. We used a target position matching task which required the participant to locate specified targets in space with their knee joint.

Research results

Results of the present study support our hypothesis and indicate that type-2 diabetics have proprioceptive errors of 46% greater magnitude than controls. Our findings suggest that fall and balance risk data on type-2 diabetics could be related to proprioceptive imbalances in the distal limb.

Research conclusions

Our novel findings indicate that proprioceptive acuity in the distal limb is disrupted in patients with type-2 diabetes. These disturbances to proprioceptive acuity may be due to degeneration of the afferent nerves commonly reported in this population. Our findings are the first to suggest that larger diameter afferent nerves are likely also influenced by the degeneration of the afferent nerve, which was previously reported as affecting only smaller diameter afferent nerves. We present data that may serve as explanation or partial explanation for the high degree of falls and loss of balance in the type-2 diabetic population as proprioception has been overwhelmingly associated with balance and stability. Furthermore, our study has provided new insights as to measurement and instrumentation of diabetic neuropathy with respect to joint position sense (JPS) testing. Future studies can incorporate JPS into measurement of disease progression, treatment and diagnosis. The application of JPS testing in a clinical setting is also warranted.

Research perspectives

We have demonstrated that proprioceptive disruption can be measured in type-2 diabetics. However, the extent of disruption could be dependent on the degree of peripheral neuropathy, number of years of exposure to the disease and or other physiologic factors. Future studies should investigate diabetic neuropathy as a spectrum with respect to JPS testing in order to establish a causal relationship. Furthermore, future studies could start to examine therapeutic effect of exercise, diet, whole body vibration and pharmacological intervention on proprioceptive acuity in type-2 diabetics.

ACKNOWLEDGEMENTS

Funding for this project was partially provided by the Murdock Charitable Trust.

REFERENCES

- Centers for Disease Control and Prevention. Crude and Age-Adjusted Percentage of Civilian, Noninstitutionalized Adults with Diagnosed Diabetes, United States, 1980-2011 [updated April 12, 2013; cited 2013 November 1]. Available from: <http://www.cdc.gov/diabetes/statistics/prev/national/figageadult.htm>
- Ogurtsova K, Fernandes JD, Huang Y. IDF Diabetes Atlas: global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pr* 2017; **128**: 40-50 [DOI: 10.1016/j.diabetes.2017.03.024]
- Sugimoto K, Murakawa Y, Sima AA. Diabetic neuropathy--a continuing enigma. *Diabetes Metab Res Rev* 2000; **16**: 408-433 [PMID: 11114101 DOI: 10.1002/1520-7560(200011/12)16:6<3.0.CO;2-R]
- Chantelau EA. Nociception at the diabetic foot, an uncharted territory. *World J Diabetes* 2015; **6**: 391-402 [PMID: 25897350 DOI: 10.4239/wjd.v6.i3.391]
- Stewart WF, Ricci JA, Chee E, Hirsch AG, Brandenburg NA. Lost productive time and costs due to diabetes and diabetic neuropathic pain in the US workforce. *J Occup Environ Med* 2007; **49**: 672-679 [PMID: 17563611 DOI: 10.1097/JOM.0b013e318065b83a]
- Malik RA. Which test for diagnosing early human diabetic neuropathy? *Diabetes* 2014; **63**: 2206-2208 [PMID: 24962918 DOI: 10.2337/db14-0492]
- Valent F. Falls requiring visit to emergency room in a population-based cohort of diabetic patients in Italy. *J Inj Violence Res* 2017; **9**: 83-90 [PMID: 28854160 DOI: 10.5249/jivr.v9i2.859]
- Brown SJ, Handsaker JC, Bowling FL, Boulton AJ, Reeves ND. Diabetic peripheral neuropathy compromises balance during daily activities. *Diabetes Care* 2015; **38**: 1116-1122 [PMID: 25765355 DOI: 10.2337/dc14-1982]
- Ledin T, Odkvist LM, Vrethem M, Möller C. Dynamic posturography in assessment of polyneuropathic disease. *J Vestib Res* 1990; **1**: 123-128 [PMID: 1670145]
- Liu MW, Hsu WC, Lu TW, Chen HL, Liu HC. Patients with type II diabetes mellitus display reduced toe-obstacle clearance with altered gait patterns during obstacle-crossing. *Gait Posture* 2010; **31**: 93-99 [PMID: 19875290 DOI: 10.1016/j.gaitpost.2009.09.005]
- Fitzpatrick R, McCloskey DI. Proprioceptive, visual and vestibular thresholds for the perception of sway during standing in humans. *J Physiol* 1994; **478** (Pt 1): 173-186 [PMID: 7965833 DOI: 10.1113/jphysiol.1994.sp020240]
- Richardson JK, Demott T, Allet L, Kim H, Ashton-Miller JA. Hip strength: ankle proprioceptive threshold ratio predicts falls and injury in diabetic neuropathy. *Muscle Nerve* 2014; **50**: 437-442 [PMID: 24282041 DOI: 10.1002/mus.24134]
- Lautenbacher S, Kunz M, Strate P, Nielsen J, Arendt-Nielsen L. Age effects on pain thresholds, temporal summation and spatial summation of heat and pressure pain. *Pain* 2005; **115**: 410-418 [PMID: 15876494 DOI: 10.1016/j.pain.2005.03.025]
- Romero-Franco N, Montaña-Munuera JA, Fernández-Domínguez JC, Jiménez-Reyes P. Validity and Reliability of a Digital Inclinator to Assess Knee Joint Position Sense in an Open Kinetic Chain. *J Sport Rehabil* 2017; **18**: 1-22 [PMID: 29252090 DOI: 10.1123/jsr.2017-0221]
- Baert IAC, Lluch E, Struyf T, Peeters G, Van Oosterwijck S, Tuynman J, Rufai S, Struyf F. Inter- and intrarater reliability of two proprioception tests using clinical applicable measurement tools in subjects with and without knee osteoarthritis. *Musculoskelet Sci Pract* 2017; pii: S2468-7812(17)30178-9 [PMID: 29174222 DOI: 10.1016/j.msksp.2017.11.011]
- King J, Harding E, Karduna A. The shoulder and elbow joints and right and left sides demonstrate similar joint position sense. *J Mot Behav* 2013; **45**: 479-486 [PMID: 24079516 DOI: 10.1080/00222895.2013.832136]
- King J, Karduna A. Joint position sense during a reaching task improves at targets located closer to the head but is unaffected by instruction. *Exp Brain Res* 2014; **232**: 865-874 [PMID: 24352607 DOI: 10.1007/s00221-013-3799-3]
- Suprak DN, Osternig LR, van Donkelaar P, Karduna AR. Shoulder joint position sense improves with elevation angle in a novel, unconstrained task. *J Orthop Res* 2006; **24**: 559-568 [PMID: 16463364 DOI: 10.1002/jor.20095]
- Suprak DN, Osternig LR, van Donkelaar P, Karduna AR. Shoulder joint position sense improves with external load. *J Mot Behav* 2007; **39**: 517-525 [PMID: 18055357 DOI: 10.3200/JMBR.39.6.517-525]
- Suprak DN, Sahlberg JD, Chalmers GR, Cunningham W. Shoulder elevation affects joint position sense and muscle activation differently in upright and supine body orientations. *Hum Mov Sci* 2016; **46**: 148-158 [PMID: 26784707 DOI: 10.1016/j.humov.2016.01.008]
- Ettinger LR, Shapiro M, Karduna A. Subacromial Anesthetics

- Increase Proprioceptive Deficit in the Shoulder and Elbow in Patients With Subacromial Impingement Syndrome. *Clin Med Insights Arthritis Musculoskelet Disord* 2017; **10**: 1179544117713196 [PMID: 28638243 DOI: 10.1177/1179544117713196]
- 22 **Meijer JW**, Smit AJ, Sonderen EV, Groothoff JW, Eisma WH, Links TP. Symptom scoring systems to diagnose distal polyneuropathy in diabetes: the Diabetic Neuropathy Symptom score. *Diabet Med* 2002; **19**: 962-965 [PMID: 12421436 DOI: 10.1046/j.1464-5491.2002.00819.x]
 - 23 **S Edwards E**, Lin YL, H King J, R Karduna A. Joint position sense - There's an app for that. *J Biomech* 2016; **49**: 3529-3533 [PMID: 27567568 DOI: 10.1016/j.jbiomech.2016.07.033]
 - 24 **Amasay TZK**, Kincl L, Hess J, Karduna A. Validation of tri-axial accelerometer for the calculation of elevation angles. *INT J IND ERGONOM* 2009; **39**: 783-789 [DOI: 10.1016/j.ergon.2009.03.005]
 - 25 **Schmidt T**. Motor Control and Learning. Third Edition ed: Human Kinetics; 1999
 - 26 **Provitera V**, Nolano M, Pagano A, Caporaso G, Stancanelli A, Santoro L. Myelinated nerve endings in human skin. *Muscle Nerve* 2007; **35**: 767-775 [PMID: 17405136 DOI: 10.1002/mus.20771]
 - 27 **Schaible HG**, Richter F. Pathophysiology of pain. *Langenbecks Arch Surg* 2004; **389**: 237-243 [PMID: 15034717 DOI: 10.1007/s00423-004-0468-9]
 - 28 **Chapman J**, Suprak DN, Karduna AR. Unconstrained shoulder joint position sense does not change with body orientation. *J Orthop Res* 2009; **27**: 885-890 [PMID: 19072986 DOI: 10.1002/jor.20813]
 - 29 **Darling WG**, Miller GF. Perception of arm orientation in three-dimensional space. *Exp Brain Res* 1995; **102**: 495-502 [PMID: 7737395 DOI: 10.1007/BF00230653]
 - 30 **Jambart S**, Ammache Z, Haddad F, Younes A, Hassoun A, Abdalla K, Selwan CA, Sunna N, Wajsbrot D, Youseif E. Prevalence of painful diabetic peripheral neuropathy among patients with diabetes mellitus in the Middle East region. *J Int Med Res* 2011; **39**: 366-377 [PMID: 21672340 DOI: 10.1177/147323001103900204]
 - 31 **Sadosky A**, McDermott AM, Brandenburg NA, Strauss M. A review of the epidemiology of painful diabetic peripheral neuropathy, postherpetic neuralgia, and less commonly studied neuropathic pain conditions. *Pain Pract* 2008; **8**: 45-56 [PMID: 18211592 DOI: 10.1111/j.1533-2500.2007.00164.x]
 - 32 **Gulpamuk B**, Tekin K, Sonmez K, Inanc M, Neselioglu S, Erel O, Yilmazbas P. The significance of thiol/disulfide homeostasis and ischemia-modified albumin levels to assess the oxidative stress in patients with different stages of diabetes mellitus. *Scand J Clin Lab Invest* 2018; **78**: 136-142 [PMID: 29298534 DOI: 10.1080/00365513.2017.1422540]
 - 33 **Christensen MS**, Lundbye-Jensen J, Geertsen SS, Petersen TH, Paulson OB, Nielsen JB. Premotor cortex modulates somatosensory cortex during voluntary movements without proprioceptive feedback. *Nat Neurosci* 2007; **10**: 417-419 [PMID: 17369825 DOI: 10.1038/nn1873]
 - 34 **Glencross DJ**, Oldfield SR. The use of ischemic nerve block procedures in the investigation of the sensory control of movements. *Biol Psychol* 1975; **2**: 227-236 [PMID: 1139020 DOI: 10.1016/0301-0511(75)90022-8]
 - 35 **Sinclair DC**. Observations on sensory paralysis produced by compression of a human limbw. *J Neurophysiol* 1948; **11**: 75-92 [PMID: 18905649 DOI: 10.1152/jn.1948.11.2.75]
 - 36 **Ziemann U**, Corwell B, Cohen LG. Modulation of plasticity in human motor cortex after forearm ischemic nerve block. *J Neurosci* 1998; **18**: 1115-1123 [PMID: 9437031 DOI: 10.1093/brain/124.6.1171]

P- Reviewer: Fourtounas C, Kin T, Raghow R, Tarantino G, Zhao JB

S- Editor: Cui LJ **L- Editor:** A **E- Editor:** Song XX





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 April 15; 9(4): 66-71



**MINIREVIEWS**

- 66 Correlation between metabolic syndrome and sleep apnea

Castaneda A, Jauregui-Maldonado E, Ratnani I, Varon J, Surani S

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Shannon Miller, Pharm D, Doctor, Pharmacotherapy Faculty, Florida Hospital East, Family Practice Residency, Orlando, FL 32822, United States

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, and Scopus.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Wen-Wen Tan*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Li-Jun Cui*
Proofing Editorial Office Director: *Ya-Juan Ma*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

Lu Qi, MD, PhD, Assistant Professor, Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, United States

Jingbo Zhao, PhD, Associate Professor, Aalborg Hospital Science and Innovation Centre, Aalborg

Hospital, Aarhus University Hospital, Aalborg 9000, Denmark

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com/1948-9358/editorialboard.htm>

EDITORIAL OFFICE
Ya-Juan Ma, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f0publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.f0publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE
April 15, 2018

COPYRIGHT
© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.f0publishing.com>

Correlation between metabolic syndrome and sleep apnea

Alejandro Castaneda, Edgar Jauregui-Maldonado, Iqbal Ratnani, Joseph Varon, Salim Surani

Alejandro Castaneda, Edgar Jauregui-Maldonado, Dorrington Medical Associates, Houston, TX 77030, United States

Iqbal Ratnani, DeBakey Heart and Vascular Center, Houston Methodist, Houston, TX 77030, United States

Joseph Varon, Department of Acute and Continuing Care. The University of Texas Health Science Center Houston, TX 77030, United States

Salim Surani, Division of Pulmonary, Critical Care and Sleep Medicine, Texas A and M University, Health Science Center, Corpus Christi, TX 78404, United States

ORCID number: Alejandro Castaneda (0000-0001-9846-947X); Edgar Jauregui-Maldonado (0000-0001-6345-7292); Iqbal Ratnani (0000-0002-1168-3041); Joseph Varon (0000-0002-7622-9974); Salim Surani (0000-0001-7106-4266).

Author contributions: Castaneda A and Jauregui-Maldonado E were involved in the preparation of the manuscript; Ratnani I, Varon J and Surani S were involved in concept generation, preparation and review of the article.

Conflict-of-interest statement: There is no declared competing conflict of interest between authors.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Salim Surani, MD, MPH, FAASM, Adjunct Professor, Division of Pulmonary, Critical Care and Sleep Medicine, Texas A and M University, Health Science Center, 701 Ayers Street, Corpus Christi, TX 78404, United States. srsurani@hotmail.com
Telephone: +1-361-8857722
Fax: +1-361-8507563

Received: March 19, 2018

Peer-review started: March 20, 2018

First decision: April 2, 2018

Revised: April 9, 2018

Accepted: April 12, 2018

Article in press: April 12, 2018

Published online: April 15, 2018

Abstract

The so-called "metabolic syndrome" (MS), constitutes a cluster of metabolic and cardiovascular abnormalities, including fasting glucose, blood pressure, triglycerides, high density lipoprotein cholesterol (HDL-C), and waist circumference that arise from insulin resistance. Obstructive sleep apnea (OSA) syndrome is characterized by recurrent episodes of partial or complete obstruction of the upper airway, involving cessation or significant decreased airflow, with intermittent hypoxemia, frequent arousals from sleep and recurrent oxyhemoglobin desaturations that interfere with normal sleep patterns generating difficulty falling asleep, unrefreshing sleep and loud snoring. The relation between these two entities is known as "Syndrome Z", and there is no question about the impact of these risk factors on health and disease. This clinical condition presents a growing epidemic Worldwide, affecting approximately 60% of the general population with both MS and OSA due to the constant increase of body mass index in humans. This article presents evidence-based data that focuses on the direct relationship between MS and OSA.

Key words: Obstructive sleep apnea; Metabolic syndrome; Sleep apnea; Obesity; Syndrome Z

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Obstructive sleep apnea (OSA), has been tightly-related to several components of metabolic syndrome (MS). However, most of the evidence documented has only evaluated individual components of the MS, or pa-

tients with a diagnosis of OSA.

Castaneda A, Jauregui-Maldonado E, Ratnani I, Varon J, Surani S. Correlation between metabolic syndrome and sleep apnea. *World J Diabetes* 2018; 9(4): 66-71 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i4/66.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i4.66>

INTRODUCTION

The term "metabolic syndrome" (MS) dates back to 1977, when Haller used it to describe the association between hypertension, dyslipidemia, obesity, and disturbed glucose metabolism^[1]. He demonstrated how the presence of multiple of these factors increased the risk of developing cardiovascular disease^[1]. Phillips, in 1978 suggested that the combination of risk factors not only predisposed to heart disease, but were also related with an increased risk for obesity^[2]. These constellation of abnormalities included glucose intolerance, hyperinsulinemia and a high level of triglycerides, glucose, cholesterol and insulin^[2]. In the late 1980's, Gerald Reaven hypothesized that insulin resistance could be the underlying factor, linking these constellations of abnormalities, which he named "Syndrome X" or also known as Metabolic X-syndrome, which is currently known as the MS^[3].

The MS remains a global epidemiological challenge^[4,5]. This is due to the exponential increase in body mass index (BMI) in humans as the result of an increase in caloric intake, increase in obesity percentage, and increased sedentary life habits^[6]. This clinical entity has a cluster of risk factors such as hypertension, central obesity, increased triglycerides, decreased high density lipoprotein cholesterol (HDL-C), increased blood glucose and insulin resistance^[7].

Taking in consideration the established guidelines by the World Health Organization (WHO) as a reference to determine the best criteria for diagnosis, specificity and sensitivity between International Diabetes Federation (IDF), revised National Cholesterol Education Program (NCEP-R), NCEP Adult Treatment Panel (ATP)-III, and American Association of Clinical Endocrinologists (AACE) were evaluated, IDF criteria obtained the best overall specificity of 81.3% and sensitivity of 71.2% for proper diagnosis^[8].

The parameters set for diagnosis include fasting glucose ≥ 100 mg/dL (or receiving drug therapy for hyperglycemia), blood pressure $\geq 130/85$ mmHg (or receiving drug therapy for hypertension), triglycerides ≥ 150 mg/dL (or receiving drug therapy for hypertriglyceridemia), HDL-C < 40 mg/dL in men or < 50 mg/dL in women (or receiving drug therapy for reduced HDL-C) and waist circumference ≥ 94 cm in men or ≥ 80 cm in women (see Table 1)^[8].

In the vast majority of cases with MS, development of clinical manifestations such as cardiovascular disease,

diabetes mellitus type 2 and obstructive sleep apnea (OSA) are known to manifest^[7,9,10].

The disorder known as OSA is not new by any means, and it is becoming more prominent due to the increase in obesity over the past few decades^[11]. Of historical interest, sleep apnea and snoring have been documented since 4000 BC^[12]. Charles Dickens in the 19th century wrote the "Pickwickian Papers", where one of the characters was "Joe, the fat boy" stating he was always asleep and snoring even on the simplest task such as errands and waiting for a table, leading way to the term "Pickwickian syndrome" in the late 19th century as apneic symptoms^[13,14]. Finally, by mid-1960's, Gastaut, and associates, formally introduced the term "Sleep apnea", when a closure of the upper airways in obese patients was found, providing the first documented relation between obesity, sleep-induced airway obstruction, sleep fragmentation, and daytime sleepiness, leading way to what is now known as "OSA"^[15,16].

These repetitive partial or, in certain advanced cases complete obstructive episodes of the upper airway during periods of sleep, are characterized by cessation of breathing for at least 10 s per minute resulting in hypopneic scenarios leading to apnea^[7,9,10]. Grading of OSA is based on the apnea-hypopnea index (AHI), which is defined as number of apnea and hypopnea episode per hour of sleep^[17]. Mild OSA has an AHI of 5-14/h, moderate OSA 15-30/h AHI, and severe OSA has an AHI of > 30 /h^[18,19] (Table 1).

Some have suggested that the MS should include OSA, as part of its clinical manifestations, and should be called "Z syndrome"^[7,18]. A study by Xu *et al*^[19] established an independent relationship between these two clinical entities.

Guidelines for the review were followed^[19,20]. The authors independently searched an electronic database (PubMed) using MeSH with the following terms "sleep apnea, obstructive sleep apnea" and "MS" to pinpoint articles up to the year 2017 excluding 2018, which were relevant to determine the correlation between MS and OSA, using the following medical subject headings and keywords: Metabolic syndrome, OSA. Overall, 321 articles were identified, and 39 studies were finally included in this review (Figure 1). All the included article reviewed were full text. Articles not related with obesity, MS, abstract only, duplicate information, comments and conference papers were excluded. Only studies in English language were included with a purpose of universal understanding. The acquired data from all qualified articles were later discussed between authors and any disagreements were resolved.

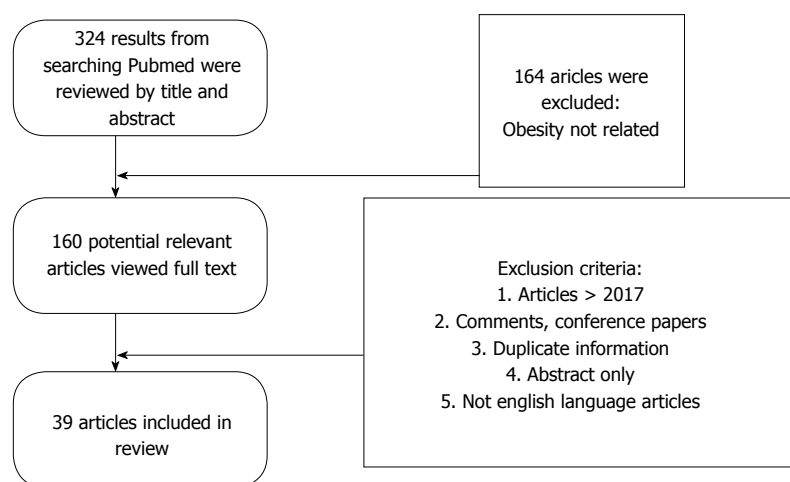
EPIDEMIOLOGY

The prevalence of the MS Worldwide is estimated to be between $< 10\%$ to as much as 84% , finding a certain correlation with developed countries, yet depends on various factors such as socioeconomic status, lifestyle, BMI, and region studied^[6,21]. Study by Khosravi-Bo-

Table 1 Current criteria for the diagnosis of the metabolic syndrome and sleep apnea severity classification

Factor	IDF
Hypertension	Current antihypertensive therapy or BP \geq 130/85 mmHg
Dyslipidemia - elevated triglycerides	Plasma triglycerides \geq 150 mg/dL or specific treatment for high triglycerides
Dyslipidemia - depressed HDL	HDL $<$ 40 mg/dL in men or $<$ 50 mg/dL in women or specific treatment for low HDL
Obesity	Waist circumference $>$ 37 inches in men or $>$ 31.5 inches in women
Glucose	Fasting glucose \geq 100 mg/dL or previously diagnosed type 2 diabetes
Requirements for diagnosis	Waist circumference criteria plus any 2 of other criteria.
No OSA	AHI $<$ 5/h
Mild	AHI 5-14/h
Moderate	AHI 15-30/h
Severe	AHI $>$ 30/h

BP: Blood pressure; IDF: International Diabetes Federation; HDL: High density lipoprotein; AHI: Apnea-hypopnea index.

**Figure 1** Flow chart implemented for the selection of articles for this review.

roujeni *et al*^[22] showed the prevalence of MS have changed from 2001-2013. They also mentioned that prevalence of diabetes has also been increasing over the years. This has been attributed to aging, life style changes, population growth, obesity and decline in physical activity. Central obesity was labelled as a critical component of the MS. The prevalence of the hypertriglyceridemia also declined, due to use of the statins, healthy eating with cutting back on trans-fat and aggressive screenings^[22].

Prevalence estimates for OSA range between 4%-24% for men and 2%-16% for women making it slightly higher amongst men, as well as most common after the age of 40 and during the postmenopausal period for women^[23]. This prevalence increases for all who have risk factors, such as obesity and diabetes ranking it a significant health care challenge for population worldwide, with the odds as high as nearly double that of normal-weight adults^[24]. While examining data, the relationship between MS and OSA was found to coexist up to 60% of cases^[25].

CORRELATIONS BETWEEN MS AND OSA

There is significant evidence of the correlation between

MS and OSA. It is clear that OSA independently leads to insulin resistance, a component of MS^[26]. There is evidence that there is lower insulin sensitivity, and a higher fasting insulin level in these patients^[27]. In these patients, there is an increase in epinephrine, norepinephrine and/or cortisol leading to an increase in gluconeogenesis and a decrease in skeletal muscle uptake of glucose^[28]. In addition, there is a concomitant elevation in systemic inflammatory markers^[29-31]. Examples of these are tumor necrosis factor alfa (TNF- α) and interleukin six (IL-6), both of which are independent of obesity and are dependent on OSA^[20]. C-reactive protein (CRP) levels are also elevated, and these directly correlates with the elevated TNF- α and IL-6, leading to insulin resistance^[26,30,31]. Furthermore, it is thought obesity and OSA have a synergistic, negative effect over glucose metabolism^[27,32]. Intermittent hypoxemia has been shown to produce beta cell dysfunction as well as insulin resistance^[32].

As noted above, OSA intermittent hypoxemia causes sympathetic excitation, decreasing insulin sensitivity, glucose uptake and stimulates hepatic gluconeogenesis. Moreover, there is activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), with subsequent production of pro-inflammatory mediators

such as TNF- α , IL-6, and interleukin eight (IL-8)^[32]. Therefore, OSA is linked to inflammation-mediated reaction with sympathetic signaling and oxidative stress, all led by NF- κ B^[33]. Obesity, on the other hand, leads to hypertrophied, dysfunctional adipocytes^[34]. These adipocytes attract cytotoxic T cells (CD8+ T) as well as macrophages with the latter producing pro-inflammatory cytokines like IL-6, TNF- α and inducible nitric oxide synthase (iNOS)^[33,34]. There is a release of free fatty acids leading impairment of insulin-signaling pathway, insulin resistance and metabolic consequences. Obesity has adipose tissue mediating pro-inflammatory reactions with compromised vascularity via hypoxemia-derived pathway^[34]. Here, the hypoxia-inducible factor 1 α is the moderator by increasing expression of pro-inflammatory adipocyte genes mediated by NF- κ B gene promoters^[32]. What appears to be interesting is that OSA may lead to MS signs and symptoms with the intermittent hypoxemia causing visceral adipose tissue inflammation in rodents or reductionist models without presence of obesity^[32].

OSA has shown to lead to early atherosclerosis by causing endothelial failure. In a study by Ciconne, 40 healthy patients were compared against 80 patients with OSA. Of the affected, 26 had mild OSA and 54 had severe OSA. The patients' demographics such as age, BMI, and neck circumference were comparable among the control group and both OSA groups. The authors hypothesized various episodes of hypoxia and re-oxygenation along with sleep deprivation would lead to systemic inflammation. They measured inflammation markers in venous blood. Patient with OSA had increased carotid intimal thickening, C-reactive protein (CRP), interleukin (IL-6), tumor necrosis factor (TNF- α) and pentraxin (PTX-3). They found CRP which promotes adhesion molecule expression and opsonizes LDL for reuptake by the macrophages within the plaque. IL-6 stimulated the production of CRP and serum amyloid A; the latter reduces HDL-C content. TNF- α stimulates monocyte adhesion to the endothelial surface with infiltration through the vascular wall, and finally their conversion to macrophages; it is also the cause of multiple atherogenic cytokine production. PTX-3 was seen to be elevated in both OSA groups. While PTX-3's role in the pathophysiology of OSA is not clear yet, it is considered as a specific marker for endothelial inflammation^[35].

In a study by Zychowski *et al.*^[36], human coronary endothelial cells were incubated with 5% serum, of control and OSA patients for 4 h. They then, performed qPCR to evaluate for endothelial inflammatory markers. OSA serum induced much more endothelial cell expression of VCAM1, ICAM1, IL8, SELP and CCL5 mRNA. This suggested another molecular role of endothelial activation and dysfunction in patients with OSA^[36].

Screening younger generations

As the estimated prevalence of OSA is 2%-3% in healthy children and between 13% and 66% among

obese adolescents, it is justified to screen for it^[26]. Children with OSA had reportedly 6.49 times increased odds of developing MS when compared with children without OSA^[34]. The principal predictor is hypoxemia. Consideration should be given to screening of obese children with MS for OSA since their outcomes are modifiable by lifestyle changes^[34].

How the MS may trigger the development of OSA

An excess in weight is more crucial for OSA than either age or gender^[37,38]. For every percent in weight reduction, there is a 3% reduction in the AHI^[27,39]. Increased waist diameter correlates with an increased incidence of OSA^[40]. Obesity is an important factor, more importantly, central obesity. Central obesity is linked to higher leptin production with resistance to said hormone and leading to increase probability of developing OSA. Metabolic abnormalities increase the chance of upper airway collapsibility. Neck circumference is a better predictor of OSA than general obesity. Central obesity impact is greater on the upper airway function when compared to peripheral obesity, as stated earlier^[40,41]. Patients with obesity and OSA have approximately a 67% more total neck fat compared to the normal person. This leads to a smaller upper airway area and greater compression on said airway while sleeping. Central obesity is more closely related to fat depositions in the neck, unlike peripheral obesity. This leads to a more notable narrowing of the upper airway while asleep^[38,42].

CONCLUSION

The presence of MS may be the trigger to the development of OSA. This is important, since it is now known that the coexistence of both pathologies within the same patient raises biomarkers, which directly cause or at least raise potential for complications. Detecting MS and OSA is very important. Similarly, known diabetic patients should be screened for OSA and complications could be avoided. Adequate treatment of OSA can help decrease insulin resistance. It is imperative for the clinicians to keep themselves updated with the recent changes in science which can translate in combating and preventing the progression of disease.

There are more pathophysiological theories of how OSA may have a synergistic negative effect with MS that is yet to be made clear. From a direct effect of OSA over the hypothalamic-pituitary-adrenal axis, adrenal medulectomy trials as a possible therapeutic procedure, or a theory of OSA affecting the microbiota of the gastrointestinal tract leading to the development or worsening of MS. The current studies are being performed in mice, but in a near future may lead to a better understanding of the relationship between OSA and MS and possibly to improved patient management.

REFERENCES

- 1 **Haller H.** Epidermology and associated risk factors of

- hyperlipoproteinemia. *Z Gesamte Inn Med* 1977; **32**: 124-128 [PMID: 883354]
- 2 **Phillips GB**. Sex hormones, risk factors and cardiovascular disease. *Am J Med* 1978; **65**: 7-11 [PMID: 356599 DOI: 10.1016/0002-9343(78)90685-X]
 - 3 **Reaven GM**. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595-1607 [PMID: 3056758 DOI: 10.2337/diabetes.37.12.1595]
 - 4 **Alam MF**, Nasreen S, Ullah E, Hussain A. The awareness and prevalence of metabolic syndrome in medical community of bahawalpur. *Oman Med J* 2011; **26**: 26-28 [PMID: 22043375 DOI: 10.5001/omj.2011.07]
 - 5 **Moreira GC**, Cipullo JP, Ciorlia LA, Cesarino CB, Vilela-Martin JF. Prevalence of metabolic syndrome: association with risk factors and cardiovascular complications in an urban population. *PLoS One* 2014; **9**: e105056 [PMID: 25180496 DOI: 10.1371/journal.pone.0105056]
 - 6 **Thounaojam MC**, Nammi S, Jadeja R. Natural Products for the Treatment of Obesity, Metabolic Syndrome, and Type 2 Diabetes 2016. *Evid Based Complement Alternat Med* 2016; **2016**: 9072345 [PMID: 27703490 DOI: 10.1155/2016/9072345]
 - 7 **Kostoglou-Athanassiou I**, Athanassiou P. Metabolic syndrome and sleep apnea. *Hippokratia* 2008; **12**: 81-86 [PMID: 18923660]
 - 8 **Onesi SO**, Ignatius UE. Metabolic syndrome: Performance of five different diagnostic criterias. *Indian J Endocrinol Metab* 2014; **18**: 496-501 [PMID: 25143905 DOI: 10.4103/2230-8210.137494]
 - 9 **Drager LF**, Lopes HF, Maki-Nunes C, Trombetta IC, Toschi-Dias E, Alves MJ, Fraga RF, Jun JC, Negrão CE, Krieger EM, Polotsky VY, Lorenzi-Filho G. The impact of obstructive sleep apnea on metabolic and inflammatory markers in consecutive patients with metabolic syndrome. *PLoS One* 2010; **5**: e12065 [PMID: 20711453 DOI: 10.1371/journal.pone.0012065]
 - 10 **Calvin AD**, Albuquerque FN, Lopez-Jimenez F, Somers VK. Obstructive sleep apnea, inflammation, and the metabolic syndrome. *Metab Syndr Relat Disord* 2009; **7**: 271-278 [PMID: 19344228 DOI: 10.1089/met.2008.0093]
 - 11 **Gibson GJ**. Obstructive sleep apnoea syndrome: underestimated and undertreated. *Br Med Bull* 2005; **72**: 49-65 [PMID: 15798032 DOI: 10.1093/bmb/ldh044]
 - 12 **Asaad T**. Sleep in Ancient Egypt. Cairo Egypt: Springer Science, 2015: 13-19 [DOI: 10.1007/978-1-4939-2089-1_2]
 - 13 **Littleton SW**, Mokhlesi B. The pickwickian syndrome-obesity hypoventilation syndrome. *Clin Chest Med* 2009; **30**: 467-478, vii-viii [PMID: 19700045 DOI: 10.1016/j.ccm.2009.05.004]
 - 14 **Bickelmann AG**, Burwell CS, Robin ED, Whaley RD. Extreme obesity associated with alveolar hypoventilation; a Pickwickian syndrome. *Am J Med* 1956; **21**: 811-818 [PMID: 13362309 DOI: 10.1016/0002-9343(56)90094-8]
 - 15 **Dempsey JA**, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. *Physiol Rev* 2010; **90**: 47-112 [PMID: 20086074 DOI: 10.1152/physrev.00043.2008]
 - 16 **Gastaut H**, Tassinari CA, Duron B. Polygraphic study of the episodic diurnal and nocturnal (hypnic and respiratory) manifestations of the Pickwickian syndrome. *Brain Res* 1966; **1**: 167-186 [PMID: 5923125 DOI: 10.1016/0006-8993(66)90117-X]
 - 17 **Wang Z**, Zhou X, Zhao W, Liu F, Ni H, Yu Z. Assessing the severity of sleep apnea syndrome based on ballistocardiogram. *PLoS One* 2017; **12**: e0175351 [PMID: 28445548 DOI: 10.1371/journal.pone.0175351]
 - 18 **Hudgel DW**. Sleep Apnea Severity Classification - Revisited. *Sleep* 2016; **39**: 1165-1166 [PMID: 27070135 DOI: 10.5665/sleep.5776]
 - 19 **Xu S**, Wan Y, Xu M, Ming J, Xing Y, An F, Ji Q. The association between obstructive sleep apnea and metabolic syndrome: a systematic review and meta-analysis. *BMC Pulm Med* 2015; **15**: 105 [PMID: 26391008 DOI: 10.1186/s12890-015-0102-3]
 - 20 **Marik PE**, Varon J. Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. *Clin Cardiol* 2009; **32**: 365-372 [PMID: 19609891 DOI: 10.1002/clc.20604]
 - 21 **Kaur J**. A comprehensive review on metabolic syndrome. *Cardiol Res Pract* 2014; **2014**: 943162 [PMID: 24711954 DOI: 10.1155/2014/943162]
 - 22 **Khosravi-Boroujeni H**, Sarrafzadegan N, Sadeghi M, Roohafza H, Talaei M, Ng SK, Phung H, Pourmogaddas A, Ahmed F. Secular Trend of Metabolic Syndrome and Its Components in a Cohort of Iranian Adults from 2001 to 2013. *Metab Syndr Relat Disord* 2017; **15**: 137-144 [PMID: 28135122 DOI: 10.1089/met.2016.0073]
 - 23 **Cizza G**, de Jonge L, Piaggi P, Mattingly M, Zhao X, Lucassen E, Rother KI, Sumner AE, Csako G; NIDDK Sleep Extension Study. Neck circumference is a predictor of metabolic syndrome and obstructive sleep apnea in short-sleeping obese men and women. *Metab Syndr Relat Disord* 2014; **12**: 231-241 [PMID: 24571423 DOI: 10.1089/met.2013.0093]
 - 24 **Romero-Corral A**, Caples SM, Lopez-Jimenez F, Somers VK. Interactions between obesity and obstructive sleep apnea: implications for treatment. *Chest* 2010; **137**: 711-719 [PMID: 20202954 DOI: 10.1378/chest.09-0360]
 - 25 **Parish JM**, Adam T, Facchiano L. Relationship of metabolic syndrome and obstructive sleep apnea. *J Clin Sleep Med* 2007; **3**: 467-472 [PMID: 17803009]
 - 26 **Nannapaneni S**, Ramar K, Surani S. Effect of obstructive sleep apnea on type 2 diabetes mellitus: A comprehensive literature review. *World J Diabetes* 2013; **4**: 238-244 [PMID: 24379913 DOI: 10.4239/wjd.v4.i6.238]
 - 27 **Ip MS**, Lam B, Ng MM, Lam WK, Tsang KW, Lam KS. Obstructive sleep apnea is independently associated with insulin resistance. *Am J Respir Crit Care Med* 2002; **165**: 670-676 [PMID: 11874812 DOI: 10.1164/ajrccm.165.5.2103001]
 - 28 **Trombetta IC**, Maki-Nunes C, Toschi-Dias E, Alves MJ, Rondon MU, Cepeda FX, Drager LF, Braga AM, Lorenzi-Filho G, Negrão CE. Obstructive sleep apnea is associated with increased chemoreflex sensitivity in patients with metabolic syndrome. *Sleep* 2013; **36**: 41-49 [PMID: 23288970 DOI: 10.5665/sleep.2298]
 - 29 **Ciftci TU**, Kokturk O, Bukan N, Bilgihan A. The relationship between serum cytokine levels with obesity and obstructive sleep apnea syndrome. *Cytokine* 2004; **28**: 87-91 [PMID: 15381186 DOI: 10.1016/j.cyto.2004.07.003]
 - 30 **Khoo MC**, Oliveira FM, Cheng L. Understanding the metabolic syndrome: a modeling perspective. *IEEE Rev Biomed Eng* 2013; **6**: 143-155 [PMID: 23232440 DOI: 10.1109/RBME.2012.2232651]
 - 31 **Yudkin JS**, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 1999; **19**: 972-978 [PMID: 10195925 DOI: 10.1161/01.ATV.19.4.972]
 - 32 **Ryan S**. Adipose tissue inflammation by intermittent hypoxia: mechanistic link between obstructive sleep apnoea and metabolic dysfunction. *J Physiol* 2017; **595**: 2423-2430 [PMID: 27901270 DOI: 10.1113/JP273312]
 - 33 **Gileles-Hillel A**, Almendros I, Khalyfa A, Nigdelioglu R, Qiao Z, Hamanaka RB, Mutlu GM, Akbarpour M, Gozal D. Prolonged Exposures to Intermittent Hypoxia Promote Visceral White Adipose Tissue Inflammation in a Murine Model of Severe Sleep Apnea: Effect of Normoxic Recovery. *Sleep* 2017; **40** [PMID: 28329220 DOI: 10.1093/sleep/zsw074]
 - 34 **Gami AS**, Caples SM, Somers VK. Obesity and obstructive sleep apnea. *Endocrinol Metab Clin North Am* 2003; **32**: 869-894 [PMID: 14711066 DOI: 10.1016/S0889-8529(03)00069-0]
 - 35 **Cicccone MM**, Scicchitano P, Zito A, Cortese F, Boninfante B, Falcone VA, Quaranta VN, Ventura VA, Zucano A, Di Serio F, Damiani MF, Resta O. Correlation between inflammatory markers of atherosclerosis and carotid intima-media thickness in Obstructive Sleep Apnea. *Molecules* 2014; **19**: 1651-1662 [PMID: 24481114 DOI: 10.3390/molecules19021651]
 - 36 **Zychowski KE**, Sanchez B, Pedrosa RP, Lorenzi-Filho G, Drager LF, Polotsky VY, Campen MJ. Serum from obstructive sleep apnea patients induces inflammatory responses in coronary artery endothelial cells. *Atherosclerosis* 2016; **254**: 59-66 [PMID: 27693879 DOI: 10.1016/j.atherosclerosis.2016.09.017]

- 37 **Jalilolghadr S**, Yazdi Z, Mahram M, Babaei F, Esmailzadehha N, Nozari H, Saffari F. Sleep architecture and obstructive sleep apnea in obese children with and without metabolic syndrome: a case control study. *Sleep Breath* 2016; **20**: 845-851 [PMID: 26711131 DOI: 10.1007/s11325-015-1291-y]
- 38 **Mortimore IL**, Marshall I, Wraith PK, Sellar RJ, Douglas NJ. Neck and total body fat deposition in nonobese and obese patients with sleep apnea compared with that in control subjects. *Am J Respir Crit Care Med* 1998; **157**: 280-283 [PMID: 9445310 DOI: 10.1164/ajrccm.157.1.9703018]
- 39 **Davies RJ**, Ali NJ, Stradling JR. Neck circumference and other clinical features in the diagnosis of the obstructive sleep apnoea syndrome. *Thorax* 1992; **47**: 101-105 [PMID: 1549815 DOI: 10.1136/thx.47.2.101]
- 40 **Young T**, Peppard PE, Taheri S. Excess weight and sleep-disordered breathing. *J Appl Physiol* (1985) 2005; **99**: 1592-1599 [PMID: 16160020 DOI: 10.1152/jappphysiol.00587.2005]
- 41 **Schwab RJ**, Pasirstein M, Pierson R, Mackley A, Hachadoorian R, Arens R, Maislin G, Pack AI. Identification of upper airway anatomic risk factors for obstructive sleep apnea with volumetric magnetic resonance imaging. *Am J Respir Crit Care Med* 2003; **168**: 522-530 [PMID: 12746251 DOI: 10.1164/rccm.200208-866OC]
- 42 **Ryan CM**, Bradley TD. Pathogenesis of obstructive sleep apnea. *J Appl Physiol* (1985) 2005; **99**: 2440-2450 [PMID: 16288102 DOI: 10.1152/jappphysiol.00772.2005]

P- Reviewer: Ciccone MM, Micheu MM **S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Tan WW





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 May 15; 9(5): 72-79



**ORIGINAL ARTICLE****Observational Study**

- 72 Adherence to self-care practices, glycemic status and influencing factors in diabetes patients in a tertiary care hospital in Delhi

Basu S, Garg S, Sharma N, Singh MM, Garg S

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Fernando Guerrero-Romero, MD, PhD, Research Fellow, Biomedical Research Unit, Mexican Social Security Institute, Dgo 34067, Mexico

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, and PubMed Central.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Wen-Wen Tan*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITOR-IN-CHIEF
Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com/1948-9358/editorialboard.htm>

www.wjgnet.com/1948-9358/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE
May 15, 2018

COPYRIGHT
© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.f6publishing.com>

Observational Study

Adherence to self-care practices, glycemic status and influencing factors in diabetes patients in a tertiary care hospital in Delhi

Saurav Basu, Suneela Garg, Nandini Sharma, M Meghachandra Singh, Sandeep Garg

Saurav Basu, Suneela Garg, Nandini Sharma, M Meghachandra Singh, Department of Community Medicine, Maulana Azad Medical College, New Delhi 110002, India

Sandeep Garg, Department of Medicine, Maulana Azad Medical College, New Delhi 110002, India

ORCID number: Saurav Basu (0000-0003-1336-8720); Suneela Garg (0000-0002-2196-1607); Nandini Sharma (0000-0001-7885-4901); M Meghachandra Singh (0000-0002-1716-746X); Sandeep Garg (0000-0001-8069-7781).

Author contributions: All authors contributed to study conception, design and approval of the final manuscript; Basu S contributed to data acquisition, data analysis, interpretation and writing of article; Garg S, Sharma N and Singh MM contributed to data analysis, interpretation, editing, reviewing; Garg S contributed to interpretation, editing, reviewing.

Institutional review board statement: Institutional ethical clearance was granted by the Institutional Ethics Committee of the hospital.

Informed consent statement: Written and informed consent was taken from all the subjects prior to enrolment in the study.

Conflict-of-interest statement: Dr. Basu has nothing to disclose.

STROBE statement: STROBE statement guidelines have been adopted.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Saurav Basu, MBBS, Doctor, Department of Community Medicine, Maulana Azad Medical College, Bahadur Shah Zafar Marg, New Delhi 110002, India. saurav.basu.mph@gmail.com
Telephone: +91-84-47527452

Received: March 6, 2018

Peer-review started: March 7, 2018

First decision: April 2, 2018

Revised: April 7, 2018

Accepted: April 15, 2018

Article in press: April 15, 2018

Published online: May 15, 2018

Abstract

AIM

To assess the adherence to self-care practices, glycemic status and influencing factors in diabetes patients.

METHODS

This was a cross-sectional observational analysis of baseline data from a quasi-experimental study conducted among 375 diabetic patients aged between 18 to 65 years at a major public tertiary care centre in New Delhi, India during February-September' 2016. The Summary of Diabetes Self-care activities measure was used to assess medical adherence in diabetic patients. Open ended questions were used to identify facilitators and inhibitors of medical adherence.

RESULTS

Mean age of the study subjects was 49.7 ± 10.2 years. A total of 201 men and 174 women were enrolled in the study. Three hundred nine (82.4%) subjects were adherent to their intake of anti-diabetic medication. On binary logistic regression, education level below primary school completion and absence of hypertension

comorbidity were found to be independent predictors of medication non-adherence. Sociocultural resistance was an important factor impeding outdoor exercise among younger women. Knowledge of diabetes in the study subjects was low with mean score of 3.1 ± 2 (maximum score = 10). Suboptimal glycemic control was found in 259 (69%) subjects which was significantly more likely in patients on Insulin therapy compared to those on Oral Hypoglycemic agents alone ($P < 0.006$).

DISCUSSION

Our study found a large gap existed between self-reported medication adherence and glycemic control. This suggests the need for enhanced physician focus for diabetic patient management.

Key words: Diabetes; Adherence; Glycemic control; Insulin; India

© **The Author(s)** 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: A cross sectional analysis was conducted in 375 adult diabetic patients in the outpatient settings of a major tertiary care government hospital in Delhi (2016). A total of 309 (82.4%) subjects reported are adherent to their prescribed anti-diabetic medication. However, optimal glycemic control was achieved by only 116 (31%) subjects. These findings suggest the presence of a high burden of clinical inertia. Furthermore, patients on insulin therapy despite reporting higher medication adherence comprised a significantly higher proportion with suboptimal glycemic control compared to those are not on insulin therapy indicating the need to effectively validate patient administration of insulin.

Basu S, Garg S, Sharma N, Singh MM, Garg S. Adherence to self-care practices, glycemic status and influencing factors in diabetes patients in a tertiary care hospital in Delhi. *World J Diabetes* 2018; 9(5): 72-79 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i5/72.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i5.72>

INTRODUCTION

Diabetes is a chronic noncommunicable disease characterized by the phenotype of hyperglycemia which afflicts an estimated 69 million people in India, the second highest burden in the world after China^[1]. It is well established that the lowering of blood glucose levels prevents or delays diabetic microvascular and macrovascular complications of diabetes^[2,3]. Achieving and maintaining optimal blood glucose levels in diabetic patients requires good medical adherence in terms of medication, diet, physical activity while ensuring regular foot-care protects against foot-ulcer and complications like amputation^[4-7].

Nevertheless, poor adherence to self-care pra-

ctices in diabetes persists as a major public health challenge globally^[8]. Non-adherence in taking anti-diabetic medication in diabetic patients can occur from to unintentional factors like irregular access to oral hypoglycemic agents and Insulin^[8-10]. Intentional factors which lower medication adherence include increasing complexity of regimen, fear of side effects of drugs and lack of patient perceived benefit from taking medication^[11-13]. Non-adherence to treatment recommendations worsen clinical parameters, risk poor patient outcomes and only permit suboptimal benefit of treatment^[6,14]. This necessitates ascertaining the burden of non-adherence to self-care practices and influencing factors in diabetes patients attending public health facilities in India. The study was thereby conducted with the objective of assessing the extent of adherence to self-care practices including medication intake and the influencing factors among diabetic patients undergoing treatment in a tertiary care hospital in Delhi.

MATERIALS AND METHODS

This was a cross-sectional observational analysis of baseline data from a quasi-experimental study conducted at the Diabetes and the Endocrinology OPDs of a major tertiary care government hospital in Central Delhi during the period from February 2016 to September 2016. Over a 1000 diabetes patient attend the OPD clinics of the hospital every week. All the prescribed diabetes related medication is provided free of cost at the hospital with refill required every 2 wk. At the time of initiation of treatment, the patients are provided dietary counselling from a dietician at the hospital while the other self-care practices are explained by the treating physician only. Furthermore, dietary counselling may be repeated in patients showing suboptimal glycemic control at the discretion of the treating physician.

Diabetic patients aged between 18 to 65 years and on diabetes treatment for at-least 1 year were included while those with serious comorbid ailments (advanced cardiovascular disease, history of cardiovascular accident, renal failure requiring dialysis, cancer, patients on psychotropic drugs, dementia and blindness) were excluded from the study. The primary outcome of this study was medication adherence which was assessed by the medication subscale of the Summary of Diabetes Self-care activities measure (SDSCA)^[15]. The license to use the SDSCA was obtained. Based on 2 previous studies, daily medication adherence equating no missed doses in any of the previous 7 d (SDSCA medication score = 7) in an urban Indian hospital-clinic setting was expected to be 60%^[16,17]. The sample size at 95% confidence levels and 5% absolute margin of error calculated by the Cochran formula, $(1.96^2 \times 0.6 \times 0.4)/0.05^2 = 369$. The patients were selected consecutively with a maximum of 12 patients being enrolled in a clinic day. Data was collected using a pretested patient interview schedule. Pretesting was conducted among 25 subjects at the medical OPD of a secondary care hospital in Delhi.

Table 1 Distribution of medical adherence in diabetes patients, Delhi, 2016 (*n* = 375)

Medical Adherence in study subjects (in previous 7 d)	mean \pm SD (d)
Medication adherence	6.25 \pm 1.5
Adhering to a healthy eating plan	5.22 \pm 1.2
Consuming \geq 1 portion of green vegetables	3.25 \pm 3
Consuming \geq 1 portion of fruits	1.8 \pm 1.8
Practicing carbohydrate spacing	4.87 \pm 1.2
Exercising for at-least 30 min in a day	2.25 \pm 2.5
Engaged in moderate physical activity as part of work, travel or household chores for at least 10 min continuously	4.42 \pm 2.1
Foot inspection	0.14 \pm 0.58

The diagnosis of diabetes, clinical characteristics and treatment details were validated from the patient medical records. Adherence to self-care practices in the patients was assessed using the SDSCA during the period preceding 7 d from the day of enrolment. The items of the questionnaire were translated into the local language, Hindi through a validated back and forth translation process. The subjects who reported missing their anti-diabetic medications on at-most 1-d in the previous 7 d equivalent to \geq 80% medication rate were classified as adherent to their prescribed anti-diabetic medication. The subjects who reported adherence to a healthy diet on at-least 5 d were classified as adherent to diet. The subjects who reported engaging in moderate physical activity as part of work, travel or household chores for at-least 150 min interspersed over 3 to 5 d were classified as adherent to physical activity. Open ended questions were used to identify facilitators and inhibitors of medical adherence.

The cut-off for optimal glycemic control in the present study was accepted at fasting blood glucose levels \leq 130 mg/dL as per the recommendations of the American Diabetes Association^[18]. The knowledge of diabetes in the patients was assessed using the 10 item Spoken Knowledge in Low Literacy in Diabetes Scale by Rothman *et al*^[19]. The socioeconomic status of the subjects was assessed using the modified Kuppuswamy classification updated for 2016 income criteria^[20].

Statistical analysis

Data was analysed using IBM SPSS Version 17. Analytical tables were used for depicting bivariate associations. The association between categorical variables was assessed using the χ^2 test and continuous variables using the Student *t* test. *P* value < 0.05 was considered as statistically significant. The variables which were significantly associated with medication non-adherence (*P* \leq 0.05) were included in a step-wise binary logistic regression model. The final model was tested for goodness-of-fit by the Hosmer-Lemeshow test.

RESULTS

A total of 375 diabetic patients were enrolled in the study.

Patient characteristics

On univariate analysis, the mean age of the study

subjects was 49.7 \pm 10.2 years (mean \pm SD). The study sample comprised of 201 (53.6%) men and 174 (46.4%) women. The educational status of the majority of the subjects was low with 132 (35.2%) educated below primary, 105 (28%) with 5-9 years of schooling and 138 (36.8%) with \geq 10 years of schooling. The Socioeconomic Status of the study participants classified as per the updated modified Kuppuswamy scale was upper (2.1%), upper middle (22.4%), lower middle (53.3%), upper lower (21.3%) and lower (0.8%). The median duration of diabetes in the study participants was 5 years.

Adherence to self-care practices

The medication adherence and self-care practices of the study subjects as per the SDSCA measure is reported in Table 1.

Medication: A total of 309 (82.4%) subjects were adherent to the intake of their prescribed anti-diabetic medication (SDSCA medication score \geq 6) while 66 (17.6%) were non-adherent. Among the adherent subjects, 254 (67.8%) reported missing none of their prescribed anti-diabetic medication on any occasion in the previous 7 d while 55 (14.2%) reporting missing their dose on only a single day. Among the reasons for medication non-adherence, 96 (25.6%) reported missing doses due to forgetfulness while 75 (20%) subjects reported self-modification of drug dosage depending upon their symptoms and sense of well-being.

Facilitators for good medication adherence observed in the study subjects were perceived benefits; "Taking medications on time keeps me well", higher self-efficacy "First medicine, then roti (bread)", "I may forget taking food but not my medicine" and the presence of family support "My daughter/daughter in law bring forth my medicines before me exactly on time". In this regard, family assistance in terms of help in remembering medication was reported by 52 (13.9%) subjects. Moreover, 42 (35.6%) subjects on insulin therapy were receiving assistance from a family member in administration of insulin.

On bivariate analysis, low education level (below primary school completion), living in joint family, patient not on insulin therapy, absence of hypertension comorbidity and lack of family assistance for taking medication were found to be significantly associated

with medication non-adherence (Table 2).

A binary logistic regression analysis was performed to assess the effects of education level, family type, insulin therapy, hypertension comorbidity and family assistance for taking anti-diabetic medication on the likelihood that the patients reported non-adherence to their anti-diabetic medication. The logistic regression model was statistically significant ($P < 0.001$) and the model correctly classified 82.4% of cases. Of the five predictor variables, only two (education level and hypertension comorbidity) were statistically significant (Table 3). Diabetic patients without hypertension comorbidity had 2.6 higher odds of reporting non-adherence to their anti-diabetic medication compared to hypertensives.

Diet: Most (84.6%) subjects reported adhering to a healthy diet prescribed for managing diabetes. However, consumption of green vegetables and fruits was low in the subjects (Table 1).

Physical activity and exercise: A total of 254 (67.7%) subjects reported low levels of physical activity. Female gender, living in joint family and low educational level (below primary school completion) were significantly associated with lower physical activity (Table 2). Furthermore, among female subjects below 40 years of age ($n = 28$), only 3 (10.7%) were exercising for the recommended duration while 9 (36%) attributed their inability to exercise to the prevailing sociocultural resistance in their communities which deterred them from exercising outdoors.

Monitoring of blood glucose: Only 66 (17.6%) subjects reported possession of a personal and functional glucometer which precluded regular self-monitoring of blood glucose in most subjects.

Foot-care practices: Most subjects did not inspect their feet for lesions even once a week (Table 1). Most (92.7%) subjects trimmed their toe nail straight. Although, 325 (86.6%) subjects reported washing their feet daily only 54 (14.4%) were cleaning and drying between the toes afterward.

Glycemic control

The mean HbA1c in the subjects was 8.39 ± 2.0 ($n = 354$). Only 116 (31%) subjects showed optimal glycemic control ($\text{FBS} \leq 130$ mg/dL). On bivariate analysis, insulin therapy was found to be significantly associated with suboptimal glycemic control ($P = 0.006$). The subjects who reported receiving family support for adhering to a recommended healthy diet were significantly more likely to exhibit good glycemic control ($P = 0.035$). The proportion of women with suboptimal glycemic status was more compared to men but the difference was not statistically significant (Table 4).

Knowledge of diabetes in the study subjects was low with mean score of 3.1 ± 2 (maximum score =

10). The knowledge score was significantly lower in patients with low education level (below primary school) compared to those educated beyond primary school ($P < 0.001$). However, the knowledge scores did not differ significantly among subjects with medication non-adherence or in those showing suboptimal glycemic control.

DISCUSSION

Diabetes has been described as a “whole life disease” since its successful management requires patients to effectively modulate several aspects of their daily living which forms the basis of diabetic self-management^[21]. Our study found nearly four-fifth of the subjects reporting being adherent to their medications (SDSCA medication score ≥ 6). This is lower than a study in a government health facility in Puducherry that reported 95.6% diabetic patients being adherent to their medications which also used the SDSCA assessment method^[22]. A previous study in government hospitals of Delhi by Basu *et al*^[10] found nearly three-fourth of the diabetic subjects showing good medication adherence assessed by the MMAS-8 scale. In our study, no missed anti-diabetic medication doses in the previous 7 d signifying daily medication adherence was reported by nearly two-third of the subjects (67.4%). The rates of daily medication adherence among diabetes subjects found in our study (67.8%) are higher compared to two South Indian clinic-based studies which reported 60% daily medication adherence rate^[16,17].

In contradiction to previous studies, low socioeconomic status was not found to be associated with medication non-adherence in the present study^[9,10]. However, our study found low educational level limited to primary school was an independent predictor of medication non-adherence while also associated with lower patient knowledge of diabetes.

Our study found hypertensive patients to be significantly more adherent to their anti-diabetic medications which could be on account of perception of increased susceptibility to disease and perceived benefit of medications in the comorbid patients.

A majority of the subjects are in our reported low physical activity and exercise levels. Apart from medical conditions like joint arthritis, environmental and sociocultural factors can also impede the levels of physical activity in diabetic populations^[23]. Environmental barriers like paucity of open public recreational spaces is known to undermine exercising behavior especially among low income groups^[24]. Similarly, the catchment areas of the health facility in the present study had a high population density with few public parks providing limited avenues for exercising. Furthermore, our study also found a high proportion of younger women reporting sociocultural resistance in their communities against engaging in outdoor exercise. This finding is in agreement with a previous study which reported the possibility of women being made uncomfortable and being judged by those

Table 2 Patient characteristics and association with medical non-adherence *n* (%)

Variable	Total (<i>n</i> = 375)	Medication non-adherence (<i>n</i> = 66)	Dietary non-adherence (<i>n</i> = 58)	Physical activity non-adherence (<i>n</i> = 253)
Age (yr)				
< 50	168 (44.8)	29 (17.2)	28 (16.6)	109
≥ 50	207 (55.2)	37 (17.8)	30 (14.5)	144
Gender				
Men	201 (53.6)	31 (15.4)	36 (18)	114 (56.7) ^b
Women	174 (46.4)	35 (20.1)	22 (12.6)	139 (80)
Education (yr)				
< 5	132 (35.2)	33 (25) ^a	21 (16)	101 (76.5) ^b
≥ 5	243 (64.8)	33 (13.5)	37 (15.2)	152 (62.5)
SES				
Upper-upper middle	92 (24.5)	14 (15.2)	14 (15.2)	56 (61)
Lower middle	200 (53.4)	34 (17)	27 (13.5)	137 (68.5)
Upper lower-lower	83 (22.1)	18 (21.7)	17 (20.5)	60 (72.3)
Family type				
Joint	289 (77)	58 (20) ^a	46 (16)	204 (70.5) ^a
Nuclear/alone	86 (23)	8 (9.3)	12 (14)	49 (57)
DM duration (yr)				
≤ 5	205 (54.7)	37 (18)	34 (16.6)	130 (63.4)
> 5	170 (45.3)	29 (17)	24 (14.1)	123 (72.3)
Hypertension				
Present	191 (51)	22 (11.5) ^b	29 (15.2)	126 (66)
Absent	184 (49)	44 (24)	29 (15.7)	127 (69)
Insulin therapy				
Present	118 (31.5)	12 (10.2) ^a	20 (17)	78 (66.1)
Absent	257 (68.5)	54 (21)	38 (14.8)	175 (68)
Family assistance with medication				
Present	71 (19)	7 (9.8) ^a	-	-
Absent	304 (81)	59 (19.4)	-	-
Family help with diet				
Present	89 (23.7)	-	8 (9)	-
Absent	286 (76.3)	-	50 (17.5)	-

^a*P* < 0.05; ^b*P* < 0.01.

Table 3 Logistic regression analysis determining factors associated with non-adherence to anti-diabetic medication (*n* = 66)

Predictor variable	Odds ratio ¹	95%CI	<i>P</i> value
Education < 5 yr	2.06	1.1-3.6	0.01
Living in nuclear family	2.1	0.95-4.8	0.06
Insulin therapy	1.7	0.83-3.5	0.14
Absence of hypertension comorbidity	2.6	1.4-4.6	0.001
Family assistance for taking medication	0.56	0.23-1.3	0.2

¹Adjusted odds with other predictor variables controlled.

around them when they tried exercising outdoors^[25]. In this regard, community directed sociocultural resistance which restricts movement of women in public spaces should also be recognized as a public health challenge. While long term efforts should be concentrated upon reducing such bias and discrimination against women in society, the short-term recommendations for exercise should take cognition of the prevailing social reality.

Foot care in our diabetic subjects was very poor compared to the findings of other Indian studies which predispose them to risk of foot ulcerations. This necessitates imparting regular health education regarding appropriate foot-care to diabetic patients at the clinics.

Similar to our study, Kassahun *et al*^[26] also observed higher fasting blood glucose levels in patients on Insulin

therapy. However, in the present study, participants on Insulin reported higher medication adherence compared to those on oral hypoglycemic agents alone (*P* ≤ 0.05). Such a finding was also reported in a previous Delhi study^[10]. These finding could result from several factors like delayed initiation of Insulin therapy, a high burden of insulin resistance, improper insulin application practices or inadequate insulin intake along with the overestimation of insulin adherence.

In conclusion, our study explored aspects of medication adherence and self-care practices in a diabetic population predominantly belonging to the lower socioeconomic classes undergoing treatment in a major tertiary care public health facility in Delhi. Our study findings have the following important implications: (1)

Table 4 Patient characteristics associated with suboptimal glycemic control *n* (%)

Variable	Total (<i>n</i> = 375)	Suboptimal glycemic control (<i>n</i> = 259)	<i>P</i> value
Age (yr)			
≤ 50	168 (44.8)	118 (70.2)	0.74
> 50	207 (55.2)	141 (68.1)	
Gender			
Men	201 (53.6)	131 (65.1)	0.09
Women	174 (46.4)	128 (73.5)	
Education (yr)			
< 5	132 (35.2)	96 (72.7)	0.29
≥ 5	243 (64.8)	163 (67)	
SES			
Upper/upper middle	92 (24.5)	57 (62)	0.24
Lower middle	200 (53.4)	143 (71.5)	
Lower middle/lower	83 (22.1)	59 (71)	
Family type			
Joint	289 (77)	205 (71)	0.18
Nuclear/alone	86 (23)	54 (62.8)	
DM duration (yr)			
≤ 5	205 (54.7)	136 (66.3)	0.22
> 5	170 (45.3)	123 (72.3)	
Hypertension			
Present	191 (51)	130 (68)	0.74
Absent	184 (49)	129 (70)	
Insulin therapy			
Present	118 (31.5)	93 (79)	0.006
Absent	257 (68.5)	166 (64.6)	
Family help with diet			
Present	89 (23.7)	53 (59.5)	0.035
Absent	286 (76.3)	206 (72)	
Medication adherence			
Non-adherent	66 (17.6)	55 (83.3)	0.005
Adherent	309 (82.4)	204 (54.4)	

Our study found more than four-fifth of the diabetic subjects undergoing treatment from outpatient settings of a government health facility which provides free of cost medication were adherent to their anti-diabetic medication which corroborates the observations in previous studies from similar health facilities^[10,22]. It is also well established that the inability to acquire medications due to economic constraints significantly reduces medication adherence^[6,8,9]. Expanding coverage of medications to all diabetic patients for achieving universal health coverage should be the primary concern for India's national program for prevention and control of non-communicable diseases including diabetes; (2) The inability to realize optimal glycemic control status despite high levels of self-reported medication adherence was found to be a major challenge for a large number of the study subjects. In our study, 82% subjects were adherent to their anti-diabetic medications but only 31% showed optimal glycemic control. There are few studies in India which have reported both medication adherence and glycemic control in diabetic populations. This high medication adherence-suboptimal glycemic control gap was also previous seen in a Delhi study^[10] although not found in a Chennai study^[16]. This phenomenon is suggestive of the presence of a high burden of clinical inertia defined as "resistance to initiate or intensify treatment in a

patient not at the evidence-based glycated hemoglobin goal^[27]. In developing nations, clinical inertia leading to accumulation of glycemic burden is often due to delayed initiation of insulin therapy arising from both patient and health system related factors^[28]. A dual burden of clinical inertia and medication non-adherence may further accelerate the worsening of health outcomes in diabetics. Enhanced physician focus is recommended for diabetic patients showing persistently suboptimal glycemic control despite good self-reported medication adherence. Future studies should also identify the factors driving clinical inertia during diabetes management in such resource-constrained settings. Furthermore, developing interventions for overcoming the barriers and challenges which drive clinical inertia and hinder the achievement of optimal glycemic control and positive health outcomes are urgently warranted; (3) Family support can facilitate medical adherence relating to medication intake, diet and physical activity in patients. Enlisting familial support when available should be prioritized by physicians especially in patients with poor health literacy; (4) Our study findings are indicative of the presence of high burden of poor diabetes knowledge associated with low educational status. Developing effective health education and patient communication strategies by mobilizing paramedical health workers including nurses and pharmacists esp-

pecially in health settings with high patient load need prioritization; and (5) Existing medication adherence scales like the SDSCA may overestimate insulin adherence. This phenomenon could be explained as an outcome of self-desirability bias or the tendency of patients to over-report adherence in order to satisfy the interviewer and shield themselves from the anticipated criticism or guilt of failing to comply with the physician or healthcare provider's recommendations. Moreover, standard (generic) medication adherence scales do not assess the validity of the patient execution of the steps of insulin administration which constitutes a significant determinant of insulin adherence^[29]. A need is perceived for development of specific scales for assessment of insulin adherence with higher reliability and validity especially in low-literacy populations.

Our study has certain limitations. First, the cross-sectional analysis did not permit observation of the trend of patient adherence towards self-care practices and glycemic control status over time. Second, geriatric patients over 65 years of age were excluded and the results cannot be generalized to them despite the high risk of non-adherence in older populations^[30]. Third, the interrelationship among the categorical variables could influence results of the logistic regression analysis due to multicollinearity.

ARTICLE HIGHLIGHTS

Research background

Nearly 80% of the global burden of diabetes is concentrated in the developing world. India has 69 million diabetic patients which is the second highest in the world after China. Management of diabetes requires lowering of blood glucose to optimal levels to prevent or delay the onset of diabetic complications which risk end organ damage. Patient adherence to anti-diabetic medication, healthy diet, and regular physical activity constitutes the mainstay of diabetes treatment. However, poor treatment adherence is a major public health challenge globally but especially in the resource-constrained settings concentrated in the developing world which undermines efforts in controlling diabetes. A complex array of factors influences medical adherence and glycemic control in diabetes patients.

Research motivation

There is paucity of evidence ascertaining the determinants of treatment adherence and glycemic control in diabetes patients attending public health facilities in the developing world.

Research objectives

The study was conducted with the objective of assessing the extent of adherence to self-care practices including medication intake and the influencing factors among diabetic patients undergoing treatment in the outpatient setting of a tertiary care hospital in Delhi. Understanding the determinants of medical adherence through this study would facilitate engineering tailored interventions promoting medical adherence and improved health outcomes among diabetic patients in resource-constrained settings.

Research methods

Diabetic patients aged between 18 to 65 years and on diabetes treatment for at-least 1 year were included while those with serious comorbid ailments (advanced cardiovascular disease, history of cardiovascular accident, renal failure requiring dialysis, cancer, patients on psychotropic drugs, dementia and blindness) were excluded from the study. The patients were selected consecutively with a maximum of 12 patients being enrolled in a clinic day. Data

was collected using a pretested patient interview schedule. The Summary of Diabetes Self-care activities measure (SDSCA) by Toobert *et al* was used to assess medical adherence in the diabetic patients. Open ended questions were used to identify facilitators and inhibitors of medication, exercise and dietary adherence. The subjects who reported missing their anti-diabetic medications on at-most 1-d in the previous 7 d equivalent to $\geq 80\%$ medication rate were classified as adherent to their prescribed anti-diabetic medication. The subjects who reported adherence to a healthy diet on at-least 5 d were classified as adherent to diet. The subjects who reported engaging in moderate physical activity as part of work, travel or household chores for at-least 150 min interspersed over 3 to 5 d were classified as adherent to physical activity. The cut-off for optimal glycemic control in the present study was accepted at fasting blood glucose levels ≤ 130 mg/dL. The knowledge of diabetes in the patients was assessed using the 10 item Spoken Knowledge in Low Literacy in Diabetes Scale by Rothman *et al*.

Research results

A total of 309 (82.4%) subjects were adherent to the intake of their prescribed anti-diabetic medication (SDSCA medication score ≥ 6) while 66 (17.6%) were non-adherent. Among the adherent subjects, 254 (67.8%) reported missing none of their prescribed anti-diabetic medication on any occasion in the previous 7 d while 55 (14.2%) reporting missing their dose on only a single day. On bivariate analysis, low education level (below primary school completion), living in joint family, patient not on insulin therapy, absence of hypertension comorbidity and lack of family assistance for taking medication were found to be significantly associated with medication non-adherence. On adjusted analysis, low education level (below primary school completion) and absence of hypertension comorbidity were found to be significant predictors of medication non-adherence. A total of 254 (67.7%) subjects reported low levels of physical activity. Female gender, living in joint family and low educational level (below primary school completion) were significantly associated with lower physical activity. The mean HbA1c in the sample population was 8.39 ± 2.0 ($n = 354$). Only 116 (31%) subjects showed optimal glycemic control (FBS ≤ 130 mg/dL). On bivariate analysis, insulin therapy was found to be significantly associated with suboptimal glycemic control ($P = 0.006$). Knowledge of diabetes in the study subjects was low with mean score of 3.1 ± 2 (maximum score = 10). The knowledge score was significantly lower in patients with low education level (below primary school) compared to those educated beyond primary school ($P < 0.001$).

Research conclusions

Our study found a large gap exists between the self-reported medication adherence (82.7%) and attainment of optimal glycemic control (31%) patients. These findings suggest the possibility of significant clinical inertia prevalent in the study setting. A dual burden of medication non-adherence and clinical inertia could undermine efforts in effective diabetes management in the resource-constrained settings of the developing world. Understanding the factors driving clinical inertia in these settings requires assessment through future studies. The present study also found patients on Insulin tend to report higher medication adherence but show suboptimal glycemic control compared to patients only on oral hypoglycemic agents. This indicates overestimation of insulin adherence when based on single item self-report measures. Future studies should assess insulin adherence through self-report based on correctness of the steps executed in the process of insulin administration. In this study, family support was found to improve medication adherence and adoption of healthy lifestyle. This indicates the need of the treating physician to enlist valuable family support whenever available for the diabetic patient.

Research perspectives

The study shows improving medication adherence in diabetic populations does not necessarily correlate with improvement in glycemic status due to the possibility of clinical inertia which requires reduction through enhanced physician focus on patient outcomes.

REFERENCES

- 1 **International Diabetes Federation.** IDF diabetes atlas. 7th ed. Brussels: International Diabetes Federation, 2015
- 2 **Fowler MJ.** Microvascular and macrovascular complications

- of diabetes. *Clin Diabetes* 2008; **26**: 77-82 [DOI: 10.2337/diaclin.26.2.77]
- 3 Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837-853 [PMID: 9742976 DOI: 10.1016/S0140-6736(98)07019-6]
- 4 **Karter AJ**, Moffet HH, Liu J, Parker MM, Ahmed AT, Ferrara A, Selby JV. Achieving good glycemic control: initiation of new antihyperglycemic therapies in patients with type 2 diabetes from the Kaiser Permanente Northern California Diabetes Registry. *Am J Manag Care* 2005; **11**: 262-270 [PMID: 15839186]
- 5 **Rhee MK**, Slocum W, Ziemer DC, Culler SD, Cook CB, El-Kebbi IM, Gallina DL, Barnes C, Phillips LS. Patient adherence improves glycemic control. *Diabetes Educ* 2005; **31**: 240-250 [PMID: 15797853 DOI: 10.1177/0145721705274927]
- 6 **Sabat   E**. Adherence to long-term therapies: evidence for action. 1st ed. Geneva: World Health Organization, 2003
- 7 **Bartus CL**, Margolis DJ. Reducing the incidence of foot ulceration and amputation in diabetes. *Curr Diab Rep* 2004; **4**: 413-418 [PMID: 15539004 DOI: 10.1007/s11892-004-0049-x]
- 8 **World Health Organization**. Global Report on Diabetes. Geneva: World Health Organization, 2016
- 9 **Sankar UV**, Lipska K, Mini GK, Sarma PS, Thankappan KR. The adherence to medications in diabetic patients in rural Kerala, India. *Asia Pac J Public Health* 2015; **27**: NP513-NP523 [PMID: 23417905 DOI: 10.1177/1010539513475651]
- 10 **Basu S**, Khobragade M, Kumar A, Raut DK. Medical adherence and its predictors in Diabetes Mellitus patients attending government hospitals in the Indian Capital, Delhi, 2013: A cross sectional study. *Int J Diabetes Dev Ctries* 2015; **35** Suppl 2: 95-101 [DOI: 10.1007/s13410-014-0232-9]
- 11 **Odegard PS**, Gray SL. Barriers to medication adherence in poorly controlled diabetes mellitus. *Diabetes Educ* 2008; **34**: 692-697 [PMID: 18669811 DOI: 10.1177/0145721708320558]
- 12 **Farmer A**, Kinmonth AL, Sutton S. Measuring beliefs about taking hypoglycaemic medication among people with Type 2 diabetes. *Diabet Med* 2006; **23**: 265-270 [PMID: 16492209 DOI: 10.1111/j.1464-5491.2005.01778.x]
- 13 **Hauber AB**, Mohamed AF, Johnson FR, Falvey H. Treatment preferences and medication adherence of people with Type 2 diabetes using oral glucose-lowering agents. *Diabet Med* 2009; **26**: 416-424 [PMID: 19388973 DOI: 10.1111/j.1464-5491.2009.02696.x]
- 14 **Osterberg L**, Blaschke T. Adherence to medication. *N Engl J Med* 2005; **353**: 487-497 [PMID: 16079372 DOI: 10.1056/NEJMr050100]
- 15 **Toobert DJ**, Hampson SE, Glasgow RE. The summary of diabetes self-care activities measure: results from 7 studies and a revised scale. *Diabetes Care* 2000; **23**: 943-950 [PMID: 10895844 DOI: 10.2337/diacare.23.7.943]
- 16 **Sasi ST**, Kodali M, Burra KC, Muppala BS, Gutta P, Bethanbhatla MK. Self Care Activities, Diabetic Distress and other Factors which Affected the Glycaemic Control in a Tertiary Care Teaching Hospital in South India. *J Clin Diagn Res* 2013; **7**: 857-860 [PMID: 23814728 DOI: 10.7860/JCDR/2013/5726.2958]
- 17 **Rajasekharan D**, Kulkarni V, Unnikrishnan B, Kumar N, Holla R, Thapar R. Self-care activities among patients with diabetes attending a tertiary care hospital in mangalore karnataka, India. *Ann Med Health Sci Res* 2015; **5**: 59-64 [PMID: 25745579 DOI: 10.4103/2141-9248.149791]
- 18 **American Diabetes Association**. Erratum. Glycemic Targets. Sec. 6. In *Standards of Medical Care in Diabetes-2017*. Diabetes Care 2017; **40** (Suppl. 1): S48-S56. *Diabetes Care* 2017; **40**: 985 [PMID: 28522557 DOI: 10.2337/dc17-er07a]
- 19 **Rothman RL**, Malone R, Bryant B, Wolfe C, Padgett P, DeWalt DA, Weinberger M, Pignone M. The Spoken Knowledge in Low Literacy in Diabetes scale: a diabetes knowledge scale for vulnerable patients. *Diabetes Educ* 2005; **31**: 215-224 [PMID: 15797850 DOI: 10.1177/0145721705275002]
- 20 **Sharma R**. Kuppaswamy's socioeconomic status scale--revision for 2011 and formula for real-time updating. *Indian J Pediatr* 2012; **79**: 961-962 [PMID: 22231776 DOI: 10.1007/s12098-011-0679-3]
- 21 **Liburd LC**. Diabetes and health disparities: community based approaches for racial and ethnic populations. 1st ed. New York: Springer Publishing Company, 2009
- 22 **Selvaraj K**, Ramaswamy G, Radhakrishnan S, Thekkur P, Chinnakali P, Roy G. Self-care practices among diabetes patients registered in a chronic disease clinic in Puducherry, South India. *J Soc Health Diabetes* 2016; **4**: 25-29 [DOI: 10.4103/2321-0656.176572]
- 23 **Basu S**, Garg S. The barriers and challenges toward addressing the social and cultural factors influencing diabetes self-management in Indian populations. *J Soc Health Diabetes* 2017; **5**: 71-76 [DOI: 10.4103/joshd.J_Soc_Health_Diabetes_3_17]
- 24 **Booth GL**, Creatore MI, Moineddin R, Gozdyra P, Weyman JT, Matheson FI, Glazier RH. Unwalkable neighborhoods, poverty, and the risk of diabetes among recent immigrants to Canada compared with long-term residents. *Diabetes Care* 2013; **36**: 302-308 [PMID: 22988302 DOI: 10.2337/dc12-0777]
- 25 **Advika TS**, Idiculla J, Kumari SJ. Exercise in patients with Type 2 diabetes: Facilitators and barriers - A qualitative study. *J Family Med Prim Care* 2017; **6**: 288-292 [PMID: 29302534 DOI: 10.4103/2249-4863.219998]
- 26 **Kassahun T**, Gesesew H, Mwanri L, Eshetie T. Diabetes related knowledge, self-care behaviours and adherence to medications among diabetic patients in Southwest Ethiopia: a cross-sectional survey. *BMC Endocr Disord* 2016; **16**: 28 [PMID: 27381349 DOI: 10.1186/s12902-016-0114-x]
- 27 **Bailey CJ**. Under-treatment of type 2 diabetes: Causes and outcomes of clinical inertia. *Int J Clin Pract* 2016; **70**: 988-995 [PMID: 28032429 DOI: 10.1111/ijcp.12906]
- 28 **Mohan V**, Shah S, Saboo B. Current glycemic status and diabetes related complications among type 2 diabetes patients in India: data from the A1chieve study. *J Assoc Physicians India* 2013; **61**: 12-15 [PMID: 24482981]
- 29 **Donnelly LA**, Morris AD, Evans JM; DARTS/MEMO collaboration. Adherence to insulin and its association with glycaemic control in patients with type 2 diabetes. *QJM* 2007; **100**: 345-350 [PMID: 17504861 DOI: 10.1093/qjmed/hcm031]
- 30 **Shruthi R**, Jyothi R, Pundarikaksha HP, Nagesh GN, Tushar TJ. A Study of Medication Compliance in Geriatric Patients with Chronic Illnesses at a Tertiary Care Hospital. *J Clin Diagn Res* 2016; **10**: FC40-FC43 [PMID: 28208878 DOI: 10.7860/JCDR/2016/21908.9088]

P- Reviewer: Beltowski J, Serhiyenko VA, Zhao J **S- Editor:** Ji FF
L- Editor: Filipodia **E- Editor:** Tan WW





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 June 15; 9(6): 80-98



**ORIGINAL ARTICLE****Basic Study**

- 80 Coagonist of glucagon-like peptide-1 and glucagon receptors ameliorates kidney injury in murine models of obesity and diabetes mellitus

Patel VJ, Joharapurkar AA, Kshirsagar SG, Sutariya BK, Patel MS, Patel HM, Pandey DK, Bahekar RH, Jain MR

EVIDENCE-BASED MEDICINE

- 92 Iranian healthcare professionals' perspectives about factors influencing the use of telemedicine in diabetes management

Ayatollahi H, Mirani N, Nazari F, Razavi N

Contents

World Journal of Diabetes
Volume 9 Number 6 June 15, 2018

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Seung-Soon Im, PhD, Associate Professor, Department of Physiology, Keimyung University, School of Medicine, Daegu 12062, South Korea

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, and PubMed Central.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Wen-Wen Tan*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITOR-IN-CHIEF
Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com/1948-9358/editorialboard.htm>

www.wjgnet.com/1948-9358/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE
May 15, 2018

COPYRIGHT
© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.f6publishing.com>

Basic Study

Coagonist of glucagon-like peptide-1 and glucagon receptors ameliorates kidney injury in murine models of obesity and diabetes mellitus

Vishal J Patel, Amit A Joharapurkar, Samadhan G Kshirsagar, Brijesh K Sutariya, Maulik S Patel, Hiren M Patel, Dheerendra K Pandey, Rajesh H Bahekar, Mukul R Jain

Vishal J Patel, Amit A Joharapurkar, Samadhan G Kshirsagar, Brijesh K Sutariya, Maulik S Patel, Hiren M Patel, Dheerendra K Pandey, Rajesh H Bahekar, Mukul R Jain, Department of Pharmacology and Toxicology, Zydus Research Centre, Cadila Healthcare Limited, Ahmedabad 382210, India

ORCID number: Vishal J Patel (0000-0002-6185-8364); Amit A Joharapurkar (0000-0003-0723-4371); Samadhan G Kshirsagar (0000-0002-5347-9348); Brijesh K Sutariya (0000-0002-3049-5994); Maulik S Patel (0000-0001-8727-4060); Hiren M Patel (0000-0002-7848-5496); Dheerendra K Pandey (0000-0001-9792-1620); Rajesh H Bahekar (0000-0002-0105-3134); Mukul R Jain (0000-0001-5730-1435).

Author contributions: Patel VJ, Joharapurkar AA and Jain MR contributed to the conception of the manuscript, design of experiments, and analysis and interpretation of the data, and writing of the manuscript; Patel VJ, Joharapurkar AA, Kshirsagar SG, Sutariya BK, Patel MS, Patel HM, Pandey DK and Bahekar RH performed the experiments, analyzed the data, and wrote the manuscript; all authors have commented on the initial and final drafts of the manuscript and are responsible for approval of the final version of the manuscript in all aspects.

Institutional review board statement: This study protocol was reviewed and approved by the Institutional Review Board of Zydus Research Centre.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care Committee of Zydus Research Centre, Ahmedabad, India (ZRC/PH/BP/003/03-2K17).

Conflict-of-interest statement: There is no conflict of interest.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Amit A Joharapurkar, PhD, Senior Scientist, Department of Pharmacology and Toxicology, Zydus Research Centre, Cadila Healthcare Limited, Sarkhej-Bavla N.H.No.8A, Moraiya, Ahmedabad 382210, India. amitjoharapurkar@zyduscadila.com
Telephone: +91-27-17665555
Fax: +91-27-17665155

Received: April 23, 2018
Peer-review started: April 24, 2018
First decision: May 7, 2018
Revised: May 10, 2018
Accepted: May 15, 2018
Article in press: May 15, 2018
Published online: June 15, 2018

Abstract

AIM

To investigate the role of glucagon-like peptide-1 (GLP-1)/glucagon receptors coagonist on renal dysfunction associated with diabetes and obesity.

METHODS

Chronic high-fat diet fed C57BL/6J mice, streptozotocin-treated high-fat diet fed C57BL/6J mice and diabetic

C57BLKS/J db/db mice were used as models of diabetes-induced renal dysfunction. The streptozotocin-treated high-fat diet fed mice and db/db mice were treated with the GLP-1 and glucagon receptors coagonist (Aib2 C24 Chimera2, 150 µg/kg, *sc*) for twelve weeks, while in chronic high-fat diet fed mice, coagonist (Aib2 C24 Chimera2, 150 µg/kg, *sc*) treatment was continued for forty weeks. Kidney function, histology, fibrosis, inflammation, and plasma biochemistry were assessed at the end of the treatment.

RESULTS

Coagonist treatment decreased body weight, plasma lipids, insulin resistance, creatinine, blood urea nitrogen, urinary albumin excretion rate and renal lipids. In kidney, expression of lipogenic genes (*SREBP-1C*, *FAS*, and *SCD-1*) was decreased, and expression of genes involved in β -oxidation (*CPT-1* and *PPAR- α*) was increased due to coagonist treatment. In plasma, coagonist treatment increased adiponectin and FGF21 and decreased IL-6 and TNF- α . Coagonist treatment reduced expression of inflammatory (*TNF- α* , *MCP-1*, and *MMP-9*) and pro-fibrotic (*TGF- β* , *COL1A1*, and α -*SMA*) genes and also improved histological derangement in renal tissue.

CONCLUSION

Coagonist of GLP-1 and glucagon receptors alleviated diabetes and obesity-induced renal dysfunction by reducing glucose intolerance, obesity, and hyperlipidemia.

Key words: Coagonist; Glucagon; Renal dysfunction; Glucagon-like peptide-1; Insulin sensitivity

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Nephropathy is a significant complication of diabetes. In this study, we have demonstrated that the coagonist of glucagon-like peptide-1 and glucagon receptors alleviates biochemical and histopathological features of nephropathy in HFSTZ and db/db mice. Coagonist reduces glucotoxicity and lipotoxicity in these animal models, which translates into benefit for prevention of nephropathy. The results provide further evidence that coagonist may be effective in the prevention of diabetic nephropathy.

Patel VJ, Joharapurkar AA, Kshirsagar SG, Sutariya BK, Patel MS, Patel HM, Pandey DK, Bahekar RH, Jain MR. Coagonist of glucagon-like peptide-1 and glucagon receptors ameliorates kidney injury in murine models of obesity and diabetes mellitus. *World J Diabetes* 2018; 9(6): 80-91 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i6/80.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i6.80>

INTRODUCTION

Diabetes mellitus is often associated with macro- and

micro-vascular complications manifested as retinopathy, chronic kidney disease (CKD), neuropathy, visual impairment and cardiovascular complications. Type 2 diabetes and obesity were closely associated and together stimulate the progression of CKD, even in the absence of other comorbidities such as hypertension^[1,2]. Thickening and glomeruli basement membrane damage are the key pathogenic events in CKD initiation^[2]. High body mass index in obesity increases glomerulus filtration to meet increased metabolic demand and enhances the risk of CKD^[3]. Elevated glucose and lipids levels cause oxidative stress. In addition it activates multiple metabolic pathways that trigger the production of inflammatory cytokines (TNF- α , IL-6, MCP-1) and growth factors (TGF- β), causing proteinuria, nodular glomerulosclerosis, and tubulointerstitial injury. As a result, glomerular filtration rate (GFR) is decreased, leading to end-stage renal disease (ESRD) owing to the fibrosis caused by excessive accumulation of extracellular matrix (mainly collagens and fibronectin)^[4]. CKD begins with an increase in GFR, intraglomerular capillary pressure, glomerulomegaly and microalbuminuria^[3]. Current therapy of CKD is aimed at controlling blood glucose, blood pressure, and lipid-induced pathologies^[5]. In case of ESRD, patient requires either dialysis or kidney transplantation^[6]. Prevention of worsening in renal function is of vital importance in CKD that is a secondary complication of diabetes or obesity. Deterioration of renal function can be reversed in CKD patients if long-term euglycemia or weight loss is achieved^[1,7].

Glucagon-like peptide-1 (GLP-1) regulates appetite, and hyperglycemia and GLP-1 based therapies are approved for treatment of diabetes and obesity^[8]. GLP-1 based therapies also demonstrate anti-inflammatory, lipid-lowering, and anti-fibrotic effects in liver and protects cardiovascular system^[9-11]. Previous studies demonstrated protective effects of GLP-1 analogs in acute renal injury and diabetic dyslipidemia-induced renal dysfunction in diabetic db/db mice by attenuating oxidative stress, renal lipid accumulation, and inflammation^[12-14]. Thus, GLP-1 signaling can modulate renal dysfunctions through multiple mechanisms. Another preproglucagon derived peptide, glucagon is used in the treatment of hypoglycemia. Glucagon receptors (GCGR) are abundantly expressed in liver and kidney suggesting a physiological involvement of glucagon in the hepatic and renal physiology^[15]. Glucagon reduces body weight and lipids^[16]. Acute administration of glucagon increases renal blood flow, GFR, and sodium excretion in humans^[17,18], while the renal function remains normal in GCGR knock-out mice^[19]. The effect of chronic administration of glucagon in renal function is unknown. Coagonist of GCGR and GLP-1 receptor (GLP-1R) is a novel approach for the treatment of obesity and type 2 diabetes. Balanced coagonist has been shown to have beneficial effects of GLP-1 and glucagon on glucose and lipid metabolism, better than GLP-1 or glucagon agonist^[20,21]. Coagonists of GCGR and GLP-1R are under clinical development for the treatment of obesity and type 2 diabetes^[22]. Chronic effect of GLP-1

agonist and glucagon analogues on renal function has not been evaluated. Reducing glucotoxicity and lipotoxicity in diabetes and obesity may attenuate the development of renal dysfunction. Coagonist is better not only in reducing insulin resistance, but also reduces lipid levels than either GLP-1 or glucagon^[21]. Coagonist also reduces inflammatory and fibrotic genes such as *TNF- α* , *MMP-9*, *MCP-1*, *TGF- β* and *α -SMA* in models of NAFLD^[23,24]. Hence, it is possible that by targeting glucotoxicity and lipotoxicity along with anti-inflammatory effects, coagonist can attenuate diabetes and obesity-induced renal dysfunctions. In the current study, we investigated the effect of coagonist of GLP-1R and GCGR on renal dysfunction in a model of obesity and diabetes.

MATERIALS AND METHODS

Chemicals and reagents

The coagonist of GCGR and GLP-1R, Aib2 C24 Chimera2 (H¹SQGT⁵FTSDY¹⁰SKYLD¹⁵EQAQK²⁰EFI-AW²⁵LMNT-NH²) was synthesized at Zydus Research Centre, Ahmedabad, India^[20]. Kits for triglycerides, cholesterol, and glucose were purchased from Avantor Performance Materials India Ltd, India. ELISA kits for IL-6 and *TNF- α* were obtained from BD Biosciences, United States; Insulin from Crystal Chem, United States; FGF21 from Wuhan Eiaab science, Co., Ltd. China; leptin and Adiponectin were purchased from B-bridge international, United States. Superoxide dismutase (SOD) and catalase activity assay kit were obtained from Cayman Chemical, United States. Creatinine, blood urea nitrogen, and albumin assay kit were obtained from Sigma-Aldrich, United States. TRIzol reagent and cDNA reverse transcription kit purchased from Invitrogen, Life Technology, United States and QIAGEN Quanti Fast SYBR Green kit were purchased from Qiagen, Germantown, United States. All other chemicals and reagents were purchased from Sigma-Aldrich chemicals, United States unless stated otherwise.

Animals and experimental protocol

Male C57BL/6J mice (6–8 wk old) and C57BLKS/J-db/db (6–8 wk old) mice were obtained from the Animal Research Facility of Zydus Research Centre, Ahmedabad, India. They had free access to food and water and were on a 12 h light–dark cycle. The protocol (ZRC/PH/BP/003/03-2K17) for animal use and experimentation were reviewed and approved by Institutional Animal Ethics Committee of Zydus Research Centre, which is an AAALAC accredited facility. All efforts were made to minimize the number of animals used and their pain or discomfort. Chow-fed control animals were maintained on chow diet (Harlan Teklad 14% Protein Rodent Diet, New Brunswick, NJ, United States).

Male C57BL/6J mice were randomly assigned to the treatment groups; vehicle control, coagonist and chow-fed control. Vehicle control and coagonist treatment

groups were fed on a high-fat diet (HFD) for 40 wk, and treatment of coagonist was started simultaneously with HFD feeding (60% fat, D12492, Research Diet Inc., United States). Coagonist was administered at 150 μ g/kg by subcutaneous (SC) route, twice a day for 40 wk. At the end, kidneys were removed, left kidney was used for measurement of lipid, and right kidney was used for histological analysis.

After eight weeks of HFD feeding, mice were given streptozotocin (STZ) at the dose of 40 mg/kg per day dissolved in freshly prepared cold 0.1 mol/L citrate buffer (pH 4.5) for five consecutive days by intraperitoneal (IP) route. The chow-fed control group (nondiabetic mice) received an intraperitoneal injection of citrate buffer alone. Only the mice with blood glucose levels ≥ 200 mg/dL, under fasting conditions were further fed on HFD for eight weeks^[25]. Streptozotocin-high-fat (HFSTZ) treated male C57BL/6J mice were randomized based on body weight (24.9 to 41.2 g) and overnight fasting glucose (189.5 to 275.3 mg/dL) to the treatment group, namely, vehicle control, and coagonist. A nondiabetic control was maintained. Coagonist was administered at 150 μ g/kg of dose by subcutaneous route (SC) twice a day for 12 wk. At the end of treatment, animals were sacrificed, and kidneys were quickly removed and weighed. The middle third of the right kidney from each mouse was fixed in 10% formaldehyde buffer and embedded in paraffin histopathological analysis. The renal cortex of the left kidney and remaining right kidney from each mouse was immediately frozen in liquid nitrogen. Fasting blood samples were collected, and the plasma was extracted and stored at -80 °C until analysis.

Male C57BLKS/J db/db mice were randomized based on body weight and plasma glucose and were assigned to treatments, namely, vehicle control and coagonist (150 μ g/kg, sc). The treatment was given twice a day for 12 wk. Body weight and total food intake were recorded. After 12 wk of treatment, mice were sacrificed, and kidneys and livers were quickly removed and weighed. The liver and left kidney were immediately frozen in liquid nitrogen and stored at -80 °C until further analysis.

Measurement of renal function and biochemical markers

At the end of the treatment, mice were housed individually in metabolic cages for 24 h to collect urine for measurements of urinary albumin. Blood samples were obtained by retro-orbital bleeding for measurement of glycosylated hemoglobin (HbA1c), triglycerides, cholesterol, glucose, creatinine, and blood urea nitrogen were assayed in blood using commercial kits. Insulin resistance (HOMA-IR) was calculated using the following formula: $\text{HOMA-IR} = [(\text{fasting insulin (ng/mL)} \times \text{fasting plasma glucose (mg/dL)})/405]$.

Determination of antioxidant status and lipid peroxidation in the kidney

SOD activity and catalase (CAT) activity were dete-

mined using a commercially available colorimetric assay kit using a microplate reader (Synergy HTX Multi-Mode Microplate Reader, BioTek Instruments, United States). Lipid peroxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS) as described earlier^[26].

Renal histopathological examination

Changes in renal morphology and fibrosis were examined in tissue sections. Tissue sections were fixed in 10% formaldehyde and embedded in paraffin. These sections (5 $\mu\text{mol/L}$ thickness) were stained with Periodic Acid-Schiff or Masson's trichrome stain. Stained sections were examined using a microscope (Olympus microscope, CX31, Tokyo, Japan) for surface area, glomerulosclerosis and tubulointerstitial fibrosis.

Tissue sections were stained with hematoxylin and eosin (H and E) assessed for morphology. The surface area (μm^2) of a minimum of ten glomerular sections from each animal was determined using the ImageJ software (WS Rasband, ImageJ, NIH, Bethesda, MD).

Periodic acid of Schiff (PAS) was used to evaluate and examine evidence of glomerulosclerosis as a glomerulosclerotic index. The degree of glomerulosclerosis, necrosis of renal tubules and thickening of the basement membrane were evaluated by a semi quantitative method^[27]. In brief, ten glomeruli in each kidney were graded in accordance with their severity of glomerular damage (0, normal; 1, slight glomerular damage, the mesangial matrix and/or hyalinosis with focal adhesion involving < 25% of the glomerulus; 2, sclerosis of 26%-50%; 3, sclerosis of 51%-75%; and 4, sclerosis of > 75% of the glomerulus). The glomerulosclerotic indexes were calculated using the following formula: glomerulosclerotic index = $(1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4) / n_0 + n_1 + n_2 + n_3 + n_4$, where n_x is the number of glomeruli in each grade of glomerulosclerosis.

Masson's trichrome stain was used to evaluate and examine evidence of tubulointerstitial fibrosis. Tubulointerstitial fibrosis was defined as tubular atrophy or dilatation, deposition of collage, and interstitial fibroblast proliferation, was evaluated by a semi quantitative method^[28]. The tubulointerstitial fibrosis index was assessed in ten different Masson's trichrome-stained sections using a light microscope on a scale of 0 to 4 (grade 0, normal; grade 1, affected area < 10%; grade 2, affected area 10%-25%; grade 3, affected area 25%-75%; grade 4, affected area greater than 75%). The average score was then calculated.

Quantitative real-time RT-PCR and gene expression

The mRNA expression of sterol regulatory element binding protein-1C (SREBP-1C), fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD-1), carnitine palmitoyltransferase I (CPT-1) and peroxisome proliferator-activated receptor alpha (PPAR- α), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- α), monocyte

chemoattractant protein-1 (MCP-1), transforming growth factor- β (TGF- β), collagen type 1A1 (COL1A1), and alpha-smooth muscle actin (α -SMA) in kidney were assessed by RT-PCR. These genes are related to glucose metabolism, lipid metabolism, kidney inflammation, and kidney fibrosis. Total RNA was extracted with TRIzol reagent (Applied Biosystem, United States) according to the manufacturer's instructions. After that, first strand cDNA synthesis was performed using the High-Capacity cDNA reverse transcription kit (Applied Biosystem, United States). The resulting cDNAs were used for quantitative PCR using the QIAGEN Quanti Fast SYBR Green kit (Cat. No. 204052, Qiagen, Germantown, MD, United States). The qPCR was run in an ABIprism-7300 (Applied Biosystems, Foster City, CA, United States). Quantitation and normalization of the mRNAs was performed using the $2^{-\Delta\Delta C_t}$ method using β -actin as a housekeeping gene. All primers and sequence details are listed in Supplementary Table 1.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism 7.03 software. A statistical review of the study was performed by a biomedical statistician. Quantitative results were expressed as the mean \pm SE ($n = 10$) for each group and $P < 0.05$ considered to be statistically significant. One way ANOVA was used to determine the level of significance among the different groups, while Dunnett's test was used for *post-hoc* analysis. *Post-hoc* tests were run only when F achieved $P < 0.05$. Histological score was evaluated using Kruskal-Wallis test followed by Dunn's test for multiple comparisons. There was no exclusion of any data in all studies.

RESULTS

Coagonist treatment prevented worsening of renal dysfunction in high-fat diet fed mice

After the chronic feeding of a high-fat diet, mice increased plasma creatinine, plasma blood urea nitrogen and urinary albumin excretion by 1.3 ± 0.4 fold, 1.6 ± 0.7 fold and 9.0 ± 1.1 fold when compared with chow-fed nondiabetic control (Table 1). Vehicle control showed increased levels of triglycerides and cholesterol in kidney, and kidney weight by 2.9 ± 1.7 , 1.6 ± 0.3 and 1.4 ± 0.5 fold, when compared with chow-fed control after 40 wk of high-fat diet exposure (Table 2). Chronic treatment with coagonist reduced plasma creatinine, plasma BUN, and urinary albumin excretion by $51.4\% \pm 15.9\%$, $48.4\% \pm 7.8\%$ and $86.0\% \pm 1.2\%$ when compared with vehicle control. While renal cholesterol, renal triglyceride, and kidney weight were decreased by $44.0\% \pm 19.9\%$, $57.1\% \pm 13.5\%$ and $24.7\% \pm 7.4\%$ when compared with vehicle control (Table 2).

Coagonist improved glucose and lipid dysfunction in db/db and HFSTZ mice

Genetically diabetic db/db mice showed increased body

Table 1 The sequence of the polymerase chain reaction primers used in the experiments

Target gene	Primers sequence (5' to 3')	
	Forward	Reverse
SERBP-1C	ATCGCAAAACAAGCTGACCTG	AGATCCAGGTTTGAGGTGGG
FAS	TTGCTGGCACTACAGAATGC	AACAGCCTCAGAGCGACAAT
SCD-1	CATCGCCTGCTCTACCCITT	GAAGAGCCGAGTCATGGAAG
CPT-1	CGCACATTACAAGGACATGG	GAAGAGCCGAGTCATGGAAG
PPAR- α	TCTGGAAGCTTTGGTTTTGC	GACTGAGGAAGGGCTGGAAG
MMP-9	ACACTCCGTCCTTACATGG	ATGAGCTCCAAGGGTGACAG
TNF- α	TGTCTCAGCCTCTTCTCATT	AGATGATCTGAGTGTGAGGG
MCP 1	ACCACAGTCCATGCCATCAC	TTGAGGTGGTTGTGGAAGAAAG
TGF- β	CACCGGAGAGCCCTGGATA	TGTACAGCTGCCGCACACA
COL1A1	TGATGGGGAAGCTGGCAAG	GAAGCCTCGGTGTCCCTTC
α -SMA	AGCATCCGACACTGCTGAC	GGGACAGCACAGCCTGAAT
β -actin	GCCTTCCTTCTGGGTATGG	GCACTGTGTTGGCATAGAGG

Table 2 Plasma biochemistry, kidney weight, and kidney lipid changes after coagonist treatment in diet-induced obese mice (46-48 wk old)

Parameter/treatment	Vehicle control	Coagonist (150 μ g/kg, sc)	Chow-fed control
Plasma creatinine (μ mol/L)	36.2 \pm 4.1	17.6 \pm 5.4 ^a	15.9 \pm 2.3 ^a
Plasma BUN (mg/dL)	48.1 \pm 5.8	24.8 \pm 2.3 ^a	18.2 \pm 4.2 ^a
Urinary albumin (μ g/d)	210.7 \pm 15.4	71.3 \pm 5.8 ^a	50.9 \pm 4.7 ^a
Renal triglycerides (μ g/mg of protein)	64.8 \pm 5.8	27.8 \pm 8.4 ^a	25.3 \pm 2.8 ^a
Renal cholesterol (μ g/mg of protein)	22.5 \pm 5.4	12.6 \pm 3.3 ^a	5.7 \pm 2.1 ^a
Kidney weight (mg)	541.2 \pm 46.8	387.4 \pm 14.8 ^a	210.3 \pm 42.4 ^a

Data are shown as the mean \pm SE. ^a P < 0.05, *vs* vehicle control. BUN: Blood urea nitrogen.

weight, fasting glucose, HbA1C, glucose intolerance and HOMA-IR when compared against a chow-fed control (Figure 1A-F and Table 3). Plasma triglycerides and cholesterol were elevated in db/db control animals (Figure 1G-H). Coagonist treatment reduced body weight gain by 17.3% \pm 2.0%, overnight glucose by 37.9% \pm 4.0%, HbA1C by 39.6% \pm 8.1%, area under curve (AUC) of glucose in IPGTT by 33.4% \pm 3.9%, AUC of glucose in ITT by 56.1% \pm 6.0% and HOMA-IR by 61.2% \pm 2.4% when compared with db/db control (Figure 1E-F and Table 3). Coagonist treatment also reduced plasma triglycerides and cholesterol by 50.8% \pm 5.8% and 31.5% \pm 3.1% respectively, when compared with db/db control (Figure 1G-H). The db/db control animals showed elevated levels of insulin, FGF21, and adiponectin when compared with chow-fed control (Figure 1I-J). Coagonist treatment reduced insulin by 37.8% \pm 1.4%, while increased levels FGF21 and adiponectin by 36.9% \pm 9.0% and 46.9% \pm 9.7%, respectively, when compared with db/db control (Figure 1I-J).

Feeding high-fat diet and STZ administration increased body weight, fasting glucose, fasting insulin, AUC of glucose after IPGTT and ITT, HbA1C and HOMA-IR when compared with chow-fed control (Figure 1A-F and Table 3).

Coagonist treatment reduced body weight by 22.8% \pm 3.0%, fasting glucose by 39.4% \pm 16.3%, fasting insulin by 39.0% \pm 9.1%, AUC of glucose in IPGTT by 24.1% \pm 5.4%, AUC of glucose in ITT by 35.7% \pm 2.8%, HbA1C by 17.8% \pm 3.9% and HOMA-

IR by 60.4% \pm 16.1%, when compared with HFSTZ control animals (Figure 1A-F). High-fat diet feeding increased plasma triglycerides and cholesterol in HFSTZ control when compared with chow-fed control (Figure 1G-H). HFSTZ control has elevated levels of FGF21 and adiponectin in plasma (Figure 1I-J). Coagonist treatment reduced plasma triglycerides and cholesterol by 39.1% \pm 4.2% and 33.1% \pm 10.9%, respectively, while increased plasma FGF21 by 147.6% \pm 11.1% and plasma adiponectin by 45.0% \pm 14.6%, when compared with HFSTZ control (Figure 1G-J).

Coagonist enhanced renal function in db/db and HFSTZ mice

We have observed that db/db control and HFSTZ control animals have increased plasma creatinine, BUN, and increased urinary albumin excretion, as compared to chow-fed control (Figure 2A-C). Coagonist treatment reduced creatinine by 43.1% \pm 4.3%, BUN by 47.6% \pm 9.2% and urinary albumin excretion by 48.2% \pm 9.1% in HFSTZ mice (Figure 2A-C). In db/db mice, coagonist reduced creatinine by 44.5% \pm 4.1%, BUN by 30.0% \pm 5.1% and urinary albumin excretion by 34.5% \pm 8.2%, when compared with db/db control (Figure 2A-C).

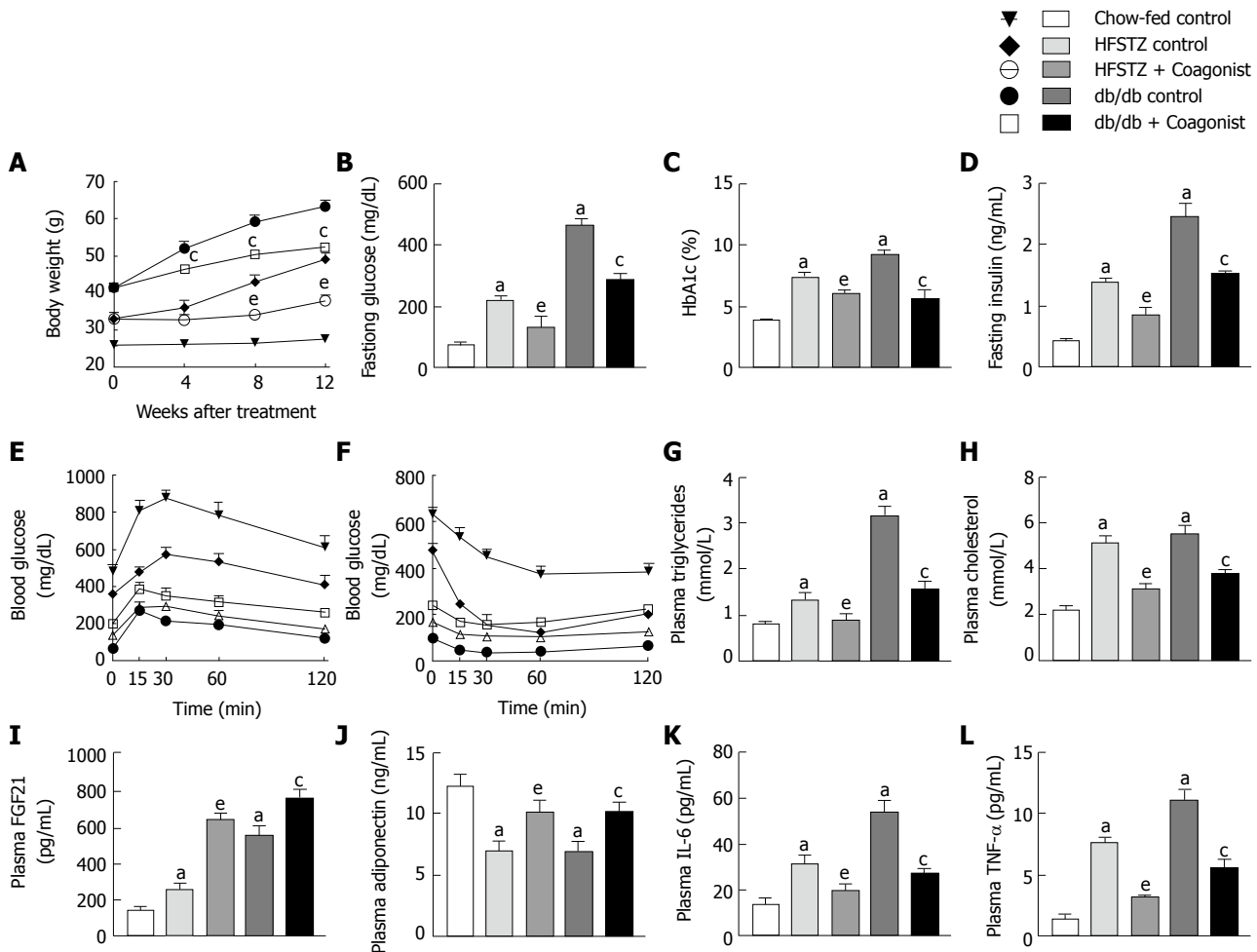
Coagonist improved renal lipid accumulation

Compared with controls, HFSTZ control and db/db control increased renal triglycerides and cholesterol. In addition, these animals showed enhanced expression

Table 3 Plasma biochemistry and kidney weight changes after coagonist treatment in streptozotocin-high-fat and db/db mice

Parameter/treatment	Chow-fed control	HFSTZ control	HFSTZ + Coagonist	db/db mice	db/db mice + Coagonist
Plasma leptin (ng/mL)	6.0 ± 1.2	12.4 ± 2.3 ^a	8.1 ± 2.2 ^e	64.3 ± 8.9 ^a	31.5 ± 5.2 ^c
HOMA-IR	2.0 ± 0.1	19.1 ± 2.5 ^a	7.5 ± 3.1 ^e	70.3 ± 2.8 ^a	27.3 ± 1.7 ^c
AUC of glucose in IPGTT (mg/dL*240 min)	22118.3 ± 1036.3	37436 ± 3526.1 ^a	28419.0 ± 2013.2 ^e	89249.0 ± 6056.1 ^a	59430.3 ± 3437.5 ^c
AUC of glucose in ITT (mg/dL*240 min)	6318.5 ± 972.5	22054.0 ± 784.8 ^a	14171.5 ± 624.7 ^e	51317.3 ± 2501.4 ^a	22548.1 ± 3054.1 ^c
Kidney weight (mg)	248.7 ± 35.6	542.8 ± 41.8 ^a	331.2 ± 41.5 ^e	367.2 ± 55.2 ^a	244.4 ± 51.3 ^c

Data are shown as the mean ± SE. ^a*P* < 0.05, *vs* chow-fed control; ^c*P* < 0.05, *vs* db/db control; ^e*P* < 0.05, *vs* HFSTZ control. HFSTZ: Streptozotocin-high-fat; AUC: Area under curve; IPGTT: Intraperitoneal glucose tolerance test; ITT: Insulin tolerance test; HOMA-IR: Homeostatic model assessment-insulin resistance.



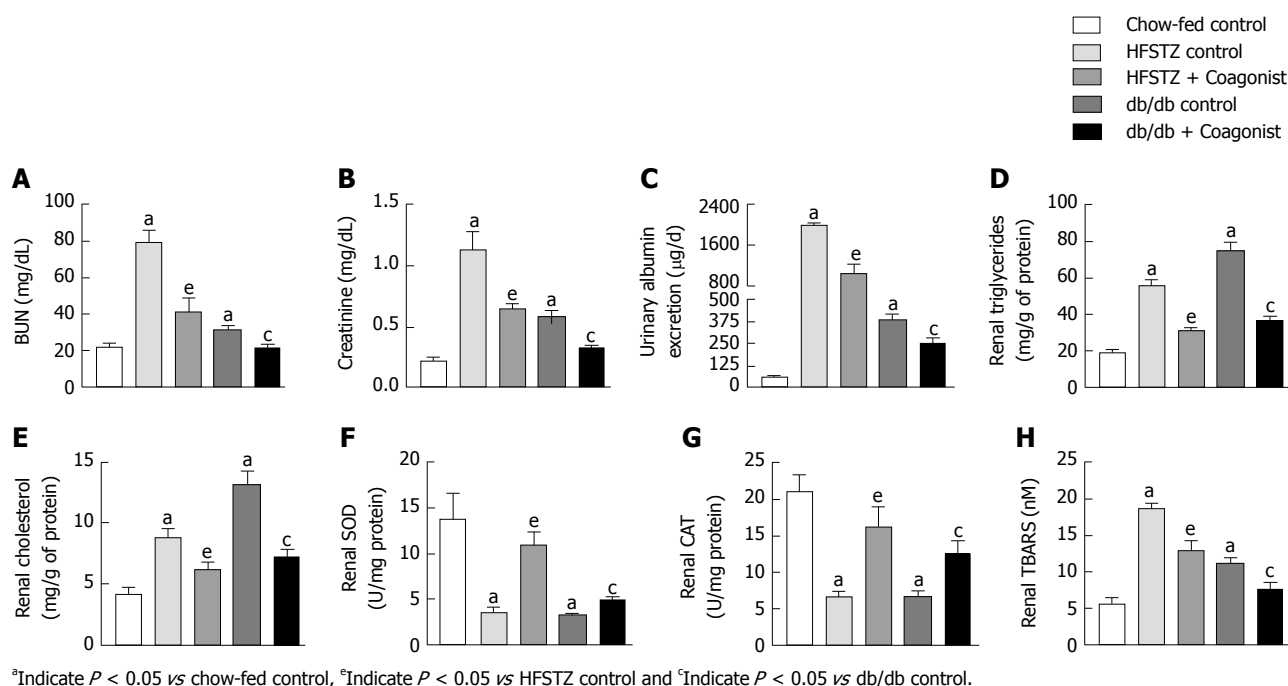
^aIndicate *P* < 0.05 *vs* chow-fed control, ^bIndicate *P* < 0.05 *vs* HFSTZ control and ^cIndicate *P* < 0.05 *vs* db/db control.

Figure 1 Plasma biochemical change after coagonist treatment in streptozotocin-high-fat and db/db mice. A: Body weight; B: Fasting glucose; C: HbA1c; D: Fasting insulin; E: OGTT; F: ITT; G: Plasma triglycerides; H: Plasma cholesterol; I: Plasma FGF21; J: Plasma adiponectin; K: Plasma IL-6; L: Plasma TNF- α . Results are shown as mean \pm SE (*n* = 10) for each group and *P* < 0.05 considered to be statistically significant. HFSTZ: Streptozotocin-high-fat; IPGTT: Intraperitoneal glucose tolerance test; ITT: Insulin tolerance test; HbA1c: Glycosylated hemoglobin; FGF21: Fibroblast growth factor 21; IL-6: Interleukin-6; TNF- α : Tumor necrosis factor α .

of the lipogenic gene including SREBP-1C, FAS, and SCD-1, while decreased β -oxidation gene including CPT-1 and PPAR- α in kidney (Figure 3A-E). Further, HFSTZ control and db/db control have elevated levels of TBARS, while reduced SOD and catalase activity in kidney (Figure 2F-H).

In HFSTZ animals, coagonist treatment reduced accumulation of triglyceride and cholesterol by 43.9%

\pm 2.9% and 29.9% \pm 6.9% in kidney, when compared with HFSTZ control. Coagonist treatment reduced renal expression of SREBP-1C, FAS, and SCD-1, while increased renal expression of CPT-1 and PPAR- α in HFSTZ mice (Figure 3A-E). Coagonist treatment reduced TBARS by 30.6% \pm 6.7%, while increased SOD and catalase activity by 210.4% \pm 39.0% and 145.4% \pm 40.6%, respectively, in the kidney when compared



^aIndicate $P < 0.05$ vs chow-fed control, ^eIndicate $P < 0.05$ vs HFSTZ control and ^cIndicate $P < 0.05$ vs db/db control.

Figure 2 Renal function after coagonist treatment in streptozotocin-high-fat and db/db mice. A: BUN; B: Creatinine; C: Urinary albumin excretion; D: Renal triglyceride; E: Renal cholesterol; F: Renal SOD; G: Renal CAT; H: Renal TBARS. Results are shown as mean \pm SE ($n = 10$) for each group and $P < 0.05$ considered to be statistically significant. HFSTZ: Streptozotocin-high-fat; BUN: Blood urea nitrogen; SOD: Superoxide dismutase; CAT: Catalase; TBARS: Thiobarbituric acid reactive substances.

with HFSTZ control (Figure 2F-H). Similarly, coagonist treatment reduced renal triglycerides and cholesterol by $51.2\% \pm 3.3\%$ and $44.5\% \pm 4.7\%$ in db/db mice (Figure 2D-E). Treatment with coagonist decreased expression of the lipogenic gene including SBREBP-1C, FAS, and SCD-1, while increased expression of the β -oxidation gene including CPT-1 and PPAR- α in these mice (Figure 3A-H). Further, coagonist treatment reduced generation of TBARS by $32.5\% \pm 9.3\%$, while increased SOD and catalase activity by $51.5\% \pm 11.2\%$ and $89.3\% \pm 26.6\%$, when compared with db/db control (Figure 2F-H).

Coagonist ameliorates renal inflammation and fibrosis in db/db and HFSTZ mice

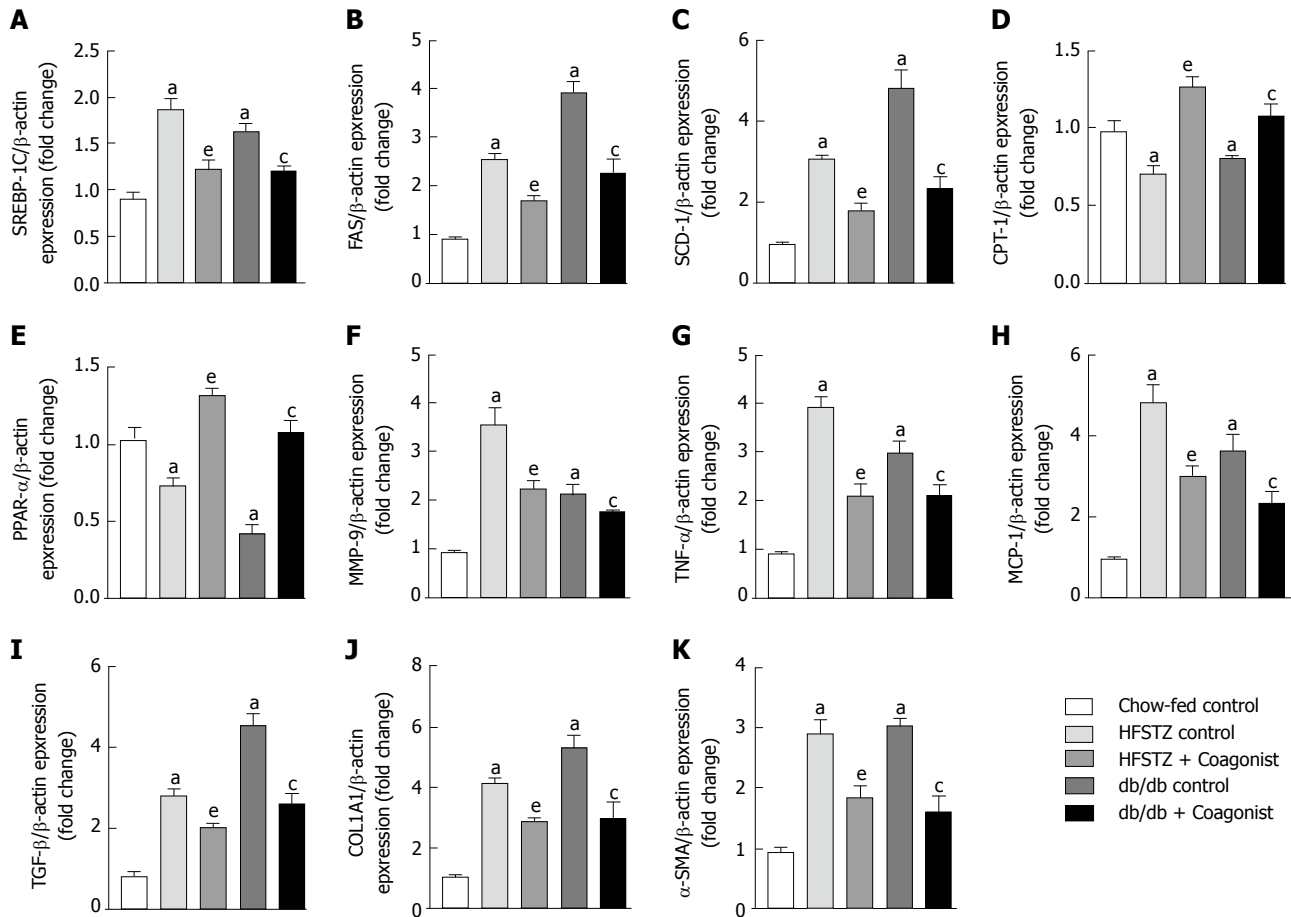
We examined the status of an inflammatory condition in circulation and kidney of HFSTZ and db/db mice. These mice showed an increased level of IL-6 and TNF- α in plasma when compared with chow-fed control (Figure 1K-L). Expression of inflammatory markers including TNF- α , MMP-9 and MCP-1 and fibrotic markers including TGF- β , COL1A1, and α -SMA were increased in liver of HFSTZ control and db/db mice (Figure 3F-K). Thus, kidney weight increased in HFSTZ and db/db control, when compared to chow-fed control.

In HFSTZ mice, coagonist treatment reduced levels of IL-6 by $36.2\% \pm 8.4\%$ and TNF- α by $58.3\% \pm 2.3\%$ in plasma, when compared with HFSTZ control (Figure 1K-L). Treatment with coagonist reduced expression of TNF- α by $42.7\% \pm 7.1\%$, MCP-1 by $37.5\% \pm 5.2\%$, MMP-9 by $37.4\% \pm 4.9\%$, TGF- β by $28.6\% \pm 4.1\%$,

COL1A1 by $30.6\% \pm 2.9\%$ and α -SMA by $36.8\% \pm 7.1\%$, when compared with HFSTZ control (Figure 3F-K). While in db/db mice, coagonist treatment showed similar in IL-6 by $49.1\% \pm 3.8\%$ and TNF- α by $50.2\% \pm 6.9\%$ level in plasma (Figure 3F-K). Renal expression of pro-inflammatory and pro-fibrotic genes including TNF- α , MCP-1, MMP-9, TGF- β , COL1A1 and α -SMA was reduced by $28.9\% \pm 6.8\%$, $35.9\% \pm 8.0\%$, $17.2\% \pm 1.6\%$, $42.9\% \pm 5.8\%$, $44.0\% \pm 10.0\%$ and $47.3\% \pm 8.7\%$, respectively after coagonist treatment in db/db mice (Figure 3F-K). Coagonist treatment reduced kidney weight by $39.1\% \pm 9.2\%$ and $33.4\% \pm 17.2\%$ in HFSTZ and db/db mice, respectively (Table 3). In HFSTZ control and db/db control mice, kidney tissues were characterized by swollen glomeruli and developed more severe glomerulosclerosis and tubulointerstitial fibrosis. Coagonist treatment markedly alleviated glomerular hypertrophy, glomerulosclerosis and collagen deposition in HFSTZ and db/db mice (Figure 4).

DISCUSSION

Diabetic nephropathy (DN) involves renal hypertrophy, fibrosis, and glomerulosclerosis, which leads to renal dysfunction. Diabetes mellitus and obesity are often associated with each other and are the basis for progression DN. The pathogenesis of DN involves both systemic and renal factors. The systemic factors include metabolic dysregulation and inflammation, which aggravates kidney dysfunction. Blocking systemic as well as renal stimuli is thus necessary to prevent det-



*Indicate $P < 0.05$ vs chow-fed control, ^eIndicate $P < 0.05$ vs HFSTZ control and ^cIndicate $P < 0.05$ vs db/db control.

Figure 3 Renal gene expression (relative to β -actin) after coagonist treatment in streptozotocin-high-fat and db/db mice. A: SREBP-1; B: FAS; C: SCD-1; D: CPT-1; E: PPAR- α ; F: MMP-9; G: TNF- α ; H: MCP-1; I: TGF- β ; J: COL1A1; K: α -SMA. Results are shown as mean \pm SE ($n = 10$) for each group and $P < 0.05$ considered to be statistically significant. HFSTZ: Streptozotocin-high-fat; SREBP-1: Sterol regulatory element-binding protein 1; FAS: Fatty acid synthase; SCD-1: Stearoyl-CoA desaturase-1; CPT1: Carnitine palmitoyltransferase 1; PPAR- α : Peroxisome proliferator-activated receptor alpha; MMP-9: Matrix metalloproteinase 9; TNF- α : Tumor necrosis factor-alpha; MCP-1: Monocyte chemoattractant protein 1; COL1A1: Collagen type I: alpha 1 chain; α -SMA: Alfa-smooth muscle actin.

eriation of DN. In this study, we have used HFSTZ and db/db mice as diabetes and obesity-induced renal dysfunction^[29]. Data shows that coagonist of GLP-1R and GCGR markedly reduces glucose and lipid levels in HFSTZ and db/db mice. Coagonist treatment also decreases plasma creatinine, plasma BUN, plasma triglycerides, plasma cholesterol, renal lipids, renal TBARS, plasma IL-6, plasma TNF- α , urinary albumin excretion and kidney weight in HFSTZ and db/db mice. The decrease in these levels was associated with reduction in expression of lipogenic genes, increase in expression of β -oxidation genes and decrease in inflammatory genes including *SREBP-1C*, *FAS*, *SCD-1*, *CPT-1*, *PPAR- α* , *TNF- α* , *MMP-9* and *MCP-1* in kidney. Coagonist increased FGF21 and adiponectin in plasma in HFSTZ and db/db mice. Coagonist treatment also improved renal histopathological and ultrastructural changes in kidney disease models associated with type 2 diabetes. We have also observed that coagonist treatment inhibits the expression of TGF- β , COL1A1, α -SMA, and increases activity of SOD and catalase. As shown in Figure 5, coagonist GLP-1R and GCGR exerts

renoprotective effects by decreasing glycemia and lipids- mediated renal injury.

Most of the obese individuals never develop renal dysfunction, and 25% of obese individuals as "metabolically healthy" suggests that obesity alone may not cause renal dysfunction, but the comorbidity associated with diabetes mellitus enhances deterioration of kidney function^[30]. Previously we have observed that chronic treatment with GLP-1R and GCGR coagonist reduces glucose intolerance, obesity and dyslipidemia in high-fat diet fed mice^[31]. We and others have also observed that feeding high-fat diet for the long term is a mild stimulus for induction of renal dysfunction^[32,33]. In the current study, we have used STZ-HFD in mice to induce overt diabetes and renal dysfunction. In addition, we have used genetically obese and diabetic db/db mice. Both of these animal models showed remarkable kidney lesions and biomarkers of renal dysfunction, oxidative stress, inflammatory and fibrotic changes.

Elevated glucose in chronic diabetes causes the release of various adipokines, chemokines and increases oxidative stress in the body. We have observed that

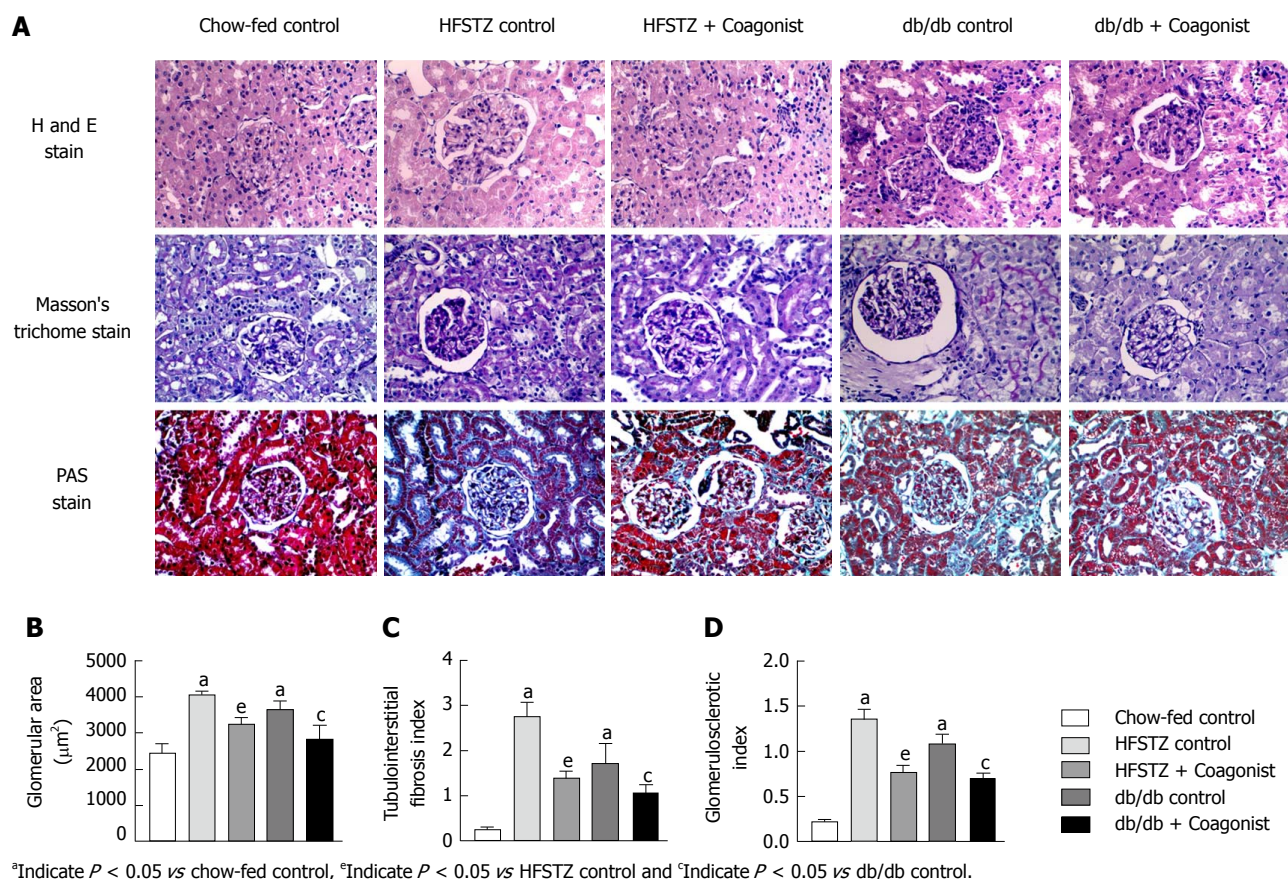


Figure 4 Histology changes in kidney after coagonist treatment in streptozotocin-high-fat and db/db mice. A: Representative photomicrographs of H and E stain, Masson's trichrome stain, and PAS stain; B: Glomerular surface area; C: Tubulointerstitial fibrosis index; D: Glomerulosclerotic index. All stain magnification: $\times 400$. HFSTZ: Streptozotocin-high-fat; H and E: Haematoxylin and Eosin; PAS: Periodic acid-Schiff.

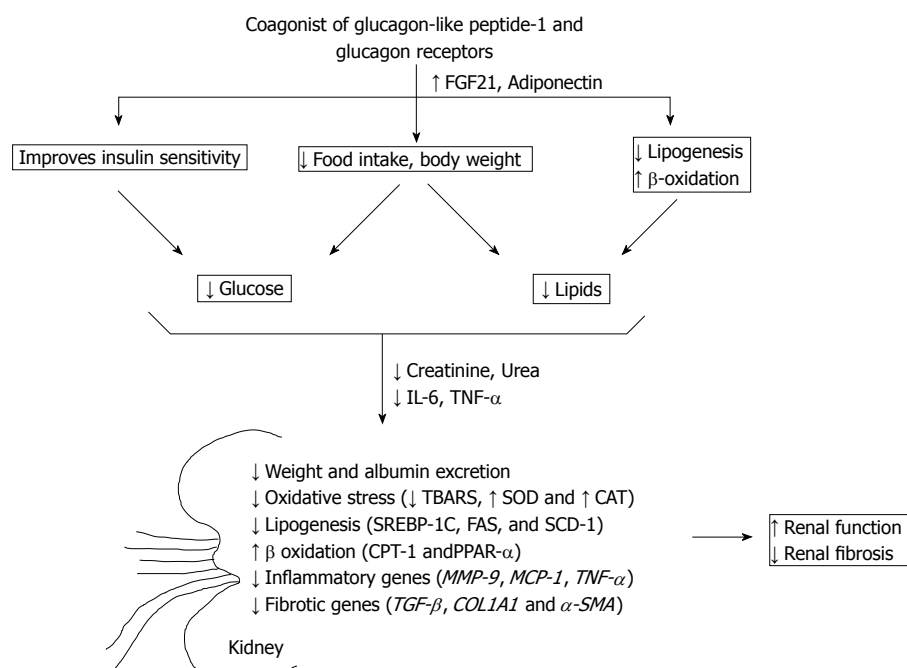


Figure 5 Effect of coagonist of glucagon-like peptide receptor and glucagon receptors on diabetic nephropathy. FAS: Fatty acid synthase; SCD-1: Stearoyl-CoA desaturase-1; TNF- α : Tumor necrosis factor-alpha.

coagonist treatment reduced glucose and improved insulin sensitivity and hence reduced subsequent consequences of elevated glucose. The exact mechanism that causes obesity-induced CKD is not clear. Increased white adipose tissue directly alters kidney function, *via* reduced adiponectin and increased leptin. Adiponectin protects against the development of albuminuria and inhibits oxidative stress, inflammation, and fibrosis in kidney through activation of AMP-activated protein kinase^[34]. We have observed that coagonist treatment increased adiponectin and leptin. Obesity is a FGF21 resistant condition, which reduces glucose uptake in adipose tissue and improves lipid levels. On the other hand, increasing FGF21 levels and sensitivity ameliorates obesity and dyslipidemia^[21,35]. Here, we have observed that coagonist increased FGF21 level, which might have resulted in amelioration of dyslipidemia and obesity.

Diabetes and obesity are known to be associated with hyperlipidemia, which can induce renal dysfunction. Elevated circulating lipids undergo oxidative modification and get entrapped in nephrons and cause glomerular and tubulointerstitial infiltration thus worsening glomerulosclerosis. Coagonist treatment in HFSTZ and db/db mice caused a decrease in dyslipidemia. Coagonist decreased lipogenesis and increased β -oxidation by regulation of SBREBP-1C, FAS, SCD-1, CPT-1 and PPAR- α in kidney. Oxidative stress plays an essential role in the pathogenesis of different types of CKD, especially in diabetic nephropathy. Hyperglycemia induces autooxidation of glucose and glycosylation of proteins by the generation of reactive oxygen species, and increases oxidative stress^[36]. Here, we have observed that coagonist increased SOD and CAT activity, and reduced TBARS content in kidney. Thus, coagonist improved the antioxidant defence and inhibited oxidative stress by lowering glucotoxicity and lipotoxicity.

Oxidative stress induces inflammation, which is an important pathogenetic mechanism of diabetic nephropathy^[37]. In this study, we found that coagonist treatment reduced inflammatory cytokines such as IL-6, MMP-9, TNF- α , and MCP-1. Elevated cytokines levels cause macrophage infiltration and release of pro-fibrotic mediators that promotes chronic inflammation and tissue destruction. In the present study, we observed that coagonist treatment decreased renal expression of TGF- β and COL1A1. TGF- β plays a vital role in fibrotic activation and mesenchymal deterioration of proximal tubule, mainly by α -SMA. It has been reported that adiponectin inhibits TGF- β mediated fibrosis^[38]. Treatment with coagonist attenuated the expression of α -SMA gene in kidney. It could be possible that by increasing adiponectin, coagonist could have suppressed α -SMA gene expression. The histopathological evaluation of kidney revealed increase in glomerular size and fibrosis observed in diabetic nephropathy^[39]. In the present study, using H and E staining of kidney sections, we have observed a marked increase in glomerular surface area in disease control group indicating presence of severe

pathology. Similarly, PAS and Masson's trichome staining which revealed presence of mild glomerulosclerosis with moderate tubulointerstitial fibrosis, characterized by glomerular basement membrane thickening and accumulation of collagen. Treatment with coagonist restored the glomerular surface area and reduced glomerulosclerosis and tubulointerstitial fibrosis.

By reducing glucose and lipid-induced oxidative stress and inflammation, coagonist leads to decrease in urinary albumin excretion rate, creatinine and blood urea nitrogen in HFSTZ and db/db mice. In current study, we have not estimated the actual protein levels of the inflammatory and fibrotic markers after coagonist treatment. Hence, we cannot rule out the possibility that alterations in actual protein and biomarker levels due to changes in body mass may affect creatinine levels. Further studies are required to investigate the role of GLP-1R and GCGR in renal dysfunction with and without associated comorbidity.

Our results demonstrate favorable effect of long-term coagonist treatment on the renal structure of diabetic HFSTZ and db/db mice. In this model of diabetes-induced renal dysfunction, coagonist of GLP-1R and GCGR alleviates structural signs of steatosis and improves renal function by modulation of glucose and lipid-induced renal dysfunction. Thus, these findings indicate that the coagonist of GLP-1R and glucagon receptors protects against diabetes and obesity-induced renal dysfunction. Preclinical study is an important initiation for translational research. Translation of these results in clinical trials would substantiate the therapeutic potential of GLP-1R and GCGR coagonist to ameliorate renal dysfunction associated with diabetes and obesity.

ARTICLE HIGHLIGHTS

Research background

Chronic kidney disease is a major complication for diabetes, mainly caused by lipotoxicity and glucotoxicity. More recent discoveries have strengthened the hypothesis that coagonist of glucagon-like peptide receptor (GLP-1R) and glucagon receptor (GCGR) is a novel therapeutic strategy for the treatment of diabetes and obesity. Preclinical and clinical data indicates that coagonist might have a beneficial effect on diabetic complications such as dyslipidemia and non-alcoholic fatty liver disease, but its potential in treating nephropathy and related complications is not yet investigated.

Research motivation

Diabetes and obesity together enhance the deterioration of renal function. Glucose and lipid both are the major causative factors for the progression of nephropathy associated with diabetes and obesity. Because coagonist reduced both glucose and lipids, it is possible that coagonist may attenuate development of nephropathy associated with diabetes and obesity.

Research objectives

We have investigated the effect of coagonist on renal dysfunction in murine model of diabetes and obesity. These models are widely used model to study diabetic complications and have better clinical translations value.

Research methods

Coagonist was administered to streptozotocin-treated and high-fat diet-fed diabetic mice, chronic high-fat diet fed obese and insulin resistant mice and

genetically diabetic and obese db/db mice. Biochemical, inflammatory and fibrotic markers of renal dysfunction was assessed. Additionally, histological assessment of kidney was performed.

Research results

Coagonist treatment reduced creatinine and urea in blood, inflammatory cytokines in blood, and expression of inflammation and fibrotic genes in kidney. Coagonist also improved histological abnormality in these mice models.

Research conclusions

Coagonist attenuated the development of renal dysfunction by improving glucose and lipid metabolism in murine model of diabetes.

Research perspectives

The results of this study provide evidence that coagonist could be a promising agent for the treatment of diabetic nephropathy. Further studies that assess the effect of coagonist on kidney structure and function in animal model of nephropathy with and without such comorbidity may substantiate our findings and pave the path for clinical translation of the therapeutic effects of GLP-1R and GCGR coagonist.

REFERENCES

- 1 **Eknoyan G.** Obesity, diabetes, and chronic kidney disease. *Curr Diab Rep* 2007; **7**: 449-453 [PMID: 18255009 DOI: 10.1007/s11892-007-0076-5]
- 2 **Amann K, Benz K.** Structural renal changes in obesity and diabetes. *Semin Nephrol* 2013; **33**: 23-33 [PMID: 23374891 DOI: 10.1016/j.semnephrol.2012.12.003]
- 3 **Maric-Bilkan C.** Obesity and diabetic kidney disease. *Med Clin North Am* 2013; **97**: 59-74 [PMID: 23290730 DOI: 10.1016/j.mcna.2012.10.010]
- 4 **Caramori ML, Mauer M.** Diabetes and nephropathy. *Curr Opin Nephrol Hypertens* 2003; **12**: 273-282 [PMID: 12698065 DOI: 10.1097/00041552-200305000-00008]
- 5 **Solini A, Ferrannini E.** Pathophysiology, prevention and management of chronic kidney disease in the hypertensive patient with diabetes mellitus. *J Clin Hypertens* (Greenwich) 2011; **13**: 252-257 [PMID: 21466620 DOI: 10.1111/j.1751-7176.2011.00446.x]
- 6 **Schieppati A, Remuzzi G.** Chronic renal diseases as a public health problem: epidemiology, social, and economic implications. *Kidney Int Suppl* 2005; **98**: S7-S10 [PMID: 16108976 DOI: 10.1111/j.1523-1755.2005.09801.x]
- 7 **Fiorotto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M.** Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med* 1998; **339**: 69-75 [PMID: 9654536 DOI: 10.1056/NEJM199807093390202]
- 8 **Heppner KM, Perez-Tilve D.** GLP-1 based therapeutics: simultaneously combating T2DM and obesity. *Front Neurosci* 2015; **9**: 92 [PMID: 25852463 DOI: 10.3389/fnins.2015.00092]
- 9 **Seufert J, Gallwitz B.** The extra-pancreatic effects of GLP-1 receptor agonists: a focus on the cardiovascular, gastrointestinal and central nervous systems. *Diabetes Obes Metab* 2014; **16**: 673-688 [PMID: 24373150 DOI: 10.1111/dom.12251]
- 10 **Okerson T, Chilton RJ.** The cardiovascular effects of GLP-1 receptor agonists. *Cardiovasc Ther* 2012; **30**: e146-e155 [PMID: 21167014 DOI: 10.1111/j.1755-5922.2010.00256.x]
- 11 **Patel VJ, Joharapurkar AA, Shah GB, Jain MR.** Effect of GLP-1 based therapies on diabetic dyslipidemia. *Curr Diabetes Rev* 2014; **10**: 238-250 [PMID: 24998439 DOI: 10.2174/1573399810666140707092506]
- 12 **Yin QH, Zhang R, Li L, Wang YT, Liu JP, Zhang J, Bai L, Cheng JQ, Fu P, Liu F.** Exendin-4 Ameliorates Lipotoxicity-induced Glomerular Endothelial Cell Injury by Improving ABC Transporter A1-mediated Cholesterol Efflux in Diabetic apoE Knockout Mice. *J Biol Chem* 2016; **291**: 26487-26501 [PMID: 27784780 DOI: 10.1074/jbc.M116.730564]
- 13 **Fujita H, Morii T, Fujishima H, Sato T, Shimizu T, Hosoba M, Tsukiyama K, Narita T, Takahashi T, Drucker DJ, Seino Y, Yamada Y.** The protective roles of GLP-1R signaling in diabetic nephropathy: possible mechanism and therapeutic potential. *Kidney Int* 2014; **85**: 579-589 [PMID: 24152968 DOI: 10.1038/ki.2013.427]
- 14 **Park CW, Kim HW, Ko SH, Lim JH, Ryu GR, Chung HW, Han SW, Shin SJ, Bang BK, Breyer MD, Chang YS.** Long-term treatment of glucagon-like peptide-1 analog exendin-4 ameliorates diabetic nephropathy through improving metabolic anomalies in db/db mice. *J Am Soc Nephrol* 2007; **18**: 1227-1238 [PMID: 17360951 DOI: 10.1681/ASN.2006070778]
- 15 **Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH.** The metabolic actions of glucagon revisited. *Nat Rev Endocrinol* 2010; **6**: 689-697 [PMID: 20957001 DOI: 10.1038/nrendo.2010.187]
- 16 **Patel V, Joharapurkar A, Kshirsagar S, Patel HM, Pandey D, Patel D, Shah K, Bahekar R, Shah GB, Jain MR.** Central and Peripheral Glucagon Reduces Hyperlipidemia in Rats and Hamsters. *Drug Res (Stuttg)* 2017; **67**: 318-326 [PMID: 28445900 DOI: 10.1055/s-0043-102405]
- 17 **Elrick H, Huffman ER, Hlad CJ Jr, Whipple N, Staub A.** Effects of glucagon on renal function in man. *J Clin Endocrinol Metab* 1958; **18**: 813-824 [PMID: 13563607 DOI: 10.1210/jcem-18-8-813]
- 18 **Parving HH, Noer J, Kehlet H, Mogensen CE, Svendsen PA, Heding L.** The effect of short-term glucagon infusion on kidney function in normal man. *Diabetologia* 1977; **13**: 323-325 [PMID: 334617 DOI: 10.1007/BF01223273]
- 19 **Charron MJ, Vuguin PM.** Lack of glucagon receptor signaling and its implications beyond glucose homeostasis. *J Endocrinol* 2015; **224**: R123-R130 [PMID: 25568163 DOI: 10.1530/JOE-14-0614]
- 20 **Day JW, Ottaway N, Patterson JT, Gelfanov V, Smiley D, Gidda J, Findeisen H, Bruemmer D, Drucker DJ, Chaudhary N, Holland J, Hembree J, Abplanalp W, Grant E, Ruehl J, Wilson H, Kirchner H, Lockie SH, Hofmann S, Woods SC, Nogueiras R, Pfluger PT, Perez-Tilve D, DiMarchi R, Tschöp MH.** A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. *Nat Chem Biol* 2009; **5**: 749-757 [PMID: 19597507 DOI: 10.1038/nchembio.209]
- 21 **Patel V, Joharapurkar A, Kshirsagar S, Patel HM, Pandey D, Patel D, Sutariya B, Patel M, Bahekar R, Jain MR.** Balanced Coagonist of GLP-1 and Glucagon Receptors Corrects Dyslipidemia by Improving FGF21 Sensitivity in Hamster Model. *Drug Res (Stuttg)* 2017; **67**: 730-736 [PMID: 28898910 DOI: 10.1055/s-0043-118808]
- 22 **Patel VJ, Joharapurkar AA, Kshirsagar SG, Patel KN, Shah GB, Jain MR.** Therapeutic potential of coagonists of glucagon and GLP-1. *Cardiovasc Hematol Agents Med Chem* 2014; **12**: 126-133 [PMID: 25330114 DOI: 10.2174/1871525712666141019194025]
- 23 **Patel V, Joharapurkar A, Kshirsagar S, Patel M, Sutariya B, Patel H, Pandey D, Patel D, Ranvir R, Kadam S, Bahekar R, Jain M.** Coagonist of glucagon-like peptide-1 and glucagon receptors ameliorates nonalcoholic fatty liver disease. *Can J Physiol Pharmacol* 2018; **9**: 587-596 [PMID: 29406832 DOI: 10.1139/cjpp-2017-0683]
- 24 **Patel V, Joharapurkar A, Kshirsagar S, Sutariya B, Patel M, Patel H, Pandey D, Patel D, Ranvir R, Kadam S, Bahekar R, Jain M.** Coagonist of GLP-1 and glucagon receptor ameliorates development of non-alcoholic fatty liver disease. *Cardiovasc Hematol Agents Med Chem* 2018 [PMID: 29357809 DOI: 10.2174/1871525716666180118152158]
- 25 **Patel V, Joharapurkar A, Dhanesha N, Kshirsagar S, Detroja J, Patel K, Gandhi T, Patel K, Bahekar R, Jain M.** Combination of omeprazole with GLP-1 agonist therapy improves insulin sensitivity and antioxidant activity in liver in type 1 diabetic mice. *Pharmacol Rep* 2013; **65**: 927-936 [PMID: 24145087 DOI: 10.1016/S1734-1140(13)71074-0]
- 26 **Sutariya B, Taneja N, Saraf M.** Betulinic acid, isolated from the leaves of *Syzygium cumini* (L.) Skeels, ameliorates the proteinuria in experimental membranous nephropathy through regulating Nrf2/NF-κB pathways. *Chem Biol Interact* 2017; **274**: 124-137 [PMID: 28711658 DOI: 10.1016/j.cbi.2017.07.011]

- 27 **Sutariya B**, Saraf M. Betanin, isolated from fruits of *Opuntia elatior* Mill attenuates renal fibrosis in diabetic rats through regulating oxidative stress and TGF- β pathway. *J Ethnopharmacol* 2017; **198**: 432-443 [PMID: 28111218 DOI: 10.1016/j.jep.2016.12.048]
- 28 **Mankhey RW**, Bhatti F, Maric C. 17 β -Estradiol replacement improves renal function and pathology associated with diabetic nephropathy. *Am J Physiol Renal Physiol* 2005; **288**: F399-F405 [PMID: 15454392 DOI: 10.1152/ajprenal.00195.2004]
- 29 **Kong LL**, Wu H, Cui WP, Zhou WH, Luo P, Sun J, Yuan H, Miao LN. Advances in murine models of diabetic nephropathy. *J Diabetes Res* 2013; **2013**: 797548 [PMID: 23844375 DOI: 10.1155/2013/797548]
- 30 **Kovesdy CP**, Furth SL, Zoccali C; World Kidney Day Steering Committee. Obesity and Kidney Disease: Hidden Consequences of the Epidemic. *Nephron* 2017; **135**: 243-251 [PMID: 28171864 DOI: 10.1159/000455698]
- 31 **Patel V**, Joharapurkar A, Dhanesha N, Kshirsagar S, Patel K, Bahekar R, Shah G, Jain M. Co-agonist of glucagon and GLP-1 reduces cholesterol and improves insulin sensitivity independent of its effect on appetite and body weight in diet-induced obese C57 mice. *Can J Physiol Pharmacol* 2013; **91**: 1009-1015 [PMID: 24289070 DOI: 10.1139/cjpp-2013-0189]
- 32 **Glastras SJ**, Chen H, Teh R, McGrath RT, Chen J, Pollock CA, Wong MG, Saad S. Mouse Models of Diabetes, Obesity and Related Kidney Disease. *PLoS One* 2016; **11**: e0162131 [PMID: 27579698 DOI: 10.1371/journal.pone.0162131]
- 33 **Kitada M**, Ogura Y, Koya D. Rodent models of diabetic nephropathy: their utility and limitations. *Int J Nephrol Renovasc Dis* 2016; **9**: 279-290 [PMID: 27881924 DOI: 10.2147/IJNRD.S103784]
- 34 **Fukushima M**, Hattori Y, Tsukada H, Koga K, Kajiwarra E, Kawano K, Kobayashi T, Kamata K, Maitani Y. Adiponectin gene therapy of streptozotocin-induced diabetic mice using hydrodynamic injection. *J Gene Med* 2007; **9**: 976-985 [PMID: 17868184 DOI: 10.1002/jgm.1104]
- 35 **Kharitonov A**, Shanafelt AB. FGF21: a novel prospect for the treatment of metabolic diseases. *Curr Opin Investig Drugs* 2009; **10**: 359-364 [PMID: 19337957 DOI: 10.1016/J.APSB.2012.06.011]
- 36 **Mahmoodnia L**, Aghadavod E, Beigrezaei S, Rafieian-Kopaei M. An update on diabetic kidney disease, oxidative stress and antioxidant agents. *J Renal Inj Prev* 2017; **6**: 153-157 [PMID: 28497094 DOI: 10.1517/jrip.2017.30]
- 37 **Navarro-González JF**, Mora-Fernández C, Muros de Fuentes M, García-Pérez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nat Rev Nephrol* 2011; **7**: 327-340 [PMID: 21537349 DOI: 10.1038/nrneph.2011.51]
- 38 **Guo X**, Zhou G, Guo M, Cheung AK, Huang Y, Beddhu S. Adiponectin retards the progression of diabetic nephropathy in db/db mice by counteracting angiotensin II. *Physiol Rep* 2014; **2**: e00230 [PMID: 24744899 DOI: 10.1002/phy2.230]
- 39 **Pourghasem M**, Shafi H, Babazadeh Z. Histological changes of kidney in diabetic nephropathy. *Caspian J Intern Med* 2015; **6**: 120-127 [PMID: 26644877]

P- Reviewer: Koch TR, Martinez-Castelao A **S- Editor:** Ji FF

L- Editor: A **E- Editor:** Wang C



Iranian healthcare professionals' perspectives about factors influencing the use of telemedicine in diabetes management

Haleh Ayatollahi, Nader Mirani, Fatemeh Nazari, Narjes Razavi

Haleh Ayatollahi, Nader Mirani, School of Health Management and Information Sciences, Iran University of Medical Sciences, Tehran 1996713883, Iran

Fatemeh Nazari, Zanzan University of Medical Sciences, Zanzan 4513956111, Iran

Narjes Razavi, School of Nursing and Midwifery, Iran University of Medical Sciences, Tehran 1996713883, Iran

ORCID number: Haleh Ayatollahi (0000-0003-3974-3648); Nader Mirani (0000-0001-9198-0507); Fatemeh Nazari (0000-0002-6939-6053); Narjes Razavi (0000-0003-3373-4505).

Author contributions: Mirani N designed the study and conducted the literature review and analysis, and drafted the manuscript; Ayatollahi H supervised the study and edited the manuscript; Nazari F and Razavi N helped with collecting the data and preparing the manuscript.

Supported by Iran University of Medical Sciences, Tehran, Iran, No. IUMS/SHMIS_94/27219.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Nader Mirani, MSc, Lecturer, School of Health Management and Information Sciences, Iran University of Medical Sciences, No. 6, Shahid Yasami St., Vali-e-Asr St., Tehran 1996713883, Iran. mirani.n@tak.iums.ac.ir
Telephone: +98-21-88794301
Fax: +98-21-88794301

Received: March 8, 2018

Peer-review started: March 8, 2018

First decision: April 4, 2018

Revised: April 30, 2018

Accepted: May 15, 2018

Article in press: May 15, 2018

Published online: June 15, 2018

Abstract

AIM

To identify factors influencing the use of telemedicine in diabetes management from the perspectives of healthcare professionals.

METHODS

This was a quantitative study that was conducted in 2016. The participants were 240 nurses and 55 physicians from three teaching hospitals as well as from one endocrinology and metabolism research center. No sampling method was used and the data were collected by using a five-point (1 to 5) Likert scale questionnaire, which had 37 questions. Descriptive and inferential statistics (Mann-Whitney *U* test) were used to analyze the data.

RESULTS

The findings showed that both physicians (4.06 ± 0.69) and nurses (4.02 ± 0.61) tended to use telemedicine technology for managing diabetes. Overall, the lowest mean value for physicians (3.79 ± 0.82) was related to the compatibility of telemedicine with other clinical activities in diabetes management. For nurses, the lowest mean value pertained to the usefulness of telemedicine in diabetes management (3.99 ± 0.53) and their attitude toward using this technology (3.99 ± 0.65).

CONCLUSION

Although physicians and nurses agreed on using telemedicine technology in diabetes management, it is necessary to consider their concerns prior to the im-

plementation and deployment of new technologies. This approach will help to improve the level of technology acceptance among the users.

Key words: Telemedicine; Diabetes mellitus; Physicians; Nurses

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: In this study, factors influencing the use of telemedicine in diabetes management were identified from the perspectives of Iranian healthcare professionals. The results revealed that both physicians and nurses intended to use telemedicine technology for managing diabetes. However, the compatibility of using telemedicine technology with other clinical activities related to diabetes management and the usefulness of this technology were two main concerns for the healthcare professionals. Therefore, it is essential to pay more attention to these factors and similar ones prior to system design and deployment.

Ayatollahi H, Mirani N, Nazari F, Razavi N. Iranian healthcare professionals' perspectives about factors influencing the use of telemedicine in diabetes management. *World J Diabetes* 2018; 9(6): 92-98 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i6/92.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i6.92>

INTRODUCTION

Diabetes is a major cause of disability and death in the world and more than 10% of the global adult population has been affected by diabetes^[1]. In addition, 59% of all deaths across the world are caused by non-communicable diseases and 9% of them are caused by diabetes^[2]. The Center for Disease Control and Prevention has estimated that 30.3 million Americans suffered from diabetes in 2015, of which 7.2 million were unaware of their disease. The direct and indirect costs of diagnosed diabetes in the United States in 2012 were estimated to be more than \$200 billion^[3].

In Iran, the prevalence of diabetes in urban areas is higher than in rural areas, and women are more affected by diabetes than men^[4]. In the early 2010s, one in every 20 Iranians had diabetes. It is estimated that in 2025, one in every seven Iranians would have the disease^[5]. In addition to the costs and the increasing number of patients, most patients experience unfavorable side effects due to late diagnosis, negligence in controlling the disease, and physical disabilities. As a result, the use of new technologies to manage the disease has been suggested^[6,7]. For example, telemedicine can be used to offer more services to patients regardless of time and place. Thus, patients with certain conditions and those who live in rural areas can benefit a lot from these services without being referred to healthcare centers^[8].

This approach reduces healthcare costs and saves time for patients as well as care providers. In addition, it will enhance independence and self-management among patients with chronic conditions^[9].

Previous studies have shown that the use of information technology, such as telemedicine, telemonitoring, sensors, decision support systems, reminders and teleconsultation is not only economic, but is also effective for managing chronic diseases like diabetes^[6,7,10-16]. Since the World Health Organization has highlighted the alarming situation pertaining to diabetes, especially in developing countries^[12], and advised different countries to take action against the disease, the use of information technology, such as telemedicine, has drawn serious focus in managing diabetes^[13]. However, before applying this technology, it is necessary to pay more attention to providing the necessary infrastructure and equipment along with training people to use telemedicine more efficiently^[11].

In the last decade, many technologies have been developed in the area of healthcare; however, less attention has been paid to the factors that influence the use of these systems^[4]. The use of telemedicine in diabetes management is one of these technologies and it is essential to know factors that influence the use of this technology. For example, the lack of telemedicine policy, inadequacy of knowledge and skills, resistance to change by clinicians^[17], the capacity of telemedicine service providers, performance expectancy (perceived usefulness), effort expectancy (perceived ease of use), and social influences were found to be some of the factors that influence the use of telemedicine in diabetes management^[18]. However, these factors can differ across the world mainly due to legal, cultural, technical, and economic differences^[19]. Identifying these factors in each country can help to strengthen the positive aspects and to overcome the constraining factors in order to implement a project successfully. Therefore, the current study aimed to identify factors that influence the use of telemedicine in diabetes management from the perspective of Iranian healthcare professionals.

MATERIALS AND METHODS

This was a quantitative study which was conducted in 2016. The research participants were 240 nurses and 55 physicians (20 general practitioners and 35 internal medicine specialists and endocrinologists) who worked in three teaching hospitals and one endocrinology and metabolism research center. As the number of potential participants was limited, no sampling method was used. To collect data, a questionnaire was designed based on the literature review^[6,8,9,15-21]. The questionnaire had 37 questions. The first six questions were related to the participants' demographic information and the duration of using a computer by a physician and a nurse, and the next 31 questions to the factors influencing the use of telemedicine technology in diabetes management.

Table 1 Cronbach's alpha for the last six parts of the questionnaire

Questionnaire sections	Cronbach's alpha
Perceived ease of use	0.83
Perceived usefulness	0.81
Intention to use	0.85
Users' attitudes	0.86
Compatibility with other clinical activities	0.80
Security and reliability of technology	0.82

Table 2 Participants' characteristics

Nurses	(%) fx	Physicians	(%) fx
134	F 120 (89.5) M 14 (10.5)	40	F 15 (37.5) M 25 (62.5)
Age (yr)	< 30 50 (37.3) 30-39 53 (39.5) 40-49 21 (15.7) 50-59 10 (7.5)	Age (yr)	< 30 14 (35.0) 30-39 20 (50.0) 40-49 6 (15.0) 50-59 0
Education	BSc 101 (75.3) MSc 33 (24.7)	Education	GP 20 (50) Specialist 20 (50)
Use of Computer	< 1 28 (20.9) 1-2 49 (36.6) > 2 57 (42.5)	Use of Compute	< 1 7 (17.5) 1-2 23 (57.5) > 2 10 (25.0)

These questions were divided into six sections: Perceived ease of use, perceived usefulness, intention to use, the users' attitudes, compatibility with other clinical activities, and security and reliability of telemedicine technology for managing diabetes (Appendix I). The validity of the questionnaire was approved by six experts in the field of health information management, health informatics and nursing. The reliability of the questionnaire was determined by calculating Cronbach's alpha coefficient ($\alpha = 0.83$). Table 1 shows the value of Cronbach's alpha for the last six parts of the questionnaire. The questionnaire was designed based on the Likert scale with the following options: strongly agree (5), agree (4), undecided (3), disagree (2), and strongly disagree (1). Data analysis was performed using SPSS software (version 23.0) and descriptive (mean value and standard deviation) and inferential statistics (Mann-Whitney *U* test) were used to analyze the data.

RESULTS

Initially, 13 incomplete questionnaires were withdrawn. Overall, 134 nurses (55.8%) and 40 physicians (72.5%) participated in the study. The findings showed that the highest frequency of nurses ($n = 120$, 89.5%) were female and the highest frequency of physicians were male ($n = 25$, 62.5%). The highest frequency of physicians ($n = 23$, 57.5%) used computers between one and two hours per day in their clinical practices and the nurses used them for more than two hours per day ($n = 57$, 42.5%). Table 2 shows the participants' characteristics.

According to the results, most of the physicians (4.19 ± 0.40) and nurses (4.60 ± 0.60) thought that telemedicine was an easy to use technology for managing diabetes. While most of the physicians (4.40 ± 0.77) and nurses (4.14 ± 0.86) perceived that the use of telemedicine technology was easy for healthcare providers, the physicians thought that it may not be easy to use for patients with diabetes (3.80 ± 1.06) and the nurses believed that it may not be easy to learn for healthcare providers (3.93 ± 0.91). The results of the Mann-Whitney *U* test showed that there was a statistically significant difference between the perspectives of the physicians and the nurses regarding the perceived ease of learning the technology ($P = 0.004$).

Concerning the technology's usefulness, the findings indicated that most of the physicians (4.17 ± 0.61) and nurses (3.99 ± 0.53) agreed on the usefulness of telemedicine technology in diabetes management. Among physicians (4.47 ± 0.78) and nurses (4.15 ± 0.88), the highest mean value was related to the usefulness of telemedicine technology in reducing unnecessary transportation costs. The lowest mean values for both physicians (3.73 ± 1.13) and nurses (3.78 ± 0.89) were related to improving patient safety after using telemedicine technology. Moreover, there was a statistically significant difference between the mean values calculated for physicians and nurses in terms of the technology's usefulness in reducing healthcare costs in hospitals ($P = 0.007$), in saving time for healthcare providers and patients with diabetes ($P = 0.012$), in reducing costs of patients referrals ($P = 0.021$), and in improving the delivery of healthcare services to the patients with diabetes in remote areas ($P = 0.028$).

In terms of intention to use, the results showed that both physicians (4.22 ± 0.72) and nurses (4.14 ± 0.56) were interested in using telemedicine technology for managing patients with diabetes. Most of the physicians (4.45 ± 0.74) and nurses (4.10 ± 0.81) agreed that using telemedicine for managing diabetes could encourage other healthcare providers to use the technology for other healthcare services as well. For physicians, the lowest mean value (3.95 ± 1.26) was related to the desirable outcomes of telemedicine technology in managing diabetes compared to the traditional methods, such as a face-to-face visit. For nurses, the lowest mean value was related to the use of telemedicine as a basis for applying new treatment methods for managing patients with diabetes (4.04 ± 0.88). The results of the Mann-Whitney *U* test showed that there was a statistically significant difference between the opinions of the physicians and the nurses regarding patient monitoring at any time of the day by using telemedicine technology ($P = 0.016$) and encouraging other healthcare providers to use the technology for other healthcare services ($P = 0.006$).

According to the findings, the attitudes of both physicians (4.08 ± 0.71) and nurses (3.99 ± 0.65) were positive about the use of telemedicine technology in

Table 3 Clinicians' attitudes towards using telemedicine technology in diabetes management

Questions	Groups	Strongly disagree	Disagree	No idea	Agree	Strongly agree	mean \pm SD	P-value
The use of telemedicine is essential for health care providers in managing patients with diabetes	Physicians	0	2 (5.0)	9 (22.5)	15 (37.5)	14 (35.0)	4.03 \pm 0.89	0.57
	Nurses	4 (3.0)	7 (5.2)	24 (17.9)	62 (46.3)	37 (27.6)	3.90 \pm 0.96	
The use of telemedicine in managing diabetes can reduce the burden of the disease	Physicians	0	3 (7.5)	6 (15.0)	16 (40.0)	15 (37.5)	4.08 \pm 0.91	0.459
	Nurses	3 (2.2)	7 (5.2)	17 (12.7)	71 (53.0)	36 (26.9)	3.97 \pm 0.90	
The use of telemedicine in diabetes management can make working with new systems easier in the future	Physicians	0	2 (5.0)	8 (20.0)	17 (42.5)	13 (32.5)	4.03 \pm 0.86	0.824
	Nurses	2 (1.5)	3 (2.2)	19 (14.9)	71 (53.0)	39 (29.1)	4.06 \pm 0.81	
The use of telemedicine in diabetes management can increase the level of satisfaction among healthcare providers	Physicians	18 (45.0)	14 (35.0)	6 (15.0)	2 (5.0)	0	4.20 \pm 0.88	0.094
	Nurses	1 (0.7)	1 (0.7)	24 (17.9)	75 (56.0)	32 (23.9)	4.02 \pm 0.72	

diabetes management. Among physicians, the highest mean value (4.20 ± 0.83) was related to an increase in the level of satisfaction among healthcare providers when using telemedicine technology for managing patients with diabetes. For nurses, the highest mean value (4.06 ± 0.81) was associated with the impact of using telemedicine technology on making the use of other systems easier in the future. In this section, the lowest mean value for both physicians (4.03 ± 0.89) and nurses (3.90 ± 0.96) was related to the necessity of using telemedicine technology for healthcare providers in managing patients with diabetes. The attitudes of the physicians and the nurses toward using telemedicine technology in diabetes management are summarized in Table 3.

The majority of physicians (3.79 ± 0.82) and nurses (4.02 ± 0.71) believed that the use of telemedicine technology was compatible with other related clinical activities in diabetes management. The lowest mean value was related to those physicians (3.60 ± 1.12) and nurses (3.92 ± 0.95) who believed that the use of telemedicine technology could cause problems in the process of care for patients with diabetes. The results of the Mann-Whitney *U* test showed that there was no statistically significant difference between the views of physicians and nurses about the compatibility of telemedicine technology with other related clinical activities in diabetes management.

The findings showed that most of the physicians (3.95 ± 0.78) and nurses (4.02 ± 0.62) perceived telemedicine technology as a secure and reliable technology in the management of diabetes. The majority of physicians (4.40 ± 0.90) believed that security policies and instructions should be provided before using telemedicine in managing diabetes and the lowest mean value (3.78 ± 1.02) was related to those physicians who thought that the risk of privacy breaches could be prevented by using this technology. For nurses, the highest mean value (4.22 ± 0.83) was related to an increase in the level of patient confidentiality by using telemedicine technology and the lowest mean value (3.93 ± 0.95) was related to the technology's security assurance based on the available infrastructure in the

country.

Finally, the findings revealed that both physicians (4.06 ± 0.69) and nurses (4.02 ± 0.61) tended to use telemedicine technology for managing diabetes. Overall, the lowest mean value for physicians (3.79 ± 0.82) was related to the compatibility of telemedicine technology with other clinical activities in diabetes management. For nurses, the lowest mean value pertained to the perceived usefulness of telemedicine technology in diabetes management (3.99 ± 0.53) and their attitude toward using this technology (3.99 ± 0.65). As Table 4 shows, no significant differences were found between the opinions of physicians and nurses in different sections of the questionnaire.

DISCUSSION

The use of technology in the health system has changed the process of healthcare delivery from hospitals and clinics to the daily lives of patients at their homes^[22]. In this regard, the use of technology for managing chronic diseases, such as diabetes, has received particular attention^[23,24]. The findings of the present study showed that physicians and nurses generally agreed on the use of telemedicine in diabetes management. However, some researchers have reported that physicians and nurses were reluctant to use telemedicine^[25].

According to the literature, technological advances and increased computer literacy among clinical staff can help in more effective use of telemedicine and similar systems^[26]. The results of the current study showed that the nurses used computers at their clinical practices more than the physicians, mainly because the nurses used the hospital information system and the nursing information system. Similarly, Asua *et al.*^[25] and Gagnon *et al.*^[16] reported that nurses used computers more than physicians.

In terms of the perceived ease of use of telemedicine technology in diabetes management, the results revealed that physicians and nurses perceived the technology as easy to use. This is in line with Brewster *et al.*^[27]'s study in which the system's ease of use was considered as an important factor for learning how to

Table 4 Comparison between the clinicians' perspectives towards factors influencing the use of telemedicine technology in diabetes management

Factors influencing the use of telemedicine technology in diabetes management	Physicians' opinions (mean \pm SD)	Nurses' opinions (mean \pm SD)	P-value
Perceived ease of use	4.19 \pm 0.54	4.06 \pm 0.60	0.448
Perceived usefulness	4.17 \pm 0.61	3.99 \pm 0.53	0.087
Intention to use	4.22 \pm 0.72	4.07 \pm 0.56	0.078
Users' attitudes	4.08 \pm 0.71	3.99 \pm 0.65	0.488
Compatibility with other related clinical activities	3.79 \pm 0.82	4.02 \pm 0.71	0.083
Perceived security and reliability	3.95 \pm 0.78	4.02 \pm 0.62	0.805

use the system. In another study, the perceived ease of use was regarded as the most important factor in the technology acceptance model^[28]. Similarly, Rho *et al.*^[29] found that a positive perspective on a technology's ease of use led to a positive perception about the technology's usefulness.

In terms of perceived usefulness, the findings showed that compared to nurses, most physicians agreed with the usefulness of telemedicine technology. According to Pai and Huang, the usefulness of technology is associated with increasing job productivity^[30]. Besides, the perceived usefulness of telemedicine is one of the important factors that encourage clinical staff to use the technology in their daily activities^[31]. Therefore, policy-makers and planners should pay more attention to demonstrating the benefits of the technology and to introduce it as an important part of the health system^[32]. The findings of the present study are in line with the findings reported by Palmas *et al.*^[33], in which both physicians and nurses agreed upon the reduction of care costs as well as unnecessary patient transportation by using of telemedicine technology.

The intention to use telemedicine technology in diabetes management was another important factor that influenced the use of technology. According to the literature, the perceived ease of use and the perceived usefulness of telemedicine technology can affect the intention to use the technology. Apart from these factors, personal abilities, availability of resources, and subjective norms are other factors that can help predicting the behavior of users in the future^[34]. The results showed that the nurses were more interested in using telemedicine technology compared to the physicians. The nurses also assumed that the successful implementation of telemedicine technology could be a basis for applying new treatment methods in diabetes management. In Maarop *et al.*^[35]'s study, the intention to use was regarded as being equal to the need for the technology. In another study, Chuttur argued that behavioral intention determined the actual behavior of an individual. In other words, if people express their feelings about something, they will apply them in the real environment^[28].

The findings of the present study revealed that overall, the physicians as well as the nurses had positive attitudes towards using telemedicine technology in diabetes management. Therefore, it can be predicted

that this factor may positively influence the use of the technology in the future. The findings of the present study also showed that the compatibility of telemedicine technology with other daily activities in diabetes management was an important factor for both physicians and nurses. Similarly, in Gagnon *et al.*^[16]'s study, paying more attention to the users' expectations and the actual performances of telemedicine technology were considered to be a major challenge for managing chronic diseases. Accordingly, if activities, workflows, and new technologies are unfitting and incompatible, the technology's acceptance will be affected negatively^[6,25].

In terms of security and reliability of telemedicine technology, some researchers have found that the lack of technology acceptance by users might be due to the lack of confidence in using the new system^[36]. Taylor *et al.* argued that before implementing a new technology, the users should receive full training; otherwise the lack of training may lead to uncertainty and the lack of confidence, which in turn would reduce the level of enthusiasm to use the technology^[37]. According to the findings of the present study, the development of security standards and guidelines and confidentiality of information were the most important challenges from the perspective of healthcare professionals. Similarly, other studies have considered the security of telemedicine technology as one of the most important concerns of clinical staff^[25,29,34,38,39].

Limitations

The current study has some limitations. One of them is related to the limited number of participants, particularly endocrinologists. However, the results showed that in most areas, the perspectives of both physicians and nurses were similar and there was no statistically significant difference between their views. This suggests that the number of participants could not affect the final results.

This study focused on the opinions of physicians and nurses about factors influencing the use of telemedicine technology in diabetes management, but ignored the perspective of patients. Therefore, conducting research to identify factors influencing the use of telemedicine from the perspective of patients is recommended. Moreover, the results showed the perspectives of Iranian healthcare professionals and thus cannot be generalized to other settings or other countries. Similar

studies can be conducted by using the questionnaire in other settings to compare the results.

In conclusion, the use of telemedicine technology is one of the solutions for reducing the costs and increasing the quality of care, particularly for managing chronic diseases. The findings of the present study showed that physicians and nurses intended to use telemedicine technology for managing diabetes. However, the physicians were concerned with the compatibility of telemedicine technology with other clinical activities related to diabetes management, while the nurses were concerned with the technology's usefulness in diabetes management. Therefore, it is essential to pay more attention to these factors and similar ones prior to system design and deployment. This approach will help in improving the level of technology acceptance among the users. The influencing factors identified in the current study can be prioritized in future research in order to enable more focus on the most important ones in the process of system design and implementation.

ARTICLE HIGHLIGHTS

Research background

The use of new technologies to manage diabetes has been suggested and telemedicine is one of these technologies that can be used to offer more services to patients regardless of time and place. However, there are a number of organizational, legal, cultural, technical, and economic factors that may influence the use of telemedicine in managing diabetes.

Research motivation

Less attention has been paid to the factors influencing the use of telemedicine in managing diabetes. These factors can be different in different countries and investigating them can help to strengthen the positive factors and to overcome the constraining factors before implementing a project successfully.

Research objectives

The research objective was to identify factors influencing the use of telemedicine in diabetes management from the perspectives of healthcare professionals in Iran.

Research methods

This was a quantitative study and the research participants included 240 nurses and 55 physicians. To collect data, a five-point Likert scale questionnaire was designed. The data analysis was performed by using descriptive and inferential statistics.

Research results

The findings showed that physicians and nurses intended to use telemedicine technology for managing diabetes. However, the physicians were concerned with the compatibility of telemedicine with other clinical activities related to diabetes management and the nurses were concerned with the technology's usefulness in diabetes management.

Research conclusions

It is essential to pay more attention to the factors influencing the use of telemedicine technology prior to system design and deployment. This approach will help to improve the level of technology acceptance among the users.

Research perspectives

The influencing factors identified in the current study can be prioritized in future research in order to enable more focus on the most important factors during system design and implementation.

REFERENCES

- Standards of Medical Care in Diabetes-2017: Summary of Revisions. *Diabetes Care* 2017; **40**: S4-S5 [PMID: 27979887 DOI: 10.2337/dc17-S003]
- Spratt SE, Batch BC, Davis LP, Dunham AA, Easterling M, Feinglos MN, Granger BB, Harris G, Lyn MJ, Maxson PJ, Shah BR, Strauss B, Thomas T, Califf RM, Miranda ML. Methods and initial findings from the Durham Diabetes Coalition: Integrating geospatial health technology and community interventions to reduce death and disability. *J Clin Transl Endocrinol* 2015; **2**: 26-36 [PMID: 29159106 DOI: 10.1016/j.jcte.2014.10.006]
- Centers for disease control and prevention. National diabetes statistics report: Estimates of diabetes and its burden in the United States. Atlanta: US Department of Health and Human Services, 2017
- Borhani F, Ranjbar H, Abbaszadeh A, Abazari F, Ranjbar F. The effect of telenursing (cell phone software) on A1c hemoglobin in patients with type 2 diabetes mellitus. *JAUMS* 2013; **11**:130-137
- Gavani R, Poursharifi H, Aliasgarzadeh A. Effectiveness of information-motivation and behavioral skill (IMB) model in improving self-care behaviors & HbA1c measure in adults with type2 diabetes in Iran-Tabriz. *Procedia Soc Behav Sci* 2010; **5**: 1868-1873 [DOI:10.1016/j.sbspro.2010.07.380]
- Saigí-Rubió F, Torrent-Sellens J, Jiménez-Zarco A. Drivers of telemedicine use: comparative evidence from samples of Spanish, Colombian and Bolivian physicians. *Implement Sci* 2014; **9**: 128 [PMID: 25293651 DOI: 10.1186/s13012-014-0128-6]
- Stamp KD, Allen NA, Lehrer S, Zagarins SE, Welch G. Telehealth program for medicaid patients with type 2 diabetes lowers hemoglobin A1c. *J Managed Care Med* 2012; **15**: 3-10
- Bashshur RL, Shannon GW, Smith BR, Alverson DC, Antonioti N, Barsan WG, Bashshur N, Brown EM, Coyle MJ, Doarn CR, Ferguson S, Grigsby J, Krupinski EA, Kvedar JC, Linkous J, Merrell RC, Nesbitt T, Poropatich R, Rheuban KS, Sanders JH, Watson AR, Weinstein RS, Yellowlees P. The empirical foundations of telemedicine interventions for chronic disease management. *Telemed J E Health* 2014; **20**: 769-800 [PMID: 24968105 DOI: 10.1089/tmj.2014.9981]
- Istepanian RS, Zitouni K, Harry D, Moutosammy N, Sungoor A, Tang B, Earle KA. Evaluation of a mobile phone telemonitoring system for glycaemic control in patients with diabetes. *J Telemed Telecare* 2009; **15**: 125-128 [PMID: 19364893 DOI: 10.1258/jtt.2009.003006]
- Hsiao JL, Chen RF. An investigation on task-technology fit of mobile nursing information systems for nursing performance. *Comput Inform Nurs* 2012; **30**: 265-273 [PMID: 22156768 DOI: 10.1097/NCN.0b013e31823eb82c]
- Wakefield BJ, Holman JE, Ray A, Scherubel M, Adams MR, Hills SL, Rosenthal GE. Outcomes of a home telehealth intervention for patients with diabetes and hypertension. *Telemed J E Health* 2012; **18**: 575-579 [PMID: 22873700 DOI: 10.1089/tmj.2011.0237]
- Dyck R, Osgood N, Lin TH, Gao A, Stang MR. Epidemiology of diabetes mellitus among First Nations and non-First Nations adults. *CMAJ* 2010; **182**: 249-256 [PMID: 20083562 DOI: 10.1503/cmaj.090846]
- Klug C, Bonin K, Bultemeier N, Rozenfeld Y, Vasquez RS, Johnson M, Cherry JC. Integrating telehealth technology into a clinical pharmacy telephonic diabetes management program. *J Diabetes Sci Technol* 2011; **5**: 1238-1245 [PMID: 22027325 DOI: 10.1177/193229681100500533]
- Jimoh L, Pate MA, Lin L, Schulman KA. A model for the adoption of ICT by health workers in Africa. *Int J Med Inform* 2012; **81**: 773-781 [PMID: 22986218 DOI: 10.1016/j.ijmedinf.2012.08.005]
- Zhai YK, Zhu WJ, Cai YL, Sun DX, Zhao J. Clinical- and cost-effectiveness of telemedicine in type 2 diabetes mellitus: a systematic review and meta-analysis. *Medicine (Baltimore)* 2014; **93**: e312 [PMID: 25526482 DOI: 10.1097/MD.0000000000000312]
- Gagnon MP, Orruño E, Asua J, Abdeljelil AB, Emparanza J. Using a modified technology acceptance model to evaluate healthcare professionals' adoption of a new telemonitoring system. *Telemed*

- J E Health* 2012; **18**: 54-59 [PMID: 22082108 DOI: 10.1089/tmj.2011.0066]
- 17 **Isabalija SR**, Mayoka KG, Rwashana AS, Mbarika VW. Factors affecting adoption, implementation and sustainability of telemedicine information systems in Uganda. *J Health Inform Dev Ctries* 2011; **72**: 299-316
 - 18 **Rho MJ**, Kim HS, Chung K, Cho IY. Factors influencing the acceptance of telemedicine for diabetes management. *Clust Comput* 2015; **18**: 321-331 [DOI: 10.1007/s10586-014-0356-1]
 - 19 **van Dyk L**. A review of telehealth service implementation frameworks. *Int J Environ Res Public Health* 2014; **11**: 1279-1298 [PMID: 24464237 DOI: 10.3390/ijerph110201279]
 - 20 **Dünnebeil S**, Sunyaev A, Blohm I, Leimeister JM, Krcmar H. Determinants of physicians' technology acceptance for e-health in ambulatory care. *Int J Med Inform* 2012; **81**: 746-760 [PMID: 22397989 DOI: 10.1016/j.ijmedinf.2012.02.002]
 - 21 **Holden RJ**, Karsh BT. The technology acceptance model: its past and its future in health care. *J Biomed Inform* 2010; **43**: 159-172 [PMID: 19615467 DOI: 10.1016/j.jbi.2009.07.002]
 - 22 **Maheu M**, Witthen P, Allen A. E-health, telehealth and telemedicine: A guide to start up and success. USA: Jossey-Bass; 2011
 - 23 **Rodrigues JJ**, Torre Diez I, Abajo BS. Telemedicine and E-Health Services, Policies, and Applications: Advancements and developments. USA: IGI Global; 2012 [DOI: 10.4018/978-1-4666-0888-7]
 - 24 **Ayatollahi H**, Hasannezhad M, Fard HS, Haghighi MK. Type 1 diabetes self-management: developing a web-based telemedicine application. *Health Inf Manag* 2016; **45**: 16-26 [PMID: 28691565 DOI: 10.1177/1833358316639456]
 - 25 **Asua J**, Orruño E, Reviriego E, Gagnon MP. Healthcare professional acceptance of telemonitoring for chronic care patients in primary care. *BMC Med Inform Decis Mak* 2012; **12**: 139 [PMID: 23194420 DOI: 10.1186/1472-6947-12-139]
 - 26 **World Health Organization**. Global Observatory for eHealth 2017. Available from: URL: <http://www.who.int/goe/en/>
 - 27 **Brewster L**, Mountain G, Wessels B, Kelly C, Hawley M. Factors affecting front line staff acceptance of telehealth technologies: a mixed-method systematic review. *J Adv Nurs* 2014; **70**: 21-33 [PMID: 23786584 DOI: 10.1111/jan.12196]
 - 28 **Chuttur MY**. Overview of the technology acceptance model: origins, developments and future directions. All Sprouts Content, 2009. Available from: URL: http://aisel.aisnet.org/cgi/viewcontent.cgi?article=1289&context=sprouts_all
 - 29 **Rho MJ**, Choi IY, Lee J. Predictive factors of telemedicine service acceptance and behavioral intention of physicians. *Int J Med Inform* 2014; **83**: 559-571 [PMID: 24961820 DOI: 10.1016/j.ijmedinf.2014.05.005]
 - 30 **Aggelidis VP**, Chatzoglou PD. Using a modified technology acceptance model in hospitals. *Int J Med Inform* 2009; **78**: 115-126 [PMID: 18675583 DOI: 10.1016/j.ijmedinf.2008.06.006]
 - 31 **Yeh RKJ**, Teng JTC. Extended conceptualization of perceived usefulness: empirical test in the context of information system use continuance. *Behav Inform Technol* 2012; **31**: 525-540 [DOI:10.1080/0144929X.2010.517272]
 - 32 **Pai FY**, Huang KI. Applying the technology acceptance model to the introduction of healthcare information systems. *Technol Forecast Soc* 2011; **78**: 650-660 [DOI: 10.1016/j.techfore.2010.11.007]
 - 33 **Palmas W**, Shea S, Starren J, Teresi JA, Ganz ML, Burton TM, Pashos CL, Blustein J, Field L, Morin PC, Izquierdo RE, Silver S, Eimicke JP, Lantigua RA, Weinstock RS; IDEATel Consortium. Medicare payments, healthcare service use, and telemedicine implementation costs in a randomized trial comparing telemedicine case management with usual care in medically underserved participants with diabetes mellitus (IDEATel). *J Am Med Inform Assoc* 2010; **17**: 196-202 [PMID: 20190064 DOI: 10.1136/jamia.2009.002592]
 - 34 **San A**, Yee C. The modified technology acceptance model for private clinical physicians: A case study in Malaysia, Penang. *Int J Acad Res Bus Soc Sci* 2013; **3**: 380-403 [DOI:10.1051/mateconf/201815401101]
 - 35 **Maarop N**, Win KT. The interplay of environmental factors in the acceptance of teleconsultation technology: A mixed methods study. *Open Int J Inform* 2011; **1**:46-58
 - 36 **Marcolino MS**, Maia JX, Alkmim MB, Boersma E, Ribeiro AL. Telemedicine application in the care of diabetes patients: systematic review and meta-analysis. *PLoS One* 2013; **8**: e79246 [PMID: 24250826 DOI: 10.1371/journal.pone.0079246]
 - 37 **Taylor J**, Coates E, Brewster L, Mountain G, Wessels B, Hawley MS. Examining the use of telehealth in community nursing: identifying the factors affecting frontline staff acceptance and telehealth adoption. *J Adv Nurs* 2015; **71**: 326-337 [PMID: 25069605 DOI: 10.1111/jan.12480]
 - 38 **Garg V**, Brewer J. Telemedicine security: a systematic review. *J Diabetes Sci Technol* 2011; **5**: 768-777 [PMID: 21722592 DOI: 10.1177/193229681100500331]
 - 39 **Ayatollahi H**, Sarabi FZ, Langarizadeh M. Clinicians' Knowledge and Perception of Telemedicine Technology. *Perspect Health Inf Manag* 2015; **12**: 1c [PMID: 26604872]

P- Reviewer: Omboni S S- Editor: Wang JL L- Editor: A
E- Editor: Wang C



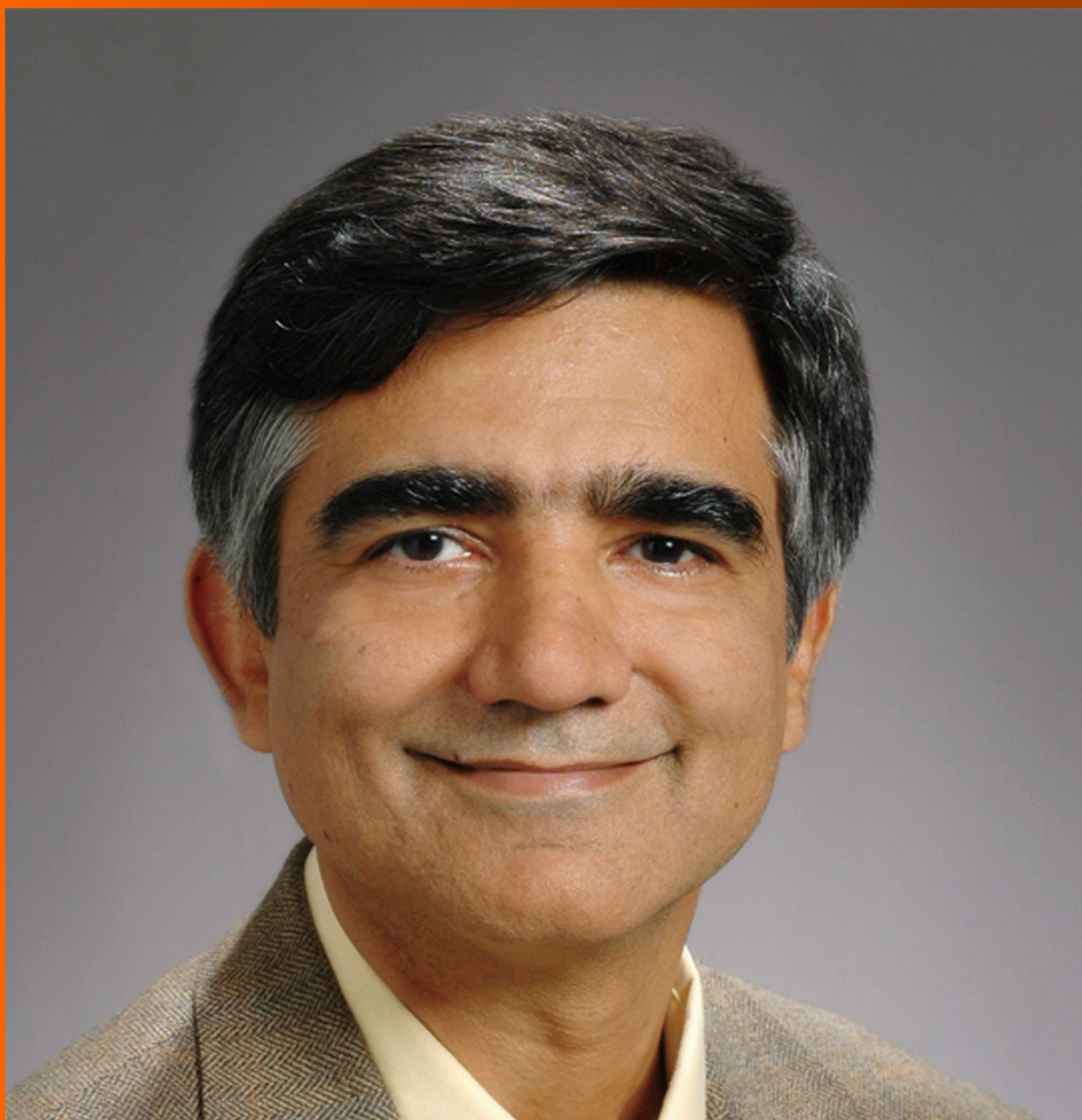


Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 July 15; 9(7): 99-137



**REVIEW**

- 99 Circadian dysrhythmia-linked diabetes mellitus: Examining melatonin's roles in prophylaxis and management

Onaolapo AY, Onaolapo OJ

- 115 Role of bisphosphonates in the management of acute Charcot foot

Durgina H, Sahoo J, Kamalanathan S, Palui R, Sridharan K, Raj H

MINIREVIEWS

- 127 Reversibility of diabetes mellitus: Narrative review of the evidence

Ang GY

ORIGINAL ARTICLE**Retrospective Study**

- 132 New-onset diabetes after kidney transplantation: Incidence and associated factors

Gomes V, Ferreira F, Guerra J, Bugalho MJ

Contents

World Journal of Diabetes
Volume 9 Number 7 July 15, 2018

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Omar Ali, MD, Associate Professor, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53226, United States

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, and PubMed Central.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Wen-Wen Tan*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITOR-IN-CHIEF
Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com/1948-9358/editorialboard.htm>

www.wjgnet.com/1948-9358/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE
July 15, 2018

COPYRIGHT
© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.f6publishing.com>

Circadian dysrhythmia-linked diabetes mellitus: Examining melatonin's roles in prophylaxis and management

Adejoke Y Onaolapo, Olakunle J Onaolapo

Adejoke Y Onaolapo, Behavioural Neuroscience/Neurobiology Unit, Department of Anatomy, Ladoke Akintola University of Technology, Ogbomosho 210211, Oyo State, Nigeria

Olakunle J Onaolapo, Behavioural Neuroscience/Neuropharmacology Unit, Department of Pharmacology, Ladoke Akintola University of Technology, Osogbo 230263, Osun State, Nigeria

ORCID number: Adejoke Y Onaolapo (0000-0001-7126-7050); Olakunle J Onaolapo (0000-0003-2142-6046).

Author contributions: Onaolapo AY and Onaolapo OJ contributed to writing sections of the review article, were also both responsible for the critical revision, editing, and the final approval of the final version.

Conflict-of-interest statement: Both authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Olakunle J Onaolapo, PhD, Professor, Behavioural Neuroscience/Neuropharmacology Unit, Department of Pharmacology, Ladoke Akintola University of Technology, PMB 4400, Osogbo 230263, Osun State, Nigeria. olakunleonaolapo@yahoo.co.uk
Telephone: +234-703-1986101

Received: April 16, 2018

Peer-review started: April 16, 2018

First decision: May 24, 2018

Revised: June 1, 2018

Accepted: June 8, 2018

Article in press: June 8, 2018

Published online: July 15, 2018

Abstract

Diabetes mellitus is a chronic, life-threatening metabolic disorder that occurs worldwide. Despite an increase in the knowledge of the risk factors that are associated with diabetes mellitus, its worldwide prevalence has continued to rise; thus, necessitating more research into its aetiology. Recent researches are beginning to link a dysregulation of the circadian rhythm to impairment of intermediary metabolism; with evidences that circadian rhythm dysfunction might play an important role in the aetiology, course or prognosis of some cases of diabetes mellitus. These evidences thereby suggest possible relationships between the circadian rhythm regulator melatonin, and diabetes mellitus. In this review, we discuss the roles of the circadian rhythm in the regulation of the metabolism of carbohydrates and other macronutrients; with emphasis on the importance of melatonin and the impacts of its deficiency on carbohydrate homeostasis. Also, the possibility of using melatonin and its analogs for the "prophylaxis" or management of diabetes mellitus is also considered.

Key words: Chronobiology; Dysmetabolism; Insulin; Pancreatic beta cell; Melatonin receptors

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Diabetes mellitus is a chronic, life-threatening metabolic disorder with a huge disease burden and rising global prevalence that is nearing epidemic proportions. Research has continued to reveal the importance of circadian rhythm and the neurohormone melatonin in the regulation of carbohydrate metabolism. More studies are also revealing the potential roles of melatonin in the pathogenesis, management and mod-

ulation of the course of diabetes mellitus; especially type 2 diabetes mellitus. Presently, an array of potential mechanisms exists for melatonin's roles in diabetes mellitus; however, a complete picture of this is yet to emerge.

Onaolapo AY, Onaolapo OJ. Circadian dysrhythmia-linked diabetes mellitus: Examining melatonin's roles in prophylaxis and management. *World J Diabetes* 2018; 9(7): 99-114 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i7/99.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i7.99>

INTRODUCTION

Diabetes mellitus is a chronic, life-threatening metabolic disorder with a huge disease burden and rising global prevalence that is nearing epidemic proportions^[1]. According to the World Health Organisation (WHO) diabetes factsheet (which was updated in November 2017), in 2014, 8.5% of adults aged 18 years and older had diabetes mellitus; also, diabetes mellitus accounted directly for about 1.6 million deaths in 2015^[1]. There have also been projections that diabetes mellitus will be the seventh leading cause of death by 2030^[1,2]. As a disorder, diabetes mellitus is associated with increasing morbidity; accounting for a two- to three-fold increase in the risk of cardiovascular and cerebrovascular disease amongst adults^[3]. About 2.6% of global blindness has been attributed to diabetes mellitus^[4], and it has also been reported to be a leading cause of chronic kidney disease^[5].

Presently, management of type 1 diabetes mellitus (T1DM) relies largely on insulin replacement, while that of T2DM is largely dependent on the use of drugs belonging to classes such as biguanides, sulfonylureas, meglitinides, intestinal brush border glucosidase inhibitors and thiazolidinediones. However, cost is a major limitation to the use of drugs (especially in low-income countries); also, there is the risk of side-effects like weight gain, heart failure and gastrointestinal disturbances^[6]. The need to drastically reduce the global prevalence of T2DM necessitates a widening of the search for aetiological factors; and over the last two decades, a growing body of evidence has increasingly suggested the role of the biological clock and multiple clock genes in metabolic homeostasis. Data from epidemiological studies have also shown a correlation between circadian dysregulation (due to urbanisation and/or shift-work) and an increase in the prevalence of cardiovascular disease, cancers, inflammatory disorders, obesity, and diabetes mellitus^[7-9]. Along this line, both human and rodent studies have demonstrated such relationships. Scheer *et al.*^[10] examined the effects of circadian misalignment between the behavioural cycle (feeding/fasting, sleep/wake) and the endogenous circadian rhythm, on metabolic and endocrine predictors of obesity, diabetes, and cardiovascular risk in humans; in their study, they demonstrated that circadian misalignment that occurs acutely (with jet lag) or chronically (with shift-work) was associated with an increased cardiometabolic risk^[10]. Genetic polymorphisms involving circadian clock genes and/or circadian locomotor output cycles *kaput* genes have been linked to the development of metabolic syndrome, obesity, T2DM and hypertension^[11-14]. *In-vivo* or *in-vitro* rodent studies have also demonstrated a possible link between the disruption of the circadian rhythm^[15] or disruption of certain components of the clock genes^[16] and the development of hypoinsulinaemia and T2DM.

Evidences demonstrating the importance of chronobiology in intermediary metabolism and the development of diabetes mellitus have also raised questions about the impact that melatonin (a regulator of the circadian rhythm) and its receptors may have on the aetiology, prognosis, prevention and treatment of diabetes mellitus. Certain studies in rodents have reported that melatonin inhibits insulin secretion from beta-cells *via* its interactions with MT1 and/or MT2 receptors on the beta cell-surface^[17]; however, in humans, studies using reverse transcription-polymerase chain reaction demonstrated that human islets expressed mRNAs coding for both melatonin (MT1 and MT2) receptors^[18]. Results of single-cell microfluorimetry have also suggested that the expression of MT1 receptor mRNA occurred only on alpha-cells and not on beta-cells^[19]. An infusion of exogenous melatonin into dissociated human islet cells and perfused human islets, increased intracellular calcium and glucagon secretion respectively^[19]. Genetic mapping and genome-wide association studies have also demonstrated strong associations between the gene for melatonin type 2 receptor (*MTNR1B*) which is expressed in the pancreatic beta-cells (amongst other tissues), and an increased risk for T2DM^[18,20,21]. Again, there have been reports of impaired glucose tolerance following acute melatonin administration^[22]. However, a few other studies have also demonstrated that melatonin receptor signalling in β -cell reduced oxidative stress response, militated against proteotoxicity-induced β -cell apoptosis, and restored glucose-stimulated insulin secretion in normal islets exposed to chronic hyperglycaemia or in type 2 diabetes islets^[23].

There is a growing body of knowledge associating alterations in circadian rhythms, circadian genes, melatonin and melatonin receptors with derangement of intermediary metabolism and the development of diabetes mellitus. While the implication of this advance in knowledge for the prevention and therapeutic management of diabetes mellitus is evolving, there are strong indications that β -cell melatonin receptor 2 signalling is relevant for the regulation of β -cell survival and function; and by extension, may also be important in T2DM^[23]. In this review, we examine relevant literature for the roles of the circadian rhythm in the physiological regulation of carbohydrates, with emphasis on the importance of melatonin in this capacity. The impacts of melatonin deficiency on carbohydrate homeostasis

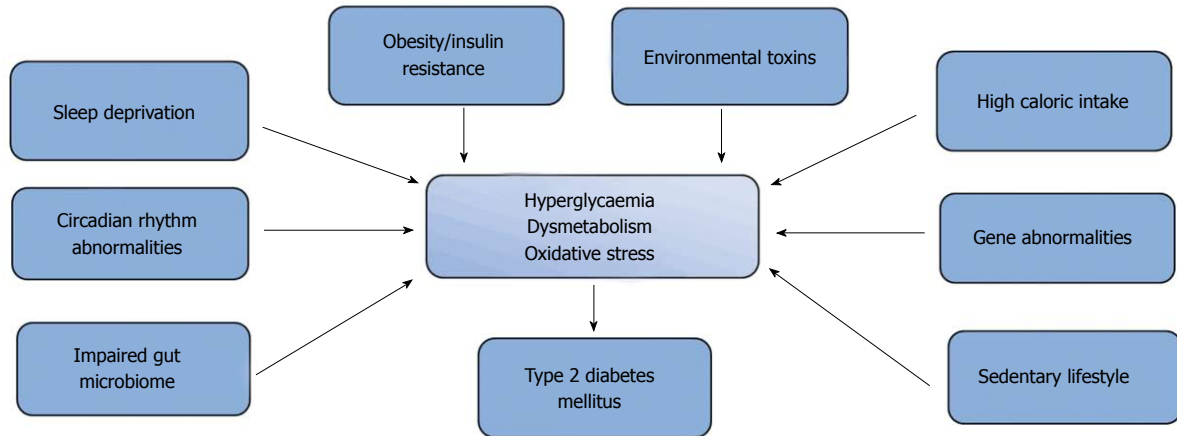


Figure 1 Pathophysiology of type 2 diabetes mellitus.

are also discussed. Finally, the possibility of utilising melatonin and its analogues for the “prophylaxis” and treatment of diabetes mellitus are also considered.

Pathogenesis and molecular basis of T2DM

T2DM, which is characterised by impaired insulin secretion (or sensitivity) and hyperglycaemia, has been reported to account for greater than 90% of the total diabetes mellitus case-load^[24,25]. It is a polygenic metabolic disorder that results from the interaction of environmental and genetic factors (Figure 1). These factors include obesity^[26,27], sedentary lifestyle, high-calorie diet^[28] and sleep deprivation^[29]. Recently, reports from epidemiological and animal studies have suggested that increased presence of endocrine disruptors like pesticides, dioxins and bisphenol A in the environment may predispose to insulin resistance, alteration of β -cell function and impairment of glucose homeostasis^[30]. While reports from genome-wide association studies have demonstrated strong associations between T2DM and over 100 gene variants that are located at four loci^[31]; the peroxisome proliferator-activated receptor gamma gene (*PPARG*), which encodes the nuclear receptor $PPAR-\gamma$ was the first candidate gene associated with T2DM^[32]. Variants of this gene that are expressed in adipose tissue have also been linked to increased transcriptional activity, increased insulin sensitivity and protection against T2DM^[22,32]. A number of candidate gene variants including the E23K polymorphisms in *KCNJ11* and P12A in *PPARG* (that have been associated with an increased risk for T2DM)^[33] have also been discovered through candidate association studies^[34,35]. Studies have also shown that loss-of-function mutations involving *KCNJ11* and *ABCC8* candidate genes are implicated in hyperinsulinemia in infancy^[36].

There have been reports that gut microbiota are important in the maintenance of gastrointestinal mucosa permeability, metabolism of dietary polysaccharides (to produce short-chain fatty acids) and the regulation of fat accumulation^[37]. These functions make them crucial to the development of obesity and obesity-related di-

seases^[38]. Differences in gut microbiome between lean and obese subjects have also been reported^[39]; with suggestions that an important role exists for gut bacteria (and possibly their end-products) in intermediary metabolism. Studies have also demonstrated that alteration in gut microbiota is associated with the development of T2DM and its complications^[40]. The importance of gut microbiome to T2DM is affirmed by studies that have shown that transplantation of faecal microbiome from lean donors to subjects with insulin-resistance results in beneficial metabolic changes^[41]. Studies in rodents have also demonstrated that modulation of the gut microbiome may also be beneficial in T2DM management^[42].

A growing body of evidence suggests an important role for adipose tissue and lipotoxicity in T2DM. Presently, adipose tissue is considered an endocrine organ which influences lipid and glucose metabolism^[43,44]. Dysfunctional adipose tissue (characterised by adipocyte hypertrophy, impaired insulin signalling and insulin resistance) results in the release of inflammatory adipokines and large amounts of free fatty acids; causing fat accumulation and lipotoxicity in organs involved in glucose metabolism such as liver, muscle and pancreatic beta cells^[45-47]. Reports from a number of human and animal studies have also demonstrated the importance of brown adipose tissue in glucose homeostasis and the regulation of energy expenditure; with the possibility of brown adipose tissue becoming a therapeutic target^[48-50].

Another area of extensive research into the pathophysiology of T2DM is the role that oxidative stress plays in the pathogenesis of micro- and macro-vascular diabetic complications^[51]. It is believed that oxidative stress (*via* a common mechanism that involves the production of superoxide, and the inactivation of endothelial nitric oxide synthase and prostacyclin synthase) leads to the development of β -cell dysfunction, insulin resistance, impaired glucose tolerance, and T2DM^[52,53]. There have also been suggestions of the involvement of this common mechanism in the development of both microvascular and macrovascular complications as-

sociated with T2DM^[53,54]. Studies have also shown that T2DM associated increase in oxidative stress occurs as a consequence of hyperglycaemia, hyperinsulinaemia, insulin resistance, and dyslipidaemia^[51].

CIRCADIAN RHYTHM

The circadian rhythms can be defined as endogenous rhythms (with behavioural and physiological components) that have a periodicity of about 24 h, and are synchronised through both photic and nonphotic stimuli^[55]. These rhythms are known to control important biological processes, including sleep-wake cycle, hormone secretion, body temperature regulation, feeding/energy homeostasis, and cell-cycle regulation^[55]. The circadian system is composed of a master clock which is located in the suprachiasmatic nucleus (SCN) of the hypothalamus and a number of peripheral clocks, which together regulate daily variations in many biological processes^[56]. The suprachiasmatic nucleus is responsible for generating the circadian rhythms and as such is referred to as the endogenous biological pacemaker^[57]. Daily adjustments of the timing of the SCN following exposure to stimuli (*zeitgebers*) which signals time of day helps to achieve synchrony with the earth's rotation. A loss of the coordination of these rhythms is known to negatively impact body physiology and behaviours^[55].

Anatomically, the SCN is a bilateral structure that contains over 20000 neurons and is a central component of the circadian timing system^[56]. It receives input pathways for light and other stimuli that are important in the synchronisation of the pacemaker to the environment; output rhythms are in turn regulated by the pacemaker^[55]. Direct (retinohypothalamic) and indirect (retinogeniculate) photic information to the SCN comes from the retina^[58]. Retinohypothalamic photic information originates from the ganglion cells of the retina (which contain melanopsin, and are regarded as the primary photoreceptors for the circadian system), nonphotic information comes from the raphe nuclei, while other afferents come from the pons, medulla, basal forebrain and posterior hypothalamus^[55]. Arising from the SCN, major efferents project to areas such as the hypothalamus (dorsomedial, subparaventricular zone and the paraventricular nucleus), thalamus, preoptic/retrochiasmatic areas, stria terminalis, lateral septum, and intergeniculate nucleus^[55]. Gamma-amino butyric acid is the dominant neurotransmitter that is found in the SCN; however, the SCN core contains vasoactive intestinal polypeptide, gastrin-releasing peptide and bombesin-containing neurons, while somatostatin and neurophysin are predominant within the shell^[55].

Circadian timing is affected by several *zeitgebers* including light, feeding schedules, activity, and the hormone melatonin; of these, light is considered of utmost importance, and the most potent stimulus^[55]. Light also modulates pineal gland melatonin secretion through

regulation by the SCN, with peak secretion occurring in the middle of the night^[57]. Another important marker of internal time (especially during periods of low ambient light) is the circadian rhythm of pineal melatonin. The timing of the endogenous circadian rhythm can be determined by dim light melatonin onset (DLMO) which is regarded as a stable marker of the circadian phase^[57]. Melatonin is also associated with the maintenance of sleep propensity rhythm in humans, and as such, it is considered a modulator of internal sleep^[59]. There is also evidence suggesting that exogenous melatonin can induce phase shifts in the circadian clock^[59].

The genetic control of the circadian rhythms is determined by a core set of *clock* genes which interact with their own products to form a number of molecular feedback loops, which regulate the circadian rhythm^[60]. These genes include three *period* (*Per*) *homolog* genes (*Per1*; *Per2*; *Per3*), two plant cryptochrome gene homologs (*Cry1* and *Cry2*), the circadian locomotor output cycles kaput gene (*Clock*) and the cycle gene (*Bmal1*)^[60]. The interactions of these genes and their products form transcription-translation (molecular) feedback loops that generate the circadian rhythm, and also controls the temporal expression of a number of clock-controlled genes^[61].

Circadian rhythm dysregulation and intermediary metabolism

The circadian rhythm is a conserved timing system that modulates behavioural and physiological process to 24-h environmental cycles^[55,62]. It is generally accepted that the circadian rhythm depends on *zeitgebers* or cues for the daily adjustments of its timing; as such, daily cycles of activity/feeding and the biological/molecular rhythm assist in the maintenance of energy homeostasis, linking the circadian clock to metabolic systems^[63]. It is known that the molecular clock is present in all metabolic tissues including the liver, intestine, adipose tissue, heart, and retina^[62]. This master clock in the SCN works in synchrony with the peripheral clocks, and together, they regulate cellular and physiological functions^[64]. Some of these functions which include metabolism and energy homeostasis occur through organs such as the liver, and other peripheral tissues. A part of this task is achieved by regulating the expression and/or activity of certain key metabolic enzymes and transport systems that are involved in the lipogenic and adipogenic pathways^[64,65]. However, this relationship is bidirectional, with the metabolic enzymes and transcription activators also interacting with and affecting the clock mechanism. An understanding of this relationship is crucial to appreciating how abnormalities such as mutations in clock genes can disrupt cellular rhythmicity and metabolic homeostasis. Also, clinical studies that focus on shift workers and obese patients further illuminate the link between the circadian clock and energy metabolism^[64,65].

There are strong indications that circadian misa-

lignment (or dysfunction) is an emerging risk factor for metabolic diseases^[62]. Studies have shown that variations in diet or dietary intake may influence the circadian rhythm of feeding/activity; and this in turn modulates the biological or molecular clock^[63]. A number of studies have also associated circadian rhythm disruption and sleep loss/deprivation with obesity^[66,67]. Studies in humans who are on night-time shift work also demonstrated that strong associations exist between alterations in circadian rhythm and metabolic parameters such as increased body mass, increased plasma lipid, and glucose levels^[68-70]. Karatsoreos *et al.*^[71] reported that chronically housing mice in an environment with shortened light/dark cycle resulted in weight gain, alteration of body temperature rhythms, and increased plasma levels of leptin and insulin^[71]. Several disorders relating to human psychology and sleep have also been associated with abnormal functioning of the master biological clock. A number of the core hormones that are involved in nutrient metabolism (including insulin, glucagon, adiponectin, corticosterone, leptin and ghrelin) have been shown to undergo circadian oscillation in their levels and activities^[72-74]. Studies have also demonstrated that the molecular clock controls mitochondrial posttranslational modification and oxidative metabolism^[75]. The molecular clock controls cellular metabolism through its ability to direct the rhythmic synthesis of nicotinamide adenine dinucleotide (NAD⁺), which is a metabolic cofactor. NAD⁺ subsequently modulates the activity of the protein deacetylase, sirtuin 1 (SIRT1), which controls cellular metabolism *via* a feedback loop^[76-78]. These nutrient sensors relay information about the cellular nutrient status to the circadian clock, and modulate the activity of clock genes. For example, while the oxidised forms of sodium dehydrogenase (NAD⁺) redox co-factor inhibits the activity of heterodimers of circadian clock genes like *Clock/Bmal1* and *Npas2/Bmal1*; the reduced forms (NADH) increases their activity^[79]. Others, like AMP kinase have also been shown to regulate expression of clock genes^[80-83]. Studies in which deletions or mutations in the clock genes result in disruption of the cellular rhythm also provide strong evidence of the cross-talk that occurs between the circadian clock and metabolism.

There are also reports suggesting that key proteins may be involved in the regulation of the core clock mechanism and adipose tissue metabolism; thereby linking the circadian rhythms with lipid metabolism^[65]. The role of the circadian clock in the regulation of adipose tissue differentiation has been considered^[84]. *In-vitro* and *in-vivo* studies have also been used to examine the role of the circadian rhythm in adipocyte physiology. Studies involving cell lines in which clock genes transcription factors like *Bmal1* or *Rev-Erb α* (a nuclear receptor which suppresses *Bmal1* expression) were knocked out reported inhibition of adipocyte differentiation^[85-87]; while those involving mutations of clock components like *Per2*^[88] or retinoid orphan receptor α ^[89,90] were associated with an increase in adipogenesis, with these effects mediated by PPAR γ ^[91,92]. Studies in male mice

have also demonstrated that the rhythm of expression of the clock genes and adipose PPAR γ are decreased by the consumption of high-fat diet^[93].

Circadian rhythm and glucose control: Like all other aspects of intermediary metabolism, blood glucose homeostasis is also under circadian regulation; with variations in blood glucose levels occurring with the changes in external synchronisers (activity/feeding and resting/starvation)^[94]. During the activity/feeding phase, blood glucose levels are maintained from dietary intake; whereas, during the resting/starvation period, there is a progressive recruitment of glucose from endogenous glucose sources in the liver to maintain blood levels within a relatively narrow margin^[94]. The liver also alternates between glycogenolysis and glycogenesis^[95,96]. Studies have also shown that daily blood glucose control is also modulated by both the central circadian clock in the SCN as well as by peripheral clocks in the pancreas, liver, muscle and white adipose tissue. This is affirmed by studies in humans, that have observed differences in glycaemic response between meal studies conducted in the morning and those in the evening^[97-99]. This alteration in glycaemic control had been attributed to circadian variations in insulin secretion and an increase in hepatic or peripheral insulin resistance^[99-101]. Studies using animal models have also shown that insulin secretion follows a rhythmicity that is regulated by peripheral pancreatic β -cell clocks^[102]. In humans there have been reports that the set-point for the regulation of the 24-h pulsatile secretion of insulin is higher in obese subjects, T2DM subjects, and their non-diabetic first degree relative^[102,103] compared to the general population. Studies using different animal models of circadian clock gene dysfunction (*Clock Δ 19*, *Cry1* and *Cry2*, *Bmal1*) have also reported evidence of hyperglycaemia, increased insulin sensitivity or impaired insulin secretion^[16,104,105].

Gut hormones which are very important in modulating gastric emptying and maintaining glucose homeostasis, like the anorexigenic peptides (glucagon, insulin, glucose inhibitory peptide, glucagon-like peptide-1, amylin, peptide YY) and the orexigenic hormone ghrelin have also been shown to fluctuate with activity/feeding and resting/starvation periods. The variations in their activity pattern are also under circadian control and as such may be altered by circadian disruptors, including altered meal times, dietary compositions and constant light exposure^[106,107].

There are evidences supporting the existence of a relationship between gut microbiota and the circadian system; and presently, it is known that intestinal microbiome is regulated by circadian rhythms through the intrinsic circadian clocks^[108]. This regulation affects host metabolic function through alteration of microbial community structure as well as their metabolic activities. Up to one-fifth of human gut bacteria exhibit diurnal variations in their activities and abundance; and some species, like *Enterobacter aerogenes* had been shown

to be responsive to the circadian fluctuations in the hormone melatonin^[108]. Alterations in the balance of this relationship can lead to changes in the activities and relative composition of gut microbiota. Finally, abnormalities in composition and activities of gut microbiota had been linked to insulin resistance and diabetes mellitus through several mechanisms, such as regulation of adiposity/obesity, regulation of the immune system, modulation of inflammatory processes, and extraction of energy from the diet^[109].

Circadian rhythm dysfunction, sleep and T2DM

A number of studies have demonstrated that a dysregulation of the internal circadian clock system or discordance with the external environmental cues has deleterious health consequences, with an associated increase in morbidity and mortality in humans^[9]. Increasingly, results from epidemiological^[110,111] and animal^[8,9,15] studies continue to show associations between circadian rhythm dysfunction (that occur due to sleep loss, shift work or nocturnal lifestyle) and the development of T2DM^[8,9,15] (Figure 2). An *in-vitro* study using rat pancreatic islets revealed that exposure of the islets to continuous light was associated with a disruption of the circadian clock function and reduction in glucose-stimulated insulin secretion, due to a decrease in insulin secretory pulse mass^[112]. Also, there have been reports that a disruption of circadian rhythm could induce abnormal insulin release in people at risk of developing T2DM. Gale *et al.*^[15] examined the metabolic and physiological changes associated with T2DM following circadian rhythm dysfunction in wild-type, Sprague Dawley and diabetes-prone human islet amyloid polypeptide transgenic rats that were exposed to prolonged episodes of normal light (or experimental disruption in the light-dark cycle), and reported that circadian rhythm disruption accelerated the development of diabetes in diabetes-prone rats, but not in wild-type rats^[15]; an effect that has been attributed to pancreatic β -cell loss and dysfunction^[15]. Marche *et al.*^[16] reported that disruption of the clock gene components (*Clock* and *Bmal1*) was associated with delays in the phase of oscillation of islet genes that were involved in islet cell growth, glucose metabolism and insulin signalling; resulting in impaired glucose tolerance, reduction in insulin secretion, and alterations in the size and proliferation of pancreatic islets^[16]. They also demonstrated that conditional ablation of the pancreatic clock resulted in the development of diabetes mellitus *via* alteration in β -cell function^[16]. Also, there have been suggestions and experimental evidence to show that the mammalian islet clock was responsible for regulating the expression of genes that are involved in sensing glucose levels, insulin secretion, as well as islet cell growth and development^[16,113].

While we gain new insights into the pathophysiology of T2DM, and continue to understand the roles played by the circadian rhythm^[15,16,113]; there is ample scientific evidence to show that a disruption of circadian rhythms

alters not only the body weight and adiposity, but it also affects glucose metabolism and glycaemic control. While the magnitude of these effects (as it relates to the development and progression of T2DM) continues to be studied, it is also important to continue to investigate their precise mechanisms, and to determine the relevance of this new knowledge to the therapy and prevention of T2DM.

There appears to be strong relationships between certain sleep parameters and the risk of development of diabetes mellitus. Along this line, numerous evidences from both epidemiological and laboratory studies have continued to reveal and support the fact that poor sleep is strongly associated with the development of glucose-intolerance, insulin resistance, and ultimately T2DM^[114].

In a community-based study of adults of both sexes in Xuzhou, China; it was found that after adjustment for a large number of possible aetiological factors, poor sleep-quality and short (≤ 6 h) sleep duration were significantly associated with increased prevalence of diabetes mellitus, when compared with the group of people with good quality of sleep and longer (6-8 h) overnight sleep duration^[115]. Again, poor sleep has been known to be associated poor glycaemic control in T2DM patients. In a Japanese study involving 3249 patients with T2DM; an assessment of sleep, using the Pittsburgh Sleep Quality Index (PSQI) showed that (independent of potential confounders) poor subjective sleep quality was associated with less-than-optimal glycaemic control^[116].

MELATONIN

Melatonin is a tryptophan-derived indoleamine which is primarily secreted by the pineal gland, with contributions from a number of other tissues including the retina, bone marrow, gastrointestinal tract, skin, ovary and placenta^[117,118]. The extra-pineal contribution to melatonin production is small when compared to secretion from the pineal gland; with suggestions that it is only triggered by some specific impulses^[119]. Melatonin secretion is regulated by the central circadian clock, as well as by seasonal variations in length of daylight. Production is acutely suppressed by exposure to light, with increased secretion occurring at night in both nocturnal and diurnal species. Plasma concentrations of endogenous melatonin also vary considerably with age^[120-122]. Melatonin is a multifunctional molecule that is capable of intracrine, paracrine or autocrine signalling^[117]. It can cross all physiological barriers and exert widespread regulatory effects on numerous body tissues. Melatonin is important in the regulation of biologic rhythms^[123]; and numerous studies in humans and rodents have reported melatonin's widespread influence on varied biological and behavioural processes^[124-126]. Melatonin plays important roles in neurogenesis, neuroprotection and the maintenance of oxidant/antioxidant balance^[127-129]. A few studies have also reported its role in diabetes control^[18].

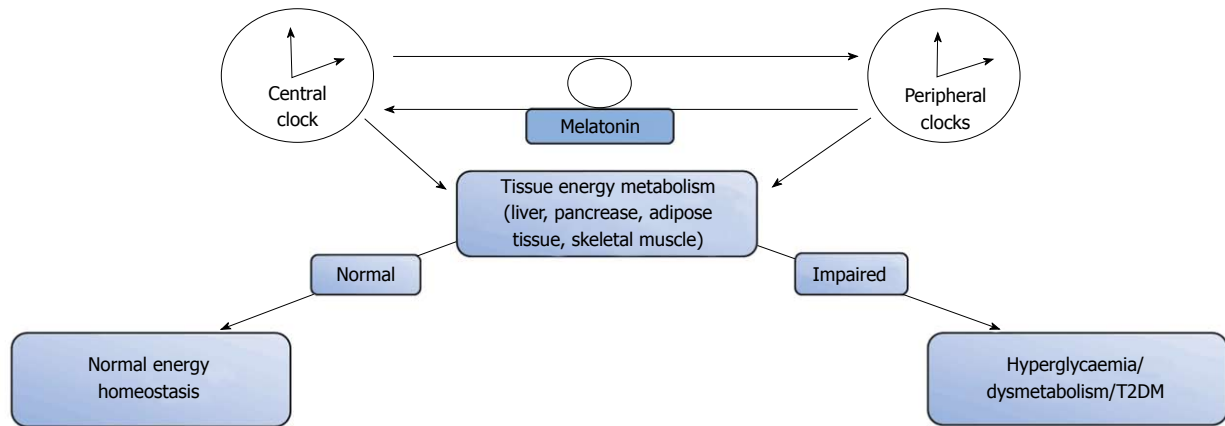


Figure 2 The role of circadian rhythm in the development of type 2 diabetes. T2DM: Type 2 diabetes mellitus.

Melatonin's role in intermediary metabolism

There is increasing scientific evidence to suggest that a derangement of melatonin rhythmicity may have adverse health implications, especially as it relates to its importance in modulating a variety of metabolic functions, as well as its role as a regulator of epigenesis^[130]. Studies have demonstrated the presence of high concentrations of extra-pineal melatonin in the gastrointestinal tract (GIT) of a number of mammals^[131]. There had also been reports suggesting that extra-pineal melatonin from the GIT contributes significantly to circulating blood melatonin levels (mostly during the day)^[131]; although there are evidences to suggest that some of the melatonin in the GIT may be pineal in origin. However; there are reports suggesting that the release of GIT melatonin may be related to the periodicity of food intake rather than being photoperiodic, as occurs with melatonin from the pineal gland^[131]. These evidences are stimulating interest in investigating the possible relationship that may exist between melatonin in the GIT and metabolism; especially, since a number of studies in vertebrates had demonstrated exogenous melatonin's ability to modulate appetite, energy metabolism, anorexigenic hormone/peptide concentration, and body weight^[132-134]. Earlier studies evaluating melatonin's relationship with the GIT and intermediary metabolism reported alterations in the overall food consumption in mice following administration of exogenous melatonin; while a few other studies also demonstrated an increase in tissue and blood melatonin levels with food intake and prolonged food deprivation^[131,135]. Studies in zebrafish (*Danio rerio*) had also demonstrated that melatonin administration induced a decrease in food intake, it also modulated the stimulation of satiety and anorexigenic signals in the liver and intestine^[136]. However, a number of studies have suggested that melatonin's roles in appetite modulation may arise from different mechanisms; with suggestions that its anorexigenic effects could be as a result of its ability to delay gastric emptying^[137,138] or *via* its stimulatory activity on fat mobilisation^[139,140]. A number of other studies in fish have also reported that melatonin's

ability to reduce food consumption may be related to circadian rhythm stimulation (*i.e.*, its ability to promote sleep), and not necessarily due to a direct effect of the hormone^[141].

Melatonin, melatonin receptors, glucose metabolism and T2DM

A number of studies have provided evidence that melatonin influences glucose metabolism. In healthy subjects, glucose homeostasis is controlled within a narrow margin *via* a complex pathway of regulatory mechanisms that involves multiple organs and tissues (Figure 3). Therefore, a disruption of normal glucose balance usually results from a sustained reduction in both pancreatic beta-cell function and insulin secretion^[142,143]. In rodents, melatonin has been shown to regulate blood glucose concentration through its ability to bind directly to melatonin receptors on hepatocytes^[144] and regulate the uptake of glucose in adipocytes, by modulating the expression of the glucose uptake transporter^[145]. Abnormalities of the nocturnal melatonin profile have also been described in diabetic patients, especially in those suffering from diabetic neuropathy^[146]. Low melatonin secretion is also independently associated with a higher risk of developing T2DM; an association that further establishes the roles of melatonin in glucose metabolism and insulin sensitivity^[147]. Post mortem studies have also indicated an association between diabetes mellitus and decreased melatonin secretion^[148]; while some *in-vivo* and *in-vitro* studies have demonstrated melatonin's ability to inhibit the secretion of insulin by pancreatic beta-cells^[149]. Presently, a growing body of evidence suggests a relationship between disturbances in melatonin production and impairment of insulin, glucose and lipid metabolism^[134,150]; and that of antioxidant capacity^[130,151,152]. Results from both *in-vivo* and *in-vitro* studies have shown that in patients with metabolic syndrome, night-time melatonin level is related to night-time insulin concentrations^[153]. There have also been reports of lower elevations in night-time melatonin levels in diabetic subjects; raising interests in the link between

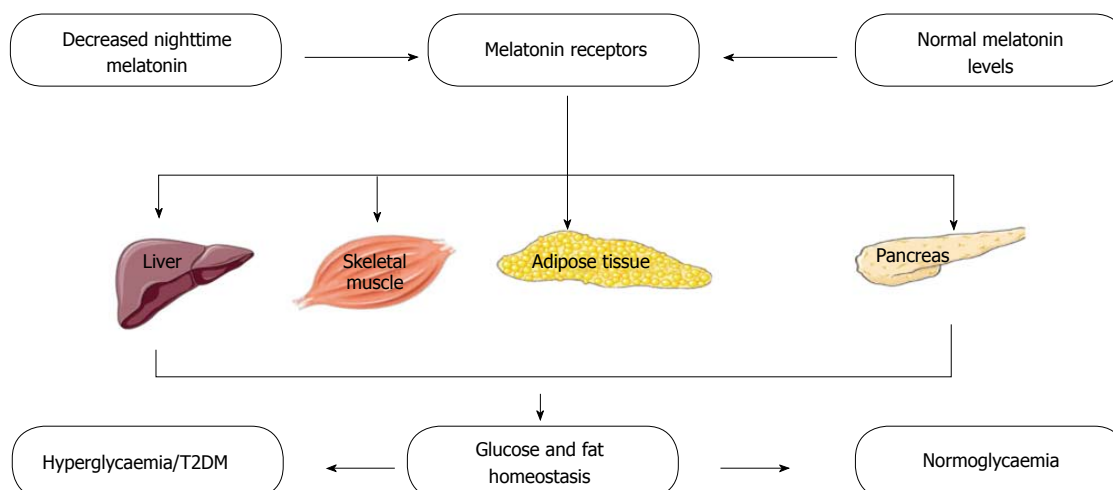


Figure 3 Melatonin, melatonin receptors, glucose and fat homeostasis and type 2 diabetes mellitus. T2DM: Type 2 diabetes mellitus.

melatonin and hyperglycaemia/diabetes mellitus^[154]. Also, melatonin has been reported to stimulate the secretion of glucagon, another hormone that is important in glucose metabolism^[155].

Melatonin receptors (MT1 and MT2) have been observed to be present in rodent^[156-158] and human^[18,19,159] pancreatic islets. The expression of these receptors also varies with the circadian rhythm and feeding status^[160]. In humans, several genetic studies have associated MT2 receptor polymorphisms with an increased risk of developing T2DM^[150]. Associations between single nucleotide polymorphisms that are situated close to (or within) the gene that encodes MT2 (MTNR1B), and an increased risk of developing T2DM^[18,161,162], diminished B-cell function^[163,164] and impaired glycaemic control^[165-167] have all been reported in cohorts of different regions and ethnicities. Studies have also demonstrated an increase in the expression of MT1 and MT2 receptors in the pancreas of diabetic rats and in subjects with T2DM^[168].

Melatonin's potential roles in prophylaxis or treatment:

Experimental and clinical data continue to suggest that both endogenous as well as exogenously-administered melatonin play crucial roles in the improvement of diabetes control. In a rat model of diabetes mellitus, long-term administration of melatonin (1.1 mg/d for 30 wk) attenuated the development of hypertriglyceridaemia, hyperinsulinaemia and hyperleptinaemia^[169]. In a study among community-dwelling diabetics, the effect of administration of 2 mg of prolonged-release melatonin (at 9-11 pm for 3 wk) on glucose and lipid metabolism was investigated^[148]. This initial administration was followed by an extended period of five months of open-label, prolonged-release melatonin administration to evaluate the effects of prolonged-release melatonin on glycosylated haemoglobin (HbA1c) levels^[148]. The results established the safety of prolonged-release melatonin with regards to parameters such as glucose, lipid metabolism, and other routine biochemical

indices; also, there were no adverse interactions with routinely-used anti-diabetic drugs, or insulin release^[148]. In an earlier study involving twenty-two postmenopausal non-diabetic women, the results suggested that glucose tolerance and insulin sensitivity are reduced following a single oral administration of melatonin 1 mg^[170]. However, in diabetic women, use of prolonged-release melatonin (in the short term or long term) did not impair insulin action or glucose tolerance; on the contrary, there was improved glycaemic control upon long-term use^[148]. A few other studies have demonstrated that melatonin plus zinc acetate alone, or in combination with metformin improved both fasting and postprandial glycaemic control in T2DM patients^[171].

Presently, research continues to unravel the multifaceted effects of melatonin on intermediary metabolism, especially that of glucose; with direct evidences of melatonin's effects on insulin secretion, pancreatic beta cell activity, hepatic glucose metabolism and insulin sensitivity^[172]. Apart from these, melatonin also combats cellular/tissue oxidative stress and inflammation. Therefore, by the modulation of several intracellular signalling pathways and tissue targets, melatonin is emerging to occupy a central role in the understanding of the aetiology and management of diabetes mellitus^[173].

Melatonin receptors (MT1 and MT2) have been shown to be present on human pancreatic islets, and the effects of melatonin on insulin secretion are mediated through these receptors^[17]. Melatonin is able to affect insulin secretion in two ways, decreasing it by inhibiting cAMP and cGMP pathways, and increasing it by activating the phospholipase C/Inositol triphosphate pathway, which mobilises calcium ions from organelles, consequently increasing insulin secretion. Melatonin also induces production of insulin growth factor and promotes insulin receptor tyrosine phosphorylation; while its supplementation attenuates glucose intolerance and insulin resistance^[17].

The use of melatonin in the pharmacotherapy of

diabetes mellitus may confer additional benefits over what is obtainable with conventional drugs alone. This is due to its ability to affect several pathways that may be involved in the pathogenesis or progression of the disease. In an experimental model designed to express obese T2DM phenotype, rats with concomitant circadian disruption and diet-induced obesity were treated daily with oral melatonin, metformin, or a combination of the two for 12 wk^[174]. It was observed that melatonin alone improved circadian activity/rhythms, attenuated induction of beta-cell failure, and enhanced glucose tolerance. Use of metformin alone only enhanced insulin sensitivity and glucose tolerance. However, combining melatonin with metformin attenuated progression of metabolic dysfunction by improving adiposity, circadian activity, insulin sensitivity, and islet cell failure^[174]. The results suggest that attenuation or arrest of circadian dysfunction may be crucial to managing metabolic dysfunction and altering the course of the disease in T2DM. In mice that were given high-fat diet (HFD), oral melatonin at 100 mg/kg per day (for 10 wk) led to a significant reduction in body weight-gain (compared to the HFD controls) and it also reduced hepatic steatosis. Also, there was improved insulin sensitivity and glucose tolerance, with down-regulation of fetuin-A (a hepatokine that is associated with insulin resistance and T2DM) and endoplasmic reticulum stress markers in the liver and serum^[175].

One of the ways by which melatonin may be beneficial in the management of T2DM and metabolic syndrome is through its ability to reduce adiposity by modulation of the gut microbiota. In mice that were fed high-fat diet, melatonin treatment significantly reversed gut microbiota dysbiosis, increasing the ratio of the bacteria that are known to be associated with a healthy mucosa while also improving markers of adiposity and inflammation^[176].

Some studies have also assessed the impact of melatonin supplementation on the development of microvascular and macrovascular complications of diabetes mellitus and concluded that melatonin has beneficial effects in repairing cardiac injury due to diabetes mellitus^[177]. Zhou *et al.*^[176] reported that inhibition of the splenic tyrosine kinase (which is activated by hyperglycaemia and contributes significantly to the development of diabetic cardiomyopathy) by melatonin supplementation reversed diabetes-related loss of myocardial function, decreased cardiac fibrosis and preserved the viability of cardiac myocytes^[176].

There have been studies that had reported the influence of melatonin on mitochondrial bioenergetics due to its ability to regulate mitochondrial fission/fusion^[178,179] and regulate mitophagy/autophagy^[180]. In view of the above, Ding *et al.*^[181] examined the possible effects of melatonin supplementation on the development of myocardial contractile dysfunction (which has been linked to an increase in mitochondrial fission in subjects with diabetes mellitus), and reported that melatonin attenuated diabetes-induced myocardial dysfunction by decreasing the expression of dynamin-related protein 1, leading to the prevention of mitochondrial fission^[181].

Melatonin administration also prevented mitochondrial fragmentation, decreased oxidative stress, and reduced apoptosis of the cardiomyocyte in streptozotocin-induced diabetic mice; however, these were not replicated in the protein deacetylase sirtuin 1 (SIRT1)^{-/-} diabetic mice^[181]. Thus, suggesting that melatonin's cardioprotective effects were exerted through its effects on SIRT1^[181].

Melatonin's antioxidant or oxidative stress-reduction effect is one of the benefits that have increased interests in its possible use in the management of diabetes mellitus and its complications. Studies in rodents have demonstrated that intraperitoneal administration of melatonin (3 mg/kg per day for 4 wk) reduced lipid peroxidation marker (malonyldialdehyde) and increased glutathione levels in the bone tissue of diabetic rats subjected to acute swimming exercise^[182]. Mehrzadi *et al.*^[183] also examined the effects of melatonin supplementation on the development of diabetes-related retinal injury in rats. Their results showed that while induction of diabetes increased oxidative stress and inflammation, treatment with melatonin for a period of seven weeks attenuated the development of retinal injury; largely through reduction of oxidative stress and inflammation^[183]. Studies in human subjects have also demonstrated that melatonin's cardioprotective effects can be attributed to its ability to reduce oxidative stress and improve cardiometabolic risk^[184]. In a randomised, double-blind, placebo-controlled trial, two groups of subjects were administered either melatonin (10 mg) or placebo, once daily for 12 wk^[184]. Results of this study showed that (compared to subjects that were administered placebo) melatonin supplementation (in addition to its beneficial effects on glycaemic control, reduction of insulin resistance and improvement of insulin sensitivity) was associated with an increase in the plasma concentration of glutathione, nitric oxide, high density lipoprotein; and a decrease in the levels of malondialdehyde and serum C-reactive protein^[184].

A few studies in rodents have also explored the possible use of melatonin as an adjunct to insulin therapy. Oliveira *et al.*^[185] reported that 8 weeks of administration of melatonin in drinking water at 0.2 mg/kg body weight (either alone or in combination with insulin (NHP, 1.5 U/100 g/d) improved glycaemic control, increased insulin sensitivity and reduced the expression of hypothalamic genes that are related to reproductive function^[185].

CONCLUSION

Research has continued to reveal the importance of circadian rhythm regulation, and the neurohormone melatonin in the regulation of carbohydrate metabolism. More studies are also revealing the potential roles of melatonin in the pathogenesis, management and modulation of the course of diabetes mellitus, especially T2DM; and as shown by these studies, an array of possible mechanisms exists for melatonin's effects.

However, a complete picture of the role(s) of melatonin in the management of DM is yet to emerge. Also, we

are yet to get to the point where melatonin and melatonin receptor agonists may be prescribed as adjuncts or alternatives to already-existing orthodox medications. Finally, we are just beginning to understand how melatonin may be used to prevent or delay the occurrence of diabetes mellitus.

REFERENCES

- 1 **World Health Organisation.** Diabetes 2017. Available from: URL: <http://www.who.int/news-room/fact-sheets/detail/diabetes>
- 2 **Shaw JE, Sicree RA, Zimmet PZ.** Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; **87**: 4-14 [PMID: 19896746 DOI: 10.1016/j.diabres.2009.10.007]
- 3 **Emerging Risk Factors Collaboration, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M, Stehouwer CD, Lewington S, Pennells L, Thompson A, Sattar N, White IR, Ray KK, Danesh J.** Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010; **375**: 2215-2222 [PMID: 20609967 DOI: 10.1016/S0140-6736(10)60484-9]
- 4 **Bourne RR, Stevens GA, White RA, Smith JL, Flaxman SR, Price H, Jonas JB, Keeffe J, Leasher J, Naidoo K, Pesudovs K, Resnikoff S, Taylor HR; Vision Loss Expert Group.** Causes of vision loss worldwide, 1990-2010: a systematic analysis. *Lancet Glob Health* 2013; **1**: e339-e349 [PMID: 25104599 DOI: 10.1016/S2214-109X(13)70113-X]
- 5 **United States Renal Data System.** 2014 USRDS annual data report: Epidemiology of kidney disease in the United States. Bethesda: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2014: 188-210
- 6 **Naveen J, Baskaran V.** Antidiabetic plant-derived nutraceuticals: a critical review. *Eur J Nutr* 2018; **57**: 1275-1299 [PMID: 29022103 DOI: 10.1007/s00394-017-1552-6]
- 7 **Knutson KL, Spiegel K, Penev P, Van Cauter E.** The metabolic consequences of sleep deprivation. *Sleep Med Rev* 2007; **11**: 163-178 [PMID: 17442599 DOI: 10.1016/j.smrv.2007.01.002]
- 8 **Bass J, Takahashi JS.** Circadian integration of metabolism and energetics. *Science* 2010; **330**: 1349-1354 [PMID: 21127246 DOI: 10.1126/science.1195027]
- 9 **Reddy AB, O'Neill JS.** Healthy clocks, healthy body, healthy mind. *Trends Cell Biol* 2010; **20**: 36-44 [PMID: 19926479 DOI: 10.1016/j.tcb.2009.10.005]
- 10 **Scheer FA, Hilton MF, Mantzoros CS, Shea SA.** Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci USA* 2009; **106**: 4453-4458 [PMID: 19255424 DOI: 10.1073/pnas.0808180106]
- 11 **Woon PY, Kaisaki PJ, Bragança J, Bihoreau MT, Levy JC, Farrall M, Gauguier D.** Aryl hydrocarbon receptor nuclear translocator-like (BMAL1) is associated with susceptibility to hypertension and type 2 diabetes. *Proc Natl Acad Sci USA* 2007; **104**: 14412-14417 [PMID: 17728404 DOI: 10.1073/pnas.0703247104]
- 12 **Sookoian S, Gemma C, Gianotti TF, Burgueño A, Castaño G, Pirola CJ.** Genetic variants of Clock transcription factor are associated with individual susceptibility to obesity. *Am J Clin Nutr* 2008; **87**: 1606-1615 [PMID: 18541547 DOI: 10.1093/ajcn/87.6.1606]
- 13 **Scott EM, Carter AM, Grant PJ.** Association between polymorphisms in the Clock gene, obesity and the metabolic syndrome in man. *Int J Obes (Lond)* 2008; **32**: 658-662 [PMID: 18071340 DOI: 10.1038/sj.ijo.0803778]
- 14 **Englund A, Kovanen L, Saarikoski ST, Haukka J, Reunanen A, Aromaa A, Lönnqvist J, Partonen T.** NPAS2 and PER2 are linked to risk factors of the metabolic syndrome. *J Circadian Rhythms* 2009; **7**: 5 [PMID: 19470168 DOI: 10.1186/1740-3391-7-5]
- 15 **Gale JE, Cox HI, Qian J, Block GD, Colwell CS, Matveyenko AV.** Disruption of circadian rhythms accelerates development of diabetes through pancreatic beta-cell loss and dysfunction. *J Biol Rhythms* 2011; **26**: 423-433 [PMID: 21921296 DOI: 10.1177/0748730411416341]
- 16 **Marcheva B, Ramsey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, Ivanova G, Omura C, Mo S, Vitaterna MH, Lopez JP, Philipson LH, Bradfield CA, Crosby SD, JeBailey L, Wang X, Takahashi JS, Bass J.** Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature* 2010; **466**: 627-631 [PMID: 20562852 DOI: 10.1038/nature09253]
- 17 **Sharma S, Singh H, Ahmad N, Mishra P, Tiwari A.** The role of melatonin in diabetes: therapeutic implications. *Arch Endocrinol Metab* 2015; **59**: 391-399 [PMID: 26331226 DOI: 10.1590/2359-3997000000098]
- 18 **Lyssenko V, Nagorny CL, Erdos MR, Wierup N, Jonsson A, Spégel P, Bugliani M, Saxena R, Fex M, Pulizzi N, Isomaa B, Tuomi T, Nilsson P, Kuusisto J, Tuomilehto J, Boehnke M, Altshuler D, Sundler F, Eriksson JG, Jackson AU, Laakso M, Marchetti P, Watanabe RM, Mulder H, Groop L.** Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. *Nat Genet* 2009; **41**: 82-88 [PMID: 19060908 DOI: 10.1038/ng.288]
- 19 **Ramracheya RD, Muller DS, Squires PE, Brereton H, Sugden D, Huang GC, Amiel SA, Jones PM, Persaud SJ.** Function and expression of melatonin receptors on human pancreatic islets. *J Pineal Res* 2008; **44**: 273-279 [PMID: 18194202 DOI: 10.1111/j.1600-079X.2007.00523.x]
- 20 **Bonnefond A, Clément N, Fawcett K, Yengo L, Vaillant E, Guillaume JL, Dechaume A, Payne F, Roussel R, Czernichow S, Hercberg S, Hadjadj S, Balkau B, Marre M, Lantieri O, Langenberg C, Bouatia-Naji N; Meta-Analysis of Glucose and Insulin-Related Traits Consortium (MAGIC), Charpentier G, Vaxillaire M, Rocheleau G, Wareham NJ, Sladek R, McCarthy MI, Dina C, Barroso I, Jockers R, Froguel P.** Rare MTNR1B variants impairing melatonin receptor 1B function contribute to type 2 diabetes. *Nat Genet* 2012; **44**: 297-301 [PMID: 22286214 DOI: 10.1038/ng.1053]
- 21 **Gaulton KJ, Ferreira T, Lee Y, Raimondo A, Mägi R, Reschen ME, Mahajan A, Locke A, Rayner NW, Robertson N, Scott RA, Prokopenko I, Scott LJ, Green T, Sparso T, Thuillier D, Yengo L, Grallert H, Wahl S, Frånberg M, Strawbridge RJ, Kestler H, Chheda H, Eisele L, Gustafsson S, Steinthorsdottir V, Thorleifsson G, Qi L, Karssen LC, van Leeuwen EM, Willems SM, Li M, Chen H, Fuchsberger C, Kwan P, Ma C, Linderman M, Lu Y, Thomsen SK, Rundle JK, Beer NL, van de Bunt M, Chalisey A, Kang HM, Voight BF, Abecasis GR, Almgren P, Baldassarre D, Balkau B, Benediktsson R, Blüher M, Boeing H, Bonnycastle LL, Bottinger EP, Burt NP, Carey J, Charpentier G, Chines PS, Cornelis MC, Couper DJ, Crenshaw AT, van Dam RM, Doney AS, Dorkhan M, Edkins S, Eriksson JG, Esko T, Eury E, Fadista J, Flannick J, Fontanillas P, Fox C, Franks PW, Gertow K, Gieger C, Gigante B, Gottesman O, Grant GB, Grarup N, Groves CJ, Hassinen M, Have CT, Herder C, Holmen OL, Hreidarsson AB, Humphries SE, Hunter DJ, Jackson AU, Jonsson A, Jørgensen ME, Jørgensen T, Kao WH, Kerrison ND, Kinnunen L, Klopp N, Kong A, Kovacs P, Kraft P, Kravic J, Langford C, Leander K, Liang L, Lichtner P, Lindgren CM, Lindholm E, Linneberg A, Liu CT, Lobbens S, Luan J, Lyssenko V, Männistö S, McLeod O, Meyer J, Mihailov E, Mirza G, Mühleisen TW, Müller-Nurasyid M, Navarro C, Nöthen MM, Oskolkov NN, Owen KR, Palli D, Pechlivanis S, Peltonen L, Perry JR, Platou CG, Roden M, Ruderfer D, Rybin D, van der Schouw YT, Sennblad B, Sigurdsson G, Stančáková A, Steinbach G, Storm P, Strauch K, Stringham HM, Sun Q, Thorand B, Tikkanen E, Tonjes A, Trakalo J, Tremoli E, Tuomi T, Wennauer R, Wiltshire S, Wood AR, Zeggini E, Dunham I, Birney E, Pasquali L, Ferrer J, Loos RJ, Dupuis J, Florez JC, Boerwinkle E, Pankow JS, van Duijn C, Sijbrands E, Meigs JB, Hu FB, Thorsteinsdottir U, Stefansson K, Lakka TA, Rauramaa R, Stumvoll M, Pedersen NL, Lind L, Keinänen-Kiukkaanniemi SM, Korpi-Hyövälti E, Saaristo TE, Saltevo J, Kuusisto J, Laakso M, Metspalu A, Erbel R, Jöcke KH, Moebus S, Ripatti S, Salomaa V, Ingelsson E, Boehm BO, Bergman RN, Collins FS, Mohlke KL, Koistinen H, Tuomilehto**

- J, Hveem K, Njølstad I, Deloukas P, Donnelly PJ, Frayling TM, Hattersley AT, de Faire U, Hamsten A, Illig T, Peters A, Cauchi S, Sladek R, Froguel P, Hansen T, Pedersen O, Morris AD, Palmer CN, Kathiresan S, Melander O, Nilsson PM, Groop LC, Barroso I, Langenberg C, Wareham NJ, O'Callaghan CA, Gloyn AL, Altshuler D, Boehnke M, Teslovich TM, McCarthy MI, Morris AP; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. *Nat Genet* 2015; **47**: 1415-1425 [PMID: 26551672 DOI: 10.1038/ng.3437]
- 22 **Rubio-Sastre P**, Scheer FA, Gómez-Abellán P, Madrid JA, Garaulet M. Acute melatonin administration in humans impairs glucose tolerance in both the morning and evening. *Sleep* 2014; **37**: 1715-1719 [PMID: 25197811 DOI: 10.5665/sleep.4088]
 - 23 **Costes S**, Boss M, Thomas AP, Matveyenko AV. Activation of Melatonin Signaling Promotes β -Cell Survival and Function. *Mol Endocrinol* 2015; **29**: 682-692 [PMID: 25695910 DOI: 10.1210/me.2014-1293]
 - 24 **Prasad RB**, Groop L. Genetics of type 2 diabetes-pitfalls and possibilities. *Genes (Basel)* 2015; **6**: 87-123 [PMID: 25774817 DOI: 10.3390/genes6010087]
 - 25 **Huang ZQ**, Liao YQ, Huang RZ, Chen JP, Sun HL. Possible role of TCF7L2 in the pathogenesis of type 2 diabetes mellitus. *Biotechnol Biotech Eq* 2018 [DOI: 10.1080/13102818.2018.1438211]
 - 26 **Muoio DM**, Newgard CB. Mechanisms of disease: Molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. *Nat Rev Mol Cell Biol* 2008; **9**: 193-205 [PMID: 18200017 DOI: 10.1038/nrm2327]
 - 27 **Day C**, Bailey CJ. Obesity in the pathogenesis of type 2 diabetes. *Br J Diabetes Vasc Dis* 2011; **11**: 55-61 [DOI: 10.1177/1474651411407418]
 - 28 **Kwak SH**, Park KS. Recent progress in genetic and epigenetic research on type 2 diabetes. *Exp Mol Med* 2016; **48**: e220 [PMID: 26964836 DOI: 10.1038/emmm.2016.7]
 - 29 **Iyer SR**. Sleep and type 2 diabetes mellitus- clinical implications. *J Assoc Physicians India* 2012; **60**: 42-47 [PMID: 23777024]
 - 30 **Alonso-Magdalená P**, Quesada I, Nadal A. Endocrine disruptors in the etiology of type 2 diabetes mellitus. *Nat Rev Endocrinol* 2011; **7**: 346-353 [PMID: 21467970 DOI: 10.1038/nrendo.2011.56]
 - 31 **Fuchsberger C**, Flannick J, Teslovich TM, Mahajan A, Agarwala V, Gaulton KJ, Ma C, Fontanillas P, Moutsianas L, McCarthy DJ, Rivas MA, Perry JRB, Sim X, Blackwell TW, Robertson NR, Rayner NW, Cingolani P, Locke AE, Tajcs JF, Highland HM, Dupuis J, Chines PS, Lindgren CM, Hartl C, Jackson AU, Chen H, Huyghe JR, van de Bunt M, Pearson RD, Kumar A, Müller-Nurasyid M, Grarup N, Stringham HM, Gamazon ER, Lee J, Chen Y, Scott RA, Below JE, Chen P, Huang J, Go MJ, Stitzel ML, Pasko D, Parker SCJ, Varga TV, Green T, Beer NL, Day-Williams AG, Ferreira T, Fingerlin T, Horikoshi M, Hu C, Huh I, Ikram MK, Kim BJ, Kim YJ, Kwon MS, Lee J, Lee S, Lin KH, Maxwell TJ, Nagai Y, Wang X, Welch RP, Yoon J, Zhang W, Barzilai N, Voight BF, Han BG, Jenkinson CP, Kuulasmaa T, Kuusisto J, Manning A, Ng MCY, Palmer ND, Balkau B, Stančáková A, Abboud HE, Boeing H, Giedraitis V, Prabhakaran D, Gottesman O, Scott J, Carey J, Kwan P, Grant G, Smith JD, Neale BM, Purcell S, Butterworth AS, Howson JMM, Lee HM, Lu Y, Kwak SH, Zhao W, Danesh J, Lam VKL, Park KS, Saleheen D, So WY, Tam CHT, Afzal U, Aguilar D, Arya R, Aung T, Chan E, Navarro C, Cheng CY, Palli D, Correa A, Curran JE, Rybin D, Farook VS, Fowler SP, Freedman BI, Griswold M, Hale DE, Hicks PJ, Khor CC, Kumar S, Lehne B, Thuillier D, Lim WY, Liu J, van der Schouw YT, Loh M, Musani SK, Puppala S, Scott WR, Yengo L, Tan ST, Taylor HA Jr, Thameem F, Wilson G Sr, Wong TY, Njølstad PR, Levy JC, Mangino M, Bonnycastle LL, Schwarzmayr T, Fadista J, Surdulescu GL, Herder C, Groves CJ, Wieland T, Bork-Jensen J, Brandslund I, Christensen C, Koistinen HA, Doney ASF, Kinnunen L, Esko T, Farmer AJ, Hakaste L, Hodgkiss D, Kravic J, Lyssenko V, Hollensted M, Jørgensen ME, Jørgensen T, Ladenvall C, Justesen JM, Käräjämäki A, Kriebel J, Rathmann W, Lannfelt L, Lauritzen T, Narisu N, Linneberg A, Melander O, Milani L, Neville M, Orho-Melander M, Qi L, Qi Q, Roden M, Rolandsson O, Swift A, Rosengren AH, Stirrups K, Wood AR, Mihailov E, Blancher C, Carneiro MO, Maguire J, Poplin R, Shakir K, Fennell T, DePristo M, de Angelis MH, Deloukas P, Gjesing AP, Jun G, Nilsson P, Murphy J, Onofrio R, Thorand B, Hansen T, Meisinger C, Hu FB, Isomaa B, Karpe F, Liang L, Peters A, Huth C, O'Rahilly SP, Palmer CNA, Pedersen O, Rauramaa R, Tuomilehto J, Salomaa V, Watanabe RM, Syvänen AC, Bergman RN, Bharadwaj D, Bottinger EP, Cho YS, Chandak GR, Chan JCN, Chia KS, Daly MJ, Ebrahim SB, Langenberg C, Elliott P, Jablonski KA, Lehman DM, Jia W, Ma RCW, Pollin TI, Sandhu M, Tandon N, Froguel P, Barroso I, Teo YY, Zeggini E, Loos RJE, Small KS, Ried JS, DeFronzo RA, Grallert H, Glaser B, Metspalu A, Wareham NJ, Walker M, Banks E, Gieger C, Ingelsson E, Im HK, Illig T, Franks PW, Buck G, Trakalo J, Buck D, Prokopenko I, Mägi R, Lind L, Farjoun Y, Owen KR, Gloyn AL, Strauch K, Tuomi T, Kooner JS, Lee JY, Park T, Donnelly P, Morris AD, Hattersley AT, Bowden DW, Collins FS, Atzmon G, Chambers JC, Spector TD, Laakso M, Strom TM, Bell GI, Blangero J, Duggirala R, Tai ES, McVean G, Hani CL, Wilson JG, Seielstad M, Frayling TM, Meigs JB, Cox NJ, Sladek R, Lander ES, Gabriel S, Burt NP, Mohlke KL, Meitinger T, Groop L, Abecasis G, Florez JC, Scott LJ, Morris AP, Kang HM, Boehnke M, Altshuler D, McCarthy MI. The genetic architecture of type 2 diabetes. *Nature* 2016; **536**: 41-47 [PMID: 27398621 DOI: 10.1038/nature18642]
 - 32 **Deeb SS**, Fajas L, Nemoto M, Pihlajamäki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J. A Pro12Ala substitution in PPARGgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 1998; **20**: 284-287 [PMID: 9806549 DOI: 10.1038/3099]
 - 33 **Hansen SK**, Nielsen EM, Ek J, Andersen G, Glümer C, Carstensen B, Mouritzen P, Drivsholm T, Borch-Johnsen K, Jørgensen T, Hansen T, Pedersen O. Analysis of separate and combined effects of common variation in KCNJ11 and PPARG on risk of type 2 diabetes. *J Clin Endocrinol Metab* 2005; **90**: 3629-3637 [PMID: 15797964 DOI: 10.1210/jc.2004-1942]
 - 34 **Hani EH**, Boutin P, Durand E, Inoue H, Permutt MA, Velho G, Froguel P. Missense mutations in the pancreatic islet beta cell inwardly rectifying K⁺ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of Type II diabetes mellitus in Caucasians. *Diabetologia* 1998; **41**: 1511-1515 [PMID: 9867219 DOI: 10.1007/s001250051098]
 - 35 **Gloyn AL**, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, Walker M, Levy JC, Sampson M, Halford S, McCarthy MI, Hattersley AT, Frayling TM. Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 2003; **52**: 568-572 [PMID: 12540637 DOI: 10.2337/diabetes.52.2.568]
 - 36 **Gloyn AL**, Cummings EA, Edghill EL, Harries LW, Scott R, Costa T, Temple IK, Hattersley AT, Ellard S. Permanent neonatal diabetes due to paternal germline mosaicism for an activating mutation of the KCNJ11 Gene encoding the Kir6.2 subunit of the beta-cell potassium adenosine triphosphate channel. *J Clin Endocrinol Metab* 2004; **89**: 3932-3935 [PMID: 15292329 DOI: 10.1210/jc.2004-0568]
 - 37 **Inturri R**, Stivala A, Furneri PM, Blandino G. Growth and adhesion to HT-29 cells inhibition of Gram-negatives by Bifidobacterium longum BB536 e Lactobacillus rhamnosus HN001 alone and in combination. *Eur Rev Med Pharmacol Sci* 2016; **20**: 4943-4949 [PMID: 27981539]
 - 38 **Muñoz-Garach A**, Diaz-Perdigones C, Tinahones FJ. Gut microbiota and type 2 diabetes mellitus. *Endocrinol Nutr* 2016; **63**: 560-568 [PMID: 27633134 DOI: 10.1016/j.endonu.2016.07.008]
 - 39 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312 DOI: 10.1038/nature05414]
 - 40 **Zhang Y**, Zhang H. Microbiota associated with type 2 diabetes and

- its related complications. *Food Science and Human Wellness* 2013; **2**: 167-172 [DOI: 10.1016/j.fshw.2013.09.002]
- 41 **Vrieze A**, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druessne A, Van Hylckama Vlieg JE, Bloks VW, Groen AK, Heilig HG, Zoetendal EG, Stroes ES, de Vos WM, Hoekstra JB, Nieuwdorp M. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; **143**: 913-6.e7 [PMID: 22728514 DOI: 10.1053/j.gastro.2012.06.031]
- 42 **Wei X**, Tao J, Xiao S, Jiang S, Shang E, Zhu Z, Qian D, Duan J. Xiexin Tang improves the symptom of type 2 diabetic rats by modulation of the gut microbiota. *Sci Rep* 2018; **8**: 3685 [PMID: 29487347 DOI: 10.1038/s41598-018-22094-2]
- 43 **Kershaw EE**, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004; **89**: 2548-2556 [PMID: 15181022 DOI: 10.1210/jc.2004-0395]
- 44 **Scherer PE**. Adipose tissue: from lipid storage compartment to endocrine organ. *Diabetes* 2006; **55**: 1537-1545 [PMID: 16731815 DOI: 10.2337/db06-0263]
- 45 **Cusi K**. The role of adipose tissue and lipotoxicity in the pathogenesis of type 2 diabetes. *Curr Diab Rep* 2010; **10**: 306-315 [PMID: 20556549 DOI: 10.1007/s11892-010-0122-6]
- 46 **Ravussin Y**, Leibel RL, Ferrante AW Jr. A missing link in body weight homeostasis: the catabolic signal of the overfed state. *Cell Metab* 2014; **20**: 565-572 [PMID: 25295786 DOI: 10.1016/j.cmet.2014.09.002]
- 47 **Scherer PE**. The Multifaceted Roles of Adipose Tissue-Therapeutic Targets for Diabetes and Beyond: The 2015 Banting Lecture. *Diabetes* 2016; **65**: 1452-1461 [PMID: 27222389 DOI: 10.2337/db16-0339]
- 48 **Lee P**, Greenfield JR, Ho KK, Fulham MJ. A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab* 2010; **299**: E601-E606 [PMID: 20606075 DOI: 10.1152/ajpendo.00298.2010]
- 49 **Sidossis L**, Kajimura S. Brown and beige fat in humans: thermogenic adipocytes that control energy and glucose homeostasis. *J Clin Invest* 2015; **125**: 478-486 [PMID: 25642708 DOI: 10.1172/JCI78362]
- 50 **Gastaldelli A**, Gaggini M, DeFronzo RA. Role of Adipose Tissue Insulin Resistance in the Natural History of Type 2 Diabetes: Results From the San Antonio Metabolism Study. *Diabetes* 2017; **66**: 815-822 [PMID: 28052966 DOI: 10.2337/db16-1167]
- 51 **Folli F**, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, Perego C, Muscogiuri G. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro- and macrovascular complications: avenues for a mechanistic-based therapeutic approach. *Curr Diabetes Rev* 2011; **7**: 313-324 [PMID: 21838680 DOI: 10.2174/157339911797415585]
- 52 **Ceriello A**, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol* 2004; **24**: 816-823 [PMID: 14976002 DOI: 10.1161/01.ATV.0000122852.22604.78]
- 53 **Wright E Jr**, Scism-Bacon JL, Glass LC. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. *Int J Clin Pract* 2006; **60**: 308-314 [PMID: 16494646 DOI: 10.1111/j.1368-5031.2006.00825.x]
- 54 **Brownlee M**. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; **414**: 813-820 [PMID: 11742414 DOI: 10.1038/414813a]
- 55 **Zee PC**, Attarian H, Videnovic A. Circadian rhythm abnormalities. *Continuum (Minneapolis)* 2013; **19**: 132-147 [PMID: 23385698 DOI: 10.1212/01.CON.0000427209.21177.aa]
- 56 **Ramkisoensing A**, Meijer JH. Synchronization of Biological Clock Neurons by Light and Peripheral Feedback Systems Promotes Circadian Rhythms and Health. *Front Neurol* 2015; **6**: 128 [PMID: 26097465 DOI: 10.3389/fneur.2015.00128]
- 57 **Golombek DA**, Rosenstein RE. Physiology of circadian entrainment. *Physiol Rev* 2010; **90**: 1063-1102 [PMID: 20664079 DOI: 10.1152/physrev.00009.2009]
- 58 **Dardente H**, Cermakian N. Molecular circadian rhythms in central and peripheral clocks in mammals. *Chronobiol Int* 2007; **24**: 195-213 [PMID: 17453843 DOI: 10.1080/07420520701283693]
- 59 **Cajochen C**, Kräuchi K, Wirz-Justice A. Role of melatonin in the regulation of human circadian rhythms and sleep. *J Neuroendocrinol* 2003; **15**: 432-437 [PMID: 12622846 DOI: 10.1046/j.1365-2826.2003.00989.x]
- 60 **Takahashi JS**, Hong HK, Ko CH, McDermarmon EL. The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nat Rev Genet* 2008; **9**: 764-775 [PMID: 18802415 DOI: 10.1038/nrg2430]
- 61 **Reppert SM**, Weaver DR. Molecular analysis of mammalian circadian rhythms. *Annu Rev Physiol* 2001; **63**: 647-676 [PMID: 11181971 DOI: 10.1146/annurev.physiol.63.1.647]
- 62 **Li MD**, Li CM, Wang Z. The role of circadian clocks in metabolic disease. *Yale J Biol Med* 2012; **85**: 387-401 [PMID: 23012586]
- 63 **Cribbet MR**, Logan RW, Edwards MD, Hanlon E, Bien Peek C, Stubblefield JJ, Vasudevan S, Ritchey F, Frank E. Circadian rhythms and metabolism: from the brain to the gut and back again. *Ann N Y Acad Sci* 2016; **1385**: 21-40 [PMID: 27589593 DOI: 10.1111/nyas.13188]
- 64 **Froy O**. Metabolism and circadian rhythms--implications for obesity. *Endocr Rev* 2010; **31**: 1-24 [PMID: 19854863 DOI: 10.1210/er.2009-0014]
- 65 **Froy O**, Garaulet M. The circadian clock in white and brown adipose tissue: mechanistic, endocrine and clinical aspects. *Endocr Rev* 2018; **39**: 261-273 [PMID: 29490014 DOI: 10.1210/er.2017-00193]
- 66 **Van Cauter E**, Spiegel K, Tasali E, Leproult R. Metabolic consequences of sleep and sleep loss. *Sleep Med* 2008; **9** Suppl 1: S23-S28 [PMID: 18929315 DOI: 10.1016/S1389-9457(08)70013-3]
- 67 **Sridhar GR**, Sanjana NS. Sleep, circadian dysrhythmia, obesity and diabetes. *World J Diabetes* 2016; **7**: 515-522 [PMID: 27895820 DOI: 10.4239/wjcd.v7.i19.515]
- 68 **Parkes KR**. Shift work and age as interactive predictors of body mass index among offshore workers. *Scand J Work Environ Health* 2002; **28**: 64-71 [PMID: 11871855 DOI: 10.5271/sjweh.648]
- 69 **Karlsson BH**, Knutsson AK, Lindahl BO, Alfredsson LS. Metabolic disturbances in male workers with rotating three-shift work. Results of the WOLF study. *Int Arch Occup Environ Health* 2003; **76**: 424-430 [PMID: 12783235 DOI: 10.1007/s00420-003-0440-y]
- 70 **Eckel-Mahan K**, Sassone-Corsi P. Metabolism and the circadian clock converge. *Physiol Rev* 2013; **93**: 107-135 [PMID: 23303907 DOI: 10.1152/physrev.00016.2012]
- 71 **Karatsoreos IN**, Bhagat S, Bloss EB, Morrison JH, McEwen BS. Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc Natl Acad Sci USA* 2011; **108**: 1657-1662 [PMID: 21220317 DOI: 10.1073/pnas.1018375108]
- 72 **La Fleur SE**, Kalsbeek A, Wortel J, Buijs RM. A suprachiasmatic nucleus generated rhythm in basal glucose concentrations. *J Neuroendocrinol* 1999; **11**: 643-652 [PMID: 10447803 DOI: 10.1046/j.1365-2826.1999.00373.x]
- 73 **Ruiter M**, La Fleur SE, van Heijningen C, van der Vliet J, Kalsbeek A, Buijs RM. The daily rhythm in plasma glucagon concentrations in the rat is modulated by the biological clock and by feeding behavior. *Diabetes* 2003; **52**: 1709-1715 [PMID: 12829637 DOI: 10.2337/diabetes.52.7.1709]
- 74 **Bodosi B**, Gardi J, Hajdu I, Szentirmai E, Obal F Jr, Krueger JM. Rhythms of ghrelin, leptin, and sleep in rats: effects of the normal diurnal cycle, restricted feeding, and sleep deprivation. *Am J Physiol Regul Integr Comp Physiol* 2004; **287**: R1071-R1079 [PMID: 15475503 DOI: 10.1152/ajpregu.00294.2004]
- 75 **Peek CB**, Affinati AH, Ramsey KM, Kuo HY, Yu W, Sena LA, Ilkayeva O, Marcheva B, Kobayashi Y, Omura C, Levine DC, Bacsik DJ, Gius D, Newgard CB, Goetzman E, Chandel NS, Denu JM, Mrksich M, Bass J. Circadian clock NAD⁺ cycle drives mitochondrial oxidative metabolism in mice. *Science* 2013; **342**: 1243417 [PMID: 24051248 DOI: 10.1126/science.1243417]

- 76 **Nakahata Y**, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, Guarente LP, Sassone-Corsi P. The NAD⁺-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* 2008; **134**: 329-340 [PMID: 18662547 DOI: 10.1016/j.cell.2008.07.002]
- 77 **Nakahata Y**, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P. Circadian control of the NAD⁺ salvage pathway by CLOCK-SIRT1. *Science* 2009; **324**: 654-657 [PMID: 19286518 DOI: 10.1126/science.1170803]
- 78 **Ramsey KM**, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, Marcheva B, Hong HK, Chong JL, Buhr ED, Lee C, Takahashi JS, Imai S, Bass J. Circadian clock feedback cycle through NAMPT-mediated NAD⁺ biosynthesis. *Science* 2009; **324**: 651-654 [PMID: 19299583 DOI: 10.1126/science.1171641]
- 79 **Rutter J**, Reick M, Wu LC, McKnight SL. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* 2001; **293**: 510-514 [PMID: 11441146 DOI: 10.1126/science.1060698]
- 80 **Kahn BB**, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab* 2005; **1**: 15-25 [PMID: 16054041 DOI: 10.1016/j.cmet.2004.12.003]
- 81 **Asher G**, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, Mostoslavsky R, Alt FW, Schibler U. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* 2008; **134**: 317-328 [PMID: 18662546 DOI: 10.1016/j.cell.2008.06.050]
- 82 **Lamia KA**, Sachdeva UM, DiTacchio L, Williams EC, Alvarez JG, Egan DF, Vasquez DS, Juguilon H, Panda S, Shaw RJ, Thompson CB, Evans RM. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science* 2009; **326**: 437-440 [PMID: 19833968 DOI: 10.1126/science.1172156]
- 83 **Huang W**, Ramsey KM, Marcheva B, Bass J. Circadian rhythms, sleep, and metabolism. *J Clin Invest* 2011; **121**: 2133-2141 [PMID: 21633182 DOI: 10.1172/JCI46043]
- 84 **Kiehn JT**, Koch CE, Walter M, Brod A, Oster H. Circadian rhythms and clocks in adipose tissues: Current Insights. *ChronoPhysiology and Therapy* 2017; **2017**: 7-17 [DOI: 10.2147/CPT.S116242]
- 85 **Fontaine C**, Dubois G, Duguay Y, Helledie T, Vu-Dac N, Gervois P, Soncin F, Mandrup S, Fruchart JC, Fruchart-Najib J, Staels B. The orphan nuclear receptor Rev-Erbalph is a peroxisome proliferator-activated receptor (PPAR) gamma target gene and promotes PPARgamma-induced adipocyte differentiation. *J Biol Chem* 2003; **278**: 37672-37680 [PMID: 12821652 DOI: 10.1074/jbc.M304664200]
- 86 **Shimba S**, Ishii N, Ohta Y, Ohno T, Watabe Y, Hayashi M, Wada T, Aoyagi T, Tezuka M. Brain and muscle Arnt-like protein-1 (BMAL1), a component of the molecular clock, regulates adipogenesis. *Proc Natl Acad Sci USA* 2005; **102**: 12071-12076 [PMID: 16093318 DOI: 10.1073/pnas.0502383102]
- 87 **Wang J**, Lazar MA. Bifunctional role of Rev-erbalph in adipocyte differentiation. *Mol Cell Biol* 2008; **28**: 2213-2220 [PMID: 18227153 DOI: 10.1128/MCB.01608-07]
- 88 **Grimaldi B**, Bellet MM, Katada S, Astarita G, Hirayama J, Amin RH, Granneman JG, Piomelli D, Leff T, Sassone-Corsi P. PER2 controls lipid metabolism by direct regulation of PPAR γ . *Cell Metab* 2010; **12**: 509-520 [PMID: 21035761 DOI: 10.1016/j.cmet.2010.10.005]
- 89 **Duez H**, Duhem C, Laitinen S, Patole PS, Abdelkarim M, Bois-Joyeux B, Danan JL, Staels B. Inhibition of adipocyte differentiation by ROR α . *FEBS Lett* 2009; **583**: 2031-2036 [PMID: 19450581 DOI: 10.1016/j.febslet.2009.05.019]
- 90 **Meissburger B**, Ukropec J, Roeder E, Beaton N, Geiger M, Teupser D, Civan B, Langhans W, Nawroth PP, Gasperikova D, Rudofsky G, Wolfrum C. Adipogenesis and insulin sensitivity in obesity are regulated by retinoid-related orphan receptor gamma. *EMBO Mol Med* 2011; **3**: 637-651 [PMID: 21853531 DOI: 10.1002/emmm.201100172]
- 91 **Rosen ED**, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, Spiegelman BM. C/EBP α induces adipogenesis through PPAR γ : a unified pathway. *Genes Dev* 2002; **16**: 22-26 [PMID: 11782441 DOI: 10.1101/gad.948702]
- 92 **Cristancho AG**, Lazar MA. Forming functional fat: a growing understanding of adipocyte differentiation. *Nat Rev Mol Cell Biol* 2011; **12**: 722-734 [PMID: 21952300 DOI: 10.1038/nrm3198]
- 93 **Kohsaka A**, Laposky AD, Ramsey KM, Estrada C, Joshi C, Kobayashi Y, Turek FW, Bass J. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab* 2007; **6**: 414-421 [PMID: 17983587 DOI: 10.1016/j.cmet.2007.09.006]
- 94 **Hutchison AT**, Wittert GA, Heilbronn LK. Matching Meals to Body Clocks-Impact on Weight and Glucose Metabolism. *Nutrients* 2017; **9**: pii: E222 [PMID: 28257081 DOI: 10.3390/nu9030222]
- 95 **Peret J**, Macaire I, Chanez M. Schedule of protein ingestion, nitrogen and energy utilization and circadian rhythm of hepatic glycogen, plasma corticosterone and insulin in rats. *J Nutr* 1973; **103**: 866-874 [PMID: 4705272 DOI: 10.1093/jn/103.6.866]
- 96 **Armstrong S**. A chronometric approach to the study of feeding behavior. *Neurosci Biobehav Rev* 1980; **4**: 27-53 [PMID: 6995873 DOI: 10.1016/0149-7634(80)90024-X]
- 97 **Bo S**, Musso G, Beccuti G, Fadda M, Fedele D, Gambino R, Gentile L, Durazzo M, Ghigo E, Cassader M. Consuming more of daily caloric intake at dinner predisposes to obesity. A 6-year population-based prospective cohort study. *PLoS One* 2014; **9**: e108467 [PMID: 25250617 DOI: 10.1371/journal.pone.0108467]
- 98 **Sonnier T**, Rood J, Gimble JM, Peterson CM. Glycemic control is impaired in the evening in prediabetes through multiple diurnal rhythms. *J Diabetes Complications* 2014; **28**: 836-843 [PMID: 24835190 DOI: 10.1016/j.jdiacomp.2014.04.001]
- 99 **Morris CJ**, Purvis TE, Mistretta J, Scheer FA. Effects of the Internal Circadian System and Circadian Misalignment on Glucose Tolerance in Chronic Shift Workers. *J Clin Endocrinol Metab* 2016; **101**: 1066-1074 [PMID: 26771705 DOI: 10.1210/jc.2015-3924]
- 100 **Morgan LM**, Aspostolou F, Wright J, Gama R. Diurnal variations in peripheral insulin resistance and plasma non-esterified fatty acid concentrations: a possible link? *Ann Clin Biochem* 1999; **36** (Pt 4): 447-450 [PMID: 10456206 DOI: 10.1177/000456329903600407]
- 101 **Van Cauter E**, Blackman JD, Roland D, Spire JP, Refetoff S, Polonsky KS. Modulation of glucose regulation and insulin secretion by circadian rhythmicity and sleep. *J Clin Invest* 1991; **88**: 934-942 [PMID: 1885778 DOI: 10.1172/JCI115396]
- 102 **Peschke E**, Peschke D. Evidence for a circadian rhythm of insulin release from perfused rat pancreatic islets. *Diabetologia* 1998; **41**: 1085-1092 [PMID: 9754828 DOI: 10.1007/s001250051034]
- 103 **Polonsky KS**, Given BD, Van Cauter E. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. *J Clin Invest* 1988; **81**: 442-448 [PMID: 3276730 DOI: 10.1172/JCI113339]
- 104 **Turek FW**, Joshi C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* 2005; **308**: 1043-1045 [PMID: 15845877 DOI: 10.1126/science.1108750]
- 105 **Ikeda H**, Yong Q, Kurose T, Todo T, Mizunoya W, Fushiki T, Seino Y, Yamada Y. Clock gene defect disrupts light-dependency of autonomic nerve activity. *Biochem Biophys Res Commun* 2007; **364**: 457-463 [PMID: 17964540 DOI: 10.1016/j.bbrc.2007.10.058]
- 106 **Gil-Lozano M**, Mingomataj EL, Wu WK, Ridout SA, Brubaker PL. Circadian secretion of the intestinal hormone GLP-1 by the rodent L cell. *Diabetes* 2014; **63**: 3674-3685 [PMID: 24789917 DOI: 10.2337/db13-1501]
- 107 **Gil-Lozano M**, Wu WK, Martchenko A, Brubaker PL. High-Fat Diet and Palmitate Alter the Rhythmic Secretion of Glucagon-Like Peptide-1 by the Rodent L-cell. *Endocrinology* 2016; **157**: 586-599 [PMID: 26646204 DOI: 10.1210/en.2015-1732]
- 108 **Voigt RM**, Forsyth CB, Green SJ, Engen PA, Keshavarzian A. Circadian Rhythm and the Gut Microbiome. *Int Rev Neurobiol* 2016; **131**: 193-205 [PMID: 27793218 DOI: 10.1016/bs.irn.2016.07.002]
- 109 **Blandino G**, Inturri R, Lazzara F, Di Rosa M, Malaguarnera L.

- Impact of gut microbiota on diabetes mellitus. *Diabetes Metab* 2016; **42**: 303-315 [PMID: 27179626 DOI: 10.1016/j.diabet.2016.04.004]
- 110 **Mikuni E**, Ohoshi T, Hayashi K, Miyamura K. Glucose intolerance in an employed population. *Tohoku J Exp Med* 1983; **141** Suppl: 251-256 [PMID: 6680494 DOI: 10.1620/tjem.141.Suppl_251]
 - 111 **Kawakami N**, Araki S, Takatsuka N, Shimizu H, Ishibashi H. Overtime, psychosocial working conditions, and occurrence of non-insulin dependent diabetes mellitus in Japanese men. *J Epidemiol Community Health* 1999; **53**: 359-363 [PMID: 10396483 DOI: 10.1136/jech.53.6.359]
 - 112 **Qian J**, Block GD, Colwell CS, Matveyenko AV. Consequences of exposure to light at night on the pancreatic islet circadian clock and function in rats. *Diabetes* 2013; **62**: 3469-3478 [PMID: 23775768 DOI: 10.2337/db12-1543]
 - 113 **Kurose T**, Hyo T, Yabe D, Seino Y. The role of chronobiology and circadian rhythms in type 2 diabetes mellitus: implications for management of diabetes. *Chronophysiology and Therapy* 2014; **4**: 41-49 [DOI: 10.2147/CPT.S44804]
 - 114 **Tang Y**, Meng L, Li D, Yang M, Zhu Y, Li C, Jiang Z, Yu P, Li Z, Song H, Ni C. Interaction of sleep quality and sleep duration on glycemic control in patients with type 2 diabetes mellitus. *Chin Med J (Engl)* 2014; **127**: 3543-3547 [PMID: 25316226 DOI: 10.3760/cma.j.issn.0366-6999.20141512]
 - 115 **Lou P**, Chen P, Zhang L, Zhang P, Yu J, Zhang N, Wu H, Zhao J. Relation of sleep quality and sleep duration to type 2 diabetes: a population-based cross-sectional survey. *BMJ Open* 2012; **2**: pii: e000956 [PMID: 22872722 DOI: 10.1136/bmjopen-2012-000956]
 - 116 **Sakamoto R**, Yamakawa T, Takahashi K, Suzuki J, Shinoda MM, Sakamaki K, Danno H, Tsuchiya H, Waseda M, Takano T, Minagawa F, Takai M, Masutani T, Nagakura J, Shigematsu E, Ishikawa M, Nakajima S, Kadonosono K, Terauchi Y. Association of usual sleep quality and glycemic control in type 2 diabetes in Japanese: A cross sectional study. Sleep and Food Registry in Kanagawa (SOREKA). *PLoS One* 2018; **13**: e0191771 [PMID: 29364963 DOI: 10.1371/journal.pone.0191771]
 - 117 **Onaolapo OJ**, Onaolapo AY. Melatonin, adolescence, and the brain: An insight into the period-specific influences of a multifunctional signaling molecule. *Birth Defects Res* 2017; **109**: 1659-1671 [PMID: 29251845 DOI: 10.1002/bdr2.1171]
 - 118 **Onaolapo OJ**, Onaolapo AY. Melatonin: Medical Uses and Role in Health and Disease. Chapter 4. Melatonin Receptors, Behaviour and Brain Function. In: Correia L and Mayers G (editors). Nova Science Publishers, 2018: 133-158
 - 119 **Hardeland R**, Pandi-Perumal SR, Cardinali DP. Melatonin. *Int J Biochem Cell Biol* 2006; **38**: 313-316 [PMID: 16219483 DOI: 10.1016/j.biocel.2005.08.020]
 - 120 **Puig-Domingo M**, Webb SM, Serrano J, Peinado MA, Corcoy R, Rusalleda J, Reiter RJ, de Leiva A. Brief report: melatonin-related hypogonadotropic hypogonadism. *N Engl J Med* 1992; **327**: 1356-1359 [PMID: 1406837 DOI: 10.1056/NEJM199211053271905]
 - 121 **Cavallo A**. Melatonin and human puberty: current perspectives. *J Pineal Res* 1993; **15**: 115-121 [PMID: 8106956 DOI: 10.1111/j.1600-079X.1993.tb00517.x]
 - 122 **Cavallo A**, Dolan LM. 6-Hydroxymelatonin sulfate excretion in human puberty. *J Pineal Res* 1996; **21**: 225-230 [PMID: 8989721 DOI: 10.1111/j.1600-079X.1996.tb00290.x]
 - 123 **Chan KH**, Wong YH. A molecular and chemical perspective in defining melatonin receptor subtype selectivity. *Int J Mol Sci* 2013; **14**: 18385-18406 [PMID: 24018885 DOI: 10.3390/ijms140918385]
 - 124 **Onaolapo AY**, Adebayo AN, Onaolapo OJ. Exogenous daytime melatonin modulates response of adolescent mice in a repeated unpredictable stress paradigm. *Naunyn Schmiedeberg's Arch Pharmacol* 2017; **390**: 149-161 [PMID: 27844092 DOI: 10.1007/s00210-016-1314-7]
 - 125 **Onaolapo OJ**, Onaolapo AY, Abiola AA, Lillian EA. Central depressant and nootropic effects of daytime melatonin in mice. *Ann Neurosci* 2014; **21**: 90-96 [PMID: 25206072 DOI: 10.5214/ans.0972.7531.210304]
 - 126 **Comai S**, Gobbi G. Unveiling the role of melatonin MT2 receptors in sleep, anxiety and other neuropsychiatric diseases: a novel target in psychopharmacology. *J Psychiatry Neurosci* 2014; **39**: 6-21 [PMID: 23971978 DOI: 10.1503/jpn.130009]
 - 127 **Pandi-Perumal SR**, Trakht I, Srinivasan V, Spence DW, Maestroni GJ, Zisapel N, Cardinali DP. Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. *Prog Neurobiol* 2008; **85**: 335-353 [PMID: 18571301 DOI: 10.1016/j.pneurobio.2008.04.001]
 - 128 **Tocharus C**, Puriboriboon Y, Junmanee T, Tocharus J, Ekthuwapranee K, Govitrapong P. Melatonin enhances adult rat hippocampal progenitor cell proliferation via ERK signaling pathway through melatonin receptor. *Neuroscience* 2014; **275**: 314-321 [PMID: 24956284 DOI: 10.1016/j.neuroscience.2014.06.026]
 - 129 **Onaolapo AY**, Aina OA, Onaolapo OJ. Melatonin attenuates behavioural deficits and reduces brain oxidative stress in a rodent model of schizophrenia. *Biomed Pharmacother* 2017; **92**: 373-383 [PMID: 28554133 DOI: 10.1016/j.biopha.2017.05.094]
 - 130 **Korkmaz A**, Reiter RJ, Topal T, Manchester LC, Oter S, Tan DX. Melatonin: an established antioxidant worthy of use in clinical trials. *Mol Med* 2009; **15**: 43-50 [PMID: 19011689 DOI: 10.2119/molmed.2008.00117]
 - 131 **Bubenik GA**. Gastrointestinal melatonin: localization, function, and clinical relevance. *Dig Dis Sci* 2002; **47**: 2336-2348 [PMID: 12395907 DOI: 10.1023/A:1020107915919]
 - 132 **Bermudez FF**, Forbes JM, Injidi MH. Involvement of melatonin and thyroid hormones in the control of sleep, food intake and energy metabolism in the domestic fowl. *J Physiol* 1983; **337**: 19-27 [PMID: 6410055 DOI: 10.1113/jphysiol.1983.sp014608]
 - 133 **Wilson AP**, Gaya H. Treatment of endocarditis with teicoplanin: a retrospective analysis of 104 cases. *J Antimicrob Chemother* 1996; **38**: 507-521 [PMID: 8889725 DOI: 10.1093/jac/38.3.507]
 - 134 **Wolden-Hanson T**, Mitton DR, McCants RL, Yellon SM, Wilkinson CW, Matsumoto AM, Rasmussen DD. Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. *Endocrinology* 2000; **141**: 487-497 [PMID: 10650927 DOI: 10.1210/endo.141.2.7311]
 - 135 **Bubenik GA**, Pang SF. The role of serotonin and melatonin in gastrointestinal physiology: ontogeny, regulation of food intake, and mutual serotonin-melatonin feedback. *J Pineal Res* 1994; **16**: 91-99 [PMID: 8014829 DOI: 10.1111/j.1600-079X.1994.tb00088.x]
 - 136 **Piccinetti CC**, Migliarini B, Olivetto I, Simoniello MP, Giorgini E, Carnevali O. Melatonin and peripheral circuitries: insights on appetite and metabolism in Danio rerio. *Zebrafish* 2013; **10**: 275-282 [PMID: 23682835 DOI: 10.1089/zeb.2012.0844]
 - 137 **Kasimay O**, Cakir B, Devseren E, Yegen BC. Exogenous melatonin delays gastric emptying rate in rats: role of CCK2 and 5-HT3 receptors. *J Physiol Pharmacol* 2005; **56**: 543-553 [PMID: 16391413]
 - 138 **Velarde E**, Alonso-Gómez AL, De Pedro N, Azpeleta L, Ortiz L, Delgado MJ. Caracterización de la actividad miométrica del intestino de *Carassius auratus* para el estudio del efecto in vitro de la melatonina en la actividad gastrointestinal. *CIVA* 2006; 249-258
 - 139 **Nieminen P**, Käkälä R, Mustonen AM, Hyvärinen H, Asikainen J. Exogenous melatonin affects lipids and enzyme activities in mink (*Mustela vison*) liver. *Comp Biochem Physiol C Toxicol Pharmacol* 2001; **128**: 203-211 [PMID: 11239833 DOI: 10.1016/S1532-0456(00)00190-3]
 - 140 **De Pedro N**, Martínez-Alvarez RM, Delgado MJ. Melatonin reduces body weight in goldfish (*Carassius auratus*): effects on metabolic resources and some feeding regulators. *J Pineal Res* 2008; **45**: 32-39 [PMID: 18284553 DOI: 10.1111/j.1600-079X.2007.00553.x]
 - 141 **Zhdanova IV**. Sleep and its regulation in zebrafish. *Rev Neurosci* 2011; **22**: 27-36 [PMID: 21615259 DOI: 10.1515/ms.2011.005]
 - 142 **Xia Q**, Chen ZX, Wang YC, Ma YS, Zhang F, Che W, Fu D, Wang XF. Association between the melatonin receptor 1B gene polymorphism on the risk of type 2 diabetes, impaired glucose regulation: a meta-analysis. *PLoS One* 2012; **7**: e50107 [PMID: 23226241 DOI: 10.1371/journal.pone.0050107]
 - 143 **Lardone PJ**, Alvarez-Sanchez SN, Guerrero JM, Carrillo-Vico A.

- Melatonin and glucose metabolism: clinical relevance. *Curr Pharm Des* 2014; **20**: 4841-4853 [PMID: 24251676 DOI: 10.2174/1381612819666131119101032]
- 144 **Poon AM**, Choy EH, Pang SF. Modulation of blood glucose by melatonin: a direct action on melatonin receptors in mouse hepatocytes. *Biol Signals Recept* 2001; **10**: 367-379 [PMID: 11721092 DOI: 10.1159/000046904]
 - 145 **Lima FB**, Machado UF, Bartol I, Seraphim PM, Sumida DH, Moraes SM, Hell NS, Okamoto MM, Saad MJ, Carvalho CR, Cipolla-Neto J. Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. *Am J Physiol* 1998; **275**: E934-E941 [PMID: 9843734 DOI: 10.1152/ajpendo.1998.275.6.E934]
 - 146 **O'Brien IA**, Lewin IG, O'Hare JP, Arendt J, Corral RJ. Abnormal circadian rhythm of melatonin in diabetic autonomic neuropathy. *Clin Endocrinol (Oxf)* 1986; **24**: 359-364 [PMID: 3742831 DOI: 10.1111/j.1365-2265.1986.tb01639.x]
 - 147 **McMullan CJ**, Schernhammer ES, Rimm EB, Hu FB, Forman JP. Melatonin secretion and the incidence of type 2 diabetes. *JAMA* 2013; **309**: 1388-1396 [PMID: 23549584 DOI: 10.1001/jama.2013.2710]
 - 148 **Garfinkel D**, Zorin M, Wainstein J, Matas Z, Laudon M, Zisapel N. Efficacy and safety of prolonged-release melatonin in insomnia patients with diabetes: a randomized, double-blind, crossover study. *Diabetes Metab Syndr Obes* 2011; **4**: 307-313 [PMID: 21887103 DOI: 10.2147/DMSO.S23904]
 - 149 **Peschke E**, Hofmann K, Pönicke K, Wedekind D, Mühlbauer E. Catecholamines are the key for explaining the biological relevance of insulin-melatonin antagonisms in type 1 and type 2 diabetes. *J Pineal Res* 2012; **52**: 389-396 [PMID: 21929683 DOI: 10.1111/j.1600-079X.2011.00951.x]
 - 150 **Espino J**, Pariente JA, Rodríguez AB. Role of melatonin on diabetes-related metabolic disorders. *World J Diabetes* 2011; **2**: 82-91 [PMID: 21860691 DOI: 10.4239/wjd.v2.i6.82]
 - 151 **Nishida S**. Metabolic effects of melatonin on oxidative stress and diabetes mellitus. *Endocrine* 2005; **27**: 131-136 [PMID: 16217126 DOI: 10.1385/ENDO.27.2.131]
 - 152 **Peschke E**. Melatonin, endocrine pancreas and diabetes. *J Pineal Res* 2008; **44**: 26-40 [PMID: 18078445 DOI: 10.1111/j.1600-079X.2007.00519.x]
 - 153 **Robeva R**, Kirilov G, Tomova A, Kumanov P. Melatonin-insulin interactions in patients with metabolic syndrome. *J Pineal Res* 2008; **44**: 52-56 [PMID: 18078448 DOI: 10.1111/j.1600-079X.2007.00527.x]
 - 154 **Peschke E**, Frese T, Chankiewitz E, Peschke D, Preiss U, Schneyer U, Spessert R, Mühlbauer E. Diabetic Goto Kakizaki rats as well as type 2 diabetic patients show a decreased diurnal serum melatonin level and an increased pancreatic melatonin-receptor status. *J Pineal Res* 2006; **40**: 135-143 [PMID: 16441550 DOI: 10.1111/j.1600-079X.2005.00287.x]
 - 155 **Bähr I**, Mühlbauer E, Schucht H, Peschke E. Melatonin stimulates glucagon secretion in vitro and in vivo. *J Pineal Res* 2011; **50**: 336-344 [PMID: 21244480 DOI: 10.1111/j.1600-079X.2010.00848.x]
 - 156 **Poirel VJ**, Cailotto C, Streicher D, Pévet P, Masson-Pévet M, Gauer F. MT1 melatonin receptor mRNA tissular localization by PCR amplification. *Neuro Endocrinol Lett* 2003; **24**: 33-38 [PMID: 12743529]
 - 157 **Stebelová K**, Anttila K, Mänttari S, Saarela S, Zeman M. Immunohistochemical definition of MT(2) receptors and melatonin in the gastrointestinal tissues of rat. *Acta Histochem* 2010; **112**: 26-33 [PMID: 19004484 DOI: 10.1016/j.acthis.2008.03.004]
 - 158 **Nagorny CL**, Sathanoori R, Voss U, Mulder H, Wierup N. Distribution of melatonin receptors in murine pancreatic islets. *J Pineal Res* 2011; **50**: 412-417 [PMID: 21355877 DOI: 10.1111/j.1600-079X.2011.00859.x]
 - 159 **Peschke E**, Fauteck JD, Musshoff U, Schmidt F, Beckmann A, Peschke D. Evidence for a melatonin receptor within pancreatic islets of neonate rats: functional, autoradiographic, and molecular investigations. *J Pineal Res* 2000; **28**: 156-164 [PMID: 10739302 DOI: 10.1034/j.1600-079X.2001.280305.x]
 - 160 **Soták M**, Mrnka L, Pácha J. Heterogeneous expression of melatonin receptor MT1 mRNA in the rat intestine under control and fasting conditions. *J Pineal Res* 2006; **41**: 183-188 [PMID: 16879325 DOI: 10.1111/j.1600-079X.2006.00355.x]
 - 161 **Staiger H**, Machicao F, Schäfer SA, Kirchhoff K, Kantartzis K, Guthoff M, Silbernagel G, Stefan N, Häring HU, Fritsche A. Polymorphisms within the novel type 2 diabetes risk locus MTNR1B determine beta-cell function. *PLoS One* 2008; **3**: e3962 [PMID: 19088850 DOI: 10.1371/journal.pone.0003962]
 - 162 **Prokopenko I**, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y, Potter SC, Erdos MR, Sanna S, Hottenga JJ, Wheeler E, Kaakinen M, Lyssenko V, Chen WM, Ahmadi K, Beckmann JS, Bergman RN, Bochud M, Bonnycastle LL, Buchanan TA, Cao A, Cervino A, Coin L, Collins FS, Crisponi L, de Geus EJ, Dehghan A, Deloukas P, Doney AS, Elliott P, Freimer N, Gateva V, Herder C, Hofman A, Hughes TE, Hunt S, Illig T, Inouye M, Isomaa B, Johnson T, Kong A, Krestyaninova M, Kuusisto J, Laakso M, Lim N, Lindblad U, Lindgren CM, McCann OT, Mohlke KL, Morris AD, Naitza S, Orrù M, Palmer CN, Pouta A, Randall J, Rathmann W, Saramies J, Scheet P, Scott LJ, Scuteri A, Sharp S, Sijbrands E, Smit JH, Song K, Steinthorsdottir V, Stringham HM, Tuomi T, Tuomilehto J, Uitterlinden AG, Voight BF, Waterworth D, Wichmann HE, Willemssen G, Witteman JC, Yuan X, Zhao JH, Zeggini E, Schlessinger D, Sandhu M, Boomsma DI, Uda M, Spector TD, Penninx BW, Altshuler D, Vollenweider P, Jarvelin MR, Lakatta E, Waeber G, Fox CS, Peltonen L, Groop LC, Mooser V, Cupples LA, Thorsteinsdottir U, Boehnke M, Barroso I, Van Duijn C, Dupuis J, Watanabe RM, Stefansson K, McCarthy MI, Wareham NJ, Meigs JB, Abecasis GR. Variants in MTNR1B influence fasting glucose levels. *Nat Genet* 2009; **41**: 77-81 [PMID: 19060907 DOI: 10.1038/ng.290]
 - 163 **Tam CH**, Ho JS, Wang Y, Lee HM, Lam VK, Germer S, Martin M, So WY, Ma RC, Chan JC, Ng MC. Common polymorphisms in MTNR1B, G6PC2 and GCK are associated with increased fasting plasma glucose and impaired beta-cell function in Chinese subjects. *PLoS One* 2010; **5**: e11428 [PMID: 20628598 DOI: 10.1371/journal.pone.0011428]
 - 164 **Müssig K**, Staiger H, Machicao F, Häring HU, Fritsche A. Genetic variants in MTNR1B affecting insulin secretion. *Ann Med* 2010; **42**: 387-393 [PMID: 20597807 DOI: 10.3109/07853890.2010.502125]
 - 165 **Sparso T**, Bonnefond A, Andersson E, Bouatia-Naji N, Holmkvist J, Wegner L, Grarup N, Gjesing AP, Banasik K, Calvacanti-Proença C, Marchand M, Vaxillaire M, Charpentier G, Jarvelin MR, Tichet J, Balkau B, Marre M, Lévy-Marchal C, Faerch K, Borch-Johnsen K, Jørgensen T, Madsbad S, Poulsen P, Vaag A, Dina C, Hansen T, Pedersen O, Froguel P. G-allele of intronic rs10830963 in MTNR1B confers increased risk of impaired fasting glycemia and type 2 diabetes through an impaired glucose-stimulated insulin release: studies involving 19,605 Europeans. *Diabetes* 2009; **58**: 1450-1456 [PMID: 19324940 DOI: 10.2337/db08-1660]
 - 166 **Takeuchi F**, Katsuya T, Chakrawarthy S, Yamamoto K, Fujioka A, Serizawa M, Fujisawa T, Nakashima E, Ohnaka K, Ikegami H, Sugiyama T, Nabika T, Kasturiratne A, Yamaguchi S, Kono S, Takayanagi R, Yamori Y, Kobayashi S, Ogihara T, de Silva A, Wickremasinghe R, Kato N. Common variants at the GCK, GCKR, G6PC2-ABCB11 and MTNR1B loci are associated with fasting glucose in two Asian populations. *Diabetologia* 2010; **53**: 299-308 [PMID: 19937311 DOI: 10.1007/s00125-009-1595-1]
 - 167 **Kan MY**, Zhou DZ, Zhang D, Zhang Z, Chen Z, Yang YF, Guo XZ, Xu H, He L, Liu Y. Two susceptible diabetogenic variants near/ in MTNR1B are associated with fasting plasma glucose in a Han Chinese cohort. *Diabet Med* 2010; **27**: 598-602 [PMID: 20536959 DOI: 10.1111/j.1464-5491.2010.02975.x]
 - 168 **Peschke E**, Stumpf I, Bazwinsky I, Litvak L, Dralle H, Mühlbauer E. Melatonin and type 2 diabetes - a possible link? *J Pineal Res* 2007; **42**: 350-358 [PMID: 17439551 DOI: 10.1111/j.1600-079X.2007.00426.x]
 - 169 **Nishida S**, Segawa T, Murai I, Nakagawa S. Long-term melatonin administration reduces hyperinsulinemia and improves the altered

- fatty-acid compositions in type 2 diabetic rats via the restoration of Delta-5 desaturase activity. *J Pineal Res* 2002; **32**: 26-33 [PMID: 11841597 DOI: 10.1034/j.1600-079x.2002.10797.x]
- 170 **Cagnacci A**, Arangino S, Renzi A, Paoletti AM, Melis GB, Cagnacci P, Volpe A. Influence of melatonin administration on glucose tolerance and insulin sensitivity of postmenopausal women. *Clin Endocrinol (Oxf)* 2001; **54**: 339-346 [PMID: 11298086 DOI: 10.1046/j.1365-2265.2001.01232.x]
 - 171 **Hussain SA**, Khadim HM, Khalaf BH, Ismail SH, Hussein KI, Sahib AS. Effects of melatonin and zinc on glycemic control in type 2 diabetic patients poorly controlled with metformin. *Saudi Med J* 2006; **27**: 1483-1488 [PMID: 17013468]
 - 172 **Forrestel AC**, Miedlich SU, Yurcheshen M, Wittlin SD, Sellix MT. Chronomedicine and type 2 diabetes: shining some light on melatonin. *Diabetologia* 2017; **60**: 808-822 [PMID: 27981356 DOI: 10.1007/s00125-016-4175-1]
 - 173 **Wojcik M**, Krawczyk M, Wojcik P, Cypriak K, Wozniak LA. Melatonin as a Pleiotropic Molecule with Therapeutic Potential for Type 2 Diabetes and Cancer. *Curr Med Chem* 2017; **24**: 3829-3850 [PMID: 28721827 DOI: 10.2174/0929867324666170718110606]
 - 174 **Thomas AP**, Hoang J, Vongbunyong K, Nguyen A, Rakshit K, Matveyenko AV. Administration of Melatonin and Metformin Prevents Deleterious Effects of Circadian Disruption and Obesity in Male Rats. *Endocrinology* 2016; **157**: 4720-4731 [PMID: 27653034 DOI: 10.1210/en.2016-1309]
 - 175 **Heo JI**, Yoon DW, Yu JH, Kim NH, Yoo HJ, Seo JA, Kim SG, Choi KM, Baik SH, Choi DS, Kim NH. Melatonin improves insulin resistance and hepatic steatosis through attenuation of alpha-2-HS-glycoprotein. *J Pineal Res* 2018; e12493 [PMID: 29607540 DOI: 10.1111/jpi.12493]
 - 176 **Zhou H**, Yue Y, Wang J, Ma Q, Chen Y. Melatonin therapy for diabetic cardiomyopathy: A mechanism involving Syk-mitochondrial complex I-SERCA pathway. *Cell Signal* 2018; **47**: 88-100 [PMID: 29601906 DOI: 10.1016/j.cellsig.2018.03.012]
 - 177 **Xu P**, Wang J, Hong F, Wang S, Jin X, Xue T, Jia L, Zhai Y. Melatonin prevents obesity through modulation of gut microbiota in mice. *J Pineal Res* 2017; **62** [PMID: 28199741 DOI: 10.1111/jpi.12399]
 - 178 **Parameyong A**, Govitrapong P, Chetsawang B. Melatonin attenuates the mitochondrial translocation of mitochondrial fission proteins and Bax, cytosolic calcium overload and cell death in methamphetamine-induced toxicity in neuroblastoma SH-SY5Y cells. *Mitochondrion* 2015; **24**: 1-8 [PMID: 26176977 DOI: 10.1016/j.mito.2015.07.004]
 - 179 **Chuang JI**, Pan IL, Hsieh CY, Huang CY, Chen PC, Shin JW. Melatonin prevents the dynamin-related protein 1-dependent mitochondrial fission and oxidative insult in the cortical neurons after 1-methyl-4-phenylpyridinium treatment. *J Pineal Res* 2016; **61**: 230-240 [PMID: 27159033 DOI: 10.1111/jpi.12343]
 - 180 **Coto-Montes A**, Boga JA, Rosales-Corral S, Fuentes-Broto L, Tan DX, Reiter RJ. Role of melatonin in the regulation of autophagy and mitophagy: a review. *Mol Cell Endocrinol* 2012; **361**: 12-23 [PMID: 22575351 DOI: 10.1016/j.mce.2012.04.009]
 - 181 **Ding M**, Feng N, Tang D, Feng J, Li Z, Jia M, Liu Z, Gu X, Wang Y, Fu F, Pei J. Melatonin prevents Drp1-mediated mitochondrial fission in diabetic hearts through SIRT1-PGC1 α pathway. *J Pineal Res* 2018; e12491 [PMID: 29575122 DOI: 10.1111/jpi.12491]
 - 182 **Bicer M**, Baltaci SB, Patlar S, Mogulkoc R, Baltaci AK. Melatonin has a protective effect against lipid peroxidation in the bone tissue of diabetic rats subjected to acute swimming exercise. *Horm Mol Biol Clin Investig* 2018; **34** [PMID: 29547389 DOI: 10.1515/hmbci-2017-0079]
 - 183 **Mehrzadi S**, Motevalian M, Rezaei Kanavi M, Fatemi I, Ghaznavi H, Shahriari M. Protective effect of melatonin in the diabetic rat retina. *Fundam Clin Pharmacol* 2018 [PMID: 29495082 DOI: 10.1111/fcp.12361]
 - 184 **Raygan F**, Ostadmohammadi V, Bahmani F, Reiter RJ, Asemi Z. Melatonin administration lowers biomarkers of oxidative stress and cardio-metabolic risk in type 2 diabetic patients with coronary heart disease: A randomized, double-blind, placebo-controlled trial. *Clin Nutr* 2017 [PMID: 29275919 DOI: 10.1016/j.clnu.2017.12.004]
 - 185 **Oliveira AC**, Andreotti S, Sertie RAL, Campana AB, de Proença ARG, Vasconcelos RP, Oliveira KA, Coelho-de-Souza AN, Donato-Junior J, Lima FB. Combined treatment with melatonin and insulin improves glycemic control, white adipose tissue metabolism and reproductive axis of diabetic male rats. *Life Sci* 2018; **199**: 158-166 [PMID: 29501522 DOI: 10.1016/j.lfs.2018.02.040]

P- Reviewer: Das U, Hamasaki H **S- Editor:** Ji FF **L- Editor:** A
E- Editor: Tan ww



Role of bisphosphonates in the management of acute Charcot foot

Harsh Durgia, Jayaprakash Sahoo, Sadishkumar Kamalanathan, Rajan Palui, Kalyani Sridharan, Henith Raj

Harsh Durgia, Jayaprakash Sahoo, Sadishkumar Kamalanathan, Rajan Palui, Kalyani Sridharan, Henith Raj, Department of Endocrinology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry 605006, India

ORCID number: Harsh Durgia (0000-0002-8404-5729); Jayaprakash Sahoo, (0000-0002-8805-143X); Sadishkumar Kamalanathan (0000-0002-2371-0625); Rajan Palui (0000-0002-2429-3595); Kalyani Sridharan (0000-0002-5867-5073); Henith Raj (0000-0002-1499-4021).

Author contributions: Durgia H, Sahoo J and Kamalanathan S designed the work and revised it critically for important intellectual content; Palui R, Sridharan K and Raj H interpreted the data and drafted the work; all authors approved the final version of the manuscript; Sahoo J is the guarantor.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited Manuscript

Correspondence to: Jayaprakash Sahoo, Associate Professor, Department of Endocrinology, Jawaharlal Institute of Postgraduate Medical Education and Research, Room No. 5444, 4th floor, Superspeciality Block, Puducherry 605006, India. jppgi@yahoo.com
Telephone: +91-962-9158368

Received: March 29, 2018

Peer-review started: March 29, 2018

First decision: April 24, 2018

Revised: April 26, 2018

Accepted: June 13, 2018

Article in press: June 14, 2018

Published online: July 15, 2018

Abstract

Diabetes mellitus is the most common cause of Charcot neuropathy affecting foot and ankle. Acute Charcot foot (CF) presents with a red and swollen foot in contrast to the painless deformed one of chronic CF. Enhanced osteoclastogenesis plays a central role in the pathogenesis of acute CF. Many studies have shown elevated levels of bone turnover markers in patients with acute CF confirming it. These findings have led clinicians to use anti-resorptive agents [bisphosphonates (BP), calcitonin, and denosumab] along with immobilization and offloading in acute CF patients. The maximum evidence among all anti-resorptive agents is available for BPs, although its quality is low. Pamidronate has been shown to reduce the markers of activity of CF like raised skin temperature, pain, edema, and bone turnover markers in the majority of studies. Intravenous BPs are known to cause acute phase reactions leading to flu-like illness following their first infusion, which can be ameliorated by oral acetaminophen. Alendronate is the only oral BP used in these patients. It needs to be taken on an empty stomach with a full glass of water to avoid esophagitis. The side-effects and contraindications to BPs should be kept in mind while treating acute CF patients with them.

Key words: Charcot foot; Diabetes mellitus; Charcot neuroarthropathy; Bisphosphonates; Pamidronate

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Bisphosphonate is an attractive treatment option for acute Charcot foot. This is based on the fact that increased osteoclastic activity plays a central role in the pathogenesis of acute Charcot foot. Among bisphosphonates, the maximum evidence in the literature is available for pamidronate. It has been shown to reduce the markers of Charcot foot activity, like raised skin temperature, pain, and edema. However, the quality of evidence is low. They should be used along

with immobilization and offloading. The side effects of bisphosphonates and their contra-indications for use should be kept in mind while treating these patients with them.

Durgia H, Sahoo J, Kamalanathan S, Palui R, Sridharan K, Raj H. Role of bisphosphonates in the management of acute Charcot foot. *World J Diabetes* 2018; 9(7): 115-126 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i7/115.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i7.115>

INTRODUCTION

Charcot foot (CF), also quoted as Charcot neuroarthropathy (CN), derives its name from Jean-Martin Charcot, who in 1868 first described neuroarthropathic changes in patients with tabes dorsalis^[1]. It was not until 68 years later, in 1936, that William Riley Jordan first established the association between diabetes mellitus (DM) and painless neuropathic arthropathy of ankle^[2]. It is a rare and devastating condition leading to the destruction of bone and joints and culminating in fractures, dislocations, deformities, and amputation of the foot in neglected cases. Virtually any condition that causes neuropathy can lead to CF, such as DM, syphilis, leprosy, spinal cord injury, meningomyelocele, syringomyelia, chronic alcoholism, and a host of other conditions like psoriasis, sarcoidosis, rheumatoid arthritis, human immunodeficiency virus, and Parkinson's disease. Currently, the world is witnessing an exponential rise in the prevalence of DM and its complications. According to the World Health Organization 2016 report, around 422 million people are living with DM^[3]. This has made DM the most common cause of CN affecting foot and ankle. The incidence of CF in diabetic patients ranges between 0.1% and 7.5%^[4].

Today, the pathophysiology of CF is still a bone of contention even after one and half centuries since its first description. Conventional theories for it include the neurovascular theory postulated by Charcot himself and the neurotraumatic theory proposed by Volkmann and Virchow^[5]. Peripheral sensorimotor neuropathy along with autonomic dysfunction is the essential factor for the development of CN. Usually a trivial trauma in the insensate foot kicks off the inflammatory cascade. Not all neuropathic patients, however, develop CF.

Recent advancements in the understanding of the pathophysiology of CF has shed light on factors like inflammatory cytokines and their interaction with receptor activator of nuclear factor kappa-B (RANK), its ligand (RANKL), and osteoprotegerin (OPG)^[6]. Long-standing hyperglycemia, with its complications ranging from neuropathy to formation of advanced glycation end products (AGEs)^[7] and protein kinase C (PKC) activation^[8], is the major culprit. Calcitonin gene-related peptide (CGRP)^[9], Wnt/beta-catenin pathway^[10], and OPG gene polymorphisms^[11] are new players in the field.

Interaction between RANKL, nuclear factor kappa-B (NF- κ B), and pro-inflammatory cytokines like tumor necrosis factor alpha (TNF- α), Interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) lead to localized osteolysis that destroys bone structure^[12]. Moreover, DM patients have lower 1,25(OH) $_2$ D $_3$ levels, leading to poor mineralization of bone^[13]. Lower calcium levels can stimulate parathyroid hormone, thus contributing to bone resorption and osteopenia^[14].

Studies have shown increased levels of osteoclastic resorption markers, such as serum carboxyterminal telopeptide of type 1 collagen (1CTP), in patients with CF^[15]. Immobilization and avoidance of physical stress by complete offloading with the help of total contact cast (TCC) is the mainstay in the management of CF^[16]. However, because of increased osteoclastic activity, the bone destruction continues unabated. Lower limb osteopenia seen in patients with CN along with increased bone resorptive markers make anti-resorptive agents like bisphosphonates (BPs), calcitonin, and denosumab reasonable treatment options, at least for adjuvant purposes. BPs are pyrophosphate (PP) analogs that have been in medical use for around half a century. First generation BPs like etidronate and clodronate have non-nitrogen containing side chains, whereas second and third generation ones like pamidronate, alendronate, ibandronate, risedronate, and zoledronate have nitrogen containing side chains. Nitrogen containing BPs are much more potent than the first generation ones and work by inhibiting farnesyl PP (FPP) synthase in the mevalonate pathway, which is crucial for function and survival of osteoclasts^[17]. In this study, we reviewed the available literature on the use of BPs in patients with acute CF.

CLINICAL PRESENTATION

Clinically, CF can present either in acute or chronic stage, and its features vary according to the stage of presentation. A high index of suspicion is required to diagnose CF in its early stage. Acute CF presents with a red and swollen foot, which is warmer than the contralateral normal foot. Patients may have mild to moderate pain or discomfort at this stage, which is much less when compared to those with a similar degree of inflammation without neuropathy^[16,18]. Skin temperature difference of $\geq 4^\circ$ Fahrenheit (or 2° Celsius) between affected and the normal foot indicates active CF^[19]. This can be measured using an infrared thermometer at the maximum point of deformity on the affected foot and at the same point on the normal foot. It is also helpful in monitoring the course of CF. Peripheral pedal pulses are typically bounding because of underlying autonomic neuropathy. Clinical presentation at this stage mimics those of deep vein thrombosis, acute gout, and cellulitis, and the diagnostic dilemma is compounded by the inability of radiographs to detect and differentiate these abnormalities. Magnetic resonance imaging can be helpful at an early stage of disease^[20]. If treatment is not

provided at this stage, it leads to further destruction of bone resulting in irreversible damage. Chronic CF is characterized by resolution of inflammation and establishment of residual deformity. Rocker bottom deformity is the classic abnormality that arises due to collapse of plantar arch in mid foot^[21]. This results in abnormal high pressure areas on the weight bearing sites of the plantar surface, making it prone to ulceration^[22].

PATHOGENESIS OF ACUTE CHARCOT FOOT

Conventional theories

Two age-old theories pertaining to the pathogenesis of CF that are still pertinent include neurovascular theory and neurotraumatic theory. Neurovascular theory^[1] suggests that damage to trophic or vasomotor nerves secondary to the underlying condition results in failure of vasoregulation, causing opening of arteriovenous shunts. This leads to the increased supply of blood to the bone, resulting in greater flux of monocytes and osteoclasts and culminating in bone resorption. Other factors, like peripheral vascular disease, are expected to co-exist with diabetic neuropathy. This leads to decreased blood flow to lower limbs, which can act as a protective factor against CF^[23]. This probably explains why CF affects only a fraction of DM patients with neuropathy.

On the other hand, Volkmann and Virchow in their neurotraumatic theory suggested that trauma to the insensate foot leads to CN^[5]. Repeated microtrauma in a patient with sensory neuropathy leads to bone destruction and deformity. Though both feet of susceptible patients have the propensity to develop CF, only the one exposed to recurrent trauma develops CF. This provides some ground for the pathogenesis of unilateral CF in the background of generalized neuropathy. However, it has been found to be bilateral in 9% to 39% of cases^[24]. With the passage of time, we have now come to know that CF results from the combination of these processes. Autonomic neuropathy weakens the bone because of increased blood supply, whereas sensory neuropathy causes loss of protective sensation leading to unperceived recurrent trauma to the abnormal bone. Muscle weakness due to motor neuropathy adds fuel to the fire, leading to joint instability and abnormal plantar pressures^[25]. These progress later to bone fracture and dislocation in foot and ankle.

Other factors that play a role in the pathogenesis of CF are: (1) inflammatory cytokines; (2) AGEs; and (3) neuropeptides and inorganic molecules. These mediators finally stimulate osteoclastogenesis, leading to bone loss *via* RANKL/OPG pathway (Figure 1).

Role of inflammatory cytokines

In addition to his neurovascular theory, Charcot recognized inflammation as one of the contributors to

CN. Christensen *et al.*^[26] in their study showed that hyperemia during an acute attack of CF was most likely secondary to the inflammation rather than sympathetic neuropathy. Thus, it is unabated inflammation in the background of neuropathy that results in the imbalance between osteoclasts and osteoblasts leading to bone resorption. This pro-inflammatory state can be triggered by repeated microtrauma. Hyperglycemia in DM can lead to increased PKC activity and formation of AGEs along with decreased phosphatidylinositol 3 kinase activity^[8]. This, in turn, results in an excessive production of pro-inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6. This storm of pro-inflammatory cytokines disturbs osteoclast-osteoblast homeostasis. Baumhauer *et al.*^[12] histologically examined 20 tissue biopsy specimens obtained from patients with CF. Immunohistochemical study of each of these biopsies showed positivity for IL-1, IL-6, and TNF- α . This was conclusive of stimulation of osteoclastic progenitor cells, leading to osteoclastogenesis by the cytokines present in the background during acute and reparative stages of CF. But inflammatory cytokines alone do not directly account for the increased osteoclastogenesis. Jeffcoate *et al.*^[27] suggested that inflammatory cytokines lead to increased osteoclastogenesis *via* increased expression of NF- κ B. This results in bone destruction, which again potentiates the inflammatory response thus culminating in a vicious cycle^[27]. Increased cytokines lead to increased activity of RANKL that in turn activates RANK, which is expressed on osteoclast precursors. Increased RANK stimulates intracellular pathways, leading to increased formation of NF- κ B. NF- κ B stimulates differentiation of osteoclast premature cells to mature osteoclasts, culminating in increased osteoclastic activity. Simultaneously, NF- κ B up regulates expression of OPG, a decoy receptor for RANKL, which effectively antagonizes its activity^[28]. Ndip *et al.*^[29] in their study showed that patients with CN have elevated RANKL/OPG ratio and illustrated that abnormal RANKL/OPG signaling plays a crucial role in increased osteoclastic bone resorption. Another bone regulating pathway involving Wnt/ β -catenin has been speculated to have some role in bone remodeling in patients with CF^[10]. To date, the RANKL/OPG pathway defect remains the most accepted theory.

Role of hyperglycemia

Glycation of collagen occurs normally with aging^[30]. Hyperglycemia accelerates this process of non-enzymatic glycation, leading to the formation of Amadori products. These products combine with amino groups on other protein molecules, ending up in formation of the AGEs, which are known to play a major role in various complications of diabetes^[31]. AGEs cause irreversible posttranslational modification of proteins, thus rendering them defective. Binding of AGEs to their receptor (RAGE) stimulates nicotinamide adenine dinucleotide phosphate oxidase^[32], resulting in the production of reactive oxygen

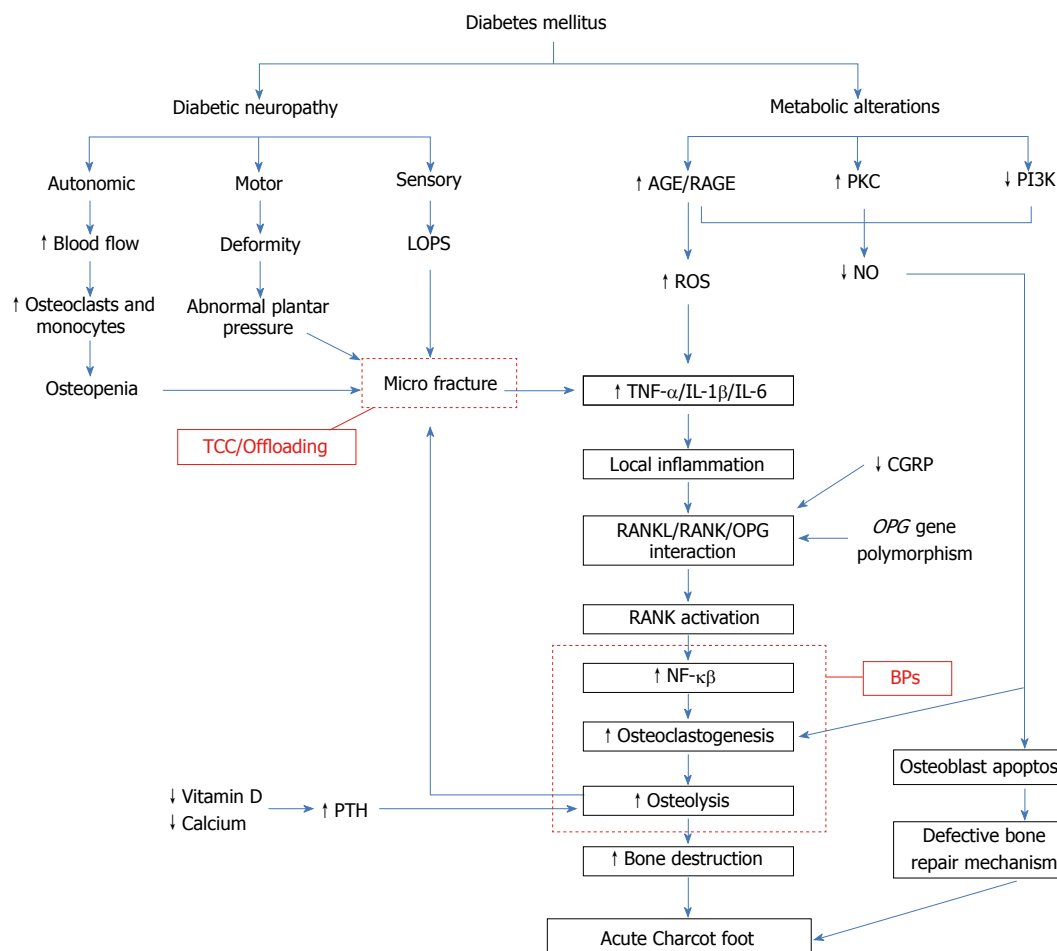


Figure 1 Pathogenesis leading to acute Charcot foot. AGE: Advanced glycation end products; RAGE: Receptor of AGE; PKC: Protein kinase C; PI3K: Phosphatidylinositol 3 kinase; LOPS: Loss of pain sensation; ROS: Reactive oxygen species; NO: Nitric oxide; TNF- α : Tumor necrosis factor- α ; IL-1 β : Interleukin-1 beta; IL-6: Interleukin-6; TCC: Total contact cast; CGRP: Calcitonin gene related peptide; NF- κ B: Nuclear factor κ B; RANKL: Receptor activator of NF- κ B ligand; OPG: Osteoprotegerin; BP: Bisphosphonates; PTH: Parathyroid hormone.

species and subsequently increased expression of NF- κ B^[33]. Katayama *et al.*^[34] elucidated the effects of AGE-modified collagen on differentiation and function of the osteoblastic cell *in vitro* and suggested that the same changes may lead to osteopenia in diabetic patients. AGEs prevent differentiation of human mesenchymal stem cells^[35]. They stimulate apoptosis of osteoblasts through mitogen activated protein kinase and cytosolic apoptotic pathways that are independent of NF- κ B activation^[36]. AGEs also cause endothelial dysfunction by extinguishing nitric oxide (NO) activity^[37]. Soluble RAGE (sRAGE) is a C-terminal splice variant of RAGE and has been shown to be cytoprotective against AGE^[38]. Witzke and colleagues in their cross-sectional study concluded that patients with CN had lower levels of sRAGE compared to healthy controls and diabetic patients without CN^[7]. They also demonstrated a positive correlation between sRAGE levels and calcaneal bone stiffness suggesting that sRAGE has a protective effect against bone resorption and loss of sRAGE defense may be one of the factors leading to CN. Thus, AGEs lead to increased osteoclastogenesis *via* the RANKL/NF- κ B pathway and

decreased bone formation by their action on osteoblasts through multiple pathways.

Role of neuropeptides and inorganic molecules

Research has shown that feedback mechanisms are abnormal in patients with DM, leading to increased expression of RANKL. One such mechanism involves CGRP secreted from the healthy neurons. It antagonizes RANKL expression by increasing the release of anti-inflammatory cytokines like IL-10^[14]. This leads to inhibition of osteoclastogenesis. The release of CGRP is reduced in peripheral and autonomic neuropathy, leading to continuous unchecked RANKL activity^[39]. NO is an inorganic molecule that plays a role in CN. AGEs, along with increased PKC expression and decreased phosphatidylinositol 3 kinase activity, results in decreased production of NO. Studies have shown that decreased NO levels can stimulate osteoclastogenesis, thereby leading to bone resorption^[40]. Endothelial NO synthase (eNOS) also regulates osteoblast proliferation and function^[41,42]. eNOS knockout animals have been shown to develop osteoporosis secondary to defective

bone formation^[42]. Both these molecules were studied by La Fontaine and colleagues in their study^[9]. They performed immunohistological analysis of bone specimens from three groups of patients with DM: group 1 included healthy patients without neuropathy, group 2 included those with neuropathy, and group 3 included those with CN stage II or III. They observed decreased levels of CGRP in patients in groups 2 and 3 when compared to group 1. They also found a statistically significant difference in the levels of eNOS, with highest levels in healthy DM patients without neuropathy (group 1) and lowest levels in DM patients with CN (group 3).

ROLE OF ANTIRESORPTIVE THERAPY IN ACUTE CHARCOT FOOT

Increased osteoclastic activity is the essence of pathogenesis leading to CF. Many studies have shown elevated levels of bone turnover markers (BTMs) in patients with acute CF pointing towards this fact.

Gough *et al.*^[15] compared BTMs between four groups of patients: acute CF, chronic CF, diabetic controls, and non-diabetic controls. They concluded that levels of serum 1CTP were significantly elevated in patients with acute CF as compared to the other three groups ($P < 0.0001$). Jostel *et al.*^[43] in their review mentioned similar results with urinary cross linked N-telopeptides of type 1 collagen, pointing towards accelerated collagen breakdown in these patients. However, levels of serum procollagen type I carboxy-terminal propeptide did not show intergroup differences.

These findings have forced researchers to use anti-resorptive agents along with traditional immobilization in acute CF patients. To date, agents like BPs have been used in multiple studies (discussed later). BPs are the principal agents in the pharmacological armamentarium against diseases, where the osteoblast-osteoclast imbalance is the underlying pathology. They are analogues of inorganic PP binding to hydroxyapatite crystals, which have extremely high affinity for bone mineral. They get deposited in mineralized bone matrix and are released at the time of bone resorption. This high affinity for bone mineral and resultant uptake by activated osteoclasts at the time of resorption ensures its toxic accumulation only in osteoclasts. First generation non-nitrogen containing BPs are metabolized to cytotoxic adenosine triphosphate analogues by osteoclasts. Intracellular deposition of these toxic non-hydrolyzable analogues causes apoptosis of osteoclasts^[44]. Unlike their predecessors, second and third generation BPs like alendronate, pamidronate, ibandronate, risedronate, and zoledronate have nitrogen side chain bound to the central carbon, which magnifies their potency manifold. The mechanism by which nitrogen containing BPs impacts osteoclast activity and survival differs from that of the first generation BPs. After getting internalized, they inhibit FPP synthase, a key enzyme in the mevalonate pathway, which is responsible for

production of cholesterol and isoprenoid lipids^[45]. As a result, isoprenylation of guanosine triphosphate binding proteins like Ras, Rho, and Rac is inhibited^[46]. These signaling proteins are important for the regulation of cell survival, proliferation, and cytoskeletal organization. Of particular importance among these is inhibition of protein prenylation and Ras signaling within osteoclasts, resulting in defective intracellular vesicle transport^[47]. Thus, osteoclasts fail to form ruffled borders, which are necessary for resorption of bone. In addition to this, FPP synthase inhibition leads to an increase in isopentenyl diphosphonate, which is further metabolized to triphosphoric acid 1-adenosin-5'-yl ester 3-[3-methylbut-3-enyl] ester, also known as ApppI. Intracellular accumulation of this ATP analogue leads to apoptosis of osteoclasts (Figure 2). Potency of BPs is decided based on the inhibition of FPP synthase activity. In this respect, zoledronate is the most potent BP followed by risedronate, ibandronate, alendronate, and pamidronate with decreasing potency^[17]. Moreover, in animal studies, BPs have shown to possess antinociceptive effects that can contribute to pain relief in patients with acute CF^[48,49].

Other anti-resorptive agents like calcitonin and denosumab have been successfully used in past. Calcitonin is a polypeptide secreted from parafollicular C cells of the thyroid. It inhibits bone resorption by its direct action on the osteoclast calcitonin receptor^[50]. Its quick action leads to loss of ruffled border of osteoclasts and decreased number of osteoclasts. It inhibits cytoplasmic motility and generates pseudopodial retraction in osteoclasts^[51]. It prevents the production and release of tartrate-resistant acid phosphatase by osteoclasts^[52]. It has also been shown that calcitonin may inhibit apoptosis of osteocytes and osteoblasts^[53]. To evaluate the effects of calcitonin on disease activity, Bem *et al.*^[54] conducted a randomized controlled trial on the effectiveness of intranasal salmon calcitonin 200 IU daily in 32 diabetic patients with acute CF. One group received intra-nasal salmon calcitonin 200 IU daily and calcium supplementation, while the other got only calcium supplements. All patients were offloaded using removable devices. Skin temperature and BTMs (measured monthly for first 3 mo and then at 6 mo) were used for monitoring the course of treatment. Nine patients with renal insufficiency, *i.e.*, serum creatinine $> 120 \mu\text{mol/L}$, were also included. Skin temperature reduced significantly at 3 mo without much inter-group difference. Significant reduction was noted in levels of 1CTP in the treatment group at 3 mo as compared to control group ($P < 0.01$). A similar trend was observed for bone-specific alkaline phosphatase (ALP) at 3 mo ($P < 0.05$), but the intergroup difference disappeared at 6 mo. The authors concluded that intranasal calcitonin not only reduces bone resorption and prevents progression of acute CF but also can be effective in patients with renal insufficiency. Calcitonin also has analgesic action mediated through central as well as peripheral mechanisms^[55,56].

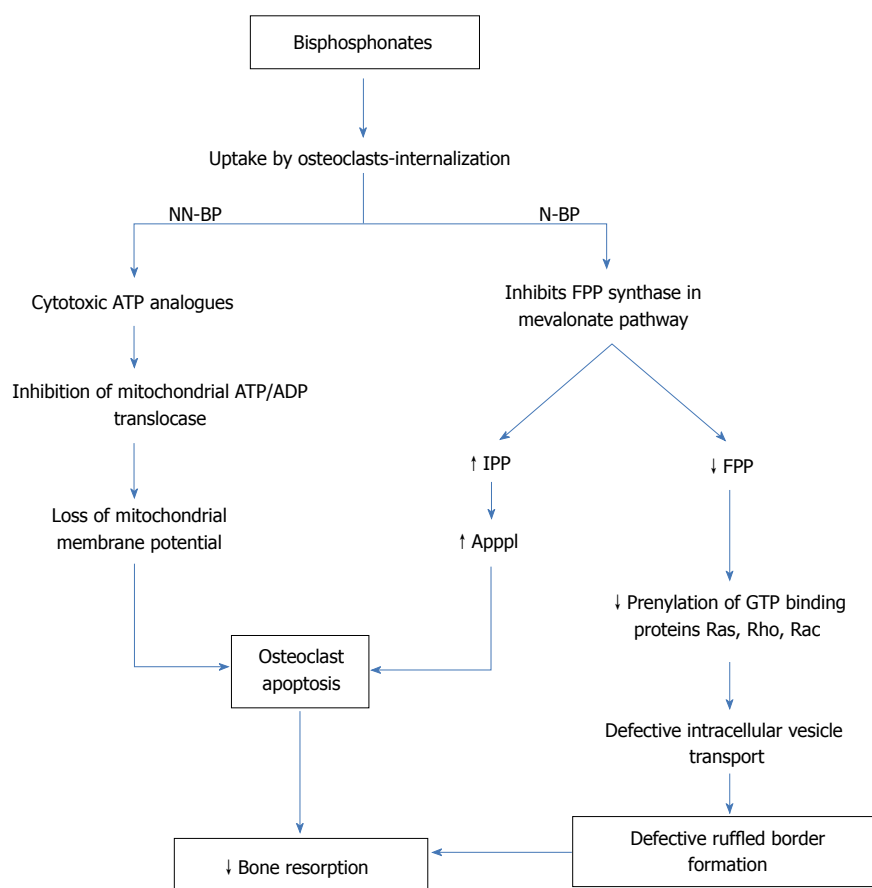


Figure 2 Molecular mechanisms of bisphosphonates. NN-BP: Non-nitrogen containing bisphosphonates; N-BP: Nitrogen containing bisphosphonates; FPP: Farnesyl pyrophosphate; IPP: Isopentenyl diphosphonate; Apppl: Triphosphoric acid 1-adenosin-5'-yl ester 3-[3-methylbut-3-enyl]ester.

As RANKL activation plays a major role in the pathogenesis of acute CF, its inhibition can be an attractive treatment option. Denosumab is a fully human monoclonal antibody that targets RANKL. It prevents interaction between RANKL and its receptor RANK. This leads to inhibition of RANKL, which in turn prevents differentiation of osteoclast precursors to mature multinucleated osteoclasts. The basic difference between BPs and denosumab is that the former act after getting internalized, while the latter works in an extracellular environment^[57]. It has been shown to reduce osteoporosis-related fracture^[58]. Taking cues from this work, Busch-Westbroek *et al.*^[59] performed an observational study to evaluate effects of denosumab in patients with acute CF. Patients seen between 2012 and 2014 were included as controls, and those from 2014 to 2016 were subjected to single subcutaneous injection of 60 mg denosumab. All the patients from 2012 to 2016 were immobilized using TCC and were supplemented with calcium and vitamin D. Fracture resolution time, as judged on radiographs and time to clinical cessation based on usage of TCC, were compared between the two groups. Both parameters were significantly shorter in the group receiving denosumab ($P < 0.01$). TCC was used until resolution of edema, and skin temperature difference between both feet decreased to less than 2°C in this study.

EVIDENCE OF BISPHOSPHONATE USE IN ACUTE CHARCOT FOOT

Case reports and case series

In 1994, Selby *et al.*^[60] first reported use of intravenous (IV) pamidronate in six diabetic patients with acute CF. Patients were treated with infusion of 30 mg of pamidronate followed by five infusions of 60 mg every 2 wk. Skin temperature, as a marker of disease activity, was monitored by an infrared thermometer. All patients reported marked improvement in their mobility and reduction in pain and swelling. Skin temperature difference between the affected and normal foot reduced from 3.4 ± 0.7 °C to 1.0 ± 0.5 °C ($P = 0.05$). Serum ALP, which was used as BTM, was also significantly reduced (by $25 \pm 3\%$, $P < 0.001$).

In 1999, Young MJ^[61] reported two diabetic patients with CF who were treated with IV infusion of 30 mg of pamidronate followed by two infusions of 60 mg every 2 wk along with immobilization measures. In both patients, skin temperature difference normalized (*i.e.*, < 2 °C), edema and pain subsided after 3 mo, and there was no deformity in the lower limbs.

In 2002, Yu *et al.*^[62] reported a case of recurrent CF in a 55-year-old diabetic patient. He was treated with serial Jones compression bandages followed by non-

weight bearing brace with a removable pneumatic walker along with three IV infusions of pamidronate each 2 wk apart. The patient improved clinically, swelling disappeared, and he resumed full weight bearing with an ankle-foot orthosis.

In 2002, Pakarinen *et al*^[63] retrospectively studied 36 CF in 32 diabetic patients. Eighteen cases received IV pamidronate 30 to 60 mg once a wk for 6 wk. They did not find any difference in casting time between patients who received pamidronate and those who did not (11 wk vs 13 wk). There was no information regarding criteria used for removal of casts or the indication for BP use in a particular patient. This along with non-uniformity in the timing of cast usage make it difficult to analyze the results of this study.

In 2007, Moreno *et al*^[64] prospectively analyzed the efficacy of pamidronate over 12 mo in four diabetic patients with acute CF. Treatment protocol comprised of three IV infusions of pamidronate each 2 mo apart. The dose used was 60 mg in patients with weight < 70 kilograms and 90 mg in patients with weight > 70 kilograms. Clinical examination, radiographs, and urine BTMs were done before and 12 mo after treatment in all patients. All patients exhibited significant clinical improvement. Urinary BTMs showed a statistically significant reduction. All patients had radiological improvement.

In 2008, Naqvi *et al*^[65] reported three diabetic patients with acute CF. First patient, a 54-year-old female was treated with three IV infusions of 90 mg of pamidronate every 2 mo. After the first infusion, the patient had marked clinical improvement in swelling, pain, erythema, and warmth. Following the second infusion, she was able to bear weight on her foot, and after the last infusion she was ambulant without the walker. The second patient was a 49-year-old African-American female, who was treated with single IV infusion of 60 mg pamidronate along with walking cast and physiotherapy. At 6 and 9 mo follow-ups, signs of inflammation disappeared. Although the natural arch of the foot was lost, she was able to walk with a boot. The third patient was an 82-year-old white woman, who was treated with a single 90 mg of pamidronate infusion. This led to significant clinical improvement, and she was able to walk with the boot at 4 wk of follow-up. One year later, she had no symptoms and was able to walk normally.

In 2002, Rajbhandari *et al*^[23] in their review revealed their anecdotal experience in patients with acute CF. They noted significant symptom relief in these patients with two IV infusions of 90 mg pamidronate.

Observational studies

In 2004, Anderson *et al*^[66] retrospectively evaluated 33 patients of acute CF who were diagnosed between October 1997 and January 2001. These patients were divided in two study groups - group 1 comprising of 18 patients who received IV pamidronate (60 to 90

mg) and group 2 comprising of 15 patients who did not receive any BPs. Both groups received standard immobilization measures. Finally, after excluding five patients each from groups 1 and 2 due to either lack of consent for treatment or bilateral CF or association with some other bone disease or infection, 13 patients from group 1 and 10 patients from group 2 were analyzed. In group 1 patients, limb temperature decreased by 2.8 °F at 48 h and 7.4 °F at 2 wk, whereas group 2 showed no reduction in temperature at 48 h and a reduction of 2.3 °F at 2 wk. The same trend was seen in serum ALP, which plummeted by 53% at 2 wk in group 1 and showed a meager reduction of 9% in group 2. Thus, this study demonstrated a statistically significant reduction in skin temperature and serum ALP in patients treated with pamidronate.

In the largest web-based observational study published in 2012, Game *et al*^[67] surveyed 288 diabetic patients with acute CF from 76 centers across the United Kingdom and Ireland. At baseline, 35% of the subjects were offloaded with the non-removable devices, while 50% were offloaded using the removable devices. Only 25% of patients received IV BPs, and around 20% received oral BPs. Follow-up data regarding resolution was available in 219 patients. The resolution was defined as a time-point when the patient starts walking in either normal or orthotic footwear. For those who received BPs, median resolution time was significantly longer than patients not receiving it (12 mo vs 10 mo, $P = 0.005$). Resolution time was significantly more in both groups as compared to other studies. One of the reasons for this can be the definition of resolution used, which required the patient to be ambulant. Regarding prolongation of resolution time with BPs, the authors have speculated the following possible explanations: first, BPs may have been used only in patients who had more severe CF or in non-responders to offloading alone. Second, BPs must have been used only if the non-removable device was unavailable. It is very tough to infer much from the results provided by this study. No data were provided regarding skin temperature or any BTMs or the type of BP used.

In 2013, Bharath *et al*^[68] were the first to compare the effects of two BPs in a prospective randomized comparative study of 45 type 2 diabetic patients with acute CF. Patients were randomized into two groups, Z and A receiving a single IV infusion of 5 mg zoledronate (diluted in 100 mL normal saline over 30 min) and oral alendronate 70 mg once weekly, respectively, until resolution of the disease. The complete clinical resolution of the disease process was defined as attainment of a temperature difference of < 1° F between two feet on two different occasions. Patients with serum creatinine ≥ 3 mg/dL or with a history of BP exposure were excluded. For patients in group Z, if serum creatinine was ≥ 2 mg/dL, the dose of zoledronate was reduced to 2.5 mg. Patients in both the groups were offloaded using TCC. Forty patients completed the study

Table 1 Table of randomized controlled trials on bisphosphonates in acute Charcot foot

Ref.	BP	Duration (mo)	Jadad score	Subjects	Outcomes			
					Skin temp	Symptom score	BTM	Others
Jude <i>et al</i> ^[69]	Pamidronate	12	5	T: <i>n</i> = 21; 90 mg single IV infusion P: <i>n</i> = 18; single IV infusion of NS	Significant reduction in both groups; more in T group at 4 wk (<i>P</i> < 0.01)	Significant improvement in T group from 3 – 12 mo (<i>P</i> < 0.01)	BSALP: Significantly greater reduction in T group till 12 wk (<i>P</i> < 0.03) uDPD crosslinks: Significant reduction in T group at 4 wk (<i>P</i> < 0.01)	
Pitocco <i>et al</i> ^[70]	Alendronate	6	1	T: <i>n</i> = 11; 70 mg once a week orally C: <i>n</i> = 9; no pharmacological treatment	Significant reduction in both groups	Signification reduction in T group at 6 mo (<i>P</i> < 0.05)	1CTP and uHP: Significant reduction in T Group (<i>P</i> < 0.05) BSALP: Greater reduction in T group (<i>P</i> = 0.06)	
Pakarinen <i>et al</i> ^[71]	Zoledronate	12	4	T: <i>n</i> = 20; 3 IV infusion of 4 mg at one monthly interval P: <i>n</i> = 19; placebo				Median immobilization time: Significantly greater in T group (<i>P</i> = 0.02)

BP: Bisphosphonate; T: Treatment group; P: Placebo group; C: Control group; IV: Intravenous; BTM: Bone turnover marker; BSALP: Bone specific alkaline phosphatase; 1CTP: Carboxyterminal telopeptide of type 1 collagen; uDPD: Urinary dehydroxypyridinoline; uHP: Urinary hydroxyproline.

(five patients withdrew), and 30 achieved complete resolution (16 patients in group Z and fourteen in group A). Five patients in each group achieved partial clinical resolution. The mean number of days required for complete healing process was around 122 d in both the groups.

Randomized controlled trials

All randomized controlled trials (RCTs) related to the use of BPs in acute CF are described in Table 1. In 2001, Jude *et al*^[69] reported a 12 mo double-blind randomized placebo-controlled trial including 39 diabetic patients with acute CF. At baseline, 21 patients received single 90 mg infusion of pamidronate over 4 h, whereas 18 patients received normal saline (placebo). All patients received standard care of foot immobilization. For the first 3 mo, all patients were followed up at 2-weekly intervals and thereafter at 6, 9, and 12 mo. At each visit, patients were assessed for clinical symptoms, and skin temperature was measured with an infrared thermometer. BTMs like bone-specific ALP and urinary dehydroxypyridinoline were measured at each visit. Skin temperature reduced significantly in both the groups with pamidronate group showing a greater reduction at 4 wk. It dipped further during the study period with no intergroup difference on subsequent visits. Both groups demonstrated symptom score improvement at 3 mo. Following this, the score remained unchanged in the control group over the next 12 mo, whereas the

pamidronate group registered further improvement (*P* < 0.01). Bone-specific ALP showed a significant reduction in the pamidronate group when compared to placebo (*P* < 0.03) at 4 wk, and this was maintained for at least 12 wk. A similar trend was observed in urinary dehydroxypyridinoline at 4 wk (*P* < 0.01). Both the BTMs gradually increased towards baseline at 12 mo.

In an observer blinded RCT, Pitocco *et al*^[70] studied the efficacy of alendronate in patients with acute CF. Eleven patients included in study group received alendronate 70 mg orally once a week while nine patients in control group received no pharmacological treatment. All patients were followed up for 6 mo and were off-loaded using a TCC boot for the first 2 mo, which was followed by a pneumatic walker in the subsequent 4 mo. BTMs like serum 1CTP, serum bone ALP, and urinary hydroxyproline were measured at baseline and at 6 mo of follow up. All these markers showed a significant reduction in the alendronate-treated group when compared to control group (*P* < 0.05), except for bone ALP (*P* = 0.06). Dual-energy x-ray absorptiometry done at baseline and at 6 mo showed statistically significant improvement in bone mineral density of total foot (*P* < 0.05) and distal phalanxes (*P* < 0.01) in the alendronate group. Visual analogue scale score for pain improved significantly in the treatment group, with no improvement in the control group (*P* < 0.05). Skin temperature reduced significantly in both groups at 6 mo.

In 2011, Pakarinen *et al.*^[71] first studied the effect of zoledronate in 39 diabetic subjects with acute CF in a double-blind randomized placebo-controlled trial. Patients were randomly assigned into two groups receiving three IV infusions of either 4 mg zoledronate or a placebo at 1-mo intervals. Patients with previous BP exposure or severe renal insufficiency were excluded. All patients were initially treated with a non-weight bearing cast and were allowed partial weight bearing when the clinical signs of active CF process subsided. Complete weight bearing was allowed only when the temperature difference between the two feet was less than 1°C for at least last 30 d with no evidence of edema or erythema. All patients were evaluated at baseline, at 2 to 4 wk intervals for the first 3 mo and then at 6, 9, and 12 mo. Finally, 35 patients who completed 12 mo follow-up were analyzed. The final endpoint of this study was median immobilization time, which was significantly longer in the zoledronate treated group as compared to the placebo group (27 wk vs 20 wk, $P = 0.02$). No information was given regarding BTMs or radiological findings at any point of time. During 12 mo follow-up, one patient relapsed in each group.

DISCUSSION

The main aims of treatment in acute CF are to relieve the patient of symptoms and to avoid complications, such as deformity and ulceration, thus preventing the progression to chronic CF. Immobilization and off-loading are the most important components of this treatment. Avoidance of repetitive microtrauma leads to the resolution of edema and swelling. Casting should be continued until the skin temperature difference between the two limbs becomes less than 2°C^[16]. However, the basic pathogenesis in CF revolves around osteolysis, which leads to subsequent bone destruction, and immobilization does not address this directly. This creates space for the adjuvant therapy that can inhibit osteolysis and hence bone resorption. BPs, calcitonin, and denosumab are the anti-resorptive agents used to date in these patients. Among BPs, maximum evidence in the literature is available for pamidronate^[72]. In a majority of case reports and series, pamidronate was shown to reduce the markers of activity of CF, like skin temperature, pain, edema, and BTMs. In the first RCT assessing the response of BP in acute CF, Jude *et al.*^[69] confirmed the beneficial effects of pamidronate in patients with acute CF. This RCT was of high methodological quality, as it was a double-blind, placebo controlled, multi-center study with proper mention about randomization process and statistical analysis. In a retrospective case-control study, Anderson *et al.*^[66] reported significant reduction in skin temperature and serum ALP in the pamidronate treated group. However, in a case series by Pakarinen *et al.*^[63], no difference was found for casting times when pamidronate was used along with conventional measures. Among other BPs,

alendronate in a RCT was shown to reduce pain and BTMs significantly in acute Charcot neuroarthropathy^[70]. Additionally, zoledronate, the most potent third generation BP, was surprisingly shown to prolong immobilization times of patients with acute CF^[71]. The limitations of this particular RCT were its underpowered nature (due to its small sample size) and the discrepancy in the immobilization times. The latest randomized comparative study evaluating the effects of zoledronate and alendronate concluded that both medications had the same response in terms of clinical resolution time and scintigraphic changes. When cost was taken into account, however, alendronate was much less expensive than zoledronate^[68].

None of these studies have ventured into the effect of BP on long-term outcome measures like avoidance of ulcerations, deformities, and amputation. The evidence from the available studies is limited because of the non-uniformity in the agent used and heterogeneity in outcome measures. Most studies, except one of Jude *et al.*^[69], have methodological flaws like open randomization, lack of blinding, and statistically small sample size. In fact, only Jude *et al.*^[69] reported the power analysis.

CLINICAL IMPLICATION

Oral alendronate and IV pamidronate have been efficacious in relieving symptoms and controlling disease activity in patients with acute CF. Oral BPs need to be taken on an empty stomach and with a full glass of water (at least 240 mL) to avoid getting it stuck in the esophagus. The patient should remain in erect posture for at least 30-60 min. Oral BPs have very poor bioavailability, with < 1% of the drug being absorbed from gastrointestinal tract^[73]. BP should be taken in the fasting state with avoidance of any food for 30-60 min after taking to prevent its absorption from decreasing further. Retained gastric contents in patients with gastroparesis may also hamper absorption. Contraindications to oral BPs include an inability to follow this strict protocol, any active esophageal pathology like achalasia, varices, or stricture, or any malabsorption disorder like celiac disease, Crohn's disease, or post gastric bypass surgery^[74]. For patients who cannot tolerate oral BPs, IV BPs can be an alternate option. IV BPs are known to cause acute phase reactions leading to flu-like illness in around 10%-30% of patients receiving their first infusion^[74]. This can be taken care of by oral acetaminophen.

Vitamin D deficiency, which is common in the diabetic population, should be treated before giving BPs. In patients with renal insufficiency, caution should be exercised while using BPs, especially if glomerular filtration rate < 30-35 mL/min^[74]. This is particularly true when given by rapid IV infusion, as it can aggravate or lead to renal dysfunction. Intranasal calcitonin can be an attractive option to treat acute CF in this group of patients. Moreover, BPs like zoledronate^[75] and al-

endronate^[76] have been linked with the occurrence of atrial fibrillation. HORIZON Pivotal Fracture Trial has shown a statistically significant increase in the incidence of serious atrial fibrillation in patients treated with zoledronate^[75]. However, a large population-based study has refuted these findings^[77].

As CN usually develops in diabetic patients with disease duration of more than 10 years, they are also expected to have gastroparesis, nephropathy, coronary artery disease, and various other complications. The above side-effects and contra-indications should be kept in mind while treating such patients with BPs.

CONCLUSION

The meteoric rise in the prevalence of DM has made it the most common cause of CN affecting foot and ankle. In a majority of the studies related to use of BPs in acute CF, pamidronate has been shown to reduce the markers of Charcot activity like skin temperature, pain, edema, and BTMs, but the quality of evidence is weak. Therefore, BPs can be considered as an adjuvant treatment option for acute CF.

REFERENCES

- 1 Charcot JM. Sur quelques arthropathies qui paraissent d'epandre d'une l'esion du cerveau ou de la moulle 'epini'ere. *Arch Physiol Norm Pathol* 1868; **1**: 161-178
- 2 Jordan WR. Neuritic manifestations in diabetes mellitus. *Arch Intern Med* 1936; **57**: 307-366 [DOI: 10.1001/archinte.1936.00170060069004]
- 3 World Health Organization. Global report on diabetes 2016. Available from: URL: http://apps.who.int/iris/bitstream/handle/10665/204871/9789241565257_eng.pdf;jsessionid=C87606F0229CFE671C7D3B12FD2581BB?sequence=1
- 4 Chisholm KA, Gilchrist JM. The Charcot joint: a modern neurologic perspective. *J Clin Neuromuscul Dis* 2011; **13**: 1-13 [PMID: 22361621 DOI: 10.1097/CND.0b013e3181c6f55b]
- 5 Ramanujam CL, Zgonis T. The Diabetic Charcot Foot from 1936 to 2016: Eighty Years Later and Still Growing. *Clin Podiatr Med Surg* 2017; **34**: 1-8 [PMID: 27865310 DOI: 10.1016/j.cpm.2016.07.001]
- 6 Mabileau G, Petrova NL, Edmonds ME, Sabokbar A. Increased osteoclastic activity in acute Charcot's osteoarthropathy: the role of receptor activator of nuclear factor-kappaB ligand. *Diabetologia* 2008; **51**: 1035-1040 [PMID: 18389210 DOI: 10.1007/s00125-008-0992-1]
- 7 Witzke KA, Vinik AI, Grant LM, Grant WP, Parson HK, Pittenger GL, Burcus N. Loss of RAGE defense: a cause of Charcot neuroarthropathy? *Diabetes Care* 2011; **34**: 1617-1621 [PMID: 21593297 DOI: 10.2337/dc10-2315]
- 8 Zhao HM, Diao JY, Liang XJ, Zhang F, Hao DJ. Pathogenesis and potential relative risk factors of diabetic neuropathic osteoarthropathy. *J Orthop Surg Res* 2017; **12**: 142 [PMID: 28969714 DOI: 10.1186/s13018-017-0634-8]
- 9 La Fontaine J, Harkless LB, Sylvia VL, Carnes D, Heim-Hall J, Jude E. Levels of endothelial nitric oxide synthase and calcitonin gene-related peptide in the Charcot foot: a pilot study. *J Foot Ankle Surg* 2008; **47**: 424-429 [PMID: 18725122 DOI: 10.1053/j.jfas.2008.05.009]
- 10 Folestad A, Ålund M, Asteberg S, Fowelin J, Aurell Y, Göthlin J, Cassuto J. Role of Wnt/ β -catenin and RANKL/OPG in bone healing of diabetic Charcot arthropathy patients. *Acta Orthop* 2015; **86**: 415-425 [PMID: 25811776 DOI: 10.3109/17453674.2015.1033606]
- 11 Pitocco D, Zelano G, Gioffrè G, Di Stasio E, Zaccardi F, Martini F, Musella T, Scavone G, Galli M, Caputo S, Mancini L, Ghirlanda G. Association between osteoprotegerin G1181C and T245G polymorphisms and diabetic charcot neuroarthropathy: a case-control study. *Diabetes Care* 2009; **32**: 1694-1697 [PMID: 19502537 DOI: 10.2337/dc09-0243]
- 12 Baumhauer JF, O'Keefe RJ, Schon LC, Pinzur MS. Cytokine-induced osteoclastic bone resorption in charcot arthropathy: an immunohistochemical study. *Foot Ankle Int* 2006; **27**: 797-800 [PMID: 17054880 DOI: 10.1177/107110070602701007]
- 13 Blakytyn R, Spraul M, Jude EB. Review: The diabetic bone: a cellular and molecular perspective. *Int J Low Extrem Wounds* 2011; **10**: 16-32 [PMID: 21444607 DOI: 10.1177/1534734611400256]
- 14 Kaynak G, Birsal O, Güven MF, Oğüt T. An overview of the Charcot foot pathophysiology. *Diabet Foot Ankle* 2013; **4**: 21117 [PMID: 23919113 DOI: 10.3402/dfa.v4i0.21117]
- 15 Gough A, Abrahams H, Li F, Purewal TS, Foster AV, Watkins PJ, Moniz C, Edmonds ME. Measurement of markers of osteoclast and osteoblast activity in patients with acute and chronic diabetic Charcot neuroarthropathy. *Diabet Med* 1997; **14**: 527-531 [PMID: 9223389 DOI: 10.1002/(SICI)1096-9136(199707)14:7<527::AID-DIA404>3.0.CO;2-Q]
- 16 Rogers LC, Frykberg RG, Armstrong DG, Boulton AJ, Edmonds M, Van GH, Hartemann A, Game F, Jeffcoate W, Jirkovska A, Jude E, Morbach S, Morrison WB, Pinzur M, Pitocco D, Sanders L, Wukich DK, Uccioli L. The Charcot foot in diabetes. *Diabetes Care* 2011; **34**: 2123-2129 [PMID: 21868781 DOI: 10.2337/dc11-0844]
- 17 Green JR. Bisphosphonates: preclinical review. *Oncologist* 2004; **9** Suppl 4: 3-13 [PMID: 15459425 DOI: 10.1634/theoncologist.9-90004-3]
- 18 Armstrong DG, Todd WF, Lavery LA, Harkless LB, Bushman TR. The natural history of acute Charcot's arthropathy in a diabetic foot specialty clinic. *Diabet Med* 1997; **14**: 357-363 [PMID: 9171250 DOI: 10.1002/(SICI)1096-9136(199705)14:5<357::AID-DIA341>3.0.CO;2-8]
- 19 Armstrong DG, Lavery LA. Monitoring healing of acute Charcot's arthropathy with infrared dermal thermometry. *J Rehabil Res Dev* 1997; **34**: 317-321 [PMID: 9239625]
- 20 Schlossbauer T, Mioc T, Sommerer S, Kessler SB, Reiser MF, Pfeifer KJ. Magnetic resonance imaging in early stage charcot arthropathy: correlation of imaging findings and clinical symptoms. *Eur J Med Res* 2008; **13**: 409-414 [PMID: 18948232]
- 21 Sella EJ, Barrette C. Staging of Charcot neuroarthropathy along the medial column of the foot in the diabetic patient. *J Foot Ankle Surg* 1999; **38**: 34-40 [PMID: 10028468 DOI: 10.1016/S1067-2516(99)80086-6]
- 22 Wolfe L, Stess RM, Graf PM. Dynamic pressure analysis of the diabetic charcot foot. *J Am Podiatr Med Assoc* 1991; **81**: 281-287 [PMID: 1920092 DOI: 10.7547/87507315-81-6-281]
- 23 Rajbhandari SM, Jenkins RC, Davies C, Tesfaye S. Charcot neuroarthropathy in diabetes mellitus. *Diabetologia* 2002; **45**: 1085-1096 [PMID: 12189438 DOI: 10.1007/s00125-002-0885-7]
- 24 Kucera T, Shaikh HH, Sponer P. Charcot Neuropathic Arthropathy of the Foot: A Literature Review and Single-Center Experience. *J Diabetes Res* 2016; **2016**: 3207043 [PMID: 27656656 DOI: 10.1155/2016/3207043]
- 25 Lee L, Blume PA, Sumpio B. Charcot joint disease in diabetes mellitus. *Ann Vasc Surg* 2003; **17**: 571-580 [PMID: 14508661 DOI: 10.1007/s10016-003-0039-5]
- 26 Christensen TM, Simonsen L, Holstein PE, Svendsen OL, Bülow J. Sympathetic neuropathy in diabetes mellitus patients does not elicit Charcot osteoarthropathy. *J Diabetes Complications* 2011; **25**: 320-324 [PMID: 21813289 DOI: 10.1016/j.jdiacomp.2011.06.006]
- 27 Jeffcoate WJ, Game F, Cavanagh PR. The role of proinflammatory cytokines in the cause of neuropathic osteoarthropathy (acute Charcot foot) in diabetes. *Lancet* 2005; **366**: 2058-2061 [PMID: 16338454 DOI: 10.1016/S0140-6736(05)67029-8]
- 28 Jeffcoate WJ. Charcot neuro-osteoarthropathy. *Diabetes Metab*

- Res Rev* 2008; **24** Suppl 1: S62-S65 [PMID: 18393327 DOI: 10.1002/dmrr.837]
- 29 **Ndip A**, Williams A, Jude EB, Serracino-Inglott F, Richardson S, Smyth JV, Boulton AJ, Alexander MY. The RANKL/RANK/OPG signaling pathway mediates medial arterial calcification in diabetic Charcot neuroarthropathy. *Diabetes* 2011; **60**: 2187-2196 [PMID: 21659498 DOI: 10.2337/db10-1220]
 - 30 **Schnider SL**, Kohn RR. Effects of age and diabetes mellitus on the solubility and nonenzymatic glucosylation of human skin collagen. *J Clin Invest* 1981; **67**: 1630-1635 [PMID: 6787079 DOI: 10.1172/JCI110198]
 - 31 **Brownlee M**. Lilly Lecture 1993. Glycation and diabetic complications. *Diabetes* 1994; **43**: 836-841 [PMID: 8194672 DOI: 10.2337/diab.43.6.836]
 - 32 **Wautier MP**, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am J Physiol Endocrinol Metab* 2001; **280**: E685-E694 [PMID: 11287350 DOI: 10.1152/ajpendo.2001.280.5.E685]
 - 33 **Mascarenhas JV**, Jude EB. The Charcot foot as a complication of diabetic neuropathy. *Curr Diab Rep* 2014; **14**: 561 [PMID: 25354828 DOI: 10.1007/s11892-014-0561-6]
 - 34 **Katayama Y**, Akatsu T, Yamamoto M, Kugai N, Nagata N. Role of nonenzymatic glycosylation of type I collagen in diabetic osteopenia. *J Bone Miner Res* 1996; **11**: 931-937 [PMID: 8797113 DOI: 10.1002/jbmr.5650110709]
 - 35 **Kume S**, Kato S, Yamagishi S, Inagaki Y, Ueda S, Arima N, Okawa T, Kojiro M, Nagata K. Advanced glycation end-products attenuate human mesenchymal stem cells and prevent cognate differentiation into adipose tissue, cartilage, and bone. *J Bone Miner Res* 2005; **20**: 1647-1658 [PMID: 16059636 DOI: 10.1359/JBMR.050514]
 - 36 **Alikhani M**, Alikhani Z, Boyd C, MacLellan CM, Raptis M, Liu R, Pischon N, Trackman PC, Gerstenfeld L, Graves DT. Advanced glycation end products stimulate osteoblast apoptosis via the MAP kinase and cytosolic apoptotic pathways. *Bone* 2007; **40**: 345-353 [PMID: 17064973 DOI: 10.1016/j.bone.2006.09.011]
 - 37 **Bucala R**, Tracey KJ, Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest* 1991; **87**: 432-438 [PMID: 1991829 DOI: 10.1172/JCI115014]
 - 38 **Yonekura H**, Yamamoto Y, Sakurai S, Petrova RG, Abedin MJ, Li H, Yasui K, Takeuchi M, Makita Z, Takasawa S, Okamoto H, Watanabe T, Yamamoto H. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem J* 2003; **370**: 1097-1109 [PMID: 12495433 DOI: 10.1042/BJ20021371]
 - 39 **Larson SA**, Burns PR. The pathogenesis of Charcot neuroarthropathy: current concepts. *Diabet Foot Ankle* 2012; **3**: 12236 [PMID: 22396834 DOI: 10.3402/dfa.v3i0.12236]
 - 40 **Collin-Osdoby P**, Rothe L, Bekker S, Anderson F, Osdoby P. Decreased nitric oxide levels stimulate osteoclastogenesis and bone resorption both in vitro and in vivo on the chick chorioallantoic membrane in association with neoangiogenesis. *J Bone Miner Res* 2000; **15**: 474-488 [PMID: 10750562 DOI: 10.1359/jbmr.2000.15.3.474]
 - 41 **Riancho JA**, Salas E, Zarrabeitia MT, Olmos JM, Amado JA, Fernández-Luna JL, González-Macías J. Expression and functional role of nitric oxide synthase in osteoblast-like cells. *J Bone Miner Res* 1995; **10**: 439-446 [PMID: 7540349 DOI: 10.1002/jbmr.5650100315]
 - 42 **van't Hof RJ**, Ralston SH. Nitric oxide and bone. *Immunology* 2001; **103**: 255-261 [PMID: 11454054 DOI: 10.1046/j.1365-2567.2001.01261.x]
 - 43 **Jostel A**, Jude EB. Medical treatment of Charcot neuroosteoarthropathy. *Clin Podiatr Med Surg* 2008; **25**: 63-69, vi-vii [PMID: 18165112 DOI: 10.1016/j.cpm.2007.09.001]
 - 44 **Drake MT**, Clarke BL, Khosla S. Bisphosphonates: mechanism of action and role in clinical practice. *Mayo Clin Proc* 2008; **83**: 1032-1045 [PMID: 18775204 DOI: 10.4065/83.9.1032]
 - 45 **Kavanagh KL**, Guo K, Dunford JE, Wu X, Knapp S, Ebetino FH, Rogers MJ, Russell RG, Oppermann U. The molecular mechanism of nitrogen-containing bisphosphonates as antiosteoporosis drugs. *Proc Natl Acad Sci U S A* 2006; **103**: 7829-7834 [PMID: 16684881 DOI: 10.1073/pnas.0601643103]
 - 46 **Luckman SP**, Hughes DE, Coxon FP, Graham R, Russell G, Rogers MJ. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. *J Bone Miner Res* 1998; **13**: 581-589 [PMID: 9556058 DOI: 10.1359/jbmr.1998.13.4.581]
 - 47 **Alakangas A**, Selander K, Mulari M, Halleen J, Lehenkari P, Mönkkönen J, Salo J, Väänänen K. Alendronate disturbs vesicular trafficking in osteoclasts. *Calcif Tissue Int* 2002; **70**: 40-47 [PMID: 11907706 DOI: 10.1007/s002230010047]
 - 48 **Bonabello A**, Galmozzi MR, Bruzzese T, Zara GP. Analgesic effect of bisphosphonates in mice. *Pain* 2001; **91**: 269-275 [PMID: 11275384 DOI: 10.1016/S0304-3959(00)00447-4]
 - 49 **Wang L**, Guo TZ, Hou S, Wei T, Li WW, Shi X, Clark JD, Kingery WS. Bisphosphonates Inhibit Pain, Bone Loss, and Inflammation in a Rat Tibia Fracture Model of Complex Regional Pain Syndrome. *Anesth Analg* 2016; **123**: 1033-1045 [PMID: 27636578 DOI: 10.1213/ANE.0000000000001518]
 - 50 **Masi L**, Brandi ML. Calcitonin and calcitonin receptors. *Clin Cases Miner Bone Metab* 2007; **4**: 117-122 [PMID: 22461211]
 - 51 **Zaidi M**, Inzerillo AM, Moonga BS, Bevis PJ, Huang CL. Forty years of calcitonin--where are we now? A tribute to the work of Iain Macintyre, FRS. *Bone* 2002; **30**: 655-663 [PMID: 11996901 DOI: 10.1016/S8756-3282(02)00688-9]
 - 52 **Yumita S**, Nicholson GC, Rowe DJ, Kent GN, Martin TJ. Biphasic effect of calcitonin on tartrate-resistant acid phosphatase activity in isolated rat osteoclasts. *J Bone Miner Res* 1991; **6**: 591-597 [PMID: 1887822 DOI: 10.1002/jbmr.5650060610]
 - 53 **Plotkin LI**, Weinstein RS, Parfitt AM, Roberson PK, Manolagas SC, Bellido T. Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. *J Clin Invest* 1999; **104**: 1363-1374 [PMID: 10562298 DOI: 10.1172/JCI6800]
 - 54 **Bem R**, Jirkovská A, Fejfarová V, Skibová J, Jude EB. Intranasal calcitonin in the treatment of acute Charcot neuroosteoarthropathy: a randomized controlled trial. *Diabetes Care* 2006; **29**: 1392-1394 [PMID: 16732029 DOI: 10.2337/dc06-0376]
 - 55 **Ito A**, Yoshimura M. Mechanisms of the analgesic effect of calcitonin on chronic pain by alteration of receptor or channel expression. *Mol Pain* 2017; **13**: 1744806917720316 [PMID: 28726540 DOI: 10.1177/1744806917720316]
 - 56 **Azria M**. Possible mechanisms of the analgesic action of calcitonin. *Bone* 2002; **30**: 80S-83S [PMID: 12008164 DOI: 10.1016/S8756-3282(02)00701-9]
 - 57 **Baron R**, Ferrari S, Russell RG. Denosumab and bisphosphonates: different mechanisms of action and effects. *Bone* 2011; **48**: 677-692 [PMID: 21145999 DOI: 10.1016/j.bone.2010.11.020]
 - 58 **Cummings SR**, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, Delmas P, Zoog HB, Austin M, Wang A, Kutilek S, Adami S, Zanchetta J, Libanati C, Siddhanti S, Christiansen C; FREEDOM Trial. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med* 2009; **361**: 756-765 [PMID: 19671655 DOI: 10.1056/NEJMoa0809493]
 - 59 **Busch-Westbroek TE**, Delpout K, Balm R, Bus SA, Schepers T, Peters EJ, Smithuis FF, Maas M, Nieuwdorp M. Effect of Single Dose of RANKL Antibody Treatment on Acute Charcot Neuroosteoarthropathy of the Foot. *Diabetes Care* 2018; **41**: e21-e22 [PMID: 29273577 DOI: 10.2337/dc17-1517]
 - 60 **Selby PL**, Young MJ, Boulton AJ. Bisphosphonates: a new treatment for diabetic Charcot neuroarthropathy? *Diabet Med* 1994; **11**: 28-31 [PMID: 8181248 DOI: 10.1111/j.1464-5491.1994.tb00225.x]
 - 61 **Young MJ**. The management of neurogenic arthropathy: a tale of two charcots. *Diabetes Metab Res Rev* 1999; **15**: 59-64 [PMID: 10398548 DOI: 10.1002/(SICI)1520-7560(199901/02)15:1<59::AID-DMRR4>3.0.CO;2-T]

- 62 **Yu GV**, Hudson JR. Evaluation and treatment of stage 0 Charcot's neuroarthropathy of the foot and ankle. *J Am Podiatr Med Assoc* 2002; **92**: 210-220 [PMID: 11961088 DOI: 10.7547/87507315-92-4-210]
- 63 **Pakarinen TK**, Laine HJ, Honkonen SE, Peltonen J, Oksala H, Lahtela J. Charcot arthropathy of the diabetic foot. Current concepts and review of 36 cases. *Scand J Surg* 2002; **91**: 195-201 [PMID: 12164523 DOI: 10.1177/145749690209100212]
- 64 **Moreno M**, Gratacós J, Casado E, Galisteo C, Orellana C, Larrosa M. [Usefulness of Pamidronate in the Treatment of Charcot's Arthropathy]. *Reumatol Clin* 2007; **3**: 257-261 [PMID: 21794443 DOI: 10.1016/S1699-258X(07)73700-2]
- 65 **Naqvi A**, Cuchacovich R, Saketkoo L, Espinoza LR. Acute Charcot arthropathy successfully treated with pamidronate: long-term follow-up. *Am J Med Sci* 2008; **335**: 145-148 [PMID: 18277124 DOI: 10.1097/MAJ.0b013e3180a5e957]
- 66 **Anderson JJ**, Woelffer KE, Holtzman JJ, Jacobs AM. Bisphosphonates for the treatment of Charcot neuroarthropathy. *J Foot Ankle Surg* 2004; **43**: 285-289 [PMID: 15480402 DOI: 10.1053/j.jfas.2004.07.005]
- 67 **Game FL**, Catlow R, Jones GR, Edmonds ME, Jude EB, Rayman G, Jeffcoate WJ. Audit of acute Charcot's disease in the UK: the CDUK study. *Diabetologia* 2012; **55**: 32-35 [PMID: 22065087 DOI: 10.1007/s00125-011-2354-7]
- 68 **Bharath R**, Bal A, Sundaram S, Unnikrishnan AG, Praveen VP, Bhavani N, Nair V, Jayakumar RV, Kumar H. A comparative study of zoledronic acid and once weekly Alendronate in the management of acute Charcot arthropathy of foot in patients with diabetes mellitus. *Indian J Endocrinol Metab* 2013; **17**: 110-116 [PMID: 23776862 DOI: 10.4103/2230-8210.107818]
- 69 **Jude EB**, Selby PL, Burgess J, Lilleystone P, Mawer EB, Page SR, Donohoe M, Foster AV, Edmonds ME, Boulton AJ. Bisphosphonates in the treatment of Charcot neuroarthropathy: a double-blind randomised controlled trial. *Diabetologia* 2001; **44**: 2032-2037 [PMID: 11719835 DOI: 10.1007/s001250100008]
- 70 **Pitocco D**, Ruotolo V, Caputo S, Mancini L, Collina CM, Manto A, Caradonna P, Ghirlanda G. Six-month treatment with alendronate in acute Charcot neuroarthropathy: a randomized controlled trial. *Diabetes Care* 2005; **28**: 1214-1215 [PMID: 15855594 DOI: 10.2337/diacare.28.5.1214]
- 71 **Pakarinen TK**, Laine HJ, Mäenpää H, Mattila P, Lahtela J. The effect of zoledronic acid on the clinical resolution of Charcot neuroarthropathy: a pilot randomized controlled trial. *Diabetes Care* 2011; **34**: 1514-1516 [PMID: 21593295 DOI: 10.2337/dc11-0396]
- 72 **Richard JL**, Almasri M, Schuldiner S. Treatment of acute Charcot foot with bisphosphonates: a systematic review of the literature. *Diabetologia* 2012; **55**: 1258-1264 [PMID: 22361982 DOI: 10.1007/s00125-012-2507-3]
- 73 **Gertz BJ**, Holland SD, Kline WF, Matuszewski BK, Freeman A, Quan H, Lasseter KC, Mucklow JC, Porras AG. Studies of the oral bioavailability of alendronate. *Clin Pharmacol Ther* 1995; **58**: 288-298 [PMID: 7554702 DOI: 10.1016/0009-9236(95)90245-7]
- 74 **Camacho PM**, Petak SM, Binkley N, Clarke BL, Harris ST, Hurley DL, Kleerekoper M, Lewiecki EM, Miller PD, Narula HS, Pessah-Pollack R, Tangpricha V, Wimalawansa SJ, Watts NB. American Association of Clinical Endocrinologists and American College of Endocrinology Clinical Practice Guidelines for the Diagnosis and Treatment of Postmenopausal Osteoporosis - 2016. *Endocr Pract* 2016; **22**: 1-42 [PMID: 27662240 DOI: 10.4158/EP161435.GL]
- 75 **Black DM**, Delmas PD, Eastell R, Reid IR, Boonen S, Cauley JA, Cosman F, Lakatos P, Leung PC, Man Z, Mautalen C, Mesenbrink P, Hu H, Caminis J, Tong K, Rosario-Jansen T, Krasnow J, Hue TF, Sellmeyer D, Eriksen EF, Cummings SR; HORIZON Pivotal Fracture Trial. Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. *N Engl J Med* 2007; **356**: 1809-1822 [PMID: 17476007 DOI: 10.1056/NEJMoa067312]
- 76 **Heckbert SR**, Li G, Cummings SR, Smith NL, Psaty BM. Use of alendronate and risk of incident atrial fibrillation in women. *Arch Intern Med* 2008; **168**: 826-831 [PMID: 18443257 DOI: 10.1001/archinte.168.8.826]
- 77 **Sørensen HT**, Christensen S, Mehnert F, Pedersen L, Chapurlat RD, Cummings SR, Baron JA. Use of bisphosphonates among women and risk of atrial fibrillation and flutter: population based case-control study. *BMJ* 2008; **336**: 813-816 [PMID: 18334527 DOI: 10.1136/bmj.39507.551644.BE]

P- Reviewer: Beltowski J, Klimontov VV, Serhiyenko VA
S- Editor: Wang JL **L- Editor:** Filipodia **E- Editor:** Tan WW



Reversibility of diabetes mellitus: Narrative review of the evidence

Gary Yee Ang

Gary Yee Ang, Health Services and Outcomes Research, National Healthcare Group, Singapore 138543, Singapore

ORCID number: Gary Yee Ang (0000-0001-9338-7299).

Author contributions: Ang GY contributed to the manuscript.

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

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Gary Yee Ang, MBBS, Doctor, Health Services and Outcomes Research, National Healthcare Group, Fusionopolis 3, Singapore 138543, Singapore. gary_ang@nhg.com.sg
Telephone: +65-64966928
Fax: +65-64966257

Received: March 26, 2018

Peer-review started: March 27, 2018

First decision: April 13, 2018

Revised: April 19, 2018

Accepted: May 31, 2018

Article in press: May 31, 2018

Published online: July 15, 2018

lilitus and stimulate interest in prolonged remission as a treatment target. The current evidence for bariatric surgery is stronger than intensive medical management and the evidence is stronger for type 2 diabetes patients compared with type 1 diabetes patients. It is also unclear whether non obese diabetes patients would benefit from such interventions and the duration of diabetes before diabetes become irreversible. Further research is needed in this area especially with regards to the subgroup of diabetes patient who will benefit from these interventions and the long term safety and efficacy remains unknown especially with intensive medical management.

Key words: Diabetes; Reversibility; Remission; Bariatric surgery; Obesity

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Diabetes mellitus is potentially reversible especially with bariatric surgery. Intensive medical management is promising but the evidence is weaker. The subgroup that is likely to go into prolonged remission is those with insulin resistance, short duration of diabetes and obesity. Further research is needed to identify those that can go into remission and how to use intensive medical management to achieve this.

Ang GY. Reversibility of diabetes mellitus: Narrative review of the evidence. *World J Diabetes* 2018; 9(7): 127-131 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i7/127.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i7.127>

Abstract

The global disease burden of diabetes mellitus is high. It is well-established that prediabetes is reversible but it is unclear whether diabetes is reversible once it has been diagnosed. The objective of this narrative review is to review the evidence of reversibility of diabetes me-

INTRODUCTION

The number of adults with diabetes in the world has increased from 108 million in 1980 to 422 million in 2014 due to rise in prevalence, population growth and

ageing^[1]. Diabetes Mellitus has been projected to become the 7th leading causes of death in 2030^[2]. It has been estimated that the direct medical costs of diabetes to the world is more than United States \$827 million^[2]. It is well-established that prediabetes is reversible^[3-7], but it is unclear whether diabetes is reversible once it has been diagnosed.

Type 2 diabetes mellitus is potentially reversible^[8]. A better term to use would be remission which is defined to be achieving glucose level below the diabetic range in the absence of active pharmacologic or surgical therapy^[9]. It can further be divided into partial or complete and if complete remission lasts for more than 5 years, it would be considered as prolonged remission^[9]. In community settings, in the absence of bariatric surgery, the 7-year cumulative incidence of partial, complete or prolonged remission was found to be 1.47% (1.40%-1.54%), 0.14% (0.12%-0.16%) and 0.007% (0.003%-0.020%)^[10] which is very low.

In this narrative review, the evidence of reversibility of diabetes mellitus will be reviewed in light of new studies recently published. This can help stimulate interest in prolonged remission as a treatment target for patients with established diabetes.

BARIATRIC SURGERY

There are several systematic reviews on the impact of bariatric surgery on diabetes mellitus^[11-13] and some have distinguished between type 1 diabetes^[14-16] and type 2 diabetes^[17-20] (Table 1). The percentage of diabetes remission after bariatric surgery is estimated to be 76.8%^[12] to 92%^[11]. However, the exact physiological and molecular mechanisms behind diabetes remission after bariatric surgery remains incompletely understood^[21,22]. (Figure 1) There are several reviews that looked at the role of bariatric surgery in managing diabetes mellitus^[23-26] and the mechanism behind reversibility of type 2 diabetes mellitus^[27,28]. All agree that diabetes remission can be an important outcome to look at after bariatric surgery and there are many risk prediction models which can predict diabetes remission^[29].

Besides diabetes remission, bariatric surgery may also reduce inflammation^[30,31], improve renal function^[31], reduce cardiovascular risk^[32] and reduce microvascular and macrovascular complications^[33]. The impact of bariatric surgery on all these remains incompletely understood.

Even if the evidence is strong for remission of diabetes after bariatric surgery, it is unlikely to be advocated at the population level due to the high cost and lack of surgeons well trained to perform bariatric surgeries. Furthermore, the indication for bariatric surgery is currently for patients with a body mass index above 35 kg/m² or between 30 and 35 kg/m² with inadequate glycemic control despite optimal medical treatment^[34]. This would not benefit diabetes patients who are non-obese (body mass index < 30 kg/m²) and the remission

rate has been shown to be much lower in non-obese diabetes patients^[18].

PANCREAS TRANSPLANTATION AND ISLET CELL TRANSPLANTATION

A recent review found that there is a need for multicenter randomized trials in pancreas transplantation to define clearly the efficacy, risks, and long term benefits due to lack of high quality evidence^[35]. The indications for pancreas transplantation alone are in patients with severe metabolic complications, incapacitating problems with exogenous insulin therapy and failure of insulin based management to prevent acute complication^[35]. It would not be to induce diabetes into remission. A systematic review on islet cell transplantation for type 1 diabetes mellitus has also concluded that there is low to very low quality evidence for all outcomes of interest such as remission of diabetes^[36].

The next question to ask is whether pancreas transplantation is able to reverse complications of diabetes such as diabetic nephropathy. A recent study has demonstrated that diabetic nephropathy may be reversible after pancreas transplantation^[37] that is contrary to current thinking. Further research is needed to look at whether it is possible to reverse diabetes and/or its complications after pancreas or islet cell transplantation.

INTENSIVE MEDICAL MANAGEMENT

There are relatively fewer studies on non-surgical remission of diabetes mellitus. A randomized controlled trial found that 40.7% of patients with type 2 diabetes for less than 3 years had complete or partial remission at 12 mo^[38]. A cluster-randomized trial found that primary care-led weight management achieved a remission rate of 46% at 12 mo in patients with type 2 diabetes for less than 6 years^[39]. A retrospective observational study of obese patients with type 2 diabetes found that 4.6 % achieved partial or complete diabetes remission after a 12-wk intensive program for diabetes weight management^[40]. These studies did not look at the long term effectiveness of such intervention of the remission of type 2 diabetes mellitus and whether the same effect could be seen in patients with type 1 diabetes, non-obese diabetes patients or those with longer duration of type 2 diabetes.

Further research is needed to evaluate the long term effectiveness and safety of intensive medical management before recommending this but the results seem promising.

CLUSTERS OF DIABETES MELLITUS

A recent study has identified 5 replicable clusters of adult-onset diabetes with different disease progression and risk of diabetes complications^[41]. The 5 clusters are

Table 1 Bariatric surgery systematic reviews

Ref.	Type of diabetes	No. of studies included	Remission percentage (95%CI)
Chang <i>et al</i> ^[11] , 2014	Not specified	164 (37 randomized clinical trials and 127 observational studies)	Randomized clinical trials: 92% (85%-97%) Observational studies: 86% (79%-92%)
Buchwald <i>et al</i> ^[12] , 2004	Not specified	136	76.8% (70.7%-82.9%)
Gloy <i>et al</i> ^[13] , 2013	Not specified	11	59.90%
Ashrafian <i>et al</i> ^[14] , 2015	Type 1 diabetes mellitus	27	Weighted mean decrease in insulin requirement: 44.5 units 78.1% (73.8%-82.3%)
Chow <i>et al</i> ^[15] , 2016	Type 1 diabetes mellitus	13	Weighted mean total daily insulin requirement decreased from 98 +/- 26 IU/d to 42 +/- 11 IU/d
Mahawar <i>et al</i> ^[16] , 2016	Type 1 diabetes mellitus	15	Not reported
Buchwald <i>et al</i> ^[17] , 2009	Type 2 diabetes mellitus	621	76.2% insulin free
Baskota <i>et al</i> ^[18] , 2015	Type 2 diabetes mellitus	10	61.8% medication free
Goh <i>et al</i> ^[19] , 2017	Type 2 diabetes mellitus	24	Remission rate Duodenal-jejunal bypass: 20%-40% Duodenal-jejunal bypass with sleeve gastrectomy: 79%-93% Duodenal-jejunal bypass sleeve: 62.5%-100%
Yan <i>et al</i> ^[20]	Type 2 diabetes mellitus	6	Ileal interposition with sleeve gastrectomy: 47%-95.7% Type 2 diabetes mellitus remission rate for roux-en-y gastric bypass <i>vs</i> medical treatment: OR: 76.4 (95%CI: 20.7-281.7)

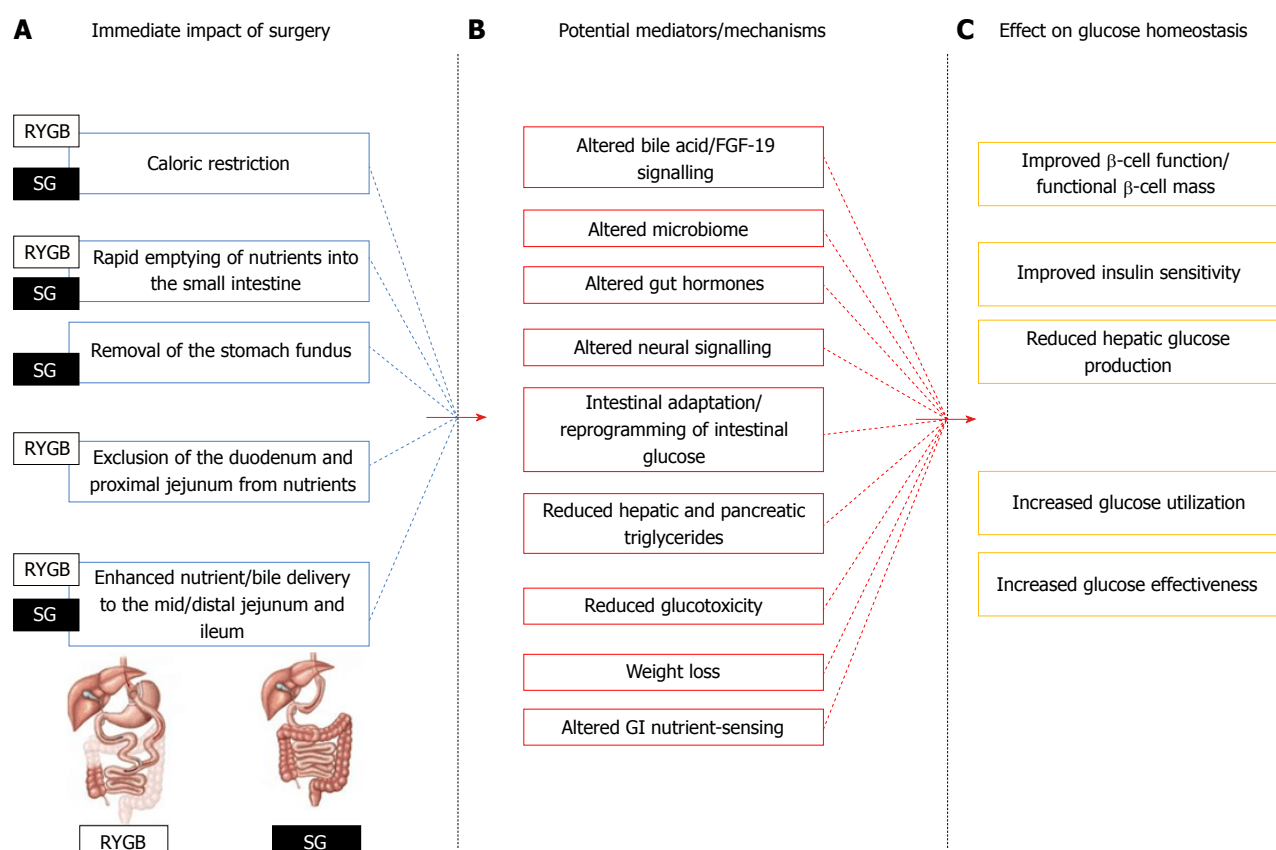


Figure 1 Potential mechanisms contributing to improved glycaemia after roux-en-Y gastric bypass and vertical sleeve gastrectomy. A: Immediate effects of improved glycaemia after roux-en-Y gastric bypass and vertical sleeve gastrectomy due to anatomical changes; B: Potential mediators/mechanisms involved. Cross talk occurs among these factors; C: Effects on glucose homeostasis^[21]; RYGB: Roux-en-Y gastric bypass; SG: Sleeve gastrectomy.

severe autoimmune diabetes (SAID), severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD) and mild age-related diabetes (MARD)^[41] (Table 2).

Of the 5, it would be interesting to see which are

more likely to go into prolonged remission with either bariatric surgery or intensive medical intervention so that clinicians can better define their treatment end-goals and treat accordingly. Based on insulin resistance, it would likely be SIRD, MOD and MARD that could go

Table 2 Subgroups of adult-onset diabetes

Subgroups	Body-mass index	Metabolic control	Insulin deficiency/resistance
Severe autoimmune diabetes	Relatively low	Poor	Insulin deficiency
Severe insulin-deficient diabetes	Relatively low	Poor	Insulin deficiency
Severe insulin-resistant diabetes	High	Fair	Insulin resistance
Mild obesity-related diabetes	High	Fair	Insulin resistance
Mild age-related diabetes	Relatively low	Fair	Insulin resistance

into prolonged remission.

Researchers may want to collect baseline data on glutamate decarboxylase antibodies, age at diagnosis, body mass index, glycated haemoglobin, and homeostatic model assessment 2 estimates of β -cell function and insulin resistance in future studies.

CONCLUSION

Diabetes Mellitus especially type 2 diabetes can go into prolonged remission *via* bariatric surgery or intensive medical therapy. The current evidence for bariatric surgery is stronger than intensive medical management but intensive medical management is likely to have a greater impact in type 2 diabetes management. More research is needed to understand the mechanism behind prolonged remission and to identify the group of diabetes patients that will benefit the most from such interventions.

REFERENCES

- 1 Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]
- 2 NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* 2016; **387**: 1513-1530 [PMID: 27061677 DOI: 10.1016/S0140-6736(16)00618-8]
- 3 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]
- 4 Aziz Z, Absetz P, Oldroyd J, Pronk NP, Oldenburg B. A systematic review of real-world diabetes prevention programs: learnings from the last 15 years. *Implement Sci* 2015; **10**: 172 [PMID: 26670418 DOI: 10.1186/s13012-015-0354-6]
- 5 Whittemore R. A systematic review of the translational research on the Diabetes Prevention Program. *Transl Behav Med* 2011; **1**: 480-491 [PMID: 24073067 DOI: 10.1007/s13142-011-0062-y]
- 6 Joiner KL, Nam S, Whittemore R. Lifestyle interventions based on the diabetes prevention program delivered via eHealth: A systematic review and meta-analysis. *Prev Med* 2017; **100**: 194-207 [PMID: 28456513 DOI: 10.1016/j.ypmed.2017.04.033]
- 7 Neamah HH, Sebert Kuhlmann AK, Tabak RG. Effectiveness of Program Modification Strategies of the Diabetes Prevention Program: A Systematic Review. *Diabetes Educ* 2016; **42**: 153-165 [PMID: 26879459 DOI: 10.1177/0145721716630386]
- 8 Steven S, Hollingsworth KG, Al-Mrabeh A, Avery L, Aribisala B, Caslake M, Taylor R. Very Low-Calorie Diet and 6 Months of Weight Stability in Type 2 Diabetes: Pathophysiological Changes in Responders and Nonresponders. *Diabetes Care* 2016; **39**: 808-815 [PMID: 27002059 DOI: 10.2337/dc15-1942]
- 9 Buse JB, Caprio S, Cefalu WT, Ceriello A, Del Prato S, Inzucchi SE, McLaughlin S, Phillips GL 2nd, Robertson RP, Rubino F, Kahn R, Kirkman MS. How do we define cure of diabetes? *Diabetes Care* 2009; **32**: 2133-2135 [PMID: 19875608 DOI: 10.2337/dc09-9036]
- 10 Karter AJ, Nundy S, Parker MM, Moffet HH, Huang ES. Incidence of remission in adults with type 2 diabetes: the diabetes & aging study. *Diabetes Care* 2014; **37**: 3188-3195 [PMID: 25231895 DOI: 10.2337/dc14-0874]
- 11 Chang SH, Stoll CR, Song J, Varela JE, Eagon CJ, Colditz GA. The effectiveness and risks of bariatric surgery: an updated systematic review and meta-analysis, 2003-2012. *JAMA Surg* 2014; **149**: 275-287 [PMID: 24352617 DOI: 10.1001/jamasurg.2013.3654]
- 12 Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrback K, Schoelles K. Bariatric surgery: a systematic review and meta-analysis. *JAMA* 2004; **292**: 1724-1737 [PMID: 15479938 DOI: 10.1001/jama.292.14.1724]
- 13 Gloy VL, Briel M, Bhatt DL, Kashyap SR, Schauer PR, Mingrone G, Bucher HC, Nordmann AJ. Bariatric surgery versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. *BMJ* 2013; **347**: f5934 [PMID: 24149519 DOI: 10.1136/bmj.f5934]
- 14 Ashrafian H, Harling L, Toma T, Athanasiou C, Nikiteas N, Efthimiou E, Darzi A, Athanasiou T. Type 1 Diabetes Mellitus and Bariatric Surgery: A Systematic Review and Meta-Analysis. *Obes Surg* 2016; **26**: 1697-1704 [PMID: 26694210 DOI: 10.1007/s11695-015-1999-6]
- 15 Chow A, Switzer NJ, Dang J, Shi X, de Gara C, Birch DW, Gill RS, Karmali S. A Systematic Review and Meta-Analysis of Outcomes for Type 1 Diabetes after Bariatric Surgery. *J Obes* 2016; **2016**: 6170719 [PMID: 27375900 DOI: 10.1155/2016/6170719]
- 16 Mahawar KK, De Alwis N, Carr WR, Jennings N, Schroeder N, Small PK. Bariatric Surgery in Type 1 Diabetes Mellitus: A Systematic Review. *Obes Surg* 2016; **26**: 196-204 [PMID: 26452482 DOI: 10.1007/s11695-015-1924-z]
- 17 Buchwald H, Estok R, Fahrback K, Banel D, Jensen MD, Pories WJ, Bantle JP, Sledge I. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med* 2009; **122**: 248-256.e5 [PMID: 19272486 DOI: 10.1016/j.amjmed.2008.09.041]
- 18 Baskota A, Li S, Dhakal N, Liu G, Tian H. Bariatric Surgery for Type 2 Diabetes Mellitus in Patients with BMI < 30 kg/m2: A Systematic Review and Meta-Analysis. *PLoS One* 2015; **10**: e0132335 [PMID: 26167910 DOI: 10.1371/journal.pone.0132335]
- 19 Goh YM, Toumi Z, Date RS. Surgical cure for type 2 diabetes by foregut or hindgut operations: a myth or reality? A systematic review. *Surg Endosc* 2017; **31**: 25-37 [PMID: 27194257 DOI: 10.1007/s00464-016-4952-4]
- 20 Yan Y, Sha Y, Yao G, Wang S, Kong F, Liu H, Zhang G, Zhang H, Hu C, Zhang X. Roux-en-Y Gastric Bypass Versus Medical Treatment for Type 2 Diabetes Mellitus in Obese Patients: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Medicine* (Baltimore) 2016; **95**: e3462 [PMID: 27124041 DOI: 10.1097/MD.00000000000003462]
- 21 Batterham RL, Cummings DE. Mechanisms of Diabetes Improvement Following Bariatric/Metabolic Surgery. *Diabetes Care* 2016; **39**: 893-901 [PMID: 27222547 DOI: 10.2337/dc16-0145]

- 22 **Haluzik M.** Bariatric surgery and the mechanism of diabetes remission: are we getting there? *J Clin Endocrinol Metab* 2013; **98**: 4336-4338 [PMID: 24194619 DOI: 10.1210/jc.2013-3698]
- 23 **Roslin MS, Cripps CN.** Bariatric surgery in managing diabetes mellitus. *Curr Opin Gastroenterol* 2016; **32**: 481-486 [PMID: 27607341 DOI: 10.1097/mog.0000000000000312]
- 24 **Ugale S, Agarwal D, Satwalekar V, Rao N, Ugale A.** Bariatric surgery as an option for diabetes mellitus prevention and treatment in obese persons. *Minerva Endocrinol* 2016; **41**: 469-476 [PMID: 27270500]
- 25 **Nguyen NT, Varela JE.** Bariatric surgery for obesity and metabolic disorders: state of the art. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 160-169 [PMID: 27899816 DOI: 10.1038/nrgastro.2016.170]
- 26 **Koliaki C, Liatis S, le Roux CW, Kokkinos A.** The role of bariatric surgery to treat diabetes: current challenges and perspectives. *BMC Endocr Disord* 2017; **17**: 50 [PMID: 28797248 DOI: 10.1186/s12902-017-0202-6]
- 27 **Perugini RA, Malkani S.** Remission of type 2 diabetes mellitus following bariatric surgery: review of mechanisms and presentation of the concept of 'reversibility'. *Curr Opin Endocrinol Diabetes Obes* 2011; **18**: 119-128 [PMID: 21522001 DOI: 10.1097/MED.0b013e3283446c1f]
- 28 **le Roux CW, Heneghan HM.** Bariatric Surgery for Obesity. *Med Clin North Am* 2018; **102**: 165-182 [PMID: 29156184 DOI: 10.1016/j.mcna.2017.08.011]
- 29 **Zhang R, Borisenko O, Telegina I, Hargreaves J, Ahmed AR, Sanchez Santos R, Pring C, Funch-Jensen P, Dillemans B, Hedenbro JL.** Systematic review of risk prediction models for diabetes after bariatric surgery. *Br J Surg* 2016; **103**: 1420-1427 [PMID: 27557164 DOI: 10.1002/bjs.10255]
- 30 **Hafida S, Mirshahi T, Nikolajczyk BS.** The impact of bariatric surgery on inflammation: quenching the fire of obesity? *Curr Opin Endocrinol Diabetes Obes* 2016; **23**: 373-378 [PMID: 27455515 DOI: 10.1097/MED.0000000000000277]
- 31 **Neff KJ, Frankel AH, Tam FW, Sadlier DM, Godson C, le Roux CW.** The effect of bariatric surgery on renal function and disease: a focus on outcomes and inflammation. *Nephrol Dial Transplant* 2013; **28** Suppl 4: iv73-iv82 [PMID: 24071659 DOI: 10.1093/ndt/gft262]
- 32 **Vest AR, Heneghan HM, Agarwal S, Schauer PR, Young JB.** Bariatric surgery and cardiovascular outcomes: a systematic review. *Heart* 2012; **98**: 1763-1777 [PMID: 23077152 DOI: 10.1136/heartjnl-2012-301778]
- 33 **Adams TD, Arterburn DE, Nathan DM, Eckel RH.** Clinical Outcomes of Metabolic Surgery: Microvascular and Macrovascular Complications. *Diabetes Care* 2016; **39**: 912-923 [PMID: 27222549 DOI: 10.2337/dc16-0157]
- 34 **Dixon JB, Zimmet P, Alberti KG, Rubino F; International Diabetes Federation Taskforce on Epidemiology and Prevention.** Bariatric surgery: an IDF statement for obese Type 2 diabetes. *Diabet Med* 2011; **28**: 628-642 [PMID: 21480973 DOI: 10.1111/j.1464-5491.2011.03306.x]
- 35 **Dean PG, Kukla A, Stegall MD, Kudva YC.** Pancreas transplantation. *BMJ* 2017; **357**: j1321 [PMID: 28373161 DOI: 10.1136/bmj.j1321]
- 36 **Health Quality Ontario.** Pancreas Islet Transplantation for Patients With Type 1 Diabetes Mellitus: A Clinical Evidence Review. *Ont Health Technol Assess Ser* 2015; **15**: 1-84 [PMID: 26644812]
- 37 **Fioritto P, Barzon I, Mauer M.** Is diabetic nephropathy reversible? *Diabetes Res Clin Pract* 2014; **104**: 323-328 [PMID: 24513120 DOI: 10.1016/j.diabres.2014.01.017]
- 38 **McInnes N, Smith A, Otto R, Vandermeij J, Punthakee Z, Sherifali D, Balasubramanian K, Hall S, Gerstein HC.** Piloting a Remission Strategy in Type 2 Diabetes: Results of a Randomized Controlled Trial. *J Clin Endocrinol Metab* 2017; **102**: 1596-1605 [PMID: 28324049 DOI: 10.1210/jc.2016-3373]
- 39 **Lean ME, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, Peters C, Zhyzhneuskaya S, Al-Mrabeh A, Hollingsworth KG, Rodrigues AM, Rehackova L, Adamson AJ, Snihotta FF, Mathers JC, Ross HM, McIlvenna Y, Stefanetti R, Trenell M, Welsh P, Kean S, Ford I, McConnachie A, Sattar N, Taylor R.** Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *Lancet* 2018; **391**: 541-551 [PMID: 29221645 DOI: 10.1016/S0140-6736(17)33102-1]
- 40 **Mottalib A, Sakr M, Shehabeldin M, Hamdy O.** Diabetes Remission after Nonsurgical Intensive Lifestyle Intervention in Obese Patients with Type 2 Diabetes. *J Diabetes Res* 2015; **2015**: 468704 [PMID: 26114120 DOI: 10.1155/2015/468704]
- 41 **Ahlqvist E, Storm P, Käräjämäki A, Martinell M, Dorkhan M, Carlsson A, Vikman P, Prasad RB, Aly DM, Almgren P, Wessman Y, Shaat N, Spégel P, Mulder H, Lindholm E, Melander O, Hansson O, Malmqvist U, Lernmark Å, Lahti K, Forsén T, Tuomi T, Rosengren AH, Groop L.** Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol* 2018; **6**: 361-369 [PMID: 29503172 DOI: 10.1016/S2213-8587(18)30051-2]

P- Reviewer: Cheungpasitporn W, Pecoraro V **S- Editor:** Cui LJ

L- Editor: A **E- Editor:** Tan WW



Retrospective Study

New-onset diabetes after kidney transplantation: Incidence and associated factors

Vânia Gomes, Florbela Ferreira, José Guerra, Maria João Bugalho

Vânia Gomes, Florbela Ferreira, Maria João Bugalho, Endocrinology, Diabetes and Metabolism Department, Santa Maria Hospital, Lisbon 1649-035, Portugal

José Guerra, Nephrology and Kidney Transplantation Department, Santa Maria Hospital, Lisbon 1649-035, Portugal

ORCID number: Vânia Gomes (0000-0002-0750-5744); Florbela Ferreira (0000-0002-2347-3658); José Guerra (0000-0001-8544-5209); Maria João Bugalho (0000-0003-0357-7350).

Author contributions: Gomes V wrote the manuscript, collected the data and performed the data analysis; Guerra J collected the data; Guerra J, Ferreira F and Bugalho MJ reviewed the manuscript for important intellectual content; all authors participated in designing the study.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of Santa Maria Hospital (No. 406/17).

Informed consent statement: Informed consent was not required for study participation or data publication because the clinical data were collected from an institutional database and had been anonymized before analysis.

Conflict-of-interest statement: All authors declare no conflicts-of-interest in relation to this article.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Vânia Gomes, MD, Doctor, Endocrinology, Diabetes and Metabolism Department, Santa Maria Hospital, Avenida Professor Egas Moniz, Lisbon 1649-035,

Portugal. vania.rodrigues.gomes@gmail.com
Telephone: +351-912-993251

Received: March 22, 2018

Peer-review started: March 23, 2018

First decision: May 8, 2018

Revised: May 24, 2018

Accepted: June 13, 2018

Article in press: June 13, 2018

Published online: July 15, 2018

Abstract

AIM

To determine the incidence and associated factors of new-onset diabetes after transplantation (NODAT) in a Portuguese central hospital.

METHODS

This single-center retrospective study involved consecutive adult nondiabetic transplant recipients, who had undergone kidney transplantation between January 2012 and March 2016. NODAT was diagnosed according to the criteria of the American Diabetes Association. Data were collected from an institutional database of the Nephrology and Kidney Transplantation Department (Santa Maria Hospital, Lisbon, Portugal) and augmented with data of laboratorial parameters collected from the corresponding patient electronic medical records. Exclusion criteria were preexisting diabetes mellitus, missing information and follow-up period of less than 12 mo. Data on demographic and clinical characteristics as well as anthropometric and laboratorial parameters were also collected. Patients were divided into two groups: With and without NODAT - for statistical comparison.

RESULTS

A total of 156 patients received kidney transplant

during the study period, 125 of who were included in our analysis. NODAT was identified in 27.2% of the patients ($n = 34$; 53% female; mean age: 49.5 ± 10.8 years; median follow-up: 36.4 ± 2.5 mo). The incidence in the first year was 24.8%. The median time to diagnosis was 3.68 ± 5.7 mo after transplantation, and 76.5% of the patients developed NODAT in the first 3 mo. In the group that did not develop NODAT ($n = 91$), 47% were female, with mean age of 46.4 ± 13.5 years and median follow-up of 35.5 ± 1.6 mo. In the NODAT group, the pretransplant fasting plasma glucose (FPG) levels were significantly higher [$101 (96.1-105.7)$ mg/dL *vs* $92 (91.4-95.8)$ mg/dL, $P = 0.007$] and pretransplant impaired fasting glucose (IFG) was significantly more frequent (51.5% *vs* 27.7%, $P = 0.01$). Higher pretransplant FPG levels and pretransplant IFG were found to be predictive risk factors for NODAT development [odds ratio (OR): 1.059, $P = 0.003$; OR: 2.772, $P = 0.017$, respectively].

CONCLUSION

NODAT incidence was high in our renal transplant recipients, particularly in the first 3 mo posttransplant, and higher pretransplant FPG level and IFG were risk factors.

Key words: New-onset diabetes after transplant; Incidence; Kidney transplantation; Impaired fasting glucose; Immunosuppression

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: New-onset diabetes mellitus after transplantation (NODAT) is a major complication of kidney transplant. The aim of this study was to evaluate the incidence and associated factors of NODAT among kidney transplant recipients in a single center. A total of 125 patients transplanted at Santa Maria Hospital (Lisbon, Portugal) were assessed, and NODAT was identified in 27.2%. The median time to diagnosis was 3.68 ± 5.7 mo after transplantation and most patients (76.5%) developed NODAT in the first 3 mo posttransplant. Higher pretransplant fasting plasma glucose level and pretransplant impaired fasting glucose were predictive risk factors for NODAT development.

Gomes V, Ferreira F, Guerra J, Bugalho MJ. New-onset diabetes after kidney transplantation: Incidence and associated factors. *World J Diabetes* 2018; 9(7): 132-137 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i7/132.htm> DOI: <http://dx.doi.org/10.4239/wjcd.v9.i7.132>

INTRODUCTION

New-onset diabetes after transplantation (NODAT) is a frequent metabolic complication of kidney transplantation, and associated with increased morbidity and

mortality^[1,2]. However, due to the absence of a standard definition of NODAT, it has been difficult to determine a reliable incidence rate. The first International Consensus Guidelines published in 2003 for the diagnosis and management of NODAT were updated in 2014 and advocate the World Health Organization (WHO) and American Diabetes Association (ADA) criteria for the diagnosis of diabetes mellitus (DM) and impaired glucose tolerance (IGT)^[3,4]. Recent studies using these criteria found incidences of NODAT to be 7%-30% in the first year after transplant^[5-8].

Increased insulin resistance and impaired insulin production are likely to contribute to the development of NODAT^[2]. Both traditional type 2 DM and transplant-related risk factors affect this condition^[9]. The NODAT risk factors can be categorized into three groups: Non-modifiable, modifiable and potentially modifiable^[10]. The non-modifiable factors include age, race/ethnicity, family history of DM, male recipient sex, the presence of certain human leukocyte antigens (HLAs; such as HLA A30, B27 and B42), increased HLA mismatches, donor-recipient mismatch, deceased donor kidney, male donor sex and history of acute rejection^[10]. Polycystic kidney disease may confer an increased risk of NODAT, although results of the related studies remain conflicting^[11]. On the other hand, the modifiable risk factors comprise obesity and type of immunosuppressive agents used to prevent or treat rejection. Finally, the potentially modifiable risk factors include pretransplant impaired fasting glucose (IFG) or IGT, and infection with hepatitis C or cytomegalovirus (CMV)^[10].

The aim of this study was to evaluate the incidence of NODAT and its associated factors among kidney transplant recipients who were treated in a transplant center of a central Portuguese hospital.

MATERIALS AND METHODS

This is a single-center retrospective study of consecutive adult nondiabetic patients, who underwent kidney transplant between January 2012 and March 2016 at Santa Maria Hospital, Lisbon, Portugal. Data were collected retrospectively from an institutional database created by the Nephrology and Kidney Transplantation Department and completed with data for laboratorial parameters collected from the respective patients' electronic medical records, in agreement with our institutional ethical recommendations.

Inclusion and exclusion criteria

NODAT was diagnosed according to the ADA criteria (2017), which involves the following: Symptoms of diabetes (*i.e.*, polyuria, polydipsia or unexplained weight loss) plus random plasma glucose of ≥ 200 mg/dL; fasting plasma glucose (FPG) of ≥ 126 mg/dL, with fasting defined as no caloric intake for at least 8 h; and 2-h plasma glucose of ≥ 200 mg/dL during an oral glucose tolerance test (OGTT). IFG was defined as FPG

between 100 mg/dL and 125 mg/dL^[3].

In the first 3 mo after transplant, glycated hemoglobin was not used as diagnostic criteria, since its validity can be affected by the processes of new hemoglobin synthesis and glycation in the posttransplant setting^[12]. The OGTT is considered the gold standard for diagnosing NODAT, enabling the identification of more patients than FPG measurement alone; likewise, it allows for diagnosis of IGT^[4]. However, in our kidney transplantation center, the OGTT is not routinely performed in transplant recipients. The NODAT diagnosis was established when the immunosuppressive therapy and kidney allograft were stable and in the absence of acute infections or other stress factors, in order to exclude patients who developed transient hyperglycemia in the early posttransplant period^[4].

Data on demographic/clinical characteristics, anthropometric and laboratorial parameters included age at transplant, sex, race, weight, height, calculated body mass index (BMI), etiology of primary renal disease, pretransplant FPG, history of hepatitis C or CMV infection, acute rejection episodes, type of transplant (deceased or living donor), type of immunosuppressive drugs for induction and maintenance therapy, follow-up time, graft loss and death. Exclusion criteria were preexisting DM, missing information (*i.e.*, pretransplant FPG) and follow-up period of less than 12 mo. A total of 156 patients were transplanted during the study period, and 125 of these were eligible for the study.

Immunosuppression regimen

All patients received induction therapy, consisting of either basiliximab (an interleukin-2 receptor monoclonal antibody; Protocol A) or rabbit antithymocyte globuline (ATG; Protocol B). Prior to the transplant, all patients received tacrolimus at 0.2 mg/kg. For Protocol A, the patient was administered 20 mg basiliximab pretransplantation and at 4 d posttransplantation; these patients also received tacrolimus at 0.075 mg/kg every 12 h and mycophenolate mofetil (1500 mg pretransplantation, followed by 1000 mg every 12 h for 1 wk posttransplantation and then 500 mg every 12 h). For Protocol B, the patient was administered 1.5-2 mg/kg ATG pretransplantation; methylprednisolone (500 mg) before ATG and tacrolimus at 0.05 mg/kg every 12 h.

All patients received 500 mg methylprednisolone intraoperatively, followed by 1 mg/kg per day for 3 d postoperatively, with progressive tapering until reaching 25 mg/d by the end of the first month after transplant. The maintenance therapy comprised corticosteroids (prednisolone), tacrolimus and mycophenolate mofetil.

Statistical analysis

Data were analyzed with SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, United States). A biomedical statistician (Nilza Gonçalves, Instituto de Medicina Molecular, Faculty of Medicine, University of Lisbon, Portugal) reviewed the study's statistics. For

comparative analysis, the patients were divided into two groups: With and without NODAT. For continuous variables, differences were analyzed using the *Mann-Whitney* test (nonparametric data) and Student's *t*-test (parametric data). For categorical variables, differences were analyzed using the χ^2 test. Multivariate analysis was performed to identify potential risk factors for NODAT by using a logistic regression test. Data were expressed as mean \pm SD or median (minimum and maximum) for continuous variables and as percentage for categorical variables. $P < 0.05$ was considered significant.

RESULTS

A total of 125 patients were enrolled for the analysis (mean age: 46.9 ± 12.9 years; 51.2% male). The majority of our patients were Caucasian, and the median follow-up was 35.7 ± 15.1 mo. NODAT was identified in 27.2% [$n = 34$; 95% confidence interval (CI): 20.17%-35.59%] of the patients; the NODAT cases were 53% female and had mean age of 49.6 ± 10.8 years. The incidence of NODAT in the first year was 24.8% (95%CI: 18.06%-33.05%).

The median time to diagnosis was 3.68 ± 5.7 mo after transplantation, with the majority of patients (76.5%) developing NODAT in the first 3 mo. NODAT diagnoses at the follow-up intervals of 3-6 mo, 6-12 mo and after 12 mo were 5.9%, 8.8% and 8.8%, respectively. The median follow-up for the NODAT group was 36.4 ± 2.5 mo. In the group that did not develop NODAT ($n = 91$), 47% were female and the mean age was 46.0 ± 13.6 years. The median follow-up was 35.5 ± 1.6 mo, which was not significantly different from that of the NODAT group ($P = 0.774$).

Table 1 compares the clinical and laboratory parameters of patients who developed NODAT with those who did not (NODAT vs non-NODAT). During the follow-up period, 1 patient in the NODAT group and 2 patients in the non-NODAT group died. There was no graft loss in the NODAT group, as opposed to the 5 cases recorded for the non-NODAT group.

In the NODAT group, the pretransplant FPG levels were significantly higher [101 (96.1 - 105.7) mg/dL vs 92 (91.4 - 95.8) mg/dL, $P = 0.007$] and the occurrence of pretransplant IFG was significantly more frequent (51.5% vs 27.7%, $P = 0.01$). Furthermore, higher pretransplant FPG levels and pretransplant IFG occurrence were identified as predictive risk factors for NODAT development [odds ratio (OR): 1.059, $P = 0.003$; OR: 2.772, $P = 0.017$, respectively].

Patients diagnosed with NODAT were more frequently of African origin (29.4% vs 22%), presented a trend for higher age (49.6 ± 10.8 years vs 46.0 ± 13.6 years) and BMI (25.2 ± 4.0 kg/m² vs 24.5 ± 4.4 kg/m²), as well as a higher frequency of hepatitis C infection (2.9% vs 1.1%), CMV infection (97% vs 93%), acute rejection (14.7% vs 8.8%) and deceased donor (100% vs 91.2%), although none of these parameters

Table 1 Clinical and laboratory parameters

	NODAT group	Non-NODAT group	P
No. of patients	34 (27.2%)	91 (72.8%)	
Age at transplant (yr)	49.6 ± 10.8	46.0 ± 13.6	0.165
Female sex	53% (18/34)	47% (43/91)	0.571
Race			
Caucasian	70.6% (24/34)	78% (71/91)	0.387
African	29.4% (10/34)	22% (20/91)	
Body mass index (kg/m ²)	25.2 ± 4.0	24.5 ± 4.4	0.418
Pre-transplant FPG (mg/dL)	101 (96.1-105.7)	92 (91.4-95.8)	0.007
Pretransplant IFG	51.5% (17/33)	27.7% (23/83)	0.01
Hepatitis C infection	2.9% (1/34)	1.1% (1/91)	0.472
CMV infection	97% (33/34)	93% (82/88)	0.672
Acute rejection	14.7% (5/34)	8.8% (8/91)	0.338
Type of transplant			
Deceased donor	100% (34/34)	91.2% (83/91)	0.106
Living donor	0% (0/34)	8.8% (8/91)	
Follow-up (mo)	36.4 ± 2.5	35.5 ± 1.6	0.774

CMV: Cytomegalovirus; FPG: Fasting plasma glucose; IFG: Impaired fasting glucose; NODAT: New-onset diabetes after transplantation.

reached statistical significance. The most frequent etiology of end-stage renal disease was hypertensive nephropathy ($n = 7$) in the NODAT group and polycystic kidney disease ($n = 17$) in the non-NODAT group.

In the NODAT group, induction therapy comprised ATG in 6 patients and basiliximab in 28; in the non-NODAT group, 24 patients received ATG and 67 received basiliximab. No statistically significant difference was found between the two groups for the induction therapies used ($P = 0.309$). In both groups, maintenance therapy consisted of immunosuppression with corticosteroids, tacrolimus and mycophenolate mofetil. Of the 34 patients diagnosed with NODAT, 44.1% ($n = 15$) needed oral hypoglycemic agents, 26.5% ($n = 9$) needed insulin and 5.9% ($n = 2$) were administered combined therapy (insulin and oral hypoglycemic agents). In the remaining 23.5% of the patients ($n = 8$), diabetes was controlled with diet and exercise alone.

DISCUSSION

Kidney transplant, besides being more cost-effective than dialysis, improves patient survival^[13]. Nevertheless, NODAT is a frequent complication of kidney transplantation and is associated with poorer outcomes, increased risk of infectious and cardiovascular complications and reduced rates of patient and graft survival^[5,14].

The reported incidence of NODAT has varied broadly between studies, probably due to the use of diverse diagnostic criteria, intensity of routine screening and follow-up length^[15]. Furthermore, variability in the immunosuppressive protocols used in different transplant centers could influence the calculated incidence rates of NODAT. For instance, it is known that tacrolimus is more diabetogenic than cyclosporine^[16]. Recent studies using the WHO/ADA criteria reported that 7%-30% of nondiabetic kidney transplant recipients develop NODAT in the first year after transplant^[5-8]. In our study,

NODAT was diagnosed in 34 patients (27.2%), with an incidence of 24.8% in the first year after transplant. Therefore, our findings are in agreement with previous studies. NODAT occurrence reportedly peaks in the first 3-6 mo posttransplant^[17,18]. Studies have also shown that the incidence is higher when higher dosages of immunosuppressive medications are used^[17]. After the 3-6 mo period, the annual incidence of diabetes is comparable to that observed in pretransplant patients^[17,18]. In the present study, the median time to diagnosis was 3.68 ± 5.7 mo, with the majority of patients (76.5%) developing NODAT in the first 3 mo, which is also consistent with the literature.

Multiple risk factors have been identified. In our study, higher pretransplant FPG levels and occurrence of pretransplant IFG were predictive risk factors for NODAT development. Other researchers have reported abnormal glucose metabolism as a NODAT risk factor. For example, Cosio *et al.*^[19] reported that high pretransplant glucose levels represent a risk factor for NODAT at 1-year posttransplant. The risk was shown to increase as pretransplant FPG levels rose. Among patients with pretransplant IFG in that study, 70% had hyperglycemia at 1 year (IFG 43% and NODAT 27%). The strongest risk factor for NODAT seems to be age^[20]. NODAT development is 2.2 times more likely to occur in patients with age above 45 years^[21]. Another independent risk factor for NODAT is obesity or overweight status. Previous studies have reported a relative risk of 1.4 and 1.8 for patients with BMI between 25-30 kg/m² and > 30 kg/m², respectively^[22]. We also found a trend for higher age and higher BMI in the NODAT group.

African-Americans have a 2-fold risk of developing NODAT compared to Caucasians. This finding can be, at least partly, related to immunosuppressive agents' pharmacokinetics variation^[15]. Hepatitis C and CMV infection are also associated with NODAT. Hepatitis C virus causes insulin resistance in the context of liver dysfunction, abnormalities in glucose metabolism and

pancreatic β cell dysfunction^[23]. Similarly, lower median insulin release has been reported for patients with CMV infection, suggesting impaired pancreatic β cell function as a possible pathogenic mechanism^[24].

History of acute rejection episodes requiring elevated doses of glucocorticoids, as well as the type of transplant (deceased donor), have also been implicated in risk of NODAT^[22]. We found higher frequencies of African-origin individuals, hepatitis C infection, CMV infection, acute rejection and deceased donors in our NODAT group, as suggested in the literature; however, the differences did not reach statistical significance. The majority of NODAT patients in our study required treatment for diabetes, with most responding to oral hypoglycemic agents, followed by insulin, and few requiring combined therapy. Nearly a quarter of the patients were able to achieve diabetes control without medication, based on lifestyle modifications.

Some limitations exist in our study design that may impact the interpretation and/or generalization of our findings. This was a retrospective study with a relatively small sample, only reflecting a single center experience. Moreover, OGTT is not currently used in our center as a NODAT screening test, which is likely to lead to underestimation of its incidence in this cohort.

The incidence of NODAT in renal transplant recipients is high, particularly in the first 3 mo. Recognition of the associated factors may help to prevent this condition. Higher pretransplant FPG levels and occurrence of pretransplant IFG were predictive risk factors for NODAT development, indicating a need for periodical blood glucose screening in patients waiting for a transplant in order to identify those at risk. Using the same rationale as for type 2 DM, early identification of impaired carbohydrate metabolism in the posttransplant setting will allow implementation of lifestyle modifications in order to minimize progression to NODAT and its potentially severe complications.

ARTICLE HIGHLIGHTS

Research background

New-onset diabetes after transplantation (NODAT) is a common complication of kidney transplantation, correlated with poorer outcomes. Its incidence varies greatly between studies, and multiple risk factors have been associated with its onset.

Research motivation

Albeit a frequent complication of kidney transplant, very few studies of NODAT in the Portuguese population have been published.

Research objectives

To evaluate the incidence and associated factors of NODAT among kidney transplant recipients in a Portuguese hospital.

Research methods

Retrospective study of consecutive adult nondiabetic patients, who underwent kidney transplant between January 2012 and March 2016 in a central Portuguese hospital.

Research results

NODAT was identified in 27.2% of the kidney transplant recipients. The median time to diagnosis was 3.68 ± 5.7 mo after transplantation. Higher pretransplant fasting plasma glucose levels and occurrence of pretransplant impaired fasting glucose (IFG) were predictive risk factors for NODAT development.

Research conclusions

Periodical blood glucose screening in patients waiting for a kidney transplant is important to identify those at risk for and to minimize progression to NODAT and its potentially severe complications.

Research perspectives

Clinicians should be aware of NODAT risk factors, namely pretransplant IFG, to perform a tighter surveillance of patients in these conditions. Multicentric studies are required to investigate other risk factors possibly implicated in NODAT development.

ACKNOWLEDGEMENTS

The authors thank Nilza Gonçalves for statistical analysis review.

REFERENCES

- 1 **Langsford D**, Dwyer K. Dysglycemia after renal transplantation: Definition, pathogenesis, outcomes and implications for management. *World J Diabetes* 2015; **6**: 1132-1151 [PMID: 26322159 DOI: 10.4239/wjd.v6.i10.1132]
- 2 **Juan Khong M**, Ping Chong Ch. Prevention and management of new-onset diabetes mellitus in kidney transplantation. *Neth J Med* 2014; **72**: 127-134 [PMID: 24846925]
- 3 **American Diabetes Association**. 2. Classification and Diagnosis of Diabetes. *Diabetes Care* 2017; **40**: S11-S24 [PMID: 27979889 DOI: 10.2337/dc17-S005]
- 4 **Sharif A**, Hecking M, de Vries AP, Porrini E, Hornum M, Rasoul-Rockenschaub S, Berlakovich G, Krebs M, Kautzky-Willer A, Scherthaner G, Marchetti P, Pacini G, Ojo A, Takahara S, Larsen JL, Budde K, Eller K, Pascual J, Jardine A, Bakker SJ, Valderhaug TG, Jenssen TG, Cohnsey S, Säemann MD. Proceedings from an international consensus meeting on posttransplantation diabetes mellitus: recommendations and future directions. *Am J Transplant* 2014; **14**: 1992-2000 [PMID: 25307034 DOI: 10.1111/ajt.12850]
- 5 **Gourishankar S**, Jhangri GS, Tonelli M, Wales LH, Cockfield SM. Development of diabetes mellitus following kidney transplantation: a Canadian experience. *Am J Transplant* 2004; **4**: 1876-1882 [PMID: 15476489 DOI: 10.1111/j.1600-6143.2004.00591.x]
- 6 **Rodrigo E**, Santos L, Piñera C, Millán JC, Quintela ME, Toyos C, Allende N, Gómez-Alamillo C, Arias M. Prediction at first year of incident new-onset diabetes after kidney transplantation by risk prediction models. *Diabetes Care* 2012; **35**: 471-473 [PMID: 22279030 DOI: 10.2337/dc11-2071]
- 7 **Yu H**, Kim H, Baek CH, Baek SD, Jeung S, Han DJ, Park SK. Risk factors for new-onset diabetes mellitus after living donor kidney transplantation in Korea - a retrospective single center study. *BMC Nephrol* 2016; **17**: 106 [PMID: 27473469 DOI: 10.1186/s12882-016-0321-8]
- 8 **Patel S**, Gohel K, Patel B. Incidences and risk factor for new onset diabetes after transplantation in live donor kidney transplantation: a prospective single centre study. *Int J Pharm Pharm Sci* 2016; **8**: 230-233
- 9 **Chakkera HA**, Hanson RL, Raza SM, DiStefano JK, Millis MP, Heilman RL, Mulligan DC, Reddy KS, Mazur MJ, Hamawi K, Moss AA, Mekeel KL, Cerhan JR. Pilot study: association of traditional and genetic risk factors and new-onset diabetes mellitus following kidney transplantation. *Transplant Proc* 2009; **41**: 4172-4177 [PMID: 20005362 DOI: 10.1016/j.transproceed.2009.08.063]

- 10 **Pham PT**, Pham PM, Pham SV, Pham PA, Pham PC. New onset diabetes after transplantation (NODAT): an overview. *Diabetes Metab Syndr Obes* 2011; **4**: 175-186 [PMID: 21760734 DOI: 10.2147/DMSO.S19027]
- 11 **Cheungpasitporn W**, Thongprayoon C, Vijayvargiya P, Anthonont P, Erickson SB. The Risk for New-Onset Diabetes Mellitus after Kidney Transplantation in Patients with Autosomal Dominant Polycystic Kidney Disease: A Systematic Review and Meta-Analysis. *Can J Diabetes* 2016; **40**: 521-528 [PMID: 27184299 DOI: 10.1016/j.cjcd.2016.03.001]
- 12 **Wilkinson A**, Davidson J, Dotta F, Home PD, Keown P, Kiberd B, Jardine A, Levitt N, Marchetti P, Markell M, Naicker S, O'Connell P, Schnitzler M, Standl E, Torregosa JV, Uchida K, Valentine H, Villamil F, Vincenti F, Wissing M. Guidelines for the treatment and management of new-onset diabetes after transplantation. *Clin Transplant* 2005; **19**: 291-298 [PMID: 15877787 DOI: 10.1111/j.1399-0012.2005.00359.x]
- 13 **Shivaswamy V**, Boerner B, Larsen J. Post-Transplant Diabetes Mellitus: Causes, Treatment, and Impact on Outcomes. *Endocr Rev* 2016; **37**: 37-61 [PMID: 26650437 DOI: 10.1210/er.2015-1084]
- 14 **Caillard S**, Eprinchard L, Perrin P, Braun L, Heibel F, Moreau F, Kessler L, Moulin B. Incidence and risk factors of glucose metabolism disorders in kidney transplant recipients: role of systematic screening by oral glucose tolerance test. *Transplantation* 2011; **91**: 757-764 [PMID: 21336240 DOI: 10.1097/TP.0b013-e31820f0877]
- 15 **Palepu S**, Prasad GV. New-onset diabetes mellitus after kidney transplantation: Current status and future directions. *World J Diabetes* 2015; **6**: 445-455 [PMID: 25897355 DOI: 10.4239/wjd.v6.i3.445]
- 16 **Luan FL**, Steffick DE, Ojo AO. New-onset diabetes mellitus in kidney transplant recipients discharged on steroid-free immunosuppression. *Transplantation* 2011; **91**: 334-341 [PMID: 21242885 DOI: 10.1097/TP.0b013e318203c25f]
- 17 **Ghisdal L**, Van Laecke S, Abramowicz MJ, Vanholder R, Abramowicz D. New-onset diabetes after renal transplantation: risk assessment and management. *Diabetes Care* 2012; **35**: 181-188 [PMID: 22187441 DOI: 10.2337/dc11-1230]
- 18 **Mourad G**, Glyda M, Albano L, Viklický O, Merville P, Tydén G, Mourad M, Löhmus A, Witzke O, Christiaans MHL, Brown MW, Undre N, Kazeem G, Kuypers DRJ; Advagraf-based immunosuppression regimen examining new onset diabetes mellitus in kidney transplant recipients (ADVANCE) study investigators. Incidence of Posttransplantation Diabetes Mellitus in De Novo Kidney Transplant Recipients Receiving Prolonged-Release Tacrolimus-Based Immunosuppression With 2 Different Corticosteroid Minimization Strategies: ADVANCE, A Randomized Controlled Trial. *Transplantation* 2017; **101**: 1924-1934 [PMID: 27547871 DOI: 10.1097/TP.0000000000001453]
- 19 **Cosio FG**, Kudva Y, van der Velde M, Larson TS, Textor SC, Griffin MD, Stegall MD. New onset hyperglycemia and diabetes are associated with increased cardiovascular risk after kidney transplantation. *Kidney Int* 2005; **67**: 2415-2421 [PMID: 15882287 DOI: 10.1111/j.1523-1755.2005.00349.x]
- 20 **Rodrigo E**, Fernández-Fresnedo G, Valero R, Ruiz JC, Piñera C, Palomar R, González-Cotorruelo J, Gómez-Alamillo C, Arias M. New-onset diabetes after kidney transplantation: risk factors. *J Am Soc Nephrol* 2006; **17**: S291-S295 [PMID: 17130277 DOI: 10.1681/ASN.2006080929]
- 21 **Cosio FG**, Pesavento TE, Osei K, Henry ML, Ferguson RM. Post-transplant diabetes mellitus: increasing incidence in renal allograft recipients transplanted in recent years. *Kidney Int* 2001; **59**: 732-737 [PMID: 11168956 DOI: 10.1046/j.1523-1755.2001.05900.2732.x]
- 22 **Kesiraju S**, Paritala P, Rao Ch UM, Sahariah S. New onset of diabetes after transplantation - an overview of epidemiology, mechanism of development and diagnosis. *Transpl Immunol* 2014; **30**: 52-58 [PMID: 24184293 DOI: 10.1016/j.trim.2013.10.006]
- 23 **Markell M**. New-onset diabetes mellitus in transplant patients: pathogenesis, complications, and management. *Am J Kidney Dis* 2004; **43**: 953-965 [PMID: 15168375 DOI: 10.1053/j.ajkd.2004.03.020]
- 24 **Hjelmestaeth J**, Sagedal S, Hartmann A, Rollag H, Egeland T, Hagen M, Nordal KP, Jenssen T. Asymptomatic cytomegalovirus infection is associated with increased risk of new-onset diabetes mellitus and impaired insulin release after renal transplantation. *Diabetologia* 2004; **47**: 1550-1556 [PMID: 15338129 DOI: 10.1007/s00125-004-1499-z]

P- Reviewer: Dinc M, Hasan M, Zhao J **S- Editor:** Ji FF
L- Editor: A **E- Editor:** Tan WW





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 August 15; 9(8): 138-140





EDITORIAL

- 138 Exercise and glucagon-like peptide-1: Does exercise potentiate the effect of treatment?

Hamasaki H

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Dimiter Avtanski, PhD, Assistant Professor, Department of Medicine, Friedman Diabetes Institute, Northwell Health, New York, NY 10022, United States

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes (*WJD*) is now abstracted and indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, Scopus, China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Wen-Wen Tan*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITOR-IN-CHIEF
Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com>

www.wjgnet.com/1948-9358/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE
August 15, 2018

COPYRIGHT
© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.f6publishing.com>

Exercise and glucagon-like peptide-1: Does exercise potentiate the effect of treatment?

Hidetaka Hamasaki

Hidetaka Hamasaki, Endocrinology and Metabolism, Internal Medicine, Hamasaki Clinic, Kagoshima 890-0046, Japan

ORCID number: Hidetaka Hamasaki (0000-0002-0124-597X).

Author contributions: Hamasaki H wrote the review.

Conflict-of-interest statement: No conflict of interest.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Hidetaka Hamasaki, MD, PhD, Doctor, Endocrinology and Metabolism, Internal Medicine, Hamasaki Clinic, 2-21-4 Nishida, Kagoshima 890-0046, Japan. hhamasaki78@gmail.com
Telephone: +81-99-2503535
Fax: +81-99-2501470

Received: June 4, 2018

Peer-review started: June 4, 2018

First decision: June 14, 2018

Revised: June 20, 2018

Accepted: June 28, 2018

Article in press: June 28, 2018

Published online: August 15, 2018

Abstract

Recently, glucagon-like peptide-1 (GLP-1) receptor agonists have become a cornerstone for the treatment of obese patients with type 2 diabetes (T2D), exhibiting favorable effects on the cardiovascular outcome. In

T2D, impaired GLP-1 secretion/function is observed, and gut microbiota dysbiosis is related to the GLP-1 resistance. Prior research has revealed that exercise increases GLP-1 levels in healthy and obese individuals; however, the efficacy of exercise on GLP-1 levels in patients with T2D remains unclear. Exercise may improve GLP-1 resistance rather than GLP-1 secretion in patients with T2D. Exercise increases the gut microbiota diversity, which could contribute to improving the GLP-1 resistance of T2D. Furthermore, the gut microbiota may play a role in the correlation between exercise and GLP-1. The combination of exercise and GLP-1-based therapy may have a synergistic effect on the treatment of T2D. Although the underlying mechanism remains unknown, exercise potentiates the efficacy of GLP-1 receptor agonist treatment in patients with T2D.

Key words: Type 2 diabetes; Exercise; Glucagon-like peptide-1; Gut microbiota; Myokine

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: The impact of exercise on glucagon-like peptide-1 (GLP-1) receptor agonists in patients with type 2 diabetes (T2D) remains unclear. Exercise could potentiate the effect of GLP-1 receptor agonists treatment and play a vital role in ameliorating GLP-1 resistance by improving gut microbiota dysbiosis and reducing the ectopic fat in patients with T2D.

Hamasaki H. Exercise and glucagon-like peptide-1: Does exercise potentiate the effect of treatment? *World J Diabetes* 2018; 9(8): 138-140 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i8/138.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i8.138>

Recently, the use of glucagon-like peptide-1 (GLP-1)

receptor agonists has become an essential treatment in obese patients with type 2 diabetes (T2D). The efficacy of GLP-1 receptor agonists has been established for all components of metabolic syndrome and hyperglycemia, which accounts for favorable effects on the cardiovascular outcome^[1]. GLP-1 is secreted by the intestinal L cells, promotes satiety, inhibits gastric emptying, stimulates insulin secretion, and suppresses glucagon secretion in response to food consumption^[2]. In patients with T2D, the incretin effect is severely diminished, suggesting that altered GLP-1 secretion/function is associated with T2D pathophysiology. The abnormality of the incretin effect in T2D could be attributed to GLP-1 resistance in β -cells^[2].

Several studies have established a marked correlation between the composition of gut microbiota and the pathophysiology of diabetes and obesity^[3]. Grasset *et al*^[4] reported that gut microbiota dysbiosis caused GLP-1 resistance in obese and diabetic mice. In addition, the relative abundance of Lactobacilli was decreased in GLP-1-resistant mice and positively correlated with the ileum GLP-1 receptor and neuronal nitric oxide synthase mRNA concentrations. Furthermore, the relative abundance of Bacteroidales, Burkholderiales, and Clostridiales was increased in diabetic mice, and that of Bacteroidales negatively correlated with the ileum GLP-1 receptor and neuronal nitric oxide synthase mRNA concentrations. Although the underlying mechanism by which gut microbiota dysbiosis induces GLP-1 resistance in the enteric nervous system remains unclear, this study suggests that the gut-brain axis plays a significant role in GLP-1-activated insulin secretion and gastric emptying.

In contrast, exercise therapy is essential for managing T2D^[5], and regular exercise offers benefits for cardiovascular, immunological, and neural systems^[6]. Reportedly, exercise increases the diversity of the gut microbiota and alters the composition of microbiota at the phylum, family, and genus levels in humans, and could regulate the immune and neural function of the gut^[7].

Studies have reported that moderate-intensity (50%-75% maximal oxygen uptake) and high-intensity (85%-90% maximal heart rate) acute exercise increases GLP-1 levels compared with controls in healthy and obese individuals^[8-11]. In addition, a 12-wk supervised chronic exercise program was reported to increase postprandial GLP-1 levels in overweight/obese individuals^[12]. Although the effect of light-intensity exercise on GLP-1 levels remains unclear, regular exercise appears to increase GLP-1 levels irrespective of its intensity. However, few studies have investigated the efficacy of exercise on GLP-1 levels in patients with T2D. Lee *et al*^[13] reported that a 12-wk high-intensity interval exercise training ($\geq 80\%$ heart rate reserve) elevated GLP-1 levels compared with the energy expenditure-matched low-intensity exercise in adolescents with T2D. Conversely, Eshghi *et al*^[14] demonstrated that moderate-intensity exercise (4.9 metabolic equivalents, 35 min) did not substantially increase the total GLP-1 levels

in patients with T2D, although metformin increased GLP-1 levels independent of exercise. A recent 16-wk, randomized, double-blind, placebo-controlled study reported that treatment using a GLP-1 receptor agonist, liraglutide, combined with exercise effectively improved glycemic control and resulted in weight loss^[15]. The exercise program comprised 60-min supervised training, including high-intensity interval training and whole-body resistance training, for three times per week. Although no changes in GLP-1 levels were observed, exercise potentiated the effect of the GLP-1 receptor agonist treatment. The combination of exercise and GLP-1 receptor agonists enhances both the β -cell function and the peripheral insulin sensitivity. In addition, short-term vigorous aerobic exercise (85% maximal heart rate) was reported to decrease fasting GLP-1 levels, whereas GLP-1 responses to glucose were considerably increased in individuals with nonalcoholic fatty liver disease^[16]. Although the impact of exercise on GLP-1 remains undetermined; Kullman *et al*^[16] suggested that exercise improves GLP-1 resistance rather than increase GLP-1 secretion in patients with T2D.

GLP-1 resistance is caused by excessive visceral fat^[17], as well as gut microbiota dysbiosis^[4]. In addition, physical inactivity is a potent risk factor for the accumulation of visceral fat, which is associated with systemic inflammation^[18]. The skeletal muscle is an endocrine organ and releases various myokines, including interleukin (IL)-6, IL-8, and IL-15. Contracting the skeletal muscle during exercise exerts anti-inflammatory effects by myokines and reduces ectopic fat^[18]. Heiskanen *et al*^[19] recently reported that both sprint interval and moderate-intensity continuous training decreased pancreatic fat and improved the β -cell function in subjects with prediabetes and T2D. Although the authors did not evaluate the incretin effect in this study, they speculated that exercise can improve the β -cell function to read potentiating incretins and neural signals.

To date, the efficacy of exercise on the GLP-1 secretion/function is only partially investigated in patients with T2D. However, exercise appears to potentiate the effect of the GLP-1 receptor agonists treatment by ameliorating GLP-1 resistance. The role played by gut microbiota in causing GLP-1 resistance is a focus of attention; however, to our knowledge, no study has investigated the effect of exercise on GLP-1 resistance in association with the alteration of the gut microbiota. Notably, the precise adjustment of various confounding factors, such as diet, medications, comorbidities, and genetic factor, is challenging in human studies. Exercise also increases microbiota-derived short chain fatty acids (SCFA)^[20] which have been shown to improve insulin sensitivity^[21]. Short chain fatty acids interact with specific G-protein coupled receptors (GPR41 and GPR43) on the intestinal L-cells^[22], and increase GLP-1 secretion^[23]. Exercise may improve GLP-1 secretion/function through the SCFA signaling mechanism. In addition, myokines are believed to play a vital role in mediating GLP-1 secretion/function during exercise. Although current

evidence is limited, a human study demonstrated that a GLP-1 receptor agonist, exenatide, treatment elevated irisin levels and enhanced the glycemic control in patients with T2D^[24]. Nevertheless, further research is warranted in the future.

REFERENCES

- 1 Chatterjee S, Ghosal S, Chatterjee S. Glucagon-like peptide-1 receptor agonists favorably address all components of metabolic syndrome. *World J Diabetes* 2016; **7**: 441-448 [PMID: 27795818 DOI: 10.4239/wjd.v7.i18.441]
- 2 Andersen A, Lund A, Knop FK, Vilsbøll T. Glucagon-like peptide 1 in health and disease. *Nat Rev Endocrinol* 2018; **14**: 390-403 [PMID: 29728598 DOI: 10.1038/s41574-018-0016-2]
- 3 Meijnikman AS, Gerdes VE, Nieuwdorp M, Herrema H. Evaluating Causality of Gut Microbiota in Obesity and Diabetes in Humans. *Endocr Rev* 2018; **39**: 133-153 [PMID: 29309555 DOI: 10.1210/er.2017-00192]
- 4 Grasset E, Puel A, Charpentier J, Collet X, Christensen JE, Tercé F, Burcelin R. A Specific Gut Microbiota Dysbiosis of Type 2 Diabetic Mice Induces GLP-1 Resistance through an Enteric NO-Dependent and Gut-Brain Axis Mechanism. *Cell Metab* 2017; **25**: 1075-1090.e5 [PMID: 28467926 DOI: 10.1016/j.cmet.2017.04.013]
- 5 Hamasaki H. Interval Exercise Therapy for Type 2 Diabetes. *Curr Diabetes Rev* 2018; **14**: 129-137 [PMID: 27804850 DOI: 10.2174/1573399812666161101103655]
- 6 Hawley JA, Hargreaves M, Joyner MJ, Zierath JR. Integrative biology of exercise. *Cell* 2014; **159**: 738-749 [PMID: 25417152 DOI: 10.1016/j.cell.2014.10.029]
- 7 Hamasaki H. Exercise and gut microbiota: clinical implications for the feasibility of Tai Chi. *J Integr Med* 2017; **15**: 270-281 [PMID: 28659231 DOI: 10.1016/S2095-4964(17)60342-X]
- 8 Ueda SY, Yoshikawa T, Katsura Y, Usui T, Fujimoto S. Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *J Endocrinol* 2009; **203**: 357-364 [PMID: 19737911 DOI: 10.1677/JOE-09-0190]
- 9 Ueda SY, Yoshikawa T, Katsura Y, Usui T, Nakao H, Fujimoto S. Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *J Endocrinol* 2009; **201**: 151-159 [PMID: 19158129 DOI: 10.1677/JOE-08-0500]
- 10 Holliday A, Blannin A. Appetite, food intake and gut hormone responses to intense aerobic exercise of different duration. *J Endocrinol* 2017; **235**: 193-205 [PMID: 28870887 DOI: 10.1530/JOE-16-0570]
- 11 Martins C, Stensvold D, Finlayson G, Holst J, Wisloff U, Kulseng B, Morgan L, King NA. Effect of moderate- and high-intensity acute exercise on appetite in obese individuals. *Med Sci Sports Exerc* 2015; **47**: 40-48 [PMID: 24824772 DOI: 10.1249/MSS.0000000000000372]
- 12 Martins C, Kulseng B, King NA, Holst JJ, Blundell JE. The effects of exercise-induced weight loss on appetite-related peptides and motivation to eat. *J Clin Endocrinol Metab* 2010; **95**: 1609-1616 [PMID: 20150577 DOI: 10.1210/jc.2009-2082]
- 13 Lee SS, Yoo JH, So YS. Effect of the low- versus high-intensity exercise training on endoplasmic reticulum stress and GLP-1 in adolescents with type 2 diabetes mellitus. *J Phys Ther Sci* 2015; **27**: 3063-3068 [PMID: 26644644 DOI: 10.1589/jpts.27.3063]
- 14 Eshghi SR, Bell GJ, Boulé NG. Effects of aerobic exercise with or without metformin on plasma incretins in type 2 diabetes. *Can J Diabetes* 2013; **37**: 375-380 [PMID: 24321717 DOI: 10.1016/j.cjcd.2013.07.030]
- 15 Mensberg P, Nyby S, Jørgensen PG, Storgaard H, Jensen MT, Sivertsen J, Holst JJ, Kiens B, Richter EA, Knop FK, Vilsbøll T. Near-normalization of glycaemic control with glucagon-like peptide-1 receptor agonist treatment combined with exercise in patients with type 2 diabetes. *Diabetes Obes Metab* 2017; **19**: 172-180 [PMID: 27717126 DOI: 10.1111/dom.12797]
- 16 Kullman EL, Kelly KR, Haus JM, Fealy CE, Scelsi AR, Pagadala MR, Flask CA, McCullough AJ, Kirwan JP. Short-term aerobic exercise training improves gut peptide regulation in nonalcoholic fatty liver disease. *J Appl Physiol* (1985) 2016; **120**: 1159-1164 [PMID: 27032902 DOI: 10.1152/japplphysiol.00693.2015]
- 17 Vendrell J, El Bekay R, Peral B, García-Fuentes E, Megia A, Macias-Gonzalez M, Fernández Real J, Jimenez-Gomez Y, Escoté X, Pachón G, Simó R, Selva DM, Malagón MM, Tinahones FJ. Study of the potential association of adipose tissue GLP-1 receptor with obesity and insulin resistance. *Endocrinology* 2011; **152**: 4072-4079 [PMID: 21862620 DOI: 10.1210/en.2011-1070]
- 18 Pedersen BK. The disease of physical inactivity--and the role of myokines in muscle-fat cross talk. *J Physiol* 2009; **587**: 5559-5568 [PMID: 19752112 DOI: 10.1113/jphysiol.2009.179515]
- 19 Heiskanen MA, Motiani KK, Mari A, Saunavaara V, Eskelinen JJ, Virtanen KA, Koivumäki M, Löytyniemi E, Nuutila P, Kalliokoski KK, Hannukainen JC. Exercise training decreases pancreatic fat content and improves beta cell function regardless of baseline glucose tolerance: a randomised controlled trial. *Diabetologia* 2018; **61**: 1817-1828 [PMID: 29717337 DOI: 10.1007/s00125-018-4627-x]
- 20 Allen JM, Mailing LJ, Niemi GM, Moore R, Cook MD, White BA, Holscher HD, Woods JA. Exercise Alters Gut Microbiota Composition and Function in Lean and Obese Humans. *Med Sci Sports Exerc* 2018; **50**: 747-757 [PMID: 29166320 DOI: 10.1249/MSS.0000000000001495]
- 21 Watterson KR, Hudson BD, Ulven T, Milligan G. Treatment of type 2 diabetes by free Fatty Acid receptor agonists. *Front Endocrinol (Lausanne)* 2014; **5**: 137 [PMID: 25221541 DOI: 10.3389/fendo.2014.00137]
- 22 Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol* 2014; **121**: 91-119 [PMID: 24388214 DOI: 10.1016/B978-0-12-800100-4.00003-9]
- 23 Christiansen CB, Gabe MBN, Svendsen B, Dragsted LO, Rosenkilde MM, Holst JJ. The impact of short chain fatty acids on GLP-1 and PYY secretion from the isolated perfused rat colon. *Am J Physiol Gastrointest Liver Physiol* 2018; **315**: G53-G65 [PMID: 29494208 DOI: 10.1152/ajpgi.00346.2017]
- 24 Liu J, Hu Y, Zhang H, Xu Y, Wang G. Exenatide treatment increases serum irisin levels in patients with obesity and newly diagnosed type 2 diabetes. *J Diabetes Complications* 2016; **30**: 1555-1559 [PMID: 27503404 DOI: 10.1016/j.jdiacomp.2016.07.020]

P- Reviewer: Das U, Fatima SS, Gómez-Sáez JM, Hill DJ, Navedo MF
S- Editor: Ji FF L- Editor: A E- Editor: Tan WW





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 September 15; 9(9): 141-164



**REVIEW**

- 141 Effects of antidiabetic drugs on epicardial fat
Xourgia E, Pazaifiropoulou A, Melidonis A

ORIGINAL ARTICLE**Basic Study**

- 149 Effects of glucagon-like peptide 1 analogs in combination with insulin on myocardial infarct size in rats with type 2 diabetes mellitus
Zykov VA, Tuchina TP, Lebedev DA, Krylova IB, Babenko AY, Kuleshova EV, Grineva EN, Bayramov AA, Galagudza MM

Case Control Study

- 157 Association of *TCF7L2* mutation and atypical diabetes in a Uruguayan population
Beloso C, Souto J, Fábregat M, Romanelli G, Javiel G, Mimbacas A

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Mark D Gorrell, BSc, PhD, Professor, Liver Enzymes in Metabolism and Inflammation Program, Centenary Institute and University of Sydney, Sydney 2006, NSW, Australia

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, and PubMed Central.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Shu-Yu Yin*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Ying Dou*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITOR-IN-CHIEF
Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com>

www.wjgnet.com/1948-9358/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE
September 15, 2018

COPYRIGHT
© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.f6publishing.com>

Effects of antidiabetic drugs on epicardial fat

Eleni Xourgia, Athanasia Papazafiropoulou, Andreas Melidonis

Eleni Xourgia, Athanasia Papazafiropoulou, Andreas Melidonis, 1st Department of Internal Medicine and Diabetes Center, Tzaneio General Hospital of Piraeus, Athens 18536, Greece

ORCID number: Eleni Xourgia (0000-0001-5766-3209); Athanasia Papazafiropoulou (0000-0002-7596-4942); Andreas Melidonis (0000-0003-0505-5708).

Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Athanasia Papazafiropoulou, MD, MSc, PhD, Attending Doctor, Research Scientist, 1st Department of Internal Medicine and Diabetes Center, Tzaneio General Hospital of Piraeus, 1 Zanni and Afentouli Street, Athens 18536, Greece. athan@ath.forthnet.gr
Telephone: +30-697-996483

Received: April 20, 2018

Peer-review started: April 21, 2018

First decision: June 8, 2018

Revised: June 19, 2018

Accepted: June 28, 2018

Article in press: June 28, 2018

Published online: September 15, 2018

Abstract

Epicardial adipose tissue is defined as a deposit of adipo-

cytes with pathophysiological properties similar to those of visceral fat, located in the space between the myocardial muscle and the pericardial sac. When compared with subcutaneous adipose tissue, visceral adipocytes show higher metabolic activity, lipolysis rates, increased insulin resistance along with more steroid hormone receptors. The epicardial adipose tissue interacts with numerous cardiovascular pathways *via* vasocrine and paracrine signalling comprised of pro- and anti-inflammatory cytokines excretion. Both the physiological differences between the two tissue types, as well as the fact that fat distribution and phenotype, rather than quantity, affect cardiovascular function and metabolic processes, establish epicardial fat as a biomarker for cardiovascular and metabolic syndrome. Numerous studies have underlined an association of altered epicardial fat morphology, type 2 diabetes mellitus (T2DM) and adverse cardiovascular events. In this review, we explore the prospect of using the epicardial adipose tissue as a therapeutic target in T2DM and describe the underlying mechanisms by which the antidiabetic drugs affect the pathophysiological processes induced from adipose tissue accumulation and possibly allow for more favourable cardiovascular outcomes though epicardial fat manipulation.

Key words: Epicardial fat; Adipose tissue; Type 2 diabetes mellitus; Antidiabetic drugs

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: In this review, we aim to create a concise overview of the pathophysiology concerning the epicardial fat deposits on a type 2 diabetic individual, while, delving into the intricacies of each antidiabetic drug and exploring the manner by which it interacts with visceral fat accumulation in the sub-pericardial space.

Xourgia E, Papazafiropoulou A, Melidonis A. Effects of antidiabetic drugs on epicardial fat. *World J Diabetes* 2018; 9(9): 141-148 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i9/141.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i9.141>

INTRODUCTION

Subcutaneous (SCAT) and visceral adipose tissue (VAT) are two extremely heterogeneous tissue types, differentiated by anatomical, molecular, cellular, physiological and clinical characteristics^[1]. Researchers have suggested that the variation of composition and function of the two tissue types is induced very early in the tissue developmental pathway, as a result of adipose stem cell distinction^[2]. VAT has an anatomically distinct distribution in the mesentery and omentum, when compared to SCAT that is mainly located in the femorogluteal area, back and abdominal wall^[1]. As a result of the anatomical differences, vascularization and innervation vary between the tissues, with VAT having superior nerve and vascular networks, as well as draining into the portal system of veins. Based on the aforementioned anatomical link, the "portal theory" of metabolic inflammation states that free fatty acids and pro-inflammatory molecules from VAT, interact with the liver, promoting hepatocellular dysfunction in the form of insulin resistance and steatosis^[3]. The dissimilarity in cellular composition between SCAT and VAT is a result of divergent ratio of large to small adipocytes between the two tissues. Large, metabolically dysfunctional, adipocytes, predominate in VAT, while SCAT is mainly composed by small adipocytes with higher free fatty acids and triglycerides capacity and increased insulin sensitivity^[4,5]. The signaling pathways activated in the two tissue types vary due to a shift in receptor distribution and adipokine synthesis^[1]. Glucocorticoid and androgen receptors present with a higher density in VAT while oestrogen receptors are more active in SCAT. Adrenergic signaling patterns are distinct for the two cell populations, with VAT being more β_3 - and α_2 - adrenoreceptor sensitive^[6]. The biologically active molecules produced by the adipose tissue, referred to as adipokines, are formed and released at different rates between VAT and SCAT. Adipokines are the basis of adipose tissue participating in and regulating endocrine and paracrine functions^[7]. The diversity of adipokines is directly linked to sympathetic excitation, metabolic regulation, including insulin sensitivity and appetite, inflammatory response and other homeostatic mechanisms. Some of the most prominent members of this family, as far as metabolic processes and cardiovascular function are examined, are: leptin, adiponectin, interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1) and tumor necrosis factor alpha (TNF- α)^[1,7]. Leptin levels are elevated in obese subjects, along with TNF- α , IL-6 and PAI-1 that are proatherogenic and prodiabetic, in contrast to plasma adiponectin that protects against vascular damage and metabolic syndrome and is reduced, as it would be expected^[8]. The variety in cytokine profile, along with the anatomic and cellular diversity that differentiate SCAT and VAT clarify and support the physiological and metabolic properties excreted by each adipocyte group. VAT cells allow for increased insulin-mediated glucose uptake and are more insulin-resistant and lipolysis-prone than those of SCAT. In contrast, the latter, exhibit a greater capacity for postprandial free fatty acid and triglyceride

uptake and storage^[1]. Taking into consideration the pivotal role of VAT in metabolic impairment, as such is supported by its aforementioned properties, it is comprehensible that studying the metabolic properties of visceral adiposity and mainly, organ-specific depositions, such as epicardial fat, has been incremental in the process of stratification of cardiometabolic risk factors. In this review, we aim to compare the morphology of epicardial fat deposits between non-diabetic individuals and subjects with type 2 diabetes mellitus (T2DM). Moreover, we will discuss the affect excreted by the antidiabetic substances in epicardial VAT, while contemplating on its clinical utility, as estimated by means of cardiovascular risk reduction.

FUNCTION AND COMPOSITION OF EPICARDIAL FAT

Epicardial adipose tissue (EAT) is an adipocyte depot of VAT with anatomical continuity to the myocardial tissue, located under the visceral layer of the pericardium^[9]. It has been suggested that it can serve as a quantifiable and modifiable therapeutic target for cardiovascular adverse events, as it can be measured with non-invasive imaging techniques such as two-dimensional echocardiography, computed tomography (CT) and magnetic resonance imaging (MRI)^[10]. Spatial imaging, as such is provided by MRI and CT scans, is preferable to that of two-dimensional echocardiography technique, in order to accurately measure the thickness of EAT. Along with technical shortcomings, operator- and subject- related variability deem echocardiographic imaging a formidable solution solely because of the rapid and cost-effective patient assessment it facilitates. Otherwise, MRI is considered to be the gold-standard method for EAT quantification and area placement, even though three dimensional image reconstruction by utilizing multidetector-row CT is slightly superior in achieving the latter^[10,11].

On a cellular level, the epicardial adipocytes are embryologically derived from the splanchnopleuric mesoderm, similarly to the mesenteric and omental adipocytes. EAT is characterized by high cellularity, defined by the concentration of adipocytes in this tissue being notably higher than that of other depots of adipose tissue^[12]. EAT is a depot of white adipocytes, cells that specialize in energy storage, as opposed to brown adipocytes that are involved in energy expenditure^[13].

EAT extends on an area exceeding 80% of the myocardial total surface in an otherwise healthy individual, spreading heterogeneously, mostly accumulating on the lateral and anterior walls of the right atrium^[14]. The physiological structure and composition of EAT varies depending on age, gender, body weight and ethnicity^[14]. The properties of EAT and its contribution in physiological and pathophysiological pathways have been extensively described. Due to its spatial distribution, EAT acts as a mechanical and thermoprotective layer for the myocardial tissue and coronary arteries. Through endocrine and paracrine function, epicardial adipocytes

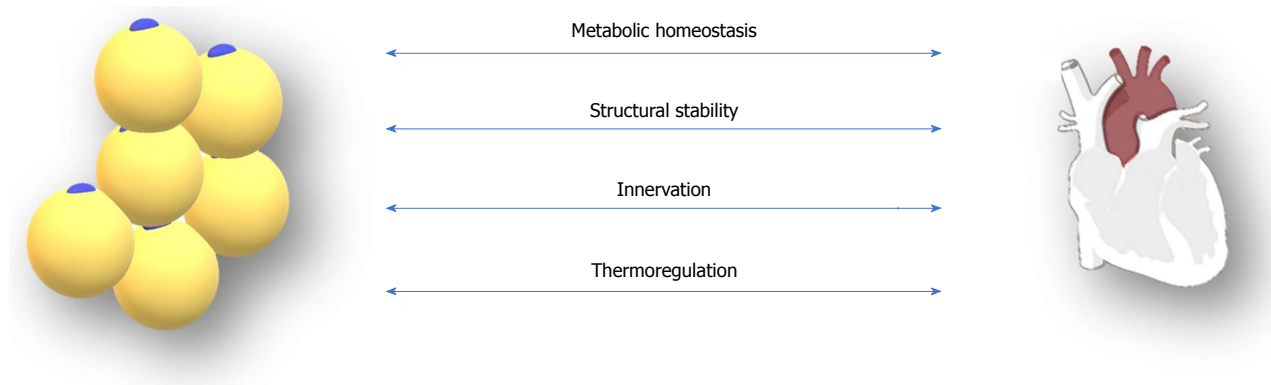


Figure 1 Mechanisms involved in the crossplay between the heart and the epicardial adipocytes.

ameliorate endothelial response of the coronaries and insulin sensitivity, while reducing oxidative stress of the cardiac tissue. Additionally, the small adipocytes of EAT are characterized by high rates of free fatty acids turnover, allowing for both energy supply and storage as demand shifts^[14].

EPICARDIAL FAT, TYPE 2 DIABETES MELLITUS AND CORONARY ARTERY DISEASE

The prevalence of type 2 diabetes mellitus (T2DM) has quadrupled in the last three decades according to International Diabetes Federation (IDF) reports. The epidemic escalation has been attributed to numerous factors including population aging as a result of improved healthcare, socioeconomic development, unhealthy diet regimes and sedentary lifestyle^[15].

EAT has been associated with numerous pathophysiological processes, such as coronary artery disease^[16-18], even though the significance of such association has not been adequately supported by all relevant studies^[19-20], electrophysiological abnormalities of the heart^[21,22], cardiovascular disease in human immunodeficiency virus treated with antiretroviral therapy^[23], amplified severity of non-alcoholic fatty liver disease^[24,25], metabolic syndrome^[26-29] and increased cardiovascular risk along with decline of renal function in individuals with T2DM^[30-34].

The pathophysiological pathways linking T2DM and EAT, support a multifactorial causative relationship between EAT attributes and structure such as volume and endocrine function and cardiovascular disease severity in the diabetic individual.

EAT deposition can be associated with coronary vascular disease pathogenesis mainly by the dysregulation of cardiac metabolic processes and the disruption of the epicardial and myocardial structural integrity. Other mechanisms that could be involved in the interaction between EAT and coronary vasculature are nerve damage and impaired cryoprotection of the heart^[35,36]. Furthermore, the epicardial adipocytes exhibit an arrhythmogenic potential, a theory suggested by many clinical trials ex-

ploring the causative relationship between EAT and atrial fibrillation^[21] (Figure 1).

EPICARDIAL FAT AND ANTIDIABETIC DRUGS

Biguanides

Metformin is the most common first-line treatment choice for T2DM and a member of the biguanides drug class. Oral administration of the substance affects the liver and gut metabolic pathways in order for its hypoglycemic attributes to be put into effect^[37]. Hepatic gluconeogenesis, glucose uptake, glycolysis and glucogen synthesis are some of the processes altered by metformin *via* AMP-activated protein kinase (AMPK)-dependent and -independent pathways^[38].

At this point in time, there seem to be no randomized controlled trials designed for clarification of the effects exerted by metformin on the volume or function of EAT. Despite the fact that metformin has not been compared with placebo, as of yet, studies conducted on sitagliptin and liraglutide as add-on therapy to metformin monotherapy, combined with epicardial fat measurement, can be used as a preliminary source of data^[39,40].

Results from these trials confirm the inferiority of metformin monotherapy when compared to metformin/sitagliptin and metformin/liraglutide for reduction of EAT volume. The findings can be either attributed to the synergy of two antidiabetic substances, affecting the EAT in a more effective manner than metformin alone, or to the complete lack of action of the biguanide class on the cardiac VAT deposits. The latter is supported by the results of the study performed by Iacobellis *et al.*^[40], that noted no EAT reduction in the metformin group during the 6-mo follow up period. Conversely, metformin has been previously shown to have positive effects on VAT, inducing its reduction on diabetic subjects^[41]. Furthermore, studies have confirmed a metformin-induced increase of plasma omentin-1 levels, an adipokine produced by epicardial fat that ameliorates insulin sensitivity, inflammatory response and cardiovascular function^[42]. Given the contradicting evidence concerning metformin, there is need for further research, as a definite conclusion on the manner by which biguanides interact with epicardial fat can only be provided

by a randomized controlled trial with EAT measurement.

Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors (α -GIs) are a class of anti-diabetic drugs acting in the epithelium of the small intestine mainly by delaying the digestion of carbohydrates through reversible and competitive inhibition of intestinal alpha-glucosidases, consequently reducing glucose absorption and attenuating postprandial hyperglycemia^[43]. Some α -GIs are acarbose, miglitol and voglibose. Similarly to the biguanide class of antidiabetic medication, there is a lack of data concerning the administration of α -GIs and their effect on EAT mass, volume or metabolic activity.

Thiazolidinediones

Thiazolidinediones (TZDs), also known as glitazones, are peroxisome proliferator-activated receptor (PPAR) agonists with numerous actions, spanning from glycemic and lipid control to inflammatory signaling and cell cycle mediation^[44]. The phenomenon of glitazone treatment and subsequent increase in body weight that has been supported by the results of numerous studies appears to be tissue-specific, since the VAT depot of the subjects remains unaffected while there is a shift of excess energy storage towards the SCAT^[45-47].

Furthermore, pioglitazone treatment in T2DM or metabolic syndrome has been shown to attenuate the inflammatory signature of EAT by means of decreased expression of proinflammatory interleukins (IL) such as IL-1 β , IL-1Ra and IL-10^[48]. In addition to the positive effect on the metabolic profile of EAT, pioglitazone can affect the epicardial fat depot directly. Nagai *et al.*^[49] recruited 97 T2DM individuals that were divided into two groups according to baseline EAT thickness and underwent therapy with pioglitazone, along with EAT thickness measurement, at the beginning and after a nine-month follow-up period. Pioglitazone reduced the EAT thickness in both groups, with more prominent results in the subjects that had a greater EAT depot at baseline.

A different TZD, rosiglitazone, when administered to mice, induced the expression of brown adipose tissue-specific proteins by the EAT, a tissue type normally presenting having a hormonal profile consistent with that of white adipose tissue^[50]. Brown adipose tissue has been linked to high rates of lipid turnover and reduced body weight, while it is essential for thermogenesis and homeostasis, in contrast to white adipose tissue that serves as an energy reservoir for the body^[51].

The data derived from the studies examining the effect of glitazones and EAT correlates with the established theory that TZD-induced weight gain is not concurrent with VAT deposition. Moreover, TZDs appear to have a favorable effect on EAT both by regulation of endocrine functions and mass reduction.

Incretins

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that delays gastric motility, suppresses appetite, stimulates

glucose-dependent insulin and decreases glucagon secretion^[52]. The enzyme dipeptidyl peptidase-4 (DPP-4) deactivates GLP-1 interrupting all incretin-stimulated signalling. DPP-4 inhibitors (DPP-4i) are one of the two categories of antidiabetic drugs acting on the incretin pathway, the other being GLP-1 receptor agonists (GLP-1 RA)^[52]. DPP-4i inhibit both GLP-1 and glucose-dependent insulintropic polypeptide (GIP) degradation, thereby increasing plasma concentrations and stimulating the pancreatic β -cell in order to better regulate glucose homeostasis.

DPP-4 inhibitors

The class of DPP-4is includes sitagliptin, vildagliptin, saxagliptin, linagliptin and alogliptin^[53]. Sitagliptin is the only DPP-4i whose effect on epicardial fat has been studied at this point in time. Lima-Martínez *et al.*^[39] formed a 24-wk interventional plan for 26 obese subjects with T2DM inadequately controlled on metformin monotherapy. Subjects meeting the inclusion criteria were introduced to a new regimen, receiving sitagliptin/metformin at a dosage of 50 mg/1000 mg respectively, twice a day. EAT deposits were reduced in size by approximately 15% (from 9.98 ± 2.63 to 8.10 ± 2.11 mm, $P = 0.001$) while the percentage of reduction in EAT was analogous to that of VAT ($r = 0.456$, $P = 0.01$).

While the aforementioned study establishes a favourable effect of sitagliptin on the mass of epicardial VAT, there is definite need for further research, in order to establish the reduction of EAT as a class effect of DPP-4is^[39].

GLP-1 receptor agonists

GLP-1 RAs utilize the "incretin effect", similarly to DPP-4 inhibitors, so as to attenuate the diabetes-induced hyperglycemia. GLP-1 RAs are divided into short- and long-acting compounds that activate the GLP-1 receptor in a manner similar to that of the endogenous GLP-1^[54]. Epicardial adipocytes have been shown to express GLP-1 and 2 receptor genes by use of RNA sequencing, while the possible quantity and dispersion pattern of the receptors in vivo has not been described^[55]. Furthermore, GLP-1 and GLP-1 receptor signaling affect the differentiation and growth of adipocytes by regulation of fatty acid synthase activity^[56]. Even though, the effects of numerous GLP-1 RAs have been studied in correlation to the metabolic regulation or mass reduction of visceral adipose tissue, the clinical trials concerning organ-specific deposits are few^[40,57-60]. Current data on EAT remodeling by GLP-1 RAs is derived by two studies, conducted with liraglutide and exenatide^[40,60].

A trial designed by Iacobellis *et al.*^[40] included 95 T2DM obese subjects with hemoglobin A1c $\leq 8\%$ while being treated with metformin. The patients were randomized into two groups to either receive a combination of metformin/liraglutide, with the latter being administered once daily, in doses up to 1.8 mg, or stay on metformin monotherapy, up to 1000 mg administered twice daily

for 6 mo. EAT thickness measurements were acquired by ultrasound imaging at baseline and at 3 and 6 mo. Subjects in the liraglutide group presented with a decline in EAT thickness, 29% and 36% reduction from baseline at 3 and 6 mo respectively. Given that there were no similar changes in the metformin group, the EAT mass reduction is considered to be an effect of the liraglutide treatment, or possibly a result of the synergy between the two antidiabetic substances.

The study involving exenatide had a broader spectrum than that of liraglutide, examining the effect of the GLP-1RA on numerous VAT depots including epicardial, myocardial, hepatic and pancreatic adipose pads. Measurements of EAT thickness were performed by magnetic resonance imaging and spectroscopy at baseline and at 26 wk. A total of 44 obese individuals with uncontrolled T2DM, originally receiving oral therapy, were randomized to two groups, either receiving exenatide or other treatment chosen according to the local guidelines. EAT was reduced by approximately 8.8% after treatment with exenatide and by 1.2% on the patients receiving oral therapy, with the difference between the two being statistically significant ($P = 0.003$)^[60].

Current research conducted on incretin treatment and ectopic adipose tissue deposition supports the theory that EAT reduction could be a class effect of GLP-1RAs and possibly a mediator of their beneficial actions on cardiovascular disease in the diabetic and obese subjects.

SGLT-2 inhibitors

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are a novel class of antidiabetic substances that bind on the SGLT2 transporter in the proximal tubule of the kidney, facilitating glucose excretion *via* hindering reabsorption. SGLT2-mediated reabsorption constitutes the main pathway by which the renal system maintains glucose homeostasis^[61]. Administration of SGLT2 inhibitors in obese individuals with T2DM has been linked with abdominal VAT size reduction^[62]. Additionally, the effects of SGLT2 inhibition on tissue-specific depots such as EAT have been clarified by studies performed on luseogliflozin, ipragliflozin, canagliflozin and dapagliflozin^[63-67].

EAT measurements following a 12-wk period of luseogliflozin administration demonstrate that treatment with luseogliflozin can reduce EAT volume in combination with adipocyte-related inflammation and metabolic dysregulation on type 2 diabetic patients. Along with EAT, numerous parameters were modified after luseogliflozin therapy including body weight, fasting plasma glucose, insulin resistance and C-reactive protein (CRP) levels. A positive correlation was established between CRP and EAT reduction ($r = 0.493$, $P = 0.019$), suggesting a concurrent effect of the SGLT2 inhibitor on both the adipose tissue mass and metabolic activity^[63].

Similar results concerning both EAT and biomarkers reduction were acquired after ipragliflozin administration, in a study designed similarly to that conducted for luseogliflozin. The two models differed in the selection

of the study population, with luseogliflozin treatment being applied to obese subjects while ipragliflozin was administered to non-obese T2DM individuals^[64].

Yagi *et al.*^[65] studied the interaction of canagliflozin and EAT during a 6-mo period of treatment. The sample consisted of type 2 diabetic individuals, each of which was administered 100 mg of canagliflozin once daily. During the follow-up period EAT was evaluated by echocardiographic imaging while VAT and SCAT size fluctuation was monitored by use of impedance methods. The mean EAT thickness values were 9.3 mm and 7.3 mm at baseline and at 6 mo, respectively, with the change observed being statistically significant ($P < 0.001$) while there was only a trend for VAT and SCAT reduction.

Dapagliflozin and epicardial adiposity were examined through two different clinical trials, studying both the shift in metabolic activity and size of the adipocytes after treatment^[66,67]. The metabolic profile of adipocytes promoted by dapagliflozin was assessed *ex vivo* on fat explants obtained from patients undergoing cardiac surgery on a trial designed by Díaz-Rodríguez *et al.*^[66]. Glucose uptake, transporter expression and adipokine secretion patterns were altered as a result of dapagliflozin application, a change indicative of a positive metabolic reform of the tissue induced by SGLT2 inhibition. Simultaneously, Sato *et al.*^[67] followed a more conventional approach, estimating the dapagliflozin-induced EAT volume reduction, by means of computed tomography imaging. Individuals receiving both dapagliflozin and other regimens for T2DM control were observed for 6 mo, with biomarker and EAT measurement at baseline and following completion of the study. While the two groups had similar EAT size measurement before the initiation of dapagliflozin therapy, the patients receiving the SGLT2 inhibitor presented with a greater reduction of epicardial VAT volume after treatment (-16.4 ± 8.3 for the dapagliflozin vs 4.7 ± 8.8 cm³ for the control group, $P = 0.01$), combined with lowered plasma levels of inflammatory adipokines.

Numerous studies conducted on many members of the SGLT2 inhibitor class of antidiabetic substances support the conclusion that EAT undergoes a multifaceted remodelling after SGLT2 inhibition, a trend that could be considered a class effect. The interconnection established between SGLT2 inhibitors and a known factor of cardiovascular risk such as epicardial adiposity could elucidate the manner by which the members of this class are cardioprotective, while, providing grounds for further therapeutic targeting of EAT (Figure 2).

CONCLUSION

Epicardial adipose tissue exhibits a unique metabolic and pathophysiologic profile, as a result of its anatomical location and its cellular composition, rendering it an appealing therapeutic target for reducing cardiovascular risk and enabling endocrine homeostasis in the dys-metabolic individual. The recent studies concerning the effect of the antidiabetic substances on the multifactorial

Biguanides	No effect/Possible synergistic effect with DPP-4 and/or GLP-1 ^[39, 40]
Alpha-Glucosidase Inhibitors	Lack of data concerning the effect of this class
Thiazolidinediones	Decreased inflammatory cytokine release and thickness of EAT (pioglitazone) modulation of cellular hormonal profile (rosiglitazone) ^[49, 50]
Dipeptidyl peptidase-4 inhibitors	Reduction of EAT thickness (sitagliptin) ^[39]
Glucagon-like peptide-1 receptor agonists	Reduction of EAT thickness (liraglutide and exenatide) ^[40, 60]
Sodium-glucose cotransporter 2 inhibitors	Reduction of EAT thickness (luseogliflozin, ipragliflozin, canagliflozin, dapagliflozin) and inflammation (luseogliflozin, ipragliflozin, dapagliflozin) ^[63-67]

Figure 2 Antidiabetic drug and their effect on epicardial adipose tissue.

cardiomyopathy of the diabetic patient and, by extension, on epicardial adiposity, have yielded interesting results that support the use of treatment for a targeted approach, in order to reduce the size and metabolic activity of ectopic adipose tissue clusters. Despite the capacity of certain treatment regimens, mostly newer agents like GLP-1 agonists and SGLT-2 inhibitors, in the manipulation of both structural and functional parameters of the epicardial adipose tissue, the clinical efficacy of this approach remains unsubstantiated for the time being. There is definite need for further research, in order to elucidate whether the targeting of epicardial adiposity facilitates the procurement of better outcomes for individuals with diabetes and cardiovascular disease, while, additionally, clarify the manner by which the antidiabetic substances can attain such results.

REFERENCES

- Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. *Obes Rev* 2010; **11**: 11-18 [PMID: 19656312 DOI: 10.1111/j.1467-789X.2009.00623.x]
- Baglioni S, Cantini G, Poli G, Francalanci M, Squecco R, Di Franco A, Borgogni E, Frontera S, Nesi G, Liotta F, Lucchese M, Perigli G, Francini F, Forti G, Serio M, Luconi M. Functional differences in visceral and subcutaneous fat pads originate from differences in the adipose stem cell. *PLoS One* 2012; **7**: e36569 [PMID: 22574183 DOI: 10.1371/journal.pone.0036569]
- Item F, Konrad D. Visceral fat and metabolic inflammation: the portal theory revisited. *Obes Rev* 2012; **13** Suppl 2: 30-39 [PMID: 23107257 DOI: 10.1111/j.1467-789X.2012.01035.x]
- Fang L, Guo F, Zhou L, Stahl R, Grams J. The cell size and distribution of adipocytes from subcutaneous and visceral fat is associated with type 2 diabetes mellitus in humans. *Adipocyte* 2015; **4**: 273-279 [PMID: 26451283 DOI: 10.1080/21623945.2015.1034920]
- Poret JM, Souza-Smith F, Marcell SJ, Gaudet DA, Tzeng TH, Braymer HD, Harrison-Bernard LM, Primeaux SD. High fat diet consumption differentially affects adipose tissue inflammation and adipocyte size in obesity-prone and obesity-resistant rats. *Int J Obes (Lond)* 2018; **42**: 535-541 [PMID: 29151595 DOI: 10.1038/s41371.2017.280]
- La Fontaine MF, Cirmigliaro CM, Kirshblum SC, McKenna C, Bauman WA. Effect of functional sympathetic nervous system impairment of the liver and abdominal visceral adipose tissue on circulating triglyceride-rich lipoproteins. *PLoS One* 2017; **12**: e0173934 [PMID: 28346471 DOI: 10.1371/journal.pone.0173934]
- Raucci R, Rusolo F, Sharma A, Colonna G, Castello G, Costantini S. Functional and structural features of adipokine family. *Cytokine* 2013; **61**: 1-14 [PMID: 23022179 DOI: 10.1016/j.cyt.2012.08.036]
- Aguiar-Valles A, Inoue W, Rummel C, Luheshi GN. Obesity, adipokines and neuroinflammation. *Neuropharmacology* 2015; **96**: 124-134 [PMID: 25582291 DOI: 10.1016/j.neuropharm.2014.12.023]
- Iacobellis G. Epicardial fat: a new cardiovascular therapeutic target. *Curr Opin Pharmacol* 2016; **27**: 13-18 [PMID: 26848943 DOI: 10.1016/j.coph.2016.01.004]
- Bertaso AG, Bertol D, Duncan BB, Foppa M. Epicardial fat: definition, measurements and systematic review of main outcomes. *Arq Bras Cardiol* 2013; **101**: e18-e28 [PMID: 23917514 DOI: 10.5935/abc.20130138]
- Gulgun M, Genç FA. Measurement of Epicardial Fat Thickness by Echocardiography Presents Challenges *Arq Bras Cardiol* 2016; **107**: 497-498 [PMID: 27982277 DOI: 10.5935/abc.20160167]
- Ouwens DM, Sell H, Greulich S, Eckel J. The role of epicardial and perivascular adipose tissue in the pathophysiology of cardiovascular disease. *J Cell Mol Med* 2010; **14**: 2223-2234 [PMID: 20716126 DOI: 10.1111/j.1582-4934.2010.01141.x]
- Giralt M, Villarroya F. White, brown, beige/brite: different adipose cells for different functions? *Endocrinology* 2013; **154**: 2992-3000 [PMID: 23782940 DOI: 10.1210/en.2013-1403]
- Nagy E, Jermendy AL, Merkely B, Maurovich-Horvat P. Clinical importance of epicardial adipose tissue. *Arch Med Sci* 2017; **13**: 864-874 [PMID: 28721155 DOI: 10.5114/aoms.2016.63259]
- Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* 2018; **14**: 88-98 [PMID: 29219149 DOI: 10.1038/nrendo.2017.151]
- Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, Vasan RS, O'Donnell CJ, Fox CS. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. *Circulation* 2008; **117**: 605-613 [PMID: 18212276 DOI: 10.1161/CIRCULATIONAHA.107.743062]
- Sato F, Maeda N, Yamada T, Namazui H, Fukuda S, Natsukawa T, Nagao H, Murai J, Masuda S, Tanaka Y, Obata Y, Fujishima Y, Nishizawa H, Funahashi T, Shimomura I. Association of Epicardial, Visceral, and Subcutaneous Fat With Cardiometabolic Diseases. *Circ J* 2018; **82**: 502-508 [PMID: 28954947 DOI: 10.1253/circj.CJ-17-0820]
- McKenney-Drake ML, Rodenbeck SD, Bruning RS, Kole A, Yancey KW, Alloosh M, Sacks HS, Sturek M. Epicardial Adipose Tissue Removal Potentiates Outward Remodeling and Arrests Coronary Atherogenesis. *Ann Thorac Surg* 2017; **103**: 1622-1630 [PMID: 28223054 DOI: 10.1016/j.athoracsur.2016.11.034]
- Wu Y, Zhang A, Hamilton DJ, Deng T. Epicardial Fat in the Maintenance of Cardiovascular Health. *Methodist Debakey Cardiovasc J* 2017; **13**: 20-24 [PMID: 28413578 DOI: 10.14797/mdcj-13-1-20]
- Nelson MR, Mookadam F, Thota V, Emani U, Al Harthi M, Lester SJ, Cha S, Stepanek J, Hurst RT. Epicardial fat: an additional measurement for subclinical atherosclerosis and cardiovascular risk stratification? *J Am Soc Echocardiogr* 2011; **24**: 339-345 [PMID: 21185148 DOI: 10.1016/j.echo.2010.11.008]
- Wong CX, Abed HS, Molae P, Nelson AJ, Brooks AG, Sharma G, Leong DP, Lau DH, Middeldorp ME, Roberts-Thomson KC, Wittert GA, Abhayaratna WP, Worthley SG, Sanders P. Pericardial fat is associated with atrial fibrillation severity and ablation outcome. *J Am Coll Cardiol* 2011; **57**: 1745-1751 [PMID: 21511110 DOI: 10.1016/j.jacc.2010.11.045]
- Thanassoulis G, Massaro JM, O'Donnell CJ, Hoffmann U, Levy D, Ellinor PT, Wang TJ, Schnabel RB, Vasan RS, Fox CS, Benjamin EJ. Pericardial fat is associated with prevalent atrial fibrillation: the

- Framingham Heart Study. *Circ Arrhythm Electrophysiol* 2010; **3**: 345-350 [PMID: 20558845 DOI: 10.1161/CIRCEP.109.912055]
- 23 **Guaraldi G**, Scaglioni R, Zona S, Orlando G, Carli F, Ligabue G, Besutti G, Bagni P, Rossi R, Modena MG, Raggi P. Epicardial adipose tissue is an independent marker of cardiovascular risk in HIV-infected patients. *AIDS* 2011; **25**: 1199-1205 [PMID: 21505301 DOI: 10.1097/QAD.0b013e3283474b9f]
 - 24 **Petta S**, Argano C, Colomba D, Cammà C, Di Marco V, Cabibi D, Tuttolomondo A, Marchesini G, Pinto A, Licata G, Craxi A. Epicardial fat, cardiac geometry and cardiac function in patients with non-alcoholic fatty liver disease: association with the severity of liver disease. *J Hepatol* 2015; **62**: 928-933 [PMID: 25445395 DOI: 10.1016/j.jhep.2014.11.030]
 - 25 **Brouha SS**, Nguyen P, Bettencourt R, Sirlin CB, Loomba R. Increased severity of liver fat content and liver fibrosis in non-alcoholic fatty liver disease correlate with epicardial fat volume in type 2 diabetes: A prospective study. *Eur Radiol* 2018; **28**: 1345-1355 [PMID: 29058029 DOI: 10.1007/s00330-017-5075-6]
 - 26 **Calabuig Á**, Barba J, Guembe MJ, Díez J, Berjón J, Martínez-Vila E, Irimia P, Toledo E. Epicardial Adipose Tissue in the General Middle-aged Population and Its Association With Metabolic Syndrome. *Rev Esp Cardiol (Engl Ed)* 2017; **70**: 254-260 [PMID: 27888013 DOI: 10.1016/j.rec.2016.08.001]
 - 27 **Pierdomenico SD**, Pierdomenico AM, Cuccurullo F, Iacobellis G. Meta-analysis of the relation of echocardiographic epicardial adipose tissue thickness and the metabolic syndrome. *Am J Cardiol* 2013; **111**: 73-78 [PMID: 23040591 DOI: 10.1016/j.amjcard.2012.08.044]
 - 28 **Fernández Muñoz MJ**, Basurto Acevedo L, Córdova Pérez N, Vázquez Martínez AL, Tepach Gutiérrez N, Vega García S, Rocha Cruz A, Díaz Martínez A, Saucedo García R, Zárate Treviño A, González Escudero EA, Degollado Córdova JA. Epicardial adipose tissue is associated with visceral fat, metabolic syndrome, and insulin resistance in menopausal women. *Rev Esp Cardiol (Engl Ed)* 2014; **67**: 436-441 [PMID: 24863591 DOI: 10.1016/j.rec.2013.10.011]
 - 29 **Rabkin SW**. The relationship between epicardial fat and indices of obesity and the metabolic syndrome: a systematic review and meta-analysis. *Metab Syndr Relat Disord* 2014; **12**: 31-42 [PMID: 24219127 DOI: 10.1089/met.2013.0107]
 - 30 **Blumensatt M**, Fahlbusch P, Hilgers R, Bekaert M, Herzfeld de Wiza D, Akhyari P, Ruige JB, Ouwens DM. Secretory products from epicardial adipose tissue from patients with type 2 diabetes impair mitochondrial β -oxidation in cardiomyocytes via activation of the cardiac renin-angiotensin system and induction of miR-208a. *Basic Res Cardiol* 2017; **112**: 2 [PMID: 27864612 DOI: 10.1007/s00395-016-0591-0]
 - 31 **Homsí R**, Sprinkart AM, Gieseke J, Meier-Schroers M, Yucel S, Fischer S, Nadal J, Dabir D, Luetkens JA, Kuetting DL, Schild HH, Thomas DK. Cardiac magnetic resonance based evaluation of aortic stiffness and epicardial fat volume in patients with hypertension, diabetes mellitus, and myocardial infarction. *Acta Radiol* 2018; **59**: 65-71 [PMID: 28440670 DOI: 10.1177/0284185117706201]
 - 32 **Cernea S**, Blendea C, Roiban AL, Benedek T. Cardio-renal Correlations and Epicardial Adipose Tissue in Patients with Type 2 Diabetes. *J Interdiscip Med* 2017 [DOI: 10.1515/jim-2017-0085]
 - 33 **Uygur B**, Celik O, Ozturk D, Erturk M, Otcu H, Ustabasioglu FE, Yildirim A. The relationship between location-specific epicardial adipose tissue volume and coronary atherosclerotic plaque burden in type 2 diabetic patients. *Kardiol Pol* 2017; **75**: 204-212 [PMID: 27958614 DOI: 10.5603/KP.a2016.0167]
 - 34 **Wang Z**, Zhang Y, Liu W, Su B. Evaluation of Epicardial Adipose Tissue in Patients of Type 2 Diabetes Mellitus by Echocardiography and its Correlation with Intimal Medial Thickness of Carotid Artery. *Exp Clin Endocrinol Diabetes* 2017; **125**: 598-602 [PMID: 28494499 DOI: 10.1055/s-0042-114035]
 - 35 **Antonopoulos AS**, Antoniadou C. The role of epicardial adipose tissue in cardiac biology: classic concepts and emerging roles. *J Physiol* 2017; **595**: 3907-3917 [PMID: 28191635 DOI: 10.1111/JP273049]
 - 36 **Psychari SN**, Rekleiti N, Papaioannou N, Varhalama E, Drakoulis C, Apostolou TS, Iliodromitis EK. Epicardial Fat in Nonalcoholic Fatty Liver Disease: Properties and Relationships With Metabolic Factors, Cardiac Structure, and Cardiac Function. *Angiology* 2016; **67**: 41-48 [PMID: 25818101 DOI: 10.1177/0003319715576672]
 - 37 **Song R**. Mechanism of Metformin: A Tale of Two Sites. *Diabetes Care* 2016; **39**: 187-189 [PMID: 26798149 DOI: 10.2337/dci15-0013]
 - 38 **Rena G**, Hardie DG, Pearson ER. The mechanisms of action of metformin. *Diabetologia* 2017; **60**: 1577-1585 [PMID: 28776086 DOI: 10.1007/s00125-017-4342-z]
 - 39 **Lima-Martínez MM**, Paoli M, Rodney M, Balladares N, Contreras M, D'Marco L, Iacobellis G. Effect of sitagliptin on epicardial fat thickness in subjects with type 2 diabetes and obesity: a pilot study. *Endocrine* 2016; **51**: 448-455 [PMID: 26233684 DOI: 10.1007/s12020-015-0710-y]
 - 40 **Iacobellis G**, Mohseni M, Bianco SD, Banga PK. Liraglutide causes large and rapid epicardial fat reduction. *Obesity (Silver Spring)* 2017; **25**: 311-316 [PMID: 28124506 DOI: 10.1002/oby.21718]
 - 41 **Tokubuchi I**, Tajiri Y, Iwata S, Hara K, Wada N, Hashinaga T, Nakayama H, Mifune H, Yamada K. Beneficial effects of metformin on energy metabolism and visceral fat volume through a possible mechanism of fatty acid oxidation in human subjects and rats. *PLoS One* 2017; **12**: e0171293 [PMID: 28158227 DOI: 10.1371/journal.pone.0171293]
 - 42 **Watanabe T**, Watanabe-Kominato K, Takahashi Y, Kojima M, Watanabe R. Adipose Tissue-Derived Omentin-1 Function and Regulation. *Compr Physiol* 2017; **7**: 765-781 [PMID: 28640441 DOI: 10.1002/cphy.c160043]
 - 43 **Bischoff H**. The mechanism of alpha-glucosidase inhibition in the management of diabetes. *Clin Invest Med* 1995; **18**: 303-311 [PMID: 8549017]
 - 44 **Davidson MA**, Mattison DR, Azoulay L, Krewski D. Thiazolidinedione drugs in the treatment of type 2 diabetes mellitus: past, present and future. *Crit Rev Toxicol* 2018; **48**: 52-108 [PMID: 28816105 DOI: 10.1080/10408444.2017.1351420]
 - 45 **Blanchard PG**, Turcotte V, Côté M, Gélinas Y, Nilsson S, Olivecrona G, Deshaies Y, Festuccia WT. Peroxisome proliferator-activated receptor γ activation favours selective subcutaneous lipid deposition by coordinately regulating lipoprotein lipase modulators, fatty acid transporters and lipogenic enzymes. *Acta Physiol (Oxf)* 2016; **217**: 227-239 [PMID: 26918671 DOI: 10.1111/apha.12665]
 - 46 **Aghamohammadzadeh N**, Niafar M, Dalir Abdollahinia E, Najafipour F, Mohammadzadeh Gharebaghi S, Adabi K, Dalir Abdollahinia E, Ahadi H. The effect of pioglitazone on weight, lipid profile and liver enzymes in type 2 diabetic patients. *Ther Adv Endocrinol Metab* 2015; **6**: 56-60 [PMID: 25941563 DOI: 10.1177/2042018815574229]
 - 47 **Fonseca V**. Effect of thiazolidinediones on body weight in patients with diabetes mellitus. *Am J Med* 2003; **115** Suppl 8A: 42S-48S [PMID: 14678865 DOI: 10.1016/j.amjmed.2003.09.005]
 - 48 **Sacks HS**, Fain JN, Cheema P, Bahouth SW, Garrett E, Wolf RY, Wolford D, Samaha J. Inflammatory genes in epicardial fat contiguous with coronary atherosclerosis in the metabolic syndrome and type 2 diabetes: changes associated with pioglitazone. *Diabetes Care* 2011; **34**: 730-733 [PMID: 21289232 DOI: 10.2337/dci10-2083]
 - 49 **Nagai H**, Ito H, Iwakura K. Abstract 710: Pioglitazone Treatment Reduces Epicardial Fat in Patients with Type 2 Diabetes Mellitus and Improves Left Ventricular Diastolic Function. *Circulation* 2008; **118**
 - 50 **Distel E**, Penot G, Cadoudal T, Balguy I, Durant S, Benelli C. Early induction of a brown-like phenotype by rosiglitazone in the epicardial adipose tissue of fatty Zucker rats. *Biochimie* 2012; **94**: 1660-1667 [PMID: 22575275 DOI: 10.1016/j.biochi.2012.04.014]
 - 51 **Salazar J**, Luzardo E, Mejías JC, Rojas J, Ferreira A, Rivas-Ríos JR, Bermúdez V. Epicardial Fat: Physiological, Pathological, and Therapeutic Implications. *Cardiol Res Pract* 2016; **2016**: 1291537 [PMID: 27213076 DOI: 10.1155/2016/1291537]
 - 52 **Vella A**. Mechanism of action of DPP-4 inhibitors--new insights. *J Clin Endocrinol Metab* 2012; **97**: 2626-2628 [PMID: 22869847 DOI: 10.1210/jc.2012-2396]

- 53 **Dicker D.** DPP-4 inhibitors: impact on glycemic control and cardiovascular risk factors. *Diabetes Care* 2011; **34** Suppl 2: S276-S278 [PMID: 21525468 DOI: 10.2337/dc11-s229]
- 54 **Meier JJ.** GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol* 2012; **8**: 728-742 [PMID: 22945360 DOI: 10.1038/nrendo.2012.140]
- 55 **Iacobellis G,** Camarena V, Sant DW, Wang G. Human Epicardial Fat Expresses Glucagon-Like Peptide 1 and 2 Receptors Genes. *Horm Metab Res* 2017; **49**: 625-630 [PMID: 28514806 DOI: 10.1055/s-0043-109563]
- 56 **Chen J,** Zhao H, Ma X, Zhang Y, Lu S, Wang Y, Zong C, Qin D, Wang Y, Yingfeng Yang Y, Wang X, Liu Y. GLP-1/GLP-1R Signaling in Regulation of Adipocyte Differentiation and Lipogenesis. *Cell Physiol Biochem* 2017; **42**: 1165-1176 [PMID: 28668964 DOI: 10.1159/000478872]
- 57 **Pastel E,** Joshi S, Knight B, Liversedge N, Ward R, Kos K. Effects of Exendin-4 on human adipose tissue inflammation and ECM remodelling. *Nutr Diabetes* 2016; **6**: e235 [PMID: 27941938 DOI: 10.1038/nutd.2016.44]
- 58 **Xu F,** Lin B, Zheng X, Chen Z, Cao H, Xu H, Liang H, Weng J. GLP-1 receptor agonist promotes brown remodelling in mouse white adipose tissue through SIRT1. *Diabetologia* 2016; **59**: 1059-1069 [PMID: 26924394 DOI: 10.1007/s00125-016-3896-5]
- 59 **Wang XC,** Gusdon AM, Liu H, Qu S. Effects of glucagon-like peptide-1 receptor agonists on non-alcoholic fatty liver disease and inflammation. *World J Gastroenterol* 2014; **20**: 14821-14830 [PMID: 25356042 DOI: 10.3748/wjg.v20.i40.14821]
- 60 **Dutour A,** Abdesselam I, Ancel P, Kober F, Mrad G, Darmon P, Ronsin O, Pradel V, Lesavre N, Martin JC, Jacquier A, Lefur Y, Bernard M, Gaborit B. Exenatide decreases liver fat content and epicardial adipose tissue in patients with obesity and type 2 diabetes: a prospective randomized clinical trial using magnetic resonance imaging and spectroscopy. *Diabetes Obes Metab* 2016; **18**: 882-891 [PMID: 27106272 DOI: 10.1111/dom.12680]
- 61 **Jabbour SA.** SGLT2 inhibitors to control glycemia in type 2 diabetes mellitus: a new approach to an old problem. *Postgrad Med* 2014; **126**: 111-117 [PMID: 24393758 DOI: 10.3810/pgm.2014.01.2731]
- 62 **Tosaki T,** Kamiya H, Himeno T, Kato Y, Kondo M, Toyota K, Nishida T, Shiroma M, Tsubonaka K, Asai H, Moribe M, Nakaya Y, Nakamura J. Sodium-glucose Co-transporter 2 Inhibitors Reduce the Abdominal Visceral Fat Area and May Influence the Renal Function in Patients with Type 2 Diabetes. *Intern Med* 2017; **56**: 597-604 [PMID: 28321056 DOI: 10.2169/internalmedicine.56.7196]
- 63 **Bouchi R,** Terashima M, Sasahara Y, Asakawa M, Fukuda T, Takeuchi T, Nakano Y, Murakami M, Minami I, Izumiya H, Hashimoto K, Yoshimoto T, Ogawa Y. Luseogliflozin reduces epicardial fat accumulation in patients with type 2 diabetes: a pilot study. *Cardiovasc Diabetol* 2017; **16**: 32 [PMID: 28253918 DOI: 10.1186/s12933-017-0516-8]
- 64 **Fukuda T,** Bouchi R, Terashima M, Sasahara Y, Asakawa M, Takeuchi T, Nakano Y, Murakami M, Minami I, Izumiya H, Hashimoto K, Yoshimoto T, Ogawa Y. Ipragliflozin Reduces Epicardial Fat Accumulation in Non-Obese Type 2 Diabetic Patients with Visceral Obesity: A Pilot Study. *Diabetes Ther* 2017; **8**: 851-861 [PMID: 28616806 DOI: 10.1007/s13300-017-0279-y]
- 65 **Yagi S,** Hirata Y, Ise T, Kusunose K, Yamada H, Fukuda D, Salim HM, Maimaituxun G, Nishio S, Takagawa Y, Hama S, Matsuura T, Yamaguchi K, Tobiume T, Soeki T, Wakatsuki T, Aihara KI, Akaike M, Shimabukuro M, Sata M. Canagliflozin reduces epicardial fat in patients with type 2 diabetes mellitus. *Diabetol Metab Syndr* 2017; **9**: 78 [PMID: 29034006 DOI: 10.1186/s13098-017-0275-4]
- 66 **Díaz-Rodríguez E,** Agra RM, Fernández ÁL, Adrio B, García-Caballero T, González-Juanatey JR, Eiras S. Effects of dapagliflozin on human epicardial adipose tissue: modulation of insulin resistance, inflammatory chemokine production, and differentiation ability. *Cardiovasc Res* 2018; **114**: 336-346 [PMID: 29016744 DOI: 10.1093/cvr/cvx186]
- 67 **Sato T,** Aizawa Y, Yuasa S, Kishi S, Fuse K, Fujita S, Ikeda Y, Kitazawa H, Takahashi M, Sato M, Okabe M. The effect of dapagliflozin treatment on epicardial adipose tissue volume. *Cardiovasc Diabetol* 2018; **17**: 6 [PMID: 29301516 DOI: 10.1186/s12933-017-0658-8]

P- Reviewer: Baptista LS, Nakhoul FM, Psychari SN
S- Editor: Cui LJ **L- Editor:** A **E- Editor:** Yin SY



Basic Study

Effects of glucagon-like peptide 1 analogs in combination with insulin on myocardial infarct size in rats with type 2 diabetes mellitus

Vladislav A Zykov, Taisiia P Tuchina, Denis A Lebedev, Irina B Krylova, Alina Y Babenko, Elvira V Kuleshova, Elena N Grineva, Alekber A Bayramov, Michael M Galagudza

Vladislav A Zykov, Taisiia P Tuchina, Denis A Lebedev, Alina Y Babenko, Elvira V Kuleshova, Elena N Grineva, Alekber A Bayramov, Michael M Galagudza, Almazov National Medical Research Centre, St-Petersburg 197341, Russia

Irina B Krylova, Institute of Experimental Medicine, St-Petersburg 197376, Russia

ORCID number: Vladislav A Zykov (0000-0002-4399-0054); Taisiia P Tuchina (0000-0003-0994-8650); Denis A Lebedev (0000-0003-1808-1331); Irina B Krylova (0000-0002-7079-3152); Alina Y Babenko (0000-0002-0559-697X); Elvira V Kuleshova (0000-0001-8808-5720); Elena N Grineva (0000-0003-0042-7680); Alekber A Bayramov (0000-0002-0673-8722); Mikhail M Galagudza (0000-0001-5129-9944).

Author contributions: Zykov VA, Tuchina TP and Lebedev DA performed the experiments and wrote the manuscript; Babenko AY, Kuleshova EV and Grineva EN performed the literature review and suggested the study concept; Krylova IB and Bayramov AA developed the experimental design and performed data analysis; Galagudza MM provided scientific consulting, coordinated experimental parts, and edited the manuscript.

Supported by Russian Science Foundation, No. 17-75-30052.

Institutional animal care and use committee statement: All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals in Almazov National Medical Research Centre, which strictly conforms to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH, Bethesda, MD). The protocol was approved by the Institutional Animal Care and Use Committee of Almazov National Medical Research Centre (protocol #2016-3).

Conflict-of-interest statement: All authors have no conflicts of interest to report.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised

according to the ARRIVE guidelines.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Alina Y Babenko, DSc, MD, PhD, Doctor, Research Scientist, Institute of Endocrinology, Almazov National Medical Research Centre, Akkuratova St., 2, St-Petersburg 197341, Russia. babenko@almazovcentre.ru
Telephone: +7-921-3314374
Fax: +7-812-7025595

Received: March 27, 2018

Peer-review started: March 27, 2018

First decision: April 23, 2018

Revised: July 3, 2018

Accepted: July 10, 2018

Article in press: July 10, 2018

Published online: September 15, 2018

Abstract

AIM

To evaluate the effects of glucagon-like peptide-1 analogs (GLP-1a) combined with insulin on myocardial ischemia-reperfusion injury in diabetic rats.

METHODS

Type 2 diabetes mellitus (T2DM) was induced in male

Wistar rats with streptozotocin (65 mg/kg) and verified using an oral glucose tolerance test. After anesthesia, the left coronary artery was occluded for 40 min followed by 80 min reperfusion. Blood glucose level was measured during surgery. Rats were randomized into six groups as follows: (1) control rats; (2) insulin (0.1 U/kg) treated rats prior to ischemia; (3) insulin (0.1 U/kg) treated rats at reperfusion; (4) GLP-1a (140 mg/kg) treated rats prior to ischemia; (5) GLP-1a (140 mg/kg) treated rats at reperfusion; and (6) rats treated with GLP-1a (140 mg/kg) prior to ischemia plus insulin (0.1 U/kg) at reperfusion. Myocardial area at risk and infarct size was measured planimetrically using Evans blue and triphenyltetrazolium chloride staining, respectively.

RESULTS

There was no significant difference in the myocardial area at risk among groups. Insulin treatment before ischemia resulted in a significant increase in infarct size ($34.7\% \pm 3.4\%$ vs $18.6\% \pm 3.1\%$ in the control rats, $P < 0.05$). Post-ischemic administration of insulin or GLP-1a had no effect on infarct size. However, pre-ischemic administration of GLP-1a reduced infarct size to $12\% \pm 2.2\%$ ($P < 0.05$). The maximal infarct size reduction was observed in the group treated with GLP-1a prior to ischemia and insulin at reperfusion ($8\% \pm 1.6\%$, $P < 0.05$ vs the control and GLP-1a alone treated groups).

CONCLUSION

GLP-1a pre-administration results in myocardial infarct size reduction in rats with T2DM. These effects are maximal in rats treated with GLP-1a pre-ischemia plus insulin at reperfusion.

Key words: Glucagon-like peptide-1 analog; Insulin; Myocardial ischemia-reperfusion injury; Infarct size; Type 2 diabetes mellitus; Rats; Experimental research

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: In addition to their glucose-lowering effects, glucagon-like peptide-1 analogs (GLP-1a) were shown to exhibit cardioprotective effects. However, the optimal protocol of GLP-1a administration for infarct size reduction has not been determined yet. Additionally, it is important to investigate the effects of GLP-1a combined with other antidiabetic drugs on myocardial infarct size. Thus, we evaluated the effects of GLP-1a with and without insulin on infarct size in rats with type 2 diabetes mellitus. We found that GLP-1a administration prior to ischemia resulted in significant infarct size reduction. Infarct size reduction was maximal in rats treated with GLP-1a before ischemia plus insulin at reperfusion.

Zykov VA, Tuchina TP, Lebedev DA, Krylova IB, Babenko AY, Kuleshova EV, Grineva EN, Bayramov AA, Galagudza MM. Effects of glucagon-like peptide 1 analogs in combination with insulin on myocardial infarct size in rats with type 2 diabetes mellitus. *World J Diabetes* 2018; 9(9): 149-156 Available from:

URL: <http://www.wjgnet.com/1948-9358/full/v9/i9/149.htm>
DOI: <http://dx.doi.org/10.4239/wjd.v9.i9.149>

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is considered a risk factor for cardiovascular diseases with an approximately three-fold increased risk of myocardial infarction (MI). Normalizing glucose variability can prevent future cardiovascular complications. Safe blood glucose levels during MI (10 mmol/L, ideally < 8.7 mmol/L, not below 4.5-5 mmol/L) are very difficult to achieve using insulin monotherapy, which is the main therapy option in the acute period of MI^[1-3].

Therefore, it is necessary to find other therapeutic options to control blood glucose levels during MI. A possible candidate is glucagon-like peptide-1 analogs (GLP-1a) due to their high efficiency and low risk for hypoglycemia^[4,5]. Moreover, accumulated data has shown that GLP-1a exhibits independent positive pleiotropic effects on the cardiovascular system^[5-13]. However, only few studies investigated the use of GLP-1 during acute MI in patients with T2DM. The most convenient way to study the pleiotropic effects of new antidiabetic drugs involves the use of experimental models^[14].

Recent studies have indicated that GLP-1a can exert beneficial effects on the cardiovascular system. The mechanisms underlying these positive effects included both indirect effects on insulin secretion, glucose uptake, and free fatty acid metabolism in the peripheral and central nervous systems, and direct effects on GLP-1 receptors in the myocardium. Although there are numerous studies on the cardiovascular effects of GLP-1a, most studies use recombinant GLP-1 infusion that is not used in clinical practice. In addition, emphasis was placed on monotherapy with GLP-1a^[10]. Therefore, it is of great interest to investigate the effects of the clinically used GLP-1a or GLP-1 mimetics on the cardiovascular system and evaluate their cardioprotective effects in combination with insulin therapy, which is more clinically applicable. In addition, it is important to study the effects of GLP-1a administration at different stages of the experiment (before and during MI) to determine the appropriate dosage regimen in patients.

Therefore, in this study, we aimed to investigate the effects of GLP-1a combined with insulin on blood glucose levels, severity of myocardial damage, and mortality in experimental MI in rats with streptozotocin-induced T2DM.

MATERIALS AND METHODS

Animals

Seventy male Wistar rats were used in this study. Both neonatal STZ-induced T2DM and MI were induced in these rats. Experimental studies were conducted at the Federal State Budget Scientific Institution "Institute of Experimental Medicine" in cooperation with the staff of the

Laboratory of Chemistry and Pharmacology of Medicine, in accordance with the "Guidelines for the Care and Use of Laboratory Animals" and "A guide to experimental (preclinical) study of new pharmacological substances," with observance of the principles of humanity, European Directives (86/609/EEC), and Helsinki Declaration.

T2DM model

We used the streptozotocin (STZ)-nicotinamide model of diabetes. Induction of diabetes in rats was carried out by a single intraperitoneal injection of STZ at 65 mg/kg, dissolved in citrate buffer (pH 5.5). For selection of rats to be used in the study, blood glucose level was measured at the age of 3 mo, followed by an oral glucose tolerance test after administration of 40 % w/v glucose solution at a dose of 3 g/kg. Diagnostic criteria for T2DM included fasting blood glucose levels from 7 to 14 mmol/L (OneTouch Select glucometer, LifeScan Inc., Milpitas, CA, United States) and two-fold increase in the area under the glucose curve of the oral glucose tolerance test, compared with that in the control group^[15,16].

Myocardial ischemia-reperfusion model

Rats were anesthetized using chloral hydrate solution (400 mg/kg), tracheotomized, and ventilated (SAR-830P, Stoelting, United States) using room air, with a tidal volume of 2 mL/100 g and a rate of approximately 60 breaths/min. The core body temperature was maintained at $37 \pm 0.5^\circ\text{C}$ using a feedback-controlled heating pad (TCAT-2LV controller, Physiotemp Instruments Inc., Clifton, NJ, United States). The left carotid artery and right femoral vein were cannulated for measurement of the mean arterial pressure (MAP) and maintenance of anesthesia, respectively. Lead II of the electrocardiograph was monitored for determination of the heart rate (HR) and arrhythmias. After a 10 min stabilization, a left thoracotomy was performed. A 6-0 polypropylene thread was placed around a prominent branch of the left coronary artery, and the ends were passed through a polyethylene tube as an occluder. Exclusion criteria were MAP < 50 mmHg and/or HR < 300 bpm at any time point during the experiment^[17].

Experimental protocol

Reperfusion was started 40 min after the onset of ischemia by removing the ligature from the coronary artery. After another 80 min, the ischemic lesion was assessed. Figure 1 shows the experimental protocol. Animals were randomly divided into six groups, as follows: Group 1: control rats with T2DM without therapy; Group 2: rats treated with insulin prior to MI (IpMI) at a dose of 0.1 U/kg 1.5 hr before induction of AMI; Group 3: rats treated with insulin after MI (IaMI) at a dose of 0.1 U/kg 40 min after coronary artery ligation; Group 4: rats treated with GLP-1a prior to MI (GLP1pMI) at a dose of 140 mg/kg 1.5 hr before ischemia; Group 5: rats treated with GLP-1a after MI (GLP1aMI) at a dose of 140 mg/kg 40 min after ischemia; Group 6: GLP1pMI + IaMI at a GLP-1a dose of

140 mg/kg 1.5 hr before ischemia and at an insulin dose of 0.1 U/kg 40 min after ischemia.

Infarct size measurement

At the end of the experiment, the left coronary artery was re-occluded, followed by administration of 0.5 mL of 5% Evans blue (MP Biomedicals, Solon, OH, United States) via the femoral vein for measurement of the area at risk (AR). The hearts were excised and cut into five 2 mm thick slices parallel to the atrioventricular groove. The basal surface of each slice was digitally photographed. The slices were immersed in 1% solution of 2,3,5-triphenyltetrazolium chloride (MP Biomedicals, Solon, OH, United States) at 37°C (pH 7.4) for 15 min and photographed again for determination of infarct area (IA). The images were digitized using Adobe Photoshop CS. The AR was expressed as a percentage of the whole slice, and the IA was expressed as a percentage of AR. Values of AR and IA for each heart were obtained by calculating mean values of the slices. Rats with AR 15% were excluded from the study. Infarct size measurement and data analyses were performed by an investigator blinded to the study groups.

Evaluation of blood glucose levels

In addition, blood glucose levels were monitored during the experiment. A blood glucose test was performed prior to T2DM induction, after T2DM induction, a three day measurement with an interval of two to three days, during the glucose tolerance test, and thereafter every week before the operation. During the operation, blood was collected for glucose monitoring according to the following protocol: 1.5 hr before MI induction, immediately before MI, and then every 20 min. Measurement of blood glucose levels was performed at all points with the Accu-Chek glucometer using diagnostic test strips^[3,18-20].

Statistical analysis

Statistical analyses were carried out using the IBM SPSS Statistics 23 program (SPSS Inc., Chicago, IL, United States) and were performed by a biomedical statistician. Data were presented as the mean \pm SD. To evaluate the differences between dependent samples, the non-parametric Wilcoxon test was used, whereas the Mann-Whitney test was used to evaluate the reliability of the differences between independent variables. *P* values < 0.05 were considered statistically significant.

RESULTS

Blood glucose levels and glycemic variability

We assessed the features of the glycemic profiles in experimental animals. Data on glycemic variability and number of episodes of hypoglycemia in rats are presented in Table 1. The highest glycemic variability was observed in the rats treated with insulin monotherapy, whereas the lowest glycemic variability was achieved in the rats receiving GLP-1a (*P* < 0.05). It is noteworthy that glycemic variability in the rats treated with combined GLP-1a and

Table 1 Number of hypoglycemia episodes and glycemic variability in experimental animals

Group	Control	IpMI	IaMI	GLP1pMI	GLP1aMI	GLP1pMI + IaMI
Hypoglycemia episodes (<i>n</i>)	0	5	3	1	2	1
Glycemic variability	4.48 ± 0.74	6.29 ± 0.9 ^a	5.57 ± 1.3	3.79 ± 0.65 ^a	3.60 ± 0.65 ^a	3.00 ± 0.42 ^a

^a*P* < 0.05, *vs* control group. Control: Rats with T2DM without therapy; IpMI: Rats treated with insulin prior to MI; IaMI: Rats treated with insulin after MI; GLP1pMI: Rats treated with GLP-1a prior to MI; GLP1aMI: Rats treated with GLP-1a after MI; GLP1pMI + IaMI: Rats treated with GLP-1a prior to MI and with insulin after MI.

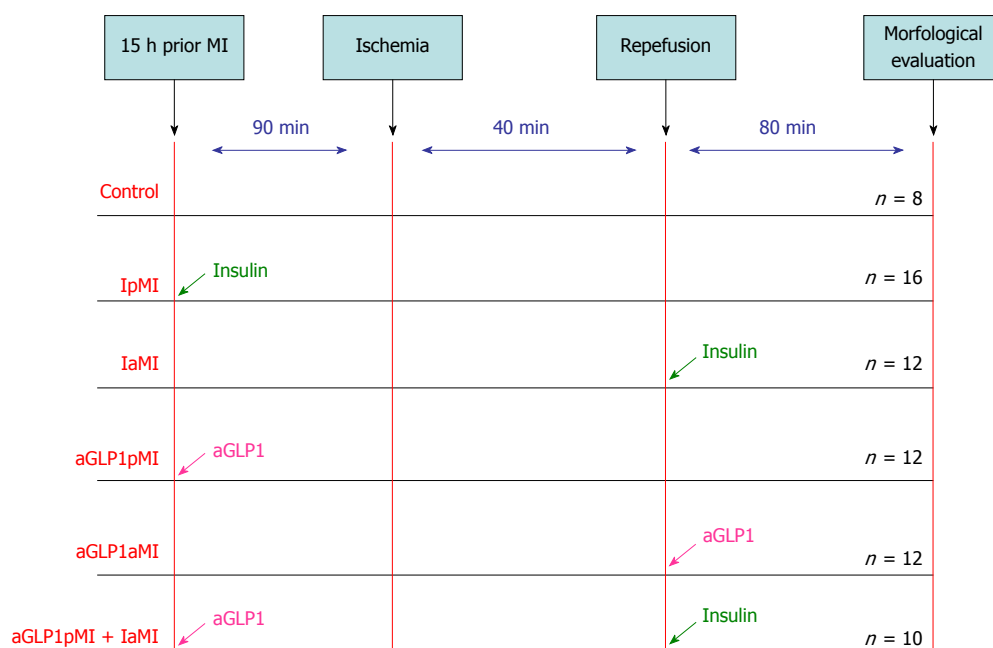


Figure 1 The experimental protocol. Group 1: Control rats with T2DM without therapy; Group 2: IpMI (rats treated with insulin prior to MI); Group 3: IaMI (rats treated with insulin after MI); Group 4: GLP1pMI (rats treated with GLP-1a prior to MI); Group 5: GLP1aMI (rats treated with GLP-1a after MI); Group 6: GLP1pMI + IaMI (rats treated with GLP-1a prior to MI and with insulin after MI).

insulin was comparable with that in the rats receiving GLP-1a monotherapy. However, it was significantly lower than that in the rats treated with insulin monotherapy and in the control group rats (*P* < 0.05). The number of episodes of hypoglycemia was also high in the groups receiving insulin monotherapy, whereas hypoglycemia was practically undetected in the groups receiving GLP-1a. Statistical analysis of hypoglycemia incidence was not performed because of the small sample size. Thus, data were expressed as absolute numbers.

Mortality

In addition, we evaluated the mortality of rats during the experiment. During the experiment, 26 rats died owing to acute heart failure and/or persistent arrhythmia (Table 1). Data on the ratios of dead and surviving rats in each group are presented in Table 1. Statistical analysis of mortality was not carried out because of the small sample size.

Area at risk and infarct size

To estimate the extent of myocardial damage, the ratio of the necrotic zone area to the ischemic zone area was calculated. The AR was expressed as a percentage of the

whole slice, and the IA was expressed as a percentage of AR. Values of AR and IA for each heart were obtained by calculating the mean values of the slices^[21]. Results are shown in Table 2.

Figures 2 and 3 show the risk and necrotic zones. The AR did not significantly differ among groups. However, the largest zone of myocardial necrosis in relation to the ischemic zone was observed in the rats receiving insulin monotherapy before induction of ischemia.

Insulin treatment before ischemia resulted in a significant increase in infarct size ($34.7\% \pm 3.4\%$ vs $18.6\% \pm 3.1\%$ in the control *P* < 0.05). Post-ischemic administration of insulin or GLP-1a had no effect on infarct size. Pre-ischemic administration of GLP-1a reduced infarct size to $12\% \pm 2.2\%$. The maximal infarct size reduction was observed in the rats treated with GLP-1a pre-ischemia and insulin at reperfusion ($8\% \pm 1.6\%$, *P* < 0.05 *vs* the control and GLP-1a alone-treated groups).

DISCUSSION

The results of this experimental study confirmed the cardioprotective effects of GLP-1a, which were reported

Table 2 Mortality of rats expressed as percentages with different antihyperglycemic therapies

Group	1 Control (n = 8)	2 IpMI (n = 16)	3 IaMI (n = 12)	4 GLP1pMI (n = 12)	5 GLP1aMI (n = 12)	6 GLP1pMI + IaMI (n = 10)
Ratio of surviving to dead rats (% of deaths)	5/3 (37.5%)	8/8 (50%)	7/5 (41.6%)	8/4 (30 %)	8/4 (30 %)	8/2 (20%)

Control: rats with T2DM without therapy; IpMI: Rats treated with insulin prior to MI; IaMI: Rats treated with insulin after MI; GLP1pMI: Rats treated with GLP-1a prior to MI; GLP1aMI: Rats treated with GLP-1a after MI; GLP1pMI + IaMI: Rats treated with GLP-1a prior to MI and with insulin after MI.

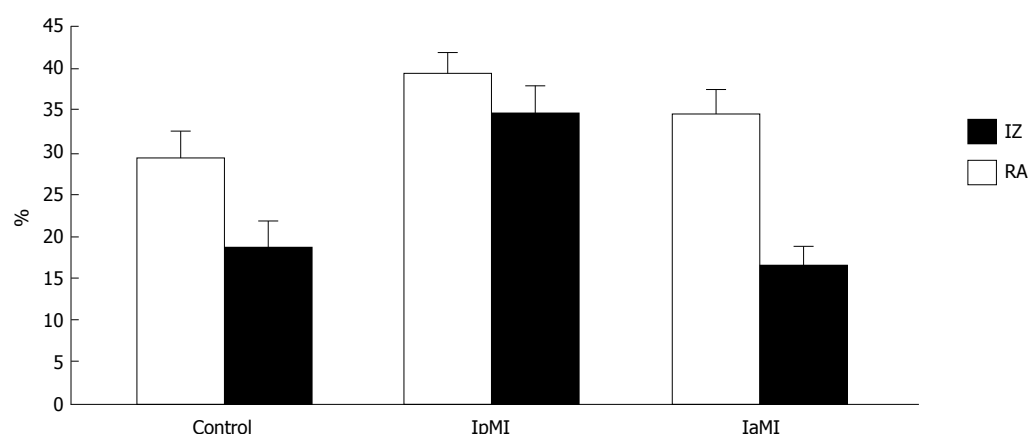


Figure 2 Comparison of the necrotic zone and zone at risk between different drugs. Control: Rats with T2DM without therapy; IpMI: Rats treated with insulin prior to MI; IaMI: Rats treated with insulin after MI.

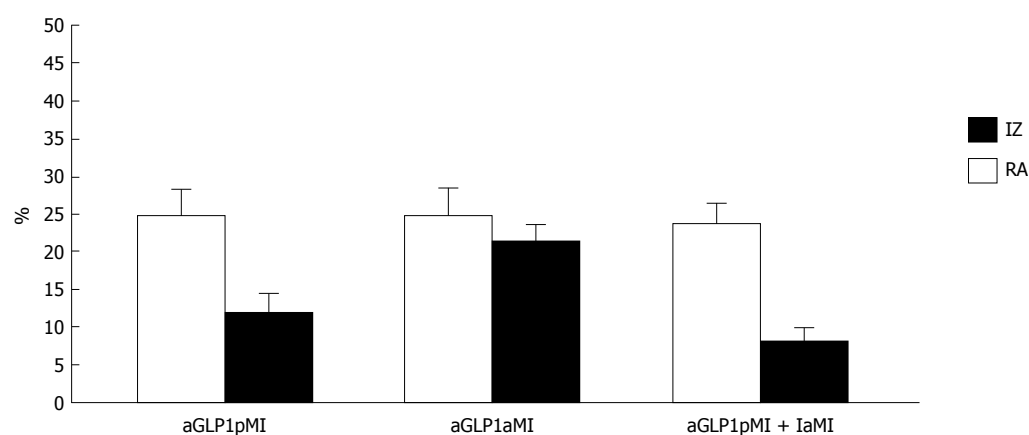


Figure 3 Comparison of the necrotic zone and zone at risk between different drugs. GLP1pMI: Rats treated with GLP-1a prior to MI; GLP1aMI: Rats treated with GLP-1a after MI; GLP1pMI + IaMI: Rats treated with GLP-1a prior to MI and with insulin after MI.

in earlier studies. In addition, we assessed the glycemic variability since inadequate control of this index has been shown to worsen the prognosis for MI in patients with T2DM^[1]. GLP-1a infusion results in a decrease in blood glucose concentration to the level of fasting glycemia. However, as soon as the level of blood glucose decreases and approaches normal values, the effects of GLP-1a on insulin secretion cease because of a feedback mechanism. In addition, GLP-1a suppresses glucagon secretion from the pancreatic α -cells by means of a glucose-dependent mechanism. Thus, the fact that GLP-1a cannot cause severe hypoglycemia is clinically important^[22,23]. The results of this study suggested that administration of GLP-

1a reduced glycemic variability, regardless of the time of administration and combination with other drugs.

Previous studies have evaluated the effects of GLP-1a in the cardiovascular system^[14]. In addition, one study investigated the effects of GLP-1 agonists on the endothelial function of blood vessels in patients with T2DM and stable angina pectoris^[24]. GLP-1a significantly improved endothelium-dependent vasodilatation of the brachial artery in samples with acetylcholine in patients with T2DM. Moreover, previous studies showed that recombinant GLP-1 infusion improved left ventricular function in patients with T2DM and severe heart failure^[25]. In addition, a significant decrease in the systolic blood

pressure was observed approximately two weeks after the initiation of therapy in LEAD studies^[26]. This anti-hypertensive effect of GLP-1a might be attributed to its vasodilator effects by increasing the expression of endothelial nitric oxide synthase. Alternatively, it could result from its natriuretic or diuretic action^[27].

Administration of GLP-1 agonists at high doses resulted in a decrease in the levels of three biomarkers of cardiovascular risk, including triglycerides, inhibitor of plasminogen-1 activator (PAI-1), and natriuretic peptide type B, compared to placebo. Accordingly, LEAD studies concluded that GLP-1a provided more effective target achievement of the final complex, combining three important parameters of metabolic control, HbA1c, systolic blood pressure, and body weight, compared with that of other hypoglycemic drugs. In addition, since GLP-1a results in an indirect increase in insulin secretion, it also achieves all the cardioprotective effects of insulin therapy. Thus, GLP-1a therapy has the advantage of maintaining the positive effects of insulin therapy by eliminating its potential complications.

Our study showed the cardioprotective effects of GLP-1a. We suggested that the use of both GLP-1a alone and in combination with insulin reduced the necrotic zone area. Our research protocol was as close as possible to the clinical situation and ensured optimal translation of the results of the present study to the clinic. Insulin-treated rats exhibited significant differences depending on whether insulin was administered prior to or after the induction of ischemia.

The mechanisms of insulin action in MI are now well studied. In particular, the cardioprotective effects of insulin at reperfusion are attributable to the increase in the production of phosphatidylinositol 3-kinase, which promotes the synthesis of antiapoptotic protein kinases, inhibits apoptosis, and promotes the survival of cardiomyocytes. In addition, it is known that insulin lowers the concentration of free fatty acids and ketone bodies in the myocardium, which increases the activity of pyruvate dehydrogenase to a certain extent and decreases the accumulation of lactate in the myocardium. These effects significantly improve the regulation of metabolic processes in the damaged myocardium and subsequently reduce the mortality and duration of hospitalization. Glucose uptake by the myocardium is significantly enhanced or even normalized with adequate insulin therapy. This, in turn, has a positive effect on prognosis and improves the systolic function and left ventricular ejection fraction. Additionally, insulin can suppress inflammation and enhance fibrinolysis (by decreasing the activity of antifibrinolytic factors) in patients with acute MI with ST segment elevation, receiving low-dose insulin infusion and fibrinolytic therapy. These effects of insulin, along with its vasodilator and antiplatelet actions, promote reperfusion at the level of the epicardium and microcirculatory bed, and thus protect the myocardium^[28].

In addition to the above evidence, our hypothesis on the putative infarct-limiting effect of insulin pretreatment was based on the data obtained in the study of Fuglestad

et al.^[29] who showed mTOR-dependent infarct size reduction after preischemic insulin administration in the Langendorff-perfused rat heart. There are, however, studies that have not confirmed this fact. Therefore, we thought to check if insulin is really cardioprotective when administered prior to ischemia. Our results showed that insulin monotherapy resulted in high glycemic variability and low survival rate in experimental animals with acute myocardial ischemia. In the experimental group treated with insulin before induction of ischemia the necrotic area was the largest among all other groups. The lowest percentage of myocardial necrosis was observed in the rats treated with GLP-1a before induction of ischemia and in rats receiving combination therapy.

The main limitation of this study was the small sample size that did not allow full-scale statistical analysis to assess the mortality of animals during the experiment and the number of episodes of hypoglycemia. However, this sample size allowed us to fulfill the main goal of this study and draw conclusions on the effects of GLP-1a in MI.

We suggest that the pronounced positive effects of GLP-1a during the course of MI therapy occurred when it was administered at the onset of infarction. This could be explained by the fact that the required drug concentration and effects in the myocardium could only be achieved when the drug was administered prior to the induction of ischemia.

In conclusion, GLP-1a pre-ischemic administration results in myocardial infarct size reduction in rats with T2DM. These effects are maximal in rats treated with GLP-1a pre-ischemia plus insulin at reperfusion.

ARTICLE HIGHLIGHTS

Research background

Type 2 diabetes mellitus (T2DM) is associated with an increased risk of myocardial infarction (MI) and poorer prognosis. Recent studies demonstrate that glucagon-like peptide-1 analogs (GLP-1a) possess infarct-limiting effects in experimental settings. However, it is not clear whether GLP-1a have beneficial effects when combined with insulin.

Research motivation

In this study, we intended to compare the cardioprotective effects of GLP-1a therapy with and without concomitant insulin in acute MI in rats with T2DM.

Research objectives

The effects of pre- and post-ischemic GLP-1a and insulin administration on infarct size were studied in the rat model of MI. The effect of a combination of GLP-1a and insulin was assessed in a separate group.

Research methods

We induced T2DM in Wistar rats with streptozotocin at a dose of 65 mg/kg. Myocardial ischemia was induced by left coronary artery occlusion. Myocardial infarct size was determined histochemically. In addition, we analyzed the number and severity of hypoglycemia episodes in the experimental groups. Animals were treated with either GLP-1a or insulin.

Research results

Results of our study show that using GLP-1a before ischemia-reperfusion significantly reduced infarct size. The maximal infarct size reduction was observed in the group treated with GLP-1a prior to ischemia and insulin at

reperfusion.

Research conclusions

We have shown that insulin infusion before ischemia increased infarct size, while GLP-1a demonstrated cardioprotective effects. Post-ischemic administration of insulin or GLP-1a had no effect on infarct size. Thus, the regimen of GLP-1a and insulin administration is crucial for expression of their cardioprotective effect.

Research perspectives

Further studies with larger sample sizes can be conducted in order to develop a clinical trial and introduce new combinations of drugs with antidiabetic activity for MI therapy in patients with T2DM.

REFERENCES

- 1 Siegelar SE, Kerr L, Jacober SJ, Devries JH. A decrease in glucose variability does not reduce cardiovascular event rates in type 2 diabetic patients after acute myocardial infarction: a reanalysis of the HEART2D study. *Diabetes Care* 2011; **34**: 855-857 [PMID: 21447661 DOI: 10.2337/dc10-1684]
- 2 Zhang X, Xu X, Jiao X, Wu J, Zhou S, Lv X. The effects of glucose fluctuation on the severity of coronary artery disease in type 2 diabetes mellitus. *J Diabetes Res* 2013; **2013**: 576916 [PMID: 23936867 DOI: 10.1155/2013/576916]
- 3 Su G, Mi SH, Tao H, Li Z, Yang HX, Zheng H, Zhou Y, Tian L. Impact of admission glycemic variability, glucose, and glycosylated hemoglobin on major adverse cardiac events after acute myocardial infarction. *Diabetes Care* 2013; **36**: 1026-1032 [PMID: 23349547 DOI: 10.2337/dc12-0925]
- 4 Monji A, Mitsui T, Bando YK, Aoyama M, Shigeta T, Murohara T. Glucagon-like peptide-1 receptor activation reverses cardiac remodeling via normalizing cardiac steatosis and oxidative stress in type 2 diabetes. *Am J Physiol Heart Circ Physiol* 2013; **305**: H295-H304 [PMID: 23709595 DOI: 10.1152/ajpheart.00990.2012]
- 5 Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, Shannon RP. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation* 2004; **109**: 962-965 [PMID: 14981009 DOI: 10.1161/01.cir.0000120505.91348.58]
- 6 Plutzky J. The incretin axis in cardiovascular disease. *Circulation* 2011; **124**: 2285-2289 [PMID: 22105195 DOI: 10.1161/CIRCULATIONAHA.111.064139]
- 7 Ryan D, Acosta A. GLP-1 receptor agonists: Nonglycemic clinical effects in weight loss and beyond. *Obesity (Silver Spring)* 2015; **23**: 1119-1129 [PMID: 25959380 DOI: 10.1002/oby.21107]
- 8 Bose AK, Mocanu MM, Carr RD, Yellon DM. Myocardial ischaemia-reperfusion injury is attenuated by intact glucagon like peptide-1 (GLP-1) in the in vitro rat heart and may involve the p70s6K pathway. *Cardiovasc Drugs Ther* 2007; **21**: 253-256 [PMID: 17541736 DOI: 10.1007/s10557-007-6030-6]
- 9 Sonne DP, Engström T, Treiman M. Protective effects of GLP-1 analogues exendin-4 and GLP-1(9-36) amide against ischemia-reperfusion injury in rat heart. *Regul Pept* 2008; **146**: 243-249 [PMID: 17976835 DOI: 10.1016/j.regpep.2007.10.001]
- 10 Lønborg J, Vejstrup N, Kelbæk H, Bøtker HE, Kim WY, Mathiasen AB, Jørgensen E, Helqvist S, Saunamäki K, Clemmensen P, Holmvang L, Thuesen L, Kruse LR, Jensen JS, Køber L, Treiman M, Holst JJ, Engström T. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J* 2012; **33**: 1491-1499 [PMID: 21920963 DOI: 10.1093/eurheartj/ehr309]
- 11 Lorber D. GLP-1 receptor agonists: effects on cardiovascular risk reduction. *Cardiovasc Ther* 2013; **31**: 238-249 [PMID: 23865382 DOI: 10.1111/1755-5922.12000]
- 12 Matsubara M, Kanemoto S, Leshnower BG, Albane EF, Hinmon R, Plappert T, Gorman JH 3rd, Gorman RC. Single dose GLP-1-Tf ameliorates myocardial ischemia/reperfusion injury. *J Surg Res* 2011; **165**: 38-45 [PMID: 19552923 DOI: 10.1016/j.jss.2009.03.016]
- 13 Terasaki M, Nagashima M, Nohtomi K, Kohashi K, Tomoyasu M, Sinmura K, Nogi Y, Katayama Y, Sato K, Itoh F, Watanabe T, Hirano T. Preventive effect of dipeptidyl peptidase-4 inhibitor on atherosclerosis is mainly attributable to incretin's actions in nondiabetic and diabetic apolipoprotein E-null mice. *PLoS One* 2013; **8**: e70933 [PMID: 23967137 DOI: 10.1371/journal.pone.0070933]
- 14 Karpov AA, Uspenskaya YK, Minasian SM, Puzanov MV, Dmitrieva RI, Bilibina AA, Anisimov SV, Galagudza MM. The effect of bone marrow- and adipose tissue-derived mesenchymal stem cell transplantation on myocardial remodeling in the rat model of ischaemic heart failure. *Int J Exp Pathol* 2013; **94**: 169-177 [PMID: 23560418 DOI: 10.1111/iep.12017]
- 15 Peterson RG, Jackson CV, Zimmerman K, de Winter W, Huebert N, Hansen MK. Characterization of the ZDS Rat: A Translational Model for the Study of Metabolic Syndrome and Type 2 Diabetes. *J Diabetes Res* 2015; **2015**: 487816 [PMID: 25961053 DOI: 10.1155/2015/487816]
- 16 Bayrasheva VK. New model of T2DM and diabetic nephropathy in rats. *Transl Med* 2016; **3**: 44-55
- 17 Galagudza MM, Sonin DL, Vlasov TD, Kurapeev DI, Shlyakhto EV. Remote vs. local ischaemic preconditioning in the rat heart: infarct limitation, suppression of ischaemic arrhythmia and the role of reactive oxygen species. *Int J Exp Pathol* 2016; **97**: 66-74 [PMID: 26990944 DOI: 10.1111/iep.12170]
- 18 Irace C, Fiorentino R, Carallo C, Scavelli F, Gnasso A. Exenatide improves glycemic variability assessed by continuous glucose monitoring in subjects with type 2 diabetes. *Diabetes Technol Ther* 2011; **13**: 1261-1263 [PMID: 21751893 DOI: 10.1089/dia.2011.0096]
- 19 Wang Z, Yang Y, Xiang X, Zhu Y, Men J, He M. [Estimation of the normal range of blood glucose in rats]. *Wei Sheng Yan Jiu* 2010; **39**: 133-137, 142 [PMID: 20459020]
- 20 Serradas P, Bailbé D, Portha B. Long-term gliclazide treatment improves the in vitro glucose-induced insulin release in rats with type 2 (non-insulin-dependent) diabetes induced by neonatal streptozotocin. *Diabetologia* 1989; **32**: 577-584 [PMID: 2528491 DOI: 10.1007/BF00285331]
- 21 Ban K, Noyan-Ashraf MH, Hofer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation* 2008; **117**: 2340-2350 [PMID: 18427132 DOI: 10.1161/CIRCULATIONAHA.107.739938]
- 22 Heine RJ, Van Gaal LF, Johns D, Mihm MJ, Widell MH, Brodows RG, GWAA Study Group. Exenatide versus insulin glargine in patients with suboptimally controlled type 2 diabetes: a randomized trial. *Ann Intern Med* 2005; **143**: 559-569 [PMID: 16230722 DOI: 10.7326/0003-4819-143-8-200510180-00006]
- 23 Bulotta A, Farilla L, Hui H, Perfetti R. The role of GLP-1 in the regulation of islet cell mass. *Cell Biochem Biophys* 2004; **40**: 65-78 [PMID: 15289644 DOI: 10.1385/CBB.40.3.65]
- 24 Nathanson D, Erdogdu O, Pernow J, Zhang Q, Nyström T. Endothelial dysfunction induced by triglycerides is not restored by exenatide in rat conduit arteries ex vivo. *Regul Pept* 2009; **157**: 8-13 [PMID: 19595708 DOI: 10.1016/j.regpep.2009.07.003]
- 25 Thrainsdottir IS, von Bibra H, Malmberg K, Rydén L. Effects of trimetazidine on left ventricular function in patients with type 2 diabetes and heart failure. *J Cardiovasc Pharmacol* 2004; **44**: 101-108 [PMID: 15175564 DOI: 10.1097/00005344-200407000-00014]
- 26 Jendle J, Torffvit O, Ridderstråle M, Ericsson Å, Nilsson B, Bøgelund M. Willingness to pay for diabetes drug therapy in type 2 diabetes patients: based on LEAD clinical programme results. *J Med Econ* 2012; **15 Suppl 2**: 1-5 [PMID: 22853443 DOI: 10.3111/13696998.2012.703633]
- 27 Sokos GG, Nikolaidis LA, Mankad S, Elahi D, Shannon RP. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure.

- J Card Fail* 2006; **12**: 694-699 [PMID: 17174230 DOI: 10.1016/j.cardfail.2006.08.211]
- 28 **Masoumi G**, Frsathkish R, Jalali A, Ziyaeifard M, Sadeghpour-Tabae A, Mansouri M. Effects of Moderate Glycemic Control in Type II Diabetes With Insulin on Arterial Blood Gas Parameters Following Coronary Artery Bypass Graft Surgery. *Res Cardiovasc Med* 2014; **3**: e17857 [PMID: 25478539 DOI: 10.5812/cardiovascmed.17857]
- 29 **Fuglestad BN**, Tiron C, Jonassen AK, Mjøs OD, Ytrehus K. Pretreatment with insulin before ischaemia reduces infarct size in Langendorff-perfused rat hearts. *Acta Physiol (Oxf)* 2009; **195**: 273-282 [PMID: 19143095 DOI: 10.1111/j.1748-1716.2008.01901.x]

P- Reviewer: Bell DSH, Robles NR **S- Editor:** Ma YJ

L- Editor: Filipodia **E- Editor:** Yin SY



Case Control Study

Association of *TCF7L2* mutation and atypical diabetes in a Uruguayan population

Carolina Beloso, Jorge Souto, Matias Fábregat, Gerardo Romanelli, Gerardo Javiel, Adriana Mimbacas

Carolina Beloso, Adriana Mimbacas, Biodiversity and Genetics Department, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo 11600, Uruguay

Jorge Souto, Cytogenetics Laboratory, Hematology and Transplant Service of Hematopoietic Progenitors, Maciel Hospital, ASSE, Montevideo 11600, Uruguay

Jorge Souto, Department of Genetics, Faculty of Medicine, UDELAR, Montevideo 11800, Uruguay

Matias Fábregat, Human Molecular Genetics Laboratory, Institut Pasteur de Montevideo 11400, Uruguay

Gerardo Romanelli, Cell Signaling and Nanobiology Laboratory, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo 11600, Uruguay

Gerardo Javiel, Unit of Diabetes Hospital Pasteur, ASSE-Ministry of Public Health, Montevideo 11400, Uruguay

Gerardo Javiel, Diabetology Service of Private Health Center, Centro de Asistencia del Sindicato Médico del Uruguay, Montevideo 11600, Uruguay

ORCID number: Carolina Beloso (0000-0003-4469-5572); Jorge Souto (0000-0002-3676-1712); Matias Fábregat (0000-0002-8248-5464); Gerardo Romanelli (0000-0002-4870-5320); Gerardo Javiel (0000-0002-5931-2055); Adriana Mimbacas (0000-0001-5302-619X).

Author contributions: Beloso C processed the samples from the atypical diabetes patients and controls, performed analysis and interpretation of the data, and participated in writing of the manuscript; Souto J contributed to laboratory processing of the samples and writing of the manuscript; Fábregat M acquired the patients' data and performed processing of the "classical" diabetes samples; Romanelli G contributed to the statistical analyses; Javiel G selected the patients for the protocol and attended to them in clinic, and made critical revisions related to the important intellectual content of the manuscript; Mimbacas A made substantial contributions to the conception and design of the study and critical revisions related to the important intellectual content of the manuscript, and gave final approval of the version of the article to be published.

Institutional review board statement: The study was approved by the Ethics Committees of each of the participating institutions (Law 18331).

Informed consent statement: All patients provided written informed consent.

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

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Adriana Mimbacas, PhD, Assistant Professor, Biodiversity and Genetics Department, Instituto de Investigaciones Biológicas Clemente Estable, Avenida Italia 3318, Montevideo 11600, Uruguay. amimbacas@iibce.edu.uy
Telephone: +598-2-4861417
Fax: +598-2-4875548

Received: March 22, 2018

Peer-review started: March 22, 2018

First decision: May 16, 2018

Revised: June 6, 2018

Accepted: July 10, 2018

Article in press: July 10, 2018

Published online: September 15, 2018

Abstract

AIM

To investigate if mutations in *TCF7L2* are associated with "atypical diabetes" in the Uruguayan population.

METHODS

Healthy, nondiabetic controls ($n = 133$) and patients with type 2 diabetes ($n = 177$) were selected from among the presenting population at level-3 referral healthcare centers in Uruguay. Patients with type 2 diabetes were subgrouped according to "atypical diabetes" ($n = 92$) and "classical diabetes" ($n = 85$). Genotyping for the rs12255372 and rs7903146 single nucleotide polymorphisms (SNPs) in the *TCF7L2* gene was carried out with TaqMan® probes. Random samples were sequenced by MacroGen Ltd. (South Korea). Statistical analysis of the SNP data was carried out with the SNPStats online tool (<http://bioinfo.iconologia.net/SNPstats>). The best inheritance model was chosen according to the lowest values of Akaike's information criterion and Bayesian information criterion. Differences between groups were determined by unpaired *t*-tests after checking the normal distribution or were converted to normalize the data. The association of SNPs was tested for matched case-control samples by using χ^2 analysis and calculation of odds ratios (ORs) with 95% confidence intervals (CIs). All statistical tests were performed using SPSS v10.0 and EpiInfo7 statistical packages. Significant statistical differences were assumed in all cases showing adjusted $P < 0.05$.

RESULTS

We genotyped two *TCF7L2* SNPs (rs7903146 and rs12255372) in a population-based sample of 310 Uruguayan subjects, including 133 healthy control subjects and 177 clinical diagnosed with type 2 diabetes. For both SNPs analyzed, the best model was the dominant type: rs12255372 = G/G *vs* G/T+T/T, OR = 0.63, 95%CI: 0.40-0.98, $P < 0.05$ and rs7903146 = C/C *vs* C/T+T/T, OR = 0.79, 95%CI: 0.41-1.55, $P = 0.3$. The rs12255372 SNP showed high association with the type 2 diabetes cases (OR = 1.60, 95%CI: 1.20-2.51, $P < 0.05$). However, when the type 2 diabetes group was analyzed according to the atypical and classical subgroupings, the association with diabetes existed only for rs12255372 and the classical subgroup (*vs* controls: OR = 2.1, 95%CI: 1.21-3.75, $P < 0.05$); no significant differences were found for either SNP or atypical diabetes.

CONCLUSION

This is the first time SNPs_ *TCF7L2* were genotyped in a diabetic population stratified by genotype instead of phenotype. Classical and atypical patients showed statistical differences.

Key words: TCF7L2; Atypical diabetes; Type 2 diabetes; Latin America; TaqMan

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: This is the first time single nucleotide polymorphisms (SNPs) of the *TCF7L2* gene were genotyped and comparatively assessed in Uruguayan type 2 diabetes patients with "atypical" and "classical" cases. The results show that these two populations are genotypically different. The only statistical association found involved

one of the SNPs, rs12255372, and classical diabetes. No association was found to exist between either of the two SNPs examined (rs7903146 and rs12255372) and atypical diabetes. The findings in this study confirm the results of our previous investigations, which indicated that atypical and classical diabetes are two separate entities of the diabetes disease.

Beloso C, Souto J, Fábregat M, Romanelli G, Javiel G, Mimbacas A. Association of *TCF7L2* mutation and atypical diabetes in a Uruguayan population. *World J Diabetes* 2018; 9(9): 157-164 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i9/157.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i9.157>

INTRODUCTION

Diabetes mellitus is a global public health problem, and the Uruguayan population poses no exception. The prevalence of diabetes in Uruguay is 8%^[1], accounting for 3.3 million of the country's inhabitants^[2]. Worldwide, type 2 diabetes (T2D) is the most common form, with incidence and prevalence having reached epidemic proportions.

Since 2009, our group has published on patients that were difficult to classify from a clinical point of view because they did not present a correlation between phenotype and genotype^[3-5]. The current international guidelines for defining the type of diabetes present in an individual are still not sufficient to diagnose atypical diabetes. Patients with atypical diabetes do not fit exactly in any of the groups defined in the guidelines because they do not precisely follow the classical presentation and disease evolution and they show poor therapeutic response.

These patients have been treated in level-3 healthcare settings. The atypical cases could be bypassed inadvertently by healthcare providers if the appropriate genetic and immunological analyses are not carried out, primarily because overweight or obese status is a gross indicator of insulin-resistance. Indeed, these visibly assessed features are the primary disorder considered in classification of type 2 diabetes in the internationally used recommendations of the American Diabetes Association (ADA)^[6]. Atypical diabetes could be a confounder in latent autoimmune diabetes of adults (LADA) but the two are distinguishable according to several specific clues. LADA includes (1) patient onset at ≥ 30 years of age (we have found children and young people with atypical diabetes); (2) an absence of metabolic syndrome along with features of obesity, high blood pressure and high cholesterol levels (all of the atypical patients we have encountered have this condition); (3) uncontrolled hyperglycemia despite using oral agents; and (4) other autoimmune diseases (with clinical evidence for diagnosis) that are not necessarily present in atypical diabetes (*i.e.*, Graves' disease and anemia)^[7].

For the work presented herein, we analyzed the association of transcription factor 7-like 2 (*TCF7L2*), one

of the major genes related to T2D, continuing with the genetic characterization of atypical diabetes patients. Our choice of this gene was based upon the remarkable amount of research that has been carried out to date on the genetic factors of diabetes, and from which *TCF7L2* has emerged as one of the strongest T2D susceptibility genes^[8-10].

TCF7L2 is a Wnt signaling-associated transcription factor, expressed in the intestine, pancreas, others tissues and plays an important role in the β -cell proliferation and insulin secretion^[11]. Publications reviewing the possible mechanisms have led to several theories on the processes by which altered *TCF7L2* production or function may cause diabetes. Among these, reduced insulinotropic effect of incretin hormones, of GLP-1 signaling in β -cells especially, impaired insulin processing or release, and decreased β -cell mass seem to be the most probable etiological mechanisms^[12-15]. The fact that genes encoding Wnt signaling pathway factors are active in β -cells or the indication that they may be involved in insulin secretion supports the notion that β -cell dysfunction is a crucial final step on the path to diabetes^[16].

The *TCF7L2* gene is located on chromosome 10q25 and is composed of two major domains: a catenin-binding domain (exon 1) and a central DNA-binding HMG domain (exons 10 and 11). Variations in this gene have been consistently associated with T2D in studies of different populations, namely those of Caucasian, Asian and African origin, and specifically involving two intronic single nucleotide polymorphisms (SNPs): rs12255372 (G>T) and rs7903146 (C>T)^[17-25]. For rs12255372, homozygous carriers of the rare T allele produce 2.5-fold higher levels of *TCF7L2* transcript than wild-type carriers, while heterozygous carriers of both alleles produce 1.5-fold higher^[26]. This SNP in particular affects diabetes through deficiency in insulin secretion, more so than through insulin resistance^[14].

For rs7903146, it is more associated with capacity of insulin secretion than insulin resistance of the β -cell^[27]. The allele T carriers permit the decrease of insulin secretion in postprandial state. This is an important characteristic because it is possible measure the cell response to glucose, aminoacid and incretins^[28].

In this study, we investigated the association between the *TCF7L2* SNPs rs12255372 and rs7903146 in a control (nondiabetic) group and a case (diabetic) group consisting of patients with classical or atypical T2D in the Uruguayan population. This study represents the first time these SNPs have been investigated by stratifying the study population according to presence or absence of HLA and nonHLA susceptibility genes to T2D in patients with body mass index (BMI) ≥ 25 kg/m².

MATERIALS AND METHODS

We analyzed a total study population of 310 individuals, including T2D patients ($n = 177$) and controls ($n = 133$) that were enrolled in the study between 2004 and 2012.

Recruitment of patients was done by selecting from two referral diabetes healthcare centers in Montevideo, Uruguay, namely the Pasteur Hospital and CASMU-IAMPP.

The study was performed in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the Ethics Committees of both participant institutions (Law 18331). All cases and controls signed an informed consent form for participation in this investigation.

T2D patients

Patients were selected according to ADA recommendations^[6]. We took into consideration another criterion, that being patients who had received a multidisciplinary care approach for their diabetes, showed good adherence to their treatment regimen (including a nutritional and physical activity plan according to their functional capabilities), and had received one or more oral antihyperglycemic drugs.

For the stratification of T2D patients into the classical and atypical diabetes groupings, we used the same classification standards as in our previous studies^[3-5]. For atypical diabetes, 92 of the patients met the following inclusion criteria: (1) BMI ≥ 25 kg/m² and categorization following the World Health Organization overweight and obesity guidelines (25-29.9 kg/m² and ≥ 30 kg/m² respectively); (2) having reached the education and nutrition plans' objectives as per international guidelines; (3) having presented doubts about their disease classification and/or not having reached a good therapeutic response (*i.e.*, no decrease of 1.5% in HbA1c levels shown in two consecutive measurements after 3 mo^[29]); and (4) presence of autoimmune-diabetes-susceptibility HLA alleles (the HLA DQB1* 0201-0302 and DR 3-4 susceptibility alleles were considered for the Uruguayan population^[30]). For classical diabetes, 85 patients fulfilled the (1) and (2) inclusion criteria listed above but did not present doubts about their diagnosis and did not show presence of autoimmune-susceptible genes.

Individuals who fit the inclusion criteria but had other metabolic disorders or were undergoing tumor processes were excluded from this study.

Control (healthy, nondiabetic) subjects

One hundred and thirty-three healthy, nondiabetic subjects were recruited from among blood donors at the Hemotherapy Department of Pasteur Hospital between 2014 and 2015. The blood donors presented for this service on a volunteer basis. Prior to the sample extraction, the attending doctors carried out an exhaustive questionnaire survey of each donor, which included information on chronic or infectious diseases and taking of any medication(s). Blood pressure was taken and weight and height were recorded in order to calculate the BMI (kg/m²). Laboratory tests were carried out and individuals with infectious diseases such as human immunodeficiency virus and hepatitis A/B/C were excluded from enrollment. At the time of sample extraction, none of the donors

Table 1 Anthropometric characteristics of the study population

	Healthy controls	Type 2 diabetes cases
<i>n</i>	133	177
Male/Female	76/57	87/90
Age (yr)	37.49 ± 13.04	63.05 ± 11.66
BMI (kg/m ²)	25.17 ± 5.35	31.73 ± 5.65

BMI: Body mass index; SD: Standard deviation.

had diabetes, but this did not rule out the possibility that they could have developed it in the future since it is a multifactorial disease. The majority of the population that donates blood has an average age of approximately 38 years.

Of the 300 individuals that were initially included as the control population, we selected those who had a BMI similar to that of the sample of patients with atypical diabetes in order to avoid a confounding variable. In addition, we had already shown in a previous study that this variable does not have a statistically significant difference between classic and atypical diabetes cases^[5].

Genetic typification

DNA was obtained from peripheral blood using the standard phenol/chloroform technique. The rs12255372 (G>T) and rs7903146 (C>T) SNPs in the *TCF7L2* gene were genotyped using *TaqMan*[®] Probes for real-time PCR in the Rotor-Gene[™] 6000 PCR machine (Corbett Research, Sydney, Australia). The primers and probes for SNP rs12255372 were designed with the Primer3Plus software (Boston, MA, United States) and AlleleID[®] software (Palo Alto, CA, United States), respectively. The primer sequences were as follows: forward, TCTGGCTTGGAAAGTGTA; reverse, GAGGCCTGAGTAATTATCAGAA. The probe sequence was as follows: FAM/HEX-CCAGGAATATCCAGGCAAGAAT[T/G]ACCA-BHQ1. The rs7903146 primers and probe were obtained from the catalog of genotyping experiments in *TaqMan*[®] SNP Genotyping Assays (Life Technologies[™], Carlsbad, CA, United States). The primer sequences were as follows: forward, GCCTCAAAACCTAGCACAGC; reverse, GTGAAGTGCCCAAGCTTCTC. The probe sequence was as follows: VIC/HEX TAGAGAGCTAAGCACTTTTATAGATA[C/T]TATATAATTTAATTGCCGTATGAGG. In both cases, a commercial genotyping kit (Platinum[®] Quantitative PCR SuperMix-UDG); Invitrogen[™] by Life Technologies[™]) was used for the genotyping procedure.

Melting curve analyses were performed using the Rotor-Gene[™] 6000 software v.1.7 (build 75) and the accompanying algorithm. Random samples were sequenced by MacroGen Ltd. (Seoul, South Korea) and were aligned using MEGA4 (Molecular Evolutionary Genetics Analysis software, Tempe, AZ, United States).

Statistical analysis

The statistical analyses for the polymorphisms were done

with the online tool for SNP analysis, SNPStats (<https://www.snpstats.net/start.htm?http://bioinfo.iconcolgia.net/SNPstats>). The best inheritance model was chosen according to the lowest values of Akaike's information criterion (AIC) and Bayesian information criterion (BIC). Continuous variables were expressed as means and standard deviations. Differences between groups were determined by unpaired *t*-tests after verification of normal distribution, or converted to normalize the data.

The association of SNPs in matched case-control samples was tested using χ^2 analysis and calculation of odds ratios (ORs) with 95% confidence intervals (CIs). All tests were performed using the SPSS statistics package version 22 (IBM Corp., Armonk, NY, United States) and Epi Info[™] statistics package version 7 (Atlanta, GA, United States). Significant statistical differences were assumed in all cases having adjusted *P* < 0.05.

RESULTS

We genotyped two *TCF7L2* SNPs-rs7903146 and rs12255372-in a population-based sample of 310 Uruguayan subjects, including 133 control subjects and 177 patients clinically diagnosed with T2D. The most relevant anthropometric values for the T2D and control groups are presented in Table 1. The T2D atypical and classical subgroups are presented in Table 2, showing the main clinical characteristics of each.

The best inheritance model was the dominant model for both SNPs analyzed: rs12255372= G/G vs G/T+T/T, AIC = 423.4, BIC = 430.8; and rs7903146 = C/C vs C/T+T/T, AIC = 426.4, BIC = 433.9. The rs12255372 SNP was the only variation that showed high association with the disease (Table 3). Comparative statistical analysis of the atypical and classical diabetes subgroups showed association only between the classical diabetes *versus* controls for rs12255372 (Table 4).

DISCUSSION

Diabetes mellitus is a complex disease, in which genetic and environmental factors are interweaved. After the discovery of *TCF7L2* as a key player in T2D etiology, several works in multiethnic populations identified two main SNPs in this gene, rs12255372 and rs7903146, and characterized them as the most relevantly associated to T2D^[9,10,17,21]. In our previous works^[3-5], we reclassified patients who are clinically diagnosed as T2D into two subgroups, representing the classical and atypical cases, as described in the Materials and Methods section. Intriguingly, these previous studies consistently found that the two case categories were different at the genetic level, involving several genes. In the current study, we amplified the genetic characterization of atypical diabetes in Uruguayans to include the analysis of two SNPs strongly related with T2D according to other populations studied and reported.

Analyzing the T2D study population in comparison to

Table 2 Clinical characteristics of the atypical and classical diabetes cases

	Atypical diabetes, <i>n</i> = 92	Classical diabetes, <i>n</i> = 85	<i>P</i>
Age (yr)	61.29 ± 13.08	65.74 ± 9.93	0.011 ^b
Age (yr)	43.36 ± 12.62	45.93 ± 14.67	0.304
BMI (kg/m ²)	32.22 ± 5.48	31.32 ± 5.96	0.288
HbA1c, %	8.27 ± 1.80	8.27 ± 1.77	0.993
Total cholesterol (mmol/L)	5.18 ± 1.14	5.53 ± 1.16	0.044 ^a
HDL (mmol/L)	1.24 ± 0.29	1.32 ± 0.33	0.080
LDL (mmol/L)	2.84 ± 1.02	3.26 ± 1.11	0.010 ^b
Triglycerides (mmol/L)	2.24 ± 1.40	2.24 ± 1.4	0.991
TG/HDL	4.47 ± 3.41	4.43 ± 4.73	0.941

^a*P* < 0.05, ^b*P* < 0.01, for all parameters. BMI: Body mass index; HbA1c: Glycated hemoglobin; HDL: High-density lipoprotein-cholesterol; LDL: Low-density lipoprotein-cholesterol; TG/HDL: Insulin resistance index.

Table 3 Genotype frequencies of rs12255372 and rs7903146 in controls and cases

SNPs	Healthy controls, %	Type 2 diabetes cases, %	OR (95%CI)	<i>P</i>
rs12255372 G > T				
G/T + T/T	48.9	60.5	1.6 (1.02-2.51)	0.04
G/G	51.1	39.5		
T allele	30	37	1.37 (0.97-1.92)	NS
G allele	70	63		
rs7903146 C > T				
C/T + T/T	46.6	52.5	1.27 (0.81-1.99)	NS
C/C	53.4	47.5		
T allele	29	34	1.22 (0.87-1.72)	NS
C allele	71	66		

CI: Confidence interval; NS: Non-significant; OR: Odds ratio; SNP: Single nucleotide polymorphism.

Table 4 Genotype frequencies of rs12255372 and rs7903146 in controls, atypical diabetes and classical diabetes patients

SNPs	Controls, %	Atypical diabetes, %	Classical diabetes, %	OR (95%CI)	<i>P</i>
rs12255372 G > T					
G/T+T/T	65	-	57	2.1 (1.21-3.75)	0.008
G/G	68	-	28		
G/T+T/T	65	50	-	1.2 (0.73-2.12)	NS
G/G	68	42	-		
rs7903146 C > T					
C/T + T/T	62	-	47	1.4 (0.82-2.45)	NS
C/C	71	-	38		
C/T+T/T	62	46	-	1.2 (0.67-1.95)	NS
C/C	71	46	-		

SNP: Single nucleotide polymorphism; CI: Confidence interval; NS: Non-significant; OR: Odds ratio; SNP: Single nucleotide polymorphism.

the control population showed us a significant association of the rs12255372 SNP. The same results have been found in other studies of different populations, and the presence of the T allele was also found to be associated to major proneness to T2D^[21,31,32]. Subsequent analysis of our results from the T2D patients upon subgrouping according to atypical and classical cases, and in comparison with controls, showed an increase in OR when we removed the atypical patients from the analysis for rs12255372. This finding could indicate that the atypical subpopulation of T2D patients could serve as a confounding factor in general analyses of T2D patients, highlighting the potential of the overall T2D population being a mixture of case subgroups. This subgroup profile may help to explain

previous results observed in different studies that used the T2D pooled population as a unique group.

The ideas expressed above are in accordance with the notion that atypical patients could be framed as a separated group from patients with classical T2D^[3]. Another perspective sets atypical diabetes in a mid-course status between type 1 diabetes and T2D, as described by Pozzilli *et al.*^[33]; as such, the atypical diabetes case would be located in the middle of the T2D disease spectrum. This concept goes along with the so-called "accelerator hypothesis", which states that β-cell loss could be variably accelerated by the conjunction and different weight of three different processes: insulin resistance, autoimmunity and constitution^[34,35]. The β-cells of those

individuals carrying the *TCF7L2* gene mutations are more susceptible than others to the metabolic demands of insulin resistance^[36], but not as susceptible as those carrying the HLA DR3/DR4 haplotype, as in the case of the atypical diabetes population, providing a combination of unfavorable genetic background, impairment in β -cell secretion and a diminished survival upon challenge with hyperglycemic stress, as well as establishing an auto-immune cell environment^[14,26,37].

Interestingly, the rs7903146 SNP did not show significant association with T2D in our analyses. The Uruguayan population has a three-hybrid admixed origin-European, African and Amerindian; the Caucasian component represents a major proportion, but there is a significant mixture degree and a noteworthy Amerindian component of maternal origin^[38]. Studies performed in different Asian populations^[39-42] have found no significant association between this SNP of the *TCF7L2* gene and T2D. Thus, one could ponder the idea that the Uruguayan population may be more related to Asian populations than expected; this idea might also be supported by the theory that the American continent was populated by Asian ancestors^[38].

Beyond the ethnic influence that has also been found in other studies of rs12255372 and rs7903146^[17-25], this study showed that the association of rs12255372 with diabetes was increased when the population was reclassified as subgroups of case types and only the classical T2D patients were compared with controls. This finding suggests the importance of taking into consideration the existence of an atypical group, which could serve to obscure the real association of SNPs in the *TCF7L2* gene. On the other hand, it is important to note that studies using populations of patients with LADA have found differences in the polymorphisms of the *TCF7L2* gene as well as with T2D^[43,44]. This finding reinforces the theory that LADA and atypical diabetes are distinct entities.

To continue the characterization of the atypical diabetes subpopulation it will be important to obtain measurements of C-peptide from the patients, so as to study if there is any difference for this marker between the subpopulations classified. The C-peptide is a precursor of insulin whose measurement shows the reserve of secretion of the same by the pancreatic β -cell. It is very useful in those patients with poor response to antihyperglycemic medication, as is the case of our atypical patients. Therefore, this technique would represent another resource to help in the classification and a more appropriate therapeutic in this population of complex patients.

Today, in our country, the investigation of C-peptide is not routinely performed and is only carried out in some specialized centers. Therefore, it becomes of paramount importance to implement the C-peptide measurement in the Healthcare Centers and Hospitals in our country. In addition, it will be important to continue characterizing the atypical diabetes subpopulation through the study of genetic markers. Identification and characterization of disease-specific genetic markers will help doctors to more

readily and more accurately classify these cases, according to an etiopathogenic base. Such could also lead us to designing and implementing a therapy that will avoid or minimize trial and error time.

At the same time, therapeutic inertia would facilitate advancement of the chronic complications of this pathology. In the group of patients investigated in this study, we took into account the presence of clinical biomarkers. Although we cannot speak from a statistical point of view (due to the short time elapsed during the study period), we have managed to individualize the therapy (data not shown). In turn, this has led our patients with atypical presentation to have greater confidence in the treatment used, such as the acceptance of a timely insulinization.

Ultimately, this study showed that the application of a translational medicine research approach provides knowledge of basic science that can be applied directly in the clinic towards the resolution of complex clinical cases.

We have studied two of the most relevant SNP variants related to T2D, in the *TCF7L2* gene, in a Uruguayan diabetic population stratified by genotype differences. The present and previous works support the idea that the combined effect of several predisposition variants would turn the atypical subpopulation into a new classification and serve as therapeutic targets^[45].

Currently, there are different classifications that encompass atypical patients, placing them within different categories. Steenkamp *et al.*^[46] refer to a group of patients who would meet some of the criteria described herein as diabetic (ketosis-prone diabetes), while other authors locate these patients within a subset of the LADA patients^[47]. Overall, this reaffirms the necessity to continue the genetic analysis of this particular population to achieve a more adequate classification and treatment of these patients.

ARTICLE HIGHLIGHTS

Research background

In a high percentage of patients, clinical presentation alone does not define the type of diabetes. This is very important, since it hinders implementation of an individualized and safe treatment. The current classification system of diabetes is useful and easy for typical patients. However, there are many situations in which it is difficult to determine what type of diabetes is presenting due to the great heterogeneity in the pathogenesis. The current classification of diabetes is not satisfactory and its revision has been under consideration for many years. Previous studies carried out in the Uruguayan population have demonstrated the existence of patients for who it is not possible to classify into any of the categories provided in the international guidelines. We continue to investigate this type of patient because it is very important to assist them appropriately and improve their quality of life. In this way, it is possible to abolish the trial stage and error that patients suffer from when not being correctly diagnosed. At this time, different researchers have proposed that the classifications of diabetes should be revised, and this is the principal objective of our work. We have emphatically proposed the inclusion of genetics determination for HLA to elucidate atypical diabetes patients. Such an approach and related data will permit correct classification and treatment for these kinds of patients.

Research motivation

To date, we have investigated genes related to type 1 diabetes in patients with atypical diabetes. In this study, we sought to analyze the major gene related to

type 2 diabetes, the *TCF7L2* gene, in the atypical diabetes patients.

Research objectives

To analyze the association of the two most important single nucleotide polymorphisms (SNPs) of the *TCF7L2* gene-rs12255372 and rs7903146-with atypical diabetes.

Research methods

This case-control study was conducted in atypical and classical cases of type 2 diabetes using genotyping with *TaqMan* probes for the rs12255372 and rs7903146SNPs of the *TCF7L2* gene.

Research results

The SNPs of the *TCF7L2* gene that were analyzed in this work showed no association with atypical diabetes; nevertheless, the rs12255372 SNP was associated with classical diabetes.

Research conclusions

As has been shown in previous studies, the genetics of atypical diabetes are different from those of classical diabetes, despite a shared phenotype.

Research perspectives

To continue the characterization of the atypical diabetes subpopulation it will be important to obtain measurements of C-peptide in these patients and to study if there is any difference for this marker between the populations classified.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the personnel of the Hemotherapy Department at the Pasteur Hospital, Montevideo, Uruguay.

REFERENCES

- 1 Ferrero R, García MV. Encuesta de prevalencia de la diabetes en Uruguay. *Arch Med Interna* 2005; **27**: 7–12
- 2 Instituto Nacional de Estadística. Censos 2011 – contame que te cuento. Available from: URL: <http://www5.ine.gub.uy/censos2011/index.html>
- 3 Mimbacas A, García L, Zorrilla P, Acosta M, Airaud C, Ferrero R, Pena A, Simonelli B, Soto E, Vitarella G, Fernandez J, Javiel G. Genotype and phenotype correlations in diabetic patients in Uruguay. *Genet Mol Res* 2009; **8**: 1352–1358 [PMID: 19937591 DOI: 10.4238/vol8-4gmr667]
- 4 Fernández M, Fabregat M, Javiel G, Mimbacas A. HLA alleles may serve as a tool to discriminate atypical type 2 diabetic patients. *World J Diabetes* 2014; **5**: 711–716 [PMID: 25317248 DOI: 10.4239/wjd.v5.i5.711]
- 5 Fabregat M, Fernandez M, Javiel G, Vitarella G, Mimbacas A. The Genetic Profile from HLA and Non-HLA Loci Allows Identification of Atypical Type 2 Diabetes Patients. *J Diabetes Res* 2015; **2015**: 485132 [PMID: 26273670 DOI: 10.1155/2015/485132]
- 6 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2014; **37** Suppl 1: S81–S90 [PMID: 24357215 DOI: 10.2337/dc14-S081]
- 7 Furlanos S, Dotta F, Greenbaum CJ, Palmer JP, Rolandsson O, Colman PG, Harrison LC. Latent autoimmune diabetes in adults (LADA) should be less latent. *Diabetologia* 2005; **48**: 2206–2212 [PMID: 16193284 DOI: 10.1007/s00125-005-1960-7]
- 8 Sanghera DK, Nath SK, Ortega L, Gambarelli M, Kim-Howard X, Singh JR, Ralhan SK, Wander GS, Mehra NK, Mulvihill JJ, Kamboh MI. TCF7L2 polymorphisms are associated with type 2 diabetes in Khatri Sikhs from North India: genetic variation affects lipid levels. *Ann Hum Genet* 2008; **72**: 499–509 [PMID: 18397358 DOI: 10.1111/j.1469-1809.2008.00443.x]
- 9 Peng S, Zhu Y, Lü B, Xu F, Li X, Lai M. TCF7L2 gene polymorphisms

- and type 2 diabetes risk: a comprehensive and updated meta-analysis involving 121,174 subjects. *Mutagenesis* 2013; **28**: 25–37 [PMID: 23188737 DOI: 10.1093/mutage/ges048]
- 10 Qiao H, Zhang X, Zhao X, Zhao Y, Xu L, Sun H, Fu S. Genetic variants of TCF7L2 are associated with type 2 diabetes in a northeastern Chinese population. *Gene* 2012; **495**: 115–119 [PMID: 22245614 DOI: 10.1016/j.gene.2011.12.055]
- 11 Shu L, Matveyenko AV, Kerr-Conte J, Cho JH, McIntosh CH, Maedler K. Decreased TCF7L2 protein levels in type 2 diabetes mellitus correlate with downregulation of GIP- and GLP-1 receptors and impaired beta-cell function. *Hum Mol Genet* 2009; **18**: 2388–2399 [PMID: 19386626 DOI: 10.1093/hmg/ddp178]
- 12 Polakis P. Wnt signaling in cancer. *Cold Spring Harb Perspect Biol* 2012; **4**: [PMID: 22438566 DOI: 10.1101/cshperspect.a008052]
- 13 Alami FM, Ahmadi M, Bazrafshan H, Tabarraei A, Khosravi A, Tabatabaiefar MA, Samaei NM. Association of the TCF7L2 rs12255372 (G/T) variant with type 2 diabetes mellitus in an Iranian population. *Genet Mol Biol* 2012; **35**: 413–417 [PMID: 22888288 DOI: 10.1590/S1415-47572012005000029]
- 14 Pearson ER. Translating TCF7L2: from gene to function. *Diabetologia* 2009; **52**: 1227–1230 [PMID: 19387612 DOI: 10.1007/s00125-009-1356-1]
- 15 Villareal DT, Robertson H, Bell GI, Patterson BW, Tran H, Wice B, Polonsky KS. TCF7L2 variant rs7903146 affects the risk of type 2 diabetes by modulating incretin action. *Diabetes* 2010; **59**: 479–485 [PMID: 19934000 DOI: 10.2337/db09-1169]
- 16 Ali O. Genetics of type 2 diabetes. *World J Diabetes* 2013; **4**: 114–123 [PMID: 23961321 DOI: 10.4239/wjd.v4.i4.114]
- 17 Jia HY, Li QZ, Lv LF. Association between transcription factor 7-like 2 genetic polymorphisms and development of type 2 diabetes in a Chinese population. *Genet Mol Res* 2016; **15** [PMID: 27323175 DOI: 10.4238/gmr.15028223]
- 18 Liu XH, Xie CG, An Y, Zhang XX, Wu WB. Meta-analysis of the association between the rs7903146 polymorphism at the TCF7L2 locus and type 2 diabetes mellitus susceptibility. *Genet Mol Res* 2015; **14**: 16856–16862 [PMID: 26681031 DOI: 10.4238/2015.December.14.12]
- 19 Assmann TS, Duarte GC, Rheinheimer J, Cruz LA, Canani LH, Crispim D. The TCF7L2 rs7903146 (C/T) polymorphism is associated with risk to type 2 diabetes mellitus in Southern-Brazil. *Arq Bras Endocrinol Metabol* 2014; **58**: 918–925 [PMID: 25627047 DOI: 10.1590/0004-2730000003510]
- 20 Carrasco Espí P, Rico Sanz J, Ortega Azorín C, González Arráez JJ, Ruiz de la Fuente S, Asensio Márquez EM, Estruch Riba R, Corella Piquer D. Consistente asociación del polimorfismo rs7903146 en el gen TCF7L2 con mayor riesgo de diabetes en población mediterránea española. *Clínica e Invest en Arterioscler* 2011; **23**: 125–132 [DOI: 10.1016/j.arteri.2011.04.002]
- 21 Wang J, Zhang J, Li L, Wang Y, Wang Q, Zhai Y, You H, Hu D. Association of rs12255372 in the TCF7L2 gene with type 2 diabetes mellitus: a meta-analysis. *Braz J Med Biol Res* 2013; **46**: 382–393 [PMID: 23579632 DOI: 10.1590/1414-431X20132677]
- 22 Luo Y, Wang H, Han X, Ren Q, Wang F, Zhang X, Sun X, Zhou X, Ji L. Meta-analysis of the association between SNPs in TCF7L2 and type 2 diabetes in East Asian population. *Diabetes Res Clin Pract* 2009; **85**: 139–146 [PMID: 19482368 DOI: 10.1016/j.diabres.2009.04.024]
- 23 Tong Y, Lin Y, Zhang Y, Yang J, Zhang Y, Liu H, Zhang B. Association between TCF7L2 gene polymorphisms and susceptibility to type 2 diabetes mellitus: a large Human Genome Epidemiology (HuGE) review and meta-analysis. *BMC Med Genet* 2009; **10**: 15 [PMID: 19228405 DOI: 10.1186/1471-2350-10-15]
- 24 Bodhini D, Radha V, Dhar M, Narayani N, Mohan V. The rs12255372(G/T) and rs7903146(C/T) polymorphisms of the TCF7L2 gene are associated with type 2 diabetes mellitus in Asian Indians. *Metabolism* 2007; **56**: 1174–1178 [PMID: 17697858 DOI: 10.1016/j.metabol.2007.04.012]
- 25 Cauchi S, El Achhab Y, Choquet H, Dina C, Kremler F, Weitgasser R, Nejari C, Patsch W, Chikri M, Meyre D, Froguel P. TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. *J Mol Med (Berl)* 2007; **85**: 777–782

- [PMID: 17476472 DOI: 10.1007/s00109-007-0203-4]
- 26 **Pang DX**, Smith AJ, Humphries SE. Functional analysis of TCF7L2 genetic variants associated with type 2 diabetes. *Nutr Metab Cardiovasc Dis* 2013; **23**: 550-556 [PMID: 22402060 DOI: 10.1016/j.numecd.2011.12.012]
 - 27 **Saxena R**, Gianniny L, Burt NP, Lyssenko V, Giuducci C, Sjögren M, Florez JC, Almgren P, Isomaa B, Orho-Melander M, Lindblad U, Daly MJ, Tuomi T, Hirschhorn JN, Ardlie KG, Groop LC, Altshuler D. Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes* 2006; **55**: 2890-2895 [PMID: 17003358 DOI: 10.2337/db06-0381]
 - 28 **Pilgaard K**, Jensen CB, Schou JH, Lyssenko V, Wegner L, Brøns C, Vilsbøll T, Hansen T, Madsbad S, Holst JJ, Vølund A, Poulsen P, Groop L, Pedersen O, Vaag AA. The T allele of rs7903146 TCF7L2 is associated with impaired insulinotropic action of incretin hormones, reduced 24 h profiles of plasma insulin and glucagon, and increased hepatic glucose production in young healthy men. *Diabetologia* 2009; **52**: 1298-1307 [PMID: 19288077 DOI: 10.1007/s00125-009-1307-x]
 - 29 **Nathan DM**, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B; American Diabetes Association; European Association for Study of Diabetes. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2009; **32**: 193-203 [PMID: 18945920 DOI: 10.2337/dc08-9025]
 - 30 **Mimbacas A**, Pérez-Bravo F, Santos JL, Pisciotto C, Grignola R, Javiel G, Jorge AM, Cardoso H. The association between HLA DQ genetic polymorphism and type 1 diabetes in a case-parent study conducted in an admixed population. *Eur J Epidemiol* 2004; **19**: 931-934 [PMID: 15575351 DOI: 10.1007/s10654-004-5176-9]
 - 31 **González-Sánchez JL**, Martínez-Larrad MT, Zabena C, Pérez-Barba M, Serrano-Rios M. Association of variants of the TCF7L2 gene with increases in the risk of type 2 diabetes and the proinsulin: insulin ratio in the Spanish population. *Diabetologia* 2008; **51**: 1993-1997 [PMID: 18712344 DOI: 10.1007/s00125-008-1129-2]
 - 32 **Ciccacci C**, Di Fusco D, Cacciotti L, Morganti R, D'Amato C, Novelli G, Sanguolo F, Spallone V, Borgiani P. TCF7L2 gene polymorphisms and type 2 diabetes: association with diabetic retinopathy and cardiovascular autonomic neuropathy. *Acta Diabetol* 2013; **50**: 789-799 [PMID: 22843023 DOI: 10.1007/s00592-012-0418-x]
 - 33 **Pozzilli P**, Buzzetti R. A new expression of diabetes: double diabetes. *Trends Endocrinol Metab* 2007; **18**: 52-57 [PMID: 17208448 DOI: 10.1016/j.tem.2006.12.003]
 - 34 **Wilkin TJ**. The accelerator hypothesis cannot be tested using the type 2 diabetes gene, TCF7L2. *Diabetologia* 2007; **50**: 1780 [PMID: 17589824 DOI: 10.1007/s00125-007-0725-x]
 - 35 **Wilkin TJ**. The accelerator hypothesis: weight gain as the missing link between Type I and Type II diabetes. *Diabetologia* 2001; **44**: 914-922 [PMID: 11508279 DOI: 10.1007/s001250100548]
 - 36 **Grant SF**, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadóttir A, Styrkarsdóttir U, Magnusson KP, Walters GB, Palsdóttir E, Jonsdóttir T, Gudmundsdóttir T, Gylfason A, Saemundsdóttir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdóttir U, Gulcher JR, Kong A, Stefansson K. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 2006; **38**: 320-323 [PMID: 16415884 DOI: 10.1038/ng1732]
 - 37 **Le Bacquer O**, Shu L, Marchand M, Neve B, Paroni F, Kerr Conte J, Pattou F, Froguel P, Maedler K. TCF7L2 splice variants have distinct effects on beta-cell turnover and function. *Hum Mol Genet* 2011; **20**: 1906-1915 [PMID: 21357677 DOI: 10.1093/hmg/ddr072]
 - 38 **Salzano FM**, Sans M. Interethnic admixture and the evolution of Latin American populations. *Genet Mol Biol* 2014; **37**: 151-170 [PMID: 24764751 DOI: 10.1590/S1415-47572014000200003]
 - 39 **Pourahmadi M**, Erfanian S, Moradzadeh M, Jahromi AS. Non-Association between rs7903146 and rs12255372 Polymorphisms in Transcription Factor 7-Like 2 Gene and Type 2 Diabetes Mellitus in Jahrom City, Iran. *Diabetes Metab J* 2015; **39**: 512-517 [PMID: 26616591 DOI: 10.4093/dmj.2015.39.6.512]
 - 40 **Saadi H**, Nagelkerke N, Carruthers SG, Benedict S, Abdulkhalek S, Reed R, Lukic M, Nicholls MG. Association of TCF7L2 polymorphism with diabetes mellitus, metabolic syndrome, and markers of beta cell function and insulin resistance in a population-based sample of Emirati subjects. *Diabetes Res Clin Pract* 2008; **80**: 392-398 [PMID: 18282631 DOI: 10.1016/j.diabres.2008.01.008]
 - 41 **Chang YC**, Chang TJ, Jiang YD, Kuo SS, Lee KC, Chiu KC, Chuang LM. Association study of the genetic polymorphisms of the transcription factor 7-like 2 (TCF7L2) gene and type 2 diabetes in the Chinese population. *Diabetes* 2007; **56**: 2631-2637 [PMID: 17579206 DOI: 10.2337/db07-0421]
 - 42 **Guo T**, Hanson RL, Traurig M, Muller YL, Ma L, Mack J, Kobes S, Knowler WC, Bogardus C, Baier LJ. TCF7L2 is not a major susceptibility gene for type 2 diabetes in Pima Indians: analysis of 3,501 individuals. *Diabetes* 2007; **56**: 3082-3088 [PMID: 17909099 DOI: 10.2337/db07-0621]
 - 43 **Zampetti S**, Spoletini M, Petrone A, Capizzi M, Arpi ML, Tiberti C, Di Pietro S, Bosi E, Pozzilli P, Giorgino F, Buzzetti R; Nirad Study Group. Association of TCF7L2 gene variants with low GAD autoantibody titre in LADA subjects (NIRAD Study 5). *Diabet Med* 2010; **27**: 701-704 [PMID: 20546291 DOI: 10.1111/j.1464-5491.2010.02997.x]
 - 44 **Szepietowska B**, Moczulski D, Wawrusiewicz-Kurylonek N, Grzeszczak W, Gorska M, Szelachowska M. Transcription factor 7-like 2-gene polymorphism is related to fasting C peptide in latent autoimmune diabetes in adults (LADA). *Acta Diabetol* 2010; **47**: 83-86 [PMID: 19533015 DOI: 10.1007/s00592-009-0133-4]
 - 45 **Schwartz SS**, Epstein S, Corkey BE, Grant SF, Gavin JR 3rd, Aguilar RB. The Time Is Right for a New Classification System for Diabetes: Rationale and Implications of the β -Cell-Centric Classification Schema. *Diabetes Care* 2016; **39**: 179-186 [PMID: 26798148 DOI: 10.2337/dc15-1585]
 - 46 **Steenkamp DW**, Alexanian SM, Sternthal E. Approach to the patient with atypical diabetes. *CMAJ* 2014; **186**: 678-684 [PMID: 24396100 DOI: 10.1503/cmaj.130185]
 - 47 **Manuel García de los Ríos A**, Pilar Durruty A, editors. Diabetes Mellitus, 3^{ed}. Rethymno: Mediterráneo, 2013

P- Reviewer: Beltowski j, Hasan m, Hssan MMA, Kusmic C

S- Editor: Ma YJ **L- Editor:** A **E- Editor:** Yin SY





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 October 15; 9(10): 165-179



**ORIGINAL ARTICLE****Retrospective Cohort Study**

- 165 Efficacy of anagliptin as compared to linagliptin on metabolic parameters over 2 years of drug consumption: A retrospective cohort study

Hamasaki H, Hamasaki Y

SYSTEMATIC REVIEWS

- 172 Topical application of platelet-rich plasma for diabetic foot ulcers: A systematic review

Hirase T, Ruff E, Surani S, Ratnani I

Contents

World Journal of Diabetes
Volume 9 Number 10 October 15, 2018

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Shannon Miller, Pharm D, Doctor, Pharmacotherapy Faculty, Florida Hospital East, Family Practice Residency, Orlando, FL 32822, United States

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, and PubMed Central, Scopus, China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Ying-Na Bian*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Jin-Lai Wang*

NAME OF JOURNAL

World Journal of Diabetes

ISSN

ISSN 1948-9358 (online)

LAUNCH DATE

June 15, 2010

FREQUENCY

Monthly

EDITOR-IN-CHIEF

Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

EDITORIAL BOARD MEMBERS

All editorial board members resources online at <http://www.wjgnet.com>

www.wjgnet.com/1948-9358/editorialboard.htm

EDITORIAL OFFICE

Jin-Lai Wang, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER

Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE

October 15, 2018

COPYRIGHT

© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS

<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION

<http://www.f6publishing.com>

Retrospective Cohort Study

Efficacy of anagliptin as compared to linagliptin on metabolic parameters over 2 years of drug consumption: A retrospective cohort study

Hidetaka Hamasaki, Yasuteru Hamasaki

Hidetaka Hamasaki, Endocrinology and Metabolism, Internal Medicine, Hamasaki Clinic, Kagoshima 890-0046, Japan

Yasuteru Hamasaki, Diabetes, Hamasaki Clinic, Kagoshima 890-0046, Japan

ORCID number: Hidetaka Hamasaki (0000-0002-0124-597X); Yasuteru Hamasaki (0000-0002-1522-0132).

Author contributions: Hamasaki H and Hamasaki Y equally contributed to study conception, data acquisition, data analysis, interpretation, and writing of article.

Institutional review board statement: This study protocol was reviewed and approved by the Japan Medical Association Ethical Review Board.

Informed consent statement: Study participants are assured that collected data will be used only for this study and will not be disclosed without the consent of the participants.

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

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.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Hidetaka Hamasaki, MD, PhD, Doctor,

Endocrinology and Metabolism, Internal Medicine, Hamasaki Clinic, Nishida 2-21-4, Kagoshima 890-0046, Japan. hhamasaki78@gmail.com
Telephone: +81-99-2503535
Fax: +81-99-2501470

Received: June 25, 2018

Peer-review started: June 25, 2018

First decision: July 9, 2018

Revised: July 12, 2018

Accepted: August 26, 2018

Article in press: August 26, 2018

Published online: October 15, 2018

Abstract

AIM

To evaluate the comparative effectiveness of anagliptin and linagliptin on the clinical parameters in patients with type 2 diabetes mellitus (T2DM).

METHODS

A 2-year retrospective cohort study was conducted in patients with T2DM who received anagliptin and linagliptin. We enrolled 234 patients (anagliptin group, 117 patients; linagliptin group, 117 patients).

RESULTS

The glycemic control considerably improved 3, 6, 12, and 24 mo after the administration of both dipeptidyl peptidase-4 (DPP-4) inhibitors. Following the administration of anagliptin, the diastolic blood pressure and serum total cholesterol levels decreased. However, serum high-density lipoprotein cholesterol levels increased and urinary albumin-creatinine ratio decreased following linagliptin administration. Furthermore, the liver function improved after the administration of linagliptin.

CONCLUSION

These findings suggest that the efficacy of DPP-4 inhibitors on the blood pressure, lipid profile, and liver function differs between anagliptin and linagliptin.

Key words: Type 2 diabetes mellitus; Dipeptidyl peptidase-4 inhibitor; Anagliptin; Linagliptin; Cholesterol

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Whether a substantial clinical difference exists in the effect of dipeptidyl peptidase-4 (DPP-4) inhibitors on metabolic parameters remains inconclusive. Although this study is a relatively small-scale, short duration, retrospective study, the findings of this study suggests that the efficacy of DPP-4 inhibitors on the blood pressure, lipid profile, and liver function differs between anagliptin and linagliptin.

Hamasaki H, Hamasaki Y. Efficacy of anagliptin as compared to linagliptin on metabolic parameters over 2 years of drug consumption: A retrospective cohort study. *World J Diabetes* 2018; 9(10): 165-171 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i10/165.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i10.165>

INTRODUCTION

Dipeptidyl peptidase-4 (DPP-4) inhibitors are extensively used in patients with type 2 diabetes mellitus (T2DM). As Asian patients with T2DM are typically characterized by β -cell dysfunction and exhibit less adiposity and insulin resistance than Caucasian patients with T2DM, DPP-4 inhibitors could be more effective in Asians^[1]. Although DPP-4 inhibitors do not exert lower cardiovascular mortality compared with glucagon-like peptide I (GLP-1) receptor agonists and sodium-glucose cotransporter 2 inhibitors^[2], they exhibit good tolerability and safety in elderly people with renal impairment and liver disease^[3]. Thus, DPP-4 inhibitors could be considered as one of the first/second preferences for treating T2DM^[1,4]. In Japan, the following seven types of once- or twice-daily DPP-4 inhibitors are currently available: Sitagliptin, vildagliptin, alogliptin, linagliptin, teneligliptin, anagliptin, and saxagliptin. However, anagliptin is not commonly available in countries other than Japan^[5], and few studies have directly compared the efficacy of anagliptin with other gliptins for the management of patients with T2DM. Anagliptin is a unique DPP-4 inhibitor because it possibly reduces low-density lipoprotein cholesterol (LDL-C) levels^[6,7]. However, it is unclear whether this effect is specific to anagliptin, and the reproducibility has not been substantially proven^[8]. Thus, this study aims to assess the comparative effectiveness of anagliptin and linagliptin on the glycemic control, blood pressure, lipid profile, and liver and renal function in Japanese patients with T2DM.

MATERIALS AND METHODS

Study design and patients

We conducted this retrospective cohort study in patients with T2DM who were treated at Hamasaki Clinic (diabetes-specialty clinic). We enrolled outpatients who were treated with anagliptin or linagliptin between April 2012 and September 2017. However, we excluded patients aged < 20 years, previously treated with DPP-4 inhibitors, changed the type of DPP-4 inhibitors, and discontinued treatment with DPP-4 inhibitors during the study period. We followed up all enrolled patients at 3, 6, 12, and 24 mo after initiation of DPP-4 inhibitors therapy. This study protocol was approved by the Japan Medical Association Ethical Review Board (Reference No. 29-6), and the study was performed in accordance with the Declaration of Helsinki.

Medical history recording

We recorded patients' duration of diabetes, smoking history, drinking habit, and history of cardiovascular disease (CVD) before commencing the treatment with DPP-4 inhibitors. In addition, we confirmed the medication adherence of study patients at every medical examination.

Anthropometric and physiological measurements

Patient height and weight were measured using a rigid stadiometer and calibrated scales, respectively. We calculated the body mass index (BMI) as body weight in kilograms divided by the square of body height in meters. In addition, the blood pressure was measured in a seated position using an automatic sphygmomanometer (KM-382; Kenzmedico Co., Ltd., Saitama, Japan).

Blood and urinary examinations

We measured plasma glucose (PG), hemoglobin A1c (HbA1c; HLC-723G9, TOSOH Co., Ltd., Tokyo, Japan), serum total cholesterol (T-C; Determiner L TC II, Kyowa Medex Co., Ltd., Tokyo, Japan), triglycerides (TG; Determiner L TG II, Kyowa Medex Co., Ltd.), high-density lipoprotein cholesterol (HDL-C; Cholestest N HDL, Sekisui Medical Co., Ltd., Tokyo, Japan), and LDL-C (Cholestest LDL, Sekisui Medical Co., Ltd.). In addition, we measured aspartate transaminase (AST), alanine aminotransferase (ALT), creatinine (Cr), and the urinary albumin-Cr ratio (UACR; N-A TIA MicroALB, Nittobo Medical Co., Ltd., Tokyo, Japan) as a marker for diabetic nephropathy.

Statistical analysis

All statistical analyses in this study were performed using SPSS version 24 (IBM Co., Ltd., Chicago, IL). Quantitative variables are presented as mean \pm SD and categorical variables are presented as numbers. We divided all participants into the anagliptin group and linagliptin group. We performed the Student's *t*-test (if normal data distribution), Mann-Whitney *U*-test (if non-normal

Table 1 Subject characteristics at baseline

	Anagliptin	Linagliptin	P-value
Age (yr)	63.5 (12.9)	62.7 (11.9)	0.63
Men/women	74/43	75/42	0.89
Height (cm)	162.1 (10.4)	162.5 (9.1)	0.77
Weight (kg)	64.4 (16.1)	66.1 (14.7)	0.39
BMI (kg/m ²)	24.3 (4.7)	24.9 (4.3)	0.23
Duration of diabetes (yr)	11.9 (8.6)	10.5 (8.5)	0.21
History of CVD (yes/no)	36/81	32/85	0.56
Smoking habit (yes/no)	44/73	46/71	0.79
Drinking habit (yes/no)	65/52	62/55	0.75
SBP (mmHg)	130.4 (18.4)	128.5 (18.7)	0.43
DBP (mmHg)	72.9 (14)	72.9 (11.4)	0.99
AST (U/L)	21.9 (9.3)	29.8 (26.7)	0.029
ALT (U/L)	24.9 (15)	33.1 (35.1)	0.069
Cr (mg/dL)	0.71 (0.19)	0.79 (0.32)	0.03
PG (mmol/L)	10.71 (4.49)	12.06 (5.25)	0.036
HbA1c (mmol/L)	11.1 (2.8)	12 (3.3)	0.053
T-C (mmol/L)	5.3 (0.88)	5.48 (1.02)	0.17
TG (mmol/L)	1.63 (1.17)	2.07 (1.5)	0.009
HDL-C (mmol/L)	1.36 (0.34)	1.33 (0.37)	0.55
LDL-C (mmol/L)	3.19 (0.8)	3.19 (0.88)	0.95
UACR (mg/gCr)	50.60 (78.4)	47.6 (92)	0.65

BMI: Body mass index; CVD: Cardiovascular disease; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; AST: Aspartate transaminase; ALT: Alanine aminotransferase; Cr: Creatinine; PG: Plasma glucose; HbA1c: Hemoglobin A1c; T-C: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; UACR: Urinary albumin creatinine ratio.

data distribution), or χ^2 test to assess the difference in clinical parameters at the baseline between groups. The Friedman test was performed to evaluate the change in clinical parameters during the study period. Furthermore, we calculated the percentage change in clinical parameters (%change) from baseline to the end of study period to compare the effectiveness of anagliptin and linagliptin. Finally, we considered $P < 0.05$ as statistically significant.

RESULTS

We enrolled 234 patients in this study. Among 117 patients in the anagliptin group, 74 were male and 43 were female. Among 117 patients in the linagliptin group, 75 were male and 42 were female. The mean age of patients was 63.5 ± 12.9 years and 62.7 ± 11.9 years, and the mean BMI was 24.3 ± 4.7 kg/m² and 24.9 ± 4.3 kg/m² in the anagliptin and linagliptin groups, respectively. No significant differences were observed in the duration of diabetes, history of CVD, smoking and drinking habits, blood pressure, plasma HbA1c levels, serum T-C levels, HDL-C and LDL-C levels, and UACR; however, PG levels and serum AST, Cr, and TG levels were higher in the linagliptin group compared with the anagliptin group (Table 1). In total, 47 patients received 100-mg anagliptin once per day, and 70 received 100-mg anagliptin twice per day. In addition, we observed no significant differences in the number of patients receiving antihypertensive agents,

cholesterol-lowering agents, insulin therapy, and other oral hypoglycemic agents between both groups. During the study period, no differences were observed in the number of patients receiving additional treatment for hypertension, dyslipidemia, and diabetes between groups (Table 2).

The glycemic control was markedly improved 3, 6, 12, and 24 mo after the administration of both DPP-4 inhibitors. HbA1c levels significantly decreased in both the anagliptin group (from 11.1 ± 2.8 mmol/L to 9.7 ± 1.9 mmol/L) and the linagliptin group (from 12 ± 3.3 mmol/L to 9.2 ± 1.8 mmol/L). However, the %change in HbA1c was lower in the anagliptin group than that in the linagliptin group (-5.6% vs -17.4% , $P = 0.004$). Notably, the diastolic blood pressure decreased 24 mo after the administration of anagliptin (from 72.9 ± 14 mmHg to 69.1 ± 9.5 mmHg); however, no change was observed in the blood pressure in the linagliptin group. The change in diastolic blood pressure was also larger in the anagliptin group than that in the linagliptin group (-9.7% vs -4.9% , $P = 0.044$). In addition, serum T-C levels declined 6 months after the administration of anagliptin (from 5.3 ± 0.88 mmol/L to 5.11 ± 0.78 mmol/L); however, serum HDL-C levels were elevated 24 mo after the administration of linagliptin (from 1.33 ± 0.37 mmol/L to 1.39 ± 0.38 mmol/L). There was also a significant difference in %change in HDL-C levels between groups (2.8% vs 5.6% , $P = 0.037$). However, we observed no changes in LDL-C levels in both groups (from 3.19 ± 0.8 mmol/L to 2.96 ± 0.53 mmol/L and from 3.19 ± 0.88 mmol/L to 2.91 ± 0.75 mmol/L, respectively). Further, no changes were observed in the liver and renal function in the anagliptin group. Nevertheless, serum ALT levels decreased and Cr levels increased in the linagliptin group. We also found a significant difference in %change in Cr levels between groups (6.8% vs 22% , $P = 0.038$); however, there was no significant difference in %change in ALT levels between groups (-2.2% vs -15.2% , $P = 0.088$). Although the UACR decreased in the linagliptin group (from 47.6 ± 92 mg/gCr to 23.8 ± 34.9 mg/gCr), it did not exhibit a substantial change in the anagliptin group (from 50.6 ± 78.4 mg/gCr to 40.7 ± 76.8 mg/gCr) (Table 3).

DISCUSSION

This study illustrates that both anagliptin and linagliptin effectively improve long-term glycemic control; however, the efficacy of DPP-4 inhibitors on the blood pressure, lipid profile, and liver function may differ between anagliptin and linagliptin. Notably, anagliptin decreased the diastolic blood pressure, and linagliptin increased serum HDL-C levels 24 mo after the administration of each DPP-4 inhibitor. However, the efficacy of DPP-4 inhibitors on the blood pressure remains debatable. The elevation in GLP-1 levels accounts for the effects of DPP-4 enzyme inhibition on the cardiovascular function, which leads to cardiac remodeling, improvement of the

Table 2 Medications in study subjects

	Anagliptin	Linagliptin	P-value
Dosage	100 mg; 47; 200 mg; 70	5 mg	-
Anti-hypertensive agents	42	46	0.59
ARB or ACE inhibitors	31	36	0.47
Cholesterol-lowering agents	41	37	0.58
Insulin	7	8	0.79
Concomitant oral hypoglycemic agents			
Metformin	48	42	0.42
Sulfonylureas	44	40	0.59
Glinides	3	4	0.70
α -glucosidase inhibitors	4	6	0.52
Pioglitazone	1	2	0.56
SGLT2 inhibitors	1	0	0.32
No medication	33	37	0.57
Additional medications for hypertension	1	2	0.56
Additional medications for dyslipidemia	3	3	1.00
Additional medications for diabetes	12	16	0.42

ARB: Angiotensin II receptor blocker; ACE: Angiotensin converting enzyme; SGLT2: Sodium-glucose cotransporter 2.

endothelial function, and lowering blood pressure^[9-11]. In our study, we observed no differences in the use of antihypertensive agents, including angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers between the anagliptin and linagliptin groups. Hence, the use of antihypertensive agents did not influence the effect of both DPP-4 inhibitors on blood pressure. Recently, Tahara *et al.*^[12] reported that anagliptin could ameliorate arterial stiffness in association with the reduction of remnant-like particle cholesterol. Although we did not observe any substantial change in LDL-C levels in patients receiving anagliptin, T-C levels decreased after anagliptin administration. The reduction in the diastolic blood pressure in the anagliptin group could be attributed to the amelioration of arterial stiffness by the lipid profile improvement. A systematic review and meta-analysis reported that treatment with DPP-4 inhibitors correlated with a reduction in T-C levels by 7.0 mg/dL^[13]. In addition, anagliptin was reported to improve the lipid profile in both fasting and postprandial conditions in men with T2DM^[14]. Goto *et al.*^[15] recently demonstrated that anagliptin decreased serum T-C and HDL-C levels in ApoE-deficient mice through DPP-4-dependent inhibition of intestinal cholesterol transport. The cholesterol-lowering effect of anagliptin could be attributed to the downregulation of hepatic cholesterol synthesis^[16]. Furthermore, a 24-wk treatment with anagliptin reduced serum apoB-100 levels in patients with T2DM^[7], suggesting that the cholesterol-lowering effect of anagliptin is caused by a reduction in hepatic cholesterol synthesis as well as intestinal cholesterol absorption.

Reportedly, long-term treatment using exenatide, a GLP-1 receptor agonist, increases HDL-C levels by 0.05 mmol/L^[17]; however, to our knowledge, no study to date has reported that DPP-4 inhibitors directly increase HDL-C levels in patients with T2DM. Individuals with metabolic syndrome have dyslipidemia, which is characterized by decreased HDL-C levels and dysfunctional HDL; such

impaired HDL-C metabolism is related to hepatic lipid deposition, such as nonalcoholic fatty liver disease through ApoI deficiency^[18]. Notably, linagliptin has a xanthine-based structure, which could cause pharmacological differences compared with other DPP-4 inhibitors^[19]. Although linagliptin is primarily eliminated by the nonrenal route, its efficacy and tolerability are not affected by hepatic impairment^[19]. In fact, linagliptin could suppress xanthine oxidase activity in patients with T2DM^[20]. In addition, an experimental animal study suggested that linagliptin suppressed adipose tissue inflammation, thus contributing to the reduction in the liver fat content and improvement of hepatic steatosis^[21]. In our study, a decline in serum ALT levels in the linagliptin group suggests that linagliptin could improve fatty liver complicated with T2DM. Furthermore, linagliptin may have increased HDL-C levels because HDL-C metabolism was improved by a reduction in the hepatic fat deposition in this study.

In T2DM, both the blood pressure and renal impairment affect UACR. However, we observed no clinical changes in the blood pressure and serum Cr levels between the baseline and 3 mo following the administration of DPP-4 inhibitors. Reportedly, linagliptin has the potential to decrease albuminuria independent of the glycemic control because of the suppression of renal inflammatory responses mediated by the GLP-1 activity, inhibition of podocyte damage, and tumor necrosis factor- α ^[22]. While the renoprotective effect of linagliptin has been suggested, an increase in serum Cr levels 24 mo after the administration of linagliptin could be attributed to the time-dependent change in the renal function in patients with T2DM. In this study, linagliptin was administered to patients whose renal function was lower than that in patients receiving anagliptin at the baseline. Linagliptin could reduce the UACR but not improve the renal function. At present, randomized controlled clinical trials investigating the effects of linagliptin on CVD and renal function-the cardiovascular outcome study of linagliptin vs glimepiride in patients

Table 3 Changes in clinical parameters after starting the dipeptidyl peptidase-4 inhibitors therapy

	Anagliptin				Linagliptin			
	3 mo	6 mo	12 mo	24 mo	3 mo	6 mo	12 mo	24 mo
Weight (kg)	64.5 (16.2)	65.4 (15.7)	64.7 (15.5)	63.7 (16.4)	66.1 (15.1)	66 (16.9)	67.3 (14.2)	66.4 (14.8)
SBP (mmHg)	128.6 (17.2)	128.2 (14.8)	126.8 (16.6)	130.1 (16.6)	129.3 (17.7)	127.1 (16.4)	128.9 (19.1)	124.9 (16)
DBP (mmHg)	70.7 (11.4)	70.9 (9.9)	69.4 (12.1)	69.1 (9.5) ^b	70.7 (11.4)	69.3 (10.8)	72.5 (11.2)	70.2 (8.6)
AST (U/L)	22.5 (9.6)	22.4 (8.8)	22.3 (10)	23.8 (12.3)	22.7 (12.7)	24.9 (17.1)	23.2 (13.1)	23.7 (12.4)
ALT (U/L)	23 (14.9) ^b	23 (14.8) ^a	22.6 (15.7) ^b	25.5 (18)	23.5 (12.9)	24.8 (14.8)	24 (13.9)	23.7 (15.4)
Cr (mg/dL)	0.74 (0.18)	0.77 (0.2)	0.77 (0.21)	0.77 (0.2)	0.85 (0.33)	0.86 (0.4)	0.9 (0.5)	0.84 (0.38) ^d
PG (mmol/L)	8.96 (3.4) ^a	8.8 (2.9)	8.91 (3.16)	9.12 (4.19) ^b	9.54 (3.82) ^d	9.28 (3.85) ^d	9.48 (3.28) ^d	9.51 (3.5) ^b
HbA1c (mmol/L)	9.3 (2.3) ^d	9.4 (2.1) ^d	9.3 (1.8) ^b	9.7 (1.9) ^b	9.3 (2) ^b	9.1 (1.8) ^d	9.6 (2.2) ^d	9.2 (1.8) ^d
T-C (mmol/L)	5.11 (0.87)	5.11 (0.78) ^b	5.19 (0.69)	5.03 (0.64)	5.11 (0.86)	5.14 (0.89)	5.31 (0.97)	5.09 (0.81)
TG (mmol/L)	1.83 (2.05)	1.73 (1.71)	1.64 (0.92)	1.51 (0.83)	2.1 (1.74)	1.85 (1.23)	2.11 (1.83)	1.76 (1.19)
HDL-C (mmol/L)	1.32 (0.3)	1.31 (0.32)	1.29 (0.27)	1.4 (0.37)	1.31 (0.37)	1.35 (0.42)	1.31 (0.34)	1.39 (0.38) ^a
LDL-C (mmol/L)	3 (0.8)	3.07 (0.78)	3.13 (0.7)	2.96 (0.53)	2.85 (0.86)	2.94 (0.83)	3.04 (0.86)	2.91 (0.75)
UACR (mg/gCr)	40.7 (76.8)	-	-	-	23.8 (34.9)	-	-	-

Superscripts indicate statistically significant changes from baseline: ^a $P < 0.05$; ^b $P < 0.01$; ^d $P < 0.001$. SBP: Systolic blood pressure; DBP: Diastolic blood pressure; AST: Aspartate transaminase; ALT: Alanine aminotransferase; Cr: Creatinine; PG: Plasma glucose; HbA1c: Hemoglobin A1c; T-C: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; UACR: Urinary albumin creatinine ratio.

with type 2 diabetes (CAROLINA) and cardiovascular safety and renal microvascular outcome study with linagliptin (CARMELINA) trials^[23,24] - are ongoing. Hence, further evidence is expected.

This study has several limitations. First, we could not adjust confounding factors, such as dietary intake and physical activity, because of the retrospective nature of this study. Second, the liver and renal function were lower in the linagliptin group than in the anagliptin group at the baseline, which may have affected the difference of serum ALT levels and UACR changes between groups. However, at the baseline, no substantial differences in serum ALT levels and UACR were observed between groups, suggesting that the study results are not because of the difference in patients' characteristics. Third, although we confirmed the medication adherence at every medical examination, we could not ensure whether the study participants kept medication adherence during the study period. Finally, we did not perform blood and urinary tests under constant conditions, which may have decreased the precision of the results, including PG and serum TG levels. Despite these limitations, this study demonstrated that anagliptin and linagliptin could have different effects on metabolic parameters in patients with T2DM.

In conclusion, this study demonstrated that anagliptin decreased diastolic blood pressure and T-C levels, and linagliptin increased HDL-C levels and decreased ALT levels and UACR beside improvement of the glycemic control in Japanese patients with T2DM. This study supports the hypothesis that there could be a drug-specific effect of DPP-4 inhibitors on metabolic parameters beyond their class effect. Thus, we cannot describe the utility of these drugs in clinical practice separately. However, a multicenter, randomized, open-label, parallel-group trial has been conducted to assess the comparative effectiveness of anagliptin and sitagliptin on LDL-C in patients with T2DM and atherosclerosis^[25].

Nevertheless, further investigations are warranted to validate the findings of this study.

ACKNOWLEDGMENTS

The authors appreciate the support of Masayo Yoshidome who collected the data.

ARTICLE HIGHLIGHTS

Research background

Dipeptidyl peptidase-4 (DPP-4) inhibitors are extensively used in patients with type 2 diabetes mellitus (T2DM). DPP-4 inhibitors can improve dyslipidemia and hypertension in addition to glycemic control.

Research motivation

Anagliptin is a unique DPP-4 inhibitor that possibly reduces the low-density lipoprotein cholesterol levels; however, it is not commonly available outside Japan. Few studies have directly compared the efficacy of anagliptin with other gliptins in the management of T2DM.

Research objectives

To assess the comparative effectiveness of anagliptin and linagliptin on the glycemic control, blood pressure, lipid profile, and liver and renal function in Japanese patients with T2DM.

Research methods

A 2-year retrospective cohort study in a diabetes-specialty clinic.

Research results

Both anagliptin and linagliptin effectively improved glycemic control for 2 years. Interestingly, diastolic blood pressure was reduced following the administration of anagliptin, and serum high-density lipoprotein cholesterol levels were increased following the administration of linagliptin. However, no significant changes in serum low-density lipoprotein cholesterol levels were observed in both the anagliptin group and the linagliptin group.

Research conclusions

This study adds to the current literature supporting that the efficacy of DPP-4 inhibitors on metabolic parameters may differ between anagliptin and linagliptin. Both DPP-4 inhibitors may have a unique effect beyond the class effect of

DPP-4 inhibitors. However, whether a substantial clinical difference exists in the effect of DPP-4 inhibitors on metabolic parameters is still inconclusive because this study is a retrospective cohort study.

Research perspectives

We suggest the need for well-designed, large-scale studies to elucidate the effect of DPP-4 inhibitors on metabolic parameters beyond the glucose-lowering effect. Furthermore, comparative efficacy of DPP-4 inhibitors for arterial stiffness should also be investigated in the future.

REFERENCES

- 1 Seino Y, Kuwata H, Yabe D. Incretin-based drugs for type 2 diabetes: Focus on East Asian perspectives. *J Diabetes Investig* 2016; **7** Suppl 1: 102-109 [PMID: 27186364 DOI: 10.1111/jdi.12490]
- 2 Zheng SL, Roddick AJ, Aghar-Jaffar R, Shun-Shin MJ, Francis D, Oliver N, Meeran K. Association Between Use of Sodium-Glucose Cotransporter 2 Inhibitors, Glucagon-like Peptide 1 Agonists, and Dipeptidyl Peptidase 4 Inhibitors With All-Cause Mortality in Patients With Type 2 Diabetes: A Systematic Review and Meta-analysis. *JAMA* 2018; **319**: 1580-1591 [PMID: 29677303 DOI: 10.1001/jama.2018.3024]
- 3 Scheen AJ. The safety of gliptins: updated data in 2018. *Expert Opin Drug Saf* 2018; **17**: 387-405 [PMID: 29468916 DOI: 10.1080/14740338.2018.1444027]
- 4 American Diabetes Association. 8. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018; **41**: S73-S85 [PMID: 29222379 DOI: 10.2337/dc18-S008]
- 5 Nishio S, Abe M, Ito H. Anagliptin in the treatment of type 2 diabetes: safety, efficacy, and patient acceptability. *Diabetes Metab Syndr Obes* 2015; **8**: 163-171 [PMID: 25834461 DOI: 10.2147/DMSO.S54679]
- 6 Kaku K. Effects of anagliptin on serum lipids in Japanese patients with type 2 diabetes-A pooled analysis of long-term therapy with anagliptin (in Japanese). *J Pharmacol Ther* 2012; **40**: 771-784
- 7 Kurozumi A, Okada Y, Arao T, Kobayashi T, Masuda D, Yamashita S, Tanaka Y. Comparison of effects of anagliptin and alogliptin on serum lipid profile in type 2 diabetes mellitus patients. *J Diabetes Investig* 2018; **9**: 360-365 [PMID: 28853228 DOI: 10.1111/jdi.12739]
- 8 Yang HK, Min KW, Park SW, Chung CH, Park KS, Choi SH, Song KH, Kim DM, Lee MK, Sung YA, Baik SH, Kim IJ, Cha BS, Park JH, Ahn YB, Lee IK, Yoo SJ, Kim J, Park IeB, Park TS, Yoon KH. A randomized, placebo-controlled, double-blind, phase 3 trial to evaluate the efficacy and safety of anagliptin in drug-naïve patients with type 2 diabetes. *Endocr J* 2015; **62**: 449-462 [PMID: 25819061 DOI: 10.1507/endocrj.EJ14-0544]
- 9 Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelis L, Stolarski C, Shen YT, Shannon RP. Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. *Circulation* 2004; **110**: 955-961 [PMID: 15313949 DOI: 10.1161/01.CIR.0000139339.85840.DD]
- 10 Basu A, Charkoudian N, Schrage W, Rizza RA, Basu R, Joyner MJ. Beneficial effects of GLP-1 on endothelial function in humans: dampening by glyburide but not by glimepiride. *Am J Physiol Endocrinol Metab* 2007; **293**: E1289-E1295 [PMID: 17711996 DOI: 10.1152/ajpendo.00373.2007]
- 11 Liu L, Liu J, Wong WT, Tian XY, Lau CW, Wang YX, Xu G, Pu Y, Zhu Z, Xu A, Lam KS, Chen ZY, Ng CF, Yao X, Huang Y. Dipeptidyl peptidase 4 inhibitor sitagliptin protects endothelial function in hypertension through a glucagon-like peptide 1-dependent mechanism. *Hypertension* 2012; **60**: 833-841 [PMID: 22868389 DOI: 10.1161/HYPERTENSIONAHA.112.195115]
- 12 Tahara N, Yamagishi SI, Bekki M, Kodama N, Nakamura T, Sugiyama Y, Oshige T, Kumashiro Y, Honda A, Tahara A, Igata S, Fukumoto Y. Anagliptin, A Dipeptidyl Peptidase-4 Inhibitor Ameliorates Arterial Stiffness in Association with Reduction of Remnant-Like Particle Cholesterol and Alanine Transaminase Levels in Type 2 Diabetic Patients. *Curr Vasc Pharmacol* 2016; **14**: 552-562 [PMID: 27357182 DOI: 10.2174/157016114666160625090212]
- 13 Monami M, Lamanna C, Desideri CM, Mannucci E. DPP-4 inhibitors and lipids: systematic review and meta-analysis. *Adv Ther* 2012; **29**: 14-25 [PMID: 22215383 DOI: 10.1007/s12325-011-0088-z]
- 14 Kakuda H, Kobayashi J, Kakuda M, Yamakawa J, Takekoshi N. The effect of anagliptin treatment on glucose metabolism and lipid metabolism, and oxidative stress in fasting and postprandial states using a test meal in Japanese men with type 2 diabetes. *Endocrine* 2015; **48**: 1005-1009 [PMID: 25115636 DOI: 10.1007/s12020-014-0376-x]
- 15 Goto M, Furuta S, Yamashita S, Hashimoto H, Yano W, Inoue N, Kato N, Kaku K. Dipeptidyl peptidase 4 inhibitor anagliptin ameliorates hypercholesterolemia in hypercholesterolemic mice through inhibition of intestinal cholesterol transport. *J Diabetes Investig* 2018 [PMID: 29754453 DOI: 10.1111/jdi.12860]
- 16 Yano W, Inoue N, Ito S, Ito T, Yasumura M, Yoshinaka Y, Hagita S, Goto M, Nakagawa T, Inoue K, Tanabe S, Kaku K. Mechanism of lipid-lowering action of the dipeptidyl peptidase-4 inhibitor, anagliptin, in low-density lipoprotein receptor-deficient mice. *J Diabetes Investig* 2017; **8**: 155-160 [PMID: 27860391 DOI: 10.1111/jdi.12593]
- 17 Simó R, Guerci B, Schernthaner G, Gallwitz B, Rosas-Guzmán J, Dotta F, Festa A, Zhou M, Kiljański J. Long-term changes in cardiovascular risk markers during administration of exenatide twice daily or glimepiride: results from the European exenatide study. *Cardiovasc Diabetol* 2015; **14**: 116 [PMID: 26338040 DOI: 10.1186/s12933-015-0279-z]
- 18 Constantinou C, Karavia EA, Xepapadaki E, Petropoulou PI, Papakosta E, Karavryaki M, Zvintzou E, Theodoropoulos V, Filou S, Hatziri A, Kalogeropoulos C, Panayiotakopoulos G, Kypreos KE. Advances in high-density lipoprotein physiology: surprises, overturns, and promises. *Am J Physiol Endocrinol Metab* 2016; **310**: E1-E14 [PMID: 26530157 DOI: 10.1152/ajpendo.00429.2015]
- 19 Ceriello A, Inagaki N. Pharmacokinetic and pharmacodynamic evaluation of linagliptin for the treatment of type 2 diabetes mellitus, with consideration of Asian patient populations. *J Diabetes Investig* 2017; **8**: 19-28 [PMID: 27180612 DOI: 10.1111/jdi.12528]
- 20 Yamagishi S, Ishibashi Y, Ojima A, Sugiura T, Matsui T. Linagliptin, a xanthine-based dipeptidyl peptidase-4 inhibitor, decreases serum uric acid levels in type 2 diabetic patients partly by suppressing xanthine oxidase activity. *Int J Cardiol* 2014; **176**: 550-552 [PMID: 25065332 DOI: 10.1016/j.ijcard.2014.07.023]
- 21 Kern M, Klötting N, Niessen HG, Thomas L, Stiller D, Mark M, Klein T, Blüher M. Linagliptin improves insulin sensitivity and hepatic steatosis in diet-induced obesity. *PLoS One* 2012; **7**: e38744 [PMID: 22761701 DOI: 10.1371/journal.pone.0038744]
- 22 Groop PH, Cooper ME, Perkovic V, Emser A, Woerle HJ, von Eynatten M. Linagliptin lowers albuminuria on top of recommended standard treatment in patients with type 2 diabetes and renal dysfunction. *Diabetes Care* 2013; **36**: 3460-3468 [PMID: 24026560 DOI: 10.2337/dc13-0323]
- 23 Marx N, Rosenstock J, Kahn SE, Zinman B, Kastelein JJ, Lachin JM, Espeland MA, Bluhmki E, Mattheus M, Ryckaert B, Patel S, Johansen OE, Woerle HJ. Design and baseline characteristics of the CARdiovascular Outcome Trial of LINAgliptin Versus Glimepiride in Type 2 Diabetes (CAROLINA®). *Diab Vasc Dis Res* 2015; **12**: 164-174 [PMID: 25780262 DOI: 10.1177/1479164115570301]
- 24 Rosenstock J, Perkovic V, Alexander JH, Cooper ME, Marx N, Pencina MJ, Toto RD, Wanner C, Zinman B, Baanstra D, Pfarr E, Mattheus M, Broedl UC, Woerle HJ, George JT, von Eynatten M, McGuire DK; CARMELINA® investigators. Rationale, design, and baseline characteristics of the CARdiovascular safety and Renal Microvascular outcome study with LINAgliptin

(CARMELINA®): a randomized, double-blind, placebo-controlled clinical trial in patients with type 2 diabetes and high cardio-renal risk. *Cardiovasc Diabetol* 2018; **17**: 39 [PMID: 29540217 DOI: 10.1186/s12933-018-0682-3]

25 Ueda S, Shimabukuro M, Arasaki O, Node K, Nomiya

T, Morimoto T. Effect of Anagliptin and Sitagliptin on Low-Density Lipoprotein Cholesterol in Type 2 Diabetic Patients with Dyslipidemia and Cardiovascular Risk: Rationale and Study Design of the REASON Trial. *Cardiovasc Drugs Ther* 2018; **32**: 73-80 [PMID: 29435776 DOI: 10.1007/s10557-018-6776-z]

P- Reviewer: Gupta Y, Nakhoul FM, Raghow R **S- Editor:** Ji FF

L- Editor: A **E- Editor:** Bian YN



Topical application of platelet-rich plasma for diabetic foot ulcers: A systematic review

Takashi Hirase, Eric Ruff, Salim Surani, Iqbal Ratnani

Takashi Hirase, Department of Orthopaedic Surgery, Houston Methodist Hospital, Houston, TX 77030, United States

Eric Ruff, Department of Plastic and Reconstructive Surgery, University of Texas Medical Branch, Galveston, TX 77555, United States

Salim Surani, Department of Medicine, Pulmonary, Critical Care and Sleep Medicine, Texas A and M University, Corpus Christi, TX 77807, United States

Iqbal Ratnani, Department of Anesthesiology and Critical Care, Houston Methodist Hospital, Houston, TX 77030, United States

ORCID number: Takashi Hirase (0000-0001-9111-0667); Eric Ruff (0000-0002-5084-8865); Salim Surani (0000-0001-7105-4266); Iqbal Ratnani (0000-0002-1168-3041).

Author contributions: Hirase T and Ruff E designed the research; Hirase T and Ruff E performed the research; Hirase T analyzed the data; Hirase T and Ruff E wrote the paper; Surani S and Ratnani I supervised the paper; all authors read and approved the final manuscript.

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

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.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Salim Surani, FACC, FACP, MD, Adjunct Clinical Professor, Department of Medicine, Pulmonary, Critical

Care and Sleep Medicine, Texas A and M University, 701 Ayers street, Corpus Christi, TX 77807, United States. srsurani@hotmail.com

Telephone: +1-361-8857722

Fax: +1-361-8857792

Received: May 2, 2018

Peer-review started: May 3, 2018

First decision: June 8, 2018

Revised: June 15, 2018

Accepted: June 28, 2018

Article in press: June 28, 2018

Published online: October 15, 2018

Abstract

AIM

To determine if topical application of platelet-rich plasma (PRP) to diabetic foot ulcers (DFUs) results in superior healing rates.

METHODS

A systematic review was registered with PROSPERO and performed using PRISMA guidelines. Level I-IV investigations of topical PRP application in DFUs were sought in multiple databases including: MEDLINE, Web of Science, and Cochrane Central Register of Controlled Trials. The search terms used were "platelet rich plasma", "diabetes", "ulcers", and "wound". The Modified Coleman Methodology Score (MCMS) was used to analyze study methodological quality. Study heterogeneity and a mostly non-comparative nature of evidence precluded meta-analysis. Only the outcome measurements used by more than 50% of the studies were included in the data synthesis to increase power of the measurement over that of individual studies. A weighted mean of healing rate per week between PRP group *vs* controls were compared using two-sample z-tests using *P*-value of less than 0.05 for significance.

RESULTS

One thousand two hundred and seventeen articles were screened. Eleven articles (322 PRP subjects, 126 controls, PRP subject mean age 58.4 ± 7.2 years, control mean age 58.7 ± 5.9 years) were analyzed. Six articles were level II evidence, four were level III, and one article was level IV. The mean MCMS was 61.8 ± 7.3 . Healing rate was significantly faster with PRP application compared to controls (0.68 ± 0.56 cm²/wk vs 0.39 ± 0.09 cm²/wk; $P < 0.001$). Mean heal time to > 90% of the original ulcer area was 7.8 ± 2.7 wk and 8.3 ± 3.7 wk for patients in the PRP group and control groups, respectively ($P = 0.115$). There were significantly lower adverse effects reported with PRP application compared to controls (7 wound infections, 1 contact dermatitis vs 14 wound infections, 1 maceration; $P < 0.001$).

CONCLUSION

The topical application of PRP for DFUs results in statistically superior healing rates and lower complication rates compared to controls.

Key words: Platelet rich plasma; Diabetes; Foot; Ulcer; Wound

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: There is growing evidence supporting the use of autologous platelet-rich plasma (PRP) to enhance the healing process of diabetic foot ulcers (DFUs). This systematic review of eleven articles (322 PRP subjects, 126 controls) showed that healing rate was significantly faster with PRP application compared to controls (0.68 ± 0.56 cm²/wk vs 0.39 ± 0.09 cm²/wk; $P < 0.001$). There were significantly lower adverse effects reported with PRP application compared to controls. The authors conclude that the topical application of PRP for DFUs results in statistically superior healing rates compared to controls with lower complication rates.

Hirase T, Ruff E, Surani S, Ratnani I. Topical application of platelet-rich plasma for diabetic foot ulcers: A systematic review. *World J Diabetes* 2018; 9(10): 172-179 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i10/172.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i10.172>

INTRODUCTION

Diabetic foot ulcers (DFUs) are among the most common complications of diabetes mellitus with a lifetime incidence of up to 15% among the diabetic population^[1]. Studies have shown that up to 80% of patients with DFUs suffer from both limb ischemia and peripheral neuropathy simultaneously^[2,3]. These conditions further delay healing of DFUs, predisposing to higher rates of complications such as cellulitis and osteomyelitis^[4]. In

spite of the high prevalence and morbidity associated with DFUs, current treatment options are limited. Current standard management consists of surgical debridement followed by frequent dressing changes with tight infection and glycemic control. Despite this comprehensive approach, complication and amputation rates remain high^[5].

In recent years, the use of autologous platelet-rich plasma (PRP) has emerged as an adjunctive method for treating DFUs^[6-16]. PRP is derived from centrifugation of whole blood, which separates into 3 layers: platelet poor plasma, platelet rich plasma, and red blood cells. Contained within these platelets are a number of hemodynamically active proteins that aid in the natural process of wound healing. Specifically, the platelet alpha-granules contain several of these molecules, including: platelet derived growth factor (PDGF), TGF- β , vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), fibrinogen, fibronectin, and vitronectin^[17-19]. In addition, platelet delta granules contain serotonin, histamine, dopamine, calcium, and adenosine, which act in tandem with the aforementioned growth factors to regulate wound healing^[20]. With increasing knowledge about the pathophysiology of refractory DFUs, alterations to the local microenvironment with PRP could play an important role in mitigating the morbidity associated with these chronic wounds.

Current studies evaluating the outcomes of topical autologous PRP on diabetic foot ulcers are limited to small randomized controlled studies and case reports. Given that there are numerous confounding variables involved with PRP use, there has been significant challenge in generating standardized protocols for patient use. Thus, the purpose of this investigation was to summarize the clinical outcomes of the topical application of autologous PRP among patients with DFUs and to determine if the method results in statistically superior outcomes compared to patients receiving conventional wound care. The authors hypothesized that the procedure results in statistically superior outcomes compared to patients receiving conventional wound care with low complication rates.

MATERIALS AND METHODS

A systematic review was registered with PROSPERO on March 9, 2017 (ID: CRD42018090780). Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines were followed^[21]. Inclusion criteria consisted of Level I-IV [via Oxford Centre for Evidence Based Medicine (CEBM)] therapeutic studies that investigated outcomes of topical applications of autologous PRP for diabetic foot ulcers among adult human patients^[22]. Studies that included non-diabetic etiology of foot ulcers and use of non-autologous PRP were excluded. Cadaveric studies, basic science and animal studies, diagnostic studies, economic studies, prognostic studies, Level V evidence expert opinion, letters to editors, and review articles were excluded.

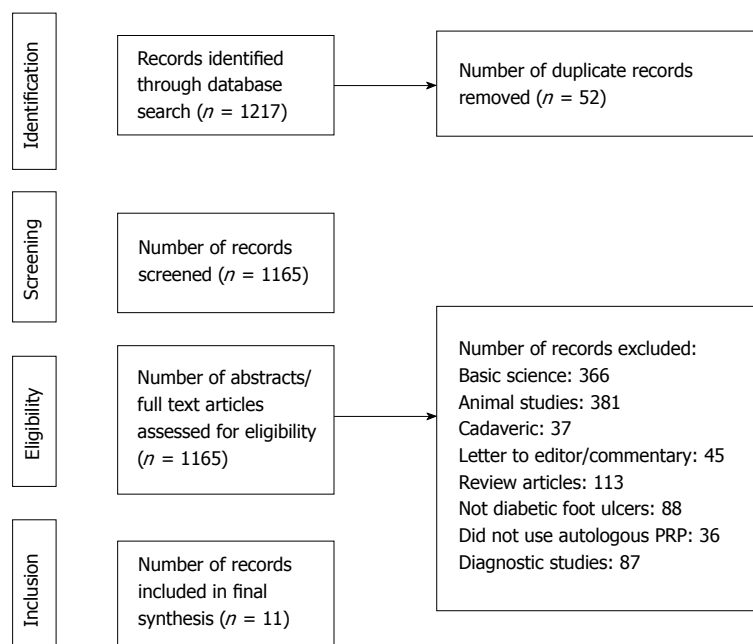


Figure 1 Flow diagram summarizing the literature search, screening, and review. PRP: Platelet-rich plasma.

Studies published in non-English languages were not excluded but were unidentified in the medical databases. In the event of different studies with duplicate subject populations, the study with the longer follow-up, higher level of evidence, greater number of subjects, or greater clarity of methods and results was included. The authors conducted separate searches of the following medical databases: MEDLINE, Web of Science, and Cochrane Central Register of Controlled Trials databases. Under the PROSPERO registration, similar prior systematic reviews and meta-analyses were sought and none were identified. The searches were performed on March 8, 2017. The search terms used were “platelet rich plasma”, “diabetes”, “ulcers”, and “wound”. The search results were reviewed for duplicates and the inclusion criteria to determine articles that were included in the final analysis (Figure 1).

Two authors independently reviewed all articles. The study design, patient populations, and procedure technique were first identified. A weighted mean of the demographics (No. of patients, age, % female gender, duration of diabetes, duration of ulcer, HbA1c, and ulcer area) between PRP group vs controls were compared using two-sample z-tests using *P*-value of less than 0.05 for significance. All reported outcome scores and complication rates were analyzed. The levels of evidence were then assigned based on the Oxford Centre for Evidence Based Medicine^[22]. Study methodological quality was analyzed using the Modified Coleman Methodology Score (MCMS)^[23]. The overall Strength-of-Recommendation Taxonomy (SORT) score was B and Grading of Recommendations Assessment, Development and Evaluation (GRADE) score was C^[24,25]. Study heterogeneity and a mostly non-comparative nature of evidence precluded meta-analysis. Thus, a best-evidence

synthesis was used instead^[26]. Only the outcome measurements used by more than 50% of the studies were included in the data synthesis to increase power of the measurement over that of individual studies. A weighted mean of healing rate per week between PRP group vs controls were compared using two-sample z-tests using *P*-value of less than 0.05 for significance.

RESULTS

One thousand two hundred and seventeen articles were screened (Figure 1). Eleven articles were included in the analysis (Table 1)^[6-16]. Six articles were level II evidence, four were level III, and one article was level IV. According to MCMS, three articles were good (scores between 70 to 84), seven articles were fair (scores between 55 to 69), and one article was poor (scores less than 55). The mean MCMS was 61.8 ± 7.3 . There were 465 patients analyzed. 322 patients were under the PRP group and 126 patients were under the control group (standard dressing changes \pm placebo gel). There were 206 males and 87 females (29 unidentified) in the PRP group and 72 males and 39 females (15 unidentified) in the control group (*P* = 0.407). Mean follow-up was 10.4 ± 3.1 wk. The mean ages were 58.4 ± 7.2 years and 58.7 ± 5.9 years under the PRP and control groups, respectively (*P* = 0.678). The mean HbA1c were 7.94 ± 1.30 and 8.74 ± 1.08 under the PRP and control groups, respectively (*P* < 0.001). The mean baseline ulcer areas were 7.7 ± 9.3 cm² and 4.6 ± 6.6 cm² under the PRP and control groups, respectively (*P* = 0.689).

Most studies prepared PRP through a single or double spinning approach and utilized Thrombin, CaCl₂, and/or calcium gluconate as activator (Table 2). Four studies reported the amount of PRP gel applied to the wound,

Table 1 Study demographics

Study	Mohammadi <i>et al.</i> ^[6] 2017	Ahmed <i>et al.</i> ^[7] 2017	Perez-Zabala <i>et al.</i> ^[8] 2016	Saad <i>et al.</i> ^[9] 2011	Kakagia <i>et al.</i> ^[10] 2007	Driver <i>et al.</i> ^[11] 2006	Li <i>et al.</i> ^[12] 2015	Saldam- accia <i>et al.</i> ^[13] 2004	Motolese <i>et al.</i> ^[14] 2015	Shan <i>et al.</i> ^[15] 2013	Kontopodis <i>et al.</i> ^[16] 2016	Weighted (mean \pm SD) ⁽⁶⁾	P-value (vs control)
Type of Study	PU	RP	CS	RP	RP	RDBP	RP	RP	PU	PU	RU	N/A	N/A
Level of evidence	III	II	IV	II	II	II	II	II	III	III	III	N/A	N/A
No. patients	70	28	2	12	17	19	59	7	15	21	72	29.3 \pm 25.4	0.104
PRP	N/A	28	N/A	12	N/A	21	58	7	N/A	N/A	N/A	25.2 \pm 20.0	
Control													
Age (mean \pm SD, yr)	53.8 \pm 10.6	43.2 \pm 18.2	65.5 \pm 2.1	NR	57.0 \pm 12.0	58.3 \pm 9.7	61.4 \pm 13.1	61.1 \pm 9.4	52.3 \pm 11.3	66.5 \pm 10.8	65	58.4 \pm 7.2	0.678
PRP	N/A	49.8 \pm 15.4	N/A	NR	N/A	55.9 \pm 8.1	64.1 \pm 9.4	58.1 \pm 7.8	N/A	N/A	N/A	58.7 \pm 5.9	
Control													
Female gender, n (%)	12 (17.1)	8 (28.6)	0 (0.0)	NR	NR	3 (15.8)	22 (37.3)	4 (57.1)	11 (73.3)	13 (61.9)	14 (19.4)	29.70%	0.407
PRP	N/A	10 (35.7)	N/A	NR	N/A	5 (23.8)	20 (34.5)	4 (57.1)	N/A	N/A	N/A	35.10%	
Control													
Duration of diabetes (mean \pm SD, yr)	16.2 \pm 7.9	NR	23.5 \pm 13.4	NR	NR	NR	7.50	16.3 \pm 7.9	38.20	6.8 \pm 6.7	NR	14.1 \pm 11.6	0.048
PRP	N/A	NR	N/A	NR	N/A	NR	10.00	19.7 \pm 9.9	N/A	N/A	N/A	11.0 \pm 6.9	
Control													
Duration of ulcer (mean \pm SD, wk)	19.6 \pm 4.7	12.5 \pm 1.0	28.3 \pm 9.5	NR	19.0 \pm 8.0	NR	4.28	NR	NR	10.1 \pm 12.0	NR	13.0 \pm 8.4	< 0.001
PRP	N/A	11.5 \pm 2.8	N/A	NR	N/A	NR	3.30	NR	N/A	N/A	N/A	6.0 \pm 5.8	
Control													
HbA1c (mean \pm SD)	6.2 \pm 0.7	7.0 \pm 0.5	9.4 \pm 3.3	NR	8.1 \pm 2.8	7.8 \pm 1.5	9.8 \pm 3.1	9.5 \pm 1.7	NR	9.1 \pm 2.2	NR	7.9 \pm 1.3	< 0.001
PRP	N/A	6.9 \pm 0.6	N/A	NR	N/A	8.1 \pm 1.8	9.80	8.8 \pm 1.7	N/A	N/A	N/A	8.7 \pm 1.2	
Control													
Ulcer area (mean \pm SD, cm ²)	6.11 \pm 4.37	6.24 \pm 0.9	10.25	NR	28.4 \pm 13.6	3.4 \pm 4.5	4.10	27.3 \pm 15.6	13.92	14.0 \pm 32.3	4.1 \pm 3.9	7.7 \pm 9.3	< 0.001
PRP	N/A	5.72 \pm 0.8	N/A	NR	N/A	3.6 \pm 4.0	2.90	17.0 \pm 8.9	N/A	N/A	N/A	4.6 \pm 6.6	
Control													

N/A: Not applicable; PU: Prospective uncontrolled; RP: Randomized prospective; CS: Case series; RDBP: Randomized double-blind prospective; RU: Retrospective uncontrolled; NR: Not recorded; PRP: Platelet-rich plasma.

and two studies reported platelet concentration. Only one study reported WBC count in the final PRP prepared.

Eight studies assessed the time to > 90% ulcer area healing and seven studies assessed healing rate per week (Table 3). Both outcome measures were included in the best evidence synthesis. Other outcome measures included percent of ulcer completely healed at 8 and/or 12 wk follow-up (6 of 11 studies), comparison of ulcer area at baseline and at final follow-up (2 of 11 studies), Resvech 2.0 measurement score at baseline and at final follow-up (1 of 11 studies), and percent of wound length/width/depth decrease at final follow-up (1 of 11 studies).

Mean heal time to > 90% of the original ulcer area was 7.8 \pm 2.7 wk and 8.3 \pm 3.7 wk for patients in the PRP group and control groups, respectively (Table 4; $P = 0.115$). Mean healing rate was significantly faster with PRP application compared to controls (0.68 \pm 0.56 cm²/wk vs 0.39 \pm 0.09 cm²/wk; $P < 0.001$). There were 8 (2.5%); 7 wound infections, 1 contact dermatitis and 15 (10.5%); 14 wound infections, 1 maceration) adverse effects reported within the PRP group and control groups respectively ($P < 0.001$).

DISCUSSION

It was determined that the topical application of PRP for DFUs resulted in statistically superior healing rate compared to patients receiving conventional wound care with low complication rates. This confirmed the authors' hypothesis that patients receiving this treatment results in significantly superior outcomes compared to patients receiving

Table 2 Platelet-rich plasma preparation

Study	Mohamm-adi <i>et al.</i> ^[6] 2017	Ahmed <i>et al.</i> ^[7] 2017	Perez-Zabala <i>et al.</i> ^[8] 2016	Saad <i>et al.</i> ^[9] 2011	Kakagia <i>et al.</i> ^[10] 2007	Driver <i>et al.</i> ^[11] 2006	Li <i>et al.</i> ^[12] 2015	Saldalam-acchia <i>et al.</i> ^[13] 2004	Motolese <i>et al.</i> ^[14] 2015	Shan <i>et al.</i> ^[15] 2013	Kontopodis <i>et al.</i> ^[16] 2016
PRP spinning approach	Single	Double	Single	Double	NR	Single	Double	NR	Single	Single	Single
Duration of spin (min)	10	5 and 5	7	NR	NR	1.5	4 and 6	NR	17	10	NR
Company	Arya Mabna Tashkhis Co, Iran	NR	NR	NR	Biomet Biologics, Warsaw, IN, United States	Cytomedix, Rockville, MD, United States	NR	NR	ThermoGenesis, Rancho Cordova, CA, United States	Haemonetics Corp, Bainbridge, MA, United States	RegenLab, Le Montsur-Lausanne, Switzerland
PRP activator	CaCl ₂	Thrombin, CaCl ₂	CaCl ₂	Thrombin, CaCl ₂	Thrombin	Thrombin	Thrombin, calcium gluconate	NR	Thrombin, CaCl ₂	Thrombin, calcium gluconate	NR
PRP amount applied	2 mL/cm ²	7 mL	3 mL	NR	NR	NR	NR	NR	5 mL	NR	NR
Platelet concentration	NR	1.0×10^6 /mL- 1.2×10^7 /mL	1.6-1.7 x baseline	NR	NR	NR	NR	NR	NR	NR	NR
WBC concentration	NR	NR	Undetectable	NR	NR	NR	NR	NR	NR	NR	NR
PRP application method	PRP gel applied on ulcers after irrigation and debridement every week covered with non-absorbing wet dressing	PRP gel applied on ulcers after irrigation with 0.9% saline twice weekly covered with non-absorbing dressing	PRP gel applied on ulcers after irrigation twice weekly covered with foam dressings	PRP gel applied on ulcers within half an hour after preparation followed by Vaseline gauze and dressing changed every 3-4 d	PRP gel applied on ulcers covered with vapor-permeable film (Tegaderm, 3M)	PRP gel applied on ulcer with contact layer dressing covered with non-absorbent foam dressing changed every 3-4 d	PRP gel applied on ulcer after irrigation and debridement covered with Suile dressing changed every 3 d. PRP gel reapplied up to 5 times in 12 wk period if wound area reduction rate < 80%	Weekly topical application of PRP gel with covered with standard dressing changed weekly	5 mL of PRP gel applied on ulcers once a week for total of 10 wk covered with non-adherent dressing and bandage	PRP gel applied on ulcers twice per week covered with occlusive dressing changed every 72 h	PRP gel applied on ulcer twice weekly after irrigation and debridement covered with standard dressings

NR: Not recorded; PRP: Platelet-rich plasma.

conventional wound management. To our knowledge, this is the first systematic review to evaluate the outcomes of topical application of PRP versus conventional management of DFUs.

All studies analyzed topical application of PRP gel to improve healing of DFUs. One of the analyzed studies by Kakagia *et al.*^[10] also utilized a biomaterial consisting of collagen and oxidized regenerated cellulose. This biomaterial designed to modify the chronic wound environment through the inactivation of proteases, free radicals and metal ions has previously been shown to be an efficient method in the management of DFUs^[27,28]. The authors found that the topical application of both the biomaterial and PRP on DFUs significantly enhances the healing rate compared to the biomaterial or PRP alone.

Various types of PRP systems exist with variable platelet, leukocyte, and growth factor concentrations. Chronic inflammatory response against foreign invaders are made possible by leukocytes including lymphocytes, monocytes, neutrophils, eosinophils, and basophils. Recent evidence has shown that leukocyte levels within PRP may have controversial effects on wound healing^[29]. Of the studies included in the review, Perez-Zabala *et al.*^[8] reported using leukocyte-poor PRP with high average healing rates 1.46 cm²/wk. However, this review was unable to develop conclusions regarding outcome differences in the use of leukocyte-rich versus leukocyte-poor PRP as no other reviewed studies reported leukocyte levels.

Complication rates after the topical application of PRP were low. Besides the 2.2% incidence of transient wound infections and 0.3% incidence of contact dermatitis no other adverse effects were reported. The complication rates were significantly lower compared to the 11.1% incidence of wound infection and 0.8% incidence of skin maceration among patients receiving conventional wound treatment. Overall, this study demonstrates that the topical application of PRP for DFUs lead to more superior clinical outcomes

Table 3 Platelet-rich plasma group individual study outcome measures

Study	Mohammadi <i>et al.</i> ^[41] 2017	Ahmed <i>et al.</i> ^[7] 2017	Perez-Zabala <i>et al.</i> ^[8] 2016	Saad <i>et al.</i> ^[9] 2011	Kakagia <i>et al.</i> ^[10] 2007	Driver <i>et al.</i> ^[11] 2006	Li <i>et al.</i> ^[12] 2015	Saldamachia <i>et al.</i> ^[13] 2004	Motolese <i>et al.</i> ^[4] 2015	Shan <i>et al.</i> ^[15] 2013	Kontopodis <i>et al.</i> ^[16] 2016
Ulcer area (mean \pm SD, cm ²)	6.11 \pm 4.37	6.24 \pm 0.9	10.3	NR	28.4 \pm 13.6	3.4 \pm 4.5	4.1	27.3 \pm 15.6	13.9	14.0 \pm 32.3	4.1 \pm 3.9
Ulcer healed, <i>n</i> (%)	NR	1.44	NR	NR	NR	NR	NR	8.0 \pm 7.5	NR	NR	NR
Resvech 2.0 measurement	NR	23 (82.1)	NR	NR	2 (11.8)	NR	NR	NR	NR	NR	NR
% wound length decrease	NR	24 (85.7)	NR	NR	NR	13 (68.4)	50 (84.8)	NR	NR	15 (71.4)	NR
% wound width decrease	NR	NR	13.5 \pm 0.7	NR	NR	NR	NR	NR	NR	NR	NR
% wound depth decrease	NR	NR	6.0 \pm 1.4	NR	NR	NR	NR	NR	NR	NR	NR
Time to > 90% ulcer area healing (mean \pm SD, wk)	NR	NR	NR	NR	14.3 \pm 7.1	NR	NR	NR	NR	NR	NR
Healing rate per week (mean, cm ²)	8.7 \pm 3.9	NR	7.0 \pm 2.8	11.5	17.4 \pm 8.0	NR	NR	NR	12.7	7.17 \pm 5.66	11.0 \pm 4.0
Adverse effects	0	NR	1.46	NR	NR	0.53	0.8	NR	1.1	1.95	0.37
	0	2 - wound infections	0	0	0	1 - contact dermatitis	5 - wound infections	0	0	0	0

NR: Not recorded.

compared to conventional treatment methods with lower complication rates. However, further higher quality studies with randomized controlled trials are necessary to justify the use of PRP over more cost-effective treatment methods.

There are several limitations among the studies included in this review. Five of the 11 articles were levels III or IV evidence, which limits the strength of the results. Only one of the studies used a double-blinded approach producing potential bias. The average study methodological quality as assessed by the MCMS was fair. Assimilation of heterogeneous low methodological quality studies with healing rates is a significant limitation. However, the authors minimized this as much as possible with strict study eligibility and inclusion criteria, despite the level III and IV evidence nature of the studies. Furthermore, the heterogeneity of outcome measures used among the studies limited the data analysis to two outcome measures. Another limitation of this review is that most reviewed studies did not include relevant baseline comorbidities including pre-existing peripheral arterial obstructive disease nor baseline home medications and were unable to be compared in this review. Future studies can improve through designing more prospective comparative trials, increasing study sizes, and standardizing clinical outcome measures such as healing rates, percentage of ulcers completely healed, and ulcer area at baseline and final follow-up. Another possible limitation of this review is that other relevant studies on this topic could have been excluded, despite conducting a systematic search.

In conclusion, topical application of autologous PRP for DFUs results in statistically superior healing rates compared to controls with lower complication rates. Further randomized controlled studies that show clinical outcome improvement in multiple parameters are necessary to evaluate the true efficacy of this treatment.

ARTICLE HIGHLIGHTS

Research background

Diabetic foot ulcers (DFUs) are among the most common complications of diabetes mellitus but current treatment options are limited. Current standard management consists of surgical debridement followed by frequent dressing changes with tight infection and glycemic control. In recent years, the use of autologous platelet-rich plasma (PRP) has emerged as an adjunctive method for treating DFUs.

Research motivation

Because current studies evaluating the outcomes of topical autologous PRP on diabetic foot ulcers are limited to small randomized controlled studies and case reports. Given that there are numerous confounding variables involved with PRP

Table 4 Average study outcome measures included in best evidence synthesis

	Time to > 90% ulcer area healing (mean \pm SD, wk)	Healing rate per week (mean \pm SD, cm ²)	Adverse effects
PRP	7.8 \pm 2.7	0.68 \pm 0.56	8 (2.5)
Control	8.3 \pm 3.7	0.39 \pm 0.09	15 (10.5)
P-value	0.115	< 0.001	< 0.001

PRP: Platelet-rich plasma.

use, there has been significant challenge in generating standardized protocols for patient use.

Research objectives

The objective was to determine if topical application of platelet-rich plasma (PRP) to diabetic foot ulcers (DFUs) results in superior healing rates. The significance of realizing this objective combined with future research consisting of further randomized controlled studies will help evaluate the true efficacy of this treatment.

Research methods

This review was registered with PROSPERO and performed using PRISMA guidelines. Level I-IV investigations of topical PRP application in DFUs were sought in multiple databases, *i.e.*, MEDLINE, Web of Science, and Cochrane Central Register of Controlled Trials. The search terms used were "platelet rich plasma", "diabetes", "ulcers", and "wound". The Modified Coleman Methodology Score (MCMS) was used to analyze study methodological quality.

Research results

One thousand two hundred and seventeen articles were screened, eleven articles were analyzed, six articles were level II evidence, four were level III, and one article was level IV. The mean MCMS was 61.8 ± 7.3 . Healing rate was significantly faster with PRP application compared to controls (0.68 ± 0.56 cm²/wk vs 0.39 ± 0.09 cm²/wk; $P < 0.001$). Mean heal time to > 90% of the original ulcer area for patients in the PRP group was significantly lower with control groups (7.8 ± 2.7 wk vs 8.3 ± 3.7 wk, $P = 0.115$). There were significantly lower adverse effects reported with PRP application compared to controls (7 wound infections, 1 contact dermatitis vs 14 wound infections, 1 maceration; $P < 0.001$).

Research conclusions

We find that the topical application of PRP for DFUs results in statistically superior healing rates and lower complication rates compared to controls. This study proposes the new theory that the use of PRP is a superior option to treating DFUs than the current standard of care. A new hypothesis that may be proposed from this study is that the use of PRP results in clinical outcome improvement in multiple parameters. Combining the findings within this study with future research consisting of further randomized controlled studies that show clinical outcome improvement in multiple parameters will provide adequate evaluation of the true efficacy of this treatment.

Research perspectives

The assimilation of heterogeneous studies allowed the development of a high quality systematic review that analyzes two outcome measures. Future studies can improve through designing more prospective comparative trials, increasing study sizes, and standardizing clinical outcome measures such as healing rates, percentage of ulcers completely healed, and ulcer area at baseline and final follow-up.

REFERENCES

- 1 Ahmad J. The diabetic foot. *Diabetes Metab Syndr* 2016; **10**: 48-60 [PMID: 26072202 DOI: 10.1016/j.dsx.2015.04.002]
- 2 Naidoo P, Liu VJ, Mautone M, Bergin S. Lower limb complications of diabetes mellitus: a comprehensive review with clinicopathological

- insights from a dedicated high-risk diabetic foot multidisciplinary team. *Br J Radiol* 2015; **88**: 20150135 [PMID: 26111070 DOI: 10.1259/bjr.20150135]
- 3 McNeely MJ, Boyko EJ, Ahroni JH, Stensel VL, Reiber GE, Smith DG, Pecoraro RF. The independent contributions of diabetic neuropathy and vasculopathy in foot ulceration. How great are the risks? *Diabetes Care* 1995; **18**: 216-219 [PMID: 7729300 DOI: 10.2337/diacare.18.2.216]
- 4 Chen Y, Ding H, Wu H, Chen HL. The Relationship Between Osteomyelitis Complication and Drug-Resistant Infection Risk in Diabetic Foot Ulcer: A Meta-analysis. *Int J Low Extrem Wounds* 2017; **16**: 183-190 [PMID: 28862037 DOI: 10.1177/1534734617728642]
- 5 Everett E, Mathioudakis N. Update on management of diabetic foot ulcers. *Ann N Y Acad Sci* 2018; **1411**: 153-165 [PMID: 29377202 DOI: 10.1111/nyas.13569]
- 6 Mohammadi MH, Molavi B, Mohammadi S, Nikbakht M, Mohammadi AM, Mostafaei S, Norooznezhad AH, Ghorbani Abdegah A, Ghavamzadeh A. Evaluation of wound healing in diabetic foot ulcer using platelet-rich plasma gel: A single-arm clinical trial. *Transfus Apher Sci* 2017; **56**: 160-164 [PMID: 27839965 DOI: 10.1016/j.transci.2016.10.020]
- 7 Ahmed M, Reffat SA, Hassan A, Eskander F. Platelet-Rich Plasma for the Treatment of Clean Diabetic Foot Ulcers. *Ann Vasc Surg* 2017; **38**: 206-211 [PMID: 27522981 DOI: 10.1016/j.avsg.2016.04.023]
- 8 Perez-Zabala E, Basterretxea A, Larrazabal A, Perez-Del-Pecho K, Rubio-Azpeitia E, Andia I. Biological approach for the management of non-healing diabetic foot ulcers. *J Tissue Viability* 2016; **25**: 157-163 [PMID: 27038643 DOI: 10.1016/j.jtvt.2016.03.003]
- 9 Saad Setta H, Elshahat A, Elsherbiny K, Massoud K, Safe I. Platelet-rich plasma versus platelet-poor plasma in the management of chronic diabetic foot ulcers: a comparative study. *Int Wound J* 2011; **8**: 307-312 [PMID: 21470370 DOI: 10.1111/j.1742-481X.2011.00797.x]
- 10 Kakagia DD, Kazakos KJ, Xarchas KC, Karanikas M, Georgiadis GS, Tripsiannis G, Manolas C. Synergistic action of protease-modulating matrix and autologous growth factors in healing of diabetic foot ulcers. A prospective randomized trial. *J Diabetes Complications* 2007; **21**: 387-391 [PMID: 17967712 DOI: 10.1016/j.jdiacomp.2007.03.006]
- 11 Driver VR, Hanft J, Fylling CP, Beriou JM; Autologel Diabetic Foot Ulcer Study Group. A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. *Ostomy Wound Manage* 2006; **52**: 68-70, 72, 74 passim [PMID: 16799184]
- 12 Li L, Chen D, Wang C, Yuan N, Wang Y, He L, Yang Y, Chen L, Liu G, Li X, Ran X. Autologous platelet-rich gel for treatment of diabetic chronic refractory cutaneous ulcers: A prospective, randomized clinical trial. *Wound Repair Regen* 2015; **23**: 495-505 [PMID: 25847503 DOI: 10.1111/wrr.12294]
- 13 Saldalamacchia G, Lapice E, Cuomo V, De Feo E, D'Agostino E, Rivellese AA, Vaccaro O. A controlled study of the use of autologous platelet gel for the treatment of diabetic foot ulcers. *Nutr Metab Cardiovasc Dis* 2004; **14**: 395-396 [PMID: 15853123 DOI: 10.1016/S0939-4753(04)80029-2]
- 14 Motolese A, Vignati F, Antelmi A, Saturni V. Effectiveness of platelet-rich plasma in healing necrobiosis lipoidica diabetorum ulcers. *Clin Exp Dermatol* 2015; **40**: 39-41 [PMID: 25266162 DOI: 10.1111/ced.12474]
- 15 Shan GQ, Zhang YN, Ma J, Li YH, Zuo DM, Qiu JL, Cheng B, Chen ZL. Evaluation of the effects of homologous platelet gel on healing lower extremity wounds in patients with diabetes. *Int J Low Extrem Wounds* 2013; **12**: 22-29 [PMID: 23509083 DOI: 10.1177/1534734613477113]
- 16 Kontopodis N, Tavlas E, Papadopoulos G, Pantidis D, Kafetzakis A, Chalkiadakis G, Ioannou C. Effectiveness of Platelet-Rich Plasma to Enhance Healing of Diabetic Foot Ulcers in Patients With Concomitant Peripheral Arterial Disease and Critical Limb Ischemia. *Int J Low Extrem Wounds* 2016; **15**: 45-51 [PMID:

- 25795280 DOI: 10.1177/1534734615575829]
- 17 **Assoian RK**, Fleurdelys BE, Stevenson HC, Miller PJ, Madtes DK, Raines EW, Ross R, Sporn MB. Expression and secretion of type beta transforming growth factor by activated human macrophages. *Proc Natl Acad Sci USA* 1987; **84**: 6020-6024 [PMID: 2888109 DOI: 10.1073/pnas.84.17.6020]
 - 18 **Banks RE**, Forbes MA, Kinsey SE, Stanley A, Ingham E, Walters C, Selby PJ. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. *Br J Cancer* 1998; **77**: 956-964 [PMID: 9528841 DOI: 10.1038/bjc.1998.158]
 - 19 **Kaplan DR**, Chao FC, Stiles CD, Antoniadou HN, Scher CD. Platelet alpha granules contain a growth factor for fibroblasts. *Blood* 1979; **53**: 1043-1052 [PMID: 444648]
 - 20 **Liao HT**, Marra KG, Rubin JP. Application of platelet-rich plasma and platelet-rich fibrin in fat grafting: basic science and literature review. *Tissue Eng Part B Rev* 2014; **20**: 267-276 [PMID: 24004354 DOI: 10.1089/ten.TEB.2013.0317]
 - 21 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**: e1000097 [PMID: 19621072 DOI: 10.1371/journal.pmed.1000097]
 - 22 **Howick J**, Chalmers I, Glasziou P, Greenhalgh T, Heneghan C, Liberati A, Moschetti I, Phillips B, Thornton H. The 2011 Oxford CEBM evidence levels of evidence (introductory document). Oxford Center for Evidence Based Medicine, 2011. Available from: URL: <https://www.cebm.net/2011/06/2011-oxford-cebm-levels-evidence-introductory-document/>
 - 23 **Coleman BD**, Khan KM, Maffulli N, Cook JL, Wark JD. Studies of surgical outcome after patellar tendinopathy: clinical significance of methodological deficiencies and guidelines for future studies. Victorian Institute of Sport Tendon Study Group. *Scand J Med Sci Sports* 2000; **10**: 2-11 [PMID: 10693606 DOI: 10.1034/j.1600-0838.2000.010001002.x]
 - 24 **Ebell MH**, Siwek J, Weiss BD, Woolf SH, Susman J, Ewigman B, Bowman M. Strength of recommendation taxonomy (SORT): a patient-centered approach to grading evidence in the medical literature. *Am Fam Physician* 2004; **69**: 548-556 [PMID: 14971837 DOI: 10.3122/jabfm.17.1.59]
 - 25 **GRADE Working Group**. Grading of Recommendations, Assessment, Development, and Evaluation, 2007. Available from: URL: <http://www.gradeworkinggroup.org/>
 - 26 **Slavin RE**. Best evidence synthesis: an intelligent alternative to meta-analysis. *J Clin Epidemiol* 1995; **48**: 9-18 [PMID: 7853053 DOI: 10.1016/0895-4356(94)00097-A]
 - 27 **Cullen B**, Watt PW, Lundqvist C, Silcock D, Schmidt RJ, Bogan D, Light ND. The role of oxidised regenerated cellulose/collagen in chronic wound repair and its potential mechanism of action. *Int J Biochem Cell Biol* 2002; **34**: 1544-1556 [PMID: 12379277 DOI: 10.1016/S1357-2725(02)00054-7]
 - 28 **Veves A**, Sheehan P, Pham HT. A randomized, controlled trial of Promogran (a collagen/oxidized regenerated cellulose dressing) vs standard treatment in the management of diabetic foot ulcers. *Arch Surg* 2002; **137**: 822-827 [PMID: 12093340 DOI: 10.1001/archsurg.137.7.822]
 - 29 **Bielecki T**, Dohan Ehrenfest DM, Everts PA, Wiczowski A. The role of leukocytes from L-PRP/L-PRF in wound healing and immune defense: new perspectives. *Curr Pharm Biotechnol* 2012; **13**: 1153-1162 [PMID: 21740376 DOI: 10.2174/138920112800624373]

P- Reviewer: Chang ST, Kita K, Zhou M **S- Editor:** Ma YJ

L- Editor: A **E- Editor:** Bian YN





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 November 15; 9(11): 180-205





EDITORIAL

- 180 Current and future impact of clinical gastrointestinal research on patient care in diabetes mellitus
Koch TR, Shope TR, Camilleri M
- 190 Unhealthy eating habits around sleep and sleep duration: To eat or fast?
Nakajima K

FIELD OF VISION

- 195 Circadian rhythms of hormone secretion and obesity
Raghow R

MINIREVIEWS

- 199 Use of sodium bicarbonate and blood gas monitoring in diabetic ketoacidosis: A review
Patel MP, Ahmed A, Gunapalan T, Hesselbacher SE

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, José Carnevalheira, MD, PhD, Associate Professor, Faculty of Medical Sciences, University of Campinas, Campinas 13083, São Paulo, Brazil

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, and PubMed Central, Scopus, China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Ying-Na Bian*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITOR-IN-CHIEF
Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com>

www.wjgnet.com/1948-9358/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE
November 15, 2018

COPYRIGHT
© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.f6publishing.com>

Current and future impact of clinical gastrointestinal research on patient care in diabetes mellitus

Timothy R Koch, Timothy R Shope, Michael Camilleri

Timothy R Koch, Timothy R Shope, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar Washington Hospital Center and Georgetown University School of Medicine, Washington, DC 20010, United States

Michael Camilleri, Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN 55905, United States

ORCID number: Timothy R Koch (0000-0002-8026-9653); Timothy R Shope (0000-0002-5189-8936); Michael Camilleri (0000-0001-6472-7514).

Author contributions: All of the authors drafted the manuscript and approved the final version of the article.

Supported by the grant from National Institutes of Health, No. R01-DK67071.

Conflict-of-interest statement: Koch TR and Shope TR have no conflict of interest to report regarding the publication of this paper. Camilleri M received support from Novo Nordisk to conduct research on liraglutide.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Corresponding author to: Timothy R Koch, MD, Doctor, Professor, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar Washington Hospital Center and Georgetown University School of Medicine, POB South, Suite 301, 106 Irving Street, NW, Washington, DC 20010, United States. timothy.r.koch@medstar.net
Telephone: +1-202-8777788
Fax: +1-877-6808198

Received: July 23, 2018

Peer-review started: July 23, 2018

First decision: August 3, 2018

Revised: August 12, 2018

Accepted: October 11, 2018

Article in press: October 11, 2018

Published online: November 15, 2018

Abstract

The worldwide rise in the prevalence of obesity supports the need for an increased interaction between ongoing clinical research in the allied fields of gastrointestinal medicine/surgery and diabetes mellitus. There have been a number of clinically-relevant advances in diabetes, obesity, and metabolic syndrome emanating from gastroenterological research. Gastric emptying is a significant factor in the development of upper gastrointestinal symptoms. However, it is not the only mechanism whereby such symptoms occur in patients with diabetes. Disorders of intrinsic pacing are involved in the control of stomach motility in patients with gastroparesis; on the other hand, there is limited impact of glycemic control on gastric emptying in patients with established diabetic gastroparesis. Upper gastrointestinal functions related to emptying and satiety are significantly associated with weight gain in obesity. Medications used in the treatment of diabetes or metabolic syndrome, particularly those related to pancreatic hormones and incretins affect upper gastrointestinal tract function and reduce hyperglycemia and facilitate weight loss. The degree of gastric emptying delay is significantly correlated with the weight loss in response to liraglutide, a glucagon-like peptide-1 analog. Network meta-analysis shows that liraglutide is one of the two most efficacious medical treatments of obesity, the other being the combination treatment phentermine-topiramate. Interventional therapies for the joint management of obesity and diabetes mellitus include newer endoscopic procedures, which require long-term follow-up and bariatric surgical

procedure for which long-term follow up shows advantages for individuals with diabetes. Newer bariatric procedures are presently undergoing clinical evaluation. On the horizon, combination therapies, in part directed at gastrointestinal functions, appear promising for these indications. Ongoing and future gastroenterological research when translated to care of individuals with diabetes mellitus should provide additional options to improve their clinical outcomes.

Key words: Obesity; Gastric emptying; Diabetes mellitus; Gastric bypass; Bariatric surgery; Weight loss

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: The worldwide prevalence of obesity continues to rise. Delayed gastric emptying and impaired gastric accommodation result in upper gastrointestinal symptoms, through intrinsic nerve and pacemaker dysfunction. Glycemic control has a limited effect on gastric emptying in diabetic gastroparesis. Treatment of diabetes with pancreatic hormones and incretins inhibits gastric emptying, reduces hyperglycemia, and facilitates weight loss. Meta-analysis shows that glucagon-like peptide-1 analog, liraglutide, is one of the two most efficacious treatments of obesity. Bariatric surgery and endoscopic interventions are efficacious in diabetes and obesity, but long term follow-up is required for endoscopic interventions as well as for newer bariatric procedures. On the horizon, combination therapies directed at gastrointestinal function appear promising for these indications.

Koch TR, Shope TR, Camilleri M. Current and future impact of clinical gastrointestinal research on patient care in diabetes mellitus. *World J Diabetes* 2018; 9(11): 180-189 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i11/180.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i11.180>

INTRODUCTION

In a recent international study of 195 countries, the prevalence of obesity doubled in more than 70 countries since 1980^[1]. This worldwide rise in the prevalence of obesity supports the need for an increased interaction between ongoing clinical research in fields of gastrointestinal medicine/surgery and diabetes mellitus. There have been a number of clinically-relevant advances in diabetes, obesity and metabolic syndrome, emanating from gastrointestinal research. These advances include newer information in pharmacological or medical care, endoscopic procedures, and bariatric surgical procedures.

The most exciting gastrointestinal research areas relevant to diabetes are focused on the stomach and on weight loss, with the goals of resolution of hyperglycemia and/or prevention of secondary complications of diabetes mellitus. Ongoing studies have focused on the

stomach because patients with diabetes develop upper gastrointestinal symptoms, including the syndrome of gastroparesis. In addition, pharmacological treatments and bariatric procedures directed to the stomach have been the most efficacious treatments of obesity. Some of these clinical gastrointestinal research observations are considered likely to impact patient care in diabetes mellitus and/or obesity and may thus lead to improved patient outcomes.

OBSERVATIONS RELATED TO UPPER GASTROINTESTINAL SYMPTOMS AND GLYCEMIC CONTROL IN DIABETES MELLITUS

Delayed stomach emptying is associated with upper gastrointestinal symptoms

Based on a systematic review of the literature, including 92 gastric emptying studies (26 breath test, 62 scintigraphy, 1 ultrasound, and 3 wireless motility capsule) there is an association between optimally measured delayed gastric emptying and upper gastrointestinal symptoms^[2]. Twenty-five of these studies provided quantitative data for meta-analysis (15 scintigraphy studies enrolling 4056 participants and 10 breath test studies enrolling 2231 participants). Evaluating the studies that used optimal gastric emptying test methodology, there were significant associations between gastric emptying and nausea, vomiting, abdominal pain, and early satiety/fullness in patients with upper gastrointestinal symptoms; gastric emptying and early satiety/fullness in patients with diabetes; and gastric emptying and nausea in patients with gastroparesis.

Gastric motor functions and other features in referred patients with diabetes with upper gastrointestinal symptoms

Among 108 adult patients with diabetes mellitus (60.2% females; median age 49.0 years; 71.3% with type 2 diabetes mellitus; one-third insulin dependent with median hemoglobin A1C 6.7%) presenting with upper gastrointestinal symptoms, the manifestations of diabetic triopathy (peripheral neuropathy, nephropathy, and retinopathy) were uncommon at the time of presentation^[3]. Nausea was the most common symptom (80.6%). Gastric emptying was rapid in 37% and slow in 19%. Gastric accommodation was abnormal in 39%. There was normal gastric accommodation and gastric emptying in 28% and 40.3% of the patients with type 2 diabetes mellitus had accelerated gastric emptying at one hour. These observations emphasize the importance of measuring these functions in patients with upper gastrointestinal symptoms in order to individualize treatment, such as with a dopamine D₂ antagonist or a 5-hydroxytryptamine receptor (5-HT₄) agonist for patients with delayed gastric emptying and a 5-HT_{1A} agonist in patients with impaired gastric accommodation.

Histopathological features and expression in the intrinsic mechanisms involved in gastric motor functions

In different morphological studies based on light microscopy examination of full-thickness gastric biopsies and immunofluorescence, there is evidence of reduction in the pacemaker cell repertoire (interstitial cells of Cajal^[4,5] and fibroblast-like cells positive for platelet-derived growth factor alpha^[6]), reduced numbers of neurons expressing nNOS^[4], and reduced numbers of M2 macrophages, which normally express the mannose receptors (CD206) and heme oxygenase-1, mediate cell repair, and have anti-inflammatory roles^[5]. Other studies show increase in CD68 immunocytes, suggesting immune-mediated damage to these pacing mechanisms^[6], and this may be aggravated in the presence of vagal denervation, a common sequel of longstanding type 1 diabetes mellitus. Normally, the efferent vagus nerve signals release of norepinephrine from splenic nerves, activating the β 2-adrenergic receptor expressed on T cells, and macrophages and other immune cells, suppressing the release of pro-inflammatory cytokines^[7]. In summary, the interplay of vagal neuropathy, intrinsic neuropathy and immune modulation are considered combination factors leading to the gastric motility disorder.

Role of hyperglycemia in diabetic gastroparesis

The role of hyperglycemia in diabetic gastroparesis is unclear. On the one hand, there is epidemiological evidence of association of glycemia with upper gastrointestinal symptoms^[8], documentation of poor glycemic control in 36% patients admitted to the hospital for exacerbations of diabetic gastroparesis^[9], kidney and pancreas transplants improve gastric emptying and associated gastrointestinal symptoms^[10]. Conversely, hemoglobin A1C was not a statistically significant predictor of abnormal (compared to normal) gastric emptying of solids in a study of 129 patients^[11], and long-term blood glucose control had no significant effect on gastric emptying in type 2 diabetes mellitus^[12].

Role of stomach emptying on glycemic control

The available literature suggests that the stomach emptying does have an impact on glycemic control, and not only in patients with gastroparesis. Some published reports confirm the notion that gastroparesis impacts glycemic control: (1) patients with gastroparesis as documented in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications cohort, gastroparesis was associated with relatively worse glycemic control, as assessed by glycosylated hemoglobin^[13]; (2) there is poor glycemic control in 36% patients admitted to the hospital for exacerbations of diabetic gastroparesis^[9]; and (3) in insulin-treated patients with gastroparesis, delayed gastric emptying may increase the potential for a mismatch the timing of exogenous, preprandial, insulin

and the actual delivery of nutrients such as glucose from the stomach to be absorbed from in the small intestine. In a study involving 11 type 1 patients, less insulin was required to achieve euglycemia during the first 120 min after a meal in the 5 with gastroparesis, and more between 180-240 min^[14]. In addition, there is also evidence that the rate of gastric emptying has a major impact on the glycemic response to carbohydrate-containing meals in health and diabetes, particularly the initial postprandial increment^[15]. Therefore, it is now appreciated that postprandial glycemic excursions make a major contribution to "overall" glycemic control as assessed by hemoglobin A1C. Delayed gastric emptying in type 1 diabetes has recently been reported to be associated with an overall increase in blood glucose during the day; this may reflect the mismatch between the preprandial insulin and the later absorption of food due to the delayed gastric emptying^[16].

OBSERVATIONS RELATED TO GASTROINTESTINAL HORMONES IN OBESITY

Role of gastric emptying in the beneficial effects of GLP-1 analogs

Liraglutide, a long-acting glucagon-like peptide-1 (GLP-1) receptor agonist, is approved for treatment of obesity; however, the mechanisms of action of liraglutide are incompletely understood and include increase in satiety, increase in resting energy expenditure, and direct effects on appetite centers in the brain^[17]. In a randomized, double-blind, placebo-controlled trial of subcutaneous liraglutide (3.0 mg) in 40 patients at Mayo Clinic, liraglutide delayed gastric emptying of solids at 5 wk and 16 wk, and there was significantly greater weight loss and lower volume of a nutrient drink to reach the maximum tolerated volume in the liraglutide group than in the placebo group. The effects of liraglutide on weight loss are associated with delay in gastric emptying of solids, and the measurement of gastric emptying (e.g., at 5 wk of treatment) may be a biomarker of responsiveness and may help to select individuals for prolonged treatment with this class of drug^[18].

The effect of GLP-1 receptor agonist on weight loss does not appear to be impacted by the presence of metabolic derangements such as type 2 diabetes. However, there is evidence of a significant correlation in the weight loss induced by liraglutide and delay of gastric emptying^[18].

Incretin combinations for obesity

Incretin and pancreatic hormones [e.g., amylin, glucagon, glucose-stimulated insulinotropic peptide (GIP), GLP-1 and peptide tyrosine tyrosine (PYY)] generally inhibit upper gastrointestinal motor function^[19] or secretion (e.g., oxyntomodulin). Moreover, many of these hormones also exert central effects that reduce appetite^[20], and some

Table 1 Bariatric endoscopic procedure

Intraluminal devices
Intragastric balloon
Orbera ^a
ReShape ^a
Obalon ^a
Duodeno-jejunal bypass sleeve (EndoBarrier)
Aspiration therapy (AspireAssist)
Intraluminal suturing
Endoscopic sleeve gastropasty
Transoral gastropasty

^aIn order of date approved by United States Food and Drug Administration.

(e.g., GLP-1 analogs or GLP-1 receptor agonists) are efficacious in the treatment of obesity^[21].

Several combined incretin hormones have been tested in the context of obesity. Co-administration of GLP-1 with glucagon in humans increased energy expenditure and reduced food intake^[22,23]. A unimolecular dual incretin consisting of PEGylated GLP-1 and GIP co-agonist maximized metabolic benefits in rodents, monkeys, and humans^[24].

The combination of GLP-1 and PYY₃₋₃₆ also exerts synergistic effects with a reduction of 30.4% of food intake compared to placebo and more than the sum of each hormone independently, suggesting a synergistic effect^[25]. Acute, continuous, subcutaneous infusion for 10.5 h/d of GLP-1, PYY, and oxyntomodulin (summarized as GOP) was administered at doses that replicate postprandial levels observed after Roux-en-Y gastric bypass in a placebo-controlled, crossover study. GOP reduced food intake with a mean reduction of 32% without significantly altering resting energy expenditure^[26].

Combined bariatric endoscopy with pharmacotherapy

One study compared the effects of an intragastric balloon in 64 patients compared to a combination of balloon plus liraglutide, up to 1.8 mg/d, in 44 patients matched for body mass index (BMI) at baseline^[27]. The mean weight loss after balloon removal was 8.3 kg greater in the balloon plus liraglutide group than in the balloon alone group, and the advantage persisted 6 mo post-balloon removal in the group receiving liraglutide^[27].

BARIATRIC ENDOSCOPIC PROCEDURES

The development of endoscopic interventions for treatment of obesity and diabetes mellitus has focused on two areas (Table 1), the placement of intraluminal devices and intraluminal suturing^[28,29].

Intraluminal devices

The first intragastric balloon, the Garren-Edwards bubble, was approved by the United States Food and Drug Administration in 1985. The United States Food and Drug Administration has now approved 3 separate intragastric

balloon systems in the past 3 years: The Orbera balloon, the ReShape balloon, and the Obalon balloon. Delayed gastric emptying has been identified as a mechanism for weight loss in individuals who have undergone insertion of a fluid-filled intragastric balloon^[29], which raises the question of their utility in individuals with diabetic gut autonomic neuropathy. After the intraluminal balloons are removed, individuals required a maintenance program (which has not yet been standardized) to prevent weight regain. Our previous concern^[29] that specialized training is needed for the use of these devices appears to be supported by three warnings (in February 2017, August 2017, and June 2018) from the United States Food and Drug Administration with regards to issues related to intragastric balloons including multiple deaths related to intragastric balloons. Two major reviews in the past two years examined the 30+ year experience with intragastric balloons^[30,31]. Brethauer *et al.*^[30] concluded that more study was required in patients with type 2 diabetes mellitus. Popov *et al.*^[31] concluded that intragastric balloons were more effective than diet alone for an initial improvement of metabolic risk factors, but that their conclusions are limited by the small number of participants and the lack of long-term follow-up data.

Some countries have the availability of a duodeno-jejunal bypass sleeve, termed the EndoBarrier. This impermeable fluoropolymer sleeve with a nitinol anchor is deployed from the duodenal bulb and into the jejunum under fluoroscopic and endoscopic guidance. A clinical trial of this device in the United States in individuals with diabetes mellitus was halted early due to the development of liver infections. Three studies involving the EndoBarrier in individuals with type 2 diabetes mellitus have been published in the past one year. Betzel *et al.*^[32] were able to implant the device in 185 out of 198 participants. Sixty-nine percent of the participants were able to complete a one year program prior to removal of the device. Hemoglobin A1C levels declined by a mean of 9%, but no long term data was available. Forner *et al.*^[33] reported their findings in 114 individuals who maintained an EndoBarrier for a mean of 51.1 wk after its placement; the authors reported that mean Hemoglobin A1C was not significantly improved but that 6 subjects developed device obstructions, 5 individuals had gastrointestinal hemorrhage, 2 individuals developed liver abscesses, and 1 individual developed acute pancreatitis. Patel *et al.*^[34] reported a multicenter trial involved EndoBarrier Placement in 45 individuals with type 2 diabetes. Thirty-one individuals (69%) completed the 12 mo study. The mean hemoglobin A1C reduction at 12 mo was 0.8% below baseline. After explant, these subjects were only followed for an additional 6 mo.

The AspireAssist (Aspire Bariatrics, King of Prussia, Pennsylvania, United States) was approved by the United States Food and Drug Administration in 2016. A specialized aspiration tube (with both an intragastric portion with holes to permit aspiration as well as a skin port) is placed percutaneously at upper endoscopy into an individual's stomach. Stomach contents are then

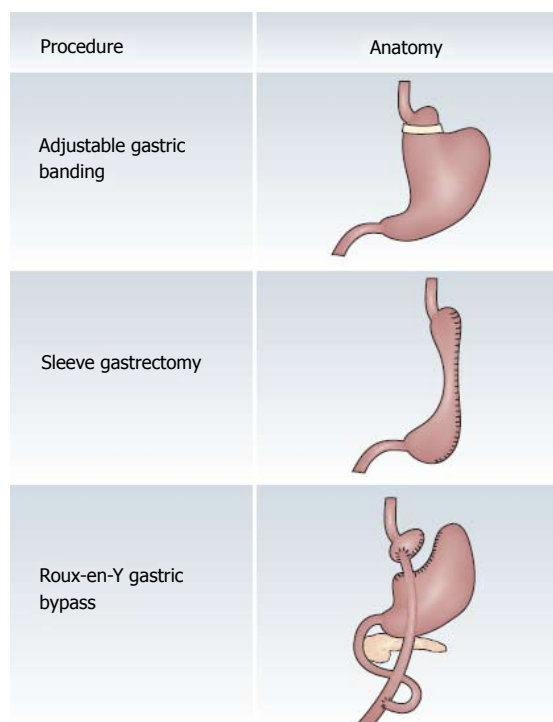


Figure 1 Comparison between bariatric surgical procedures. The top cartoon depicts the adjustable gastric band which limits the types of food consumed postoperatively by patients. The middle cartoon depicts the vertical sleeve gastrectomy which limits the volume of food consumed postoperatively by patients. The lower cartoon depicts the Roux-en-Y gastric bypass which limits volume of food consumed, may alter absorption of macronutrients, and alters release of intestinal incretins (Reproduced with the permission of Nature Publishing Group from Bal *et al.* *Nature Rev Endocrinol* 2012; 8: 544-556).

aspirated 20 min after a meal containing more than 200 kcal. A recent European trial examining the aspiration tube described a decrease in Hemoglobin A1C from 7.8% at baseline to 6.8% at only 1 year post-placement and mean percent weight loss of 19.2% at 4 years post-placement^[35].

As described above, long-term weight loss and metabolic results are not available for these endoscopic devices. Therefore their role in the treatment of obese individuals with diabetes mellitus remains to be defined.

Intraluminal suturing

Formation of an endoscopic sleeve gastropasty or a transoral gastropasty has been described by using intraluminal suturing devices during upper endoscopy. The Mayo Clinic, Rochester, Minnesota United States reported a method in 2013 for an endoscopic sleeve gastropasty. This procedure involves the use of a commercially available suturing device (OverStitch, Apollo Endosurgery, Austin, Texas United States), and the endoscopic sleeve gastrectomy is the predominant intraluminal technique presently described in the literature. Development of an endoscopic procedure to mimic the surgical vertical sleeve gastrectomy could reduce the risks of a gastric leak or perforation and of general anesthesia. However, results from the surgical literature support the importance of obtaining long-

term weight loss data. When the Mayo Clinic, Rochester, Minnesota, United States reported their results from the surgical non-banded vertical gastropasty, only 31% of patients were judged to have persistent excess weight lost after 4 years^[36]. A proposed mechanism for weight loss after the endoscopic sleeve gastrectomy is slowing of gastric emptying, which raises the question of its utility in individuals with diabetic gut autonomic neuropathy. Further research should better define the potential long-term role of intraluminal suturing in weight loss and the treatment of obese individuals with diabetes mellitus.

BARIATRIC SURGERY

Bariatric surgical procedures

The well described and worldwide utilized bariatric surgical procedures^[37] include the adjustable gastric band, the vertical sleeve gastrectomy, and the Roux-en-Y gastric bypass (Figure 1). By 2014, there were 579000 yearly bariatric surgical procedures of which 45.9% were the vertical sleeve gastrectomy, 39.6% were the Roux-en-Y gastric bypass, and 7.4% were the adjustable gastric band^[38]. The adjustable gastric band systems now use a soft, silicone ring which is placed around the upper part of the stomach approximately 4 cm below the gastroesophageal junction and is connected to an access port by tubing to adjust the band volume. Restriction of the proximal stomach is altered by addition or removal of sterile saline through the access port and there is no cutting or stapling of the stomach or bypass of small intestine. The vertical sleeve gastrectomy or gastric sleeve resection can be completed with a single step restrictive operation. By resection of 60% to 80% of the stomach along the greater curvature, multiple staplers can produce a tubular gastric pouch. Weight loss after vertical sleeve gastrectomy appears to involve several potential mechanisms in addition to restriction in the size of meal portions^[39]. In the Roux-en-Y gastric bypass, there is complete division of native stomach with production of a gastric pouch of less than 30 mL. The surgeon divides the jejunum 30 to 70 cm distal to the junction of the duodenum with the jejunum. The location of the jejuno-enteric anastomosis determines the lengths of the Roux limb (*e.g.*, the gastric pouch to the jejuno-enteric anastomosis) and the common channel (*e.g.*, the jejuno-enteric anastomosis to the ileocecal valve). A common channel that is shorter than 120 cm can induce a severe malabsorptive disorder. The mechanisms of weight loss after gastric bypass are complex and can include upper gut bacterial overgrowth, a common intestinal disorder in individuals with diabetes mellitus, as well as glucose malabsorption^[40]. Studies of glucose malabsorption after gastric bypass are of interest because jejunal administration of glucose appears to suppress plasma levels of the orexigenic hormone, acyl ghrelin^[41]. Further studies of the mechanisms of weight loss after bariatric surgery are clearly important since a proportion of individuals have poor long-term weight loss^[42].

Table 2 Long term weight loss after bariatric surgery

Ref.	Study ^a	Type of surgery ^b	Follow up	Result (%) ¹
[52]	MA	AGB	≥ 10 yr	EWL: 47.4
[52]	MA	VSG	≥ 5 yr	EWL: 53.2
[52]	MA	RYGB	≥ 10 yr	EWL: 63.5
[44]	MCS	AGB	15 yr	MWL: 13.0
[44]	MCS	RYGB	15 yr	MWL: 27.0
[53]	SCS	VSG	8 yr	EWL: 67.0
[54]	SCS	VSG	8 yr	EWL: 51.1
[55]	MCS	VSG	10 yr	EWL: 70.5
[56]	SR	AGB	3-5 yr	EWL: 45.0
[56]	SR	VSG	3-5 yr	EWL: 64.5
[56]	SR	RYGB	3-5 yr	EWL: 65.7

^aMA: Meta-analysis; MCS: Multi-center study; SCS: Single-center study; SR: Systematic review; ^bAGB: Adjustable gastric band; VSG: Vertical sleeve gastrectomy; RYGB: Roux-en-Y gastric bypass; ¹EWL: Mean percentage excess weight loss; MWL: Mean weight loss.

Long term mortality after bariatric surgery

The potential importance of bariatric surgery in individuals with diabetes mellitus was well publicized following a 2007 report that after a mean follow up of 7.1 years, individuals who underwent gastric bypass surgery had a 40% decrease in their adjusted long-term mortality (but by 92% for diabetes) compared to the control group^[43]. This landmark study has been supported by a report of decreased mortality compared to usual care at 16 years in the Swedish Obese Subjects trial^[44]. In a national study from Israel, bariatric surgery at a mean follow up of 4.5 years was shown to lower all-cause mortality compared to usual care obesity management^[45]. Finally, in a recent examination of the American National Health and Nutrition Examination Survey, it was reported that bariatric surgery can result in a relevant reduction of mortality in the United States obese population^[46].

Long term weight loss after bariatric surgery

Evaluation of the results reported for the different major bariatric surgical procedures may vary dependent upon whether single center data or multicenter data is examined. The largest discrepancy appears in reports concerning the adjustable gastric band, which may in part explain its decreased worldwide utilization. Several major reports in the past one year have supported major weaknesses of the adjustable band. In a single center report from Switzerland, after over 10 years of follow up, 71% of patients had lost their gastric band and only 15% of patients had good to excellent results^[47]. In a French national study of 52868 patients up to 7 years after adjustable gastric banding, the band removal rate was about 6% per year^[48]. In a study from the state of New York, among 16444 patients who underwent adjustable gastric banding, with at least four years of follow up the rate of revisions/conversions was 26.0%^[49]. An early meta-analysis reported that bariatric surgery does result in a weight loss of 20 to 30 kg, which is maintained for

up to 10 years^[50]. A follow up meta-analyses in 2013 by Gloy and associates reported in a shorter follow up that individuals allocated to bariatric surgery lost a mean of 26 kg more body weight^[51]. Representative reports of long-term weight loss after bariatric surgery are summarized in Table 2. The most effective bariatric surgical procedure for weight loss at up to 15 years of post-operative follow up is the Roux-en-Y gastric bypass surgery^[44,52]. The least effective major bariatric surgical procedure in long term studies of weight loss is the adjustable gastric band^[44,52,56].

Long term control of diabetes mellitus after bariatric surgery

A joint statement by international diabetes organizations supports consideration of bariatric surgery in individuals with diabetes mellitus and: BMI ≥ 40 kg/m², BMI 35-39.9 kg/m² and inadequate control of hyperglycemia with optimal medical therapy, or BMI 30-34.9 kg/m² and inadequate control of hyperglycemia with oral or injectable medications^[59]. In the United States, individuals with diabetes mellitus considering bariatric surgery are evaluated if they fulfill National Institutes of Health criteria, which is a BMI of ≥ 35 kg/m², while in other countries individuals with diabetes and a BMI as low as 25 kg/m² may be considered for Roux-en-Y gastric bypass. Specific bariatric surgical procedures such as the vertical sleeve gastrectomy may not be effective for treatment of individuals with type 1 diabetes mellitus^[60]. A meta-analysis comparing non-surgical treatment for obesity with bariatric surgery concluded that individuals allocated to bariatric surgery had a higher remission rate of type 2 diabetes^[51]. A second meta-analysis with 5 years of follow-up confirmed a significant decline in the relative risk of diabetes after bariatric surgery^[61]. Representative reports of long-term control of diabetes mellitus after bariatric surgery are summarized in Table 3. The most effective bariatric surgical procedure for remission of diabetes mellitus at up to 6 years of post-operative follow up is the Roux-en-Y gastric bypass surgery^[56,62]. The least effective major bariatric surgical procedure in long term studies of remission of diabetes mellitus is the adjustable gastric band^[56,62]. The importance of remission of diabetes is supported by a report of decreased incidence of microvascular and macrovascular complications in post-operative bariatric patients compared to controls^[58]. These published results do support the importance of ongoing development of more effective bariatric surgical procedures for the treatment of individuals with obesity and type 2 diabetes mellitus.

BARIATRIC PROCEDURES UNDER DEVELOPMENT

Gastrointestinal surgeons who specialize in bariatrics have seen an improvement in weight related comorbidities for decades. Encouraged by mounting evidence of resolution

Table 3 Long term control of diabetes mellitus after bariatric surgery

Ref.	Type of study ^a	Surgery ^b	Follow up	Result ¹
[53]	SCS	VSG	8 yr	NoRMRxDM: 43.4%
[54]	SCS	VSG	8 yr	NoRMRxDM: 37%
[56]	SR	AGB	3-5 yr	NoRMRxDM: 28.6%
[56]	SR	RYGB	3-5 yr	NoRMRxDM: 66.7%
[57]	SCS	RYGB	9 yr	NoRMRxDM: 73%
[58]	MCS	AGB	15 yr	NoRMRxDM: 38%
[58]	MCS	RYGB	15 yr	NoRMRxDM: 35%
[62]	NPBCS	AGB	6 yr	NoRMRxDM: 32%
[62]	NPBCS	VSG	6 yr	NoRMRxDM: 41%
[62]	NPBCS	RYGB	6 yr	NoRMRxDM: 58%
[63]	SCS	AGB	10 yr	NoRMRxDM: 18%

^aSCS: Single-center study; SR: Systematic review; MCS: Multi-center study; NPBCS: Nationwide population-based cohort study; ^bAGB: Adjustable gastric band; VSG: Vertical sleeve gastrectomy; RYGB: Roux-en-Y gastric bypass; ¹NoRMRxDM: No requirement for medical therapy for diabetes mellitus.

or substantial improvement in diseases such as diabetes mellitus, hyperlipidemia, and hypertension, leaders in the field created a paradigm shift by renaming the American Society of Bariatric Surgery as the American Society of Metabolic and Bariatric Surgery in 2007 (ASMBS.org; accessed on July 15, 2018). Gastrointestinal surgeons continue to manipulate the gastrointestinal tract in an effort to maximize the physiologic benefit to the individual patient. Restrictive procedures (*i.e.*, vertical sleeve gastrectomy, adjustable gastric banding, and the largely abandoned vertical banded gastroplasty) provide a benefit usually in proportion to the absolute weight loss achieved. Newer endoscopic and surgical procedures including the intragastric balloon, vBloc (described below), and aspirational therapy have more modest results.

Procedures that combine restriction with malabsorption by bypassing a portion of the foregut and midgut do provide measurable changes in comorbid conditions out of proportion to absolute weight loss. The traditional "gold standard" of surgical weight loss procedures, the Roux-en-Y gastric bypass, can provide rapid glucose control for patients. This procedure may take advantage of the "foregut hypothesis" that bypassing the foregut reduces or suppresses the secretion of anti-incretin hormones, which in turn leads to improvement of blood glucose control^[64]. Proponents of the "hindgut hypothesis" feel this improvement is more likely secondary to rapid delivery of nutrients to the distal small intestine, which facilitates the release of hormones such as GLP-1^[64,65]. Procedures which take advantage of this scenario include the biliopancreatic diversion/duodenal switch. Based upon these previous observations and notions, more recent modifications to this malabsorption procedure have shown favorable results in treatment of metabolic diseases, with up to 4 years of follow up^[66,67]. Further refinements in these procedures will likely yield more promising results which may be able to be individualized for specific patient needs.

Another area of ongoing, active clinical research has been vagal nerve stimulation, based on the important role the vagus nerve plays in regulation pathways

involving short-term regulation of dietary intake. The therapy termed vBloc for Vagal BLOCking therapy (ReShape Lifesciences, San Clemente, California United States) uses intermittent intra-abdominal high-frequency electrical currents for vagal blocking. At laparoscopy, electrodes are placed on the two vagal trunks near the gastroesophageal junction. There is no anatomical modification and an external controller is used to program the device. Vagal nerve stimulation with vBloc in a 2 year study has shown promise for weight loss (mean of 21% of excess weight loss), but with only an marginal impact on diabetes (only a 0.3% decline in hemoglobin A1C)^[68]. Further evaluation is therefore required to determine which patients with obesity and diabetes mellitus may benefit from this bariatric procedure.

CONCLUSION

The worldwide prevalence of obesity continues to rise. This rise increases the incidence of type 2 diabetes mellitus with subsequent requirements for additional health care in countries across the world. This supports the need for an increased interaction between ongoing clinical research in the allied fields of gastrointestinal medicine/surgery and diabetes mellitus. Among the clinically-relevant advances in diabetes, obesity, and metabolic syndrome emanating from gastroenterological research, delayed gastric emptying and impaired gastric accommodation result in upper gastrointestinal symptoms, through intrinsic nerve and pacemaker dysfunction. Glycemic control has a limited effect on gastric emptying in diabetic gastroparesis. Treatment of diabetes with pancreatic hormones and incretins inhibits gastric emptying, reduces hyperglycemia, and facilitates weight loss. The GLP-1 analog, liraglutide, is one of the two most efficacious treatments of obesity. New bariatric endoscopic procedures have been developed for weight loss in individuals with obesity, but long term follow-up with regards to maintenance of weight loss and control of hyperglycemia in individuals with diabetes is required prior to mass introduction of these endoscopic interventions. Bariatric surgical procedures are efficacious

in diabetes and obesity, but a proportion of individuals have poor long-term weight loss after bariatric surgery. On the horizon, combination therapies directed at gastrointestinal function and newer bariatric surgical procedures appear promising for individuals with obesity and type 2 diabetes mellitus.

REFERENCES

- GBD 2015 Obesity Collaborators**, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, Marczak L, Mokdad AH, Moradi-Lakeh M, Naghavi M, Salama JS, Vos T, Abate KH, Abbafati C, Ahmed MB, Al-Aly Z, Alkerwi A, Al-Raddadi R, Amare AT, Amberbir A, Amegah AK, Amini E, Amrock SM, Anjana RM, Ärnlöv J, Asayesh H, Banerjee A, Barac A, Baye E, Bennett DA, Beyene AS, Biadgilign S, Biryukov S, Bjertness E, Boneya DJ, Campos-Nonato I, Carrero JJ, Cecilio P, Cercy K, Ciobanu LG, Cornaby L, Damtew SA, Dandona L, Dandona R, Dharmaratne SD, Duncan BB, Eshrati B, Esteghamati A, Feigin VL, Fernandes JC, Fürst T, Gebrehiwot TT, Gold A, Gona PN, Goto A, Habtewold TD, Hadush KT, Hafezi-Nejad N, Hay SI, Horino M, Islami F, Kamal R, Kasaiean A, Katikireddi SV, Kengne AP, Kesavachandran CN, Khader YS, Khang YH, Khubchandani J, Kim D, Kim YJ, Kinfu Y, Kosen S, Ku T, Defo BK, Kumar GA, Larson HJ, Leinsalu M, Liang X, Lim SS, Liu P, Lopez AD, Lozano R, Majeed A, Malekzadeh R, Malta DC, Mazidi M, McAlinden C, McGarvey ST, Mengistu DT, Mensah GA, Mensink GBM, Mezgebe HB, Mirakhorimov EM, Mueller UO, Noubiap JJ, Obermeyer CM, Ogbo FA, Owolabi MO, Patton GC, Pourmalek F, Qorbani M, Rafay A, Rai RK, Ranabhat CL, Reinig N, Safiri S, Salomon JA, Sanabria JR, Santos IS, Sartorius B, Sawhney M, Schmidhuber J, Schutte AE, Schmidt MI, Sepanlou SG, Shamsizadeh M, Sheikhbahaei S, Shin MJ, Shiri R, Shiue I, Roba HS, Silva DAS, Silverberg JI, Singh JA, Stranges S, Swaminathan S, Tabarés-Seisdedos R, Tadese F, Tedla BA, Tegegne BS, Terkawi AS, Thakur JS, Tonelli M, Topor-Madry R, Tyrovolas S, Ukwaja KN, Uthman OA, Vaezghasemi M, Vasankari T, Vlassov VV, Vollset SE, Weiderpass E, Werdecker A, Wesana J, Westerman R, Yano Y, Yonemoto N, Yonga G, Zaidi Z, Zenebe ZM, Zipkin B, Murray CJL. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med* 2017; **377**: 13-27 [PMID: 28604169 DOI: 10.1056/NEJMoa1614362]
- 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* 2018 [PMID: 29860241 DOI: 10.1136/gutjnl-2018-316405]
- 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* 2018 [PMID: 30166634 DOI: 10.1038/s41395-018-0234-1]
- Grover M**, Farrugia G, Lurken MS, Bernard CE, Fausone-Pellegrini MS, Smyrk TC, Parkman HP, Abell TL, Snape WJ, Hasler WL, Ünalp-Arida A, Nguyen L, Koch KL, Calles J, Lee L, Tonascia J, Hamilton FA, Pasricha PJ; NIDDK Gastroparesis Clinical Research Consortium. Cellular changes in diabetic and idiopathic gastroparesis. *Gastroenterology* 2011; **140**: 1575-85.e8 [PMID: 21300066 DOI: 10.1053/j.gastro.2011.01.046]
- Bernard CE**, Gibbons SJ, Mann IS, Froschauer L, Parkman HP, Harbison S, Abell TL, Snape WJ, Hasler WL, McCallum RW, Sarosiek I, Nguyen LA, Koch KL, Tonascia J, Hamilton FA, Kendrick ML, Shen KR, Pasricha PJ, Farrugia G; NIDDK Gastroparesis Clinical Research Consortium (GpCRC). Association of low numbers of CD206-positive cells with loss of ICC in the gastric body of patients with diabetic gastroparesis. *Neurogastroenterol Motil* 2014; **26**: 1275-1284 [PMID: 25041465 DOI: 10.1111/nmo.12389]
- Herring BP**, Hoggatt AM, Gupta A, Griffith S, Nakeeb A, Choi JN, Idrees MT, Nowak T, Morris DL, Wo JM. Idiopathic gastroparesis is associated with specific transcriptional changes in the gastric muscularis externa. *Neurogastroenterol Motil* 2018; **30**: e13230 [PMID: 29052298 DOI: 10.1111/nmo.13230]
- Han B**, Li X, Hao J. The cholinergic anti-inflammatory pathway: An innovative treatment strategy for neurological diseases. *Neurosci Biobehav Rev* 2017; **77**: 358-368 [PMID: 28392244 DOI: 10.1016/j.neubiorev.2017.04.002]
- 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]
- Uppalapati SS**, Ramzan Z, Fisher RS, Parkman HP. Factors contributing to hospitalization for gastroparesis exacerbations. *Dig Dis Sci* 2009; **54**: 2404-2409 [PMID: 19760157 DOI: 10.1007/s10620-009-0975-1]
- Gaber AO**, Oxley D, Karas J, Cardoso S, Hathaway D, Shokouh-Amiri MH, Jensen SL, Abell TL. Changes in gastric emptying in recipients of successful combined pancreas-kidney transplants. *Dig Dis* 1991; **9**: 437-443 [PMID: 1804583 DOI: 10.1159/000171334]
- Bharucha AE**, Camilleri M, Forstrom LA, Zinsmeister AR. Relationship between clinical features and gastric emptying disturbances in diabetes mellitus. *Clin Endocrinol (Oxf)* 2009; **70**: 415-420 [PMID: 18727706 DOI: 10.1111/j.1365-2265.2008.03351.x]
- Holzäpfel A**, Festa A, Stacher-Janotta G, Bergmann H, Shnawa N, Brannath W, Schernthaner G, Stacher G. Gastric emptying in Type II (non-insulin-dependent) diabetes mellitus before and after therapy readjustment: no influence of actual blood glucose concentration. *Diabetologia* 1999; **42**: 1410-1412 [PMID: 10651258 DOI: 10.1007/s001250051311]
- Bharucha AE**, Batey-Schaefer B, Cleary PA, Murray JA, Cowie C, Lorenzi G, Driscoll M, Harth J, Larkin M, Christofi M, Bayless M, Wimmergren N, Herman W, Whitehouse F, Jones K, Kruger D, Martin C, Ziegler G, Zinsmeister AR, Nathan DM; Diabetes Control and Complications Trial-Epidemiology of Diabetes Interventions and Complications Research Group. Delayed Gastric Emptying Is Associated With Early and Long-term Hyperglycemia in Type 1 Diabetes Mellitus. *Gastroenterology* 2015; **149**: 330-339 [PMID: 25980755 DOI: 10.1053/j.gastro.2015.05.007]
- Ishii M**, Nakamura T, Kasai F, Onuma T, Baba T, Takebe K. Altered postprandial insulin requirement in IDDM patients with gastroparesis. *Diabetes Care* 1994; **17**: 901-903 [PMID: 7956640 DOI: 10.2337/diacare.17.8.901]
- Phillips LK**, Deane AM, Jones KL, Rayner CK, Horowitz M. Gastric emptying and glycaemia in health and diabetes mellitus. *Nat Rev Endocrinol* 2015; **11**: 112-128 [PMID: 25421372 DOI: 10.1038/nrendo.2014.202]
- Parthasarathy G**, Kudva YC, Low PA, Camilleri M, Basu A, Bharucha AE. Relationship Between Gastric Emptying and Diurnal Glycemic Control in Type 1 Diabetes Mellitus: A Randomized Trial. *J Clin Endocrinol Metab* 2017; **102**: 398-406 [PMID: 27880079 DOI: 10.1210/jc.2016-2809]
- Horowitz M**, Flint A, Jones KL, Hindsberger C, Rasmussen MF, Kapitza C, Doran S, Jax T, Zdravkovic M, Chapman IM. Effect of the once-daily human GLP-1 analogue liraglutide on appetite, energy intake, energy expenditure and gastric emptying in type 2 diabetes. *Diabetes Res Clin Pract* 2012; **97**: 258-266 [PMID: 22446097 DOI: 10.1016/j.diabres.2012.02.016]
- Halawi H**, Khemani D, Eckert D, O'Neill J, Kadouh H, Grothe K, Clark MM, Burton DD, Vella A, Acosta A, Zinsmeister AR, Camilleri M. Effects of liraglutide on weight, satiation, and gastric functions in obesity: a randomised, placebo-controlled pilot trial. *Lancet Gastroenterol Hepatol* 2017; **2**: 890-899 [PMID: 28958851 DOI: 10.1016/S2468-1253(17)30285-6]
- Camilleri M**. Integrated upper gastrointestinal response to food intake. *Gastroenterology* 2006; **131**: 640-658 [PMID: 16890616 DOI: 10.1053/j.gastro.2006.03.023]
- Zanchi D**, Depoorter A, Egloff L, Haller S, Mählmann L, Lang

- UE, Drewe J, Beglinger C, Schmidt A, Borgwardt S. The impact of gut hormones on the neural circuit of appetite and satiety: A systematic review. *Neurosci Biobehav Rev* 2017; **80**: 457-475 [PMID: 28669754 DOI: 10.1016/j.neubiorev.2017.06.013]
- 21 **Khera R**, Murad MH, Chandar AK, Dulai PS, Wang Z, Prokop LJ, Loomba R, Camilleri M, Singh S. Association of Pharmacological Treatments for Obesity With Weight Loss and Adverse Events: A Systematic Review and Meta-analysis. *JAMA* 2016; **315**: 2424-2434 [PMID: 27299618 DOI: 10.1001/jama.2016.7602]
 - 22 **Tan TM**, Field BC, McCullough KA, Troke RC, Chambers ES, Salem V, Gonzalez Maffe J, Baynes KC, De Silva A, Viardot A, Alsafi A, Frost GS, Ghatei MA, Bloom SR. Coadministration of glucagon-like peptide-1 during glucagon infusion in humans results in increased energy expenditure and amelioration of hyperglycemia. *Diabetes* 2013; **62**: 1131-1138 [PMID: 23248172 DOI: 10.2337/db12-0797]
 - 23 **Cegla J**, Troke RC, Jones B, Tharakan G, Kenkre J, McCullough KA, Lim CT, Parvizi N, Hussein M, Chambers ES, Minnion J, Cuenco J, Ghatei MA, Meeran K, Tan TM, Bloom SR. Coinfusion of low-dose GLP-1 and glucagon in man results in a reduction in food intake. *Diabetes* 2014; **63**: 3711-3720 [PMID: 24939425 DOI: 10.2337/db14-0242]
 - 24 **Finan B**, Ma T, Ottaway N, Müller TD, Habegger KM, Heppner KM, Kirchner H, Holland J, Hembree J, Raver C, Lockie SH, Smiley DL, Gelfanov V, Yang B, Hofmann S, Bruemmer D, Drucker DJ, Pfluger PT, Perez-Tilve D, Gidda J, Vignati L, Zhang L, Hauptman JB, Lau M, Brecheisen M, Uhles S, Riboulet W, Hainaut E, Sebokova E, Conde-Knape K, Konkar A, DiMarchi RD, Tschöp MH. Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Sci Transl Med* 2013; **5**: 209ra151 [PMID: 24174327 DOI: 10.1126/scitranslmed.3007218]
 - 25 **Schmidt JB**, Gregersen NT, Pedersen SD, Arentoft JL, Ritz C, Schwartz TW, Holst JJ, Astrup A, Sjödin A. Effects of PYY3-36 and GLP-1 on energy intake, energy expenditure, and appetite in overweight men. *Am J Physiol Endocrinol Metab* 2014; **306**: E1248-E1256 [PMID: 24735885 DOI: 10.1152/ajpendo.00569.2013]
 - 26 **Tan T**, Behary P, Tharakan G, Minnion J, Al-Najim W, Albrechtsen NJW, Holst JJ, Bloom SR. The Effect of a Subcutaneous Infusion of GLP-1, OXM, and PYY on Energy Intake and Expenditure in Obese Volunteers. *J Clin Endocrinol Metab* 2017; **102**: 2364-2372 [PMID: 28379519 DOI: 10.1210/jc.2017-00469]
 - 27 **Mosli MM**, Elyas M. Does combining liraglutide with intragastric balloon insertion improve sustained weight reduction? *Saudi J Gastroenterol* 2017; **23**: 117-122 [PMID: 28361843]
 - 28 **Rashti F**, Gupta E, Ebrahimi S, Shope TR, Koch TR, Gostout CJ. Development of minimally invasive techniques for management of medically-complicated obesity. *World J Gastroenterol* 2014; **20**: 13424-13445 [PMID: 25309074 DOI: 10.3748/wjg.v20.i37.13424]
 - 29 **Koch TR**, Shope TR, Gostout CJ. Organization of future training in bariatric gastroenterology. *World J Gastroenterol* 2017; **23**: 6371-6378 [PMID: 29085186 DOI: 10.3748/wjg.v23.i35.6371]
 - 30 **Brethauer SA**, Chang J, Galvao Neto M, Greve JW. Gastrointestinal devices for the treatment of type 2 diabetes. *Surg Obes Relat Dis* 2016; **12**: 1256-1261 [PMID: 27568475 DOI: 10.1016/j.soard.2016.02.031]
 - 31 **Popov VB**, Ou A, Schulman AR, Thompson CC. The Impact of Intragastric Balloons on Obesity-Related Co-Morbidities: A Systematic Review and Meta-Analysis. *Am J Gastroenterol* 2017; **112**: 429-439 [PMID: 28117361 DOI: 10.1038/ajg.2016.530]
 - 32 **Betzl B**, Homan J, Aarts EO, Janssen IMC, de Boer H, Wahab PJ, Groenen MJM, Berends FJ. Weight reduction and improvement in diabetes by the duodenal-jejunal bypass liner: a 198 patient cohort study. *Surg Endosc* 2017; **31**: 2881-2891 [PMID: 27804045 DOI: 10.1007/s00464-016-5299-6]
 - 33 **Forner PM**, Ramacciotti T, Farey JE, Lord RV. Safety and Effectiveness of an Endoscopically Placed Duodenal-Jejunal Bypass Device (EndoBarrier®): Outcomes in 114 Patients. *Obes Surg* 2017; **27**: 3306-3313 [PMID: 29018990 DOI: 10.1007/s11695-017-2939-4]
 - 34 **Patel N**, Mohanaruban A, Ashrafian H, Le Roux C, Byrne J, Mason J, Hopkins J, Kelly J, Teare J. EndoBarrier®: a Safe and Effective Novel Treatment for Obesity and Type 2 Diabetes? *Obes Surg* 2018; **28**: 1980-1989 [PMID: 29450844 DOI: 10.1007/s11695-018-3123-1]
 - 35 **Nyström M**, Machytka E, Norén E, Testoni PA, Janssen I, Turró Homedes J, Espinos Perez JC, Turro Arau R. Aspiration Therapy As a Tool to Treat Obesity: 1- to 4-Year Results in a 201-Patient Multi-Center Post-Market European Registry Study. *Obes Surg* 2018; **28**: 1860-1868 [PMID: 29388050 DOI: 10.1007/s11695-017-3096-5]
 - 36 **Hocking MP**, Kelly KA, Callaway CW. Vertical gastropasty for morbid obesity: clinical experience. *Mayo Clin Proc* 1986; **61**: 287-291 [PMID: 3951259 DOI: 10.1016/S0025-6196(12)61932-6]
 - 37 **Bal BS**, Finelli FC, Shope TR, Koch TR. Nutritional deficiencies after bariatric surgery. *Nat Rev Endocrinol* 2012; **8**: 544-556 [PMID: 22525731 DOI: 10.1038/nrendo.2012.48]
 - 38 **Angrisani L**, Santonicola A, Iovino P, Vitiello A, Zundel N, Buchwald H, Scopinaro N. Bariatric Surgery and Endoluminal Procedures: IFSO Worldwide Survey 2014. *Obes Surg* 2017; **27**: 2279-2289 [PMID: 28405878 DOI: 10.1007/s11695-017-2666-x]
 - 39 **Sharbaugh ME**, Shope TR, Koch TR. Upper gut bacterial overgrowth is a potential mechanism for glucose malabsorption after vertical sleeve gastrectomy. *New Insights Obes Gent Beyond* 2017; **1**: 30-35 [DOI: 10.29328/journal.hodms.1001006]
 - 40 **Andalib I**, Shah H, Bal BS, Shope TR, Finelli FC, Koch TR. Breath Hydrogen as a Biomarker for Glucose Malabsorption after Roux-en-Y Gastric Bypass Surgery. *Dis Markers* 2015; **2015**: 102760 [PMID: 26538792 DOI: 10.1155/2015/102760]
 - 41 **Tamboli RA**, Sidani RM, Garcia AE, Antoun J, Isbell JM, Albaugh VL, Abumrad NN. Jejunal administration of glucose enhances acyl ghrelin suppression in obese humans. *Am J Physiol Endocrinol Metab* 2016; **311**: E252-E259 [PMID: 27279247 DOI: 10.1152/ajpendo.00082.2016]
 - 42 **Pucci A**, Batterham RL. Mechanisms underlying the weight loss effects of RYGB and SG: similar, yet different. *J Endocrinol Invest* 2018; **1-12** [PMID: 29730732 DOI: 10.1007/s40618-018-0892-2]
 - 43 **Adams TD**, Gress RE, Smith SC, Halverson RC, Simper SC, Rosamond WD, Lamonte MJ, Stroup AM, Hunt SC. Long-term mortality after gastric bypass surgery. *N Engl J Med* 2007; **357**: 753-761 [PMID: 17715409 DOI: 10.1056/NEJMoa066603]
 - 44 **Sjöström L**. Review of the key results from the Swedish Obese Subjects (SOS) trial - a prospective controlled intervention study of bariatric surgery. *J Intern Med* 2013; **273**: 219-234 [PMID: 23163728 DOI: 10.1111/joim.12012]
 - 45 **Reges O**, Greenland P, Dicker D, Leibowitz M, Hoshen M, Gofer I, Rasmussen-Torvik LJ, Balicer RD. Association of Bariatric Surgery Using Laparoscopic Banding, Roux-en-Y Gastric Bypass, or Laparoscopic Sleeve Gastrectomy vs Usual Care Obesity Management With All-Cause Mortality. *JAMA* 2018; **319**: 279-290 [PMID: 29340677 DOI: 10.1001/jama.2017.20513]
 - 46 **Gaeta M**, Rausa E, Malavazos AE, Bonavina L, Smuts CM, Ricci C. Bariatric Surgery to Reduce Mortality in US Adults. A Public Health Perspective from the Analysis of the American National Health and Nutrition Examination Survey Linked to the US Mortality Register. *Obes Surg* 2018; **28**: 900-906 [PMID: 29080041 DOI: 10.1007/s11695-017-2981-2]
 - 47 **Vinzens F**, Kilchenmann A, Zumstein V, Slawik M, Gebhart M, Peterli R. Long-term outcome of laparoscopic adjustable gastric banding (LAGB): results of a Swiss single-center study of 405 patients with up to 18 years' follow-up. *Surg Obes Relat Dis* 2017; **13**: 1313-1319 [PMID: 28602794 DOI: 10.1016/j.soard.2017.04.030]
 - 48 **Lazzati A**, De Antonio M, Paolino L, Martini F, Azoulay D, Iannelli A, Katsahian S. Natural History of Adjustable Gastric Banding: Lifespan and Revisional Rate: A Nationwide Study on Administrative Data on 53,000 Patients. *Ann Surg* 2017; **265**: 439-445 [PMID: 27433894 DOI: 10.1097/SLA.0000000000001879]
 - 49 **Altieri MS**, Yang J, Nie L, Blackstone R, Spaniolas K, Pryor A. Rate of revisions or conversion after bariatric surgery over 10 years in the state of New York. *Surg Obes Relat Dis* 2018; **14**: 500-507

- [PMID: 29496440 DOI: 10.1016/j.soard.2017.12.019]
- 50 **Maggard MA**, Shugarman LR, Suttrop M, Maglione M, Sugerman HJ, Livingston EH, Nguyen NT, Li Z, Mojica WA, Hilton L, Rhodes S, Morton SC, Shekelle PG. Meta-analysis: surgical treatment of obesity. *Ann Intern Med* 2005; **142**: 547-559 [PMID: 15809466 DOI: 10.7326/0003-4819-142-7-200504050-00013]
 - 51 **Gloy VL**, Briel M, Bhatt DL, Kashyap SR, Schauer PR, Mingrone G, Bucher HC, Nordmann AJ. Bariatric surgery versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. *BMJ* 2013; **347**: f5934 [PMID: 24149519 DOI: 10.1136/bmj.f5934]
 - 52 **Golzarand M**, Toolabi K, Farid R. The bariatric surgery and weight losing: a meta-analysis in the long- and very long-term effects of laparoscopic adjustable gastric banding, laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy on weight loss in adults. *Surg Endosc* 2017; **31**: 4331-4345 [PMID: 28378086 DOI: 10.1007/s00464-017-5505-1]
 - 53 **Noel P**, Nedelcu M, Eddballi I, Manos T, Gagner M. What are the long-term results 8 years after sleeve gastrectomy? *Surg Obes Relat Dis* 2017; **13**: 1110-1115 [PMID: 28755888 DOI: 10.1016/j.soard.2017.03.007]
 - 54 **Kowalewski PK**, Olszewski R, Walędziak MS, Janik MR, Kwiatkowski A, Gałązka-Świderek N, Cichoń K, Brągoszewski J, Paśnik K. Long-Term Outcomes of Laparoscopic Sleeve Gastrectomy-a Single-Center, Retrospective Study. *Obes Surg* 2018; **28**: 130-134 [PMID: 28707172 DOI: 10.1007/s11695-017-2795-2]
 - 55 **Chang DM**, Lee WJ, Chen JC, Ser KH, Tsai PL, Lee YC. Thirteen-Year Experience of Laparoscopic Sleeve Gastrectomy: Surgical Risk, Weight Loss, and Revision Procedures. *Obes Surg* 2018; **28**: 2991-2997 [PMID: 29931481 DOI: 10.1007/s11695-018-3344-3]
 - 56 **Puzziferri N**, Roshek TB 3rd, Mayo HG, Gallagher R, Belle SH, Livingston EH. Long-term follow-up after bariatric surgery: a systematic review. *JAMA* 2014; **312**: 934-942 [PMID: 25182102 DOI: 10.1001/jama.2014.10706]
 - 57 **MacDonald KG Jr**, Long SD, Swanson MS, Brown BM, Morris P, Dohm GL, Pories WJ. The gastric bypass operation reduces the progression and mortality of non-insulin-dependent diabetes mellitus. *J Gastrointest Surg* 1997; **1**: 213-220; discussion 220 [PMID: 9834350 DOI: 10.1016/S1091-255X(97)80112-6]
 - 58 **Sjöström L**, Peltonen M, Jacobson P, Ahlin S, Andersson-Assarsson J, Anveden Å, Bouchard C, Carlsson B, Karason K, Lönnroth H, Näslund I, Sjöström E, Taube M, Wedel H, Svensson PA, Sjöholm K, Carlsson LM. Association of bariatric surgery with long-term remission of type 2 diabetes and with microvascular and macrovascular complications. *JAMA* 2014; **311**: 2297-2304 [PMID: 24915261 DOI: 10.1001/jama.2014.5988]
 - 59 **Rubino F**, Nathan DM, Eckel RH, Schauer PR, Alberti KG, Zimmet PZ, Del Prato S, Ji L, Sadikot SM, Herman WH, Amiel SA, Kaplan LM, Taroncher-Oldenburg G, Cummings DE; Delegates of the 2nd Diabetes Surgery Summit. Metabolic Surgery in the Treatment Algorithm for Type 2 Diabetes: a Joint Statement by International Diabetes Organizations. *Obes Surg* 2017; **27**: 2-21 [PMID: 27957699 DOI: 10.1007/s11695-016-2457-9]
 - 60 **Al Sabah S**, Al Haddad E, Muzaffar TH, Almulla A. Laparoscopic Sleeve Gastrectomy for the Management of Type 1 Diabetes Mellitus. *Obes Surg* 2017; **27**: 3187-3193 [PMID: 28653181 DOI: 10.1007/s11695-017-2777-4]
 - 61 **Ricci C**, Gaeta M, Rausa E, Asti E, Bandera F, Bonavina L. Long-term effects of bariatric surgery on type II diabetes, hypertension and hyperlipidemia: a meta-analysis and meta-regression study with 5-year follow-up. *Obes Surg* 2015; **25**: 397-405 [PMID: 25240392 DOI: 10.1007/s11695-014-1442-4]
 - 62 **Thereaux J**, Lesuffleur T, Czernichow S, Basdevant A, Msika S, Nocca D, Millat B, Fagot-Campagna A. Association Between Bariatric Surgery and Rates of Continuation, Discontinuation, or Initiation of Antidiabetes Treatment 6 Years Later. *JAMA Surg* 2018; **153**: 526-533 [PMID: 29450469 DOI: 10.1001/jamasurg.2017.6163]
 - 63 **Wentworth JM**, Cheng C, Laurie C, Skinner S, Burton PR, Brown WA, O'Brien PE. Diabetes Outcomes More than a Decade Following Sustained Weight Loss After Laparoscopic Adjustable Gastric Band Surgery. *Obes Surg* 2018; **28**: 982-989 [PMID: 28975466 DOI: 10.1007/s11695-017-2944-7]
 - 64 **Mingrone G**, Castagneto-Gissey L. Mechanisms of early improvement/resolution of type 2 diabetes after bariatric surgery. *Diabetes Metab* 2009; **35**: 518-523 [PMID: 20152737 DOI: 10.1016/S1262-3636(09)73459-7]
 - 65 **Strader AD**, Vahl TP, Jandacek RJ, Woods SC, D'Alessio DA, Seeley RJ. Weight loss through ileal transposition is accompanied by increased ileal hormone secretion and synthesis in rats. *Am J Physiol Endocrinol Metab* 2005; **288**: E447-E453 [PMID: 15454396 DOI: 10.1152/ajpendo.00153.2004]
 - 66 **Cottam A**, Cottam D, Zaveri H, Cottam S, Surve A, Medlin W, Richards C. An Analysis of Mid-Term Complications, Weight Loss, and Type 2 Diabetes Resolution of Stomach Intestinal Pylorus-Sparing Surgery (SIPS) Versus Roux-En-Y Gastric Bypass (RYGB) with Three-Year Follow-Up. *Obes Surg* 2018; **28**: 2894-2902 [PMID: 29790130 DOI: 10.1007/s11695-018-3309-6]
 - 67 **Zaveri H**, Surve A, Cottam D, Cottam A, Medlin W, Richards C, Belnap L, Cottam S, Horsley B. Mid-term 4-Year Outcomes with Single Anastomosis Duodenal-Ileal Bypass with Sleeve Gastrectomy Surgery at a Single US Center. *Obes Surg* 2018; **28**: 3062-3072 [PMID: 29909514 DOI: 10.1007/s11695-018-3358-x]
 - 68 **Apovian CM**, Shah SN, Wolfe BM, Ikramuddin S, Miller CJ, Tweden KS, Billington CJ, Shikora SA. Two-Year Outcomes of Vagal Nerve Blocking (vBloc) for the Treatment of Obesity in the ReCharge Trial. *Obes Surg* 2017; **27**: 169-176 [PMID: 27506803 DOI: 10.1007/s11695-016-2325-7]

P- Reviewer: Reggiani GM, Tziomalos K S- Editor: Dou Y

L- Editor: A E- Editor: Bian YN



Unhealthy eating habits around sleep and sleep duration: To eat or fast?

Kei Nakajima

Kei Nakajima, School of Nutrition and Dietetics, Faculty of Health and Social Services, Kanagawa University of Human Services, Yokosuka 238-8522, Kanagawa, Japan

Kei Nakajima, Department of Endocrinology and Diabetes, Saitama Medical Center, Saitama Medical University, Kawagoe 350-8550, Saitama, Japan

ORCID number: Kei Nakajima (0000-0002-1788-3896).

Author contributions: Nakajima K conceived, designed and wrote this article.

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

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Corresponding author to: Kei Nakajima, MD, PhD, Doctor, Professor, School of Nutrition and Dietetics, Faculty of Health and Social Services, Kanagawa University of Human Services, 1-10-1 Heisei-cho, Yokosuka 238-8522, Kanagawa, Japan. nakajima-rsh@kuhs.ac.jp
Telephone: +81-46-8282660
Fax: +81-46-8282661

Received: July 30, 2018

Peer-review started: July 30, 2018

First decision: August 27, 2018

Revised: September 15, 2018

Accepted: October 11, 2018

Article in press: October 11, 2018

Published online: November 15, 2018

Abstract

Traditionally, breakfast skipping (BS), and recently late-night dinner eating (LNDE), have attracted attention in public health because they can predispose to cardiometabolic conditions such as obesity and type 2 diabetes. Intriguingly, it has become evident that short duration of sleep elicits similar health risks. As LNDE, BS, and short sleep can be closely related and can aggravate each other, these three should not be considered separately. In this context, LNDE (or its equivalents, snacking or heavy alcohol consumption after dinner) and BS may be representative unhealthy eating habits around sleep (UEHAS). While it is important to take energy in the early morning for physical and intellectual activities, attaining a fasting state is essential for metabolic homeostasis. Our previous UEHAS studies have shown that BS without LNDE, *i.e.*, BS alone, is not associated with obesity and diabetes, suggesting the possibility that BS or taking a very low energy breakfast, which could yield fasting for a while, may prevent obesity and diabetes in people with inevitable LNDE. Further studies considering UEHAS and short sleep simultaneously are needed to elucidate the effects of these unhealthy lifestyles on cardiometabolic diseases.

Key words: Breakfast skipping; Late-night dinner eating; Sleep; Fasting; Hungry

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Breakfast skipping (BS), late-night dinner eating (LNDE), and short duration of sleep have attracted attention because they elicit similar health risks: Obesity and type 2 diabetes. However, to-date these factors have been considered separately in terms of their health risks. LNDE and BS may be representative unhealthy eating habits around sleep

(UEHAS). It is important to take energy in the early morning, whereas attaining a fasting state is essential for metabolic homeostasis. Therefore, BS or taking a very low energy breakfast may prevent obesity and diabetes in people with LNDE. Consideration of UEHAS and short sleep deserves further study.

Nakajima K. Unhealthy eating habits around sleep and sleep duration: To eat or fast? *World J Diabetes* 2018; 9(11): 190-194 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i11/190.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i11.190>

MAIN CONTENT

Traditionally, breakfast skipping (BS) has been considered to contribute to various cardiometabolic conditions including obesity and type 2 diabetes not only in children and adolescents, but also in adults including the elderly^[1-5]. However, conflicting results have been reported^[6-8], probably because definitions of breakfast and BS have not been established yet^[9,10]. Multiple confounding factors, including age, sex, morbidities, and dietary culture, may also affect the outcomes, although these confounders are usually statistically adjusted for. Moreover, for the past decade, it has been argued that BS may result from conditions in the preceding night, such as late-night dinner eating (LNDE), eating snacks after dinner, or drinking alcohol until immediately before going to bed^[11-15].

Simultaneously, the quality and quantity of sleep, which usually manifest as short or long duration of sleep, may affect conditions in the early morning such as appetite for the breakfast meal^[16-19]. Of note, short duration of sleep has been robustly associated with similar cardiometabolic conditions to those associated with BS and LNDE, such as obesity, diabetes, and metabolic syndrome, as well as increased mortality^[20-23]. Taken together, the effect of short sleep on the above health risks may include the effect of BS, and vice versa. However, short sleep (or possibly long sleep) and BS have rarely been considered together. Circadian misalignment may be prevalent in individuals with LNDE, BS, and short sleep. Because LNDE, BS, and short sleep can be closely related and can aggravate each other^[9,11,13,15], these three factors should not be considered separately in terms of health and cardiometabolic conditions. In this context, LNDE and BS may be representative unhealthy eating habits around sleep (UEHAS) (Figure 1). Taken together, specific features can form when UEHAS and short sleep (UEHASs) are combined because of the relationships between them, whereas sleep and UEHAS are sufficiently independent in individuals with good sleep and healthy eating-habits around sleep (SHEHAS).

It is reasonable to assume that BS prolongs the fasting state and results in a lack of energy in the morning, which can result in hampered physical and intellectual activities and possibly in larger meal con-

sumption later in the day^[9,24], though conflicting outcomes have been reported^[25-28]. However, this theory may be exclusively applicable to healthy people without LNDE, *i.e.*, those who do not take dinner late at night. Of note, LNDE may be associated with hyperglycemia^[12,29-31], which remains until early morning. A time period of less than 6 h from LNDE to the end of a short sleep falls short of the 8-10 h criteria commonly used for overnight fasting^[32,33], although the definition of overnight fasting has not been definitively established. Theoretically, therefore, LNDE within 2 h of going to bed combined with a short (< 6 h) sleep does not yield a fasting state in the early morning. If people with LNDE sleep for a normal length of time, the opportunity for breakfast consumption may be missed because they do not have enough time to take a breakfast meal (Figure 1).

As mentioned by numerous experts, it is important to take energy in the early morning for healthy physical activity, whereas attaining a fasting state for a certain period in the day, usually during sleep because sleep involves equal or lower energy expenditure than resting energy expenditure^[34,35], is essential for metabolic homeostasis. Adequate fasting especially during sleep can enable plasma glucose to return to the preprandial level and plasma insulin to decline to baseline level, which prevents over-secretion of insulin and has a protective action for β -cell function in the pancreas. Having an appetite, *i.e.*, a feeling of hunger, for breakfast may be inappropriate if the body is not in a fasting state (etymologically, taking a breakfast without fasting beforehand does not constitute a break of fasting). Consumption of breakfast without adequate fasting may lead to an absence of the fasting state throughout the day, which results in sustained hyperglycemia and elevated insulin secretion.

Meanwhile, in view of the time course, LNDE can affect the quantity and the quality of the following sleep^[36,37], which may in turn affect the conditions of the next morning, *i.e.*, eating breakfast. Studies concerning the effect of LNDE on sleep are limited and the underlying mechanisms remain poorly understood. LNDE can deteriorate circadian rhythms and the secretion of leptin, peptide-YY, melatonin, orexins, and ghrelin^[38-41]. LNDE, which can result in sleep with a full stomach, may cause gastroesophageal reflux disease^[42,43] and reduced diet-induced thermogenesis^[38,44], both of which reduce the quality of sleep. Additionally, higher circulation volumes consisting of a large volume of water and high concentrations of sodium and glucose in the trunk circulation may burden the heart, vessels, and kidney, possibly resulting in arrhythmia and incidents of proteinuria, as observed in our previous studies^[11,45].

Shorter time periods between dinner and sleep, and between sleep and breakfast, can intensify the plausible effect of the postprandial condition after LNDE on sleep and the effect of poor sleep on breakfast, respectively (Figure 1). In addition, LNDE may affect conditions in the early morning after wakeup, especially when the duration

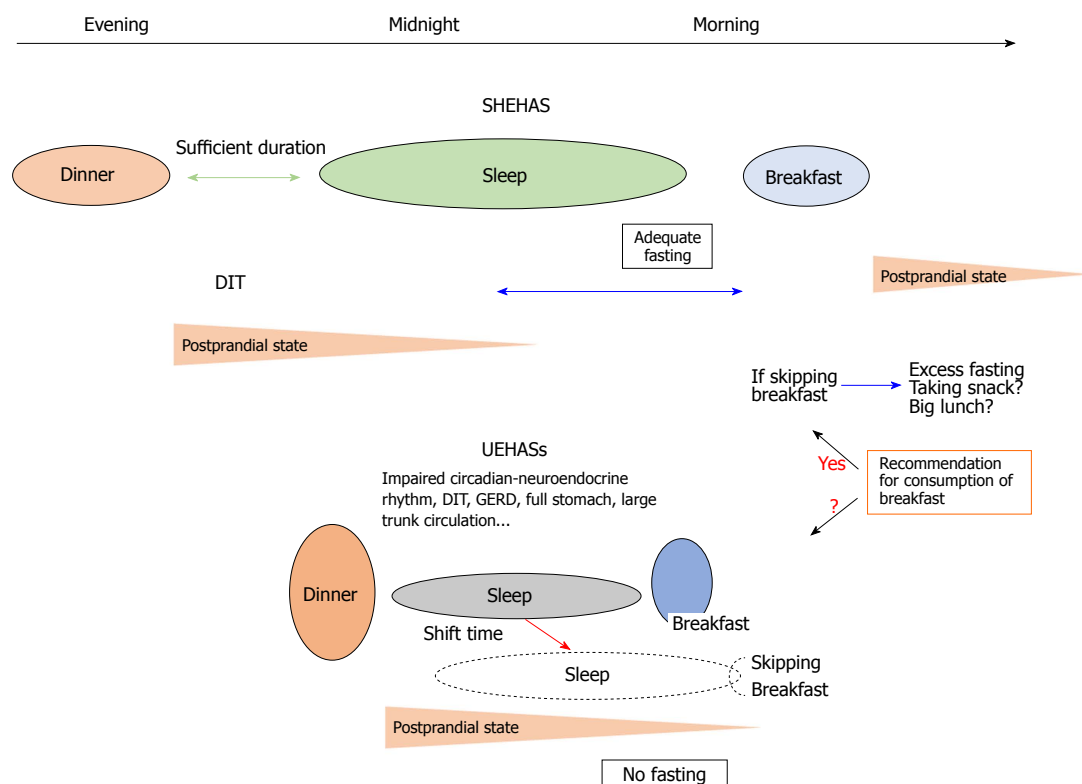


Figure 1 Comparison between unhealthy eating-habits around sleep and short sleep and sleep and healthy eating-habits around sleep. Sufficient time periods exist around sleep and sufficient fasting occurs before the breakfast meal in sleep and healthy eating-habits around sleep (SHEHAS), but not in unhealthy eating-habits around sleep and short sleep (UEHASs). If individuals with late-night dinner eating (LNDE) delay their bedtime to allow for a certain time period before sleep, or prolong the duration of sleep, the opportunity for taking a breakfast may be missed owing to delayed wakeup. DIT: Diet-induced thermogenesis; GERD: Gastroesophageal reflux disease.

of sleep is shorter. Therefore, it is best to refrain from LNDE for a healthy sleep and for optimal conditions in early morning. However, if it is impossible to prevent LNDE because of compulsory shift work or family/individual reasons, the dinner should have less energy and consist of a small amount of easy-to-digest ingredients. Alternatively, instead of completely skipping breakfast, consumption of a very low-calorie meal of less than 200 kcal including water, minimum minerals, and vitamins^[46,47] may be an effective option for avoiding potential fasting and adverse reactions such as hypoglycemia and dehydration. Simultaneously, healthy sleep habits may be necessary for conditions the next morning.

Our previous UEHAS cross-sectional studies^[11,12] have shown that BS without LNDE, *i.e.*, BS alone, was not associated with obesity or diabetes. Therefore, these results suggest a paradoxical possibility that BS or taking a very low energy breakfast might prevent obesity and diabetes in people with habitual LNDE. Otherwise, hunger, but not fasting, occurs throughout the day in individuals with LNDE. It is possible that BS or taking a smaller breakfast in children with LNDE^[13] may be a natural physiological response that manages to avoid the sustained metabolic abnormalities such as hyperglycemia caused by LNDE.

Importantly, the timing of meals substantially affects peripheral clocks existing in multiple organs, including

liver, adrenal gland, stomach, intestines, pancreas, kidney, heart, and lungs^[48-50]. Therefore, UEHAS may disrupt the peripheral circadian rhythm and thereby affect the central circadian rhythm, regulated by a master circadian clock located in the suprachiasmatic nucleus of the anterior hypothalamus, *via* irregular secretion of hormones including cortisol, ghrelin, leptin, insulin, glucagon, and glucagon-like peptide-1^[48]. This metabolic feedback can be mediated through so-called circadian-endocrine cross-talk^[49]. In particular, LNDE may enhance the desynchrony between the peripheral and central circadian rhythms, possibly by shortening the duration of sleep, besides unfavorable effects of LNDE on the secretion of incretins. Intriguingly, plasma insulin has been reported to be fundamentally regulated by pancreatic autonomous circadian oscillators, independent of the suprachiasmatic nucleus^[51]. In this regard, however, the composition of a meal, for instance the proportion of energy as carbohydrate, can also affect the peripheral circadian rhythm because insulin is usually secreted in greater quantities following a carbohydrate rich meal. This topic therefore warrants further study.

In conclusion, taking a breakfast is recommended primarily for people without LNDE to take sufficient energy for intellectual and physical activities in the morning (Figure 1). In contrast, taking a breakfast, especially of a full amount, may not be recommended

for people with habitual LNDE to allow them to attain a fasting state for a certain period per day. However, a well-considered meal for late-night dinner or breakfast can ameliorate the conditions above and the metabolic abnormalities in people with LNDE and/or BS, in harmony with the autonomous circadian-endocrine system. Health professionals such as physicians and dieticians should carefully consider individuals' backgrounds and chrononutrition, and UEHAs. Further integrated studies are needed to elucidate the effects of eating behaviors and sleep on health and cardiometabolic diseases in view of scientific and public interests.

REFERENCES

- Pereira MA, Erickson E, McKee P, Schrankler K, Raatz SK, Lytle LA, Pellegrini AD. Breakfast frequency and quality may affect glycemia and appetite in adults and children. *J Nutr* 2011; **141**: 163-168 [PMID: 21123469 DOI: 10.3945/jn.109.114405]
- Horikawa C, Kodama S, Yachi Y, Heianza Y, Hirasawa R, Ibe Y, Saito K, Shimano H, Yamada N, Sone H. Skipping breakfast and prevalence of overweight and obesity in Asian and Pacific regions: a meta-analysis. *Prev Med* 2011; **53**: 260-267 [PMID: 21925535 DOI: 10.1016/j.ypmed.2011.08.030]
- Bi H, Gan Y, Yang C, Chen Y, Tong X, Lu Z. Breakfast skipping and the risk of type 2 diabetes: a meta-analysis of observational studies. *Public Health Nutr* 2015; **18**: 3013-3019 [PMID: 25686619 DOI: 10.1017/S1368980015000257]
- Otaki N, Obayashi K, Saeiki K, Kitagawa M, Tone N, Kurumatani N. Relationship between Breakfast Skipping and Obesity among Elderly: Cross-Sectional Analysis of the HEIJO-KYO Study. *J Nutr Health Aging* 2017; **21**: 501-504 [PMID: 28448079 DOI: 10.1007/s12603-016-0792-0]
- Okada C, Tabuchi T, Iso H. Association between skipping breakfast in parents and children and childhood overweight/obesity among children: a nationwide 10.5-year prospective study in Japan. *Int J Obes (Lond)* 2018; **42**: 1724-1732 [PMID: 29686380 DOI: 10.1038/s41366-018-0066-5]
- McCrorry MA. Meal skipping and variables related to energy balance in adults: a brief review, with emphasis on the breakfast meal. *Physiol Behav* 2014; **134**: 51-54 [PMID: 24825781 DOI: 10.1016/j.physbeh.2014.05.005]
- Lee JS, Mishra G, Hayashi K, Watanabe E, Mori K, Kawakubo K. Combined eating behaviors and overweight: Eating quickly, late evening meals, and skipping breakfast. *Eat Behav* 2016; **21**: 84-88 [PMID: 26824682 DOI: 10.1016/j.eatbeh.2016.01.009]
- Dhurandhar EJ. True, true, unrelated? A review of recent evidence for a causal influence of breakfast on obesity. *Curr Opin Endocrinol Diabetes Obes* 2016; **23**: 384-388 [PMID: 27584010 DOI: 10.1097/MED.0000000000000281]
- Zilberter T, Zilberter EY. Breakfast: to skip or not to skip? *Front Public Health* 2014; **2**: 59 [PMID: 24918099 DOI: 10.3389/fpubh.2014.00059]
- Gibney MJ, Barr SI, Bellisle F, Drewnowski A, Fagt S, Livingstone B, Masset G, Varela Moreiras G, Moreno LA, Smith J, Vieux F, Thielecke F, Hopkins S. Breakfast in Human Nutrition: The International Breakfast Research Initiative. *Nutrients* 2018; **10**: pii: E559 [PMID: 29723985 DOI: 10.3390/nu10050559]
- Kutsuma A, Nakajima K, Suwa K. Potential Association between Breakfast Skipping and Concomitant Late-Night-Dinner Eating with Metabolic Syndrome and Proteinuria in the Japanese Population. *Scientifica (Cairo)* 2014; **2014**: 253581 [PMID: 24982814 DOI: 10.1155/2014/253581]
- Nakajima K, Suwa K. Association of hyperglycemia in a general Japanese population with late-night-dinner eating alone, but not breakfast skipping alone. *J Diabetes Metab Disord* 2015; **14**: 16 [PMID: 25874189 DOI: 10.1186/s40200-015-0147-0]
- Karatzi K, Moschonis G, Choupi E, Manios Y. Healthy Growth Study group. Late-night overeating is associated with smaller breakfast, breakfast skipping, and obesity in children: The Healthy Growth Study. *Nutrition* 2017; **33**: 141-144 [PMID: 27461167 DOI: 10.1016/j.nut.2016.05.010]
- Teixeira GP, Mota MC, Crispim CA. Eveningness is associated with skipping breakfast and poor nutritional intake in Brazilian undergraduate students. *Chronobiol Int* 2018; **35**: 358-367 [PMID: 29219626 DOI: 10.1080/07420528.2017.1407778]
- Azami Y, Funakoshi M, Matsumoto H, Ikota A, Ito K, Okimoto H, Shimizu N, Tsujimura F, Fukuda H, Miyagi C, Osawa S, Osawa R, Miura J. Long working hours and skipping breakfast concomitant with late evening meals are associated with suboptimal glycemic control among young male Japanese patients with type 2 diabetes. *J Diabetes Investig* 2018 [PMID: 29667372 DOI: 10.1111/jdi.12852]
- Pot GK. Sleep and dietary habits in the urban environment: the role of chrono-nutrition. *Proc Nutr Soc* 2018; **77**: 189-198 [PMID: 29065932 DOI: 10.1017/S0029665117003974]
- Chaput JP, Dutil C. Lack of sleep as a contributor to obesity in adolescents: impacts on eating and activity behaviors. *Int J Behav Nutr Phys Act* 2016; **13**: 103 [PMID: 27669980 DOI: 10.1186/s12966-016-0428-0]
- Li W, Sekine M, Yamada M, Fujimura Y, Tatsuse T. Lifestyle and overall health in high school children: Results from the Toyama birth cohort study, Japan. *Pediatr Int* 2018; **60**: 467-473 [PMID: 29513391 DOI: 10.1111/ped.13548]
- Ogilvie RP, Lutsey PL, Widome R, Laska MN, Larson N, Neumark-Sztainer D. Sleep indices and eating behaviours in young adults: findings from Project EAT. *Public Health Nutr* 2018; **21**: 689-701 [PMID: 29208064 DOI: 10.1017/S1368980017003536]
- Xi B, He D, Zhang M, Xue J, Zhou D. Short sleep duration predicts risk of metabolic syndrome: a systematic review and meta-analysis. *Sleep Med Rev* 2014; **18**: 293-297 [PMID: 23890470 DOI: 10.1016/j.smrv.2013.06.001]
- Dashti HS, Scheer FA, Jacques PF, Lamon-Fava S, Ordovas JM. Short sleep duration and dietary intake: epidemiologic evidence, mechanisms, and health implications. *Adv Nutr* 2015; **6**: 648-659 [PMID: 26567190 DOI: 10.3945/an.115.008623]
- Wu Y, Gong Q, Zou Z, Li H, Zhang X. Short sleep duration and obesity among children: A systematic review and meta-analysis of prospective studies. *Obes Res Clin Pract* 2017; **11**: 140-150 [PMID: 27269366 DOI: 10.1016/j.orcp.2016.05.005]
- Medic G, Wille M, Hemels ME. Short- and long-term health consequences of sleep disruption. *Nat Sci Sleep* 2017; **9**: 151-161 [PMID: 28579842 DOI: 10.2147/NSS.S134864]
- Betts JA, Chowdhury EA, Gonzalez JT, Richardson JD, Tsintzas K, Thompson D. Is breakfast the most important meal of the day? *Proc Nutr Soc* 2016; **75**: 464-474 [PMID: 27292940 DOI: 10.1017/S0029665116000318]
- Iovino I, Stuff J, Liu Y, Brewton C, Dovi A, Kleinman R, Nicklas T. Breakfast consumption has no effect on neuropsychological functioning in children: a repeated-measures clinical trial. *Am J Clin Nutr* 2016; **104**: 715-721 [PMID: 27465375 DOI: 10.3945/ajcn.116.132043]
- Chowdhury EA, Richardson JD, Tsintzas K, Thompson D, Betts JA. Effect of extended morning fasting upon ad libitum lunch intake and associated metabolic and hormonal responses in obese adults. *Int J Obes (Lond)* 2016; **40**: 305-311 [PMID: 26278005 DOI: 10.1038/ijo.2015.154]
- Zakrzewski-Fruer JK, Plekhanova T, Mandila D, Lekatis Y, Tolfrey K. Effect of breakfast omission and consumption on energy intake and physical activity in adolescent girls: a randomised controlled trial. *Br J Nutr* 2017; **118**: 392-400 [PMID: 28901889 DOI: 10.1017/S0007114517002148]
- Yoshimura E, Hatamoto Y, Yonekura S, Tanaka H. Skipping breakfast reduces energy intake and physical activity in healthy women who are habitual breakfast eaters: A randomized crossover trial. *Physiol Behav* 2017; **174**: 89-94 [PMID: 28284879 DOI: 10.1016/j.physbeh.2017.03.008]
- Sato M, Nakamura K, Ogata H, Miyashita A, Nagasaka S, Omi

- N, Yamaguchi S, Hibi M, Umeda T, Nakaji S, Tokuyama K. Acute effect of late evening meal on diurnal variation of blood glucose and energy metabolism. *Obes Res Clin Pract* 2011; **5**: e169-e266 [PMID: 24331104 DOI: 10.1016/j.orcp.2011.02.001]
- 30 **Sakai R**, Hashimoto Y, Ushigome E, Miki A, Okamura T, Matsugasumi M, Fukuda T, Majima S, Matsumoto S, Senmaru T, Hamaguchi M, Tanaka M, Asano M, Yamazaki M, Oda Y, Fukui M. Late-night-dinner is associated with poor glycemic control in people with type 2 diabetes: The KAMOGAWA-DM cohort study. *Endocr J* 2018; **65**: 395-402 [PMID: 29375081 DOI: 10.1507/endocrj.EJ17-0414]
- 31 **Kadowaki T**, Haneda M, Ito H, Sasaki K, Hiraide S, Matsukawa M, Ueno M. Relationship of Eating Patterns and Metabolic Parameters, and Teneligliptin Treatment: Interim Results from Post-marketing Surveillance in Japanese Type 2 Diabetes Patients. *Adv Ther* 2018; **35**: 817-831 [PMID: 29777520 DOI: 10.1007/s12325-018-0704-2]
- 32 **Mayer KH**, Stamler J, Dyer A, Freinkel N, Stamler R, Berkson DM, Farber B. Epidemiologic findings on the relationship of time of day and time since last meal to glucose tolerance. *Diabetes* 1976; **25**: 936-943 [PMID: 976602 DOI: 10.2337/diab.25.10.936]
- 33 **Pongsuthana S**, Tivatsunakul N. Optimal Fasting Time before Measurement of Serum Triglyceride Levels in Healthy Volunteers. *J Med Assoc Thai* 2016; **99** Suppl 2: S42-S46 [PMID: 27266215]
- 34 **Garby L**, Kurzer MS, Lammert O, Nielsen E. Energy expenditure during sleep in men and women: evaporative and sensible heat losses. *Hum Nutr Clin Nutr* 1987; **41**: 225-233 [PMID: 3610667]
- 35 **Jung CM**, Melanson EL, Frydendall EJ, Perreault L, Eckel RH, Wright KP. Energy expenditure during sleep, sleep deprivation and sleep following sleep deprivation in adult humans. *J Physiol* 2011; **589**: 235-244 [PMID: 21059762 DOI: 10.1113/jphysiol.2010.197517]
- 36 **Crispin CA**, Zimberg IZ, dos Reis BG, Diniz RM, Tufik S, de Mello MT. Relationship between food intake and sleep pattern in healthy individuals. *J Clin Sleep Med* 2011; **7**: 659-664 [PMID: 22171206 DOI: 10.5664/jcsn.1476]
- 37 **Brown RF**, Thorsteinsson EB, Smithson M, Birmingham CL, Aljarallah H, Nolan C. Can body temperature dysregulation explain the co-occurrence between overweight/obesity, sleep impairment, late-night eating, and a sedentary lifestyle? *Eat Weight Disord* 2017; **22**: 599-608 [PMID: 28929462 DOI: 10.1007/s40519-017-0439-0]
- 38 **McHill AW**, Melanson EL, Higgins J, Connick E, Moehlman TM, Stothard ER, Wright KP Jr. Impact of circadian misalignment on energy metabolism during simulated nightshift work. *Proc Natl Acad Sci USA* 2014; **111**: 17302-17307 [PMID: 25404342 DOI: 10.1073/pnas.1412021111]
- 39 **Gallant A**, Lundgren J, Drapeau V. Nutritional Aspects of Late Eating and Night Eating. *Curr Obes Rep* 2014; **3**: 101-107 [PMID: 26626471 DOI: 10.1007/s13679-013-0081-8]
- 40 **Medic G**, Korchagina D, Young KE, Toumi M, Postma MJ, Wille M, Hemels M. Do payers value rarity? An analysis of the relationship between disease rarity and orphan drug prices in Europe. *J Mark Access Health Policy* 2017; **5**: 1299665 [PMID: 28473888 DOI: 10.1080/20016689.2017.1299665]
- 41 **Sieminski M**, Szypienbejl J, Partinen E. Orexins, Sleep, and Blood Pressure. *Curr Hypertens Rep* 2018; **20**: 79 [PMID: 29992504 DOI: 10.1007/s11906-018-0879-6]
- 42 **Fujiwara Y**, Arakawa T, Fass R. Gastroesophageal reflux disease and sleep disturbances. *J Gastroenterol* 2012; **47**: 760-769 [PMID: 22592763 DOI: 10.1007/s00535-012-0601-4]
- 43 **Takeshita E**, Furukawa S, Sakai T, Niiya T, Miyaoka H, Miyake T, Yamamoto S, Senba H, Yamamoto Y, Arimitsu E, Yagi S, Utsunomiya H, Tanaka K, Ikeda Y, Matsuura B, Miyake Y, Hiasa Y. Eating Behaviours and Prevalence of Gastroesophageal Reflux Disease in Japanese Adult Patients With Type 2 Diabetes Mellitus: The Dogo Study. *Can J Diabetes* 2018; **42**: 308-312 [PMID: 29056257 DOI: 10.1016/j.cjcd.2017.07.007]
- 44 **Romon M**, Edme JL, Boulenguez C, Lescroart JL, Frimat P. Circadian variation of diet-induced thermogenesis. *Am J Clin Nutr* 1993; **57**: 476-480 [PMID: 8460600 DOI: 10.1093/ajcn/57.4.476]
- 45 **Nakajima K**, Suwa K, Oda E. Atrial fibrillation may be prevalent in individuals who report late-night dinner eating and concomitant breakfast skipping, a complex abnormal eating behavior around sleep. *Int J Cardiol* 2014; **177**: 1124-1126 [PMID: 25147065 DOI: 10.1016/j.ijcard.2014.08.058]
- 46 **Anderson JW**, Hamilton CC, Brinkman-Kaplan V. Benefits and risks of an intensive very-low-calorie diet program for severe obesity. *Am J Gastroenterol* 1992; **87**: 6-15 [PMID: 1728126]
- 47 **Johansson K**, Neovius M, Hemmingsson E. Effects of anti-obesity drugs, diet, and exercise on weight-loss maintenance after a very-low-calorie diet or low-calorie diet: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2014; **99**: 14-23 [PMID: 24172297 DOI: 10.3945/ajcn.113.070052]
- 48 **Patton DF**, Mistlberger RE. Circadian adaptations to meal timing: neuroendocrine mechanisms. *Front Neurosci* 2013; **7**: 185 [PMID: 24133410 DOI: 10.3389/fnins.2013.00185]
- 49 **Tsang AH**, Astiz M, Friedrichs M, Oster H. Endocrine regulation of circadian physiology. *J Endocrinol* 2016; **230**: R1-R11 [PMID: 27106109 DOI: 10.1530/JOE-16-0051]
- 50 **Jiang P**, Turek FW. Timing of meals: when is as critical as what and how much. *Am J Physiol Endocrinol Metab* 2017; **312**: E369-E380 [PMID: 28143856 DOI: 10.1152/ajpendo.00295.2016]
- 51 **Damiola F**, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev* 2000; **14**: 2950-2961 [PMID: 11114885 DOI: 10.1101/gad.183500]

P- Reviewer: Hamasaki H, Hamaguchi M, Serhiyenko VA, Surani S, Tziomalos K **S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Bian YN



Circadian rhythms of hormone secretion and obesity

Rajendra Raghaw

Rajendra Raghaw, Department of Veterans Affairs Medical Center, Memphis, TN 38104, United States

Rajendra Raghaw, Department of Pharmacology, University of Tennessee Health Science Center, Memphis, TN 38163, United States

ORCID number: Rajendra Raghaw (0000-0002-4709-7669).

Author contributions: Raghaw R solely wrote this paper.

Conflict-of-interest statement: Rajendra Raghaw declares that there is neither a conflict of interest with regard to the publication discussed in this FOV communication nor with respect to a commercial entity.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Corresponding author to: Rajendra Raghaw, PhD, Professor, Department of Veterans Affairs Medical Center, 1030 Jefferson Avenue, Memphis, TN 38104, United States. r.ghaw@uthsc.edu
Telephone: +1-901-5238990
Fax: +1-901-5237274

Received: July 19, 2018
Peer-review started: July 19, 2018
First decision: August 9, 2018
Revised: October 8, 2018
Accepted: October 24, 2018
Article in press: October 24, 2018
Published online: November 15, 2018

Abstract

The adipose tissue homeostasis is profoundly affected

by circadian rhythms of corticosteroid secretion and chronic loss of hormonal oscillations is associated with obesity. How adipose tissue differentially responds to pulsatile *vs* continuous presence of glucocorticoids is poorly defined. To address this question, Bahrami-Nejad *et al* studied differentiation of pre-adipocytes, containing endogenously tagged CCAAT/enhancer binding protein and peroxisome proliferator-activated receptor (PPAR) γ (key regulators of adipocyte differentiation), in response to corticosteroids that were delivered either in an oscillatory fashion or continuously. The authors show that the bi-stable state of differentiation of pre-adipocytes and adipocytes was regulated by a combination of fast and slow positive feedback networks, that determined unique threshold of PPAR γ in these cells. Evidently, pre-adipocytes used the fast feedback loop to reject differentiation cues of oscillating pulses of glucocorticoids and failed to differentiate into fat cells. In contrast, when glucocorticoids were delivered continuously, precursor cells exploited the slow feedback loop to embark on a path of maximal differentiation. This differential differentiation response of pre-adipocytes to pulsatile *vs* continuous exposure to glucocorticoids was corroborated *in vivo*. Thus, mice receiving non-oscillating doses of exogenous glucocorticoids, for 21 d, elicited excessive accumulation of visceral and subcutaneous fat. These data shed new light on the mechanisms of obesity caused by putative misalignment of circadian secretion of glucocorticoids or their persistently high levels due to chronic stress or Cushing's disease.

Key words: Circadian rhythms; Glucocorticoids; Adipose tissue; Pre-adipocytes; Stem cells; Terminal differentiation

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Bahrami-Nejad *et al* examined pre-adipocytes for their ability to differentiate into fat cells in response to hormonal stimuli that were presented either in a pulsatile manner, mimicking circadian rhythms, or delivered continuously. These experiments revealed that

adipocyte differentiation program, made up of slow and fast feedback circuits, was able to distinguish between the oscillating and continuous hormonal signals. The authors showed that pre-adipocytes apparently used the fast, positive feedback network to reject the oscillating hormonal cues. In contrast, if delivered continuously, similar strength glucocorticoids impinged on the slow positive feedback circuit to trigger maximal differentiation of pre-adipocytes into bone fide fat cells. The pulsatile *vs* continuous hormone stimuli were similarly discriminated *in vivo* since mice receiving glucocorticoids in a non-oscillating manner for 21 d elicited increased accumulation of subcutaneous and visceral fat. These data elucidate a potential mechanism underling the development of obesity associated with chronic stress or Cushing's disease.

Raghow R. Circadian rhythms of hormone secretion and obesity. *World J Diabetes* 2018; 9(11): 195-198 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i11/195.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i11.195>

COMMENTARY ON HOT TOPICS

Disturbance of diurnal rhythms of day and night, as experienced by night-shift workers, has been linked to obesity and type 2 diabetes mellitus. However, the mechanistic connection between circadian misalignment and obesity are poorly defined. Persistent interruption of diurnal rhythms leads to dysfunctional patterns of secretion of hormones, including corticosteroids, which adversely affect many tissues that include the adipose tissue.

Circadian secretion of glucocorticoids is pivotally involved in the mechanisms of adipose tissue homeostasis^[1]. Adipocyte stem cells, pre-adipocytes, embedded in the subcutaneous and visceral adipose tissues comprise about 20% of the cell population^[2]. Although pre-adipocytes are exposed to diurnal pulses of glucocorticoids, their terminal differentiation occurs at a very slow rate. For instance, in healthy humans, on a given day, approximately 1% pre-adipocytes embark on the process of differentiation which is completed in about 12 d^[3]. This behavior of pre-adipocytes is even more puzzling since these cells mount a robust, dose-dependent differentiation response to glucocorticoids *in vitro*. Sustained non-pulsatile exposure to glucocorticoids, as occurs during chronic stress or in patients with Cushing's disease, leads to the development of visceral obesity^[4-6]. This raises an important mechanistic question: How does the machinery of adipocyte differentiation distinguish between the physiological (diurnal glucocorticoid oscillations) and pathological (persistently high glucocorticoid levels) presence of glucocorticoids? This question was recently addressed by Bahrami-Nejad *et al*^[7] via a series of elegant *in vitro* and *in vivo* experiments. To further supplant brief methodological

and conceptual description contained in my FOV commentary, motivated readers should consult the original publication and its' Graphical Abstract.

The cellular and molecular underpinnings of how pre-adipocytes differentiate into bona fide fat cells have been studied in model cell lines and in stem cells isolated from adipose^[3]. These studies, facilitated by methods of molecular biology, quantitative mass spectrometry and single cell imaging, combined with computer modeling, indicate that differentiation of pre-adipocytes into adipocytes involves key cell-intrinsic elements and their interactions with hormones such as glucocorticoids, insulin, ghrelin, and others. It is also evident from these studies that unique gene expression signatures distinguish pre-adipocytes from bone fide fat cells; apparently, these bi-stable phenotypes are maintained by unique thresholds of CCAAT/enhancer binding protein α (CEBPA) and peroxisome proliferator-activated receptor γ (PPARG). A positive feedback loop between CEBPA and PPARG is thought to interact with additional feedback networks to induce adipocyte differentiation in response to different hormonal inputs^[8]. Hierarchical interactions among putative gene regulatory networks and their temporal regulation during *de novo* adipogenesis are poorly defined.

Since unique thresholds of PPARG and CEBPA proteins are thought to distinguish pre-adipocytes from bona fide fat cells^[8,9], Bahrami-Nejad *et al*^[7], created a clone of murine pre-adipocytes (OP9 cells) that harbored fluorescently tagged CEBPA and PPARG genes. These model pre-adipocytes enabled the authors to simultaneously monitor the expression of CEBPA and PPARG and their relationship with a progressive emergence of canonical markers of adipocyte differentiation^[10] in live cells, over a period of several days. When cultured in medium (DMI) containing a cocktail of differentiation inducing factors (1 μ mol/L of dexamethasone, 250 μ mol/L of IBMX and 1.75 nmol/L of insulin) OP9 cells (and stromal vascular fraction-associated primary pre-adipocytes) vigorously differentiated into mature fat cells. Progressively longer exposure to either dexamethasone (a synthetic glucocorticoid) or corticosterone (a physiological corticosteroid), for 12, 24, 36 and 48 h, induced a correspondingly larger fraction of pre-adipocytes to differentiate. However, when glucocorticoid-containing DMI was presented in oscillating pulses, only a small fraction of pre-adipocytes elicited terminal differentiation. Thus, the differentiation program seemed to reject the circadian rhythms of glucocorticoid treatment, but responded robustly to sustained presence of glucocorticoids in the DMI. In contrast, rosiglitazone (a direct activator of PPARG) induced adipocyte differentiation in a dose-dependent manner, regardless of the mode of temporal delivery. These data were interpreted to mean that filtering of temporal glucocorticoid signals occurred either prior to or simultaneously with the induction of PPARG gene expression.

To further explore the regulatory behavior of the putative bi-stable switch separating pre-adipocytes from adipocytes, Bahrami-Nejad *et al*^[7], tracked the expression

of fluorescently tagged PPARG in OP9 cells continuously over a 4-d period. Interestingly, the abundance of nuclear PPARG in individual cells was positively correlated with their progressive phenotypic transformation into bona fide fat cells. When cells were exposed to glucocorticoids in repeated 12-h on/12-h off cycles, a small fraction of OP9 and primary pre-adipocytes (SVF cells) concomitantly traversed a high threshold of PPARG and underwent terminal differentiation. In contrast, if glucocorticoid stimuli of similar strength were applied in a sustained manner, a high proportion of pre-adipocytes differentiated into fat cells.

Since the of PPARG gene expression is known to be activated by the transcription factor CCAAT/enhancer binding protein β (CEBPB), the authors tracked expression of CEBPB in live cells while they were cultured in DMI. These experiments revealed that nuclear abundance of CEBPB was dynamically regulated, and even more importantly, temporal expression CEBPB closely mirrored the oscillations of glucocorticoids. Since CEBPB is known to have a rapid rate of turnover, the authors reasoned that CEBPB could potentially form a fast feedback loop with PPARG. In response to circadian glucocorticoid stimuli, such a fast responsive system will not allow nuclear levels of PPARG to reach the threshold needed to initiate differentiation. An additional feature of such regulation would be that the positive feedback loop will become independent of external differentiation stimuli once a certain threshold of PPARG was reached. Quantification of nuclear abundance of PPARG *via* continuous, live cell imaging of OP9 cells revealed that an irreversible PPARG threshold was indeed reached after 36–48 h exposure to DMI^[7]. Although, an involvement of a fast feedback loop between CEBPB and PPARG was confirmed by experiments assessing differentiation of pre-adipocytes in response to diurnally oscillating glucocorticoids, such a fast feedback loop could not explain how a progressive build-up of PPARG occurred in response to continuous, days-long exposure to glucocorticoids.

The mode of differentiation of pre-adipocytes in response to prolonged, non-oscillating exposure to glucocorticoids predicted the existence of a slow positive feedback loop regulating nuclear abundance of PPARG. Such a slow positive feedback circuit would enable adipocyte precursors to discriminate between oscillating vs continuous signals and mount a differential differentiation response.

From a number of candidate genes that might be linked to PPARG in a slow positive feedback loop^[8], the authors favored FABP4, a known regulator of PPARG with a long half-life^[11]. It was noted that the rate of turnover of FABP4 was low, and even more importantly, accumulation of FABP4 in the cytoplasm and nuclear abundance of PPARG followed similar kinetics. Thus, authors posited that FABP4, *via* its ability to transport fatty acid ligands that activate PPARG, formed a slow positive feedback loop with PPARG; involvement of such a slow positive feedback regulatory loop would enable

pre-adipocytes to mount a differential response to oscillatory vs continuous stimuli. Of course, these data did not rule out the existence of additional regulatory circuits that could further modulate the mechanisms of *de novo* adipogenesis.

Experimental findings derived from *in vitro* differentiation of pre-adipocyte cell lines were subjected to computer simulations. The authors used an ordinary differentiation equation model to test the predicted dynamics of nuclear abundance of PPARG, *via* actions of the combined fast and slow positive feedback circuits. Indeed, such simulations could explain the observed rejection of single and repetitive pulses of differentiation stimuli. However, this model failed to explain why a small fraction of cells still differentiated in response to oscillating signals and why prolonged pulses of < 12 h induced a larger fraction of cells to undergo differentiation. To reconcile these observations, the authors modified their theoretical model of combined fast and slow positive feedbacks by adding a factor of stochastic variation in PPARG levels in the population of pre-adipocytes. These analyses revealed that a regulatory system consisting of fast and slow positive feedback links, when combined with putative stochastic abundance of nuclear PPARG (a cell-intrinsic property), could not only explain the variable delay in cells reaching PPARG threshold but also why low differentiation rates were seen in response to daily oscillations of glucocorticoids.

To corroborate these *in vitro* findings *in vivo*, the authors implanted continuous release pellets of cortisone in 8-wk old C57BL/6J mice. Experimental flattening of circadian rhythms of cortisone secretion, for 21 d, led to enhanced accumulation of subcutaneous (inguinal) and epididymal (visceral) fat in mice. Mice with cortisone implants had significantly larger adipocytes compared with animals containing sham implants. Moreover, the visceral adipose tissues of mice exposed to sustained high levels of cortisone had more numerous adipocytes. Thus, persistently high circulating levels of cortisone not only enhanced *de novo* adipogenesis but also led to increased volume of adipocytes

In summary, Bahrami-Nejad *et al*^[7], have shown that adipocyte differentiation was underpinned by cell-autonomous factors and their differential interaction with cortisone, depending on whether it was delivered continuously or in a circadian manner. While low signal variability was a key stochastic determinant that prevented de-differentiation of bone fide adipocytes, a high cell-to-cell signal variability was needed to ensure that only a small fraction of pre-adipocytes underwent differentiation in response to circadian delivery of glucocorticoids. The results of Bahrami-Nejad *et al*^[7], in addition to elucidating how circadian exposure to glucocorticoid affects adipocyte differentiation, have broader clinical implications. It was demonstrated in a recent study that misaligned circadian rhythms had adverse effects on insulin sensitivity and energy metabolism in the skeletal muscles of healthy young adults^[12]. Since mechanisms of repair and regeneration,

via recruitment of stem cells and their differentiation *in situ*, are central to tissue homeostasis across the animal kingdom^[13], a role of circadian secretion of growth and differentiation factors in these processes is warranted.

REFERENCES

- 1 **Weitzman ED**, Fukushima D, Nogueira C, Roffwarg H, Gallagher TF, Hellman L. Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J Clin Endocrinol Metab* 1971; **33**: 14-22 [PMID: 4326799 DOI: 10.1210/jcem-33-1-14]
- 2 **Tchoukalova YD**, Sarr MG, Jensen MD. Measuring committed preadipocytes in human adipose tissue from severely obese patients by using adipocyte fatty acid binding protein. *Am J Physiol Regul Integr Comp Physiol* 2004; **287**: R1132-R1140 [PMID: 15284082 DOI: 10.1152/ajpregu.00337.2004]
- 3 **Rosen ED**, Spiegelman BM. What we talk about when we talk about fat. *Cell* 2014; **156**: 20-44 [PMID: 24439368 DOI: 10.1016/j.cell.2013.12.012]
- 4 **Rebuffé-Scrive M**, Walsh UA, McEwen B, Rodin J. Effect of chronic stress and exogenous glucocorticoids on regional fat distribution and metabolism. *Physiol Behav* 1992; **52**: 583-590 [PMID: 1409924 DOI: 10.1016/0031-9384(92)90351-2]
- 5 **Campbell JE**, Peckett AJ, D'souza AM, Hawke TJ, Riddell MC. Adipogenic and lipolytic effects of chronic glucocorticoid exposure. *Am J Physiol Cell Physiol* 2011; **300**: C198-C209 [PMID: 20943959 DOI: 10.1152/ajpcell.00045.2010]
- 6 **Lee MJ**, Pramyothin P, Karastergiou K, Fried SK. Deconstructing the roles of glucocorticoids in adipose tissue biology and the development of central obesity. *Biochim Biophys Acta* 2014; **1842**: 473-481 [PMID: 23735216 DOI: 10.1016/j.bbdis.2013.05.029]
- 7 **Bahrani-Nejad Z**, Zhao ML, Tholen S, Hunerdosse D, Tkach KE, van Schie S, Chung M, Teruel MN. A Transcriptional Circuit Filters Oscillating Circadian Hormonal Inputs to Regulate Fat Cell Differentiation. *Cell Metab* 2018; **27**: 854-868.e8 [PMID: 29617644 DOI: 10.1016/j.cmet.2018.03.012]
- 8 **Ahrends R**, Ota A, Kovary KM, Kudo T, Park BO, Teruel MN. Controlling low rates of cell differentiation through noise and ultrahigh feedback. *Science* 2014; **344**: 1384-1389 [PMID: 24948735 DOI: 10.1126/science.1252079]
- 9 **Park BO**, Ahrends R, Teruel MN. Consecutive positive feedback loops create a bistable switch that controls preadipocyte-to-adipocyte conversion. *Cell Rep* 2012; **2**: 976-990 [PMID: 23063366 DOI: 10.1016/j.celrep.2012.08.038]
- 10 **Tontonoz P**, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem* 2008; **77**: 289-312 [PMID: 18518822 DOI: 10.1146/annurev.biochem.77.061307.091829]
- 11 **Spangenberg L**, Shigunov P, Abud AP, Cofré AR, Stimamiglio MA, Kuligovski C, Zych J, Schittini AV, Costa AD, Rebelatto CK, Brofman PR, Goldenberg S, Correa A, Naya H, Dallagiovanna B. Polysome profiling shows extensive posttranscriptional regulation during human adipocyte stem cell differentiation into adipocytes. *Stem Cell Res* 2013; **11**: 902-912 [PMID: 23845413 DOI: 10.1016/j.scr.2013.06.002]
- 12 **Wefers J**, van Moorsel D, Hansen J, Connell NJ, Havekes B, Hoeks J, van Marken Lichtenbelt WD, Duez H, Phielix E, Kalsbeek A, Boekschoten MV, Hooiveld GJ, Hesselink MKC, Kersten S, Staels B, Scheer FAJL, Schrauwen P. Circadian misalignment induces fatty acid metabolism gene profiles and compromises insulin sensitivity in human skeletal muscle. *Proc Natl Acad Sci USA* 2018; **115**: 7789-7794 [PMID: 29987027 DOI: 10.1073/pnas.1722295115]
- 13 **Wells JM**, Watt FM. Diverse mechanisms for endogenous regeneration and repair in mammalian organs. *Nature* 2018; **557**: 322-328 [PMID: 29769669 DOI: 10.1038/s41586-018-0073-7]

P- Reviewer: Biswas SK, Karras SN, Klimontov VV, Senol MG, Surani

S- Editor: Ji FF **L- Editor:** A **E- Editor:** Bian YN



Use of sodium bicarbonate and blood gas monitoring in diabetic ketoacidosis: A review

Mit P Patel, Ali Ahmed, Tharini Gunapalan, Sean E Hesselbacher

Mit P Patel, Ali Ahmed, Tharini Gunapalan, Sean E Hesselbacher, Department of Internal Medicine, Eastern Virginia Medical School, Norfolk, VA 23501, United States

Sean E Hesselbacher, Medicine Service, Hampton Veterans Affairs Medical Center, Hampton, VA 23667, United States

ORCID number: Mit P Patel (0000-0002-3315-0756); Ali Ahmed (0000-0003-0821-2983); Tharini Gunapalan (0000-0003-4850-6089); Sean E Hesselbacher (0000-0002-4133-2517).

Author contributions: All authors equally contributed to this paper with literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Corresponding author to: Sean E Hesselbacher, FCCP, MD, Assistant Professor, Medicine Service, Hampton Veterans Affairs Medical Center, 100 Emancipation Drive, Hampton, VA 23667, United States. hesselse@evms.edu
Telephone: +1-757-7229961
Fax: +1-757-7283187

Received: July 22, 2018

Peer-review started: July 22, 2018

First decision: August 9, 2018

Revised: August 30, 2018

Accepted: October 9, 2018

Article in press: October 9, 2018

Published online: November 15, 2018

Abstract

Diabetic ketoacidosis (DKA) is a severe and too-common complication of uncontrolled diabetes mellitus. Acidosis is one of the fundamental disruptions stemming from the disease process, the complications of which are potentially lethal. Hydration and insulin administration have been the cornerstones of DKA therapy; however, adjunctive treatments such as the use of sodium bicarbonate and protocols that include serial monitoring with blood gas analysis have been much more controversial. There is substantial literature available regarding the use of exogenous sodium bicarbonate in mild to moderately severe acidosis; the bulk of the data argue against significant benefit in important clinical outcomes and suggest possible adverse effects with the use of bicarbonate. However, there is scant data to support or refute the role of bicarbonate therapy in very severe acidosis. Arterial blood gas (ABG) assessment is an element of some treatment protocols, including society guidelines, for DKA. We review the evidence supporting these recommendations. In addition, we review the data supporting some less cumbersome tests, including venous blood gas assessment and routine chemistries. It remains unclear that measurement of blood gas pH, via arterial or venous sampling, impacts management of the patient substantially enough to warrant the testing, especially if sodium bicarbonate administration is not being considered. There are special circumstances when serial ABG monitoring and/or sodium bicarbonate infusion are necessary, which we also review. Additional studies are needed to determine the utility of these interventions in patients with severe DKA and pH less than 7.0.

Key words: Diabetic ketoacidosis; Sodium bicarbonate; Blood gas analysis; Acidosis; Ketosis; Ketone bodies; Hyperglycemia

© The Author(s) 2018. Published by Baishideng Publishing

Group Inc. All rights reserved.

Core tip: Serial arterial blood gas measurements and intravenous sodium bicarbonate are often used to assess and correct acidosis associated with diabetic ketoacidosis. The available literature, primarily in patients with mild to moderately severe acidosis, does not support the routine use of sodium bicarbonate. Additionally, arterial sampling for blood gas measurement may not be necessary, nor does it appear to substantially add to the care of these patients. While neither intervention may be needed on a routine basis, there are special circumstances when either, or both, of these modalities is indicated and useful.

Patel MP, Ahmed A, Gunapalan T, Hesselbacher SE. Use of sodium bicarbonate and blood gas monitoring in diabetic ketoacidosis: A review. *World J Diabetes* 2018; 9(11): 199-205 Available from: URL: <http://www.wjgnet.com/1948-9358/full/v9/i11/199.htm> DOI: <http://dx.doi.org/10.4239/wjd.v9.i11.199>

INTRODUCTION

Diabetic ketoacidosis (DKA) represents one of the most serious complications of uncontrolled diabetes mellitus (DM)^[1]. It is responsible for more than 500000 hospital days per year and is estimated to generate \$2.4 billion in healthcare costs per year^[2]. Furthermore, epidemiological studies have shown that hospital admissions for DKA in the United States are increasing at a rate even faster than the overall rate of the diagnosis of DM^[1]. Insulin and intravenous hydration are the mainstays of therapy in the management of DKA. For severe cases, adjunctive therapies such as bicarbonate administration and protocols that call for serial blood gas monitoring have been more controversial. This article will review the evidence regarding bicarbonate administration and the utility of arterial and venous blood gas (VBG) monitoring.

PATHOPHYSIOLOGY

Metabolic derangements during an episode of DKA, depicted in Figure 1, can lead to profound consequences if left untreated. A myriad of events can occur which can lead to hyperglycemia; insulin deficiency, peripheral insulin resistance, and increased counter-regulatory hormones such as cortisol, growth hormone and catecholamines, all contribute to deteriorating clinical status and underlie the pathophysiology of DKA^[3]. Furthermore, these effects are compounded by increased gluconeogenesis, glycogenolysis and impaired glucose uptake by peripheral tissue. The unfavorable combination of insulin resistance and counter-regulatory hormones leads to the release of free fatty acids (FFA) from adipose tissue via lipolysis and decreased lipogenesis, which ultimately results in ketogenesis and the production of beta-hydroxybutyrate and acetoacetate^[4,5]. Overproduction

of these strong ketoacids leads to excessive hydrogen ion production upon dissociation, overwhelming the human body's buffering capacity, depleting bicarbonate stores, and ultimately generating an anion gap metabolic acidosis^[6]. In addition, this process generates glycerol and alanine, which serve as substrates in the production of glucose in the liver, which propagates the cycle of hyperglycemia. Unchecked, this can lead to an osmotic diuresis that leads to marked urinary losses of free water and derangement of electrolytes. Urinary ketone losses will drive excretion of both sodium and potassium^[5]. Serum sodium may fall drastically due to natriuresis or rise due to large losses of free water. As a response to acidosis, potassium shifts to the extracellular space via the proton-potassium exchange channel, resulting in normal or elevated serum potassium concentrations despite a severe total body deficit. To counter these metabolic derangements, aggressive intravenous volume and electrolyte repletion along with parenteral insulin administration are implemented and represent the foundation of treatment of patients in DKA.

SODIUM BICARBONATE INFUSION IN DKA

The use of sodium bicarbonate infusion in the setting of DKA has been a controversial topic for many years. Early on, the administration of bicarbonate to patients in severe DKA had been largely empiric. As clinical and experimental data emerged that failed to demonstrate therapeutic value, concerns arose regarding the efficacy and safety of this treatment modality. Controversy regarding its use in severe DKA persists to this day, resulting in varied practice pattern.

The acidemia that plagues these patients is often quite severe and perhaps multifactorial. Ketone-generated acidosis may be compounded by lactate acidosis resulting from impaired tissue perfusion due to volume contraction and adrenergic response to the underlying precipitating illness, such as infection^[7]. Tissue acidosis can lead to profound organ dysfunction, including reduced myocardial contractility and cardiac output^[7]. Additionally, the oxyhemoglobin dissociation curve may shift via the Bohr Effect, with concurrently lowering levels of 2,3-diphosphoglycerate (2,3-DPG) increasing hemoglobin-oxygen affinity; thus, metabolic acidosis influences tissue oxygenation and inhibits key rate limiting intracellular enzymes which can alter metabolic pathways and result in vital organ dysfunction^[8-10]. Furthermore, severe acidosis impairs the ability of insulin to utilize glucose, with a lower pH conferring high insulin resistance^[11]. Table 1 outlines many of the known consequences of significant acidosis. The fate of bicarbonate in the body can be illustrated by the following equation: $H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$. Given that the direct observable end products of this pathway are benign, its implementation was thought to be non-harmful. As a result, the mainstay of therapy in the past

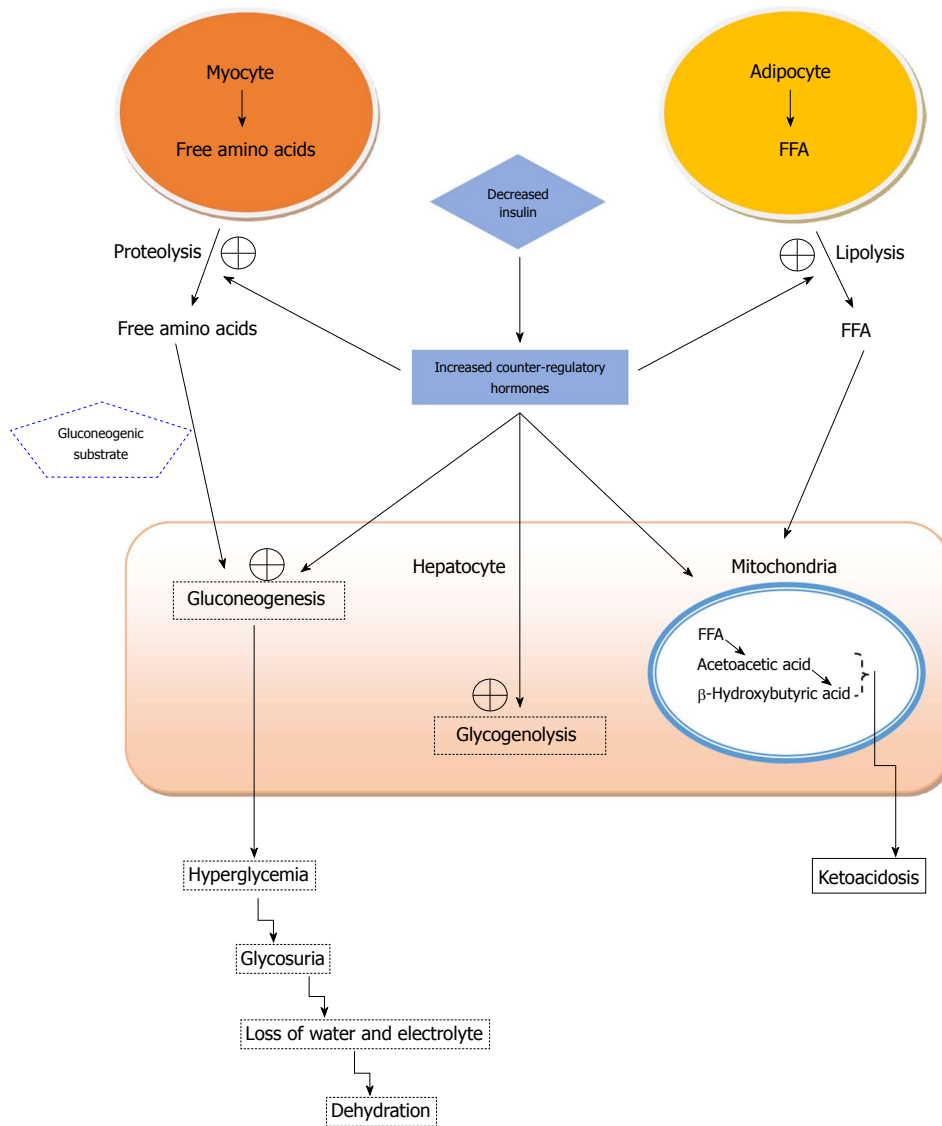


Figure 1 The pathophysiology of diabetic ketoacidosis. Decreased insulin sensitivity leads to increased concentrations of counter-regulatory hormones which promote catabolism of proteins and adipocytes. The production of free amino acids leads to the stimulation of gluconeogenesis and glycogenolysis leading to hyperglycemia. Free fatty acids undergo oxidation in the mitochondria and result in ketone production leading to acidosis. FFA: Free fatty acids.

placed great emphasis on the rapid reversal of acute acidemia in concordance with intravenous hydration and insulin administration. This physiological paradigm led to the widespread acceptance of intravenous bicarbonate administration in this setting.

There is robust data suggesting that the use of bicarbonate in patients with moderate DKA, in whom the pH is greater than 7.0, is not associated with improved outcomes as compared to saline-treated counterparts^[12-15]. However, in patients with severe DKA (pH less than 7.0), there is a deficit of data that incorporates large, randomized controlled trial (RCT) designs. Several smaller studies failed to show benefit, albeit in only a handful of patients. Morris *et al*^[15] showed in a randomized trial of 21 DKA patients with initial pH ranging between 6.90 to 7.14 that bicarbonate therapy did not improve morbidity or mortality. Additionally, the time to resolution of acidosis and bicarbonate

regeneration was not significantly different. As of the writing of this review article, there have not been any results reported from prospective randomized trials concerning the use of bicarbonate in severe DKA with pH less than 6.90.

In a well-executed systematic review that included 44 articles including three RCTs, Chua *et al*^[12] demonstrated a lack of consensus in pH threshold, time, concentration and amount of bicarbonate administration in various studies. There was no evidence of improved outcomes or glycemic control. Bicarbonate administration did not result in any significant benefit in duration of hospitalization, mortality, resolution of ketosis and/or acidosis, electrolyte imbalance, tissue oxygenation, or cerebrospinal fluid (CSF) acidosis^[12]. It is worth noting that two adult RCTs demonstrated a shorter reversal time of acidosis at two hours after therapy in the bicarbonate arm^[14,16], which was not sustained at 24 h

Table 1 Clinical effects of metabolic acidosis^[27,28]

System	Clinical effects
Cardiovascular	Depressed myocardium contractility Changes in SVR Acidosis-aided catecholamine release opposes acidosis-mediated vasodilation. Net SVR depends on the sum of both effects Conduction defects and dysrhythmias
Pulmonary	Impaired response to exogenous vasopressors Increased work of breathing and respiratory failure Compensatory alveolar hyperventilation Dyspnea (Kussmaul's breathing) Acute decrease in hemoglobin oxygen affinity (Bohr Effect) Temporary: Affinity rises after 36 h due to depletion of RBC 2,3-DPG
Renal	Pseudo-hyperkalemia Hyperuricemia Hypercalcemia
Hematological effect	Impaired coagulation Thrombocytopenia Reduced fibrinogen and thrombin formation Impaired clotting factor function Factor V a Factor VIIa Factor VIIa/tissue factor complex
Endocrine	Insulin resistance Catecholamine, cortisol, PTH and aldosterone stimulation Bone demineralization Protein wasting Free radical formation
Musculoskeletal system	Anti-anabolic effect on the bone growth centers in chronic metabolic acidosis Muscle fatigue
Central nerve system	Cerebral edema Depressed sensorium
Immune system	Impaired leukocyte function Increased susceptibility to infections

SVR: Systemic vascular resistance; RBC: Red blood cell; 2,3-DPG: 2,3 diphosphoglycerate; PTH: Parathyroid hormone.

follow up mark^[16] and led to no clinical difference. The vast majority of retrospective adult studies failed to show improvement in acidosis resolution^[12]. A composite of nine small studies totaling 434 patients with DKA (217 treated with bicarbonate plus standard care and 178 with standard care) mirrors previous findings in a lack of benefit in outcomes^[17].

There are several concerns that come into play when considering the role of bicarbonate infusion for DKA. Okuda *et al*^[18] demonstrated a rise in serum ketoacid anion levels and a delay in ketosis resolution in patients treated with bicarbonate infusion. Animal data suggests acceleration in ketogenesis with bicarbonate administration^[18]. In addition, if bicarbonate infusion is able to increase serum bicarbonate levels acutely, this may lead to a paradoxical worsening of acidosis in the central nervous system. Increased partial pressure of carbon dioxide (pCO₂) quickly and readily crosses the blood-brain barrier as compared to arterial bicarbonate, which can lead to a fall in cerebral pH and clinical neurological deterioration. In an RCT, adults receiving bicarbonate infusion had a non-significant trend toward a larger decline in CSF pH at 6-8 h compared with controls^[15]. In the pediatric population, multiple non-randomized studies have implicated bicarbonate

therapy as a risk factor for the development of cerebral edema^[12] and retrospective evidence suggests that it is associated with prolonged hospitalization. Several studies, including one double-blinded adult RCT^[16], identified a need for more aggressive potassium replacement in patients receiving bicarbonate infusion over 24 h. Given that patients in DKA are already at a total body deficit of potassium, implementation of bicarbonate may compound the problem and perhaps lead to fatal arrhythmia. These studies did not report any fatal outcomes secondary to hypokalemia; however, the theoretical risk is of substantial concern, especially when considering the widespread use of this intervention. Acute reversal of acidosis with bicarbonate has previously been linked to worsening tissue oxygenation. Acidosis will induce the Bohr effect and reduce total hemoglobin-oxygen affinity. However, it also lowers the concentration of 2,3-DPG in erythrocytes which leads to a counter-active increased hemoglobin-oxygen affinity. There exists a delicate balance in favor of the Bohr effect in the initial presentation of DKA, which theoretically can be pushed towards lower 2,3-DPG levels with bicarbonate administration and abrupt acidemia reversal. However, there is evidence to suggest that this may occur regardless of bicarbonate administration, and

Table 2 Key findings and conclusions regarding the use of sodium bicarbonate in diabetic ketoacidosis

Sodium bicarbonate use in mild to moderate acidemia (pH \geq 7.0) is associated with
No benefit in mortality or duration of hospitalization ^[12]
Possible transient benefit in reversal of acidosis ^[12,14,16]
Delay in resolution of ketosis ^[18]
Trend toward worsening of central nervous system acidosis ^[15]
Increased need for potassium supplementation ^[16]
Worsened tissue hypoxia ^[19]
Cerebral edema and prolonged hospitalization in pediatric patients ^[12]
Post-treatment metabolic alkalosis
Sodium bicarbonate use in severe acidemia (pH < 7.0) has not been well-studied
No improvement in morbidity or mortality in a small, randomized trial ^[15]
Routine use of sodium bicarbonate in diabetic ketoacidosis is not supported by the available literature
Several situations exist in which the use of sodium bicarbonate may be warranted
Severe acidosis
Life-threatening hyperkalemia
Recovery from saline-induced metabolic acidosis

levels of 2,3-DPG remain quite low for several days beyond the treatment of acidosis^[19]. Finally, bicarbonate administration can lead to post-treatment metabolic alkalosis as insulin mediated ketoacid metabolism leads to both spontaneous bicarbonate generation and resolution of metabolic acidosis.

Although no prospective randomized trials have been conducted on patients with severe DKA, the American Diabetes Association recommends the administration of 100 mmol sodium bicarbonate in 400 mL sterile water with 20 mEq of KCl to patients with a pH of less than 6.90 until the pH rises above 7.00^[5]. This is largely due to the concern of cardiovascular compromise in the setting of severe acidemia^[8]. Additionally, bicarbonate administration is reasonable in the setting of life threatening hyperkalemia, since its administration may shift potassium into cells. Another potential setting in which bicarbonate therapy may be helpful is during the recovery phase. Intravenous hydration therapy with 0.9% sodium chloride, widely implemented in the treatment of DKA, contributes to the development of hyperchloremic metabolic acidosis. Also contributing to hyperchloremia is the preferential renal excretion of ketones over chloride anions. This may lead to reduced renal bicarbonate genesis in the setting of concomitant kidney injury and volume related hyperchloremic acidosis. This is perhaps the mechanism of the initial favorable physiologic outcome in the two previously discussed RCTs^[14,16] with bicarbonate therapy as it may represent a reduced risk of hyperchloremic acidosis. However, the evidence is weak at best: the effect was transient and of uncertain clinical significance.

Taken in context of patient care, the theoretical benefits that provided the rational basis of rapid acidemia reversal with bicarbonate administration failed to provide any significant clinical differences or improved outcomes. This holds true for patients with severe DKA as well, albeit their sparse involvement in trials precludes any robust, evidence-based conclusion. Transient paradoxical worsening of ketosis and increased need for potassium replacement were the major clinical issues

found to be of concern. In the pediatric population, retrospective analysis yielded evidence of clinical harm including increased risk of cerebral edema and prolonged hospitalization with bicarbonate administration. The findings and conclusions drawn from the available literature are summarized in Table 2.

ARTERIAL AND VBG MONITORING

Modern medicine has evolved to quite an extent so as to provide a wide complement of tools that are available for use in the diagnosis and management of any disease process. The most fundamental element upon which all else is built is a thorough history and physical exam. Patients who present with DKA characteristically develop a rapid onset of signs and symptoms that prompt initial evaluation. Classically, complaints of polyuria, polydipsia, weight loss, nausea and vomiting, abdominal pain and generalized weakness are among the most common symptoms. Physical findings can include dry mucus membranes and poor skin turgor, tachycardia, Kussmaul respirations, fruity odor, and diffuse abdominal tenderness to palpation^[5]. Caution needs to be exercised to assess for infection, as it is the most common cause of DKA. Other factors such as medication compliance, changes in medications or dosages, myocardial infarction, and pancreatitis must be assessed as well.

The triad of hyperglycemia, anion gap metabolic acidosis and ketonemia are the hallmark findings that help establish the diagnosis. The American Diabetes Association have proposed diagnostic criteria which stratify DKA severity based on pH, bicarbonate levels, and anion gap in addition to mental status changes^[5]. As such, the measurement of arterial pH in the diagnosis of DKA became an important aspect of the management of these patients. Many protocols for the management of these patients, including the guidelines set forth by the American Diabetes Association, call for the serial measurement of several laboratory parameters including serum chemistry and blood gases as often as every two hours^[5].

Table 3 Key findings and conclusions regarding blood gas monitoring in diabetic ketoacidosis

<p>Venous blood is similar to arterial sampling in measuring</p> <p>pH^[21-25]</p> <p>Bicarbonate^[21,24]</p> <p>Lactate^[21]</p> <p>Base excess^[21]</p> <p>Venous blood gas measurement may be used in place of arterial blood for the purposes of stratifying disease severity in diabetic ketoacidosis</p> <p>Blood gas measurement does not often change management of diabetic ketoacidosis, especially when routine chemistries (including bicarbonate level) and ketone body identification are available^[25]</p> <p>Routine use of arterial and/or venous blood gas measurement may not be necessary in the evaluation and management of diabetic ketoacidosis</p> <p>Exceptions where blood gas analysis would likely alter management include</p> <ul style="list-style-type: none"> Abnormal baseline serum bicarbonate levels Chronic respiratory failure Renal tubular acidosis Acute respiratory compromise Adequacy of respiratory compensation for metabolic acidosis Respiratory muscle fatigue and failure
--

As such, attention shifted to the possible role of VBG sampling in the monitoring of DKA in an effort to avoid the complications and patient discomfort that accompanies repeated arterial punctures. Multiple studies comparing arterial to venous blood gases parameters in a wide array of patient population and co-morbidities including DKA demonstrate a close agreement for the values of pH, bicarbonate, lactate, and base excess with an acceptably narrow 95% limits of agreement^[20-25]. The authors universally agree that VBG analysis for pH and bicarbonate is an acceptable alternative of arterial blood gas (ABG) analysis. Despite strong data to support its use, many centers still engage in ABG usage for assessment of acid-base status.

An interesting and perhaps more thought-provoking element of management is to question the role of blood gas monitoring itself. While ABG and VBG may accurately measure the parameters in question, the impact on disease management is less clear, when taken in the context of the larger clinical picture and other available laboratory parameters. An interesting observational study by Ma *et al*^[25], looked at two hundred consecutive patients who presented to the emergency department with suspected DKA and had ABG, VBG and a chemistry panel drawn before treatment. Attending physicians indicated a tentative treatment plan and disposition on a standardized form before and after reviewing results of the blood gases, and found that this additional information rarely led to a change in diagnosis, treatment, management, or disposition^[26]. Additionally, they mirrored the data cited from previous studies regarding the correlation of venous to arterial pH and drew similar conclusions regarding its use as a substitute.

In most patients, routine measurement of pH may not necessarily add more information to the clinical picture, as the presence of metabolic acidosis can be established by routine measurement of venous bicarbonate level and identification of abnormal ketone bodies. Previously cited studies have demonstrated a strong correlation between pH and bicarbonate levels^[21-25]; as such, information from a blood gas will add little, if any, diagnostic value to

serum bicarbonate levels in both the initial presentation and subsequent management of DKA patients. Some exceptions may be found in patients with known or suspected abnormal baseline serum bicarbonate levels, as in chronic respiratory failure or renal tubular acidosis; a single measurement of arterial or VBG may confirm this abnormality. In select cases, measurement of an ABG may be of value in seeking information about the respiratory status of the patient. The value of pCO₂ may help assess the adequacy of respiratory compensation for the ongoing metabolic acidosis, and potentially identify those patients who may require mechanical ventilator support due to respiratory muscle fatigue^[26]. However, perhaps the same information can be attained with serial physical examination and close clinical monitoring of the patient. The findings and conclusions drawn from the available literature are summarized in Table 3.

CONCLUSION

It is clear from the increasing rate of hospital admission for DKA, healthcare providers will need to be weary of following dogmatic policies of previous decades and turn to evidence-based practices to improve outcomes. The role of sodium bicarbonate administration has been fraught with controversy for many years now; however, an increasing volume of evidence reflects a lack of benefit in its role for the treatment of DKA. Some evidence suggests that the use of bicarbonate is associated with delayed ketone clearance and worsened hypokalemia. In children, bicarbonate has been associated with prolonged hospitalizations and a higher risk of cerebral edema. However, to draw more definitive conclusions, prospective RCTs that include severely acidotic patients need to be performed on a large scale. As far as blood gas sampling, a plethora of data is available that faithfully correlates VBG sampling, including pH and bicarbonate, to their corresponding arterial samples. However, the additional value that a blood gas sample may provide is questionable and, guidelines notwithstanding, may not be necessary in all patients who present with DKA.

REFERENCES

- 1 **National Center for Health Statistics.** National hospital discharge and ambulatory surgery data. Accessed 19 June 2018. Available from: URL: <http://www.cdc.gov/nchs/about/major/hdasd/nhds.htm>
- 2 **Kim S.** Burden of hospitalizations primarily due to uncontrolled diabetes: implications of inadequate primary health care in the United States. *Diabetes Care* 2007; **30**: 1281-1282 [PMID: 17290038 DOI: 10.2337/dc06-2070]
- 3 **Kitabchi AE, Umpierrez GE, Murphy MB, Kreisberg RA.** Hyperglycemic crises in adult patients with diabetes: a consensus statement from the American Diabetes Association. *Diabetes Care* 2006; **29**: 2739-2748 [PMID: 17130218 DOI: 10.2337/dc06-9916]
- 4 **Gosmanov AR, Gosmanova EO, Dillard-Cannon E.** Management of adult diabetic ketoacidosis. *Diabetes Metab Syndr Obes* 2014; **7**: 255-264 [PMID: 25061324 DOI: 10.2147/DMSO.S50516]
- 5 **Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN.** Hyperglycemic crises in adult patients with diabetes. *Diabetes Care* 2009; **32**: 1335-1343 [PMID: 19564476 DOI: 10.2337/dc09-9032]
- 6 **Nyenwe EA, Razavi LN, Kitabchi AE, Khan AN, Wan JY.** Acidosis: the prime determinant of depressed sensorium in diabetic ketoacidosis. *Diabetes Care* 2010; **33**: 1837-1839 [PMID: 20484127 DOI: 10.2337/dc10-0102]
- 7 **Zimmet PZ, Taft P, Ennis GC, Sheath J.** Acid production in diabetic acidosis; a more rational approach to alkali replacement. *Br Med J* 1970; **3**: 610-612 [PMID: 4990378 DOI: 10.1136/bmj.3.5723.610]
- 8 **Mitchell JH, Wildenthal K, Johnson RL Jr.** The effects of acid-base disturbances on cardiovascular and pulmonary function. *Kidney Int* 1972; **1**: 375-389 [PMID: 4599247 DOI: 10.1038/ki.1972.48]
- 9 **Kono N, Kuwajima M, Tarui S.** Alteration of glycolytic intermediary metabolism in erythrocytes during diabetic ketoacidosis and its recovery phase. *Diabetes* 1981; **30**: 346-353 [PMID: 6451463 DOI: 10.2337/diabetes.30.4.346]
- 10 **Adrogué HJ, Madias NE.** Management of life-threatening acid-base disorders. First of two parts. *N Engl J Med* 1998; **338**: 26-34 [PMID: 9414329 DOI: 10.1056/NEJM199801013380106]
- 11 **Walker BG, Phear DN, Martin FI, Baird CW.** Inhibition of insulin by acidosis. *Lancet* 1963; **2**: 964-965 [PMID: 14059049 DOI: 10.1016/S0140-6736(63)90670-6]
- 12 **Chua HR, Schneider A, Bellomo R.** Bicarbonate in diabetic ketoacidosis - a systematic review. *Ann Intensive Care* 2011; **1**: 23 [PMID: 21906367 DOI: 10.1186/2110-5820-1-23]
- 13 **Lever E, Jaspán JB.** Sodium bicarbonate therapy in severe diabetic ketoacidosis. *Am J Med* 1983; **75**: 263-268 [PMID: 6309004 DOI: 10.1016/0002-9343(83)91203-2]
- 14 **Hale PJ, Crase J, Natrass M.** Metabolic effects of bicarbonate in the treatment of diabetic ketoacidosis. *Br Med J (Clin Res Ed)* 1984; **289**: 1035-1038 [PMID: 6091840 DOI: 10.1136/bmj.289.6451.1035]
- 15 **Morris LR, Murphy MB, Kitabchi AE.** Bicarbonate therapy in severe diabetic ketoacidosis. *Ann Intern Med* 1986; **105**: 836-840 [PMID: 3096181 DOI: 10.7326/0003-4819-105-6-836]
- 16 **Gamba G, Oseguera J, Castrejón M, Gómez-Pérez FJ.** Bicarbonate therapy in severe diabetic ketoacidosis. A double blind, randomized, placebo controlled trial. *Rev Invest Clin* 1991; **43**: 234-238 [PMID: 1667955]
- 17 **Viallon A, Zeni F, Lafond P, Venet C, Tardy B, Page Y, Bertrand JC.** Does bicarbonate therapy improve the management of severe diabetic ketoacidosis? *Crit Care Med* 1999; **27**: 2690-2693 [PMID: 10628611 DOI: 10.1097/00003246-199912000-00014]
- 18 **Okuda Y, Adrogué HJ, Field JB, Nohara H, Yamashita K.** Counterproductive effects of sodium bicarbonate in diabetic ketoacidosis. *J Clin Endocrinol Metab* 1996; **81**: 314-320 [PMID: 8550770 DOI: 10.1210/jcem.81.1.8550770]
- 19 **Alberti KG, Emerson PM, Darley JH, Hockaday TD.** 2,3-Diphosphoglycerate and tissue oxygenation in uncontrolled diabetes mellitus. *Lancet* 1972; **2**: 391-395 [PMID: 4115219 DOI: 10.1016/S0140-6736(72)91793-X]
- 20 **Taylor D, Durward A, Tibby SM, Thorburn K, Holton F, Johnstone IC, Murdoch IA.** The influence of hyperchloreaemia on acid base interpretation in diabetic ketoacidosis. *Intensive Care Med* 2006; **32**: 295-301 [PMID: 16447033 DOI: 10.1007/s00134-005-0009-1]
- 21 **Middleton P, Kelly AM, Brown J, Robertson M.** Agreement between arterial and central venous values for pH, bicarbonate, base excess, and lactate. *Emerg Med J* 2006; **23**: 622-624 [PMID: 16858095 DOI: 10.1136/emj.2006.035915]
- 22 **Malatesha G, Singh NK, Bharija A, Rehani B, Goel A.** Comparison of arterial and venous pH, bicarbonate, PCO₂ and PO₂ in initial emergency department assessment. *Emerg Med J* 2007; **24**: 569-571 [PMID: 17652681 DOI: 10.1136/emj.2007.046979]
- 23 **Kelly AM, McAlpine R, Kyle E.** Venous pH can safely replace arterial pH in the initial evaluation of patients in the emergency department. *Emerg Med J* 2001; **18**: 340-342 [PMID: 11559602 DOI: 10.1136/emj.18.5.340]
- 24 **Brandenburg MA, Dire DJ.** Comparison of arterial and venous blood gas values in the initial emergency department evaluation of patients with diabetic ketoacidosis. *Ann Emerg Med* 1998; **31**: 459-465 [PMID: 9546014 DOI: 10.1016/S0196-0644(98)70254-9]
- 25 **Ma OJ, Rush MD, Godfrey MM, Gaddis G.** Arterial blood gas results rarely influence emergency physician management of patients with suspected diabetic ketoacidosis. *Acad Emerg Med* 2003; **10**: 836-841 [PMID: 12896883 DOI: 10.1197/aemj.10.8.836]
- 26 **Gokel Y, Paydas S, Koseoglu Z, Alparslan N, Seydaoglu G.** Comparison of blood gas and acid-base measurements in arterial and venous blood samples in patients with uremic acidosis and diabetic ketoacidosis in the emergency room. *Am J Nephrol* 2000; **20**: 319-323 [PMID: 10970986 DOI: 10.1159/000013607]
- 27 **Al-Jaghbeer M, Kellum JA.** Acid-base disturbances in intensive care patients: etiology, pathophysiology and treatment. *Nephrol Dial Transplant* 2015; **30**: 1104-1111 [PMID: 25213433 DOI: 10.1093/ndt/gfu289]
- 28 **Ronco C, Bellomo R, Kellum JA.** Critical Care Nephrology. 2nd ed. Canada: Elsevier Health Sciences, 2009: 1848

P- Reviewer: Jiang L, Surani S S- Editor: Ji FF L- Editor: A
E- Editor: Bian YN





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



World Journal of *Diabetes*

World J Diabetes 2018 December 15; 9(12): 206-257





EDITORIAL

- 206 Role of vitamin C in diabetic ketoacidosis: Is it ready for prime time?
Casillas S, Pomerantz A, Surani S, Varon J

REVIEW

- 209 Treatment approach to type 2 diabetes: Past, present and future
Blaslov K, Naranda FS, Kruljac I, Pavlić Renar I

MINIREVIEWS

- 220 Unexpected alliance between syndecan-1 and innate-like T cells to protect host from autoimmune effects of interleukin-17
Jaiswal AK, Sadasivam M, Hamad ARA
- 226 Guidelines and controversies in the management of diabetic ketoacidosis – A mini-review
Islam T, Sherani K, Surani S, Vakil A
- 230 Effects of glucose-lowering agents on cardiorespiratory fitness
Hamasaki H

ORIGINAL ARTICLE

Basic Study

- 239 Pathological changes in the cellular structures of retina and choroidea in the early stages of alloxan-induced diabetes
Danilova I, Medvedeva S, Shmakova S, Cheresheva M, Sarapultsev A, Sarapultsev P

Observational Study

- 252 Using real world data to assess cardiovascular outcomes of two antidiabetic treatment classes
Stapff MP

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Fernando Cordido, MD, PhD, Professor, Department of Medicine, University A Coruña, Coruña 15006, Spain

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJD covers topics concerning α , β , δ and PP cells of the pancreatic islet, the effect of insulin and insulinresistance, pancreatic islet transplantation, adipose cells and obesity.

We encourage authors to submit their manuscripts to *WJD*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Diabetes is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, and PubMed Central, Scopus, China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Han Song*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITOR-IN-CHIEF
Timothy R Koch, MD, Doctor, Professor, (E-mail: timothy.r.koch@medstar.net) Georgetown University School of Medicine, Department of Surgery, Center for Advanced Laparoscopic General and Bariatric Surgery, MedStar-Washington Hospital Center, Washington, DC 20010, United States

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <https://www.wjgnet.com>

www.wjgnet.com/1948-9358/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <https://www.f6publishing.com/helpdesk>
<https://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <https://www.f6publishing.com/helpdesk>
<https://www.wjgnet.com>

PUBLICATION DATE
December 15, 2018

COPYRIGHT
© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

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

ONLINE SUBMISSION
<https://www.f6publishing.com>

Role of vitamin C in diabetic ketoacidosis: Is it ready for prime time?

Sebastian Casillas, Alan Pomerantz, Salim Surani, Joseph Varon

Sebastian Casillas, Alan Pomerantz, Dorrington Medical Associates, PA, Houston, TX 77030, United States

Salim Surani, Division of Pulmonary, Critical Care and Sleep Medicine, Texas A and M University, Health Science Center, Corpus Christi, TX 78414, United States

Joseph Varon, Acute and Continuing Care, The University of Texas Health Science Center at Houston, Houston, TX 77030, United States

Joseph Varon, Department of Medicine, the University of Texas, Medical Branch at Galveston, Houston, TX 77030, United States

Joseph Varon, Critical Care Services, United Memorial Medical Center/United General Hospital, Houston, TX 77030, United States

ORCID number: Sebastian Casillas (0000-0002-7465-1768); Alan Pomerantz (0000-0001-6609-1417); Salim Surani (0000-0001-7105-4266); Joseph Varon (0000-0002-7622-9974).

Author contributions: All authors have contributed to the preparation of manuscript, literature search and review for this editorial.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Open-Access: This is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Corresponding author to: Joseph Varon, FACP, FCCP, MD, Professor, Acute and Continuing Care, The University of Texas Health Science Center at Houston, 2219 Dorrington Street, Houston, TX 77030, United States. joseph.varon@uth.tmc.edu
Telephone: +1-713-6691670

Fax: +1-713-6691671

Received: August 28, 2018

Peer-review started: August 28, 2018

First decision: October 16, 2018

Revised: October 20, 2018

Accepted: November 2, 2018

Article in press: November 3, 2018

Published online: December 15, 2018

Abstract

Diabetic ketoacidosis (DKA) is life-threatening acute metabolic complication of diabetes mellitus (DM) that is characterized by acidosis, ketosis, and hyperglycemia, currently affecting mostly patients under 30 years of age with diabetes mellitus type 1. In both, DM and DKA, a pro-inflammatory state exists. This clinical entity occurs as a result of hyperglycemia-induced disturbances, resulting in an increased oxidative metabolism. For the latter reason, the use of vitamin C seems promising in DKA due to its antioxidant role in reducing the superoxide radicals that are consequence of the oxidative stress. This can decrease the pro-inflammatory state and avoids complications. Vitamin C, or also known as ascorbic acid, has been widely used in several illnesses, such as common cold, tissue healing, fertility, atherosclerosis, cancer prevention, immunity restoration, neuro-degenerative disease and also has been suggested to decrease the risk of DM, and this reason is giving place to believe that vitamin C can have an important role in treating diabetic complications such as DKA. In order to counteract these oxidative disturbances in DKA patients, we analyzed the current data regarding vitamin C and evaluate its role in any type treatment of this complication in the near future.

Key words: Vitamin C; Diabetes complications; Ascorbic acid; Diabetic ketoacidosis; Diabetes mellitus

© The Author(s) 2018. Published by Baishideng Publishing

Group Inc. All rights reserved.

Core tip: The use of vitamin C in diabetic ketoacidosis (DKA) has remained controversial due to insufficient clinical data. The lack of concrete evidence, and no randomized controlled trials available on the use of vitamin C for DKA has caused significant controversies and debate. Some preliminary data, however, has shown a decrease in lipid peroxidation and limitation of endothelial damage. There is a significant need for a large randomized clinical trial to evaluate the role of vitamin C in patients with diabetes mellitus and specifically in those with DKA.

Casillas S, Pomerantz A, Surani S, Varon J. Role of vitamin C in diabetic ketoacidosis: Is it ready for prime time? *World J Diabetes* 2018; 9(12): 206-208

URL: <https://www.wjgnet.com/1948-9358/full/v9/i12/206.htm>

DOI: <https://dx.doi.org/10.4239/wjd.v9.i12.206>

INTRODUCTION

Diabetes mellitus (DM) continues to present a global challenge, with a large number of individuals being diagnosed daily around the world. It is estimated that the number of patients with DM in the world will be 366 million, or approximately 4.4% of the population by the year 2030^[1]. A life-threatening complication of DM is diabetic ketoacidosis (DKA), which is an acute metabolic complication marked by acidosis, ketosis, and hyperglycemia. It results from lack of insulin, or insulin resistance along with increased levels of cortisol, glucagon, catecholamine and growth hormone. In addition, this clinical entity may be precipitated by an inadequate insulin administration, infection or other comorbidities (such as acute myocardial infarction, hyperthyroidism, stress)^[2].

In the United States, most patients with DKA (54%-76%) are less than 30 years of age and have type 1 DM, with a mortality rate of less than 1% in hospitalized patients^[3]. In these critically ill patients, an increase in the oxidative metabolism is commonly seen^[4].

Ascorbic acid, most commonly known as vitamin C, is a water-soluble antioxidant, which has a role in scavenging superoxide radicals, and has been reported to inhibit low-density lipoprotein oxidation and stabilize the endothelium^[4,5]. Vitamin C is essential for the normal physiological function of the body by playing a role in the synthesis and metabolism of tyrosine, tryptophan and folic acid, in addition to hydroxylation of proline, glycine and catecholamine. This vitamin also helps in lowering the cholesterol level by conversion of cholesterol into bile acid^[6,7]. Vitamin C has also been widely used in the treatment of common cold, tissue healing, fertility, atherosclerosis, cancer prevention, immunity restoration, and neurodegenerative disease and

has been suggested to decrease the risk of developing DM^[7]. Furthermore, vitamin C is known to participate in the regeneration of antioxidants molecules such as tocopherol, glutathione, carotenes and urate^[8].

DISCUSSION

Diabetes is characterized by a pro-inflammatory state, which leads to oxidative stress that results in the production of free radicals^[9]. This has been studied in the context of DKA. For example, Lee *et al*^[4] studied the degree of oxidative stress by determining the levels of fatty acids in six patients before, during and after DKA, as well as, the levels of vitamin A, C and E during these periods. In this study, lipid peroxidation was noted 24 to 72 h after correction of DKA; In addition, the levels of vitamin C and E were also decreased 24 to 72 h post correction of DKA. These authors suggested that vitamin C and E may play and important role in the presence of oxidative stress in DKA^[4].

Recently, vitamin C has been shown to be beneficial in-patient with septic shock, opening a new era of interest in the role of vitamin C on many other diseases. There are several studies that have clearly documented vitamin C deficiency among patients who are critically ill with sepsis and septic shock^[10-12]. To our knowledge, no randomized clinical trial analyzing the role of vitamin C in DM complications, such as DKA, is being done. Prior studies have shown that vitamin C ingestion interferes with testing devices that monitor glucose and ketones, giving false-positive results^[13].

Cerioti *et al*^[14] showed that vitamin C exhibited falsely elevated readings for glucose and beta-hydroxybutyrate in hospitalized patients. Moreover, the use of vitamin C in diabetic patients has remained questionable due to a prior study performed by Beckman *et al*^[15] showing that oral intake of vitamin C achieved a low concentration of plasma level, being unlikely to scavenge extracellular superoxide anion.

CONCLUSION

The use of vitamin C in DKA has remained controversial due to insufficient data collected in recent years. For the latter reason, it has not been applied in the clinical field. We believe that based on the data mentioned above vitamin C supplementation may have a role in patients with DKA. A large randomized controlled clinical trial aimed to identify if vitamin C supplementation in patients with DKA modifies their outcome is needed.

REFERENCES

- 1 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]
- 2 Meas T, Taboulet P, Sobngwi E, Gautier JF. Is capillary ketone determination useful in clinical practice? In which circumstances? *Diabetes Metab* 2005; **31**: 299-303 [PMID: 16142023 DOI: 10.117

- 7/2042018816681706]
- 3 **Fazeli Farsani S**, Brodovicz K, Soleymanlou N, Marquard J, Wissinger E, Maiese BA. Incidence and prevalence of diabetic ketoacidosis (DKA) among adults with type 1 diabetes mellitus (T1D): a systematic literature review. *BMJ Open* 2017; **7**: e016587 [PMID: 28765134 DOI: 10.1136/bmjopen-2017-016587]
- 4 **Lee DM**, Hoffman WH, Carl GF, Khichi M, Cornwell PE. Lipid peroxidation and antioxidant vitamins prior to, during, and after correction of diabetic ketoacidosis. *J Diabetes Complications* 2002; **16**: 294-300 [PMID: 12126788 DOI: 10.1016/S1056-8727(01)00215-X]
- 5 **Dalton RR**, Hoffman WH, Passmore GG, Martin SL. Plasma C-reactive protein levels in severe diabetic ketoacidosis. *Ann Clin Lab Sci* 2003; **33**: 435-442 [PMID: 14584758]
- 6 **Dunitz JD**. Linus Carl Pauling: 28 February 1901 - 19 August 1994. *Biogr Mem Fellows R Soc* 1996; **42**: 317-338 [PMID: 11619334 DOI: 10.1098/rsbm.1996.0020]
- 7 **Chambial S**, Dwivedi S, Shukla KK, John PJ, Sharma P. Vitamin C in disease prevention and cure: an overview. *Indian J Clin Biochem* 2013; **28**: 314-328 [PMID: 24426232 DOI: 10.1007/s12291-013-0375-3]
- 8 **Ashor AW**, Werner AD, Lara J, Willis ND, Mathers JC, Siervo M. Effects of vitamin C supplementation on glycaemic control: a systematic review and meta-analysis of randomised controlled trials. *Eur J Clin Nutr* 2017; **71**: 1371-1380 [PMID: 28294172 DOI: 10.1038/ejcn.2017.24]
- 9 **Gillani SW**, Sulaiman SAS, Abdul MIM, Baig MR. Combined effect of metformin with ascorbic acid versus acetyl salicylic acid on diabetes-related cardiovascular complication; a 12-month single blind multicenter randomized control trial. *Cardiovasc Diabetol* 2017; **16**: 103 [PMID: 28807030 DOI: 10.1186/s12933-017-0584-9]
- 10 **Marik PE**, Hooper MH. Doctor-your septic patients have scurvy! *Crit Care* 2018; **22**: 23 [PMID: 29378661 DOI: 10.1186/s13054-018-1950-z]
- 11 **Fowler AA 3rd**, Syed AA, Knowlson S, Sculthorpe R, Farthing D, DeWilde C, Farthing CA, Larus TL, Martin E, Brophy DF, Gupta S; Medical Respiratory Intensive Care Unit Nursing, Fisher BJ, Natarajan R. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. *J Transl Med* 2014; **12**: 32 [PMID: 24484547 DOI: 10.1186/1479-5876-12-32]
- 12 **Reynolds PS**, Fisher BJ, McCarter J, Sweeney C, Martin EJ, Middleton P, Ellenberg M, Fowler E, Brophy DF, Fowler AA 3rd, Spiess BD, Natarajan R. Interventional vitamin C: A strategy for attenuation of coagulopathy and inflammation in a swine multiple injuries model. *J Trauma Acute Care Surg* 2018; **85**: S57-S67 [PMID: 29538225 DOI: 10.1097/TA.0000000000001844]
- 13 **Plüddemann A**, Heneghan C, Price CP, Wolstenholme J, Thompson M. Point-of-care blood test for ketones in patients with diabetes: primary care diagnostic technology update. *Br J Gen Pract* 2011; **61**: 530-531 [PMID: 21801574 DOI: 10.3399/bjgp11X588600]
- 14 **Cerioti F**, Kaczmarek E, Guerra E, Mastrantonio F, Lucarelli F, Valgimigli F, Mosca A. Comparative performance assessment of point-of-care testing devices for measuring glucose and ketones at the patient bedside. *J Diabetes Sci Technol* 2015; **9**: 268-277 [PMID: 25519295 DOI: 10.1177/1932296814563351]
- 15 **Beckman JA**, Goldfine AB, Gordon MB, Garrett LA, Keaney JF Jr, Creager MA. Oral antioxidant therapy improves endothelial function in Type 1 but not Type 2 diabetes mellitus. *Am J Physiol Heart Circ Physiol* 2003; **285**: H2392-H2398 [PMID: 12881209 DOI: 10.1152/ajpheart.00403.2003]

P- Reviewer: Dabla PK, Quintos JB **S- Editor:** Ma RY
L- Editor: A **E- Editor:** Song H



Treatment approach to type 2 diabetes: Past, present and future

Kristina Blaslov, Fran Stjepan Naranda, Ivan Kruljac, Ivana Pavlić Renar

Kristina Blaslov, Ivan Kruljac, Department of Endocrinology, Diabetes and Metabolic Diseases Mladen Sekso, University Hospital Center Sestre Milosrdnice, Zagreb 10000, Croatia

Fran Stjepan Naranda, Ivana Pavlić Renar, School of Medicine, University of Zagreb, Zagreb 10000, Croatia

ORCID number: Kristina Blaslov (0000-0002-9747-8742); Fran Stjepan Naranda (0000-0003-0689-4028); Ivan Kruljac (0000-0003-0323-0154); Ivana Pavlić Renar (0000-0003-3495-7153).

Author contributions: Blaslov K and Pavlić Renar I conceived of and designed the study; Kruljac I and Naranda SF searched the literature; Blaslov K and Kruljac I drafted the article; all authors revised the article for important intellectual content; Pavlić Renar I gave final approval for the article.

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

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Corresponding author to: Kristina Blaslov, MD, PhD, Doctor, Department of Endocrinology, Diabetes and Metabolic Diseases Mladen Sekso, University Hospital Center Sestre Milosrdnice, Vinogradska cesta 29, Zagreb 10000, Croatia. kblaslov@gmail.com
Telephone: +385-1-3787111
Fax: +385-1-3769067

Received: August 29, 2018

Peer-review started: August 29, 2018

First decision: October 5, 2018

Revised: November 20, 2018

Accepted: November 26, 2018

Article in press: November 26, 2018

Published online: December 15, 2018

Abstract

Type 2 diabetes mellitus (DM) is a lifelong metabolic disease, characterized by hyperglycaemia which gradually leads to the development and progression of vascular complications. It is recognized as a global burden disease, with substantial consequences on human health (fatality) as well as on health-care system costs. This review focuses on the topic of historical discovery and understanding the complexity of the disease in the field of pathophysiology, as well as development of the pharmacotherapy beyond insulin. The complex interplay of insulin secretion and insulin resistance developed from previously known "ominous triumvirate" to "ominous octet" indicate the implication of multiple organs in glucose metabolism. The pharmacological approach has progressed from biguanides to a wide spectrum of medications that seem to provide a beneficial effect on the cardiovascular system. Despite this, we are still not achieving the target treatment goals. Thus, the future should bring novel antidiabetic drug classes capable of acting on several levels simultaneously. In conclusion, given the raising burden of type 2 DM, the best present strategy that could contribute the most to the reduction of morbidity and mortality should be focused on primary prevention.

Key words: Type 2 diabetes mellitus; Physical activity; Hyperglycaemia; Insulin resistance; Hypoglycaemic agents

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Type 2 diabetes mellitus (DM) is a global burden disease and one of the leading all-cause mortality causes due to cardiovascular (CV) complications. The rapid raise in the understanding of its pathogenesis resulted in treatment approach options beyond insulin that also provide beneficial CV effect. We discuss this scientific pathological and pharmacological development through a comprehensive historical approach. The wide spectrum of therapeutic agents currently used in type 2 DM treatment result in a CV mortality reduction which is not exclusively in correlation with glucose-lowering potency but is linked to its mechanism of action.

Blaslov K, Naranda FS, Kruljac I, Pavlić Renar I. Treatment approach to type 2 diabetes: Past, present and future. *World J Diabetes* 2018; 9(12): 209-219

URL: <https://www.wjgnet.com/1948-9358/full/v9/i12/209.htm>

DOI: <https://dx.doi.org/10.4239/wjd.v9.i12.209>

INTRODUCTION

Diabetes mellitus (DM) is chronic, lifelong progressive metabolic disease characterized by hyperglycaemia due to absolute or relative insulinopaenia. There are several different types of DM and each are caused by a complex interplay between genetic predisposition and environmental factors. The metabolic dysregulation that contributes to hyperglycaemia includes diminished insulin secretion, impaired glucose utilization or increased glucose production, and eventually causes pathophysiological changes in multiple organs and organ systems^[1]. Despite all the scientific advances in the field of pathophysiology, diagnosis and treatment, the prevalence of DM has shown a dramatic rise over the past 200 years. Nowadays, DM represents a global burden disease with fatal consequences on human health, and significant impact on health-care system costs. It is estimated that in 2017, there were 451 million people (ages 18-99 years) with diabetes worldwide^[2], and this number is expected to rise, mostly due to type 2 DM. Thus, we review herein the longitudinal history and therapeutic approaches beyond insulin for type 2 DM.

HISTORY AND CLASSIFICATION OF DM

The first documented symptoms of DM were recorded by ancient physicians in 1552 B.C. in a 3rd Dynasty Egyptian papyrus, being described as a rare mysterious disease characterized by excessive urination which leads to emaciation and death^[3,4]. Around year 150 of the new era, the term "diabetes mellitus" meaning "honey" and "siphon" was introduced by an ancient Greek physician Aretaeus, reflecting the sweet urine taste in affected individuals^[5]. However, its recognition as what is called a "clinical entity" - a condition that has separate

and distinct existence from any known underlying cause or specific treatment option - occurred in an 1822 publication in the *New England Journal of Medicine and Surgery*^[6].

The idea beyond this disease was not clarified until 1889, when Josph von Mering and Oskar Minkowski found that pancreatectomy performed on a dog resulted in fatal diabetes^[7,8]. In 1910, Edward Albert Sharpey-Schafer hypothesized that this might be due to the lack of a single pancreatic chemical, which he called "insulin"^[6]. His hypothesis was confirmed by the discovery of insulin in 1921 by Frederick Banting and Charles Best^[9,10]. After initially reversing diabetes in a dog using an extract from pancreatic islets of a healthy dog, together with James Collip and John Macleod they purified the hormone from bovine pancreas and used it to treat diabetes in humans^[11].

From that point of time, DM has represented a fertile ground for scientific research^[12]. Since 1923, 10 scientists have received a Nobel prize for diabetes-related investigation^[6]. Over the past two centuries it became clear that DM does not represent a unique clinical condition with a common pathophysiological background. Namely, insulin transformed the lives of children and young adults with diabetes but had limited impact upon the survival of those diagnosed at the age of 50^[6].

The classification of DM is primarily based on the pathogenic process that results in hyperglycaemia. In brief, it is now well known that severe insulin deficiency accounts for about 10% of all DM cases and is characterized by selective autoimmune destruction of insulin producing pancreatic β -cells, which are classified as type 1 DM, usually occurring in younger, lean individuals^[1]. The majority of patients, however, belong to the group with insulin resistance as the core pathophysiological disorder rather than insulin deficiency^[1], classified as type 2 DM. This type of DM is phenotypically often accompanied by central obesity, hypertension and dyslipidaemia.

The different pathophysiological background of hyperglycaemia was first proposed by Himsworth^[13] in the year 1936, who tested the ability of injected insulin to clear an oral glucose load from the circulation. He concluded that there were insulin sensitive patients whose diabetes was due to insulin deficiency and insulin insensitive patients whose diabetes was due to resistance to insulin. These findings were strengthened by the research of Claude Bernard, who showed that blood glucose is also regulated by the non-glucose precursors driven by the liver^[14-16], which probably represent the core of diabetes classification that was, however, not adopted until the 1970s^[17] under the terms "maturity onset diabetes" and "non-insulin-dependent diabetes" (NIDDM). Those were abandoned in favour of the newer terminology between 1980 and the 1990s. The categorization of DM involving two principal groups, namely type 1 and type 2 DM, raises its own concerns,

especially in terms of type 2 DM because of diversity in clinical presentation and the natural course of the disease requiring an individual therapeutic approach^[18].

PATOPHYSIOLOGY OF TYPE 2 DM: WHAT HAVE WE LEARNED IN THE LAST CENTURY?

Type 2 DM (formerly known as NIDDM) is a common metabolic disorder characterized by insulin resistance, relative impairment in insulin secretion, and certain degree of genetic predisposition, the prevalence of which markedly rises with the degree of obesity^[1]. It is often accompanied by hypertension and dyslipidaemia: high serum low density lipoprotein concentrations and low serum high density lipoprotein concentrations that increase cardiovascular (CV) risk. The constellation of these clinical conditions is referred to as metabolic syndrome. Although, the risk factors associated with this type of DM were observed as early on as the 1920s and the term "metabolic syndrome" was coined in the 1950s when the French physician Jean Vague noticed that upper body obesity seemed to be associated with an increased risk for the conditions of atherosclerosis, diabetes, kidney stones and gout^[19]. He also noticed that these patients show significant improvement in their diabetes, high blood cholesterol and high triglycerides following a low-calorie and low-carbohydrate diet^[20]. The term became commonly used in the 1970s, and in the 1988, Gerald Reaven hypothesized that insulin resistance could be the underlying factor linking this constellation of abnormalities, which he went on to name "syndrome X"^[21,22].

Indeed, it is now well known that type 2 DM usually presents with varying degrees of insulin resistance, consequent relative insulin deficiency, and hyperglycaemia which further impair pancreatic β -cell function, resulting in a vicious cycle of metabolic state worsening^[23]. In addition, it is now well known that the majority of type 2 DM patients have genetic risk for its development. Its importance is supported primarily due to the observation that normoglycaemic offspring of type 2 DM parents have reduced non-oxidative glucose metabolism associated with increased muscle intracellular lipid content and reduced muscle glycogen synthesis^[1]. This is due to a complex interaction among many genes and environmental factors (*i.e.*, a complex polygenic interplay which finally results in insulin resistance, namely decreased insulin sensitivity) that represents a core pathophysiological factor in type 2 DM development.

The exact molecular mechanism leading to insulin resistance has not been elucidated so far. Although the amount of insulin receptor expression on target tissues is diminished due to insulin's cellular internalization and reduced tyrosine kinase activity, the different expression probably represents the secondary and not the

primary defect. It is considered that the post-receptor alterations in insulin receptor substrate-1 (IRS-1), regulating phosphorylation and dephosphorylation, might play a predominant role in this condition. Precisely, there is an imbalance between IRS-1 tyrosine and serine phosphorylation (Figure 1)^[24]. Diminished IRS-1 tyrosine phosphorylation results in reduced translocation of glucose transporter type 4 (GLUT-4) to the plasma membrane, which enables glucose influx into the cells. Simultaneously, enhanced IRS-1 serine phosphorylation activates mitogen-activated proteins, whose action is not involved in metabolic but in mitotic insulin activity and proinflammatory pathways activation which result in intramitochondrial stress and further enhance insulin resistance. It is also implicated in the diabetes-related micro- and macrovascular complications' development. In brief, insulin resistance consists of two tightly coupled mechanisms: lack of suppression of glucose production and lack of glucose uptake by peripheral tissues, primarily muscles. Skeletal muscles usually utilize more than 80% of the circulating glucose in the presence of circulating insulin, while in the condition of insulin resistance this effect is diminished^[19-22,24,25].

Furthermore, during the overnight fast, there is a substantial (1.8-2.0 mg/kg per min) glucose production, essential to meet the needs of the brain and other neural tissues whose uptake accounts for 50%-60% of total glucose disposal and is insulin independent. In euglycaemic individuals, the hepatic glucose production is suppressed following the glucose influx into the portal vein due to rise in insulin and inhibition in glucagon release^[1]. In type 2 DM, this mechanism is diminished, which then results in both fasting as well as postprandial hyperglycaemia. The mechanisms involved in hepatic glucose production include hyperglucagonaemia, increased levels of circulating glucose precursors, free fatty acid oxidation, enhanced sensitivity to glucagon and decreased sensitivity to insulin^[1,24].

A gradual increase in insulin resistance requires a notable higher amount of insulin in order to overcome hyperglycaemia. Consequently, as pancreatic β -cells start to release insulin from its secretory granules, a higher amount of amylin appears in higher concentration in circulation but also in pancreas itself^[1,23]. Circulating amylin decreases glucose uptake in peripheral tissues, *i.e.*, enhances insulin resistance, while the pancreatic amylin further decreases pancreatic insulin secretion contributing to the hyperglycaemic state in both cases^[1]. Thus, this complex interplay of insulin secretion and insulin resistance in the liver and the skeletal muscle, also known as "ominous triumvirate"^[1], represented the first proposed fundamental mechanism of type 2 DM development and progression over the last two decades, *i.e.*, ever since it was established by a prospective study carried out by Jallut *et al.*^[26] in 1990 and later supported by many prospective studies carried out in diverse ethnic populations^[27,28].

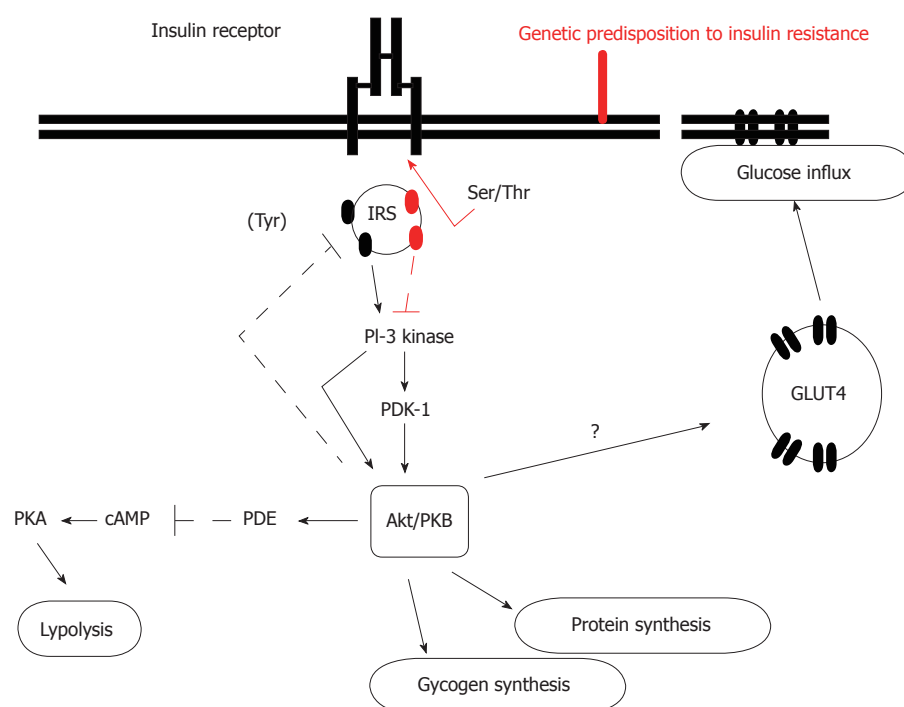


Figure 1 The molecular mechanism of insulin resistance. In insulin resistance, the binding of insulin to its receptor does not result in serine phosphorylation of insulin receptor substrate-1 and activation of the cascade of intracellular substrates' activation which result in glucose influx, glucagon and protein synthesis, and lipolysis inhibition. IRS: Insulin receptor substrate; Ser/Thr: Serine/threonine protein kinase; Tyr: Tyrosine kinase; PI-3: Phosphatidylinositol 3; PDK-1: Phosphoinositide-dependent protein kinase-1; Akt/PKB: AKT serine/threonine kinase 1 (protein kinase B family); PDE: Phosphodiesterase; cAMP: Cyclic adenosine monophosphate; PKA: Protein kinase A; GLUT4: Glucose transporter type 4.

However, since 1990 and until 2009, due to technological and pharmacological advances, there was a growing body of literature suggesting that the “ominous triumvirate” was not the sole pathophysiological disturbance in type 2 DM. An exponential growth of experimental and clinical studies suggested the possible implications of other organs: primarily pancreatic glucagon producing cells (α -cells), visceral adipose tissue, gut, kidneys and central nervous system. This was finally defined by DeFronzo^[29] in 2009 under the term “ominous octet”. As it gradually became evident that persons affected by type 2 DM have mostly a specific adipose tissue topography, *i.e.*, that visceral obesity is often accompanying type 2 DM, the two might be considered a part of this pathogenic process. It has been shown that they are also insulin resistant, resulting in the antilipolytic effect and thus leading to daylong elevation in the plasma free-fatty acid concentration^[30-36], which not only further disrupts pancreatic β -cell function^[37,38] but also promotes hepatic and muscle insulin resistance^[39-41] and stimulates gluconeogenesis^[42,43]. Moreover, the visceral adipocytes have a secretory capacity for a number of biological active products, including the adipokines, namely adiponectin, leptin, resistin, tumour necrosis factor- α , interleukine-1 β , plasminogen activator inhibitor-1, retinol binding protein 4, *etc.*, which are all associated with the function of intermediary metabolism^[30,44].

After the basic elucidation of these so-called “dysharmonius quartet”, at the beginning of the 2000s,

the concept of type 2 DM pathophysiology was further expanded to include the gastrointestinal tissues as the fifth member of the “quintessential quintet”. Even back in 1932, it was observed that the administration of an extract from the upper intestine could produce a fall in blood glucose, and the presumed hormone was named “incretin”^[45]. Moreover, it was noticed that oral glucose administration resulted in a much greater insulin secretion, as compared to intravenous glucose infusion in a concentration that mimicked orally-absorbed plasma glucose concentration^[46-48]; the phenomena was called “incretin effect”. The discovery of two gastrointestinal peptides - glucose-dependent insulintrophic peptide secreted by the K-cells of the more proximal small intestine, and glucagon like peptide-1 (GLP-1) secreted by the L-cells of the distal small intestine - probably mediate > 99% of this “incretin effect”. They have been shown to delay gastric emptying, stimulate insulin and suppress glucagon secretion in a glucose-dependent manner. In fact, the diminished “incretin effect” has been repeatedly shown in type 2 DM patients^[49-52].

The sixth member of the “octet” is the pancreatic α -cell. We have already mentioned glucagon, *i.e.*, the lack of glucagon suppression in the context of hepatic insulin resistance as well as in the incretin part, so it does not come as surprise that the higher fasting plasma glucose has been documented in several clinical studies in type 2 DM individuals back from the 1970s^[53-58]. It has been demonstrated that higher concentrations of fasting glucagon closely correlate with

the increase in fasting hepatic glucose production as described in detail earlier in the paper. Furthermore, the authors showed a simultaneous decrease following somatostatin infusion. Thus, it is clear that hyperglucagonemia merits being considered as one of the key features in the pathogenesis type 2 DM.

In addition to muscle, liver, pancreatic α -cell and β -cells, adipocytes and the gut, the kidney is one of the two most recent members implicated in the pathophysiology of type 2 DM. Although excessive urination was one of the first described characteristics of DM^[3-5] and the effort of its reduction was one of the first therapeutic approaches to DM while the sweet urine taste represented one of the first diagnostic approaches for DM, its pivotal role in type 2 DM pathogenesis was only recently explained. Physiologically, a total of approximately 162 g of glucose is filtered by kidneys on daily basis, and 90% of that amount is reabsorbed by the high capacity of sodium-glucose-transporter-2 (SGLT2) in the renal proximal tubule, while the remaining 10% of the filtered glucose is reabsorbed by the SGLT1 transporter in the descending part of the proximal tubule^[59]. The result is that no glucose appears in the urine in healthy individuals. During the last two decades it became evident that in both type 1 and type 2 DM the maximal renal tubular reabsorptive capacity is increased^[60,61]. This mechanism is due to markedly increased levels of SGLT2 mRNA and protein itself in the proximal renal tubular cells in type 2 DM^[62]. In conclusion, the overactivation of an adaptive response by the kidney to conserve glucose seems to play an important role in hyperglycaemia development in type 2 DM.

Finally, the last component of the "ominous octet" is the central nervous system. Following functional magnetic resonance imaging investigations, it became evident that certain hypothalamic areas, precisely those which are involved in appetite regulation, show diminished inhibitory response in insulin-resistant type 2 DM individuals compared to normal glucose tolerant subjects, even though the plasma insulin response was noted as markedly increased in the obese group. This indicates that besides peripheral tissues, the insulin resistance, a common feature of all type 2 DM subjects, exists in the central nervous system.

Therefore, it is evident that the last few decades provided better and comprehensive knowledge on the pathophysiology of type 2 DM, which certainly had repercussion on the pharmacological treatment approach.

LONGITUDINAL HISTORY OF TREATMENT OPTIONS IN TYPE 2 DM

From the very beginning, even back in the times when frequent and excessive urination was considered a hallmark of DM, physicians tried to understand how it could be managed. They initially used the methods that

could decrease this process, and there are documents reporting that horseback riding often was one of the first proposed methods^[5]. Centuries later, Rollo^[63] established the link between consumed food and the amount of glucose in the urine. He observed that carbohydrates increased glucose levels, while animal products' consumption resulted in less glucose^[3,4,64]. Thus, he proposed that DM treatment should be based on a high fat and protein rich diet with low carbohydrates. This modification of diet became the recommended treatment for diabetes until the discovery of insulin^[65].

Since the first proposal in 1877, a series of modifications and even individualized approaches, such as "oat-cure", "potato therapy" and the "starvation diet", persisted until 1916. This was the year when a Boston scientist, Elliot Joslin, was established as one of the leading diabetes experts upon creating *The Treatment of Diabetes Mellitus* textbook, wherein he reported that fasting diet combined with regular but moderate physical activity could significantly reduce the risk of death in diabetes^[66]. Although the diet and physical activity concept underwent numerous changes, especially over the last two decades, they still represent two official fundamental treatment approaches for type 2 DM, beyond pharmacological treatment^[67], and probably will remain so in the future.

The pharmacological treatment options in type 2 DM, however, did not occur until after the 1900s, and in the century of their development resulted in a wide spectrum of insulin and non-insulin hypoglycaemic agents experiencing an exponential growth. Although the discovery of insulin is considered the beginning of the pharmacological "DM-treatment" era, it is less known that the discovery and the use of oral hypoglycaemic agents (OHL) started 60 years ago, with an OHL class of biguanides that now represent the basic, first-line pleiotropic agent known as metformin^[67]. Namely, during the medieval times, the French lilac plant (*Galega officinalis*) was used to relieve DM symptoms in Southern and Eastern Europe^[68]. At the beginning of the 20th century, the anti-hyperglycaemic compound of the plant, guanidine, was isolated, synthesized and named Synthalin^[69]. The further development of Synthalin and other guanidine homologs was stopped since they were hepatotoxic, and completely ended with the discovery and global use of insulin^[70]. However, a resurgence of interest in the biguanides occurred several decades later, as the pathophysiology of DM as well as the difference in its clinical presentation (*i.e.*, before-mentioned classification) became clearer. Although metformin was introduced in 1959, its wide clinical use started two decades after and it was not approved in the United States until 1990^[68-71]; meanwhile, other agents from this class - phenformin and buformin - resulted in a significant number of lactic acidosis, which led to their withdrawal^[70]. Nowadays, metformin represents the only clinically significant biguanide whose

primary mechanism of action is the ability to reduce hepatic gluconeogenesis and glycogenolysis due to enhancement of insulin resistance^[72].

The sulfonylureas (SUs) represent the second and latest class of OHL, the discovery of which was triggered by the observation of an accidental hypoglycaemic effect. All the other OHL classes developed rapidly during the last two to three decades, accompanied by a deeper understanding of the complex pathophysiology of type 2 DM at the molecular level. The history of SUs starts in 1937, with the observation of hypoglycaemic activity of the synthetic sulphur compounds^[73] and which was further confirmed by hypoglycaemia occurrence in typhoid patients treated with antibiotic para-amino-sulfonamide-isopropylthiodiazole^[74]. In 1946, Auguste Loubatieres confirmed that aryl SU compounds stimulated release of insulin and, therefore, they require the remaining pancreatic β -cell function to achieve the effect^[73,74]. Soon after, in the 1950s, the first SU - tolbutamide - was marketed in Germany^[74], followed by the introduction of the other first-generation SU agents-chlorpropamide, acetohexamide, and tolazamide. The next advancement in SU therapy was the release of the more potent second-generation agents, back in 1984 - glipizide, glyburide, and gliklazide - and the third-generation agent glimepiride which was released in 1995^[73-75]. Nowadays, the SUs are widely used, since they are generally safe, inexpensive, and relatively predictable, with hypoglycaemia as the primarily use-limiting side effect. In addition, due to research advances in seeking an agent that could be used with less fear of hypoglycaemia, the class of meglitinides was released on the market in 2000^[76,77]. The meglitinides act similar to SUs, *i.e.*, they enhance insulin secretion but their effect is diminished at low glucose concentrations. Thus, they might cause hypoglycaemia but less frequently and less severe than the "conventional" SUs^[74].

The class of thiazolidinediones (TZDs) was initially introduced to the market in the middle of 1990. As a peroxisome proliferator-activated receptor- γ drug class activator, they were recognized soon after the discovery that the activation of this precise cell surface receptor enhances skeletal muscle insulin sensitivity and reduces hepatic glucose production^[31,78]. This class of agents was thought to possess a similar but more durable effect than metformin. Troglitazone was the first TZD approved by the United States' Federal Drug Administration (FDA), and was soon accompanied by pioglitazone and rosiglitazone^[79]. Troglitazone was removed from the market 4 years after its release, in 2000. This was due to the FDA having received reports of 63 hepatic failure cases with lethal outcome in patients treated with troglitazone^[80]. Two other TZDs, rosiglitazone and pioglitazone, have been linked to fluid retention, which limited their use in patients with congestive heart failure. Pioglitazone has been shown to have a potentially modest beneficial impact on CV disease but has also been associated with a possible

increase in the incidence of bladder cancer^[79]. However, the wide use of rosiglitazone was soon associated with an increased risk of myocardial infarction, which led to its temporary market restriction, *i.e.*, it remained available only in the United States. However, in November 2013, there was a change in position based on the findings of the large "Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycemia in Diabetes" (RECORD) study, demonstrating that individuals treated with rosiglitazone did not have an elevated risk of myocardial infarction compared to those taking other antihyperglycemic agents^[81,82]. Despite this, pioglitazone remains the most used TZD at the present time, taking its place in all the available diabetes management guidelines as the first to second option, but one cannot speculate whether rosiglitazone will dominate the market in the near future.

Before the endocrine role of gut itself in the "incretin effect" became a clear and important pathophysiological player in type 2 DM development (which then led to introduction of so called "incretin" group of antihyperglycaemic agents), a class of drugs targeting gut, precisely small intestine α -glucosidase and thereby decreasing glucose absorption, was introduced into clinical practice^[74-78]. The first drug in this category of α -glucosidase inhibitors that reached the market was acarbose, back in 1995, followed a year later by miglitol. Despite the logical background of drug development, neither drug has ever been used widely, probably because of the modest impact on A1C and their gastrointestinal side effects. However, following the discovery and elucidation of the incretin-insulin pathway, the "incretin based" class of agents became an intriguing and rapid growing area of research and development in the pharmaceutical industry.

At first, researchers became interested in the development of dipeptidyl-peptidase inhibitors - agents that could be taken orally and would prolong the circulating half-life of endogenous incretins^[45-47,74]. First among these was sitagliptin, in 2006^[83], this was soon followed by saxagliptin, linagliptin, vildagliptin and alogliptin, comprising a separate OHL class, the "gliptines". Obviously, these drugs showed good results in the post-market clinical trials, as nowadays they are recommended even as second-line diabetes therapy^[67]. Parallel to those, GLP-1 analogues were developed. The first analogue, exenatide was produced from exendin-4, which was isolated from the salivary gland venom of the Gila monster (*Heloderma suspectum*)^[45-47,74]. This drug became available for clinical use in 2005^[74]. A second GLP-1 receptor agonist, liraglutide, was approved in 2010. Soon after, *i.e.*, over the last 8 years, the market of GLP-1 analogues has grown exponentially, starting with the once-weekly form of exenatide, dulaglutide, lixisenatide and albiglutide, to lately with semaglutide^[74]. Although these drugs major advantage is weight loss, they also show cardio- and neuro-protective effects^[67,84,85]. However, the exact mechanism of these post-market results remains to be elucidated.

And finally, after the “ominous octet” was completed with the elucidation of overactivation or renal tubular glucose reabsorptive capacity^[59-62] due to a markedly increased level of SGLT2 in the proximal renal tubular cells^[86], the selective SGLT-2 inhibitors were developed. More precisely, when SGLT-2 is antagonized, the excess of glucose in the renal tubules is not reabsorbed but instead secreted in the urine. Canagliflozin was the first SGLT-2 inhibitor to be approved by the FDA, in March 2013^[87], which was followed by dapagliflozin in early 2014 and finally by empagliflozin.

Thus, here we described a wide spectrum of non-insulin therapeutic agents currently used in type 2 DM treatment. The official recommendations on their efficacy and safety, indications and contraindications, and effective combinations change almost on a yearly basis; yet, simultaneously we are experiencing an increase in diabetes-related complications, especially those leading to CV death^[2,67]. This would, however, come as surprise if we kept in mind the results from the “Action to Control Cardiovascular Risk in Diabetes” trial^[81] which clearly demonstrated that aggressive glycaemic control does not reduce the risk of CV death, despite the reduction in myocardial infarction.

NON-INSULIN THERAPEUTIC APPROACH AND CV RISK

As aforementioned in detail, due to the complex pathophysiological background of type 2 DM with the wide spectrum of CV risk factors that coexist in addition to the hyperglycaemia itself. It is important to emphasize here that the promising effects in terms of CV risk reduction were first published back in the late 90’s, as an observation in the “STOP-NIDDM Trial” (an international study on the efficacy of an alpha-glucosidase inhibitor to prevent type 2 DM in a population with impaired glucose tolerance)^[88]. The primary outcome of the study that clearly demonstrated that acarbose can prevent or delay the progression of impaired glucose tolerance to type 2 DM was recently confirmed in the “Acarbose Cardiovascular Evaluation” (ACE) trial^[89], but it showed no effect in CV risk reduction. Thus, the “Empagliflozin Cardiovascular Outcome Event Trial in type 2 DM Patients—Removing Excess Glucose” (EMPA-REG OUTCOME) was the first clinical study that demonstrated superiority of a glucose lowering agent, in CV disease, heart failure, and renal and mortality endpoints compared to placebo^[90]. Given the glycated haemoglobin (HbA1c) reduction of 0.45%, blood pressure in approximately 5/2 mmHg, and reduction in body weight by approximately 2%, the question arose: Could these results have been predicted based on what we knew about the mode of action?

Thus, shortly after the EMPA-REG OUTCOME trial was published, Ferrannini *et al.*^[90] developed a so-called “thrifty substrate” hypothesis which posits that in conditions of mild but persistent hyperketonaemia,

β -hydroxybutyrate is taken up by the heart and oxidized into fatty acids. This selection of substrates improves the transduction process of oxygen consumption into work efficiency in the myocytes. In addition, the enhanced oxygen release to the myocardium through haemoconcentration-driven by the diuresis^[91,92] might affect a powerful synergy with the substrate shift. The rationale for this hypothesis came from experimental studies in diet-induced obese rats treated with dapagliflozin^[93], ipragliflozin^[94], or tofogliflozin^[95] that demonstrated accelerated lipolysis and increased circulating ketone body levels, especially in the fasting state or when animals were fed in pairs. In addition, this was also confirmed in patients with type 2 DM following a 4-wk course of treatment with 25 mg of empagliflozin^[96]. Concomitantly, both fasting and post-meal plasma β -hydroxybutyrate concentrations were increased 2-fold to 3-fold and these changes were similar in time course, though attenuated in extent, in a group of non-diabetic volunteers receiving the drug. This hypothesis of the thrifty substrate might also explain the similar outcome obtained by the “Combined results from the Canagliflozin Cardiovascular Assessment Study” (CANVAS)^[97]. Nevertheless, the post-market period has been too short to support any general conclusion at this time.

And finally, some of the GLP-1 agonists, *i.e.*, liraglutide^[98] and semaglutide^[99] have shown a significant relative risk reduction compared with placebo for the 3-point major adverse CV event primary outcome and relative risk in CV as well as in all-cause mortality, having low-to-moderate between-trial statistical heterogeneity. However, the concerns about their potential in pancreatic cell proliferation observed in experimental studies has not been elucidated so far, due to the relatively short post-market period^[100].

CONCLUSION

In this narrative review, we described the rapid development of the pathophysiology type 2 DM concept accompanied by lifesaving treatment options beyond insulin that have dramatically enhanced the quality of life and life expectancy of affected individuals. The nephroprotective effects of angiotensin-receptor blockade, angiotensin-converting enzyme inhibition and protein restriction have been shown^[99-104], while laser photocoagulation has preserved the vision of millions of patients with diabetic retinopathy^[105]. The target hyperglycaemic agents’ development has resulted in better glycaemic control, which has increased the focus of their potential in the context of development of diabetic complication preventive strategies.

It is now known that better gluco-regulation results in development and progression of microvascular complications, according to the large, population-based studies of Diabetes Control and Complications Trial^[106] as well as United Kingdom Prospective Diabetes

Study^[107]. Additionally, the follow-up ACCORD study has showed reduced myocardial infarction with improved glycaemic control, but it didn't provide an all-cause CV mortality rate. This finding raised awareness that an exclusively glucose-centric approach to diabetes will most likely not lead to reduction in all-cause CV disease mortality^[98]. This finding was further strengthened by the Steno-2 trial^[108,109], which demonstrated up to 50% CVD mortality with a multifactorial, instead of glucose-centric, control.

Thus, the present position statement for type 2 DM treatment comprises the simultaneous approach to control of glucose along with lipids, blood pressure and obesity^[109,110]. Since obesity accompanies more than 80% of the type 2 DM population and contributes to other targeted factors' improvement, its treatment strategy should be a priority in the comprehensive assessment of diabetes care. According to the American Diabetes Association Standards of Care from 2016, bariatric surgery should be considered in obesity management, in addition to behaviour modification and pharmacotherapy^[111].

However, despite all the knowledge and all the pharmaceutical agents that are available, there will always be a need for more effective treatment options in order to affect the disease in an even more precise pathophysiological pathway, in the near future. For instance, we can expect a completely novel antidiabetic drug class-oxidative phosphorylation blockers, currently represented by imeglimin^[112]. The underlying mechanism of action of this drug class consists of balancing bioenergetics in mitochondria and consequent insulin resistance to result in balance of insulin secretion and utilization as well as hepatic gluconeogenesis suppression. Thus, it is important to emphasize that this is not the sole promising novel drug class. This is indicated by findings from the research on the first-in-class drug, an adenosine monophosphate (AMP)-activated protein kinase activator, targeting one of the key players in the process of energy balance preservation, especially during caloric disturbances^[113], and, finally, findings on the first monoclonal antibody, bimagrumab, that blocks the myostatin type II receptor, which results in fat reduction^[114].

In conclusion, given the raising burden of type 2 DM and CV mortality due to diabetes, despite all of the therapeutic options that are available or will become available in due time, we should be focused on primary prevention, *i.e.*, targeting preventive public health policies and in the rigorous evidence-based initiatives to introduce dietary products that will address metabolic disturbances.

ACKNOWLEDGMENTS

The authors acknowledge Dr. Lora Stanka Kirigin Biloš, a native English speaker for assistance on the English language editing process.

REFERENCES

- 1 **Powers AC.** Diabetes Mellitus. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J. Harrison's Principles of Internal Medicine, 18th ed. New York: McGraw-Hill, 2012
- 2 **International Diabetes Federation.** IDF Diabetes Atlas, 8th ed. Brussels: International Diabetes Federation, 2017
- 3 **Guthrie DW, Humphreys SS.** Diabetes urine testing: an historical perspective. *Diabetes Educ* 1988; **14**: 521-526 [PMID: 3061764 DOI: 10.1177/014572178801400615]
- 4 **Eknoyan G, Nagy J.** A history of diabetes mellitus or how a disease of the kidneys evolved into a kidney disease. *Adv Chronic Kidney Dis* 2005; **12**: 223-229 [PMID: 15822058 DOI: 10.1053/j.ackd.2005.01.002]
- 5 **Reece E.** The history of diabetes mellitus. In: Reece E, Coustan D, editors. Diabetes Mellitus in Pregnancy. New York: Churchill Livingstone, 1995
- 6 **Polonsky KS.** The past 200 years in diabetes. *N Engl J Med* 2012; **367**: 1332-1340 [PMID: 23034021 DOI: 10.1056/NEJMra1110560]
- 7 **von Mering J, Minkowski O.** Diabetes mellitus nach Pankreas extirpation. *Arch Exp Pathol Pharmacol* 1890; **26**: 371-387 [DOI: 10.1007/BF01831214]
- 8 **Brogard JM, Vetter T, Blicke JF.** Discovery of pancreatic diabetes in Strasbourg. *Diabete Metab* 1992; **18**: 104-114 [PMID: 1511752]
- 9 **Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA.** Pancreatic Extracts in the Treatment of Diabetes Mellitus. *Can Med Assoc J* 1922; **12**: 141-146 [PMID: 20314060]
- 10 **Bliss M.** The discovery of insulin: the inside story. *Publ Am Inst Hist Pharm* 1997; **16**: 93-99 [PMID: 11619903]
- 11 **Geyelin HR, Harrop G, Murray MF, Corwin E.** The use of insulin in juvenile diabetes. *J Metabolic Res* 1922; **2**: 767-792 [DOI: 10.1001/jama.1924.02660240028008]
- 12 **Dublin LI.** The facts of life from birth to death. Macmillan: New York, 1951
- 13 **Himsworth HP.** Diabetes mellitus: its differentiation into insulin-sensitive and insulin-insensitive types. *Diabet Med* 2011; **28**: 1440-1444 [PMID: 22092505 DOI: 10.1111/j.1464-5491.2011.3508.x]
- 14 **Cori CF, Cori GT.** Carbohydrate metabolism. *Annu Rev Biochem* 1946; **15**: 193-218 [PMID: 20995968 DOI: 10.1146/annurev.bi.15.070146.001205]
- 15 **Houssay BA, Smyth FS, Foglia VG, Houssay AB.** Comparative diabetogenic action of the hypophysis from various animals. *J Exp Med* 1942; **75**: 93-106 [PMID: 19871171 DOI: 10.1084/jem.75.1.93]
- 16 **Fischer EH.** Phosphorylase and the origin of reversible protein phosphorylation. *Biol Chem* 2010; **391**: 131-137 [PMID: 20030590 DOI: 10.1515/BC.2010.011]
- 17 **From the NIH:** Successful diet and exercise therapy is conducted in Vermont for "diabesity". *JAMA* 1980; **243**: 519-520 [PMID: 7351780 DOI: 10.1001/jama.1980.03300320015011]
- 18 **Gale EA.** Is type 2 diabetes a category error? *Lancet* 2013; **381**: 1956-1957 [PMID: 23725732 DOI: 10.1016/S0140-6736(12)62207-7]
- 19 **NHS.** Metabolic syndrome. Accessed August 27, 2018 Available from: URL: <https://www.nhs.uk/conditions/metabolic-syndrome/>
- 20 **The Globe diabetes community.** Metabolic syndrome. Accessed August 27, 2018 Available from: URL: <https://www.diabetes.co.uk/diabetes-and-metabolic-syndrome.html>
- 21 **BBC.** Metabolic syndrome. Accessed August 27, 2018 Available from: URL: http://www.bbc.co.uk/radio4/science/casenotes_20080122.shtml
- 22 **International Diabetes Federation.** Guidelines. Accessed August 27, 2018 Available from: URL: <https://www.idf.org/e-library/guidelines.html>
- 23 **Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA.** beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. *J Clin Endocrinol Metab* 2005; **90**: 493-500 [PMID: 15483086 DOI: 10.1210/jc.2004-1133]
- 24 **Petersen MC, Shulman GI.** Mechanisms of Insulin Action and Insulin Resistance. *Physiol Rev* 2018; **98**: 2133-2223 [PMID: 29811111 DOI: 10.1152/physrev.00011.2018]

- 30067154 DOI: 10.1152/physrev.00063.2017]
- 25 **DeFronzo RA.** Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 1988; **37**: 667-687 [PMID: 3289989 DOI: 10.2337/diab.37.6.667]
- 26 **Jallut D,** Golay A, Munger R, Frascarolo P, Schutz Y, Jéquier E, Felber JP. Impaired glucose tolerance and diabetes in obesity: a 6-year follow-up study of glucose metabolism. *Metabolism* 1990; **39**: 1068-1075 [PMID: 2215253 DOI: 10.1016/0026-0495(90)90168-C]
- 27 Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837-853 [PMID: 9742976 DOI: 10.1016/S0140-6736(98)07019-6]
- 28 **Levy J,** Atkinson AB, Bell PM, McCance DR, Hadden DR. Beta-cell deterioration determines the onset and rate of progression of secondary dietary failure in type 2 diabetes mellitus: the 10-year follow-up of the Belfast Diet Study. *Diabet Med* 1998; **15**: 290-296 [PMID: 9585393 DOI: 10.1002/(SICI)1096-9136(199804)15:4<290::AID-DIA570>3.0.CO;2-M]
- 29 **DeFronzo RA.** Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 2009; **58**: 773-795 [PMID: 19336687 DOI: 10.2337/db09-9028]
- 30 **Groop LC,** Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA. Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. *J Clin Invest* 1989; **84**: 205-213 [PMID: 2661589 DOI: 10.1172/JCI114142]
- 31 **Groop LC,** Saloranta C, Shank M, Bonadonna RC, Ferrannini E, DeFronzo RA. The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1991; **72**: 96-107 [PMID: 1986032 DOI: 10.1210/jcem-72-1-96]
- 32 **Bays H,** Mandarino L, DeFronzo RA. Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. *J Clin Endocrinol Metab* 2004; **89**: 463-478 [PMID: 14764748 DOI: 10.1210/jc.2003-030723]
- 33 **Coelho M,** Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. *Arch Med Sci* 2013; **9**: 191-200 [PMID: 23671428 DOI: 10.5114/aoms.2013.33181]
- 34 **Bonadonna RC,** De Fronzo RA. Glucose metabolism in obesity and type 2 diabetes. *Diabete Metab* 1991; **17**: 112-135 [PMID: 1936466]
- 35 **DeFronzo RA.** Dysfunctional fat cells, lipotoxicity and type 2 diabetes. *Int J Clin Pract Suppl* 2004; 9-21 [PMID: 16035392 DOI: 10.1111/j.1368-504X.2004.00389.x]
- 36 **Fraze E,** Donner CC, Swislocki AL, Chiou YA, Chen YD, Reaven GM. Ambient plasma free fatty acid concentrations in noninsulin-dependent diabetes mellitus: evidence for insulin resistance. *J Clin Endocrinol Metab* 1985; **61**: 807-811 [PMID: 3900120 DOI: 10.1210/jcem-61-5-807]
- 37 **Kashyap S,** Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, Bajaj M, Mandarino L, DeFronzo R, Cusi K. A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. *Diabetes* 2003; **52**: 2461-2474 [PMID: 14514628 DOI: 10.2337/diabetes.52.10.2461]
- 38 **Carpentier A,** Mittelman SD, Bergman RN, Giacca A, Lewis GF. Prolonged elevation of plasma free fatty acids impairs pancreatic beta-cell function in obese nondiabetic humans but not in individuals with type 2 diabetes. *Diabetes* 2000; **49**: 399-408 [PMID: 10868961 DOI: 10.2337/diabetes.49.3.399]
- 39 **Thiébaud D,** DeFronzo RA, Jacot E, Golay A, Acheson K, Maeder E, Jéquier E, Felber JP. Effect of long chain triglyceride infusion on glucose metabolism in man. *Metabolism* 1982; **31**: 1128-1136 [PMID: 6752642 DOI: 10.1016/0026-0495(82)90163-9]
- 40 **Felber JP,** Vannotti A. Effects of fat infusion on glucose tolerance and insulin plasma levels. *Med Exp Int J Exp Med* 1964; **10**: 153-156 [PMID: 14129851]
- 41 **Roden M,** Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI. Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 1996; **97**: 2859-2865 [PMID: 8675698 DOI: 10.1172/JCI118742]
- 42 **Williamson JR,** Kreisberg RA, Felts PW. Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. *Proc Natl Acad Sci USA* 1966; **56**: 247-254 [PMID: 4381783 DOI: 10.1073/pnas.56.1.247]
- 43 **Bevilacqua S,** Bonadonna R, Buzzigoli G, Boni C, Ciociaro D, Maccari F, Giorico MA, Ferrannini E. Acute elevation of free fatty acid levels leads to hepatic insulin resistance in obese subjects. *Metabolism* 1987; **36**: 502-506 [PMID: 3553852 DOI: 10.1016/0026-0495(87)90051-5]
- 44 **Bays HE,** González-Campoy JM, Bray GA, Kitabchi AE, Bergman DA, Schorr AB, Rodbard HW, Henry RR. Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. *Expert Rev Cardiovasc Ther* 2008; **6**: 343-368 [PMID: 18327995 DOI: 10.1586/14779072.6.3.343]
- 45 **Holst JJ.** Glucagon-like peptide-1: from extract to agent. The Claude Bernard Lecture, 2005. *Diabetologia* 2006; **49**: 253-260 [PMID: 16416146 DOI: 10.1007/s00125-005-0107-1]
- 46 **Drucker DJ.** The biology of incretin hormones. *Cell Metab* 2006; **3**: 153-165 [PMID: 16517403 DOI: 10.1016/j.cmet.2006.01.004]
- 47 **Drucker DJ,** Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006; **368**: 1696-1705 [PMID: 17098089 DOI: 10.1016/S0140-6736(06)69705-5]
- 48 **Meier JJ,** Nauck MA. Incretins and the development of type 2 diabetes. *Curr Diab Rep* 2006; **6**: 194-201 [PMID: 16898571 DOI: 10.1007/s11892-006-0034-7]
- 49 **Vilsbøll T,** Holst JJ. Incretins, insulin secretion and Type 2 diabetes mellitus. *Diabetologia* 2004; **47**: 357-366 [PMID: 14968296 DOI: 10.1007/s00125-004-1342-6]
- 50 **Gutniak M,** Orskov C, Holst JJ, Ahrén B, Efendic S. Anti-diabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 1992; **326**: 1316-1322 [PMID: 1348845 DOI: 10.1056/NEJM199205143262003]
- 51 **Nathan DM,** Schreiber E, Fogel H, Moysov S, Habener JF. Insulinotropic action of glucagonlike peptide-I-(7-37) in diabetic and nondiabetic subjects. *Diabetes Care* 1992; **15**: 270-276 [PMID: 1547685 DOI: 10.2337/diacare.15.2.270]
- 52 **Zander M,** Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 2002; **359**: 824-830 [PMID: 11897280 DOI: 10.1016/S0140-6736(02)07952-7]
- 53 **Consoli A,** Nurjhan N, Reilly JJ Jr, Bier DM, Gerich JE. Mechanism of increased gluconeogenesis in noninsulin-dependent diabetes mellitus. Role of alterations in systemic, hepatic, and muscle lactate and alanine metabolism. *J Clin Invest* 1990; **86**: 2038-2045 [PMID: 2254458 DOI: 10.1172/JCI114940]
- 54 **Matsuda M,** DeFronzo RA, Glass L, Consoli A, Giordano M, Bressler P, Delprato S. Glucagon dose-response curve for hepatic glucose production and glucose disposal in type 2 diabetic patients and normal individuals. *Metabolism* 2002; **51**: 1111-1119 [PMID: 12200754 DOI: 10.1053/meta.2002.34700]
- 55 **Unger RH,** Aguilar-Parada E, Müller WA, Eisentraut AM. Studies of pancreatic alpha cell function in normal and diabetic subjects. *J Clin Invest* 1970; **49**: 837-848 [PMID: 4986215 DOI: 10.1172/JCI106297]
- 56 **Reaven GM,** Chen YD, Golay A, Swislocki AL, Jaspan JB. Documentation of hyperglucagonemia throughout the day in nonobese and obese patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1987; **64**: 106-110 [PMID: 3536980 DOI: 10.1210/jcem-64-1-106]
- 57 **Wise JK,** Hendler R, Felig P. Evaluation of alpha-cell function by infusion of alanine in normal, diabetic and obese subjects. *N*

- Engl J Med* 1973; **288**: 487-490 [PMID: 4567490 DOI: 10.1056/NEJM197303082881003]
- 58 **Boden G**, Soriano M, Hoeldtke RD, Owen OE. Counterregulatory hormone release and glucose recovery after hypoglycemia in non-insulin-dependent diabetic patients. *Diabetes* 1983; **32**: 1055-1059 [PMID: 6357904 DOI: 10.2337/diab.32.11.1055]
- 59 **Abdul-Ghani MA**, DeFronzo RA. Inhibition of renal glucose reabsorption: a novel strategy for achieving glucose control in type 2 diabetes mellitus. *Endocr Pract* 2008; **14**: 782-790 [PMID: 18996802 DOI: 10.4158/EP.14.6.782]
- 60 **Noonan WT**, Shapiro VM, Banks RO. Renal glucose reabsorption during hypertonic glucose infusion in female streptozotocin-induced diabetic rats. *Life Sci* 2001; **68**: 2967-2977 [PMID: 11411796 DOI: 10.1016/S0024-3205(01)01090-6]
- 61 **Kamran M**, Peterson RG, Dominguez JH. Overexpression of GLUT2 gene in renal proximal tubules of diabetic Zucker rats. *J Am Soc Nephrol* 1997; **8**: 943-948 [PMID: 9189862]
- 62 **Rahmouni H**, Thompson PW, Ward JM, Smith CD, Hong G, Brown J. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. *Diabetes* 2005; **54**: 3427-3434 [PMID: 16306358 DOI: 10.2337/diabetes.54.12.3427]
- 63 **Bernard MC**. Lecons sur Le Diabete et La Glycogenese Animale. Paris: Bailliere et Fils, 1877
- 64 **Papaspapros NS**. The History of Diabetes Mellitus, 2nd ed. Stuttgart: Georg Thieme Verlag, 1964 [DOI: 10.1017/S0025727300030623]
- 65 **Allan FN**. Diabetes before and after insulin. *Med Hist* 1972; **16**: 266-273 [PMID: 4595523 DOI: 10.1017/S0025727300017750]
- 66 **Joslin EP**. The Treatment of Diabetes Mellitus. *Can Med Assoc J* 1916; **6**: 673-684 [PMID: 20310820]
- 67 Introduction: *Standards of Medical Care in Diabetes-2018*. *Diabetes Care* 2018; **41**: S1-S2 [PMID: 29222369 DOI: 10.2337/dc18-Sint01]
- 68 **White JR**, Campbell RK, White JR. Overview of the medications used to treat type 2 diabetes. In: White JR, Campbell RK, editors. Medications for the Treatment of Diabetes. Alexandria: American Diabetes Association, 2008: 5-15
- 69 **Frank E**, Nothmann M, Wagner A. Über synthetische dargestellte Körper mit Insulinartiger Wirkung auf den normalen und diabetischen Organismus. *Klin Wchnschr* 1926; **5**: 2011-2107 [DOI: 10.1007/BF01736560]
- 70 **Jarvis B**, Elkinson S. Agents in development for type 2 diabetes. *Drugs R D* 1999; **2**: 95-99 [PMID: 10820648 DOI: 10.2165/00126839-199902020-00002]
- 71 **Alberti KGMM**, Zimmet P, DeFronzo RA: International Textbook of Diabetes Mellitus, 2nd ed. New York: John Wiley & Sons, 1997
- 72 **Rodbard HW**, Blonde L, Braithwaite SS, Brett EM, Cobin RH, Handelsman Y, Hellman R, Jellinger PS, Jovanovic LG, Levy P, Mechanick JI, Zangeneh F; AACE Diabetes Mellitus Clinical Practice Guidelines Task Force. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the management of diabetes mellitus. *Endocr Pract* 2007; **13** Suppl 1: 1-68 [PMID: 17613449 DOI: 10.4158/EP.13.S1.1]
- 73 **Levine R**. Sulfonylureas: background and development of the field. *Diabetes Care* 1984; **7** Suppl 1: 3-7 [PMID: 6376027]
- 74 **Quianzon CC**, Cheikh IE. History of current non-insulin medications for diabetes mellitus. *J Community Hosp Intern Med Perspect* 2012; **2** [PMID: 23882374 DOI: 10.3402/jchimp.v2i3.19081]
- 75 **Galloway JA**. Diabetes Mellitus, 9th ed. Indianapolis: Eli Lilly and Company, 1988
- 76 **US Food and Drug Administration**. Prandin. Accessed August 27, 2018 Available from: URL: <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=SearchDrugDetails>
- 77 **US Food and Drug Administration**. Precose. Accessed August 27, 2018 Available from: URL: <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=SearchDrugDetails>
- 78 **Inzucchi SE**, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR; American Diabetes Association (ADA); European Association for the Study of Diabetes (EASD). Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2012; **35**: 1364-1379 [PMID: 22517736 DOI: 10.2337/dc12-0413]
- 79 **Kendall DM**. Thiazolidinediones: the case for early use. *Diabetes Care* 2006; **29**: 154-157 [PMID: 16373917 DOI: 10.2337/diacare.29.01.06.dc05-0711]
- 80 **US Food and Drug Administration**. Trogliatone: presentation to advisory committee. Accessed August 27, 2018 Available from: URL: <http://www.fda.gov/ohrms/dockets/ac/00/slides/3615s1a.PPT>
- 81 **ACCORD Study Group**, Gerstein HC, Miller ME, Genuth S, Ismail-Beigi F, Buse JB, Goff DC Jr, Probstfield JL, Cushman WC, Ginsberg HN, Bigger JT, Grimm RH Jr, Byington RP, Rosenberg YD, Friedewald WT. Long-term effects of intensive glucose lowering on cardiovascular outcomes. *N Engl J Med* 2011; **364**: 818-828 [PMID: 21366473 DOI: 10.1056/NEJMoa1006524]
- 82 **Nature world news**. FDA eases restrictions on Glaxo diabetes drug Avandia. Accessed August 27, 2018 Available from: URL: <http://www.natureworldnews.com/articles/5075/20131126/fda-eases-restrictions-glaxos-drug.htm>
- 83 **Neumiller JJ**. Differential chemistry (structure), mechanism of action, and pharmacology of GLP-1 receptor agonists and DPP-4 inhibitors. *J Am Pharm Assoc* (2003) 2009; **49** Suppl 1: S16-S29 [PMID: 19801361 DOI: 10.1331/JAPhA.2009.09078]
- 84 **Hausenloy DJ**, Yellon DM. GLP-1 therapy: beyond glucose control. *Circ Heart Fail* 2008; **1**: 147-149 [PMID: 19808284 DOI: 10.1161/CIRCHEARTFAILURE.108.810887]
- 85 **MacDonald PE**, El-Kholy W, Riedel MJ, Salapatek AM, Light PE, Wheeler MB. The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. *Diabetes* 2002; **51** Suppl 3: S434-S442 [PMID: 12475787 DOI: 10.2337/diabetes.51.2007.S434]
- 86 **Forbes**. FDA approves first SGLT2 inhibitor for diabetes. Accessed August 27, 2018 Available from: URL: <http://www.forbes.com/sites/larryhusten/2013/03/29/fda-approves-first-sgl2-inhibitor-for-diabetes>
- 87 **Chiasson JL**, Gomis R, Hanefeld M, Josse RG, Karasik A, Laakso M. The STOP-NIDDM Trial: an international study on the efficacy of an alpha-glucosidase inhibitor to prevent type 2 diabetes in a population with impaired glucose tolerance: rationale, design, and preliminary screening data. Study to Prevent Non-Insulin-Dependent Diabetes Mellitus. *Diabetes Care* 1998; **21**: 1720-1725 [PMID: 9773737 DOI: 10.2337/diacare.21.10.1720]
- 88 **Holman RR**, Coleman RL, Chan JCN, Chiasson JL, Feng H, Ge J, Gerstein HC, Gray R, Huo Y, Lang Z, McMurray JJ, Rydén L, Schröder S, Sun Y, Theodorakis MJ, Tendera M, Tucker L, Tuomilehto J, Wei Y, Yang W, Wang D, Hu D, Pan C; ACE Study Group. Effects of acarbose on cardiovascular and diabetes outcomes in patients with coronary heart disease and impaired glucose tolerance (ACE): a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol* 2017; **5**: 877-886 [PMID: 28917545 DOI: 10.1016/S2213-8587(17)30309-1]
- 89 **Zinman B**, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 2117-2128 [PMID: 26378978 DOI: 10.1056/NEJMoa1504720]
- 90 **Ferrannini E**, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A "Thriftly Substrate" Hypothesis. *Diabetes Care* 2016; **39**: 1108-1114 [PMID: 27289126 DOI: 10.2337/dc16-0330]
- 91 **McMurray J**. EMPA-REG - the "diuretic hypothesis". *J Diabetes Complications* 2016; **30**: 3-4 [PMID: 26597600 DOI: 10.1016/j.jdiacomp.2015.10.012]
- 92 **Devenny JJ**, Godonis HE, Harvey SJ, Rooney S, Cullen MJ, Pellemounter MA. Weight loss induced by chronic dapagliflozin treatment is attenuated by compensatory hyperphagia in diet-induced obese (DIO) rats. *Obesity* (Silver Spring) 2012; **20**: 1645-1652 [PMID: 22402735 DOI: 10.1038/oby.2012.59]

- 93 **Yokono M**, Takasu T, Hayashizaki Y, Mitsuoka K, Kihara R, Muramatsu Y, Miyoshi S, Tahara A, Kurosaki E, Li Q, Tomiyama H, Sasamata M, Shibasaki M, Uchiyama Y. SGLT2 selective inhibitor ipragliflozin reduces body fat mass by increasing fatty acid oxidation in high-fat diet-induced obese rats. *Eur J Pharmacol* 2014; **727**: 66-74 [PMID: 24486393 DOI: 10.1016/j.ejphar.2014.01.040]
- 94 **Suzuki M**, Takeda M, Kito A, Fukazawa M, Yata T, Yamamoto M, Nagata T, Fukuzawa T, Yamane M, Honda K, Suzuki Y, Kawabe Y. Tofogliflozin, a sodium/glucose cotransporter 2 inhibitor, attenuates body weight gain and fat accumulation in diabetic and obese animal models. *Nutr Diabetes* 2014; **4**: e125 [PMID: 25000147 DOI: 10.1038/nutd.2014.20]
- 95 **Ferrannini E**, Baldi S, Frascerra S, Astiarraga B, Heise T, Bizzotto R, Mari A, Pieber TR, Muscelli E. Shift to Fatty Substrate Utilization in Response to Sodium-Glucose Cotransporter 2 Inhibition in Subjects Without Diabetes and Patients With Type 2 Diabetes. *Diabetes* 2016; **65**: 1190-1195 [PMID: 26861783 DOI: 10.2337/db15-1356]
- 96 **Neal B**, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erond N, Shaw W, Law G, Desai M, Matthews DR, CANVAS Program Collaborative Group. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N Engl J Med* 2017; **377**: 644-657 [PMID: 28605608 DOI: 10.1056/NEJMoa1611925]
- 97 **Marso SP**, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, Nissen SE, Pocock S, Poulter NR, Ravn LS, Steinberg WM, Stockner M, Zinman B, Bergenstal RM, Buse JB; LEADER Steering Committee; LEADER Trial Investigators. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2016; **375**: 311-322 [PMID: 27295427 DOI: 10.1056/NEJMoa1603827]
- 98 **Marso SP**, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, Lingvay I, Rosenstock J, Seufert J, Warren ML, Woo V, Hansen O, Holst AG, Pettersson J, Vilsbøll T; SUSTAIN-6 Investigators. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 2016; **375**: 1834-1844 [PMID: 27633186 DOI: 10.1056/NEJMoa1607141]
- 99 **Azoulay L**, Filion KB, Platt RW, Dahl M, Dormuth CR, Clemens KK, Durand M, Juurlink DN, Targownik LE, Turin TC, Paterson JM, Ernst P; Canadian Network for Observational Drug Effect Studies Investigators. Incretin based drugs and the risk of pancreatic cancer: international multicentre cohort study. *BMJ* 2016; **352**: i581 [PMID: 26888382 DOI: 10.1136/bmj.i581]
- 100 **Barnett AH**, Bain SC, Bouter P, Karlberg B, Madsbad S, Jervell J, Mustonen J; Diabetics Exposed to Telmisartan and Enalapril Study Group. Angiotensin-receptor blockade versus converting-enzyme inhibition in type 2 diabetes and nephropathy. *N Engl J Med* 2004; **351**: 1952-1961 [PMID: 15516696 DOI: 10.1056/NEJMoa042274]
- 101 **Lewis EJ**, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 1993; **329**: 1456-1462 [PMID: 8413456 DOI: 10.1056/NEJM19931113292004]
- 102 **Brenner BM**, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S; RENAAL Study Investigators. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; **345**: 861-869 [PMID: 11565518 DOI: 10.1056/NEJMoa011161]
- 103 **Lewis EJ**, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I; Collaborative Study Group. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 2001; **345**: 851-860 [PMID: 11565517 DOI: 10.1056/NEJMoa011303]
- 104 **Parving HH**, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P; Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 2001; **345**: 870-878 [PMID: 11565519 DOI: 10.1056/NEJMoa011489]
- 105 **Klahr S**, Levey AS, Beck GJ, Caggiula AW, Hunsicker L, Kusek JW, Striker G. The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. *N Engl J Med* 1994; **330**: 877-884 [PMID: 8114857 DOI: 10.1056/NEJM199403313301301]
- 106 **Frank RN**. Diabetic retinopathy. *N Engl J Med* 2004; **350**: 48-58 [PMID: 14702427 DOI: 10.1056/NEJMra021678]
- 107 **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]
- 108 Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 854-865 [PMID: 9742977 DOI: 10.1016/S0140-6736(98)07037-8]
- 109 **Gaede P**, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 2003; **348**: 383-393 [PMID: 12556541 DOI: 10.1056/NEJMoa021778]
- 110 **Gaede P**, Lund-Andersen H, Parving HH, Pedersen O. Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Engl J Med* 2008; **358**: 580-591 [PMID: 18256393 DOI: 10.1056/NEJMoa0706245]
- 111 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]
- 112 **Vuylsteke V**, Chastain LM, Maggu GA, Brown C. Imeglimin: A Potential New Multi-Target Drug for Type 2 Diabetes. *Drugs R D* 2015; **15**: 227-232 [PMID: 26254210 DOI: 10.1007/s40268-015-0099-3]
- 113 **Kim KH**, Lee IS, Park JY, Kim Y, An EJ, Jang HJ. Cucurbitacin B Induces Hypoglycemic Effect in Diabetic Mice by Regulation of AMP-Activated Protein Kinase Alpha and Glucagon-Like Peptide-1 via Bitter Taste Receptor Signaling. *Front Pharmacol* 2018; **9**: 1071 [PMID: 30298009 DOI: 10.3389/fphar.2018.01071]
- 114 **Garito T**, Roubenoff R, Hompesch M, Morrow L, Gomez K, Rooks D, Meyers C, Buchsbaum MS, Neelakantham S, Swan T, Filosa LA, Laurent D, Petricoul O, Zakaria M. Bimagrimumab improves body composition and insulin sensitivity in insulin-resistant individuals. *Diabetes Obes Metab* 2018; **20**: 94-102 [PMID: 28643356 DOI: 10.1111/dom.13042]

P- Reviewer: Barzilay J, Parikh M **S- Editor:** Ma RY
L- Editor: A **E- Editor:** Song H



Unexpected alliance between syndecan-1 and innate-like T cells to protect host from autoimmune effects of interleukin-17

Anil Kumar Jaiswal, Mohanraj Sadasivam, Abdel Rahim A Hamad

Anil Kumar Jaiswal, Department of Pathobiology, Auburn University, Auburn, AL 36849, United States

Mohanraj Sadasivam, Abdel Rahim A Hamad, Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, MD 21205, United States

ORCID number: Anil Kumar Jaiswal (0000-0002-8169-0335); Mohanraj Sadasivam (0000-0002-3132-2948); Abdel Rahim A Hamad (0000-0003-3148-4020).

Author contributions: All authors contributed to conception and writing of this article.

Conflict-of-interest statement: Authors declare no conflict of interest.

Open-Access: This is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Corresponding author to: Abdel Rahim A Hamad, PhD, Associate Professor, Department of Pathology, School of Medicine, Johns Hopkins University, Ross 66G, 720 Rutland Ave, Baltimore, MD 21205, United States. ahamad@jhmi.edu
Telephone: +1-410-6143021
Fax: +1-410-6143548

Received: September 1, 2018

Peer-review started: September 3, 2018

First decision: October 16, 2018

Revised: October 23, 2018

Accepted: November 26, 2018

Article in press: November 26, 2018

Published online: December 15, 2018

Abstract

Innate-like T cells, namely natural killer T (NKT) and $\gamma\delta$ T cells, play critical roles in linking innate and adaptive immune responses through rapid production of cytokines. Prominent among these cytokines is interleukin-17 (IL-17), which is a potent proinflammatory cytokine that plays a critical role in host defense against fungi and extracellular bacteria. However, excessive IL-17-production promotes autoimmune diseases, including psoriasis, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and systemic lupus erythematosus. IL-17 has also been implicated in regulating body fat, which is highly relevant given rises in obesity and type 2 diabetes. NKT cells, $\gamma\delta$ T cells and mucosal-associated invariant T cells (MAIT) are the major sources of IL-17 involved in protection of mucosal surfaces from opportunistic infections and causing autoimmunity when become dysregulated. Given the pathogenic effects of IL-17, efforts have been directed towards understanding mechanisms that guard against IL-17 overproduction. One novel potent mechanism is mediated by the heparan sulfate proteoglycan, syndecan-1 (sdc1), which is selectively expressed by IL-17-producing subsets of NKT and $\gamma\delta$ T cells. This unexpected role for sdc1 is uncovered by analysis of NKT and $\gamma\delta$ T cells in sdc1-deficient mice. In this mini-review, we discuss selective expression of sdc1 by these innate T cells and consequences of its absence on IL-17 homeostasis and pathological implications.

Key words: Natural killer T cell; Natural killer T 17 cells; $\gamma\delta$ 17 cells; Syndecan-1; Interleukin-17

© The Author(s) 2018. Published by Baishideng Publishing

Group Inc. All rights reserved.

Core tip: Interleukin-17 (IL-17) is a potent proinflammatory cytokine that plays a critical role in host defense against fungi and extracellular bacteria. Excessive production of IL-17, however, has been implicated in pathogenesis of many autoimmune diseases. Our recent findings show that natural killer T (NKT) cells and $\gamma\delta$ T cells employ syndecan-1 (sdc1), a heparan sulfate proteoglycan that is predominantly expressed by epithelia, to prevent out of control expansion of IL-17-producing subsets of NKT (NKT17) cell and $\gamma\delta$ ($T\gamma\delta 17$) cells. In this mini-review, we highlight these findings and briefly discuss their significance for developing new strategies to prevent IL-17-mediated autoimmune diseases.

Jaiswal AK, Sadasivam M, Hamad ARA. Unexpected alliance between syndecan-1 and innate-like T cells to protect host from autoimmune effects of interleukin-17. *World J Diabetes* 2018; 9(12): 220-225

URL: <https://www.wjgnet.com/1948-9358/full/v9/i12/220.htm>
DOI: <https://dx.doi.org/10.4239/wjd.v9.i12.220>

INTRODUCTION

Recent data show that innate-like T cells utilize sdc1 to regulate interleukin (IL)-17 production. Significance of this alliance is uncovered by analysis of homeostasis of IL-17 production by natural killer T (NKT) and $\gamma\delta$ T cells in sdc1-deficient mice. The results show significant increases in specific subsets of these innate-like T cells that specialized in production of IL-17 in the thymus and in peripheral organs in mice lacking sdc1 as illustrated (Figure 1). In this minireview, we briefly describe the three players forming this axis and how deficiency of sdc1 dysregulates homeostasis of IL-17 production by NKT and $\gamma\delta$ T cells and the consequences in autoimmunity.

Syndecan family

The syndecan (sdc) family is comprised of four transmembrane heparan sulfate proteoglycans (HSPGs)^[1]. These four HSPGs are sdc1, 2, 3, and 4. The structures of these sdcs are highly conserved with high sequence homology in vertebrates and invertebrates^[2,3]. Sdc2 is primarily expressed on cells of mesenchymal cells^[4]; sdc3 is primarily expressed by neuronal tissue and cartilage^[5], and sdc4 is ubiquitously expressed in most tissues^[6]. On the other hand, sdc1 is a heparan sulfate that is ubiquitously expressed on epithelial cells, hepatocytes, endothelium. Sdc1 ectodomain interacts with various ligands (including growth factors, chemokines, cytokines and their receptors, and pathogens) to modulate various functions, including differentiation, migration, survival, and proliferation^[7]. It is reported that sdc1 is a target of Blimp-1, the trans-

cription factor that regulates differentiation of B cells into plasma cells. Sdc1 is also involved in the growth and metastasis of multiple myeloma *in vivo*^[8]. In contrast, there is very limited information on the role of sdc1 in the adaptive immune cells except as a marker for plasma and myeloma cells and regulators of their survival^[9,10]. More recently, however, we have identified sdc1 as a marker of IL-17-producing subsets of NKT cells and $\gamma\delta$ T cells, (NKT17 and $T\gamma\delta 17$), respectively. The other members of sdcs family, however, in the regulation of cytokines including IL-17 are not well documented.

IL-17

IL-17 (also called as IL-17A) is a member of the IL-17 family. The family of IL-17 consists of six members: namely IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. IL-17A is commonly known as IL-17^[11], is a potent proinflammatory cytokine that has been strongly associated with pathology, especially autoimmunity. IL-17-mediated recruitment of inflammatory cells in response to bacterial or fungal infections is vital for the clearance of infections and if not discontinued it leads to the initiation of chronic inflammation and autoimmunity. Indeed, increased production of IL-17 has been associated with a wide range of inflammatory diseases, including rheumatoid arthritis^[12], inflammatory bowel disease^[13], diabetes^[14], cancer^[15], and allergic asthma^[16]. Although the Th17 subset of conventional T cells was the first to be identified^[17], subsequent studies identified several types of innate immune cells that are important sources of IL-17. Prominent among them are specialized subsets (NKT17 cell and $T\gamma\delta 17$ cell) of NKT and $\gamma\delta$ T cells. Mucosal associated invariant T cells (MAIT) cells is another innate like T cell that is a significant producer of IL-17. They comprise up to 5% of human peripheral T cells and they express a semi-invariant TCR alpha chain (V α 7.2) which recognizes antigens in the context of the nonpolymorphic major histocompatibility complex (MHC)-related protein 1 (MR1)^[18]. Production of IL-17 by MAIT cells has been implicated in the pathogenesis of various diseases like multiple sclerosis^[18,19].

Here we will discuss the selective expression of sdc1 on innate-like T cells and its potential implications.

SELECTIVE EXPRESSION OF SDC1 ON IL-17-PRODUCING NKT CELLS

NKT cells represent a distinct lineage of $\alpha\beta$ T cells that expresses an invariant TCR and specializes in recognizing self and foreign glycolipids as antigens in the context of the CD1d MHC class Ib molecule. They are experimentally stimulated using the synthetic glycolipid, α GalCer (α -Galactosylceramide)^[20] and fluorochrome-conjugated α GalCer/CD1d tetramers are routinely used to stain and identify NKT cells by flow cytometry. Thus, there are fundamental differences between NKT cells

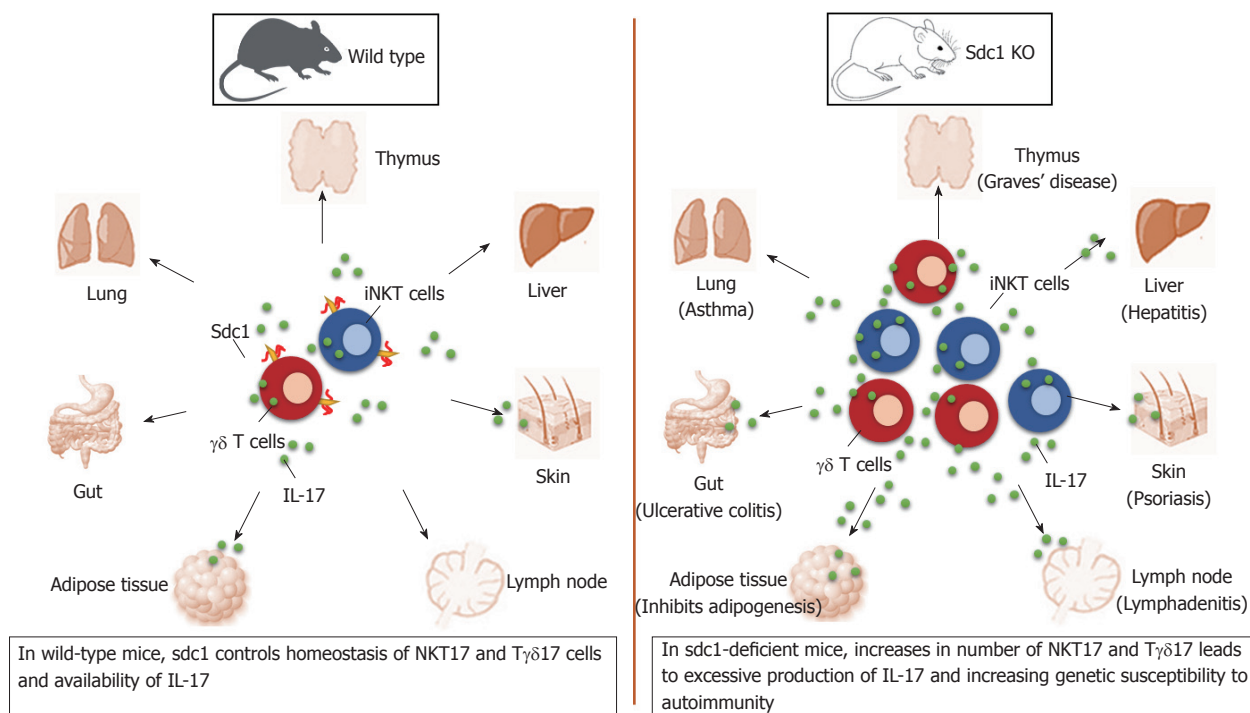


Figure 1 Innate-like T cells employ syndecan-1 to regulate interleukin-17 production. Homeostasis of interleukin (IL)-17 production by natural killer T (NKT) and $\gamma\delta$ T cells in wild type (left) and syndecan-1-deficient (right) mice. The diagram illustrated significant increases in numbers of NKT17 and $\gamma\delta$ 17 subsets in the thymus and in peripheral organs thereby increasing genetic susceptibility to IL-17-driven autoimmune diseases. IL-17: Interleukin-17; NKT: Natural killer T cells; Sdc1: Syndecan-1; KO: Knockout; NKT17: Interleukin-17-producing subsets of natural killer T cells; $\gamma\delta$ 17: Interleukin-17-producing subsets of $\gamma\delta$ T cells; iNKT: Invariant natural killer T cells.

and conventional T cells (which recognize peptides as antigens and express highly diverse TCR repertoire). NKT cells are considered innate-like T cells as they are selected through the agonist selection pathway that is favored by autoreactive T cell receptors (TCRs) and they acquire their effector functions while developing in the thymus by differentiating into three distinct subsets that produce interferon- γ (IFN- γ) (NKT1), IL-4 (NKT2) or IL-17 (NKT17), respectively^[21]. Upon stimulation, NKT cells produce massive amount of two of the most potent proinflammatory cytokines (IL-17 and IFN- γ). NKT cells are important early sources of these key cytokines that play central roles as first line of defense and in shaping adaptive immune responses, including differentiation of CD4 T cells into T helper (Th)1, Th2 and Th17 programs. These cells possess both protective and pathogenic roles in many microbial infections, autoimmune disease, allergic disease and cancer^[22]. Moreover, other innate-like T cells in this regard are $\gamma\delta$ T-cells. Both NKT cells, $\gamma\delta$ T-cells develop in the thymus where a subpopulation specially $\gamma\delta$ 17 cells, acquires the effector ability to produce IL-17 rapidly^[9]. $\gamma\delta$ 17 cells predominantly localize in peripheral lymph nodes and skin of the mice^[23].

SDC1 DEFICIENCY LEADS TO EXPANSION OF NKT17 CELLS

We and others^[24,25] have identified sdc1 as a phenotypic

marker of NKT17 cells. Apart from being specific marker for NKT17 cell, sdc1 is a regulator of NKT17 subset. Deletion of sdc1 significantly increases the frequency of NKT17 at the expense of NKT1 cells, which was reflected in systemic increase in production of IL-17 in sdc1-knockout (KO) mice as compared to WT mice upon α -Galcer stimulation^[26]. These results uncover a critical role for sdc1 expression in regulating homeostasis of NKT17 and consequently production of IL-17.

An intriguing aspect of NKT cells is their selective residence in metabolic organs with NKT1 residing mainly in liver and NKT17 cells in visceral adipose tissue^[21,26]. Furthermore, whereas a great deal is known about specific roles of Th1, Th2 and Th17 subsets, the precise roles of NKT cells remain poorly undefined and the specific functions of its three distinct effector subsets and their relationships to one another remain unclear. The relationship between NKT17 cells and adipose tissue, however, has been difficult to dissect even though IL-17 inhibits adipogenesis and causes insulin resistance^[27]. Moreover, attempts to understand overall metabolic role of NKT cells produced conflicting data that ranged from tolerogenic to pathogenic or no role^[28]. A main likely reason, in our opinion, is the complex nature of NKT cells and studying them as one whole even though they are comprised of distinct subsets with clearly opposing functions. Therefore, our ability to sort NKT cells into viable NKT17 and NKT1 using sdc1 expression present new opportunities to study their

specific properties, how they modulate one another, and to generate adoptive hosts bearing exclusively NKT17 or NKT1 cells to examine their specific effects on VAT separately. Sdc1 deficiency is associated with reduced body fat and insulin resistance in chow-fed mice. Kasza *et al.*^[29] reported that sdc1KO Balb/c mice have reduced intradermal fat and that their VAT is also significantly reduced in 12-wk-old mice.

SELECTIVE EXPRESSION OF SDC1 ON T $\gamma\delta$ 17 CELLS

T $\gamma\delta$ T cells are a population of lymphocytes expressing γ and δ TCR chains and these innate immune T cells are considered as link between innate and adaptive immune responses. In the mouse, T $\gamma\delta$ T cells primarily develop in the thymus into completely functional subsets which further secrete high levels of pro-inflammatory cytokines, such as IFN- γ or IL-17, upon activation in the periphery^[30,31]. T $\gamma\delta$ T cells are abundant in the skin (dermis and epidermis), lymph node, respiratory mucosa such as nasal mucosa, bronchial mucosa, and lung^[32]. Moreover, T $\gamma\delta$ T cell have been characterized in several epithelial tissues for the selective tissue homing and retention and involved in immune surveillance and immune defense. There is abundant evidence that T $\gamma\delta$ T cells are involved in allergic and inflammatory settings and suggest that they can both drive and regulate immune responses through different mechanisms. Here we will discuss the selective expression of sdc1 on innate T cells (NKT17 cell and Tgd17 cell) and its potential implications.

T $\gamma\delta$ T cells are the main source of early IL-17 in various murine models of infection, inflammation, and autoimmunity^[33,34]. T $\gamma\delta$ 17 cells develop in the thymus where a subset acquires the innate effector ability of rapidly producing IL-17. In the periphery, T $\gamma\delta$ 17 cells localize to lymph nodes, mucosal tissues such as the intestine, skin and lung^[35,36]. In human, T $\gamma\delta$ 17 cells have been found to increase in patients with tuberculosis, bacterial meningitis, ankylosing spondylitis, and psoriasis^[32,37]. These findings provide a potential explanation that IL-17-producing T $\gamma\delta$ T cells are a key component in the pathogenesis of various inflammatory and autoimmune diseases. Recently, we have found that sdc1 is selectively expressed on IL-17-producing T $\gamma\delta$ T subset, including those in the thymus, lymph nodes and skin^[23]. Given selective expression of sdc1 by NKT17 cells, its specific expression of on T $\gamma\delta$ 17 subset indicate a special relationship between sdc1 and innate-like T cells, which are major sources of IL-17 production. Therefore, sdc1 serves at least two roles on T $\gamma\delta$ T cells: (1) Acts as a surface marker for T $\gamma\delta$ 17; and (2) A negative regulator of T $\gamma\delta$ 17 cells.

SDC1 NEGATIVELY REGULATES HOMEOSTASIS OF T $\gamma\delta$ 17 CELLS

T $\gamma\delta$ 17 cells play an important role in early host defense

against fungal and bacterial infections. Early reports suggested the functional involvement of T $\gamma\delta$ 17 cells as a critical source of IL-17 that drives autoimmune disease including psoriasis^[38]. Thus, identifying the factors that control homeostasis of T $\gamma\delta$ 17 cells is important and could be useful for developing strategies to prevent pathogenic production of IL-17. Therefore, studies addressing the roles of sdc1 expressing T $\gamma\delta$ 17 cell may provide an alternative approach to understanding its role in autoimmune diseases. Sdc1 expression on T $\gamma\delta$ 17 might be useful for clear understanding of their biology and their physiologic role in steady state and disease condition.

In concordance and in light of our findings, that sdc1 is selectively expressed and negatively regulates homeostasis NKT17 cells^[26], we thought to determine whether sdc1 is also expressed controls homeostasis of T $\gamma\delta$ 17 cells. That turned out to be the case and as in NKT17, deletion of sdc1, significantly and selectively increased the numbers of T $\gamma\delta$ 17 cells in thymus, lymph nodes and skin, in steady state^[23]. Sdc1 deficiency significantly exacerbated imiquimod (IMQ)-induced psoriasiform dermatitis and significantly increased T $\gamma\delta$ 17 cells, accompanied by increased skin inflammation in sdc1KO mice than wild type. Therefore, these findings suggest that targeting sdc1 could represent a novel strategy to control IL-17 production by NKT and T $\gamma\delta$ T cells.

DOES SDC1 REGULATE PRODUCTION OF IL-17 BY OTHER INNATE-LIKE T CELLS OR INNATE CELLS?

As mentioned above, the other major innate-like T cells that produce IL-17 are MAIT cells. However, whether sdc1 is also involved in regulation of IL-17 by MAIT cells is currently unknown and worthy of future investigation. Furthermore, innate-like lymphocyte 3 are major producers of IL-17^[39] and need to be investigated for expression of sdc1 in future studies.

CONCLUSION

In summary, the discovery of selective expression of sdc1 on NKT17 and T $\gamma\delta$ 17 reveals a previously unexpected role for sdc1 in regulating IL-17 by innate-like T cells. The results provide an impetus for future experiments aimed at understanding specific mechanisms by which sdc1 regulates IL-17 production by innate-like T cells. In addition, sdc1-deficient mouse strains provide new model for of the role of innate-like T cells in IL-17-mediated autoimmune diseases. Such efforts may lead to new therapeutic strategies for autoimmune diseases where IL-17 plays a central role.

REFERENCES

- 1 Bernfield M, Götte M, Park PW, Reizes O, Fitzgerald ML,

- Lincecum J, Zako M. Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem* 1999; **68**: 729-777 [PMID: 10872465 DOI: 10.1146/annurev.biochem.68.1.729]
- 2 **Chakravarti R**, Adams JC. Comparative genomics of the syndecans defines an ancestral genomic context associated with matrilins in vertebrates. *BMC Genomics* 2006; **7**: 83 [PMID: 16620374 DOI: 10.1186/1471-2164-7-83]
 - 3 **Chen L**, Couchman JR, Smith J, Woods A. Molecular characterization of chicken syndecan-2 proteoglycan. *Biochem J* 2002; **366**: 481-490 [PMID: 12038962 DOI: 10.1042/BJ20020711]
 - 4 **Marynen P**, Zhang J, Cassiman JJ, Van den Berghe H, David G. Partial primary structure of the 48- and 90-kilodalton core proteins of cell surface-associated heparan sulfate proteoglycans of lung fibroblasts. Prediction of an integral membrane domain and evidence for multiple distinct core proteins at the cell surface of human lung fibroblasts. *J Biol Chem* 1989; **264**: 7017-7024 [PMID: 2523388]
 - 5 **Carey DJ**, Evans DM, Stahl RC, Asundi VK, Conner KJ, Garbes P, Cizmeci-Smith G. Molecular cloning and characterization of N-syndecan, a novel transmembrane heparan sulfate proteoglycan. *J Cell Biol* 1992; **117**: 191-201 [PMID: 1556152 DOI: 10.1083/jcb.117.1.191]
 - 6 **David G**, van der Schueren B, Marynen P, Cassiman JJ, van den Berghe H. Molecular cloning of amphiglycan, a novel integral membrane heparan sulfate proteoglycan expressed by epithelial and fibroblastic cells. *J Cell Biol* 1992; **118**: 961-969 [PMID: 1500433 DOI: 10.1083/jcb.118.4.961]
 - 7 **Teng YH**, Aquino RS, Park PW. Molecular functions of syndecan-1 in disease. *Matrix Biol* 2012; **31**: 3-16 [PMID: 22033227 DOI: 10.1016/j.matbio.2011.10.001]
 - 8 **Yang Y**, MacLeod V, Dai Y, Khotskaya-Sample Y, Shriver Z, Venkataraman G, Sasisekharan R, Naggi A, Torri G, Casu B, Vlodavsky I, Suva LJ, Epstein J, Yaccoby S, Shaughnessy JD Jr, Barlogie B, Sanderson RD. The syndecan-1 heparan sulfate proteoglycan is a viable target for myeloma therapy. *Blood* 2007; **110**: 2041-2048 [PMID: 17536013 DOI: 10.1182/blood-2007-04-082495]
 - 9 **Sanderson RD**, Epstein J. Myeloma bone disease. *J Bone Miner Res* 2009; **24**: 1783-1788 [PMID: 19839769 DOI: 10.1359/jbmr.090901]
 - 10 **McCarron MJ**, Park PW, Fooksman DR. CD138 mediates selection of mature plasma cells by regulating their survival. *Blood* 2017; **129**: 2749-2759 [PMID: 28381397 DOI: 10.1182/blood-2017-01-761643]
 - 11 **Chang SH**, Dong C. Signaling of interleukin-17 family cytokines in immunity and inflammation. *Cell Signal* 2011; **23**: 1069-1075 [PMID: 21130872 DOI: 10.1016/j.cellsig.2010.11.022]
 - 12 **Lubberts E**, van den Bersselaar L, Oppers-Walgreen B, Schwarzenberger P, Coenen-de Roo CJ, Kolls JK, Joosten LA, van den Berg WB. IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kappa B ligand/osteoprotegerin balance. *J Immunol* 2003; **170**: 2655-2662 [PMID: 12594294 DOI: 10.4049/jimmunol.170.5.2655]
 - 13 **Zhang Z**, Zheng M, Bindas J, Schwarzenberger P, Kolls JK. Critical role of IL-17 receptor signaling in acute TNBS-induced colitis. *Inflamm Bowel Dis* 2006; **12**: 382-388 [PMID: 16670527 DOI: 10.1097/01.MIB.0000218764.06959.91]
 - 14 **Ankathatti Munegowda M**, Deng Y, Chibbar R, Xu Q, Freywald A, Mulligan SJ, van Drunen Littel-van den Hurk S, Sun D, Xiong S, Xiang J. A distinct role of CD4+ Th17- and Th17-stimulated CD8+ CTL in the pathogenesis of type 1 diabetes and experimental autoimmune encephalomyelitis. *J Clin Immunol* 2011; **31**: 811-826 [PMID: 21674137 DOI: 10.1007/s10875-011-9549-z]
 - 15 **McAllister F**, Bailey JM, Alsina J, Nirschl CJ, Sharma R, Fan H, Rattigan Y, Roesser JC, Lankapalli RH, Zhang H, Jaffee EM, Drake CG, Housseau F, Maitra A, Kolls JK, Sears CL, Pardoll DM, Leach SD. Oncogenic Kras activates a hematopoietic-to-epithelial IL-17 signaling axis in preinvasive pancreatic neoplasia. *Cancer Cell* 2014; **25**: 621-637 [PMID: 24823639 DOI: 10.1016/j.ccr.2014.03.014]
 - 16 **Allen JE**, Sutherland TE, Rückerl D. IL-17 and neutrophils: unexpected players in the type 2 immune response. *Curr Opin Immunol* 2015; **34**: 99-106 [PMID: 25794823 DOI: 10.1016/j.coi.2015.03.001]
 - 17 **Damsker JM**, Hansen AM, Caspi RR. Th1 and Th17 cells: adversaries and collaborators. *Ann N Y Acad Sci* 2010; **1183**: 211-221 [PMID: 20146717 DOI: 10.1111/j.1749-6632.2009.05133.x]
 - 18 **Walker LJ**, Kang YH, Smith MO, Tharmalingham H, Ramamurthy N, Fleming VM, Sahgal N, Leslie A, Oo Y, Geremia A, Scriba TJ, Hanekom WA, Lauer GM, Lantz O, Adams DH, Powrie F, Barnes E, Klennerman P. Human MAIT and CD8 $\alpha\alpha$ cells develop from a pool of type-17 precommitted CD8+ T cells. *Blood* 2012; **119**: 422-433 [PMID: 22086415 DOI: 10.1182/blood-2011-05-353789]
 - 19 **Dusseau M**, Martin E, Serriari N, Péguillet I, Premel V, Louis D, Milder M, Le Bourhis L, Soudais C, Treiner E, Lantz O. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood* 2011; **117**: 1250-1259 [PMID: 21084709 DOI: 10.1182/blood-2010-08-303339]
 - 20 **Sullivan BA**, Kronenberg M. Activation or anergy: NKT cells are stunned by alpha-galactosylceramide. *J Clin Invest* 2005; **115**: 2328-2329 [PMID: 16138189 DOI: 10.1172/JCI26297]
 - 21 **McDonald BD**, Constantinides MG, Bendelac A. Polarized effector programs for innate-like thymocytes. *Nat Immunol* 2013; **14**: 1110-1111 [PMID: 24145782 DOI: 10.1038/ni.2739]
 - 22 **Van Kaer L**, Parekh VV, Wu L. Invariant natural killer T cells as sensors and managers of inflammation. *Trends Immunol* 2013; **34**: 50-58 [PMID: 23017731 DOI: 10.1016/j.it.2012.08.009]
 - 23 **Jaiswal AK**, Sadasivam M, Archer NK, Miller RJ, Dillen CA, Ravipati A, Park PW, Chakravarti S, Miller LS, Hamad ARA. Syndecan-1 Regulates Psoriasiform Dermatitis by Controlling Homeostasis of IL-17-Producing $\gamma\delta$ T Cells. *J Immunol* 2018; **201**: 1651-1661 [PMID: 30045969 DOI: 10.4049/jimmunol.1800104]
 - 24 **Lee YJ**, Starrett GJ, Lee ST, Yang R, Henzler CM, Jameson SC, Hogquist KA. Lineage-Specific Effector Signatures of Invariant NKT Cells Are Shared amongst $\gamma\delta$ T, Innate Lymphoid, and Th Cells. *J Immunol* 2016; **197**: 1460-1470 [PMID: 27385777 DOI: 10.4049/jimmunol.1600643]
 - 25 **Georgiev H**, Ravens I, Benarafa C, Förster R, Bernhardt G. Distinct gene expression patterns correlate with developmental and functional traits of iNKT subsets. *Nat Commun* 2016; **7**: 13116 [PMID: 27721447 DOI: 10.1038/ncomms13116]
 - 26 **Dai H**, Rahman A, Saxena A, Jaiswal AK, Mohamood A, Ramirez L, Noel S, Rabb H, Jie C, Hamad AR. Syndecan-1 identifies and controls the frequency of IL-17-producing naïve natural killer T (NKT17) cells in mice. *Eur J Immunol* 2015; **45**: 3045-3051 [PMID: 26300525 DOI: 10.1002/eji.201545532]
 - 27 **Zúñiga LA**, Shen WJ, Joyce-Shaikh B, Pyatnova EA, Richards AG, Thom C, Andrade SM, Cua DJ, Kraemer FB, Butcher EC. IL-17 regulates adipogenesis, glucose homeostasis, and obesity. *J Immunol* 2010; **185**: 6947-6959 [PMID: 21037091 DOI: 10.4049/jimmunol.1001269]
 - 28 **Mathis D**. Immunological goings-on in visceral adipose tissue. *Cell Metab* 2013; **17**: 851-859 [PMID: 23747244 DOI: 10.1016/j.cmet.2013.05.008]
 - 29 **Kasza I**, Suh Y, Wollny D, Clark RJ, Roopra A, Colman RJ, MacDougald OA, Shedd TA, Nelson DW, Yen MI, Yen CL, Alexander CM. Syndecan-1 is required to maintain intradermal fat and prevent cold stress. *PLoS Genet* 2014; **10**: e1004514 [PMID: 25101993 DOI: 10.1371/journal.pgen.1004514]
 - 30 **Jensen KD**, Su X, Shin S, Li L, Youssef S, Yamasaki S, Steinman L, Saito T, Locksley RM, Davis MM, Baumgarth N, Chien YH. Thymic selection determines gammadelta T cell effector fate: antigen-naïve cells make interleukin-17 and antigen-experienced cells make interferon gamma. *Immunity* 2008; **29**: 90-100 [PMID: 18585064 DOI: 10.1016/j.immuni.2008.04.022]
 - 31 **Ribot JC**, deBarros A, Pang DJ, Neves JF, Peperzak V, Roberts SJ, Girardi M, Borst J, Hayday AC, Pennington DJ, Silva-Santos B. CD27 is a thymic determinant of the balance between interferon-gamma- and interleukin 17-producing gammadelta T cell subsets. *Nat Immunol* 2009; **10**: 427-436 [PMID: 19270712 DOI: 10.1038/ni.1717]
 - 32 **Cai Y**, Shen X, Ding C, Qi C, Li K, Li X, Jala VR, Zhang HG, Wang T, Zheng J, Yan J. Pivotal role of dermal IL-17-producing $\gamma\delta$

- T cells in skin inflammation. *Immunity* 2011; **35**: 596-610 [PMID: 21982596 DOI: 10.1016/j.immuni.2011.08.001]
- 33 **Hamada S**, Umemura M, Shiono T, Tanaka K, Yahagi A, Begum MD, Oshiro K, Okamoto Y, Watanabe H, Kawakami K, Roark C, Born WK, O'Brien R, Ikuta K, Ishikawa H, Nakae S, Iwakura Y, Ohta T, Matsuzaki G. IL-17A produced by gammadelta T cells plays a critical role in innate immunity against listeria monocytogenes infection in the liver. *J Immunol* 2008; **181**: 3456-3463 [PMID: 18714018 DOI: 10.4049/jimmunol.181.5.3456]
 - 34 **Petermann F**, Rothhammer V, Claussen MC, Haas JD, Blanco LR, Heink S, Prinz I, Hemmer B, Kuchroo VK, Oukka M, Korn T. $\gamma\delta$ T cells enhance autoimmunity by restraining regulatory T cell responses via an interleukin-23-dependent mechanism. *Immunity* 2010; **33**: 351-363 [PMID: 20832339 DOI: 10.1016/j.immuni.2010.08.013]
 - 35 **Malik S**, Want MY, Awasthi A. The Emerging Roles of Gamma-Delta T Cells in Tissue Inflammation in Experimental Autoimmune Encephalomyelitis. *Front Immunol* 2016; **7**: 14 [PMID: 26858718 DOI: 10.3389/fimmu.2016.00014]
 - 36 **Martin B**, Hirota K, Cua DJ, Stockinger B, Veldhoen M. Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. *Immunity* 2009; **31**: 321-330 [PMID: 19682928 DOI: 10.1016/j.immuni.2009.06.020]
 - 37 **Kenna TJ**, Davidson SI, Duan R, Bradbury LA, McFarlane J, Smith M, Weedon H, Street S, Thomas R, Thomas GP, Brown MA. Enrichment of circulating interleukin-17-secreting interleukin-23 receptor-positive $\gamma\delta$ T cells in patients with active ankylosing spondylitis. *Arthritis Rheum* 2012; **64**: 1420-1429 [PMID: 22144400 DOI: 10.1002/art.33507]
 - 38 **Sutton CE**, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* 2009; **31**: 331-341 [PMID: 19682929 DOI: 10.1016/j.immuni.2009.08.001]
 - 39 **Sedda S**, Marafini I, Figliuzzi MM, Pallone F, Monteleone G. An overview of the role of innate lymphoid cells in gut infections and inflammation. *Mediators Inflamm* 2014; **2014**: 235460 [PMID: 25061260 DOI: 10.1155/2014/235460]

P- Reviewer: Tao R, Wakao H **S- Editor:** Ma RY
L- Editor: A **E- Editor:** Song H



Guidelines and controversies in the management of diabetic ketoacidosis – A mini-review

Tasnim Islam, Khalid Sherani, Salim Surani, Abhay Vakil

Tasnim Islam, Khalid Sherani, Salim Surani, Abhay Vakil,
Department of Pulmonary and Critical Care, Corpus Christi
Medical Center, Corpus Christi, TX 78412, United States

ORCID number: Tasnim Islam (0000-0002-8088-172X); Khalid
Sherani (0000-0003-0078-6376); Salim Surani (0000-0001-71
05-4266); Abhay Vakil (0000-0003-4947-0233).

Author contributions: All authors have contributed equally to
the conception, design, literature review, drafting and revision of
this paper.

Conflict-of-interest statement: None of the authors have any
conflict of interest to disclose.

Open-Access: This article is an open-access article which was
selected by an in-house editor and fully peer-reviewed by external
reviewers. It is distributed in accordance with the Creative
Commons Attribution Non Commercial (CC BY-NC 4.0) license,
which permits others to distribute, remix, adapt, build upon this
work non-commercially, and license their derivative works on
different terms, provided the original work is properly cited and
the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Corresponding author to: Tasnim Islam, MD, Doctor,
Resident Physician, Department of Pulmonary and Critical
Care, Corpus Christi Medical Center, 3315 S Alameda Street,
Corpus Christi, TX 78412,
United States. tasnim.islam@live.unthsc.edu
Telephone: +1-361-8857722
Fax: +1-361-8857726

Received: August 6, 2018
Peer-review started: August 7, 2018
First decision: October 5, 2018
Revised: October 16, 2018
Accepted: November 15, 2018
Article in press: November 16, 2018
Published online: December 15, 2018

Abstract

Diabetic ketoacidosis (DKA) is a complication seen in patients with both type 1 and type 2 diabetes. Due to its large, growing economic impact with associated morbidity, closer look at proper management is important. Factors involved in appropriate management involves fluid resuscitation, insulin regimen, and electrolyte replacement including types of fluid and insulin treatment. The caveat with generalized protocol is application to special populations such as renal or heart failure patients the sequelae of complications due to pathophysiology of the disease processes. This leads to complications and longer length of stay in the hospital, therefore, possibly increased cost and resource utilization during the hospitalization. This review takes a closer look at current guidelines of DKA management and resource utilization, the drawbacks of current management protocols and the cost associated with it. Therefore, a need for amendment to existing protocol or initiation of a newer guideline that properly manages DKA should incorporate special populations and appropriate regimen of fluid resuscitation, insulin therapy and electrolyte management.

Key words: Diabetic ketoacidosis management; Fluid resuscitation; Insulin regimen; Electrolyte replacement

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Diabetic ketoacidosis (DKA) management in both type 1 and type 2 has been in practice for many years, yet the complications and cost associated with it is ever increasing. Treatment with proper resource utilization is the key to appropriate management of DKA, decreased complications and length of stay, therefore, decreased cost of treatment. This review aims to review previous guidelines, choice of therapy, cost

associated with it and need for amendments to existing protocols to increase efficacy of DKA treatment, decrease complications and decrease economic burden due to mismanagement of DKA.

Islam T, Sherani K, Surani S, Vakil A. Guidelines and controversies in the management of diabetic ketoacidosis – A mini-review. *World J Diabetes* 2018; 9(12): 226-229
URL: <https://www.wjgnet.com/1948-9358/full/v9/i12/226.htm>
DOI: <https://dx.doi.org/10.4239/wjcd.v9.i12.226>

INTRODUCTION

Diabetic ketoacidosis (DKA) is a metabolic disorder that is common but preventable complication of diabetes often, as a result of insulin deficiency^[1]. The metabolic disorder itself is hyperglycemia with increase in ketones circulating in the body leading to ketoacidosis^[2]. DKA is a significant contributor of mortality and morbidity in type 1 diabetes mellitus (T1DM) patients and most common reason for hospitalization of T1DM patients and therefore contributes significantly to hospital costs. Healthcare costs in events of ketosis are high in patients with T1DM patients, adults and children alike. The number of hospital admissions secondary to DKA have steadily increased worldwide with a decrease in total length of stay^[3]. The in-hospital mortality has decreased, however the cost of hospitalizations has increased significantly^[4]. This literature review focuses to analyze the current practice and guidelines followed for the management of DKA.

SEARCH

MEDLINE (*via* PubMed) and EMBASE databases were searched for articles published between 1 January 2000 and 31 December 2017 (date of search execution) by a single review author. The terms used to search the databases for relevant articles were - "Management of DKA", "Guidelines for DKA" and "Cost/Burden of DKA". Only human studies published in English were included.

TREATMENT STRATEGY FOR DKA

Management of DKA includes optimizing volume status, glucose levels, ketoacidosis, electrolyte abnormalities and precipitating factors. The current protocols for DKA management calls for fluid resuscitation with goal of volume repletion within 24-36 h with 50% of resuscitation fluid administered within first 8-12 h of presentation^[5]. Protocols are in place to optimize DKA management. Prior to protocol implementation, the mean intensive care unit (ICU) unit stays were 44+/-28 h, and hospital lengths of stay were 91+/-73 h. After implementation of protocols, ICU stays have decreased

23% to 34+/-18 h. and mean hospital lengths of stay have decreased 30% to 64+/-41 h of stay^[6].

Current guidelines recommend initiating volume repletion with isotonic saline (0.9% NaCl). Further volume repletion with the type of IV fluid is based on corrected serum sodium. To manage hyperglycemia, current guideline has options for insulin use either *via* intravenous or Subcutaneous/Intramuscular route. The other focus of therapy is to correct electrolytes (particularly sodium, potassium, phosphorus and magnesium) as necessary and avoid over correction. With management of DKA comes management of acid/base level as well. Bicarbonate supplementation is recommended only for pH < 6.9.

CONTROVERSIES

Although the mainstay in DKA management is regular insulin either *via* either IV continuous infusion or frequent subcutaneous or intramuscular injections, the question remains regarding the bolus dose of insulin, ideal route of insulin therapy and the cost associated with it. According to current guidelines of DKA management, an IV insulin bolus dose is recommended followed by continuous infusion. However, in a prospective observational study it was observed that administration of an initial bolus dose of insulin was not associated with significant benefit to DKA patients and was noted to be on similar efficacy and results when compared to patients who were not administered bolus dose of insulin^[7]. On the other hand, for most practicing clinicians and experts, IV regular insulin after initial bolus dose of insulin still remains the preferred route due to delayed onset of action^[8]. Although treating patients with IV insulin causes rapid decline in plasma glucose and ketone levels, the cost of DKA treatment with IV insulin is higher due to management of DKA requiring ICU admission or specialized care unit requiring continuous IV insulin infusion^[9]. In a recent small randomized study including 3 studies in adults and 1 study including pediatric population, it was observed, patients with mild-to-moderate DKA, SC insulin lispro every 1 to 2 h conferred an alternative to continuous IV regular insulin^[10].

EFFICACY OF PROTOCOL DRIVEN TREATMENT

Studies have shown that protocol driven management of DKA is safe and efficient with decreased length of stay^[11]. In a retrospective study, the efficacy of protocol driven management of DKA was studied in teaching hospital in the United States based on 2009 American Diabetes Associations guidelines. Patients undergoing this protocol had resolution of DKA within approximately 10 h^[12]. However, the protocol driven care of DKA differs based on different institutions. For example,

retrospective study in the United Kingdom showed that universal protocol was not adhered to for reasons including patient and clinician factors^[13]. Other studies revealed that low adherence was prevalent as a result of discontinuation of medical care, staffing issues^[14].

SPECIAL POPULATION

Another deficit of following current protocols is the failure to address DKA management in special patient populations such as patient with chronic kidney disease or congestive heart failure or both. For example, in chronic kidney diseases (CKD) patients, osmotic diuresis due to hyperglycemia fails to occur, therefore leading to extracellular volume expansion. If according to current protocol fluid resuscitation is undertaken fatal consequences can pursue. Similarly, potassium replacement is vital in management of DKA. Potassium excretion is often impaired in patients with renal injury or failure. Therefore, potassium supplementation according to current protocol can result in life-threatening hyperkalemia. In addition, insulin is renally excreted, therefore, dose adjustment of insulin is needed in CKD patients^[15]. Application of general DKA protocol to all patient populations can be dangerous to patients leading to complications and longer hospital stays. General DKA protocol in renal or heart failure patients can be detrimental due to over treatment with fluids and exacerbating fluid status and the sequelae associated in such fluid sensitive patient populations. Therefore, increasing costs of for hospitalization and treatment of DKA.

NEW GUIDELINES/RECONSIDERATION

In a review study of efficacy of DKA treatment according to Joint British Diabetes Society protocol, it was revealed that guideline adherence in DKA management is of benefit in the immediate stage of treatment. But inadequate fluid or electrolyte management, inadequate metabolic monitoring, iatrogenic hypoglycemia continues to be area of concern^[16]. This often precludes to avoidable consequences that leads to longer duration of hospital course, cost, both health and economic, associated with mismanagement of DKA for patients. Therefore, it seems appropriate to highlight the need for nursing education on timely administration of fluids, hourly laboratory draws, and administration of insulin.

Due to hyperglycemia in DKA causing osmotic diuresis and severe dehydration, the mainstay of treatment is rehydration. Traditional treatment as mentioned above is "one bag protocol (1 liter/bag)" with normal saline and supplemental electrolytes vs "two bag protocol (1 liter/bag)" that includes two bags of fluids, one containing saline and supplemental electrolytes and another bag containing same solution with additional 10% dextrose. Closure of anion gap was noted to be

earlier (10 h) with "two bag protocol" compared to "one bag protocol" (14 h). Hyperglycemia was also noted to improve faster in "two bag protocol" (7 h) compared to "one bag protocol" (9 h)^[17]. Whether or not this affects length of hospital stay in the long term is difficult to assess but should be explored in future prospective studies. Similarly, prospective studies on efficacy and cost effect on treatment of DKA with subcutaneous insulin vs IV insulin infusion need to be pursued.

CONCLUSION

Given the increasing cost burden on management of diabetes, with large proportion attributed to DKA management and hospitalization, it is appropriate to readdress guidelines for management of DKA. While current protocol for DKA management has been standard use, it is important to address the efficacy of it. Therefore, there is a need for new protocol where treatment with subcutaneous insulin vs IV insulin infusion, "one bag protocol" vs "two bag protocol," and management of DKA in special populations should be addressed.

REFERENCES

1. Seth P, Kaur H, Kaur M. Clinical Profile of Diabetic Ketoacidosis: A Prospective Study in a Tertiary Care Hospital. *J Clin Diagn Res* 2015; **9**: OC01-OC04 [PMID: 26266145 DOI: 10.7860/JCDR/2015/8586.5995]
2. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2017. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Department of Health and Human Services; 2017
3. Maldonado MR, Chong ER, Oehl MA, Balasubramanyam A. Economic impact of diabetic ketoacidosis in a multiethnic indigent population: analysis of costs based on the precipitating cause. *Diabetes Care* 2003; **26**: 1265-1269 [PMID: 12663608 DOI: 10.2337/diacare.26.4.1265]
4. Dhatariya KK, Skedgel C, Fordham R. The cost of treating diabetic ketoacidosis in the UK: a national survey of hospital resource use. *Diabet Med* 2017; **34**: 1361-1366 [PMID: 28727175 DOI: 10.1111/dme.13427]
5. Rosival V. Management of adult diabetic ketoacidosis. *Diabetes Metab Syndr Obes* 2014; **7**: 571-573 [PMID: 25489249 DOI: 10.2147/DMSO.S73896]
6. Bull SV, Douglas IS, Foster M, Albert RK. Mandatory protocol for treating adult patients with diabetic ketoacidosis decreases intensive care unit and hospital lengths of stay: results of a nonrandomized trial. *Crit Care Med* 2007; **35**: 41-46 [PMID: 17095944 DOI: 10.1097/01.CCM.0000249825.18677.D2]
7. Goyal N, Miller JB, Sankey SS, Mossallam U. Utility of initial bolus insulin in the treatment of diabetic ketoacidosis. *J Emerg Med* 2010; **38**: 422-427 [PMID: 18514472 DOI: 10.1016/j.jemermed.2007.11.033]
8. Fisher JN, Shahshahani MN, Kitabchi AE. Diabetic ketoacidosis: low-dose insulin therapy by various routes. *N Engl J Med* 1977; **297**: 238-241 [PMID: 406561 DOI: 10.1056/NEJM197708042970502]
9. Vincent M, Nobécourt E. Treatment of diabetic ketoacidosis with subcutaneous insulin lispro: a review of the current evidence from clinical studies. *Diabetes Metab* 2013; **39**: 299-305 [PMID: 23642642 DOI: 10.1016/j.diabet.2012.12.003]
10. Kearney T, Dang C. Diabetic and endocrine emergencies. *Postgrad Med J* 2007; **83**: 79-86 [PMID: 17308209 DOI: 10.1136/

- pgmj.2006.049445]
- 11 **Waller SL**, Delaney S, Strachan MW. Does an integrated care pathway enhance the management of diabetic ketoacidosis? *Diabet Med* 2007; **24**: 359-363 [PMID: 17298587 DOI: 10.1111/j.1464-5491.2007.02102.x]
 - 12 **Hara JS**, Rahbar AJ, Jeffres MN, Izuora KE. Impact of a hyperglycemic crises protocol. *Endocr Pract* 2013; **19**: 953-962 [PMID: 23807521 DOI: 10.4158/EP13077.OR]
 - 13 **Devalia B**. Adherence to protocol during the acute management of diabetic ketoacidosis: would specialist involvement lead to better outcomes? *Int J Clin Pract* 2010; **64**: 1580-1582 [PMID: 20846206 DOI: 10.1111/j.1742-1241.2010.02348.x]
 - 14 **Agarwal A**, Yadav A, Gutch M, Consul S, Kumar S, Prakash V, Gupta AK, Bhattacharjee A. Prognostic Factors in Patients Hospitalized with Diabetic Ketoacidosis. *Endocrinol Metab* (Seoul) 2016; **31**: 424-432 [PMID: 27586452 DOI: 10.3803/EnM.2016.31.3.424]
 - 15 **Varma R**, Karim M. Lesson of the month 1: Diabetic ketoacidosis in established renal failure. *Clin Med (Lond)* 2016; **16**: 392-393 [PMID: 27481389 DOI: 10.7861/clinmedicine.16-4-392]
 - 16 **Sidana JK**, Phillips C, Sinha A, Levy N. Therapeutic challenges in the management of diabetic ketoacidosis. *J Intensive Care Soc* 2016; **17**: 353-355 [PMID: 28979521 DOI: 10.1177/1751143716638376]
 - 17 **Munir I**, Fargo R, Garrison R, Yang A, Cheng A, Kang I, Motabar A, Xu K, Loo LK, Kim DI. Comparison of a 'two-bag system' versus conventional treatment protocol ('one-bag system') in the management of diabetic ketoacidosis. *BMJ Open Diabetes Res Care* 2017; **5**: e000395 [PMID: 28878933 DOI: 10.1136/bmjdr-2017-000395]

P- Reviewer: Koch TR, Liu SH **S- Editor:** Dou Y
L- Editor: A **E- Editor:** Song H



Effects of glucose-lowering agents on cardiorespiratory fitness

Hidetaka Hamasaki

Hidetaka Hamasaki, Endocrinology and Metabolism, Internal Medicine, Hamasaki Clinic, Kagoshima 890-0046, Japan

ORCID number: Hidetaka Hamasaki (0000-0002-0124-597X).

Author contributions: Hamasaki H wrote the review.

Conflict-of-interest statement: No conflict of interest.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Corresponding author to: Hidetaka Hamasaki, MD, PhD, Doctor, Endocrinology and Metabolism, Internal Medicine, Hamasaki Clinic, 2-21-4 Nishida, Kagoshima 890-0046, Japan. hhamasaki78@gmail.com
Telephone: +81-99-2503535
Fax: +81-99-2501470

Received: August 27, 2018

Peer-review started: August 27, 2018

First decision: October 5, 2018

Revised: October 15, 2018

Accepted: November 26, 2018

Article in press: November 27, 2018

Published online: December 15, 2018

Abstract

Exercise therapy is essential for the management of type 2 diabetes (T2D). However, patients with T2D show lower physical activity and reduced cardiorespiratory fitness than healthy individuals. It would be ideal for

clinicians to co-prescribe glucose-lowering agents that improve cardiorespiratory fitness or exercise capacity in conjunction with exercise therapy. Metformin does not improve cardiorespiratory fitness and may attenuate any beneficial effect of exercise in patients with T2D. In contrast, thiazolidinediones appear to improve cardiorespiratory fitness in patients with T2D. Although evidence is limited, sodium-glucose cotransporter 2 (SGLT2) inhibitors may improve cardiorespiratory fitness in patients with heart failure, and the effect of glucagon-like peptide-1 (GLP-1) receptor agonists on cardiorespiratory fitness is controversial. Recent clinical trials have shown that both SGLT2 inhibitors and GLP-1 receptor agonists exert a favorable effect on cardiovascular disease. It becomes more important to choose drugs that have beneficial effects on the cardiovascular system beyond glucose-lowering effects. Further studies are warranted to determine an ideal glucose-lowering agent combined with exercise therapy for the treatment of T2D.

Key words: Type 2 diabetes; Glucagon-like peptide 1 receptor agonist; Cardiorespiratory fitness; Exercise capacity; Metformin; Thiazolidinedione; Sodium-glucose cotransporter 2 inhibitors

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: What is the most effective combination of drugs and exercise for the treatment of type 2 diabetes? It has become increasingly important for clinicians to prescribe drugs that reduce cardiovascular disease and mortality in addition to their glucose-lowering effects. This review summarized the current literature investigating the effect of glucose-lowering agents on cardiorespiratory fitness. Thiazolidinediones, sodium-glucose cotransporter 2 inhibitors, and glucagon-like peptide-1 receptor agonists have the potential to improve cardiorespiratory fitness; however, further research will be needed to confirm.

Hamasaki H. Effects of glucose-lowering agents on cardiorespiratory fitness. *World J Diabetes* 2018; 9(12): 230-238
 URL: <https://www.wjgnet.com/1948-9358/full/v9/i12/230.htm>
 DOI: <https://dx.doi.org/10.4239/wjd.v9.i12.230>

INTRODUCTION

More than 400 million people worldwide suffer from diabetes. Diabetes can lead to microvascular and macrovascular complications and increase the physical and psychological burden in patients^[1]. Nutrition and exercise therapy are essential for the management of diabetes, and patients with type 1 and 2 diabetes are recommended to engage in regular moderate-to-vigorous intensity aerobic exercise and resistance training^[2]. In addition, higher levels of physical activity are associated with reduced risk of breast cancer (14%), colon cancer (21%), ischemic heart disease (25%), and stroke (26%)^[3]. Exercise is a standard component of chronic disease prevention and management^[4]. However, patients with diabetes typically exhibit lower energy expenditure, physical activity duration^[5], skeletal muscle mass^[6], and cardiorespiratory fitness^[7], and it can be challenging to effectively and safely incorporate exercise therapy in diabetes patients also presenting with vascular complications and comorbidities. Combined diet and exercise therapy is effective against diabetes; however, in more severe cases, drugs are usually required to intensively improve glycemic control. There are currently nine different groups of glucose-lowering agents available: metformin, thiazolidinediones, sulfonylureas, glinides, α -glucosidase inhibitors, dipeptidyl peptidase-4 (DPP-4) inhibitors, sodium-glucose cotransporter 2 (SGLT2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and insulin. Of these, metformin^[8], SGLT2 inhibitors^[9,10], and a GLP-1 receptor agonists^[11] have beneficial effects on cardiovascular disease (CVD) as well as glycemic control, making these the drugs of choice for type 2 diabetes (T2D) treatment^[12].

Exercise is important in the primary and secondary prevention of CVD^[13] and, thus, should be an integral part of the strategy to reduce CVD risk. Individuals with low cardiorespiratory fitness (< 7.9 metabolic equivalent; MET) have a 1.70-fold and 1.56-fold increased risk of all-cause mortality and cardiovascular events, respectively, compared with those with high cardiorespiratory fitness (≥ 10.8 MET)^[14]. Ideally, clinicians should preferably prescribe drugs that improve cardiorespiratory fitness. However, the optimal combination of exercise and glucose-lowering agents remains unclear as the effects of glucose-lowering agents on exercise capacity/cardiorespiratory fitness are not well understood.

This review summarizes the current literature regarding the effects of glucose-lowering agents on cardio-

respiratory fitness in humans and aims to highlight the optimum drug selection in the treatment of patients with diabetes who engage in regular exercise.

METFORMIN AND CARDIORESPIRATORY FITNESS

Metformin is the most widely used oral glucose-lowering drug with known beneficial effects on macrovascular complications in T2D^[15]. While the mechanisms of action of metformin remain unclear, it is known to activate the cellular energy sensor, AMP-activated protein kinase (AMPK), suppress proinflammatory cytokine secretion, inhibit hepatic gluconeogenesis and lipogenesis, and stimulate GLP-1 secretion by modulating the gut microbiota^[16]. Metformin is a complex drug with multiple mechanisms of action. While it is the first-line medication recommended by the American Diabetes Association and the European Association of the Study of Diabetes^[17], clinicians usually also co-prescribe metformin with exercise therapy. It is important to understand whether metformin affects cardiorespiratory fitness/exercise capacity, and the interaction between metformin and exercise has been well studied^[18-25].

Johnson *et al*^[18] examined the acute effects of metformin on maximal oxygen consumption ($\text{VO}_{2\text{max}}$) during exercise. A cycle ergometer was used for graded maximal exercise tests. Participants cycled at 75–80 rpm with a resistance of 2.0 kp, which was increased by 0.5 kp every 3 min until volitional exhaustion. A single dose (1000 mg) of metformin increased mean VO_2 (2.9 ± 0.5 L/min vs 2.8 ± 0.5 L/min) during exercise but not $\text{VO}_{2\text{max}}$ (4.00 ± 0.58 L/min vs 4.00 ± 0.66 L/min). Braun *et al*^[19] investigated the effect of metformin on aerobic capacity in healthy individuals. Peak aerobic capacity ($\text{VO}_{2\text{peak}}$) was measured 7–9 d after administration of either metformin or placebo. An incremental exercise test began using a cycle ergometer at 50–150 W or a treadmill at 6.4–9.6 km/h. The cycle resistance (+25–50 W) and treadmill grade (+2%) were increased every 2 min until exhaustion. The initial dose of metformin was 500 mg/d, which was increased every second day to a maximum of 2000 mg/d. Metformin treatment reduced $\text{VO}_{2\text{peak}}$ (3.53 ± 0.29 L/min vs 3.63 ± 0.9 L/min for metformin and placebo, respectively; -2.7%), and there was no significant association between the decrease in $\text{VO}_{2\text{peak}}$ and baseline cardiorespiratory fitness. Although the effect was physiologically subtle, short-term treatment with metformin had a negative effect on cardiorespiratory fitness. The same authors also examined the effect of metformin on fat oxidation during and after exercise^[20]. Fat oxidation, which was calculated from respiratory gas composition (volume of oxygen consumption (VO_2) and volume of carbon dioxide production (VCO_2), was higher with metformin compared with placebo treatment during exercise but lower during recovery. In contrast, metformin increased carbohydrate

Table 1 Effects of metformin on cardiorespiratory fitness in healthy individuals

Ref.	Study design	Subjects	Metformin dose and intervention	Results
Johnson <i>et al</i> ^[18] , 2008	Randomized, double-blind, placebo-controlled, crossover study	11 healthy and active men Age: 29.9 ± 3.7 yr Sex: All men BMI: 25.2 ± 2.8 kg/m ²	1000 mg/d Cycle ergometer at the mean intensity of 69 ± 5.5% of VO _{2max}	VO _{2max} →, ventilator threshold→, maximal heart rate→, time to fatigue→ Lactate↓, blood glucose concentrations↓
Braun <i>et al</i> ^[19] , 2008	Non-randomized, placebo-controlled study	18 healthy subjects Age: 27.9 ± 3.3 yr Sex: 11 men and 7 women BMI: 24.1 ± 3.6 kg/m ²	2000 mg/d Treadmill or cycle ergometer	VO _{2peak} ↓, peak heart rate↓, peak ventilation↓, peak respiratory exchange ratio↓, exercise duration↓ Rating of perceived exertion→
Malin <i>et al</i> ^[20] , 2010	Non-randomized, double-blind, counterbalanced crossover study	15 healthy and active subjects Age: 25 ± 4.4 yr Sex: 7 men and 8 women BMI: 22.8 ± 2.7 kg/m ²	2000 mg/d Cycle exercise at 5 submaximal cycle workloads	VO ₂ → During exercise: Fat oxidation↑ Postexercise: Fat oxidation↓
Learsi <i>et al</i> ^[21] , 2015	Randomized, placebo-controlled, counterbalanced study	10 healthy men Age: 23.5 ± 3.6 yr Sex: All men BMI: No description (height: 170.4 ± 4.8 cm, weight: 66.4 ± 6.5 kg)	500 mg/d Cycle ergometer: An incremental test, 6 submaximal workload test at 40%–90% VO _{2max} , 2 supramaximal tests at 110% VO _{2max}	VO ₂ →, maximal accumulated oxygen deficit→, lactate concentrations→ Time to exhaustion↑, VO ₂ recovery↑

BMI: Body mass index; VO₂: Oxygen consumption.

oxidation after exercise. Oxygen consumption was not different at rest or during exercise with metformin. Therefore, metformin may increase the rate of fat oxidation during exercise *via* activation of AMPK, but appears to have no effect on cardiorespiratory fitness. Learsi *et al*^[21] examined the effect of metformin on high-intensity, short-duration exercise on anaerobic capacity. Exercise tests comprised a maximal incremental test to evaluate VO_{2 max}, six workload tests with submaximal intensities (40%–90% of maximal power output), and two supramaximal intensity tests (110% of maximal power output). Participants took low-dose metformin (500 mg) or placebo prior to the supramaximal test. Time to exhaustion was improved with metformin (191 ± 33 s vs 167 ± 32 s for metformin and placebo, respectively), but VO₂ during the supramaximal test was not different between the groups. Maximum O₂ deficit and lactate concentrations did not differ between the groups. The authors concluded that metformin improves exercise performance by mediating the alactic anaerobic system. Table 1 summarizes the effects of metformin on cardiorespiratory fitness in healthy individuals. However, what is known about the interaction between metformin and cardiorespiratory fitness in patients with T2D or insulin resistance? A noteworthy study by Boulé *et al*^[22] investigated the interaction between metformin and exercise on the hormonal response to a standardized meal. The authors studied 10 patients with mild T2D who took metformin or placebo for 28 d, and measured exercise capacity, glucose, lactate, non-esterified fatty acids, insulin, and glucagon levels on the last two days. Resistance and aerobic exercise tests were conducted

using an isokinetic dynamometer and treadmill. After performing resistance exercise (leg extensions and flexions), the patients started three bouts of aerobic exercise comprising walking at 3.5 km/h with 0% gradient for 15 min, then increasing the speed and gradient until just below the ventilatory threshold, followed by walking at an intensity above the ventilator threshold for 5 min. The mean respiratory exchange ratio (0.96 ± 0.02 vs 0.98 ± 0.02) was lower, and the mean heart rate (124 ± 9 vs 118 ± 8 beats per min) was higher in the metformin group. Mean VO₂ was not affected. As expected, metformin improved glycemic response but glycemic response was attenuated in combination with exercise. In addition, glucagon levels were highest in the metformin plus exercise group. It is surprising that exercise has an opposing effect on the glucose-lowering effect of metformin. High-intensity exercise increases insulin counterregulatory hormones, such as epinephrine, norepinephrine, cortisol, and growth hormone, as well as glucagon, which may further deteriorate glucose response in T2D. Boulé *et al*^[23] also investigated the long-term effects of metformin on glycemic control and physical fitness in participants in the Diabetes Aerobic and Resistance Exercise trial^[26]. Subjects were randomly assigned to four groups, namely, aerobic exercise, resistance training, combined aerobic exercise and resistance training, and control. The exercise group performed progressive aerobic exercise, increasing to an intensity of 75% of maximum heart rate for 45 min. Resistance training included seven exercises: abdominal crunches, seated row, seated biceps curls, supine bench presses, leg presses,

Table 2 Effects of metformin on cardiorespiratory fitness in patients with type 2 diabetes and metabolic syndrome

Ref.	Study design	Subjects	Metformin dose and intervention	Results
Boulé <i>et al</i> ^[22] , 2011	Randomized, placebo-controlled, crossover study	10 patients with type 2 diabetes Age: 58 ± 6 yr Sex: 8 men and 2 women BMI: 28.6 ± 5.3 kg/m ² HbA1c: 6.5 ± 0.6%	2000 mg/d Exercise mode: Treadmill at three different submaximal intensities Study duration: 22 wk	VO ₂ →, respiratory exchange ratio↓ Heart rate↑, lactate↑, rating of perceived exertion↑
Boulé <i>et al</i> ^[23] , 2013	Randomized controlled trial (Diabetes Aerobic and Resistance Exercise) trial	251 patients with type 2 diabetes (143 patients treated with metformin and 82 patients treated without metformin) Age: 54.9 ± 7.1 yr vs 53.1 ± 6.9 yr Sex (Men/Women): 100/43 vs 46/36 BMI: 33.3 ± 5.5 kg/m ² vs 33.3 ± 6.4 kg/m ² HbA1c: 7.78 ± 0.9% vs 7.47 ± 0.77%	Approximately 1600 mg/d Exercise mode: Aerobic training, resistance training, and combined aerobic and resistance training Study duration: 4 wk	VO _{2peak} →
Cadeddu <i>et al</i> ^[25] , 2014	Non-randomized, non-controlled trial	75 patients with insulin resistance Age: 46.2 ± 11 yr Sex: 35 men and 40 women BMI: 29.8 ± 4.1 kg/m ²	1000 mg/d 30–50 min of cycle exercise at the intensity of 60%–80% of the heart rate reserve Study duration: 12 wk	VO _{2peak} →
Paul <i>et al</i> ^[26] , 2017	Prospective observational study	15 patients with metabolic syndrome Age: No description Sex: No description BMI: No description (weight: 75.4 ± 12.08 kg)	1000 mg/d No intervention Study duration: 6 wk	VO _{2max} ↓

BMI: Body mass index; HbA1c: Hemoglobin A1c; VO₂: Oxygen consumption.

shoulder presses, and leg extensions. VO_{2peak} increased in the aerobic group by 0.16 L/min and in the combined exercise group by 0.11 L/min without metformin. However, VO_{2peak} did not change in any of the metformin groups. In the aerobic exercise group, HbA1c levels were reduced with metformin. In the combined exercise group, fasting glucose levels decreased with metformin. There were no significant differences in changes in HbA1c and glucose levels with or without metformin. The study concluded that metformin did not impair physical fitness or glycemic control when combined with exercise. The findings of this study are inconsistent with previous short-term studies that have shown that the addition of exercise to metformin showed a negative effect on cardiorespiratory fitness and glycemia. The authors speculated that difference in the characteristics of the study participants, such as duration of metformin treatment and glycemic control at baseline, may explain this discrepancy.

Two clinical studies have investigated metformin and cardiorespiratory fitness in individuals with insulin resistance and metabolic syndrome. Cadeddu *et al*^[25] investigated the effect of metformin, exercise alone, or a combination of metformin and exercise on exercise capacity. Study participants had impaired glucose tolerance and/or impaired fasting glucose and were allocated to one of the three groups. The exercise program comprised 30–50 min cycle ergometry with an intensity of 60%–80% of heart rate reserve based on the age of the subjects. After a 12-wk intervention, the exercise only group had improved VO_{2peak}, whereas

the metformin plus exercise therapy group did not. Moreover, metformin plus exercise therapy did not show an improved aerobic threshold compared with the exercise along group. The combination of metformin and exercise was not superior to exercise alone with regard to cardiorespiratory fitness. A recent study in India showed a negative effect of metformin on exercise capacity in patients with newly diagnosed metabolic syndrome^[25]. This study was a simple observational study to evaluate changes in VO₂, ventilatory anaerobic threshold, and other indicators of cardiorespiratory fitness in response to metformin treatment for 6 wk, and showed that VO_{2max} decreased from 1.10 ± 0.44 to 0.9 ± 0.39 L/min and ventilatory anaerobic threshold decreased by 1.5 mL/min per kilogram. However, these studies were non-randomized, non-controlled observational studies, and thus, the study design was suboptimal (Table 2).

Metformin improves energy metabolism in skeletal muscle and has a cardioprotective effect *via* AMPK activation^[27]. Metformin also inhibits mitochondrial respiratory-chain complex 1 and decreases ATP production^[27], which could potentially reduce oxygen consumption during exercise. In addition, metformin increases lactate concentrations and reduces the lactate threshold during exercise^[28]; however, lactate accumulation may have a protective effect on skeletal muscle rather than cause fatigue^[29]. Previous studies have suggested that the effect of metformin on cardiorespiratory fitness is clinically subtle. However, treatment with metformin does not appear to have a synergetic effect on cardiorespiratory

fitness in combination with exercise therapy.

THIAZOLIDINEDIONES AND CARDIORESPIRATORY FITNESS

The mechanism of action of thiazolidinediones is mediated by peroxisome proliferator-activated receptors (PPARs)^[30]. Thiazolidinediones exert an insulin-sensitizing effect by promoting fatty acid uptake and modulation of secretion of adipokines, such as interleukin-6, tumor necrosis factor- α , and adiponectin^[31]. PPAR- γ overactivation by thiazolidinediones increases body weight *via* fluid retention^[30] and stimulatory effect on adipogenesis and adipose tissue accumulation^[32]; thus, thiazolidinediones may be associated with increased cardiovascular risk in some patients. However, these drugs appear to improve cardiorespiratory fitness in patients with T2D.

In 2005, a randomized, double-blind, placebo-controlled study reported that rosiglitazone, a thiazolidinedione, improved exercise capacity *via* improvement in endothelial function in patients with T2D^[33]. Twenty patients were divided into rosiglitazone (4 mg/d) and placebo groups. After a 4-mo intervention, $\text{VO}_{2\text{ max}}$ increased from 1902 ± 603 mL/min (19.8 ± 5.3 mL/kg per minute) to 2074 ± 585 mL/min (21.2 ± 5.1 mL/kg per minute) in rosiglitazone-treated patients, but showed no improvement in controls. In addition, the change in $\text{VO}_{2\text{ max}}$ negatively correlated with changes in fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR) was positively correlated with insulin sensitivity, as measured by hyperinsulinemic-euglycemic clamp. Thiazolidinediones may improve $\text{VO}_{2\text{ max}}$ *via* multiple mechanisms. First, thiazolidinediones enhance gene transcription that promotes adipocyte differentiation and increases fatty acid transport, synthesis, and storage in the adipose tissue by binding to PPAR γ . This reduces ectopic fat accumulation in muscle and liver, and improves both cellular lipotoxicity and insulin sensitivity. Second, thiazolidinediones may also activate AMPK, which leads to increased fat oxidation and PPAR γ coactivator 1 α expression, regulating mitochondrial biogenesis^[34]. Mitochondrial dysfunction in patients with T2D is attenuated by thiazolidinediones^[35], which may result in an improvement in cardiorespiratory fitness.

Another randomized controlled study investigating the effect of rosiglitazone on cardiorespiratory fitness in patients with T2D was conducted in Greece^[36]. Seventy patients (28 men and 42 women) with T2D were randomly assigned to a rosiglitazone (8 mg/d) treatment group or a control group. Rosiglitazone treatment for 6 mo increased $\text{VO}_{2\text{ peak}}$ from 24.47 ± 3.98 to 26.39 ± 4.04 mL/kg per minute. Changes in adiponectin, HOMA-IR, and HbA1c levels were independent predictors of incremental increase in $\text{VO}_{2\text{ peak}}$. Rosiglitazone, a PPAR γ activator, may improve cardiorespiratory fitness *via*

upregulation of adiponectin. Recently, Yokota *et al*^[37] showed that pioglitazone improves cardiorespiratory fitness in Japanese patients with metabolic syndrome. Fourteen male patients with metabolic syndrome received 15 mg/d of pioglitazone for four months. Pioglitazone increased $\text{VO}_{2\text{ peak}}$ from 25.1 ± 4.9 to 27.2 ± 3.9 mL/kg per minute, and the anaerobic threshold from 12.7 ± 1.9 to 13.6 ± 0.6 mL/kg per minute. Pioglitazone also decreased the intramyocellular lipid content in resting calf muscle by 26%, with no concurrent change in the cross-sectional area of the muscle. There was an inverse correlation between the increase in anaerobic threshold and the decrease in intramyocellular lipid content. These data suggest that pioglitazone improves cardiorespiratory fitness *via* skeletal muscle fatty acid metabolism. In addition, pioglitazone decreased muscle phosphocreatinine loss during exercise, suggesting that altered mitochondrial function contributes to the improvement in skeletal muscle energy metabolism. Taken together, these studies indicate that thiazolidinediones have a beneficial effect on cardiorespiratory fitness in patients with T2D and metabolic syndrome (Table 3).

INCRETIN-RELATED DRUGS AND CARDIORESPIRATORY FITNESS

GLP-1 is secreted by the intestine and has multiple physiological effects, including brain neuroprotection, suppressing appetite, cardiovascular protection, improving cardiac function, slowing gastric emptying, decreasing glucose production in the liver, increasing glucose uptake in adipose tissue and skeletal muscle, stimulating insulin secretion, suppressing glucagon secretion, promoting pancreatic β -cell proliferation, and inhibiting pancreatic β -cell apoptosis^[38]. Secretion and function of GLP-1 is severely diminished in patients with T2D, and GLP-1 receptor agonists effectively improve diabetes and obesity *via* pleiotropic effects. Additionally, there could be an interaction between exercise and GLP-1 in patients with T2D^[39]. The effect of GLP-1 receptor agonists on exercise capacity/cardiorespiratory fitness remains controversial. Lepore *et al*^[40] investigated whether albiglutide, a long-acting GLP-1 receptor agonist, improved cardiac function and exercise performance in patients with chronic heart failure. Eighty-one patients participated in this multicenter, randomized, placebo-controlled study, and received either 30 mg of albiglutide or placebo for 12 wk. The albiglutide group showed improved $\text{VO}_{2\text{ peak}}$ (from 16.2 ± 0.9 to 17.1 ± 1 mL/kg per minute), an increase of 1.5 mL/min per kilogram compared with the placebo group. However, no significant improvement in cardiac function, 6-min walk test, myocardial glucose, and oxygen use was observed. The authors stated that the improvement in cardiorespiratory fitness may have been mediated by a physiological effect rather

Table 3 Effects of thiazolidinediones on cardiorespiratory fitness in patients with type 2 diabetes and metabolic syndrome

Ref.	Study design	Subjects	Thiazolidinedione dose	Results
Regensteiner <i>et al</i> ^[33] , 2005	Randomized controlled trial	20 patients with type 2 diabetes (10 patients received rosiglitazone and 10 patients received a placebo) Age: 55 ± 7 yr vs 56 ± 1 yr Sex (Men/Women): 5/5 vs 5/5 BMI: 32.2 ± 5.6 kg/m ² vs 30.4 ± 5.8 kg/m ² HbA1c: 7.2 ± 1.1% vs 7.2 ± 1.0%	Rosiglitazone, 4 mg/d	VO ₂ ↑, insulin sensitivity↑, endothelial function↑
Kadoglou <i>et al</i> ^[36] , 2008	Randomized controlled trial	70 patients with type 2 diabetes (35 patients received rosiglitazone and 35 patients received a placebo) Age: 63.8 ± 7.3 yr vs 66.7 ± 9.6 yr Sex (Men/Women): 14/21 vs 16/19 BMI: 29.5 ± 3.8 kg/m ² vs 29.9 ± 4.3 kg/m ² HbA1c: 8.2 ± 1.2% vs 8 ± 0.8%	Rosiglitazone, 8 mg/d	VO _{2peak} ↑, duration of the exercise test↑, oxygen pulse↑ Insulin resistance↓, diastolic blood pressure↓
Yokota <i>et al</i> ^[37] , 2017	Before-after study	14 patients with metabolic syndrome Age: 52 ± 11 yr Sex: All men BMI: 26.6 ± 3.3 kg/m ² HbA1c: 5.7 ± 0.6%	Pioglitazone, 15 mg/d	VO _{2peak} ↑, anaerobic threshold↑ Intramyocellular lipid content↓, muscle phosphocreatinine loss during exercise↓

BMI: Body mass index; HbA1c: Hemoglobin A1c; VO₂: Oxygen consumption.

than cardiac function due to the administration of albiglutide. Scalzo *et al*^[41] investigated the effect of exenatide on functional exercise capacity in patients with T2D after 3-mo treatment of 10 µg twice-daily exenatide. Exenatide did not improve VO_{2peak} or endothelial function, but diastolic cardiac function and arterial stiffness improved.

The controversial results from these studies may be attributed to patient characteristics. One study was conducted using patients with chronic heart failure (without diabetes) and the other used patients with mild T2D (without heart failure). Although the underlying mechanisms are unknown, the baseline cardiac function may have influenced the change in cardiorespiratory fitness due to the GLP-1 receptor agonist treatment.

A randomized, placebo-controlled, double-blind, parallel group, phase IV trial which aims at examining the effect of liraglutide on physical performance in patients with T2D is currently underway^[42], with promising results.

To the best of our knowledge, to date, no human studies have reported the effect of DPP-4 inhibitors on exercise capacity/cardiorespiratory fitness. However, one animal study suggested that exercise capacity and mitochondrial biogenesis in skeletal muscle are improved by the administration of a DPP-4 inhibitor in mice with heart failure^[43]. DPP-4 inhibitors may also have the potential to improve exercise capacity/cardiorespiratory fitness in humans.

SGLT2 INHIBITORS AND CARDIORESPIRATORY FITNESS

SGLT2 inhibitors decrease glucose reabsorption at the proximal renal tubules, which increases urinary glucose excretion and improves glycemic control. SGLT2

inhibitors also exert various metabolic effects, including weight loss, insulin sensitivity improvement, blood pressure lowering, renal hemodynamic modulation, and reduction in albuminuria, which leads to cardiovascular and renal protection^[44]. Treatment using empagliflozin resulted in a 35% risk reduction in hospitalization for heart failure compared with placebo^[9], suggesting that SGLT2 inhibitors also have an effect on cardiorespiratory fitness in patients with T2D.

To date, two pilot studies have investigated whether empagliflozin improves cardiorespiratory fitness in patients with T2D with heart failure. Núñez *et al*^[45] showed that short-term (4 wk) empagliflozin treatment increased VO_{2peak} by 1.21 mL/kg per minute (11.1%) from baseline. Conversely, Carbone *et al*^[46] showed that empagliflozin treatment for 4 wk did not significantly improve VO_{2peak} (14.5 mL/kg vs 15.8 mL/kg per minute). Intriguingly, patients concomitantly treated with loop diuretics demonstrated improved VO_{2peak} (+0.9 mL/kg per minute), whereas those without loop diuretics demonstrated a decrease in VO_{2peak} (−0.9 mL/kg per minute). Indeed, all patients in the study by Núñez *et al*^[45] received loop diuretics. The authors hypothesized that empagliflozin acts on the proximal renal tubules by interacting with sodium/hydrogen exchangers, thereby increasing sodium delivery at the distal renal tubules and enhancing the effect of loop diuretics^[47,48]. Carbone *et al*^[46] also speculated that empagliflozin improves cardiorespiratory fitness in patients concomitantly treated with loop diuretics by reducing the activity of the rennin-angiotensin-aldosterone system. Empagliflozin may exert cardiovascular and renal benefits *via* changes in myocardial and renal energy metabolism. Empagliflozin increases ketone oxidation instead of fat and glucose oxidation, which can improve cardiac and renal work efficiency^[49]. Taken together, these studies suggest that SGLT2 inhibitors improve cardiorespiratory

Table 4 Effects of sodium–glucose cotransporter 2 inhibitors on cardiorespiratory fitness in patients with type 2 diabetes

Ref.	Study design	Subjects	SGLT2 inhibitors dose	Results
Núñez <i>et al</i> ^[45] , 2017	Before-after study	19 patients with type 2 diabetes and heart failure Age (median): 72 yr Sex: 14 men and 5 women BMI: 30.6 ± 5.5 kg/m ² HbA1c: No description	Empagliflozin, 10 mg/d	VO _{2peak} ↑, ventilatory efficiency during exercise↑, 6-minute walking distance↑, ↓ antigen carbohydrate 125
Carbone <i>et al</i> ^[46] , 2018	Before-after study	15 patients with type 2 diabetes and heart failure Age (median): 60 yr Sex: 7 men and 8 women BMI (median): 34 kg/m ² HbA1c (median): 7.8%	Empagliflozin, 10 mg/d	VO _{2peak} ↑ in patients using loop diuretics VO _{2peak} ↓ in patients without loop diuretics

SGLT2: Sodium–glucose cotransporter 2; BMI: Body mass index; HbA1c: Hemoglobin A1c; VO₂: Oxygen consumption.

Table 5 Effect of glucose-lowering agents on cardiorespiratory fitness

Glucose-lowering agents	Cardiorespiratory fitness
Metformin	→ or ↓
Thiazolidinediones	↑
DPP-4 inhibitors	Unknown (↑ in mice with heart failure)
GLP-1 receptor agonists	↑ in patients with heart failure → in patients with type 2 diabetes
SGLT2 inhibitors	↑ in patients treated with loop diuretics

DPP-4: Dipeptidyl peptidase-4; GLP-1: Glucagon-like peptide-1; SGLT2: Sodium–glucose cotransporter 2.

fitness in patients with T2D with heart failure (Table 4).

CONCLUSION

Metformin does not improve cardiorespiratory fitness and may attenuate a beneficial effect of exercise on cardiorespiratory fitness in patients with T2D. In contrast, thiazolidinediones appear to improve cardiorespiratory fitness in patients with T2D. The effect of GLP-1 receptor agonists on cardiorespiratory fitness remains controversial and is not fully understood. Notably, SGLT2 inhibitors may improve cardiorespiratory fitness in patients with heart failure by modulating cardiac energy metabolism or *via* a synergetic effect with loop diuretics. Unfortunately, no human studies have examined the effect of DPP-4 inhibitors, sulfonylureas, glinides, or α -glucosidase inhibitors on cardiorespiratory fitness (Table 5). This review cannot recommend the optimal combination of exercise and glucose-lowering agents with regard to cardiorespiratory fitness in patients with T2D; however, thiazolidinediones, GLP-1 receptor agonists, and SGLT2 inhibitors have the potential to improve both glycemic control and cardiorespiratory fitness without interfering with exercise therapy. Further studies are warranted to demonstrate the clinical benefits of glucose-lowering agents for cardiorespiratory fitness, and to elucidate the underlying mechanisms of action.

REFERENCES

- Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. *Lancet* 2017; **389**: 2239–2251 [PMID: 28190580 DOI: 10.1016/S0140-6736(17)30058-2]
- American Diabetes Association. 4. Lifestyle Management: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018; **41**: S38–S50 [PMID: 29222375 DOI: 10.2337/dc18-S004]
- Kyu HH, Bachman VF, Alexander LT, Mumford JE, Afshin A, Estep K, Veerman JL, Delwiche K, Iannarone ML, Moyer ML, Cercy K, Vos T, Murray CJ, Forouzanfar MH. Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: systematic review and dose-response meta-analysis for the Global Burden of Disease Study 2013. *BMJ* 2016; **354**: i3857 [PMID: 27510511 DOI: 10.1136/bmj.i3857]
- American College of Sports Medicine. Exercise is Medicine®: A Global Health Initiative. Available from: URL: <http://www.exerciseismedicine.org/>
- Fagour C, Gonzalez C, Pezzino S, Florenty S, Rosette-Narece M, Gin H, Rigalleau V. Low physical activity in patients with type 2 diabetes: the role of obesity. *Diabetes Metab* 2013; **39**: 85–87 [PMID: 23159129 DOI: 10.1016/j.diabet.2012.09.003]
- Park SW, Goodpaster BH, Lee JS, Kuller LH, Boudreau R, de Rekeneire N, Harris TB, Kritchevsky S, Tyllavsky FA, Nevitt M, Cho YW, Newman AB; Health, Aging, and Body Composition Study. Excessive loss of skeletal muscle mass in older adults with type 2 diabetes. *Diabetes Care* 2009; **32**: 1993–1997 [PMID: 19549734 DOI: 10.2337/dc09-0264]
- Ozdirenç M, Biberoğlu S, Özcan A. Evaluation of physical fitness in patients with Type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2003; **60**: 171–176 [PMID: 12757989 DOI: 10.1016/S0168-8227(03)00064-0]
- Anabtawi A, Miles JM. Metformin: nonglycemic effects and potential novel indications. *Endocr Pract* 2016; **22**: 999–1007 [PMID: 27579542 DOI: 10.4158/EP151145.RA]
- Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 2117–2128 [PMID: 26378978 DOI: 10.1056/NEJMoa1504720]
- Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erond N, Shaw W, Law G, Desai M, Matthews DR; CANVAS Program Collaborative Group. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N Engl J Med* 2017; **377**: 644–657 [PMID: 28605608 DOI: 10.1056/NEJMoa1611925]
- Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, Nissen SE, Pocock S, Poulter NR, Ravn LS, Steinberg WM, Stockner M, Zinman B, Bergenstal RM, Buse JB; LEADER Steering Committee; LEADER Trial Investigators. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2016; **375**: 311–322 [PMID: 27295427 DOI: 10.1056/

- NEJMoA1603827]
- 12 **American Diabetes Association.** 8. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018; **41**: S73-S85 [PMID: 29222379 DOI: 10.2337/dc18-S008]
- 13 **Alves AJ,** Viana JL, Cavalcante SL, Oliveira NL, Duarte JA, Mota J, Oliveira J, Ribeiro F. Physical activity in primary and secondary prevention of cardiovascular disease: Overview updated. *World J Cardiol* 2016; **8**: 575-583 [PMID: 27847558 DOI: 10.4330/wjc.v8.i10.575]
- 14 **Kodama S,** Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, Sugawara A, Totsuka K, Shimano H, Ohashi Y, Yamada N, Sone H. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA* 2009; **301**: 2024-2035 [PMID: 19454641 DOI: 10.1001/jama.2009.681]
- 15 Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 854-865 [PMID: 9742977 DOI: 10.1016/S0140-6736(98)07037-8]
- 16 **Rena G,** Hardie DG, Pearson ER. The mechanisms of action of metformin. *Diabetologia* 2017; **60**: 1577-1585 [PMID: 28776086 DOI: 10.1007/s00125-017-4342-z]
- 17 **Inzucchi SE,** Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR; American Diabetes Association (ADA); European Association for the Study of Diabetes (EASD). Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2012; **35**: 1364-1379 [PMID: 22517736 DOI: 10.2337/dc12-0413]
- 18 **Johnson ST,** Robert C, Bell GJ, Bell RC, Lewanczuk RZ, Boulé NG. Acute effect of metformin on exercise capacity in active males. *Diabetes Obes Metab* 2008; **10**: 747-754 [PMID: 17970761 DOI: 10.1111/j.1463-1326.2007.00805.x]
- 19 **Braun B,** Eze P, Stephens BR, Hagobian TA, Sharoff CG, Chipkin SR, Goldstein B. Impact of metformin on peak aerobic capacity. *Appl Physiol Nutr Metab* 2008; **33**: 61-67 [PMID: 18347654 DOI: 10.1139/H07-144]
- 20 **Malin SK,** Stephens BR, Sharoff CG, Hagobian TA, Chipkin SR, Braun B. Metformin's effect on exercise and postexercise substrate oxidation. *Int J Sport Nutr Exerc Metab* 2010; **20**: 63-71 [PMID: 20190353 DOI: 10.1123/ijsem.20.1.63]
- 21 **Learsi SK,** Bastos-Silva VJ, Lima-Silva AE, Bertuzzi R, De Araujo GG. Metformin improves performance in high-intensity exercise, but not anaerobic capacity in healthy male subjects. *Clin Exp Pharmacol Physiol* 2015; **42**: 1025-1029 [PMID: 26250859 DOI: 10.1111/1440-1681.12474]
- 22 **Boulé NG,** Robert C, Bell GJ, Johnson ST, Bell RC, Lewanczuk RZ, Gabr RQ, Brocks DR. Metformin and exercise in type 2 diabetes: examining treatment modality interactions. *Diabetes Care* 2011; **34**: 1469-1474 [PMID: 21602430 DOI: 10.2337/dc10-2207]
- 23 **Boulé NG,** Kenny GP, Larose J, Khandwala F, Kuzik N, Sigal RJ. Does metformin modify the effect on glycaemic control of aerobic exercise, resistance exercise or both? *Diabetologia* 2013; **56**: 2378-2382 [PMID: 23975325 DOI: 10.1007/s00125-013-3026-6]
- 24 **Sigal RJ,** Kenny GP, Boulé NG, Wells GA, Prud'homme D, Fortier M, Reid RD, Tulloch H, Coyle D, Phillips P, Jennings A, Jaffey J. Effects of aerobic training, resistance training, or both on glycemic control in type 2 diabetes: a randomized trial. *Ann Intern Med* 2007; **147**: 357-369 [PMID: 17876019 DOI: 10.7326/0003-4819-147-6-200709180-00005]
- 25 **Cadeddu C,** Nocco S, Cugusi L, Deidda M, Bina A, Fabio O, Bandinu S, Cossu E, Baroni MG, Mercurio G. Effects of metformin and exercise training, alone or in association, on cardio-pulmonary performance and quality of life in insulin resistance patients. *Cardiovasc Diabetol* 2014; **13**: 93 [PMID: 24884495 DOI: 10.1186/1475-2840-13-93]
- 26 **Paul AA,** Dkhar SA, Kamalanathan S, Thabab MM, George M, Chandrasekaran I, Gunaseelan V, Selvarajan S. Effect of metformin on exercise capacity in metabolic syndrome. *Diabetes Metab Syndr* 2017; **11** Suppl 1: S403-S406 [PMID: 28283393 DOI: 10.1016/j.dsx.2017.03.025]
- 27 **Foretz M,** Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: from mechanisms of action to therapies. *Cell Metab* 2014; **20**: 953-966 [PMID: 25456737 DOI: 10.1016/j.cmet.2014.09.018]
- 28 **DeFronzo R,** Fleming GA, Chen K, Bicsak TA. Metformin-associated lactic acidosis: Current perspectives on causes and risk. *Metabolism* 2016; **65**: 20-29 [PMID: 26773926 DOI: 10.1016/j.metabol.2015.10.014]
- 29 **Lindinger MI.** Combating muscle fatigue: extracellular lactic acidosis and catecholamines. *J Physiol* 2007; **581**: 419 [PMID: 17379626 DOI: 10.1113/jphysiol.2007.132209]
- 30 **Nanjan MJ,** Mohammed M, Prashantha Kumar BR, Chandrasekar MJN. Thiazolidinediones as antidiabetic agents: A critical review. *Bioorg Chem* 2018; **77**: 548-567 [PMID: 29475164 DOI: 10.1016/j.bioorg.2018.02.009]
- 31 **Lehrke M,** Lazar MA. The many faces of PPARgamma. *Cell* 2005; **123**: 993-999 [PMID: 16360030 DOI: 10.1016/j.cell.2005.11.026]
- 32 **Hauner H.** The mode of action of thiazolidinediones. *Diabetes Metab Res Rev* 2002; **18** Suppl 2: S10-S15 [PMID: 11921433 DOI: 10.1002/dmrr.249]
- 33 **Regensteiner JG,** Bauer TA, Reusch JE. Rosiglitazone improves exercise capacity in individuals with type 2 diabetes. *Diabetes Care* 2005; **28**: 2877-2883 [PMID: 16306548 DOI: 10.2337/diacare.28.12.2877]
- 34 **LeBrasseur NK,** Ruderman NB. Why might thiazolidinediones increase exercise capacity in patients with type 2 diabetes? *Diabetes Care* 2005; **28**: 2975-2977 [PMID: 16306565 DOI: 10.2337/diacare.28.12.2975]
- 35 **Colca JR,** McDonald WG, Kletzien RF. Mitochondrial target of thiazolidinediones. *Diabetes Obes Metab* 2014; **16**: 1048-1054 [PMID: 24774061 DOI: 10.1111/dom.12308]
- 36 **Kadoglou NP,** Iliadis F, Angelopoulou N, Perrea D, Liapis CD, Alevizos M. Beneficial effects of rosiglitazone on novel cardiovascular risk factors in patients with Type 2 diabetes mellitus. *Diabet Med* 2008; **25**: 333-340 [PMID: 18307460 DOI: 10.1111/j.1464-5491.2007.02375.x]
- 37 **Yokota T,** Kinugawa S, Hirabayashi K, Suga T, Takada S, Omokawa M, Kadoguchi T, Takahashi M, Fukushima A, Matsushima S, Yamato M, Okita K, Tsutsui H. Pioglitazone improves whole-body aerobic capacity and skeletal muscle energy metabolism in patients with metabolic syndrome. *J Diabetes Investig* 2017; **8**: 535-541 [PMID: 27930876 DOI: 10.1111/jdi.12606]
- 38 **Gallwitz B.** Glucagon-like peptide-1 analogues for Type 2 diabetes mellitus: current and emerging agents. *Drugs* 2011; **71**: 1675-1688 [PMID: 21902291 DOI: 10.2165/11592810-000000000-00000]
- 39 **Hamasaki H.** Exercise and glucagon-like peptide-1: Does exercise potentiate the effect of treatment? *World J Diabetes* 2018; **9**: 138-140 [PMID: 30147850 DOI: 10.4239/wjd.v9.i8.138]
- 40 **Lepore JJ,** Olson E, Demopoulos L, Haws T, Fang Z, Barbour AM, Fossler M, Davila-Roman VG, Russell SD, Gropler RJ. Effects of the Novel Long-Acting GLP-1 Agonist, Albiglutide, on Cardiac Function, Cardiac Metabolism, and Exercise Capacity in Patients With Chronic Heart Failure and Reduced Ejection Fraction. *JACC Heart Fail* 2016; **4**: 559-566 [PMID: 27039125 DOI: 10.1016/j.jchf.2016.01.008]
- 41 **Scalzo RL,** Moreau KL, Ozemek C, Herlache L, McMillin S, Gilligan S, Huebschmann AG, Bauer TA, Dorosz J, Reusch JE, Regensteiner JG. Exenatide improves diastolic function and attenuates arterial stiffness but does not alter exercise capacity in individuals with type 2 diabetes. *J Diabetes Complications* 2017; **31**: 449-455 [PMID: 27884660 DOI: 10.1016/j.jdiacomp.2016.10.003]
- 42 **Wagner AM,** Miranda-Calderín G, Ugarte-Lopetegui MA, Marrero-Santiago H, Suárez-Castellano L, Alberiche-Ruano MD, Castillo-García N, López-Madrado MJ, Alemán C, Martínez-Mancebo C, López-Ríos L, Díez Del Pino A, Nóvoa-Mogollón FJ. Effect of liraglutide on physical performance in type 2 diabetes

- (LIPER2): A randomised, double-blind, controlled trial. *Contemp Clin Trials Commun* 2016; **4**: 46-51 [PMID: 29736469 DOI: 10.1016/j.conctc.2016.06.007]
- 43 **Takada S**, Masaki Y, Kinugawa S, Matsumoto J, Furihata T, Mizushima W, Kadoguchi T, Fukushima A, Homma T, Takahashi M, Harashima S, Matsushima S, Yokota T, Tanaka S, Okita K, Tsutsui H. Dipeptidyl peptidase-4 inhibitor improved exercise capacity and mitochondrial biogenesis in mice with heart failure via activation of glucagon-like peptide-1 receptor signalling. *Cardiovasc Res* 2016; **111**: 338-347 [PMID: 27450980 DOI: 10.1093/cvr/cvw182]
 - 44 **Heerspink HJ**, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium Glucose Cotransporter 2 Inhibitors in the Treatment of Diabetes Mellitus: Cardiovascular and Kidney Effects, Potential Mechanisms, and Clinical Applications. *Circulation* 2016; **134**: 752-772 [PMID: 27470878 DOI: 10.1161/CIRCULATIONAHA.116.021887]
 - 45 **Núñez J**, Palau P, Domínguez E, Mollar A, Núñez E, Ramón JM, Miñana G, Santas E, Fácila L, Górriz JL, Sanchis J, Bayés-Genís A. Early effects of empagliflozin on exercise tolerance in patients with heart failure: A pilot study. *Clin Cardiol* 2018; **41**: 476-480 [PMID: 29663436 DOI: 10.1002/clc.22899]
 - 46 **Carbone S**, Canada JM, Billingsley HE, Kadariya D, Dixon DL, Trankle CR, Buckley LF, Markley R, Vo C, Medina de Chazal H, Christopher S, Buzzetti R, Van Tassell BW, Abbate A. Effects of empagliflozin on cardiorespiratory fitness and significant interaction of loop diuretics. *Diabetes Obes Metab* 2018; **20**: 2014-2018 [PMID: 29603546 DOI: 10.1111/dom.13309]
 - 47 **Packer M**, Anker SD, Butler J, Filippatos G, Zannad F. Effects of Sodium-Glucose Cotransporter 2 Inhibitors for the Treatment of Patients With Heart Failure: Proposal of a Novel Mechanism of Action. *JAMA Cardiol* 2017; **2**: 1025-1029 [PMID: 28768320 DOI: 10.1001/jamacardio.2017.2275]
 - 48 **Perrone-Filardi P**, Avogaro A, Bonora E, Colivicchi F, Fioretto P, Maggioni AP, Sesti G, Ferrannini E. Mechanisms linking empagliflozin to cardiovascular and renal protection. *Int J Cardiol* 2017; **241**: 450-456 [PMID: 28395981 DOI: 10.1016/j.ijcard.2017.03.089]
 - 49 **Mudaliar S**, Alloju S, Henry RR. Can a Shift in Fuel Energetics Explain the Beneficial Cardiorespiratory Outcomes in the EMPA-REG OUTCOME Study? A Unifying Hypothesis. *Diabetes Care* 2016; **39**: 1115-1122 [PMID: 27289124 DOI: 10.2337/dc16-0542]

P- Reviewer: Beltowski J, Jiang L, Raghow R, Reggiani GM

S- Editor: Ji FF **L- Editor:** A **E- Editor:** Song H



Basic Study

Pathological changes in the cellular structures of retina and choroidea in the early stages of alloxan-induced diabetes

Irina Danilova, Svetlana Medvedeva, Svetlana Shmakova, Margarita Chereshneva, Alexey Sarapultsev, Petr Sarapultsev

Irina Danilova, Svetlana Medvedeva, Svetlana Shmakova, Department of Biology and Fundamental Medicine, Institute of Natural Sciences and Mathematics, Ural Federal University Named After the First Pres. of Russia B.N. Yeltsin, Ekaterinburg 620002, Russia

Irina Danilova, Svetlana Medvedeva, Svetlana Shmakova, Laboratory of Morphology and Biochemistry, Institute of Immunology and Physiology, Ural Division of Russian Academy of Sciences, Ekaterinburg 620049, Russia

Margarita Chereshneva, Laboratory of Immunophysiology and Immunopharmacology, Institute of Immunology and Physiology, Ural Division of Russian Academy of Sciences, Ekaterinburg 620049, Russia

Alexey Sarapultsev, Petr Sarapultsev, Institute of Chemical Engineering, Ural Federal University Named After the First Pres. of Russia B.N. Yeltsin, Ekaterinburg 620002, Russia

Alexey Sarapultsev, Petr Sarapultsev, Laboratory of Immunophatophysiology, Institute of Immunology and Physiology, Ural Division of Russian Academy of Sciences, Ekaterinburg 620049, Russia

ORCID number: Irina Danilova (0000-0001-6841-1197); Svetlana Medvedeva (0000-0002-0691-7579); Svetlana Shmakova (0000-0002-6552-7327); Margarita Chereshneva (0000-0002-6169-7978); Alexey Sarapultsev (0000-0003-3101-9655); Petr Sarapultsev (0000-0002-4093-4380).

Author contributions: Danilova I, Chereshneva M, Sarapultsev P, and Sarapultsev A contributed to the conception of the manuscript, design of experiments, and analysis and interpretation of the data, and wrote the manuscript; Danilova I, Medvedeva S, Shmakova S, and Sarapultsev A performed the experiments, analyzed the data, and wrote the manuscript; all authors have commented on the initial and final drafts of the manuscript and are responsible for approval of the final version of the manuscript in all aspects.

Supported by the Russian Science Foundation, No. 16-15-00039.

Institutional review board statement: This study protocol was reviewed and approved by the Institutional Review Board of Institute of Immunology and Physiology of Ural Branch of RAS, Ekaterinburg, Russia.

Institutional animal care and use committee statement: All institutional and national guidelines for the care and use of laboratory animals were followed. All experimental procedures with the animals were approved by the Institute of Animal Care and Use Committee at the Institute of Immunology and Physiology of the Ural Division of RAS (diab-1-04-2016).

Conflict-of-interest statement: The authors declare that there is no conflict of interest regarding the publication of this paper.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Corresponding author to: Alexey Sarapultsev, MD, PhD, Senior Researcher, Laboratory of Immunophatophysiology, Institute of Immunology and Physiology, Ural Division of Russian Academy of Sciences, 106 Pervomayskaya Street, Ekaterinburg 620049, Russia. a.sarapultsev@gmail.com
Telephone: +7-343-3740070
Fax: +7-343-3740070

Received: June 26, 2018
Peer-review started: June 26, 2018
First decision: July 19, 2018
Revised: September 1, 2018
Accepted: November 2, 2018
Article in press: November 3, 2018
Published online: December 15, 2018

Abstract

AIM

To investigate the temporal sequence of pathological changes in the cellular structures of retina and choroidea in the early stages of diabetes in laboratory animals.

METHODS

Experimental type 1 diabetes was modeled by three intraperitoneal injections of an alloxan solution into 30 male nonlinear rats at 16 wk of age. The 30th and 60th days from the final alloxan injection were chosen as the endpoints. Light and electron microscopy and morphometric and immunohistochemical studies were performed on histological slices of eyeballs from experimental animals.

RESULTS

Diabetic disturbances progressed to 60 d of the experiment. Thus, in the retina, a partial destruction of photoreceptors accompanied by interstitial edema was observed. The morphometric analysis revealed a reduction in the thickness of the retina. A reduction in the number of blood vessels of the choroid with disturbances of the endothelial cells and the vascular walls and a persistent reduction in the number of melanocytes were observed. The number of proliferating Ki-67 positive cells decreased, and the number of macrophages increased with diabetes development.

CONCLUSION

The starting point in the development of destructive changes involves early reduction in the number of melanocytes of the choroidea and alterations in the retinal pigment epithelium.

Key words: Alloxan; Diabetes; Diabetic retinopathy; Early stage; Morphology; Histological changes

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Diabetic retinopathy is the most frequent microvascular complication of diabetes. However, most of therapeutic approaches being developed do not address the early and potentially reversible failure of retinal perfusion. Thus, we examined pathological changes in the cellular structures of retina and choroidea in the early stages of diabetes in laboratory animals. According to the obtained results, the starting point in

the development of destructive changes involves the early reduction in the number of melanocytes of the choroidea and the destruction of the retinal pigment epithelium, accompanied by an inflammatory process, which may represent a potential therapeutic target.

Danilova I, Medvedeva S, Shmakova S, Chereshneva M, Sarapultsev A, Sarapultsev P. Pathological changes in the cellular structures of retina and choroidea in the early stages of alloxan-induced diabetes. *World J Diabetes* 2018; 9(12): 239-251

URL: <https://www.wjgnet.com/1948-9358/full/v9/i12/239.htm>

DOI: <https://dx.doi.org/10.4239/wjd.v9.i12.239>

INTRODUCTION

Diabetic retinopathy (DR) is one of the major complications associated with diabetes, and has equally been implicated as one of the leading causes of visual impairment and blindness globally. Because of this, DR is in the limelight of most clinical studies^[1-4]. Hyperglycemia, hypertension, renal disease, and dyslipidemia, which are typical conditions in the manifestation of diabetes, have all been linked to the pathogenesis of DR^[5,6]. According to the prevailing point of view, the leading causes of DR development include metabolic disturbances and vascular bed abnormalities, which accompany diabetes development^[7-11]. In diabetes, hyperglycemia and associated oxidative stress trigger the pathological cascade underlying the vascular injury (micro- and macroangiopathy development)^[12-14]. Due to the subsequent disturbances of vessel walls, the permeability of the hemato-retinal barrier breaks down, and hypoxia appears, leading to trophic retinal degeneration and photoreceptor cell death^[15-17]. The subsequent progression of the developed retinopathy leads to retinal neovascularization, vitreous hemorrhages, and the formation of fibrous tissue in the foci of preretinal hemorrhages, which forms the pathogenomic picture of diabetic complications^[18-20].

However, despite the seeming transparency of DR pathogenesis and the progress in its treatment observed in recent years, a number of issues remain that warrant further study^[6,21-23]. One of them is the temporal sequence of pathological changes in DR development^[19-22]. Studies in rodents have highlighted that biomarkers of inflammation, such as leukostasis, overexpression of adhesion molecules in retinal vascular endothelial cells and leukocytes, vascular permeability alteration, and aggravated production of nitric oxide, prostaglandins, cytokines, and other inflammatory mediators appears in the retina during 1-6 mo of diabetes crisis^[5]. Most developed therapies for DR, have primarily focused on the terminal stage of this disease, and as thus, failed to address the early potentially reversible stage of this disease. In addition, most of

Table 1 Level of glucose and glycosylated hemoglobin in the blood of experimental animals (mmol/L)

Biochemical parameters	Control animals (Group 1)	Diabetes, 30 d (2 nd group)	Diabetes, 60 d (2 nd group)
Glucose (mmol/L)	5.99 ± 0.33	25.98 ± 1.84 ^a	32.60 ± 0.80 ^a
Hb A1c (%)	5.12 ± 0.24	7.10 ± 0.60 ^a	6.45 ± 0.29 ^a
Insulin (μg/L)	1.28 ± 0.19	0.47 ± 0.05 ^a	0.36 ± 0.04 ^a

^aDifferences to control animals were significant at $P < 0.05$.

these therapies have been associated with severe sight-threatening side effects^[6].

With that, understanding of the temporal sequence and stages of pathological disturbances of DR development is of great prognostic and scientific value, as it might contribute to improvements to current methods or even the development of new methods of diagnosis and treatment of such a serious complication of diabetes. Thus, this work investigated the temporal sequence of pathological changes in the cellular structures of retina and choroidea in the early stages of diabetes in laboratory animals.

MATERIALS AND METHODS

Animal preparation

Healthy, sexually matured male Wister rats were used for the purpose of this experiment. The animals employed in this study were quarantined in the vivarium of the Institute of Immunology and Physiology of the Ural Division of RAS (Ekaterinburg, Russia). Only animals showing no symptoms of any disease were selected. All experimental animals were housed in similar conditions, and fed according to a customary schedule. All the experimental procedures conducted on the animals were approved by the Institute of Animal Care and Use Committee at the Institute of Immunology and Physiology of the Ural Division of RAS (diab-1-04-2016), and implemented in compliance with the principles formulated in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, France, 18.03.1986), APS's Guiding Principles in the Care and Use of Vertebrate Animals in Research and Training, and the Laboratory Practice Regulations of Russia Federation (Ministry of Public Health Order No. 267 from 19.06.2003).

Experimental model of type 1 diabetes

Experimental type 1 diabetes was modeled by three intraperitoneal injections (10 mg/100 g of weight) of an alloxan solution (Sigma-Aldrich, St. Louis, MO, United States) dissolved in physiological saline at 1 d intervals (total dose of alloxan 30 mg/100 g) according to a modified version of the standard model of diabetes in rats^[24,25]. Alloxan is a toxic glucose analogue that has been employed to induce experimental diabetes. This compound accumulates in pancreatic cells and

selectively destroys the insulin producing beta-cells^[26,27].

Experimental protocol

The experiments were conducted on 30 male nonlinear rats of the same age (16-wk-old). The 30th and 60th days from the final alloxan injection were chosen as the endpoints of the experiment. This duration of diabetes in rats corresponds to a duration of diabetes in humans approximately equal to 4.25 and 8.5 years, which is a sufficient time for the development of diabetes complications, including neurodegenerative complications^[17,28]. Thirty rats with body weight of 190–220 g were randomly divided into three groups ($n = 10$ in each group): control (group 1), diabetes 30 d (group 2), and diabetes 60 d (group 3). The control animals (group 1) received *i.p.* saline injections at day 1 and between 30–60 d (20 injections in total). The diabetes 30 d animals (group 2), weighing approximately 207 ± 10 g, were rendered diabetic after 16 h fasting conditions, by a single *i.p.* administration of alloxan monohydrate (Sigma-Aldrich, St. Louis, MO, United States) at a dose of 300 mg/kg of body weight, dissolved in 10 mmol/L of sodium citrate (pH 4.5). Afterwards, the animals were housed in standard conditions until the end of the 30 d experimental duration of the group. The diabetes 60 d animals (group 3), weighing 207 ± 10 g, received a single *i.p.* dose of 300 mg/kg alloxan monohydrate and were housed in similar conditions for 60 d. Peripheral blood glucose from the tail vein was obtained to determine glycemia in all experimental groups (Table 1).

On the respective sacrifice dates of each animal, they were first anaesthetized with 40 mg/kg pento-barbital sodium administered intraperitoneally. Blood samples (approximately 3 mL) were collected by heart puncture for biochemical and enzyme immunoassay investigations. Histological, immunohistochemical, and light and electron microscopy methods were used to study the rat's eye slices.

Laboratory blood tests

Plasma glucose levels were determined with a standard glucose oxidase test kit (Novogluk-R, "VektorBest", Russia)^[29,30]. The plasma insulin level was determined using a standard ELISA Rat assay (Insulin ELISA, Mercodia AB, Switzerland). Biochemical testing was carried out with a DU-800 spectrophotometer (Beckman Coulter Int S.A., Switzerland).

HbA1c measurement was performed by affinity chromatography ("Diabetes-test", (HbA1c) TOR 9398240-16404416-01, Fosfosorb OJSC, Russian Federation), according to the manufacturer's instructions ("Fosfosorb" OJSC, Russia)^[31].

Histological studies

A neutral buffered solution of 10% formalin was used to preserve the eye samples for 24 h, then paraffinized through a series of solutions^[30]. The standard dehydration procedure was performed. The tissue was processed and embedded in paraffin wax using the autoprocessor Leica EG 1160. Hematoxylin and eosin (HE) staining of the 3-5 micron thick sections were performed for morphological and morphometric studies. The remaining sections were placed in a buffer for antigen unmasking and further immunohistochemical studies.

Immunohistochemical studies

For immunohistochemical evaluation, tissues were first fixed in formalin, then embedded in paraffin, and sectioned at 3 μm . The antibody staining of the tissues was performed with the Autostainer DAKO, according to a standard protocol. High-temperature treatment in a citrate buffer (pH = 6) using Pascal DAKO^[32-34], was employed for the unmasking procedure of antigens. The visualization of antigen-reactive cells was performed using the Novolink™ Polymer Detection System (Novocastra Lab., Ltd), with its buffer solution consisting of a chromogenic agent 3,3'-diaminobenzidine (DAB). Macrophages were visualized with anti-CD68 antibodies (clone KP1, Thermo Scientific). The assessment of proliferation was performed with mouse anti-rat monoclonal antibodies to the Ki-67 marker (clone MM1, Leica Microsystems).

Morphometric analysis

Using sections of eyeballs stained with HE, the number of vessels and melanocytes per unit area (0.01 mm^2 tissue of choroid) ($N/0.01 \text{ mm}^2$) was estimated in the choroidea, whereas the total thickness and the thickness of separate layers (in μm) were estimated in the retina.

The number of proliferating cells in the ganglionic and internal nuclear retinal layers was estimated on sections stained with the Ki-67 proliferation marker, the ratio of the total proliferating cells to total number of cells in the retina layer was subsequently calculated. Using sections stained with CD68 marker, the number of CD68 positive cells per unit area (1 mm^2 tissue) (N/mm^2) was determined in the choroidea and the retina.

Optical-microscopic examination

Optical-microscopic examination was conducted with the microscope (Leica DM 2500), and the analysis of

the image was done using Video Test "Morphology" 5.0 program (VideoTest, St. Petersburg, Russia).

Electron microscopy examination

For ultramicroscopic examination after enucleation of the eyeball, the lens of the eye and the posterior wall of the eyeball containing the retina and the choroid were fixed in a 2.5% solution of glutaraldehyde followed by postfixation in a 1% solution of osmium tetroxide (OsO_4). After thorough washing, dehydration in alcohols of increasing concentrations (50%, 70%, 96% and 100%) was performed followed polymerization in an araldite resin at a temperature of 60 $^\circ\text{C}$ ^[35]. Slices were created using ultramicrotome (Leica EM UC6), contrasted with lead citrate, and examined with the aid of a digital transmission electron microscope (Morgagni™ 268).

Statistical analysis

Analysis of data was performed using Statistica 6.0 software (StatSoft, United States), variables showing results with a heterogeneous distribution were analyzed using the nonparametric (*U*) Mann-Whitney test. All analysis was carried out at 0.05% significance level of probability.

RESULTS

Confirmation of diabetes development

The development of diabetes in experimental animals was confirmed by biochemical study. According to the results, a significant increase in the levels of glucose and glycosylated hemoglobin (HbA1c) and a decrease in the level of insulin were detected after alloxan administration in the animals of experimental groups 2 and 3 compared to the control group (Table 1).

Experimental diabetes: Thirty days

Retina: Histological examination of the retina and choroid of animals in the control group exhibited no structural disturbances (Figures 1 and 2A). However, in experimental group 2, moderately pronounced interstitial edema and fullness of dome capillaries in the ganglionic and inner nuclear layers of the retina were observed (Figure 3A).

Electron microscopic examination confirmed the presence of edema in the form of an expansion of the spaces between the layers of rods and cones and their partial deformation and disorganization of the outer and inner segments of the photoreceptors (Figures 3B and 4). In the outer nuclear layer, round-shaped nuclei with irregular intervals between them were observed. This feature was attributed to the developing interstitial edema. The contours of the nuclei were even. The chromatin was osmiophilic in the center of the nucleus and bright on the periphery. The monolayer of cells

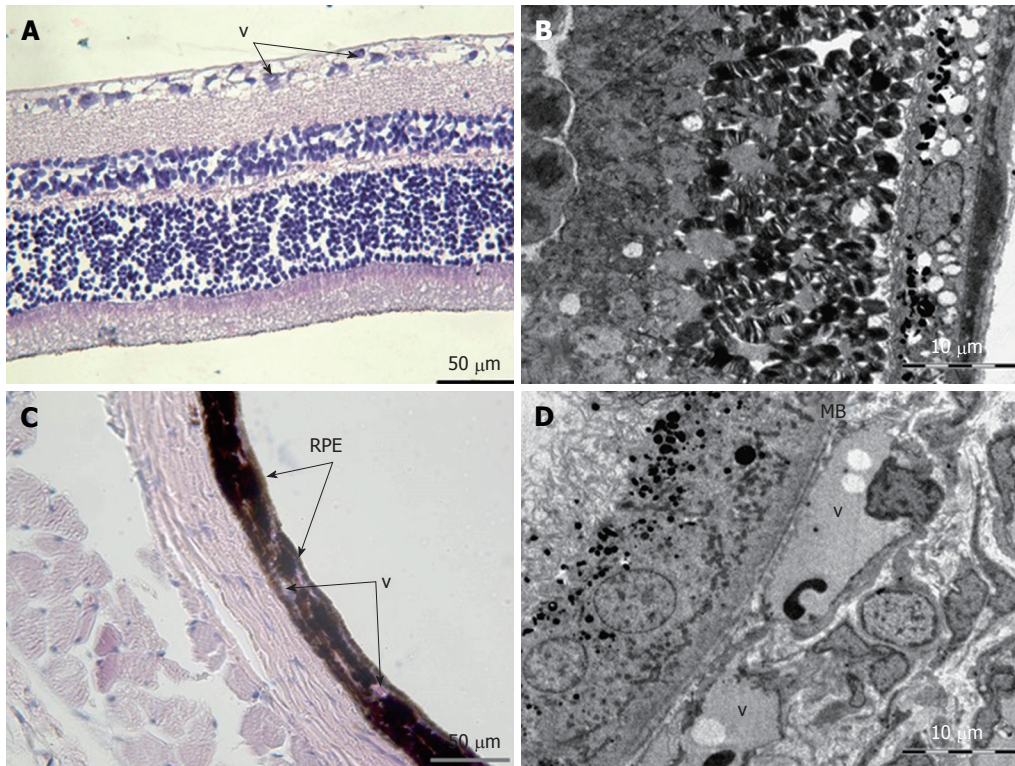


Figure 1 Back of the eye of a control animal. A: Light microscopy visualization of the retina. v: blood vessels; B: Electron microscopy of the outer layers of the retina; C: Light microscopy of the choroid and sclera of the eye. v: choroid vessels; RPE: pigment epithelium of the retina; D: Electron microscopy of the retinal pigment epithelium and choroid. v: choroid vessels; Light microscopy: staining with hematoxylin and eosin, magnification $\times 400$, bar 50 μm ; Electron microscopy: bar 10 μm .

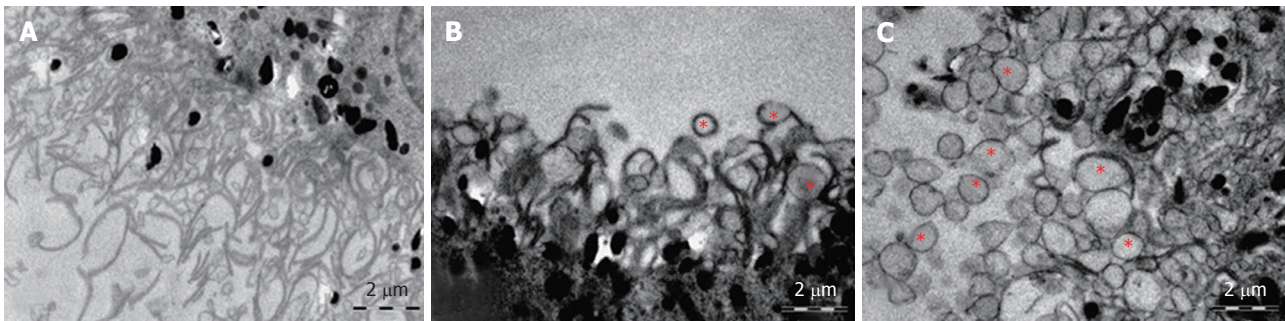


Figure 2 Cell processes of retinal pigment epithelium. A: Control animals (group 1); B: Diabetes at 30 d (group 2); C: Diabetes at 60 d (group 3); Bar 2 μm . *: vacuolation of cell processes.

of retinal pigment epithelium adhered to the Bruch's membrane. In the cytoplasm of the pigment epithelium, an uneven distribution with a quantitative decrease of pigment granules was detected (Figure 2B). Electron microscopy revealed loosening of the membranes of the pigment epithelium nuclei, mitochondrial swelling, the destruction of the crista, and the enlightenment of the mitochondrial matrix (Figure 3D).

Morphometric examination of the retina revealed changes in the thickness of different layers. Thus, a decrease in the total thickness of the retina and in the rods and cones, outer nuclear and ganglionic layers was revealed, indicating the development of dystrophic

processes during the time course of diabetes (Table 2).

Choroidea: Morphometric analysis of the choroidea revealed a decrease in the number of blood vessels per unit area in group 2 (1.79 ± 0.07) compared to the control animals (2.62 ± 0.33) (Table 3, Figures 5 and 6).

According to the results of optical microscopic examination, alterations of the microcirculatory vessels in the choroidea were detected accompanied by desquamation and swelling of endothelial cells. These features led to the occlusion of small capillaries, the expansion of their lumen, and the development of edema (Figure 2C).

Electron microscopic examination revealed a pro-

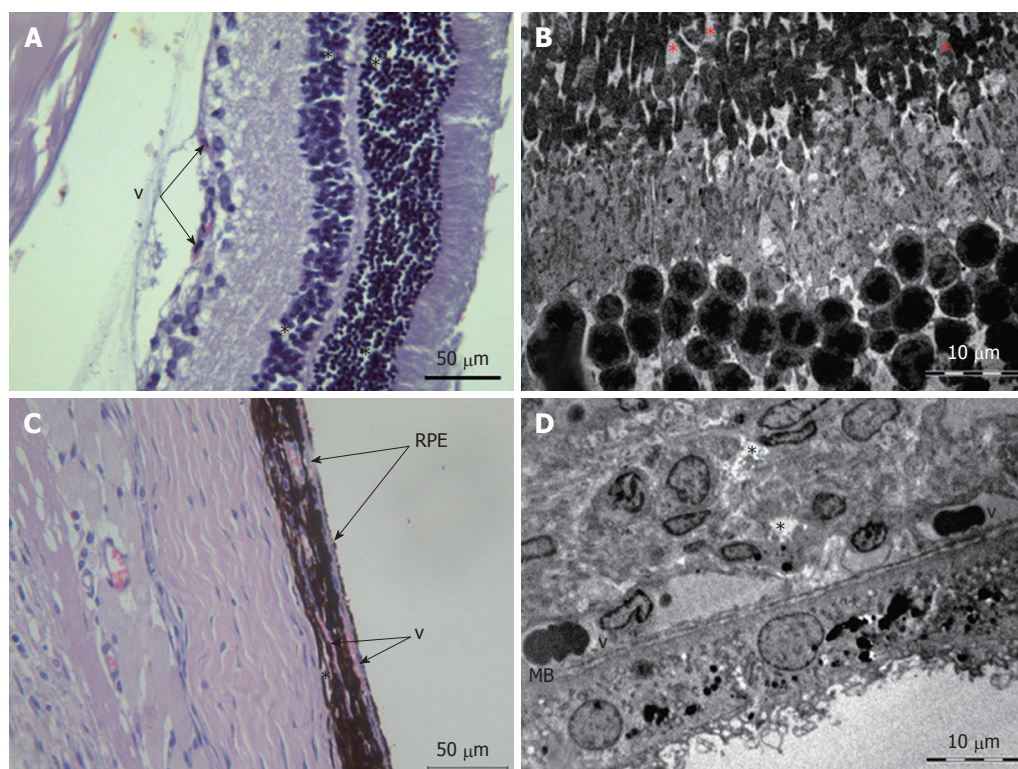


Figure 3 Posterior wall of the eye of an animal with diabetes at 30 d. A: Light microscopy of the retina; *: interstitial edema; v: full blood vessels; B: Electron microscopy of the outer layers of the retina; *: destroying rods and cones; C: Light microscopy of the choroid and sclera of the eye; *: interstitial edema; v: full blood vessels; RPE: destructive changes in retinal pigment epithelial cells; D: Electron microscopy of the retinal pigment epithelium and choroid; *: interstitial edema; v: choroid vessels with sludge complexes; MB: unevenly thickened Bruch's membrane; Light microscopy: staining with hematoxylin and eosin, magnification $\times 400$, bar 50 μm ; Electron microscopy: bar 10 μm .

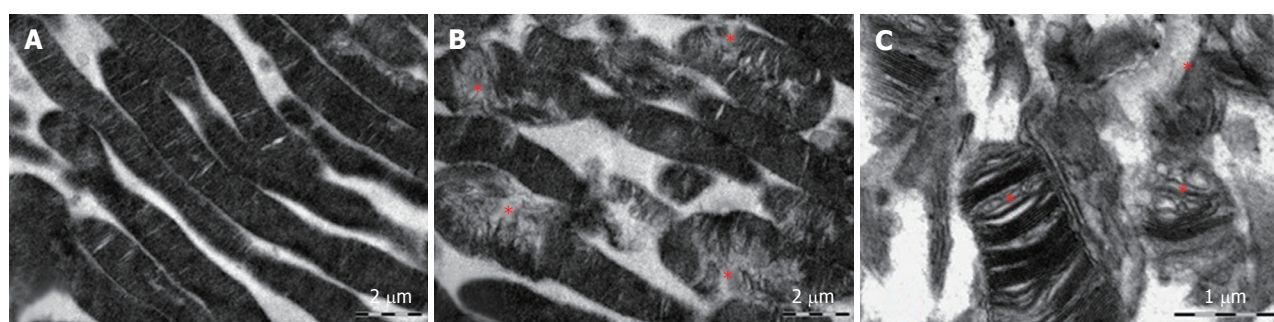


Figure 4 Photoreceptors of the retina. A: control animals (group 1), bar 2 μm ; B: diabetes at 30 d (group 2), bar 2 μm ; C: diabetes at 60 d (group 3), bar 1 μm . *: destruction of photoreceptors.

nounced loosening of the connective tissue with the formation of edema foci in the perivascular zone. The choroid was hypovascularized, and only a small number of vessels that were generally small in diameter were detected. In vessels, various alterations of the integrity of basal membranes as well as endothelial cell swelling and their partial destruction were clearly defined. The sluggish erythrocytes were visible in the lumen of capillaries (Figure 3D).

Based on light microscopy, the pigmented layer of the choroid after 30 d of experimental diabetes was characterized by pronounced dystrophic changes in melanocytes with the destruction of their cytoplasmic

membrane and the release of pigment granules into the intercellular space.

According to the results of optical microscopic examination, the layer of melanocytes in the choroid was characterized by pronounced dystrophic changes in melanocytes with the destruction of their cytoplasmic membranes and signs of pigment granule release into the intercellular space. Melanocytes located perivascularly were characterized by the presence of pronounced dystrophic changes in their ultrastructure: the destruction of mitochondria and endoplasmic reticulum and the output of secretory granules to the extracellular space. The number of choroidal melanocytes was significantly

Table 2 Characteristics of the thickness of the retina and its individual layers (μm , $M \pm m$)

Group	Retinal layers						Total thickness of the retina
	Layer rods and cones	Outer nuclear layer	Outer plexiform layer	Inner nuclear layer	Inner plexiform layer	Ganglion cell layer	
Control (group 1)	36.31 ± 5.11	56.43 ± 1.72	9.85 ± 1.68	25.82 ± 0.76	38.93 ± 4.79	17.82 ± 0.72	185.16 ± 9.42
Diabetes at 30 d (group 2)	28.65 ± 3.44 ^a	51.62 ± 6.51 ^a	11.46 ± 1.59	26.1 ± 1.55	40.21 ± 7.14	15.98 ± 1.37 ^a	174.00 ± 2.93 ^a
Diabetes at 60 d (group 3)	28.38 ± 1.43 ^a	56.87 ± 5.30	9.69 ± 1.04 ^c	26.24 ± 0.95 ^a	39.94 ± 7.10	14.65 ± 2.05 ^a	175.77 ± 5.22 ^a

^aDifferences compared to control animals were significant at $P < 0.05$; ^cDifferences compared to animals with diabetes at 30 d were significant at $P < 0.05$.

reduced per unit area (20.5 ± 0.39) compared to the control animals (10.1 ± 2.42) (Table 4).

Immunohistochemical study results: Proliferating cells are localized in the inner nuclear and ganglionic layers or retina, where glia cells capable of proliferating are present. Ki-67 positive cells were reduced in the inner nuclear and ganglionic layers of the retina in both the absolute and relative indices, and the decrease was more pronounced in the ganglionic layer (Table 4, Figures 7 and 8).

Immunohistochemical staining of the choroid and retina with anti-CD68 antibodies revealed a decrease in the number of macrophages in the retina, both in the ganglionic and inner nuclear layers compared to control animals. No significant changes were observed in the choroidea (Table 5).

Experimental diabetes: Sixty days

Retina: Histological examination of the retina of experimental animals from group 3 revealed an increase in dystrophic changes of photoreceptor and pigment epithelium layers compared to the histological features of group 2 animals (Figure 4). A plethora of capillaries of the retinal ganglionic layer and foci of angiomatosis in the inner nuclear layer were also observed (Figure 9).

Morphometric examination of the retina revealed changes in the thickness of different layers. Thus, a decrease in the thickness of the photoreceptor layer, internal nuclear, ganglionic, and outer reticular layers was revealed, indicating the dynamics of the development of dystrophic processes during the time course of diabetes (Table 2 and Figure 6).

Electron microscopic examination revealed signs of partial destruction of the layer of rods and cones. The remains of the membrane discs were observed, some of which were clearly visualized. In the inner nuclear layer, small diameter vessels of the sinusoidal type were observed (Figure 9B). Cells of the pigment epithelium of the retina were arranged on Bruch's membrane, exhibiting a folded, uneven shape with invagination sites (Figure 2C). The nuclei of the pigment cells and pigment granules were determined extracellularly, and cell outgrowths were in a state of destruction (Figure 9D).

Choroidea: Melanocyte dystrophy (a redistribution of melanin granules with a decrease in the total number of cells), which was described in group 2, was preserved (Table 3 and Figure 9C).

In the connective tissue layer, focal vascular fullness with the formation of sludge complexes was revealed and accompanied by the occlusion of some vessels, endothelial cell swelling, and the destruction of the basal membrane. The number of vessels per unit area corresponded to the values obtained at 30 d (Table 3).

Electron microscopy examination revealed loosening of connective tissue and massive perivascular edema. Most of the observed vessels were characterized by an enlarged lumen with swollen endothelial cells. The cytoplasmic membrane of the endothelial cells and their nuclei were uneven and folded. Swollen mitochondria with a visible matrix and the remnants of crista were detected inside the cells.

Results of immunohistochemical study: The immunohistochemical study of Ki-67 positive cells revealed that their quantity did not decrease and were similar to group 2 (Table 4, Figures 7 and 8).

Table 3 Average number of blood vessels and pigment cells in the choroid of the eyes of experimental animals (per unit area, $S = 0.01 \text{ mm}^2$)

	Control (group 1)	Diabetes at 30 d (group 2)	Diabetes at 60 d (group 3)
No. of blood vessels	2.62 ± 0.33	1.79 ± 0.07^a	1.59 ± 0.22^a
No. of pigment cells	35.23 ± 5.69	20.5 ± 0.39^a	10.1 ± 2.42^a

^aDifferences compared to control animals were significant at $P < 0.05$.

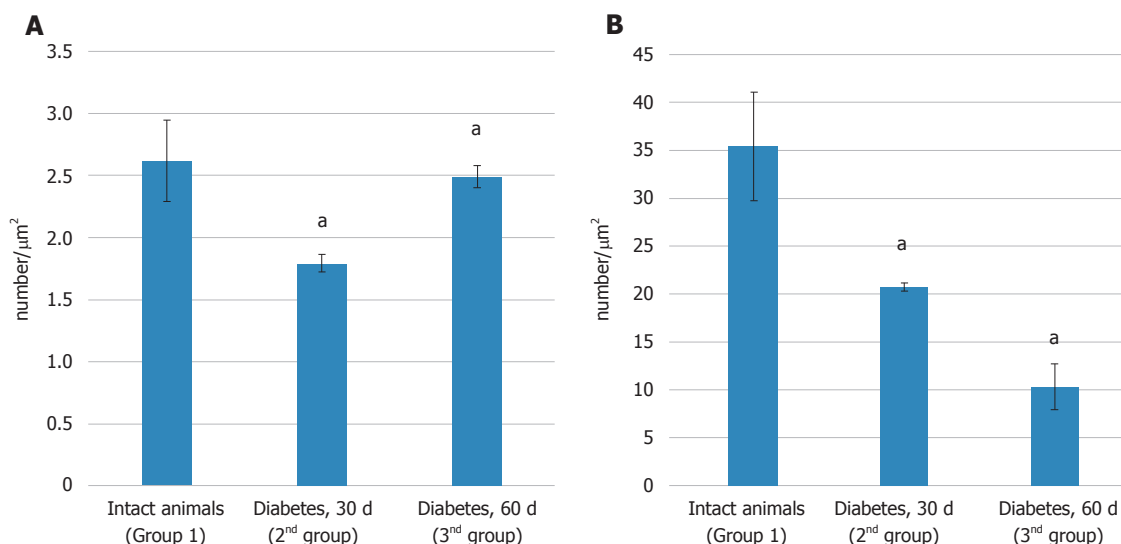


Figure 5 Morphometric examination of the choroid of the eyes of animals in experimental groups. A: The average number of choroidal vessels per unit area (0.01 mm^2); B: Average number of pigment cells of the choroid per unit area (0.01 mm^2). ^aDifferences compared to the control animals were significant at $P < 0.05$.

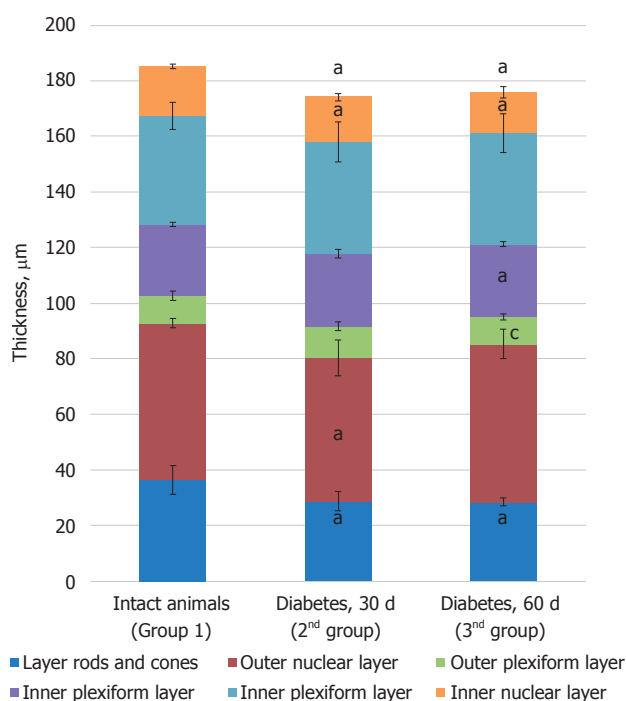


Figure 6 Morphometric examination of retinal layers of experimental groups. ^aDifferences compared to control animals were significant at $P < 0.05$; ^cDifferences to animals with diabetes for 30 d were significant at $P < 0.05$.

retina with anti-CD68 antibodies revealed an increase in the number of macrophages in choroidea compared to group 1 and group 2. The quantity of macrophages in the inner layer of the retina was similar to group 2. In the ganglionic layer, an increase in the number of macrophage was equal to the control group (Table 5).

DISCUSSION

A plethora of evidence obtained over the past 20 years based on different clinical studies and experimental data have shed more light on the development and pathogenesis of DR and how it develops^[6,10,11,36,37]. However, the complexity of pathogenic pathways that lead to the development of DR is beyond the scope of this article and are reviewed elsewhere^[5,6,10,11,36]. The typical histological picture of diabetes characterized by the destruction of stroma and cell elements was also described in a number of studies^[37].

The aim of the present study was to supplement this picture with the use of immunohistochemical and morphometric methods of investigation to estimate the numbers and proliferation status of individual cellular elements (melanocytes), thus providing information about the time course of destructive processes with the focus on the early stages of diabetes development.

In the present study, the alloxan-induced diabetes model demonstrated that in the early stages of the

Immunohistochemical staining of the choroidea and

Table 4 Number of Ki-67 positive cells in the layers of the retina ($M \pm m$)

Group	Layers of the retina					
	Inner nuclear layer			Ganglion cell layer		
	All cells	Ki-67 positive cells	% of Ki-67 positive cells	All cells	Ki-67 positive cells	% of Ki-67 positive cells
Control (group 1)	28.60 \pm 2.11	7.25 \pm 0.93	25.46 \pm 3.53	7.71 \pm 1.01	0.99 \pm 0.3	12.98 \pm 3.24
Diabetes at 30 d (group 2)	27.94 \pm 1.14	4.92 \pm 0.92 ^a	17.82 \pm 3.79 ^a	5.45 \pm 0.78 ^a	0.42 \pm 0.18 ^a	7.83 \pm 3.11 ^a
Diabetes at 60 d (group 3)	29.24 \pm 2.56	4.55 \pm 1.5 ^a	15.4 \pm 4.76 ^a	6.19 \pm 0.79 ^a	0.59 \pm 0.3 ^a	9.95 \pm 5.12

^aDifferences compared to control animals were significant at $P < 0.05$.

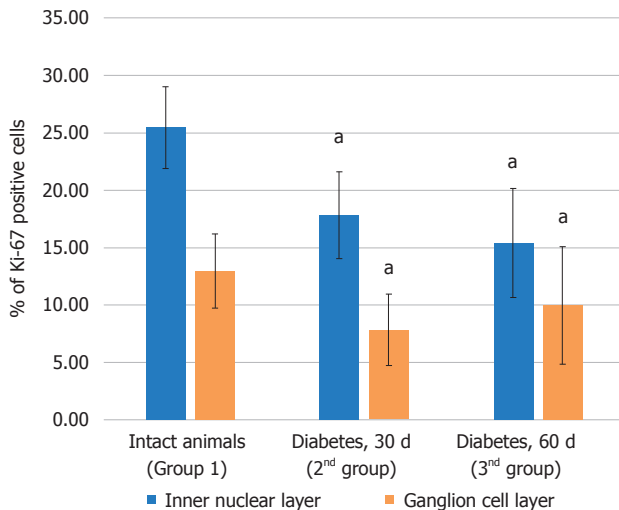


Figure 7 Relative content of Ki-67 positive cells from the total number of cells in the inner nuclear and ganglionic layers of the retina. ^aDifferences compared to control animals were significant at $P < 0.05$.

disease (30 d), diabetic alterations in the structures of the retina and choroid are present, and these alterations progress slightly after 60 d.

In the retina, these disorders manifest themselves as a partial destruction of the structural-functional elements, namely, photoreceptors and are accompanied by a stromal reaction in the form of the development of interstitial edema, which was confirmed by the histological and electron microscope images of the examined structures^[38]. In addition, morphometric analysis revealed a reduction in the thickness of the retina due to photoreceptor destruction. Moreover, in retinal layers that are capable of proliferation (the inner nuclear layer and ganglionic layer), the number of Ki-67 positive cells decreased with the development of diabetes.

The choroidea consists of a network of chorio-capillaries and stroma. Similar to other types of connective tissue, mast cells, macrophages, and lymphocytes are present in the stroma^[39]. It is believed that the vascular membrane fulfills the function of supplying the outer layers of the retina with oxygen and nutrients. Thus, disruption of the choriocapillary structure causes degenerative changes in the latter and its neovascularization^[39-41]. However, the precise cellular mechanisms leading to retinal dysfunction under high

glucose levels remain unclear.

According to these results, a reduction in the number of blood vessels of the choroid with the pathological alterations of endothelial cells and vascular walls were observed. Moreover, the described changes develop during early stages of the disease (30 d) and generally do not change as time progresses.

Pathological changes in the number and state of cellular elements of the stroma of choroidea (melanocytes and macrophages) complete the picture of DR. Thus, the persistent reduction in the number of melanocytes in the choroidea (1.5-fold at 30 d and 3-fold at 60 d) was observed. Moreover, the pigment epithelium of the retina exhibited signs of dystrophic changes in the ultrastructure of cells accompanied by a reduction in the amount and redistribution of melatonin granules in these cells. Moreover, given that melanocytes release the key factors of angiogenesis, such as fibromodulin, a reduction in melanocytes may be one of the factors that leads to the above described reduction in the number of capillaries in the choroidea^[42].

Macrophages are present in the choroidea under normal conditions, performing homeostatic functions^[42]. However, in DR macrophages play a key role in the development of the inflammatory response, releasing pro-inflammatory cytokines that lead to capillary degeneration^[43]. Moreover, according to Aveleira *et al.*^[44], the proapoptotic effect of inflammatory cytokines is significantly increased with hyperglycemia. According to our results, an increase in the number of macrophages (3.5-fold) in the choroidea was observed in diabetes^[44]. Apparently, such a pronounced macrophage infiltration was caused by the recruitment of cells of the monocyte-macrophage lineage from the blood stream, as evidenced by their perivascular localization. The initiating factor of the observed migration of macrophages into the choroid was the development of destructive disorders (inflammation) in the latter^[45].

Finally, a significant reduction (3.5-fold) in the number of pigment cells was also observed, which corresponds to findings reported in the literature^[46]. This feature characterized the progression of pathological changes in the choroidea and led to further disruption of the integrity of the hemato-retinal barrier^[47].

In general, based on the results of our study, it can be assumed that the starting point in the development

Table 5 Quantitative distribution of macrophages in the eyes based on the structures ($M \pm m / 1 \text{ mm}^2$)

Structure of the eye		Control (group 1)	Diabetes at 30 d (group 2)	Diabetes at 60 d (group 3)
Choroidea		4.16 ± 3.31	4.99 ± 2.84	14.4 ± 6.69 ^{a,c}
Retina	Ganglionic layer	18.46 ± 2.66	9.42 ± 1.00 ^a	16.25 ± 5.30 ^c
	Inner nuclear layer	11.25 ± 3.71	6.6 ± 2.59 ^a	7.87 ± 1.71 ^a

^aDifferences compared to control animals were significant at $P < 0.05$; ^cDifferences compared to animals with diabetes at 30 d were significant at $P < 0.05$.

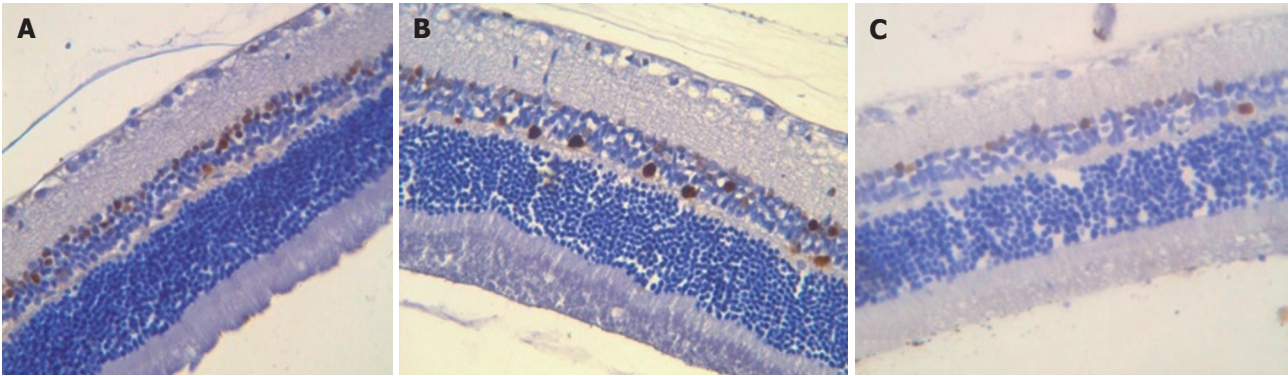


Figure 8 Ki-67 staining of the retina. A: Control animals; B: Diabetes at 30 d; C: Diabetes at 60 d.

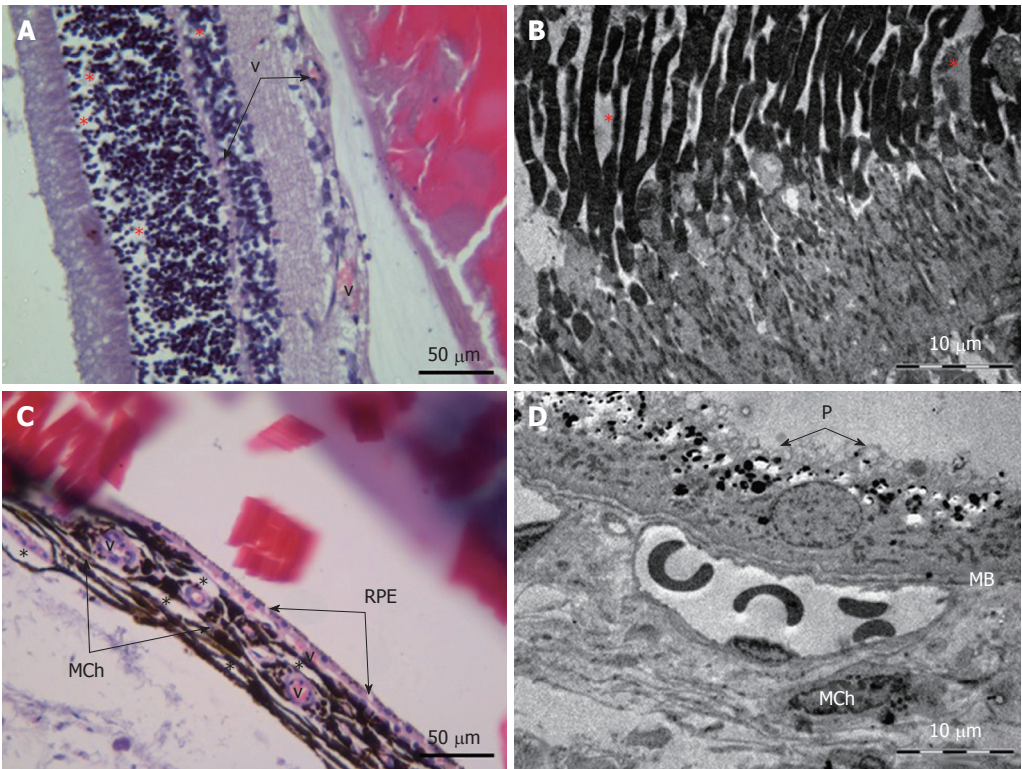


Figure 9 Posterior wall of the eye of an animal with diabetes at 60 d. A: Light microscopy of the retina; *: interstitial edema; v: full blood vessels; B: Electron microscopy of the outer layers of the retina; *: destruction of rods and cones; C: Light microscopy of the choroid of the eye; *: interstitial edema; v: full blood vessels with swollen endothelial cells; RPE: destructive changes in retinal pigment epithelial cells; MCh: destruction of pigment cells of the choroid; D: Electron microscopy of the retinal pigment epithelium and choroid; MB: uneven Bruch's membrane with invagination sites; MCh: choroidal melanocyte with reduced amount of pigment granules; P: destructive changes in the outgrowths of retinal pigment epithelial cells; Light microscopy: staining with hematoxylin and eosin, magnification $\times 400$, bar 50 μm ; Electron microscopy: bar 10 μm .

of destructive changes in DR involves the early reduction in the number of melanocytes of the choroidea and the

destruction of the retinal pigment epithelium, which are the primary components of the hematoretinal barrier.

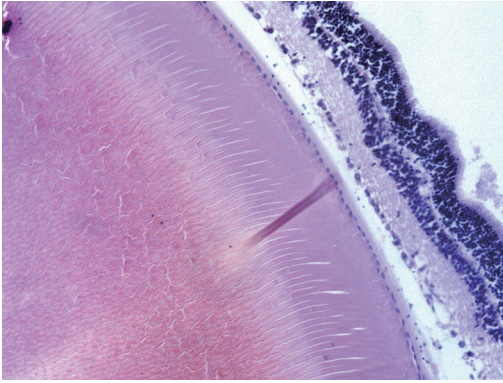


Figure 10 Fragment of the eye of an animal after alloxan administration at the dose of 30 mg/kg at 14 d. No visible structural changes are detected. Light microscopy: staining with hematoxylin and eosin, magnification $\times 400$.

Limitations of the study

According to the literature, the direct toxic effects of alloxan on the retina, rather than secondary changes from diabetes, have been described^[48-50]. Some teratogenic effects of alloxan in mice have been observed, including abnormalities of the lens and iris^[49]. However, according to our results, the injection of alloxan in the total dose of 30 mg/100 g did not cause any disturbances at 14 d that could be observed via optical microscopy (Figure 10).

ARTICLE HIGHLIGHTS

Research background

Diabetic retinopathy (DR) is a disease commonly associated with diabetes complications. It is known as one of the primary causes of visual impairment and blindness globally. More recent discoveries have shown that indicators of inflammation, altered vascular permeability, and increased production of inflammatory mediators occurs in the retina after 1-6 mo of the presence of diabetes. However, most of therapeutic approaches being developed do not address the early and potentially reversible failure of retinal perfusion.

Research motivation

Better understanding of the temporal sequence and stages of pathological disturbances of DR development is of scientific value, as it might contribute to improvements to current methods or even the development of new methods of diagnosis and treatment of the early and potentially reversible failure of retinal perfusion.

Research objectives

We have investigated the temporal sequence of pathological changes in the cellular structures of retina and choroidea in a rat model of alloxan-induced diabetes in the early stages of disease.

Research methods

Alloxan accumulates in pancreatic cells, resulting in selective β -cell necrosis and diabetes. Experimental diabetes was modeled by three intraperitoneal injections (10 mg/100 g of weight) of an alloxan solution dissolved in physiological saline at 1-d intervals (total dose of alloxan 30 mg/100 g). The 30th and 60th days from the final alloxan injection were chosen as the endpoints of the experiment. Biochemical and enzyme immunoassay were performed. Furthermore, histological, immunohistochemical, and electron microscopy methods were employed to evaluate the rat's eye slices. Similarly, light microscopy and morphometric analyses of slides were also conducted.

Research results

In the present study, the alloxan-induced diabetes model demonstrated that in the early stages of the disease, diabetic alterations in the structures of the retina and choroid are present, and these alterations progress with time. In the retina, DR manifest itself as a partial destruction of the structural-functional elements, namely, photoreceptors and are accompanied by a stromal reaction in the form of the development of interstitial edema and a reduction in the thickness of the retina due to photoreceptor destruction. The reduction in the number of blood vessels of the choroid, melanocytes, and pigment cells along with an increase in the number of macrophages were also observed at early stages of the disease.

Research conclusions

The results of this study provide evidence that DR manifests itself at the early stages of diabetes. The starting point in the development of DR involves the early reduction in the number of melanocytes of the choroidea and the destruction of the retinal pigment epithelium, which are the primary components of the hematoretinal barrier.

Research perspectives

Further studies that estimated vascular endothelial growth factor, prostate-derived Ets transcription factor, cytokines, NO, and antioxidants and correlated them with blood glucose levels and changes in the retina in various experimental models and at different time periods will contribute to the improvements and the development of new methods of diagnosis and treatment of DR.

REFERENCES

- 1 **Constantino MI**, Molyneaux L, Wu T, Twigg SM, Wong J, Yue DK. Data collection on retinopathy as a public health tool: The Hubble telescope equivalent of looking back in time. *J Diabetes Complications* 2017; **31**: 721-725 [PMID: 28161385 DOI: 10.1016/j.jdiacomp.2016.12.016]
- 2 **Vujosevic S**, Pucci P, Casciano M, Daniele A, Bini S, Berton M, Cavarzeran F, Avogaro A, Lapolla A, Midena E. A decade-long telemedicine screening program for diabetic retinopathy in the north-east of Italy. *J Diabetes Complications* 2017; **31**: 1348-1353 [PMID: 28551296 DOI: 10.1016/j.jdiacomp.2017.04.010]
- 3 **Das A**. Diabetic retinopathy: Battling the global epidemic. *Indian J Ophthalmol* 2016; **64**: 2-3 [PMID: 26953017 DOI: 10.4103/0301-4738.178155]
- 4 **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]
- 5 **Chen M**, Stitt A. Animal Models of Diabetic Retinopathy. In: Chan CC, editor. *Animal Models of Ophthalmic Diseases*. Cham: Springer International Publishing, 2016: 67-83 [DOI: 10.1007/978-3-319-19434-9_5]
- 6 **Stitt AW**, Lois N, Medina RJ, Adamson P, Curtis TM. Advances in our understanding of diabetic retinopathy. *Clin Sci (Lond)* 2013; **125**: 1-17 [PMID: 23485060 DOI: 10.1042/CS20120588]
- 7 **Duță I**, Fica S, Ion DA. The Association between Insulin Resistance and Proliferative Retinopathy in Type 1 Diabetes. *Rom J Intern Med* 2015; **53**: 261-266 [PMID: 26710502 DOI: 10.1515/rjim-2015-0034]
- 8 **Kandarakis SA**, Piperi C, Topouzis F, Papavassiliou AG. Emerging role of advanced glycation-end products (AGEs) in the pathobiology of eye diseases. *Prog Retin Eye Res* 2014; **42**: 85-102 [PMID: 24905859 DOI: 10.1016/j.preteyeres.2014.05.002]

- 9 **Stem M**, Boynton G, Thompson A, Khan NW, Jackson GR, Pop-Busui R, Gardner TW. Inner retinal sensory neuropathy in persons with type 1 diabetes mellitus. *Invest Ophthalmol Vis Sci* 2014; **55**: 4426-4426
- 10 **Antonetti DA**, Klein R, Gardner TW. Diabetic retinopathy. *N Engl J Med* 2012; **366**: 1227-1239 [PMID: 22455417 DOI: 10.1056/NEJMr1005073]
- 11 **Kern TS**. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Exp Diabetes Res* 2007; **2007**: 95103 [PMID: 18274606 DOI: 10.1155/2007/95103]
- 12 **Madsen-Bouterse SA**, Kowluru RA. Oxidative stress and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. *Rev Endocr Metab Disord* 2008; **9**: 315-327 [PMID: 18654858 DOI: 10.1007/s11154-008-9090-4]
- 13 **Barber AJ**, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest* 1998; **102**: 783-791 [PMID: 9710447 DOI: 10.1172/JCI2425]
- 14 **Roy S**, Kern TS, Song B, Stuebe C. Mechanistic Insights into Pathological Changes in the Diabetic Retina: Implications for Targeting Diabetic Retinopathy. *Am J Pathol* 2017; **187**: 9-19 [PMID: 27846381 DOI: 10.1016/j.ajpath.2016.08.022]
- 15 **Barber AJ**, Gardner TW, Abcouwer SF. The significance of vascular and neural apoptosis to the pathology of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2011; **52**: 1156-1163 [PMID: 21357409 DOI: 10.1167/iovs.10-6293]
- 16 **Barber AJ**. A new view of diabetic retinopathy: a neurodegenerative disease of the eye. *Prog Neuropsychopharmacol Biol Psychiatry* 2003; **27**: 283-290 [PMID: 12657367 DOI: 10.1016/S0278-5846(03)00023-X]
- 17 **Énzsöly A**, Szabó A, Kántor O, Dávid C, Szalay P, Szabó K, Szél Á, Németh J, Lukáts Á. Pathologic alterations of the outer retina in streptozotocin-induced diabetes. *Invest Ophthalmol Vis Sci* 2014; **55**: 3686-3699 [PMID: 24845643 DOI: 10.1167/iovs.13-13562]
- 18 **Zampetaki A**, Willeit P, Burr S, Yin X, Langley SR, Kiechl S, Klein R, Rossing P, Chaturvedi N, Mayr M. Angiogenic microRNAs Linked to Incidence and Progression of Diabetic Retinopathy in Type 1 Diabetes. *Diabetes* 2016; **65**: 216-227 [PMID: 26395742 DOI: 10.2337/db15-0389]
- 19 **Schorr SG**, Hammes HP, Müller UA, Abholz HH, Landgraf R, Bertram B. The Prevention and Treatment of Retinal Complications in Diabetes. *Dtsch Arztebl Int* 2016; **113**: 816-823 [PMID: 28073426 DOI: 10.3238/arztebl.2016.0816]
- 20 **Ford JA**, Lois N, Royle P, Clar C, Shyangdan D, Waugh N. Current treatments in diabetic macular oedema: systematic review and meta-analysis. *BMJ Open* 2013; **3**: pii: e002269 [PMID: 23457327 DOI: 10.1136/bmjopen-2012-002269]
- 21 **El Rami H**, Barham R, Sun JK, Silva PS. Evidence-Based Treatment of Diabetic Retinopathy. *Semin Ophthalmol* 2017; **32**: 67-74 [PMID: 27700224 DOI: 10.1080/08820538.2016.1228397]
- 22 **Lechner J**, O'Leary OE, Stitt AW. The pathology associated with diabetic retinopathy. *Vision Res* 2017; **139**: 7-14 [PMID: 28412095 DOI: 10.1016/j.visres.2017.04.003]
- 23 **Rajalakshmi R**, Prathiba V, Mohan V. Does tight control of systemic factors help in the management of diabetic retinopathy? *Indian J Ophthalmol* 2016; **64**: 62-68 [PMID: 26953026 DOI: 10.4103/0301-4738.178146]
- 24 **Danilova IG**, Sarapultsev PA, Medvedeva SU, Gette IF, Bulavintceva TS, Sarapultsev AP. Morphological restructuring of myocardium during the early phase of experimental diabetes mellitus. *Anat Rec (Hoboken)* 2015; **298**: 396-407 [PMID: 25251897 DOI: 10.1002/ar.23052]
- 25 **Radenković M**, Stojanović M, Prostran M. Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. *J Pharmacol Toxicol Methods* 2016; **78**: 13-31 [PMID: 26596652 DOI: 10.1016/j.vascn.2015.11.004]
- 26 **Lenzen S**. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 2008; **51**: 216-226 [PMID: 18087688 DOI: 10.1007/s00125-007-0886-7]
- 27 **Szkudelski T**. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res* 2001; **50**: 537-546 [PMID: 11829314]
- 28 **Gelashvily OA**. Variant of periodization of biologically similar stages of human and rat's ontogenesis (in Russian). *Saratov J Med Sci Res* 2008; **4**: 125-126
- 29 **Karpischev AI**. Medical laboratory technology (in Russian). St. Petersburg: Intermedika, 2002
- 30 **Yakovleva GE**. The enzymes in clinical biochemistry (in Russian). Novosibirsk: Vector-Best, 2005
- 31 **Jeppsson JO**, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, Miedema K, Mosca A, Mauri P, Paroni R, Thienpont L, Umemoto M, Weykamp C; International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Approved IFCC reference method for the measurement of HbA1c in human blood. *Clin Chem Lab Med* 2002; **40**: 78-89 [PMID: 11916276 DOI: 10.1515/CCLM.2002.016]
- 32 **Kumar GL**, Rudbeck L. Education guide. Immunohistochemical (IHC) staining methods. California: Dako North America, Carpinteria, 2009: 224. Available from: URL: http://www.kanidis.gr/common/files/ANOSISTOCHIMIA/DETECTION/ihc_staining_methods_5ed.pdf
- 33 **Taylor PR**, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S. Macrophage receptors and immune recognition. *Annu Rev Immunol* 2005; **23**: 901-944 [PMID: 15771589 DOI: 10.1146/annurev.immunol.23.021704.115816]
- 34 **Brochhausen C**, Schmitt VH, Mamilos A, Schmitt C, Planck CN, Rajab TK, Hierlemann H, Kirkpatrick CJ. Expression of CD68 positive macrophages in the use of different barrier materials to prevent peritoneal adhesions-an animal study. *J Mater Sci Mater Med* 2017; **28**: 15 [PMID: 27995493 DOI: 10.1007/s10856-016-5821-3]
- 35 **Weakley BS**. A beginner's handbook in biological transmission electron microscopy. Edinburgh: Churchill Livingstone, 1981: 264
- 36 **Robinson R**, Barathi VA, Chaurasia SS, Wong TY, Kern TS. Update on animal models of diabetic retinopathy: from molecular approaches to mice and higher mammals. *Dis Model Mech* 2012; **5**: 444-456 [PMID: 22730475 DOI: 10.1242/dmm.009597]
- 37 **Capitão M**, Soares R. Angiogenesis and Inflammation Crosstalk in Diabetic Retinopathy. *J Cell Biochem* 2016; **117**: 2443-2453 [PMID: 27128219 DOI: 10.1002/jcb.25575]
- 38 **Park SH**, Park JW, Park SJ, Kim KY, Chung JW, Chun MH, Oh SJ. Apoptotic death of photoreceptors in the streptozotocin-induced diabetic rat retina. *Diabetologia* 2003; **46**: 1260-1268 [PMID: 12898017 DOI: 10.1007/s00125-003-1177-6]
- 39 **Nickla DL**, Wallman J. The multifunctional choroid. *Prog Retin Eye Res* 2010; **29**: 144-168 [PMID: 20044062 DOI: 10.1016/j.pret.2009.12.002]
- 40 **Hua R**, Li Q, Wong IY, Ning H, Wang H. Choroidal microvascular proliferation secondary to diabetes mellitus. *Oncotarget* 2017; **8**: 2034-2036 [PMID: 28008140 DOI: 10.18632/oncotarget.14020]
- 41 **Melancia D**, Vicente A, Cunha JP, Abegão Pinto L, Ferreira J. Diabetic choroidopathy: a review of the current literature. *Graefes Arch Clin Exp Ophthalmol* 2016; **254**: 1453-1461 [PMID: 27109344 DOI: 10.1007/s00417-016-3360-8]
- 42 **Adini I**, Ghosh K, Adini A, Chi ZL, Yoshimura T, Benny O, Connor KM, Rogers MS, Bazinet L, Birsner AE, Bielenberg DR, D'Amato RJ. Melanocyte-secreted fibromodulin promotes an angiogenic microenvironment. *J Clin Invest* 2014; **124**: 425-436 [PMID: 24355922 DOI: 10.1172/JCI69404]
- 43 **Cherepanoff S**, McMenamin P, Gillies MC, Kettle E, Sarks SH. Bruch's membrane and choroidal macrophages in early and advanced age-related macular degeneration. *Br J Ophthalmol* 2010; **94**: 918-925 [PMID: 19965817 DOI: 10.1136/bjo.2009.165563]
- 44 **Aveleira CA**, Lin CM, Abcouwer SF, Ambrósio AF, Antonetti DA. TNF- α signals through PKC ζ /NF- κ B to alter the tight junction complex and increase retinal endothelial cell permeability. *Diabetes*

- 2010; **59**: 2872-2882 [PMID: 20693346 DOI: 10.2337/db09-1606]
- 45 **Nita M**, Grzybowski A, Ascaso FJ, Huerva V. Age-related macular degeneration in the aspect of chronic low-grade inflammation (pathophysiological parainflammation). *Mediators Inflamm* 2014; **2014**: 930671 [PMID: 25214719 DOI: 10.1155/2014/930671]
- 46 **Aizu Y**, Oyanagi K, Hu J, Nakagawa H. Degeneration of retinal neuronal processes and pigment epithelium in the early stage of the streptozotocin-diabetic rats. *Neuropathology* 2002; **22**: 161-170 [PMID: 12416555 DOI: 10.1046/j.1440-1789.2002.00439.x]
- 47 **Rizzolo LJ**. Barrier properties of cultured retinal pigment epithelium. *Exp Eye Res* 2014; **126**: 16-26 [PMID: 24731966 DOI: 10.1016/j.exer.2013.12.018]
- 48 **Grant WM**, Schuman JS. Toxicology of the Eye: Effects on the Eyes and Visual System from Chemicals, Drugs, Metals and Minerals, Plants, Toxins, and Venoms; Also, Systemic Side Effects from Eye med (4th edition). Springfield, Ill., USA: Charles C Thomas Pub Ltd., 1993: 1608
- 49 **Koskenoja M**. Alloxan diabetes in the pregnant mouse. Its effect on the offspring and particularly on their eyes. *Acta Ophthalmol Suppl* 1961; Suppl 68: 1-92 [PMID: 14458651]
- 50 **Emanuelli G**. Retinal ultrastructural alterations induced by alloxan in the rat (In Italian). *Rass Ital Ottalmol* 1964; **33**: 62-70

P- Reviewer: Das U, Koch TR **S- Editor:** Ji FF
L- Editor: A **E- Editor:** Song H



Observational Study

Using real world data to assess cardiovascular outcomes of two antidiabetic treatment classes

Manfred Paul Stapff

Manfred Paul Stapff, CMO, TriNetX Inc., Cambridge, MA 02140, United States

ORCID number: Manfred Paul Stapff (0000-0002-6127-1109).

Author contributions: Stapff MP developed the scientific concept, literature search, study design, applied the data querying, result interpretation, scientific discussion, and prepared the manuscript.

Institutional review board statement: As a federated network TriNetX received a waiver from Western IRB since only aggregated counts, statistical summaries of de-identified information, but no protected health information is received, and no study specific activities are performed in retrospective analyses.

Informed consent statement: This was an observational study based on analyses of anonymized electronic medical records describing real world treatment. No intervention or any study specific activity was done. Therefore, no informed consent was necessary and would even have been not feasible considering the anonymized and retrospective character of the analysis.

Conflict-of-interest statement: The author is employee of TriNetX Inc., the data network and analytics platform used for this publication. TriNetX as a company was not involved in the design of the study; the collection, analysis, and interpretation of data; writing the report; or the decision to submit the report for publication. The author does not declare conflicting interests (including but not limited to commercial, personal, political, intellectual, or religious interests).

STROBE statement: The author has read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on

different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Corresponding author to: Manfred Paul Stapff, MD, PhD, Chief Medical Officer, CMO, TriNetX Inc., 125 Cambridgepark Drive, Ste 500, Cambridge, MA 02140, United States. manfred.stapff@trinetx.com
Telephone: +1-857-2856043

Received: August 22, 2018

Peer-review started: August 22, 2018

First decision: October 4, 2018

Revised: October 10, 2018

Accepted: November 15, 2018

Article in press: November 15, 2018

Published online: December 15, 2018

Abstract

AIM

To evaluate the effect on cardiovascular outcomes of sodium-glucose co-transporter-2 (SGLT2) inhibitors in a real world setting by analyzing electronic medical records.

METHODS

We used TriNetX, a global federated research network providing statistics on electronic health records (EHR). The analytics subset contained EHR from approximately 38 Million patients in 35 Health Care Organizations in the United States. The records of 46,909 patients who had taken SGLT2 inhibitors were compared to 189,120 patients with dipeptidyl peptidase (DPP) 4 inhibitors. We identified five potential confounding factors and built respective strata: elderly, hypertension, chronic kidney disease (CKD), and co-medication with either insulin or metformin. Cardiovascular events were counted

as stroke (ICD10 code: I63) or myocardial infarction (ICD10: I21) occurring within three years after the first instance of the respective medication in the patients' records.

RESULTS

Of the 46909 patients with SGLT2 inhibitors in their EHR, 1667 patients (3.6%) had an ICD code for stroke or for myocardial infarction within the first three years after the first instance of the medication. In the control group, there were 10680 events of 189120 patients (5.6%), which represents a risk ratio of 0.63 (95%CI: 0.60-0.66). The overall incidence of stroke or myocardial infarction in the strata with a potential confounding risk factor reached from 4.9% in patients taking metformin to 12.5% in the stratum with the highest risk (concomitant CKD). In all strata, the difference in risk of experiencing a cardiovascular event was similarly in favor of SGLT2 *vs* control, with Risk Ratio ranging from 0.62 to 0.81.

CONCLUSION

Real world data replicated the results from randomized clinical trials, confirmed the cardiovascular advantages of SGLT2 inhibitors, and showed its applicability to the US population.

Key words: Sodium-glucose co-transporter-2 inhibitors; Cardiovascular events; Clinical trials; Electronic medical records; Dipeptidyl peptidase 4 inhibitors; Real world evidence; Diabetes

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Cardiovascular advantages of sodium-glucose co-transporter-2 (SGLT2) inhibitors were shown in complex clinical trials or in countries with large registries. However, it was unclear whether these findings could be applied to routine medical practice in the US. This real world analysis from 46909 patients with SGLT2 inhibitors revealed a 0.63 (95%CI: 0.60-0.66) risk ratio of SGLT2 inhibitors compared to 189120 patients with dipeptidyl peptidase 4 inhibitors. This analysis of electronic health records could replicate the results of randomized clinical trials, which supports the usefulness of such real world studies (*e.g.*, for long-term outcome or safety observations).

Stapff MP. Using real world data to assess cardiovascular outcomes of two antidiabetic treatment classes. *World J Diabetes* 2018; 9(12): 252-257

URL: <https://www.wjgnet.com/1948-9358/full/v9/i12/252.htm> DOI: <https://dx.doi.org/10.4239/wjd.v9.i12.252>

INTRODUCTION

An estimated 30.3 million people of all ages (or 9.4% of

the United States population) had diabetes in 2015^[1]. It is expected that the world prevalence of diabetes among adults will increase to 7.7%, or 439 million adults, by 2030. Between 2010 and 2030, there will be a 69% increase in the number of adults with diabetes in developing countries, and a 20% increase in developed countries^[2].

While short-term treatment targets focus on the normalization of values for glucose and hemoglobin A1c, the long-term objective is to avoid late-stage complications of diabetes and end-organ damage. Up to 70% of patients with diabetes type II (T2DM) also have arterial hypertension^[1] and are thus exposed to an increased risk of experiencing a stroke or heart attack. It is therefore important that treatment paradigms for T2DM consider the long-term cardiovascular risk.

In 2015, the EMPA-REG OUTCOME trial found a significant mortality benefit of sodium-glucose co-transporter-2 (SGLT2) inhibitors *vs* placebo^[3]. Because the findings were unexpected, unprecedented and not linked to obvious mechanistic pathways, it was suggested that the results be replicated in future investigations^[4]. Recently, CVD-REAL Nordic, a multinational observational study, analyzed the cardiovascular mortality and morbidity in patients with T2DM following initiation SGLT2 inhibitors^[5]. CVD-REAL Nordic was an observational analysis of individual patient-level data from national registries in three Scandinavian countries, showing that SGLT2 inhibitor use was associated with reduced cardiovascular disease and cardiovascular mortality.

The objective of the following analysis was to support or contradict the results of EMPA-REG OUTCOME and CVD-REAL Nordic by using electronic medical records (EMR) from a predominately United States-based research network, thus evaluating the representativity of these results outside the experimental setting of a randomized clinical trial and beyond a European population, respectively.

MATERIALS AND METHODS

We used TriNetX, a global federated research network providing access to statistics on EMR (diagnoses, procedures, medications, laboratory values, genomic information). The analytics subset allowed the analysis of approximately 38 million patients in 35 large Health Care Organizations predominately in the United States. As a federated network, TriNetX received a waiver from Western IRB, since only aggregated counts, statistical summaries of de-identified information, and no protected health information is received. In addition, no study-specific activities are performed in retrospective analyses. Details of the network have been described elsewhere^[6-8]. All analyses were done in the TriNetX "Analytics" network using the browser-based real-time analytics features. At the time of the analysis in June 2018, we analyzed the EMR of 46909 patients in the network who had an instance of any SGLT2 inhibitor

Table 1 Patient characteristics and results before correcting for potential confounding factors

	SGLT2	Control
<i>n</i>	46909	189120
Mean age	59	66
SD age	11	13
Percent male	53%	52%
Comorbidities		
Hypertension (I10)	45%	41%
CKD (N18)	4%	8%
Co-medication		
On insulin	32%	19%
On metformin	52%	33%
LDL cholesterol (mg/dL)	91.6	93.1
HDL cholesterol (mg/dL)	43.6	43.2
After index event		
Total stroke (I63) or MI (I21)	12347 (5.2%)	
<i>n</i> in group	1667	10680
Percent in group	3.60%	5.60%
RR SGLT2 vs control	0.63	

SGLT2: Sodium-glucose co-transporter-2; RR: Risk ratio; SD: Standard deviation; CKD: Chronic kidney disease; LDL: Low density lipoprotein; HDL: High density lipoprotein; MI: Myocardial infarction.

(empagliflozin, dapagliflozin or canagliflozin) any time within the past ten years in their electronic medical record. As a comparison group, we chose patients who had taken dipeptidyl peptidase (DPP) 4 inhibitors (linagliptin, alogliptin, sitagliptin or saxagliptin) during the same time, and found 189120 patients. Using a Bayesian statistical approach^[9] on demographics and pre-existing (baseline) comorbidities of the two groups, we identified five potential confounding factors and built strata with the following criteria: age \geq 60 years, presence of hypertension [International Classification of Diseases (ICD)10 code I10], presence of CKD (ICD10 code N18), co-medication with insulin, and co-medication with metformin. Separately analyzing strata allowed us to address potential bias in the federated data model without direct access to the individual data sets on the patient level.

Cardiovascular events were counted by selecting any stroke (ICD10 code I63) or myocardial infarction (ICD10 code I21) occurring during a three-year observation period after the first instance of the above mentioned medications in the patients' records.

The risks of experiencing an event in each stratum were calculated by dividing the number of patients with an event (numerator) by the total number of patients with the respective medication in each stratum (denominator). The risk ratios for SGLT2 inhibitors vs the comparison group were calculated by dividing the risk for each SGLT2 stratum by the risk in each corresponding DPP4 stratum.

RESULTS

Of the 46909 patients taking SGLT2 inhibitors, 1667 patients (3.6%) had an ICD code for stroke or myo-

cardial infarction during their three-year observation period, compared to 10680 of 189120 (5.6%) in the control group (Table 1). This translates into a risk ratio of 0.63 without any correction for potential bias ($P < 0.001$; 95%CI: 0.60-0.66).

SGLT2 inhibitors carry a contra-indication for renal insufficiency^[10]. Indeed, the percentage of patients with CKD was only 4% in the SGLT2 group, compared to 8% in the control group. While the groups were similar in gender distribution (53% and 52% male, respectively) and low density lipoprotein, as well as high density lipoprotein levels, the SGLT2 group was younger than the control group (mean age 59 vs 66) and had more patients with concomitant hypertension (45% vs 41%). There were also differences in the use of insulin (32% vs 19%) and metformin (52% vs 33%). To balance for these potential confounding factors, strata were built for age \geq 60 years, CKD, hypertension, and antidiabetic co-medication (insulin and metformin). The overall incidence of stroke or myocardial infarction in each stratum reached from 4.9% to 12.5%. In all strata, the difference in the risk of experiencing a cardiovascular event in the SGLT2 group vs control was similarly in favor of SGLT2, with risk ratios ranging from 0.62 (co-medication insulin) to 0.81 (patients with CKD) (Table 2).

DISCUSSION

Drug therapy of type II diabetes mellitus should both bring glucose and hemoglobin A1c values into an acceptable and stable range, and reduce the likelihood of end organ damage or cardiovascular events.

Several studies and meta-analyses have suggested a positive effect on cardiovascular outcomes by the SGLT2 inhibitor class^[11,12]. EMPA-REG OUTCOME and CANVAS were randomized placebo controlled prospective trials that used empagliflozin^[4] and canagliflozin^[13], respectively.

A recent observational cohort study observed protective effects of SGLT2 inhibitors compared to sulfonylureas by a database analysis^[14]. Another study, CVD-REAL Nordic, was the first large observational analysis performed in real world settings in three Scandinavian countries that evaluated the cardiovascular benefits of this class, which also showed that SGLT2 inhibitor use was associated with reduced cardiovascular disease and cardiovascular mortality compared with the use of other glucose-lowering drugs^[4]. Such real-world studies are less complicated and significantly less costly than traditional prospective randomized clinical outcomes trials. In addition, the reduced number of eligibility criteria ensures that the study results are representative and applicable to a much wider population. Recently, another study confirmed that real-world data analyses of patients receiving routine care provide findings similar to those found in a randomized clinical trial, and may even support (supplemental) regulatory applications^[15]. Real world evidence can sometimes complement or

Table 2 Results from the patient subgroups (strata) with potential confounding factors

	Stratum 1 > 60 yr		Stratum 2 hypertension		Stratum 3 CKD		Stratum 4 insulin		Stratum 5 metformin	
	SGLT2	control	SGLT2	control	SGLT2	control	SGLT2	control	SGLT2	control
<i>n</i>	23594	131219	27499	115703	3786	34388	24395	90978	37762	136569
patients with stroke or MI	9784 (6.3%)		10827 (7.6%)		4755 (12.5%)		8976 (7.8%)		8629 (4.9%)	
<i>n</i> in group	1077	8707	1452	9375	391	4364	1275	7701	1394	7235
percent in group	4.60%	6.60%	5.30%	8.10%	10.30%	12.70%	5.20%	8.50%	3.70%	5.30%
RR SGLT2 vs control	0.69		0.65		0.81		0.62		0.7	

SGLT2: Sodium-glucose co-transporter-2; MI: Myocardial infarction; RR: Risk ratio; CKD: Chronic kidney disease.

even replace randomized controlled trials, but prejudices and reservations so far have limited their acceptance^[16].

Therefore, the underlying data sources must be reliable, and the methods used have to be defined in advance to avoid "data dredging" based on the findings^[17]. Furthermore, the data usually come from non-consented patients and therefore the highest standards of data privacy must be ensured.

The present study was undertaken to evaluate whether the results of the EMPA-REG OUTCOME and CVD-REAL Nordic studies can be replicated in a federated network of EHR, and if they can be applied to a predominantly United States American population. As controls, we chose DPP4 inhibitors that represent another homogeneous and relatively new non-metformin class. We found a significantly lower incidence of stroke or myocardial infarction in the SGLT2 group within the three-year observational period compared with the control group.

In a federated data network, individual data sets never leave the source (*i.e.*, the data warehouse of a healthcare organization). Instead, the analyses are done based on aggregated statistical counts. At the time of this analysis, our platform limited the methods that could be applied to correct for potential confounding factors, such as pair matching or propensity score matching (PSM). While PSM is a popular method of preprocessing data for causal inference, it is controversial since it may accomplish the opposite of its intended goal, such as increasing imbalance or bias^[18]. In addition, the censoring by PSM that excludes certain patients from the analysis, reduces the sample size and the representation of a diverse patient population, thus re-introducing the criticism often applied to randomized clinical trials regarding their very restrictive eligibility criteria.

We therefore chose to build subgroups of the study population according to the presence of potentially confounding factors, and to test these strata individually. SGLT2 inhibitors have a contraindication for renal insufficiency and are a relatively new class of antidiabetics with less long-term experience than comparator classes, such as metformin or DPP4 inhibitors. One can therefore assume that the treatment decision by prescribing physicians may be driven by a patient's renal function, patient age, and other potential risk factors. Indeed, we found a lower mean

age in the SGLT2 group, similar to CVD-REAL Nordic before matching. Furthermore, the SGLT2 group had fewer patients with CKD than the comparison group. In prospective randomized clinical trials, such factors usually get balanced by randomization, which must be corrected for when a retrospective analysis is done. We therefore created five strata, based on age ≥ 60 years, hypertension, CKD, insulin therapy or metformin therapy, and tested the event rates individually in each of these subgroups. The fact that the overall highest event rate was found in the higher risk stratum (patients with CKD) provides internal validation for the selection of the strata.

All strata showed very similar hazard ratios for cardiovascular events (according to our definition using ICD10 codes for myocardial infarction or stroke), which were consistently in favor of the SGLT2 inhibitor group, *i.e.*, between 0.62 and 0.81. This generally confirms the findings of the CVD-REAL Nordic study, where the risk ratio for cardiovascular mortality and for major cardiovascular events was in a similar range of 0.53 and 0.78, respectively.

Limitations

Due to the nature of the design (retrospective, non-randomized) and data analysis (federated, aggregated strata), this study could be done very quickly, simplistically and with minimal cost, but may have several limitations. Non-randomized comparisons bear the risk that patients' disease state influence the treatment decision and thus introduce imbalances. We limited balancing for confounders to five major factors and did not further correct for residual, potentially confounding factors like other co-morbidities, duration of diabetes, glucose or HbA1c values, concomitant medications or length of exposure to concomitant treatment. Our outcome criteria were simply the ICD10 codes for myocardial infarction or stroke, relying on correct coding at the source without differentiation between morbidity and mortality. Despite the fact that one specific compound numerically dominated in each group (SGLT2: canagliflozin 78%, DPP4: sitagliptin 69%), we consider the results as representative of a class but not robust enough for a comparison of two individual compounds.

Real world studies depend on the prescribing and documentation behavior of the data-providing institutions. We used EHR in structured form rather than

Table 3 Data density in the two comparator cohorts

	SGLT2	Control
Total facts	54852092	261813664
Avg facts per patient	1143	1325
Avg diagnosis facts per patient	231	262
Eastern United States, patients (%)	68	69
Western United States, patients (%)	32	31

SGLT2: Sodium-glucose co-transporter-2; Avg: Average.

claims data. This has the advantage of complete medical information coming from the respective Health Care Organization, but data may be lacking if a patient visits another institution. This especially applies to medication and prescription refills. While we defined an observational period of three years, we could not validate whether the patients actually stayed with their medication for the whole period, as we defined the treatment group based on one documentation of SGLT2 or DPP4 in their records. Insofar as a difference in compliance or persistence between the groups could introduce a potential imbalance, the approach would be similar to the intent-to-treat principle, which is applied to randomized clinical trials.

Furthermore, differences in the completeness of medical records between comparison groups need to be taken into consideration as well. In searching for a potential documentation bias, we found similar data density in the SGLT2 cohort compared to control (Table 3).

Theoretically, one could assume that more events had been found in the control group simply because this patient cohort was better documented. In real world studies, consideration of different therapeutic settings and documentation completeness is important, e.g., when comparing oral vs injectable medication, or inpatient vs outpatient procedures. However, SGLT2 inhibitors and DPP4 inhibitors are both taken orally and prescribed in similar settings. In addition, our data overall found about 20% more events in the DPP4 group, but the density of facts per patient in the documentation of this group was only 6% higher. Therefore, a documentation bias as an explanation for the difference in CV events in this study is very unlikely.

In conclusion, this study was conducted by analyzing EHR of approximately 38 million patients from 35 health-care organizations, mainly from the United States. This real world clinical setting allows the analysis of data from patients with a much broader cardiovascular risk profile than the highly selective population in randomized clinical trials. The federated structure of this network ensures the highest level of data privacy standards, but poses some restrictions on the possible analytics, such as matching by propensity scores. Despite these limitations: (1) this analysis could replicate the results from much more complex and costly studies on the same topic, which validates our methods and the quality of data in the network; (2) our analysis shows that the cardiovascular advantages of SGLT2 inhibitors found

in the Scandinavian CVD-REAL Nordic study can be applied to the United States American population.

ARTICLE HIGHLIGHTS

Research background

Therapy for diabetes mellitus intends to control blood glucose values, to prevent or delay diabetic complications such as chronic kidney disease or retinopathy, and to reduce the likelihood of cardiovascular events like myocardial infarction or stroke. Several randomized clinical trials and sophisticated European registries have suggested that sodium-glucose co-transporter-2 (SGLT2) inhibitors may have an advantage in preventing cardiovascular events.

Research motivation

Randomized clinical trials are conducted on highly selected patient populations and follow very artificial treatment protocols. This makes it sometimes questionable whether the results are representative and can be applied to routine medical practice.

Research objectives

To determine whether positive results from randomized clinical trials with SGLT2 inhibitors can be confirmed by real world data from actual routine medical practice in the United States.

Research methods

A federated research network was used, allowing analyses of electronic medical records (EMR) from 38 million patients in 35 large Health Care Organizations predominately in the United States. Cardiovascular events occurring during a three-year observation period after start of a therapy with an SGLT2 inhibitor were counted and compared to a control group starting dipeptidyl peptidase 4 inhibitors. Comorbidity strata were created to address potential confounders.

Research results

In the overall cohort and in all comorbidity strata, the risk of experiencing a cardiovascular event was similarly in favor of SGLT2, with risk ratios ranging from 0.62 to 0.81.

Research conclusions

The analysis of data from patients with a much broader cardiovascular risk profile than the selected population in randomized clinical trials could replicate the results of such trials. This validates the methods and quality of data in the network, and allows extrapolation of the trial results to the general patient population.

Research perspectives

Sophisticated analyses of high quality EMR can complement costly, complex and lengthy randomized clinical trials, can assess their representativity for actual medical practice in the real world, and may even, in certain instances, be able to replace them.

REFERENCES

- 1 **Centers for Disease Control and Prevention.** National Diabetes Statistics Report. 2017. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services; 2017 Available from: URL: <https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf>
- 2 **Cinek O,** Kramna L, Mazankova K, Odeh R, Allassaf A, Ibekwe MU, Ahmadov G, Elmahi BME, Mekki H, Lebl J, Abdullah MA. The bacteriome at the onset of type 1 diabetes: A study from four geographically distant African and Asian countries. *Diabetes Res Clin Pract* 2018; **144**: 51-62 [PMID: 30121305 DOI: 10.1016/j.diabetes.2018.08.010]
- 3 **Bhatt AS.** Digesting New Developments in Biosensors. *N Engl J Med* 2018; **379**: 686-688 [PMID: 30110595 DOI: 10.1056/NEJMcibr1806952]

- 4 **Kaul S.** Is the Mortality Benefit With Empagliflozin in Type 2 Diabetes Mellitus Too Good To Be True? *Circulation* 2016; **134**: 94-96 [PMID: 27400894 DOI: 10.1161/CIRCULATIONAHA.116.022537]
- 5 **Birkeland KI,** Jørgensen ME, Carstensen B, Persson F, Gulseth HL, Thuresson M, Fenici P, Nathanson D, Nyström T, Eriksson JW, Bodegård J, Norhammar A. Cardiovascular mortality and morbidity in patients with type 2 diabetes following initiation of sodium-glucose co-transporter-2 inhibitors versus other glucose-lowering drugs (CVD-REAL Nordic): a multinational observational analysis. *Lancet Diabetes Endocrinol* 2017; **5**: 709-717 [PMID: 28781064 DOI: 10.1016/S2213-8587(17)30258-9]
- 6 **Stacey J,** Mehta M. Using EHR Data Extraction to Streamline the Clinical Trial Process. *Clinical Researcher* 2017; **4**: 2-7 [DOI: 10.14524/CR-17-0004]
- 7 **Stapff M.** Use of Electronic Health Data in Clinical Development. Pharm. Ind. **79**, Nr. 2, 204-210. ECV Editio Cantor Verlag, Aulendorf, Germany (2017) Available from: <https://www.trinetx.com/wp-content/uploads/2018/05/Use-of-Electronic-Health-Data-in-Clinical-Development.pdf>
- 8 **Stapff M.** Use of Electronic Health Records for Development and Feasibility Testing of Clinical Trial Protocols. DIA 28th EuroMeeting, April 6-8, 2016, Hamburg, Germany Available from: https://www.diaglobal.org/productfiles/4124219/16101_pgm.pdf
- 9 **Oliphant TE.** A Bayesian perspective on estimating mean, variance, and standard-deviation from data. *Faculty Publications* 2006; 278 Available from: URL: <http://hdl.lib.byu.edu/1877/438>
- 10 Prescribing information canagliflozin 07/2017, Available from: URL: <https://www.invokana.com/prescribing-information.pdf>
- 11 **Sonesson C,** Johansson PA, Johnsson E, Gause-Nilsson I. Cardiovascular effects of dapagliflozin in patients with type 2 diabetes and different risk categories: a meta-analysis. *Cardiovasc Diabetol* 2016; **15**: 37 [PMID: 26895767 DOI: 10.1186/s12933-016-0356-y]
- 12 **Wu JH,** Foote C, Blomster J, Toyama T, Perkovic V, Sundström J, Neal B. Effects of sodium-glucose cotransporter-2 inhibitors on cardiovascular events, death, and major safety outcomes in adults with type 2 diabetes: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* 2016; **4**: 411-419 [PMID: 27009625 DOI: 10.1016/S2213-8587(16)00052-8]
- 13 **Neal B,** Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erond N, Shaw W, Law G, Desai M, Matthews DR; CANVAS Program Collaborative Group. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N Engl J Med* 2017; **377**: 644-657 [PMID: 28605608 DOI: 10.1056/NEJMoa1611925]
- 14 **Matsuda H,** Mullapudi ST, Yang YHC, Masaki H, Hesselson D, Stainier DYR. Whole-Organism Chemical Screening Identifies Modulators of Pancreatic β -Cell Function. *Diabetes* 2018; **67**: 2268-2279 [PMID: 30115653 DOI: 10.2337/db18-1493-P]
- 15 **Fralick M,** Kesselheim AS, Avorn J, Schneeweiss S. Use of Health Care Databases to Support Supplemental Indications of Approved Medications. *JAMA Intern Med* 2018; **178**: 55-63 [PMID: 29159410 DOI: 10.1001/jamainternmed.2017.3919]
- 16 **Franklin JM,** Schneeweiss S. When and How Can Real World Data Analyses Substitute for Randomized Controlled Trials? *Clin Pharmacol Ther* 2017; **102**: 924-933 [PMID: 28836267 DOI: 10.1002/cpt.857]
- 17 **Berger ML,** Sox H, Willke RJ, Brixner DL, Eichler HG, Goettsch W, Madigan D, Makady A, Schneeweiss S, Tarricone R, Wang SV, Watkins J, Daniel Mullins C. Good practices for real-world data studies of treatment and/or comparative effectiveness: Recommendations from the joint ISPOR-ISPE Special Task Force on real-world evidence in health care decision making. *Pharmacoepidemiol Drug Saf* 2017; **26**: 1033-1039 [PMID: 28913966 DOI: 10.1002/pds.4297]
- 18 **King G,** Nielsen R. Why propensity scores should not be used for matching. 2016 Available from: <http://gking.harvard.edu/files/gking/files/psnot.pdf>. Accessed June 28, 2018

P- Reviewer: Avtanski D, Senol MG **S- Editor:** Dou Y
L- Editor: Filipodia **E- Editor:** Song H





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <https://www.f6publishing.com/helpdesk>
<https://www.wjgnet.com>

