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REVIEW

Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment

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Abstract

Cardiac autonomic neuropathy (CAN) is a serious complication 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, dihomo-γ-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

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.

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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 Microalbuminunia (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 n 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; Gastrointestinal, 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: Excercise 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 unstability	↑ 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 my 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 managment 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 of 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 lability

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 QTi 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-operatory 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), [^{11}C]-meta-hydroxyephedrine (HED), 6-[^{18}F] dopamine, and [^{11}C]-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 [^{11}C]-epinephrine parallels those of [^{11}C]-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)
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	Adrenal insufficiency 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 paradoxic orthostatic bradycardia syndrome and the vasoconstrictor syndrome (paradoxic 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 B_{12}, 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 (baroreflex 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 B_1; 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 glycose 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 simmetrial 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 evolucumab 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 (mipomersen *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 dihomo-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 autoxidation 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 $^{\bullet}O_2^-$ 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]. Itra- and intermolecular 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, ubichinol, and uric acid or derivatives of the diet such as vitamins A, C and E, carotenoids, lipoic acid (LA); coenzyme Q_{10} (CoQ_{10}); and cofactors like albumin, vitamins B_1, B_3, 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, B_1, B_3, 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 B_1: 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 B_3): 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 B_3 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 H_2O_2 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]. CoQ_{10} 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 CoQ_{10} in the body. "Total CoQ_{10}" refers to the sum of both forms, since CoQ_{10} 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 CoQ_{10} within the electron transport chain, where it transports electrons from complex Ⅰ and Ⅱ to complex Ⅲ. CoQ_{10} 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 CoQ_{10} is often seen in patients with T2DM[171]. CoQ_{10} 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 CoQ_{10} (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 H_2O_2 and potentially act as an anticarcinogen (as noted in human lymphocytes at least)[171].

Deficiency in CoQ_{10} is often present among patients with T2DM due to various reasons. As a potent antioxidant, CoQ_{10} 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 CoQ_{10} could potentially alleviate OS, preserve mitochondrial function, and eventually lead to improvement of glycemic control[171]. In DM, CoQ_{10} has been reported to show promising therapeutic potential[171]. The standard dose for CoQ_{10} 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 VO_2max, 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 israpidine 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 neurvorum 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 A_2 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.

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Basic Study

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

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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 [^{3}H]-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

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 (H_2O_2) 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 μmol/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 (*n*)	14.1 ± 1.3 (7)
Cell lipid content/glucose uptake assay, mg (*n*)	15.9 ± 1.3 (10)
Lipolytic responsiveness (fold increase over basal glycerol release, *n* = 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, *n* = 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). [^{3}H]-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 stimulation.

For hexose uptake assays, incubations of the tested agents with fat cell suspensions lasted 45 min at 37 ℃ before 10 min exposure to 0.1 mmol/L [^{3}H]-2-deoxyglucose (2-DG) as previously described[19]. Separation of internalized hexose was performed on 200 μL aliquots by centrifugation through dynonylphtalate 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 ℃ under 5% CO_2 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

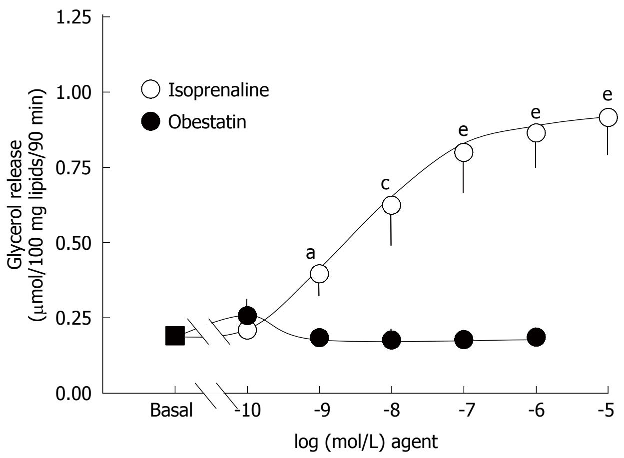

Figure 1 Effects of isoprenaline and of obestatin on lipolysis activation in human adipocytes. Fat cells were incubated for 90 min with isoprenaline (open circles) or obestatin (closed circles) at the indicated concentrations. Mean ± SEM of 7 experiments. In several occurrences, SEM bar lies within the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

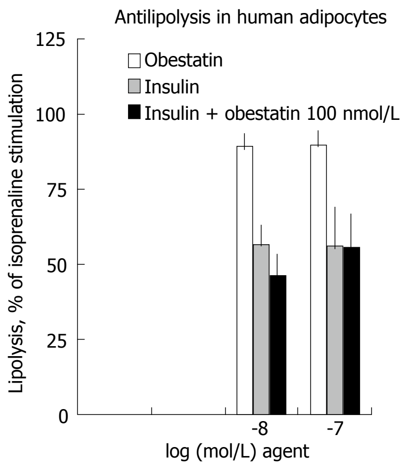

Figure 2 Effects of obestatin and of insulin on isoprenaline-induced lipolysis in human adipocytes. The submaximal stimulation of lipolysis by 5 nmol/L isoprenaline was set at 100 % (control) and determined in the presence of indicated doses of obestatin (open columns), insulin (shaded columns), or the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

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 [^{3}H]-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.

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 µmol/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

	n	Treatment	Control	Obestatin 1 nmol/L	Obestatin 10 nmol/L	Obestatin 100 nmol/L	UK14304 1 μmol/L
Lipolysis, μmol glycerol/ 100 mg lipid/90 min	3	Isoprenaline	0.64 ± 0.10	0.61 ± 0.09	0.62 ± 0.10	0.62 ± 0.10	0.29 ± 0.04[a]
Glucose transport, nmol 2-DG/100 mg lipids/10 min	10	Insulin	0.46 ± 0.14	0.45 ± 0.09	0.45 ± 0.09	0.43 ± 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 ± SEM. Different from corresponding control at: [a]P < 0.05. ND: Not determined.

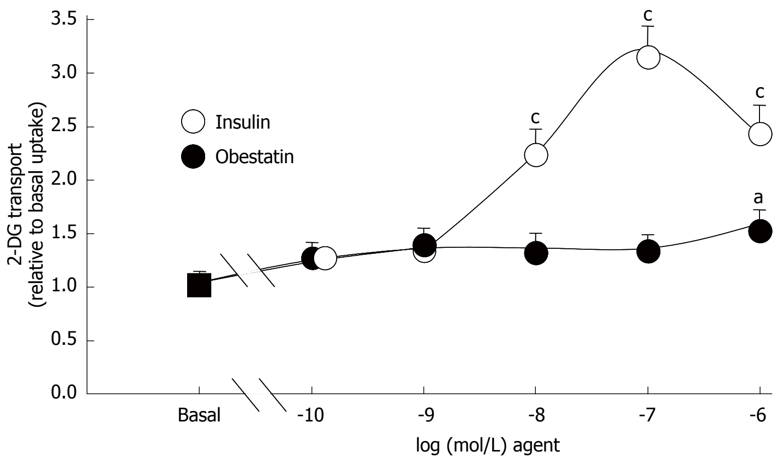

Figure 3 Effects of obestatin and insulin on glucose transport in human adipocytes. 2-deoxyglucose uptake (2-DG) was measured after 45-min incubation without (basal) and with the indicated doses of human insulin (open circles), or obestatin (closed circles). Mean ± SEM of 10 experiments. Statistically 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 μmol/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 μmol/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 μmol/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 μmol/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 μmol/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_2-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 characterization 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 prediabetic 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_2-adrenergic agonist bromoxidine surpasses the insulin-induced inhibition of lipolysis in human fat cells. At 1 μmol/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.

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ORIGINAL ARTICLE

Observational Study

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

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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.

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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/m^2, and 139.4 ± 19.4 mmHg respectively. Fifty-six percent were females, 85% were whites, 15% were blacks, 18% were smokers. The mean ± 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

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/wjd.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 ± 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 use 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/m^2, 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 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 ⩾ 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 polyneuropathies 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

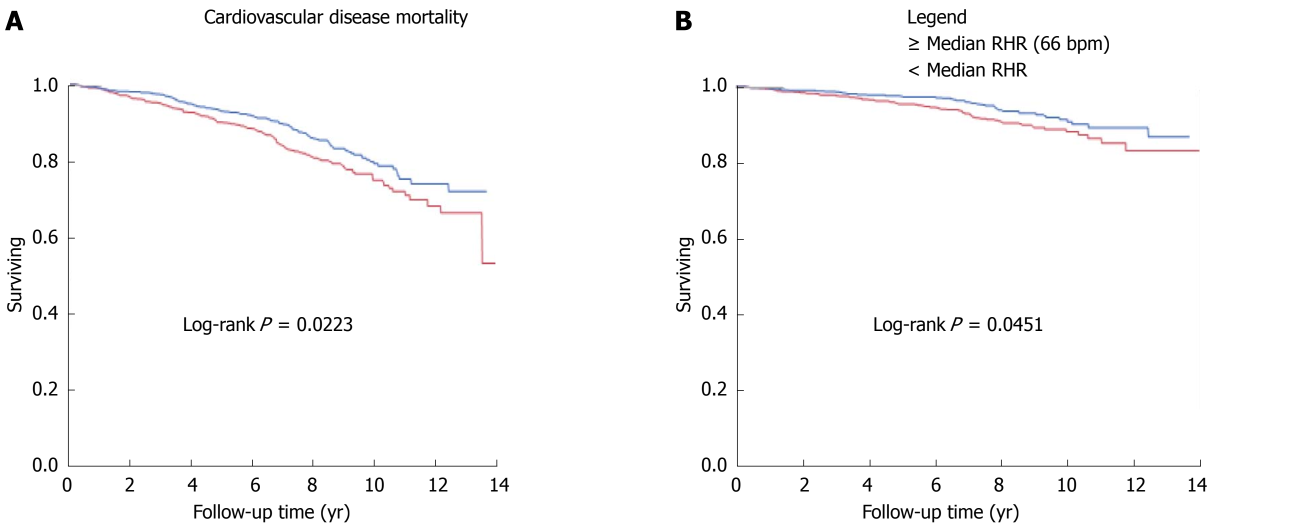

Figure 1 Result of Kaplan Meier curves. A: Kaplan Meier curves showing the Mortality free- survival of Type-Diabetics with resting heart rate (RHR) above and below the median (Median RHR = 69 bpm) in the Diabetes Heart Study; B: Kaplan Meier curves showing the Cardiovascular disease Mortality free- survival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

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META-ANALYSIS

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

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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

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.

Babu GR, Murthy GVS, Ana Y, Patel P, Deepa R, Benjamin-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 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 BMI (≥ 30) Waist-hip ratio (> 0.80 for females and > 0.90 for males) Waist circumference (≥ 90 cm, > 88 cm for female and > 102 cm for male)	Hypertension (JNC VII criteria) SBP greater than or equal to 140 mmHg or DBP greater than or equal to 90 mmHg respectively	T2DM 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)

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% × (Q - df)/Q, where Q is Cochrane'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

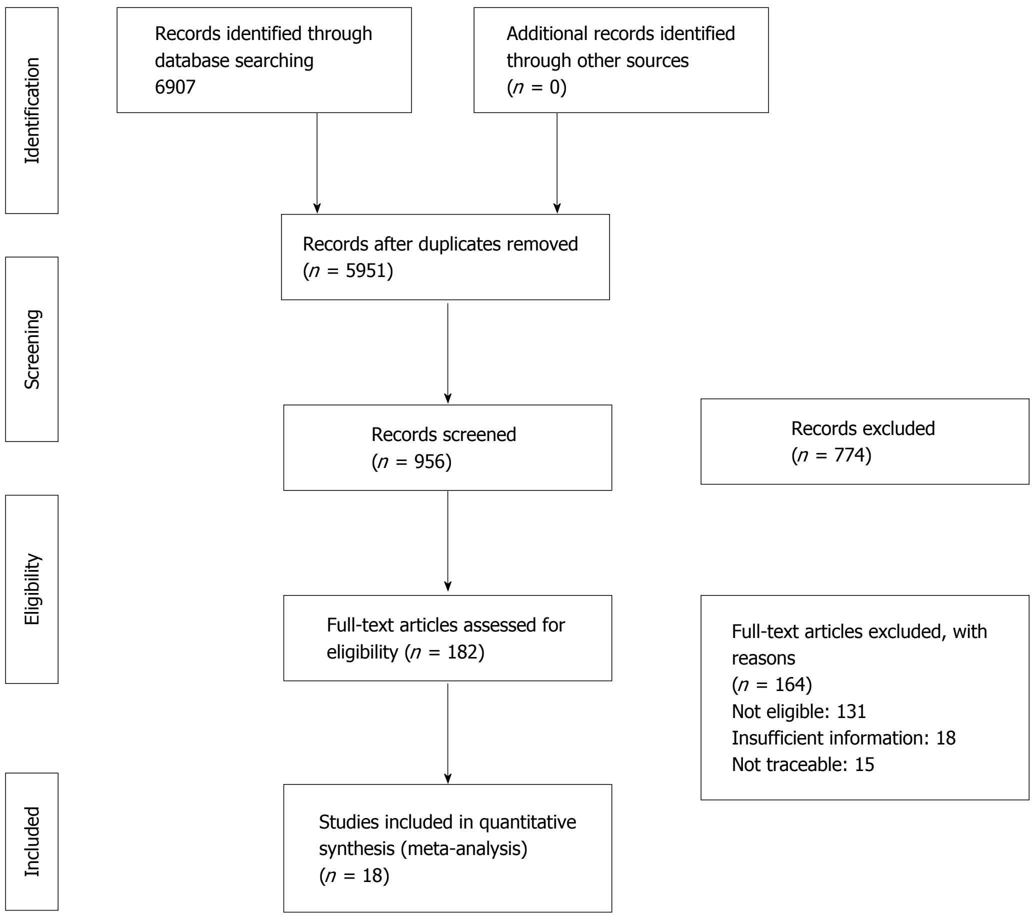

Figure 1 Preferred reporting Items for systematic reviews and meta-analysis study flow diagram.

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 *et al*[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 *et al*[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 *et al*[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 *et al*[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 *et al*[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 *et al*[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%

[1]Important 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 mm Hg 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 *et al*[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 *et al*[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 *et al*[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 *et al*[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 *et al*[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 *et al*[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 *et al*[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 *et al*[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 *et al*[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 *et al*[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 *et al*[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%
Vijayakumar *et al*[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%

[1]Important 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.

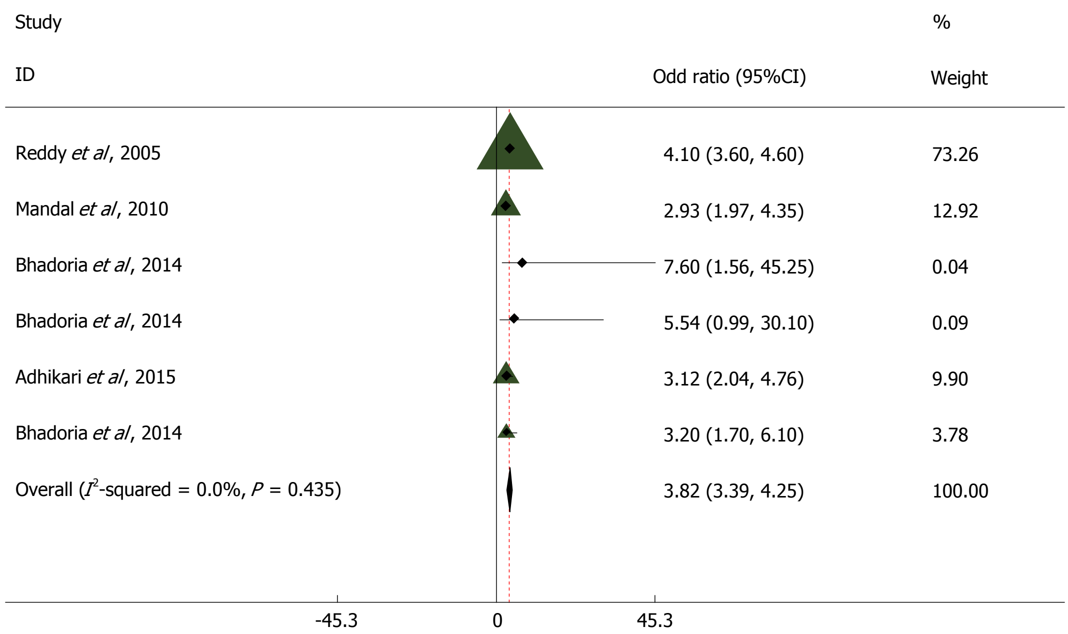

Figure 2 Meta-analysis of studies exploring association between obesity and hypertension in India.

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 under-valued. 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

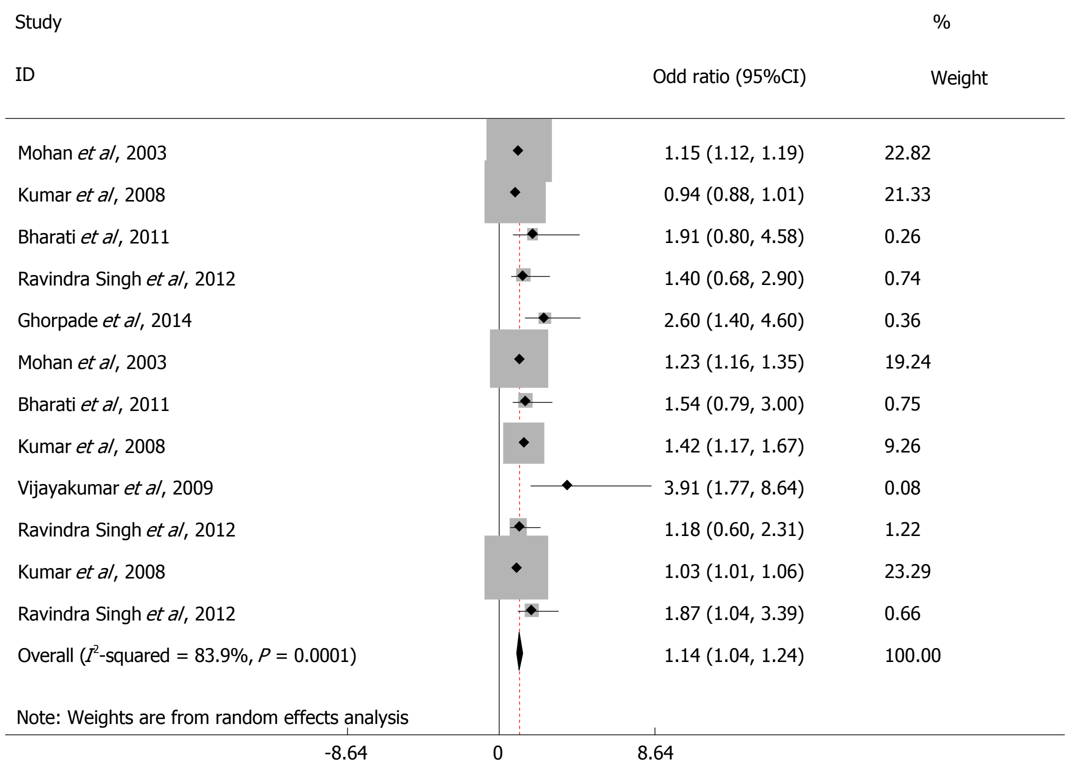

Figure 3 Meta-analysis of studies exploring association between obesity and type 2 diabetes mellitus in India.

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 route 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 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.

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