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REVIEW

Insulin resistance is associated with subclinical vascular disease in humans

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Abstract

Insulin resistance is associated with subclinical vascular disease that is not justified by conventional cardiovascular risk factors, such as smoking or hypercholesterolemia. Vascular injury associated to insulin resistance involves functional and structural damage to the arterial wall that includes impaired vasodilation in response to chemical mediators, reduced distensibility of the arterial wall (arterial stiffness), vascular calcification, and increased thickness of the arterial wall. Vascular dysfunction associated to insulin resistance is present in asymptomatic subjects and predisposes to cardiovascular diseases, such as heart failure, ischemic heart disease, stroke, and peripheral vascular disease. Structural and functional vascular disease associated to insulin resistance is highly predictive of cardiovascular morbidity and mortality. Its pathogenic mechanisms remain undefined. Prospective studies have demonstrated that animal protein consumption increases the risk of developing cardiovascular disease and predisposes to type 2 diabetes (T2D) whereas vegetable protein intake has the opposite effect. Vascular disease linked to insulin resistance begins to occur early in life. Children and adolescents with insulin resistance show an injured arterial system compared with youth free of insulin resistance, suggesting that insulin resistance plays a crucial role in the development of initial vascular damage. Prevention of the vascular dysfunction related to insulin resistance should begin early in life. Before the clinical onset of T2D, asymptomatic subjects endure a long period of time characterized by insulin resistance. Latent vascular dysfunction begins to develop during this phase, so that patients with T2D are at increased cardiovascular risk long before the diagnosis of the disease.

Key words: Diabetes; Cardiovascular risk; Arterial stiffness; Arterial elasticity; Intima-media thickness; Vascular calcification; Insulin resistance; Animal protein; Vegetable protein

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Core tip: Vascular injury associated to insulin resistance includes impaired vasodilation in response to chemical mediators, reduced distensibility of the arterial wall (arterial stiffness), vascular calcification, and increased thickness of the arterial wall. Vascular dysfunction associated to insulin resistance is present in asymptomatic subjects and predisposes to cardiovascular diseases, such as heart failure, ischemic heart disease, stroke, and peripheral vascular disease. Structural and functional vascular disease associated to insulin resistance is highly predictive of cardiovascular morbidity and mortality.

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INTRODUCTION

Cardiovascular disease is a major cause of morbidity and mortality particularly in patients with diabetes. Cardiovascular risk in this population group begins decades prior the clinical diagnosis of the disease and is not fully explained by traditional risk factors such as hypercholesterolemia and smoking. Multiple investigations provide compelling evidence of an association between insulin resistance by itself and cardiovascular risk in the general population and patients with diabetes. More insulin-resistant subjects endure higher cardiovascular risk compared to those who are more insulin-sensitive[1]. A causative link between insulin resistance by itself and vascular disease is very likely to exist, but the pathogenic mechanisms that explain the vascular dysfunction related to insulin resistance remain elusive. There is conclusive evidence that dietary habits that include animal protein increase the risk of type 2 diabetes (T2D) and cardiovascular disease whereas dietary patterns with elevated content of vegetable protein reduce the risk of both disorders[2]. Population groups that change their dietary routine to augment animal protein intake experience a dramatic increase in the rate of T2D and cardiovascular events[3]. Animal protein consumption activates glucagon secretion. Glucagon is the primary hormone that opposes insulin action. Animal protein ingestion may predispose to T2D and cardiovascular events by intensifying insulin resistance via glucagon secretion (Figure 1)[4].

Asymptomatic individuals with insulin resistance experience striking vascular damage that is not justified by traditional cardiovascular risk factors, such as hypercholesterolemia or smoking. Vascular injury related to insulin resistance develops progressively in asymptomatic subjects during a period of time that may begin during childhood. A long phase of insulin resistance and latent vascular injury precedes the clinical onset of T2D increasing cardiovascular risk before the diagnosis of the disease[5-7]. Accordingly, subclinical vascular dysfunction is evident in patients with screen-detected T2D[8]. Vascular damage associated with insulin resistance includes functional and structural vascular injury, such as impaired vasodilation, loss of elasticity of the arterial wall (arterial stiffness), increased intima-media thickness of the arterial wall, and vascular calcification. (Figure 2) The presence of subclinical vascular disease associated with insulin resistance is highly predictive of future cardiovascular events[9-12].

INSULIN RESISTANCE IS INDEPENDENTLY ASSOCIATED WITH SUBCLINICAL IMPAIRMENT OF VASCULAR REACTIVITY

Vascular smooth muscle cells normally undergo contraction or relaxation to regulate the magnitude of the blood flow according to physiological conditions. Normal endothelial cells generate vasoactive substances that modulate the reactivity of vascular smooth muscle cells. Among them, nitric oxide is a short-lived gas that

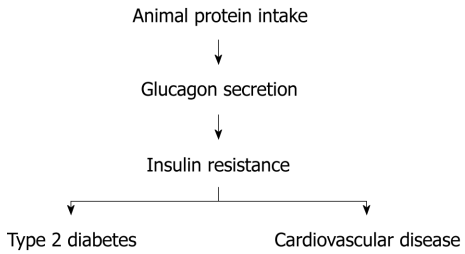

Figure 1 A simplified proposed mechanism underlying vascular disease associated with insulin resistance.

induces vasodilation. Acetylcholine is an endogenous transmitter that activates endothelial nitric oxide production by acting on muscarinic receptors. Acetylcholine induces endothelium-dependent vasodilation while exogenous sources of nitric oxide (such as nitroglycerin and sodium nitroprusside) induce endothelium-independent vasodilation. In response to increased blood flow, vascular smooth muscle cells normally relax to produce vasodilation and accommodate the elevated blood flow. Flow-mediated vasodilation is attributed to nitric oxide release by endothelial cells. The degree of flow-mediated vasodilation is considered a measure of endothelium-dependent vasodilation and can be determined by ultrasonography performed at the brachial artery[13-15].

A number of investigations show that insulin resistance is independently associated with blunted flow-mediated arterial vasodilation in asymptomatic healthy individuals compared to control subjects[5,16,17].

Similarly, insulin resistance is associated with limited vasodilation in response to metacholine chloride, a muscarinic agent. The increment in blood flow in response to metacholine is lower in insulin-resistant subjects compared to insulin-sensitive controls[18].

Likewise, arterial response to exogenous sources of nitric oxide, such as nitroglycerin, sodium nitroprusside, and nitrates is impaired in subjects with insulin resistance compared to control subjects[10,16,18].

Similarly to healthy subjects, flow-mediated vasodilation is defective in nondiabetic patients with coronary heart disease, compared to control subjects. On multivariate analysis, the extent of flow-mediated vasodilation is correlated with serum high-density lipoprotein (HDL)-c, but not with low-density lipoprotein (LDL)-c or total cholesterol levels[10].

Impairment of flow-mediated vasodilation associated with insulin resistance is already apparent in childhood. Obese children show impaired arterial vasodilation compared to control children. Further, regular exercise over 6 mo restores abnormal vascular dysfunction in obese children. The improvement in flow-mediated vasodilation after 6-mo exercise program correlates with enhanced insulin sensitivity, reflected by reduced body mass index (BMI), waist-to-hip ratio, systolic blood pressure, fasting insulin, triglycerides, and LDL/HDL ratio[19].

In normal weight and overweight adolescents, there is a gradual deterioration of flow-mediated vasodilation with worsening of insulin resistance evaluated by the euglycemic hyperinsulinemic clamp[20].

INSULIN RESISTANCE IS INDEPENDENTLY ASSOCIATED WITH SUBCLINICAL ARTERIAL STIFFNESS

Loss of distensibility of the arterial wall (arterial stiffness) leads to elevated systolic blood pressure and consequently increases cardiac afterload resulting in left ventricular hypertrophy that contributes to the development of congestive heart failure. In addition, arterial stiffness leads to reduced diastolic blood pressure, which may deteriorate diastolic coronary blood flow contributing to ischemic heart disease[21,22] (Figure 3). Arterial stiffness is associated with wide pulse pressure (systolic blood pressure minus diastolic blood pressure)[7,23].

Parameters that estimate arterial stiffness include blood pressure, pulse pressure, pulse-wave velocity, augmentation index, coefficients of distensibility and compliance, and the Young's elastic modulus, which includes intima-media thickness and estimates arterial stiffness controlling for arterial wall thickness[6]. Pulse-wave velocity is the speed of the pressure wave generated by left ventricular contraction. Arterial stiffness impairs the ability of the arterial wall to cushion the pressure wave and increases pulse-wave velocity[21]. Augmentation is the pressure difference

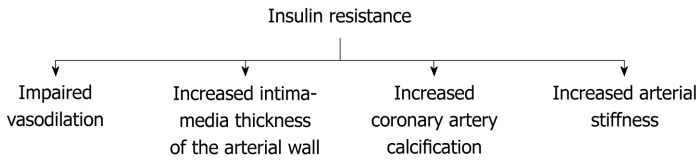

Figure 2 Pathophysiological changes associated with insulin resistance-mediated vascular disease.

between the second and first systolic peaks of the central arterial pressure waveform. Increased augmentation reflects arterial stiffness[24,25]. The augmentation index has been defined as augmentation divided by pulse pressure, being a measure of peripheral wave reflection. A higher augmentation index reflects increased arterial stiffness[26,27].

Age is consistently associated with arterial stiffness, but the loss of arterial elasticity related with age is not justified by conventional cardiovascular risk factors. Insulin resistance becomes deeper with age and may be a major pathophysiological determinant of arterial stiffness in the elderly population[12,28,29].

Numerous investigations document an association between insulin resistance and subclinical arterial stiffness in nondiabetic individuals across all ages. Arterial stiffness related to insulin resistance begins early in life and progresses in asymptomatic subjects during a latent period of time before the diagnosis of cardiovascular disease. Subclinical arterial stiffness associated with insulin resistance strongly predicts future cardiovascular events. Conventional cardiovascular risk factors do not explain the loss of arterial elasticity related to insulin resistance[7,22].

Arterial stiffness is apparent in asymptomatic subjects with insulin resistance ascertained either by its clinical expression, the metabolic syndrome, or by estimates of insulin sensitivity.

Estimates of insulin resistance are associated with subclinical arterial stiffness

In a variety of population groups, insulin resistance identified by different estimates is consistently associated with measures of arterial stiffness independently of classic cardiovascular risk factors (Table 1).

The Atherosclerosis Risk in Communities study is a prospective population-based trial with African American and Caucasian participants. A cross-sectional analysis showed an independent association between insulin resistance (assessed by glucose tolerance tests) and arterial stiffness. Subjects with insulin resistance had stiffer arteries compared to those with normal glucose tolerance after adjustment for confounding factors[6].

Similarly, insulin resistance (glucose tolerance tests) in individuals from the general population was independently associated with arterial stiffness estimated by distensibility and compliance of the carotid, femoral and brachial arteries, compared to normal glucose tolerance. Arterial stiffness worsened with deteriorating glucose tolerance[22].

Comparable findings were obtained in healthy Chinese subjects. Insulin resistance (impaired glucose tolerance) was independently associated with arterial stiffness (estimated by brachial-ankle pulse-wave velocity) compared to normal glucose tolerance. Normoglycemic subjects with altered glucose metabolism have increased arterial stiffness[30].

Likewise, arterial stiffness (brachial artery pulse-wave velocity) is positively correlated with postprandial glucose and negatively correlated with plasma adiponectin level, suggesting that arterial stiffness is greater in patients with insulin resistance compared to those with normal glucose tolerance[17].

Assessment of insulin resistance with the euglycemic hyperinsulinemic clamp is also independently associated with subclinical arterial stiffness of the common carotid and femoral arteries evaluated by pulse-wave velocity in asymptomatic healthy adults[21]. In patients with hypertension, insulin resistance (glucose tolerance tests) is independently associated with arterial stiffness (carotid-femoral pulse-wave velocity and pulse pressure) as well[31,32].

Several studies document an association between insulin resistance evaluated by the homeostasis model assessment (HOMA) index and arterial stiffness in asymptomatic individuals from different population groups. In healthy subjects and in Korean post-menopausal women, insulin resistance is independently associated with increased arterial stiffness (evaluated by brachial-ankle, aortic and peripheral pulse-wave velocity). Arterial stiffness increases sequentially with the degree of insulin resistance[33,34]. Analogous findings are observed in normotensive normoglycemic first-degree relatives of patients with diabetes. Arterial stiffness

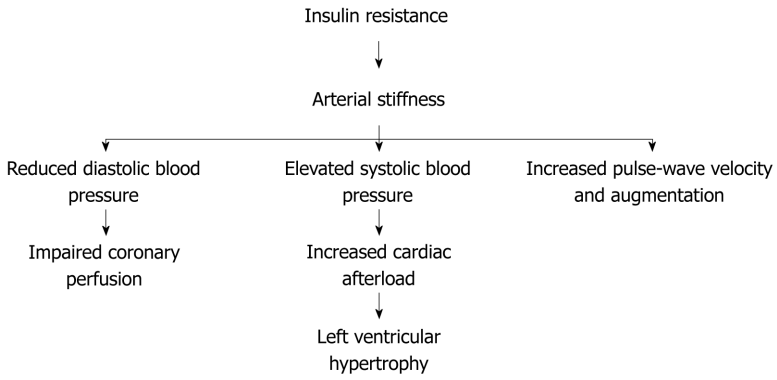

Figure 3 Cardiovascular disease associated to arterial stiffness.

(carotid-femoral pulse-wave velocity) is increased in the relatives with insulin resistance compared to those more insulin-sensitive[35]. Insulin resistance and arterial stiffness (augmentation index and pulse-wave velocity) were compared in Indigenous Australians (a population group with elevated rate of T2D) and European Australians. The Indigenous population group had higher HOMA-IR values and increased arterial stiffness compared to their European counterparts, suggesting that intensified insulin resistance among Indigenous participants contributes to explain increased arterial stiffness in this group[4].

Subclinical arterial stiffness is already present in children and adolescents with insulin resistance, compared to insulin-sensitive control subjects. In healthy children and adolescents from the general population of different countries, insulin resistance (HOMA-IR values) is independently associated with increased arterial stiffness evaluated by carotid-femoral pulse-wave velocity or brachial artery distensibility compared to control subjects[11,36,37]. In obese children and adolescents, a profound independent effect of insulin resistance on vascular compliance has been observed. Insulin-resistant subjects (HOMA-IR) experience increased vascular stiffness (aortic pulse-wave velocity) compared to control individuals[38-40]. In normal weight and overweight adolescents, insulin resistance assessed by euglycemic hyperinsulinemic clamp is associated with higher augmentation index, indicating that insulin resistance in adolescents is related to increased arterial stiffness[20].

Clinical manifestations of insulin resistance are associated with subclinical arterial stiffness

The metabolic syndrome is a cluster of clinical features that reflects insulin resistance, including obesity, systolic hypertension, dyslipemia (hypertriglyceridemia and low HDL-c), and hyperinsulinemia. The metabolic syndrome and its individual components have been independently associated with arterial stiffness. Patients with any clinical expression of insulin resistance experience subclinical arterial stiffness that is not explained by conventional cardiovascular risk factors. Arterial stiffness has been considered a further clinical manifestation of insulin resistance[7] (Table 2).

The metabolic syndrome is associated with arterial stiffness: The longitudinal association between the metabolic syndrome and arterial stiffness was investigated in the Cardiovascular Health Study. Metabolic syndrome at baseline (obesity, systolic hypertension, hyperinsulinemia and hypertriglyceridemia) independently predicted increased arterial stiffness (aortic pulse-wave velocity) at follow-up[28].

In the Atherosclerosis Risk in Communities study, the joint effect of elevated glucose, hyperinsulinemia and hypertriglyceridemia (reflecting insulin resistance) is independently associated with arterial stiffness in subjects from the general population[6]. Similarly, the clustering of at least three components of the metabolic syndrome is related with increased carotid artery stiffness among healthy participants across all age groups in the Baltimore Longitudinal Study on Aging independently of other cardiovascular risk factors[41].

Likewise, the metabolic syndrome is strongly and independently associated with reduced distensibility of the common carotid artery in healthy women from the general population[9]. In 12517 subjects with no history of cardiovascular disease, systolic hypertension, obesity, hypertriglyceridemia, and hyperuricemia are independent determinants for arterial stiffness (brachial-ankle pulse-wave velocity) on multiple regression analysis[29]. Arterial stiffness (augmentation index and pulse-wave velocity) was compared in Indigenous and European Australians. Factor

Table 1 Studies that find an independent association between insulin resistance and subclinical arterial stiffness unexplained by classic cardiovascular risk factors

Ref.	Population group	Insulin resistance	Arterial stiffness
Salomaa *et al*[6]	African American and Caucasian	IGT	Arterial compliance, Young's elastic modulus
Henry *et al*[22]	General population	IGT	Arterial compliance
Shin *et al*[30]	Healthy Chinese subjects	IGT	Brachial-ankle PWV
Liye *et al*[17]	IGT versus normal glucose tolerance	IGT, serum adiponectin levels	Brachial artery PWV
Giltay *et al*[21]	Healthy subjects	Hyperinsulinemic euglycemic clamp	Carotid-femoral PWV
Vyssoulis *et al*[32]	Patients with hypertension	IGT	Carotid-femoral PWV
Sengstock *et al*[31]	Patients with hypertension	Frequently sampled IV tolerance test	Aortic PWV, pulse pressure
Kasayama *et al*[33]	Healthy adults	HOMA	Brachial-ankle PWV
Park *et al*[34]	Postmenopausal women	HOMA-IR	Aortic and peripheral PWV
Maple-Brown *et al*[4]	Indigenous Australians	HOMA-IR	Augmentation index
Scuteri *et al*[35]	Family history of diabetes	HOMA-IR	Carotid-femoral PWV
Sakuragi *et al*[36]	Prepubescent children	HOMA-IR	Carotid-femoral PWV
Whincup *et al*[11]	British children	HOMA-IR	Brachial artery distensibility
Gungor *et al*[38]	Children and adolescents	HOMA-IR	Aortic PWV
Iannuzzi *et al*[39]	Children and adolescents	HOMA-IR	Aortic PWV
Tomsa *et al*[20]	Adolescents	Hyperinsulinemic euglycemic clamp	Augmentation index

IGT: Impaired glucose tolerance; PWV: Pulse-wave velocity; HOMA: Homeostasis model assessment; HOMA-IR: Homeostasis model assessment-insulin resistance.

analysis revealed that metabolic syndrome components clustered with Indigenous Australian participants. Arterial stiffness was more pronounced among Indigenous compared to European Australians[4].

Subclinical arterial stiffness is already present in children and adolescents with the metabolic syndrome, suggesting that insulin resistance plays an important role in the early pathogenesis of vascular disease. British and Chinese children and adolescents with the metabolic syndrome have increased arterial stiffness compared to control children after adjustment for covariates. There is a strong graded inverse relationship between the number of metabolic syndrome components and brachial artery distensibility[11,37]. In obese children, common carotid artery stiffness is more prominent in the group with the metabolic syndrome compared to the control group[42]. Normoglycemic young adults (mean age 20 years) with a positive family history of T2D have higher BMI and fasting insulin and increased arterial stiffness (aortic pulse-wave velocity) than their counterparts without T2D relatives[43]. The longitudinal relationship between the metabolic syndrome identified in childhood and arterial elasticity assessed in adulthood was investigated in a prospective population-based cohort study with 21 years of follow-up, the Cardiovascular Risk in Young Finns Study. Childhood metabolic syndrome (obesity, systolic hypertension, hypertriglyceridemia and hyperinsulinemia) predicts independently carotid artery stiffness in adulthood[44].

Obesity is associated with arterial stiffness: Longitudinal and cross-sectional studies consistently show that measures of adiposity (BMI, waist circumference, waist-to-hip ratio, body fat, and abdominal fat) are independently associated with estimates of arterial stiffness in diverse population groups. This association is already apparent during childhood and cannot be explained by traditional cardiovascular risk factors. In a population-based setting, adulthood obesity (BMI and waist-to-hip ratio) is associated with increased stiffness of carotid, femoral, and brachial arteries after adjusting for cardiovascular risk factors. Arterial distensibility consistently decreased with higher BMI[9,45]. Similarly, obesity (BMI and waist circumference) is independently related to increased arterial stiffness (augmentation index) in Indigenous Australians free of T2D compared to European Australians[46]. In female twins, abdominal adiposity is a determinant of arterial stiffness (augmentation index) independent of genetic effects and other confounding factors[47].

The association between adiposity parameters and increased arterial stiffness begins during childhood. In obese children, there is a marked effect of insulin resistance associated with obesity on vascular compliance. Obese children are more insulin-resistant and have stiffer arteries compared with lean controls[39,40]. In a population-based setting, childhood obesity (BMI and waist circumference) is

Table 2 Studies that find an independent association between the clinical expression of insulin resistance and subclinical arterial stiffness unexplained by classic cardiovascular risk factors

Ref.	Population group	Insulin resistance	Arterial stiffness
Mackey *et al*[28]	Elderly	Metabolic syndrome	Aortic pulse-wave velocity
Salomaa *et al*[6]	General population	Metabolic syndrome	Arterial compliance, Young's elastic modulus
		Hyperinsulinemia	
Scuteri *et al*[41]	Healthy subjects	Metabolic syndrome	Carotid artery stiffness
Van-Popele *et al*[9]	Women	Metabolic syndrome	Carotid artery stiffness
		Obesity	
		Dyslipemia	
Tomiyama *et al*[29]	Healthy subjects	Metabolic syndrome	Brachial-ankle pulse-wave velocity
		Systolic hypertension	
Maple-Brown *et al*[4]	Indigenous versus European Australians	Metabolic syndrome	Augmentation index, pulse-wave velocity
Whincup *et al*[11]	British children	Metabolic syndrome	Brachial artery distensibility
		Obesity	
		Hyperinsulinemia	
Xi *et al*[37]	Chinese children	Metabolic syndrome	Brachial artery distensibility
Ianuzzi *et al*[42]	Obese children	Metabolic syndrome	Carotid artery stiffness
Hopkins *et al*[43]	Relatives of patients with type 2 diabetes	Metabolic syndrome	Aortic pulse-wave velocity
Juonala *et al*[44]	Children	Metabolic syndrome	Carotid artery stiffness
		Hyperinsulinemia	
Zebekakis *et al*[45]	General population	Obesity	Carotid, femoral, and brachial arteries stiffness
Maple-Brown *et al*[46]	Indigenous versus European Australians	Obesity	Augmentation index
Greenfield *et al*[47]	Female twins	Abdominal obesity	Augmentation index
Sakuragi *et al*[35]	Children	Obesity	Brachial artery distensibility
		Dyslipemia	
		Hyperinsulinemia	
Gungor *et al*[38]	Adolescents and young adults	Obesity	Aortic pulse-wave velocity
Jourdan *et al*[47]		Dyslipemia	
Urbina *et al*[49]			
Kappus *et al*[50]			
Wildman *et al*[51]	Young and older adults	Obesity	Aortic pulse-wave velocity
Iannuzzi *et al*[39]		Systolic hypertension	Aortic pulse-wave velocity
Kasayama *et al*[33]		Dyslipemia	
Ceceija *et al*[12]		Hyperinsulinemia	
Urbina *et al*[52]		Triglyceride/HDL-c	Aortic pulse-wave velocity

associated with increased arterial stiffness after adjustment for confounding factors. There is a strong graded inverse relationship between BMI and brachial artery distensibility, This association is apparent even at BMI levels below those considered to represent obesity[11,36]. Similar results are observed in adolescents and young adults. Obesity is associated with subclinical arterial stiffness independently of cardiovascular risk factors[38,48-50].

The association between obesity and arterial stiffness (aortic pulse-wave velocity) was evaluated in young adults (20 to 40 years, 50% African American) and older adults (41 to 70 years, 33% African American). Obesity parameters (BMI, waist circumference, hip circumference, and waist-to-hip ratio) were strongly correlated with higher aortic pulse-wave velocity, independently of risk factors. Obesity is an independent and strong predictor of aortic stiffness for both races and age groups[51].

Systolic hypertension, dyslipemia, and hyperinsulinemia are associated with arterial stiffness: Other clinical manifestations of insulin resistance, including systolic hypertension[12,29,39,40], dyslipemia[9,33,36,38-40,52], and hyperinsulinemia[6,11,36,40,44] are also consistently associated with different measures of arterial stiffness independently of other cardiovascular risk factors, in diverse population groups, across all ages. Longitudinal studies such as the Atherosclerosis Risk in Communities study and the Multi-Ethnic Study of Atherosclerosis have shown that arterial stiffness predicts the development of systolic hypertension[53,54]. In healthy subjects 10 to 26 years old,

triglyceride-to-HDL-c ratio is an independent predictor of arterial stiffness after adjustment for cardiovascular risk factors, particularly in the obese. Arterial stiffness rose progressively across tertiles of triglyceride-to-HDL-c ratio[52].

INSULIN RESISTANCE IS INDEPENDENTLY ASSOCIATED WITH SUBCLINICAL STRUCTURAL CHANGES OF THE ARTERIAL WALL

Similarly to arterial stiffness, a gradual increase in carotid intima-media thickness occurs with age. A systematic review documents a strong association between age and carotid intima-media thickness in healthy subjects and individuals with cardiovascular disease. This relationship is not affected by cardiovascular risk factors. Ageing is associated with magnification of insulin resistance that may explain the increase in intima-media thickness[55].

Insulin resistance either ascertained by estimates or by its clinical expression is associated with increased intima-media thickness and increased calcification of the arterial wall in asymptomatic subjects. This association is not mediated by classic cardiovascular risk factors, suggesting that insulin resistance plays a crucial role in the development of initial vascular damage (Table 3).

Estimates of insulin resistance are associated with increased thickness of the arterial wall and increased coronary calcification

Increased thickness of the arterial wall: In healthy subjects from the Kuopio Ischemic Heart Disease Risk Factor study, insulin resistance was determined by the euglycemic hyperinsulinemic clamp technique and the presence of subclinical vascular disease in the femoral and carotid arteries was evaluated by ultrasonography. Subjects with asymptomatic vascular disease were more insulin-resistant compared to control subjects[56].

The association between insulin resistance and subclinical vascular disease was confirmed in healthy Swedish men. Insulin resistance was determined by the hyperinsulinemic euglycemic clamp in subjects with high cardiovascular risk (hypercholesterolemia, smoking) and subjects with no cardiovascular risk factors. Asymptomatic vascular disease was evaluated by B-mode ultrasound of the common carotid artery. A negative correlation between insulin sensitivity and carotid intima-media thickness was observed in both population groups (high and low cardiovascular risk). Participants with insulin resistance had greater carotid wall thickness compared to insulin-sensitive subjects[57].

A similar association between insulin resistance and subclinical vascular disease (increased intima-media thickness of the arterial wall) was observed in healthy Caucasian participants of the Insulin Resistance Atherosclerosis Study. Insulin sensitivity was evaluated by the frequently sampled intravenous glucose tolerance test with analysis by the minimal model of Bergman. Asymptomatic vascular disease was assessed by the measurement of intima-media thickness of the carotid artery by B-mode ultrasonography. In Caucasian men, insulin resistance is associated with a subclinical increase in carotid intima-media thickness, after adjustment for traditional cardiovascular risk factors[58].

The independent association between insulin resistance (HOMA-IR) and subclinical vascular disease (increased carotid intima-media thickness) has been confirmed in healthy subjects of four ethnic groups (non-Hispanic Whites, African-Americans, Hispanic Americans, and Chinese Americans) from the Multi-Ethnic Study of Atherosclerosis[59].

In asymptomatic patients with impaired glucose tolerance, insulin resistance (calculated by the insulin sensitivity check index) is strongly associated with severe carotid atherosclerosis (assessed by ultrasonography) on multiple regression analysis after adjustment for confounders. Carotid intima-media thickness correlated inversely with insulin sensitivity[60].

The association between insulin resistance and asymptomatic increased intima-media thickness is apparent in childhood. In healthy children, insulin resistance measured with the euglycemic hyperinsulinemic clamp is associated with higher carotid intima-media thickness[61]. Likewise, obese children aged 6-14 years with higher HOMA-IR had increased carotid intima-media thickness compared to control children[39].

Increased coronary artery calcification: Insulin resistance is also associated with subclinical coronary artery calcification. Asymptomatic subjects with insulin resistance (HOMA-IR) have increased coronary calcification score (derived from

Table 3 Studies that find an independent association between insulin resistance and subclinical vascular calcification or increased intima-media thickness of the arterial wall unexplained by traditional cardiovascular risk factors

Ref.	Population group	Insulin resistance	Vascular disease
Laakso *et al*[56]	Healthy subjects	Euglycemic hyperinsulinemic clamp	Increased carotid IMT
Agewall *et al*[57]	Healthy men	Euglycemic hyperinsulinemic clamp	Increased carotid wall thickness
Howard *et al*[58]	Healthy Caucasians	Frequently sampled IV glucose tolerance test	Increased carotid IMT
Bertoni *et al*[59]	Multiethnic healthy subjects	HOMA-IR	Increased carotid IMT, elevated coronary calcium
Rajala *et al*[60]	Healthy subjects	Insulin sensitivity check index	Increased carotid IMT
Iannuzzi *et al*[39]	Obese children	HOMA-IR	Increased carotid IMT
Ryder *et al*[61]	Healthy children	Euglycemic hyperinsulinemic clamp	Increased carotid IMT
Arad *et al*[62]	Healthy subjects	HOMA-IR	Elevated coronary calcium score
Ong *et al*[63]	Healthy subjects	HOMA-IR	Elevated coronary calcium score
Meigs *et al*[64]	Healthy subjects	Glucose tolerance tests	Coronary artery calcification
Dabelea *et al*[65]	Healthy and type 1 diabetes children	Glucose disposal rate	Coronary artery calcification
Reilly *et al*[66]	Family history of cardiovascular disease	HOMA-IR	Coronary artery calcification
Qasim *et al*[67]	Family history of cardiovascular disease	HOMA-IR	Coronary artery calcification
Young *et al*[68]	Patients with coronary artery disease	Glucose tolerance test	Coronary artery calcification
Shinozaki *et al*[69]	Family history of cardiovascular disease	Glucose tolerance test	Coronary artery calcification

HOMA-IR: Homeostasis model assessment-insulin resistance; IMT: Intima-media thickness.

electron-beam computed tomography) that is not explained by traditional cardiovascular risk factors[59,62,63].

In the Framingham Offspring Study, there is a graded increase in subclinical coronary artery calcification with worsening insulin resistance (impaired glucose tolerance) among asymptomatic subjects[64].

The association between insulin resistance (estimated glucose disposal rate) and coronary artery calcification was examined among patients with type 1 diabetes and healthy subjects in the Coronary Artery Calcification in Type 1 Diabetes study. Insulin resistance was independently associated with coronary artery calcification (electron-beam computed tomography) in both population groups[65].

In the Study of Inherited Risk of Coronary Atherosclerosis, insulin resistance (HOMA-IR) is associated with coronary artery calcification after adjustment for confounding factors in asymptomatic subjects with a family history of premature cardiovascular disease. The HOMA-IR index predicts coronary artery calcification scores beyond other cardiovascular risk factors in this population group[66,67].

In normoglycemic patients with coronary artery disease, insulin resistance (glucose tolerance tests) is associated with severity of the coronary disease documented by coronary arteriography compared to control subjects. Nondiabetic patients with coronary artery disease are insulin-resistant compared to control subjects[68,69].

Clinical manifestations of insulin resistance are associated with subclinical structural damage to the arterial wall

The metabolic syndrome and its individual components are associated with subclinical structural vascular disease that is not explained by conventional cardiovascular risk factors.

The metabolic syndrome: In healthy participants of several studies, including the Atherosclerosis Risk in Communities study, the Baltimore Longitudinal Study on Aging study, and the Multi-Ethnic Study of Atherosclerosis, the metabolic syndrome is independently associated with asymptomatic increased carotid intima-media thickness across all age groups and ethnicities[6,41,59,70]. Likewise, the metabolic syndrome is associated with coronary artery calcification independently of other cardiovascular risk factors in asymptomatic subjects with a family history of premature cardiovascular disease participants of the Study of Inherited Risk of Coronary Atherosclerosis[66].

Subclinical vascular damage is detectable at young age in the presence of metabolic

syndrome. Asymptomatic carotid intima-media thickness is increased in children with metabolic syndrome as compared with healthy control children, after adjustment for confounders[71]. Regular exercise over 6 mo improves the metabolic syndrome and reduces carotid intima-media thickness in obese children compared to control subjects[19].

In analyses from four cohort studies (Cardiovascular Risk in Young Finns study, Bogalusa Heart study, Princeton Lipid Research study, Insulin study) with a mean follow-up of 22.3 years, the presence of the metabolic syndrome during childhood is associated with higher carotid intima-media thickness in adulthood[72].

In the Bogalusa Heart study, postmortem examinations performed in children and adolescents from a biracial (African American and Caucasian) community showed that the antemorten presence of the metabolic syndrome (obesity, dyslipemia, and hypertension) strongly predicted the extent of vascular disease in the aorta and coronary arteries[73].

In the Pathobiological Determinants of Atherosclerosis in Youth study, arteries collected from autopsies aged 15-34 years whose deaths were accidental showed that vascular disease in the aorta and right coronary artery is associated with the presence of impaired glucose tolerance, obesity, hypertension, and low HDL-c level. This association is not explained by hypercholesterolemia or smoking[74].

Obesity: In healthy asymptomatic adults, greater BMI and waist-to-hip ratio are independently associated with increased carotid intima-media thickness[70,75]. Increased diameter of the arterial wall associated with obesity is present in several areas of the arterial system, including carotid, femoral and brachial arteries. Across a wide age range, intima-media thickness of several arteries increased with higher BMI in a population-based sample of participants[45].

The independent relationship between obesity and subclinical increased intima-media thickness of carotid and femoral arteries is present in children and adolescents. Obese children have increased carotid and femoral intima-media thickness compared to control children[39,48,50]. In a prospective cohort of children and adolescents, BMI assessed at 11, 15, and 18 years was associated with higher carotid intima-media thickness after controlling for confounders. Overweight/obese subjects had higher carotid intima-media thickness compared to subjects with normal BMI[76].

In analyses from four cohort studies (Cardiovascular Risk in Young Finns study, Bogalusa Heart study, Princeton Lipid Research study, Insulin study) with a mean follow-up of 22.3 years, childhood BMI was associated with higher carotid intima-media thickness in adulthood[72].

Systolic hypertension, dyslipemia, and hyperinsulinemia: Fasting hyperinsulinemia is independently associated with greater carotid intima-media thickness and coronary artery calcification in asymptomatic healthy subjects[6,62,64,70]. The association between hyperinsulinemia and increased carotid intima-media thickness is similar in African American and Caucasian subjects[6,70]. The Mexico City Diabetes study investigated the longitudinal relationship between systolic hypertension and vascular damage in a population-based prospective trial. In normotensive subjects who progress to hypertension (prehypertensive subjects), baseline carotid intima-media thickness increased in comparison with subjects who remained normotensive. After adjusting for multiple cardiovascular risk factors, converter status was independently associated with a higher carotid intima-media thickness[77].

Autopsy examinations from the Pathobiological Determinants of Atherosclerosis in Youth study show that systolic hypertension is associated with greater vascular injury in both the aorta and right coronary artery (particularly fibrous plaques) in subjects throughout the 15-34 year age span. The association of hypertension with vascular damage remained after adjusting for BMI and glycohemoglobin[74].

Longitudinal autopsy studies conducted in children and adults show that low HDL-c is independently associated with vascular disease. The degree of vascular lesions in both the aorta and right coronary artery is negatively associated with serum HDL-c on multiple regression analysis[73,74,78,79].

SUBCLINICAL VASCULAR DISEASE ASSOCIATED WITH INSULIN RESISTANCE PREDICTS CARDIOVASCULAR DISEASE

Subclinical structural and functional vascular dysfunction associated with insulin resistance in otherwise healthy subjects is highly predictive of future cardiovascular events. Reduced vasodilation, loss of arterial distensibility, and increased arterial

intima-media thickness in asymptomatic subjects are all associated with future cardiovascular disease.

In a systematic review and meta-analysis of prospective studies, impaired brachial flow-mediated vasodilatation was associated with future cardiovascular events both in asymptomatic and diseased population groups[15]. Impaired nitroglycerin-mediated vasodilatation of the brachial artery has been independently associated with coronary artery calcification in a population-based study[80]. In a prospective study, impaired coronary vasoreactivity was independently associated with a higher incidence of cardiovascular events. Baseline coronary vasoreactivity in response to several stimuli (acetylcholine, sympathetic activation, increased blood flow, and nitroglycerin) predicted incident cardiovascular events at follow-up, after adjustment for traditional cardiovascular risk factors[81].

The ability of arterial stiffness to predict cardiovascular events independently of other cardiovascular risk factors has been documented in cross-sectional and prospective studies, systemic reviews and meta-analyses.

Prospective studies show that increased arterial stiffness (estimated by wide pulse pressure, carotid-femoral pulse-wave velocity, and common carotid distensibility) is a powerful predictor of incident cardiovascular events in asymptomatic individuals from the general population, patients with hypertension, subjects with impaired glucose tolerance, and patients with T2D beyond classic cardiovascular risk factors[82-84]. A systematic review of cross-sectional studies concludes that arterial stiffness is highly predictive of cardiovascular events[12]. A systematic review and meta-analysis of longitudinal studies that followed-up 15877 subjects for a mean of 7.7 years concludes that aortic stiffness (expressed as aortic pulse-wave velocity) is a strong predictor of future cardiovascular events, cardiovascular mortality, and all-cause mortality, independently of classic cardiovascular risk factors. The predictive value of increased arterial stiffness is larger in patients with higher baseline cardiovascular risk states, such as renal disease, coronary artery disease, or hypertension compared with low-risk subjects (general population)[85].

The prospective association between arterial stiffness and postmortem vascular damage was investigated among elderly subjects. There was a weak correlation between baseline arterial stiffness (pulse-wave velocity) and the degree of vascular damage observed at autopsy[86].

A large cross-sectional study with 10828 participants investigated the ability of brachial-ankle pulse-wave velocity for screening cardiovascular risk in the general population. On multivariate analysis, brachial-ankle pulse-wave velocity was associated with cardiovascular risk independently from conventional risk factors[87].

In a population-based cohort study in the elderly (Rotterdam study), arterial stiffness had a strong positive association with structural vascular disease. Aortic and carotid stiffness (assessed by carotid-femoral pulse-wave velocity and common carotid distensibility) was associated with carotid intima-media thickness after adjustment for cardiovascular risk factors[88].

Subclinical carotid intima-media thickness predicts cardiovascular events in healthy subjects and patients with coronary artery disease. A systematic review and meta-analysis concluded that carotid intima-media thickness is a strong independent predictor of future vascular events, although data for younger individuals are limited[89]. A prospective cohort study of women shows that increased carotid intima-media thickness predicts cardiovascular events during 7-year follow-up regardless of glucose tolerance and other cardiovascular risk factors[90]. In a systematic review, population groups with cardiovascular disease had a higher carotid intima-media thickness compared to population groups free of cardiovascular disease[55].

CONCLUSION

Numerous studies provide compelling evidence of an association between insulin resistance and subclinical cardiovascular disease that is not explained by traditional cardiovascular risk factors, such as hypercholesterolemia or smoking. Pathogenic mechanisms underlying vascular damage linked to insulin resistance are undefined. Vascular injury associated with insulin resistance begins early in life and includes impaired vasodilation, loss of arterial distensibility, increased intima-media thickness of the arterial wall and increased arterial calcification. Subclinical vascular dysfunction associated with insulin resistance in otherwise healthy subjects is highly predictive of future cardiovascular events. Reduced vasodilation, loss of arterial distensibility, increased arterial intima-media thickness and vascular calcification in asymptomatic subjects are associated with future cardiovascular disease.

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REFERENCES

1 **Eddy D**, Schlessinger L, Kahn R, Peskin B, Schiebinger R. Relationship of insulin resistance and related metabolic variables to coronary artery disease: a mathematical analysis. *Diabetes Care* 2009; **32**: 361-366 [PMID: 19017770 DOI: 10.2337/dc08-0854]
2 **Satija A**, Bhupathiraju SN, Rimm EB, Spiegelman D, Chiuve SE, Borgi L, Willett WC, Manson JE, Sun Q, Hu FB. Plant-Based Dietary Patterns and Incidence of Type 2 Diabetes in US Men and Women: Results from Three Prospective Cohort Studies. *PLoS Med* 2016; **13**: e1002039 [PMID: 27299701 DOI: 10.1371/journal.pmed.1002039]
3 **Lillioja S**, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 1993; **329**: 1988-1992 [PMID: 8247074 DOI: 10.1056/NEJM199312303292703]
4 **Maple-Brown LJ**, Piers LS, O'Rourke MF, Celermajer DS, O'Dea K. Increased arterial stiffness in remote Indigenous Australians with high risk of cardiovascular disease. *J Hypertens* 2007; **25**: 585-591 [PMID: 17278975 DOI: 10.1097/HJH.0b013e328011f766]
5 **Jaap AJ**, Hammersley MS, Shore AC, Tooke JE. Reduced microvascular hyperaemia in subjects at risk of developing type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1994; **37**: 214-216 [PMID: 8163058 DOI: 10.1007/s001250050096]
6 **Salomaa V**, Riley W, Kark JD, Nardo C, Folsom AR. Non-insulin-dependent diabetes mellitus and fasting glucose and insulin concentrations are associated with arterial stiffness indexes. The ARIC Study. Atherosclerosis Risk in Communities Study. *Circulation* 1995; **91**: 1432-1443 [PMID: 7867184 DOI: 10.1161/01.CIR.91.5.1432]
7 **Stehouwer CD**, Henry RM, Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. *Diabetologia* 2008; **51**: 527-539 [PMID: 18239908 DOI: 10.1007/s00125-007-0918-3]
8 **Johansen NB**, Charles M, Vistisen D, Rasmussen SS, Wiinberg N, Borch-Johnsen K, Lauritzen T, Sandbæk A, Witte DR. Effect of intensive multifactorial treatment compared with routine care on aortic stiffness and central blood pressure among individuals with screen-detected type 2 diabetes: the ADDITION-Denmark study. *Diabetes Care* 2012; **35**: 2207-2214 [PMID: 22787176 DOI: 10.2337/dc12-0176]
9 **van Popele NM**, Westendorp IC, Bots ML, Reneman RS, Hoeks AP, Hofman A, Grobbee DE, Witteman JC. Variables of the insulin resistance syndrome are associated with reduced arterial distensibility in healthy non-diabetic middle-aged women. *Diabetologia* 2000; **43**: 665-672 [PMID: 10855542 DOI: 10.1007/s001250051356]
10 **Zhang X**, Zhao SP, Li XP, Gao M, Zhou QC. Endothelium-dependent and -independent functions are impaired in patients with coronary heart disease. *Atherosclerosis* 2000; **149**: 19-24 [PMID: 10704610 DOI: 10.1016/S0021-9150(99)00288-9]
11 **Whincup PH**, Gilg JA, Donald AE, Katterhorn M, Oliver C, Cook DG, Deanfield JE. Arterial distensibility in adolescents: the influence of adiposity, the metabolic syndrome, and classic risk factors. *Circulation* 2005; **112**: 1789-1797 [PMID: 16172286 DOI: 10.1161/CIRCULATIONAHA.104.532663]
12 **Cecelja M**, Chowienczyk P. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension: a systematic review. *Hypertension* 2009; **54**: 1328-1336 [PMID: 19884567 DOI: 10.1161/HYPERTENSIONAHA.109.137653]
13 **McVeigh GE**, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, Andrews JW, Hayes JR. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1992; **35**: 771-776 [PMID: 1511805]
14 **Adams MR**, Robinson J, McCredie R, Seale JP, Sorensen KE, Deanfield JE, Celermajer DS. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. *J Am Coll Cardiol* 1998; **32**: 123-127 [PMID: 9669259 DOI: 10.1016/S0735-1097(98)00206-X]
15 **Ras RT**, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. *Int J Cardiol* 2013; **168**: 344-351 [PMID: 23041097 DOI: 10.1016/j.ijcard.2012.09.047]
16 **Su Y**, Liu XM, Sun YM, Wang YY, Luan Y, Wu Y. Endothelial dysfunction in impaired fasting glycemia, impaired glucose tolerance, and type 2 diabetes mellitus. *Am J Cardiol* 2008; **102**: 497-498 [PMID: 18678313 DOI: 10.1016/j.amjcard.2008.03.087]
17 **Liye H**, Lvyun Z, Guangyao S, Luping R. Investigation of early change of endothelial function and related factors in individuals with hyperglycemia. *Diabetes Res Clin Pract* 2011; **92**: 194-197 [PMID: 21334758 DOI: 10.1016/j.diabres.2011.01.018]
18 **Steinberg HO**, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J Clin Invest* 1996; **97**: 2601-2610 [PMID: 8647954 DOI: 10.1172/JCI118709]
19 **Meyer AA**, Kundt G, Lenschow U, Schuff-Werner P, Kienast W. Improvement of early vascular changes and cardiovascular risk factors in obese children after a six-month exercise program. *J Am Coll Cardiol* 2006; **48**: 1865-1870 [PMID: 17084264 DOI: 10.1016/j.jacc.2006.07.035]
20 **Tomsa A**, Klinepeter Bartz S, Krishnamurthy R, Krishnamurthy R, Bacha F. Endothelial Function in Youth: A Biomarker Modulated by Adiposity-Related Insulin Resistance. *J Pediatr* 2016; **178**: 171-177 [PMID: 27546204 DOI: 10.1016/j.jpeds.2016.07.025]
21 **Giltay EJ**, Lambert J, Elbers JM, Gooren LJ, Asscheman H, Stehouwer CD. Arterial compliance and distensibility are modulated by body composition in both men and women but by insulin sensitivity only in women. *Diabetologia* 1999; **42**: 214-221 [PMID: 10064102 DOI: 10.1007/s001250051141]
22 **Henry RM**, Kostense PJ, Spijkerman AM, Dekker JM, Nijpels G, Heine RJ, Kamp O, Westerhof N, Bouter LM, Stehouwer CD; Hoorn Study. Arterial stiffness increases with deteriorating glucose tolerance

status: the Hoorn Study. *Circulation* 2003; **107**: 2089-2095 [PMID: 12695300 DOI: 10.1161/01.CIR.0000065222.34933.FC]

23 **Dart AM**, Kingwell BA. Pulse pressure--a review of mechanisms and clinical relevance. *J Am Coll Cardiol* 2001; **37**: 975-984 [PMID: 11263624 DOI: 10.1016/S0735-1097(01)01108-1]

24 **Nichols WW**, Singh BM. Augmentation index as a measure of peripheral vascular disease state. *Curr Opin Cardiol* 2002; **17**: 543-551 [PMID: 12357133 DOI: 10.1097/00001573-200209000-00016]

25 **Fukui M**, Kitagawa Y, Nakamura N, Mogami S, Ohnishi M, Hirata C, Ichio N, Wada K, Kamiuchi K, Shigeta M, Sawada M, Hasegawa G, Yoshikawa T. Augmentation of central arterial pressure as a marker of atherosclerosis in patients with type 2 diabetes. *Diabetes Res Clin Pract* 2003; **59**: 153-161 [PMID: 12560165 DOI: 10.1016/S0168-8227(02)00204-8]

26 **Brooks B**, Molyneaux L, Yue DK. Augmentation of central arterial pressure in type 1 diabetes. *Diabetes Care* 1999; **22**: 1722-1727 [PMID: 10526742 DOI: 10.2337/diacare.22.10.1722]

27 **Shah AS**, Wadwa RP, Dabelea D, Hamman RF, D'Agostino R, Marcovina S, Daniels SR, Dolan LM, Fino NF, Urbina EM. Arterial stiffness in adolescents and young adults with and without type 1 diabetes: the SEARCH CVD study. *Pediatr Diabetes* 2015; **16**: 367-374 [PMID: 25912292 DOI: 10.1111/pedi.12279]

28 **Mackey RH**, Sutton-Tyrrell K, Vaitkevicius PV, Sakkinen PA, Lyles MF, Spurgeon HA, Lakatta EG, Kuller LH. Correlates of aortic stiffness in elderly individuals: a subgroup of the Cardiovascular Health Study. *Am J Hypertens* 2002; **15**: 16-23 [PMID: 11824854 DOI: 10.1016/S0895-7061(01)02228-2]

29 **Tomiyama H**, Yamashina A, Arai T, Hirose K, Koji Y, Chikamori T, Hori S, Yamamoto Y, Doba N, Hinohara S. Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement--a survey of 12517 subjects. *Atherosclerosis* 2003; **166**: 303-309 [PMID: 12535743 DOI: 10.1016/S0021-9150(02)00332-5]

30 **Shin JY**, Lee HR, Lee DC. Increased arterial stiffness in healthy subjects with high-normal glucose levels and in subjects with pre-diabetes. *Cardiovasc Diabetol* 2011; **10**: 30 [PMID: 21492487 DOI: 10.1186/1475-2840-10-30]

31 **Sengstock DM**, Vaitkevicius PV, Supiano MA. Arterial stiffness is related to insulin resistance in nondiabetic hypertensive older adults. *J Clin Endocrinol Metab* 2005; **90**: 2823-2827 [PMID: 15728211 DOI: 10.1210/jc.2004-1686]

32 **Vyssoulis G**, Pietri P, Vlachopoulos C, Alexopoulos N, Kyvelou SM, Terentes-Printzios D, Stefanadis C. Early adverse effect of abnormal glucose metabolism on arterial stiffness in drug naive hypertensive patients. *Diab Vasc Dis Res* 2012; **9**: 18-24 [PMID: 21994165 DOI: 10.1177/1479164111422827]

33 **Kasayama S**, Saito H, Mukai M, Koga M. Insulin sensitivity independently influences brachial-ankle pulse-wave velocity in non-diabetic subjects. *Diabet Med* 2005; **22**: 1701-1706 [PMID: 16401315 DOI: 10.1111/j.1464-5491.2005.01718.x]

34 **Park JS**, Nam JS, Cho MH, Yoo JS, Ahn CW, Jee SH, Lee HS, Cha BS, Kim KR, Lee HC. Insulin resistance independently influences arterial stiffness in normoglycemic normotensive postmenopausal women. *Menopause* 2010; **17**: 779-784 [PMID: 20215975 DOI: 10.1097/gme.0b013e3181cd3d60]

35 **Scuteri A**, Tesauro M, Rizza S, Iantorno M, Federici M, Lauro D, Campia U, Turriziani M, Fusco A, Cocciolillo G, Lauro R. Endothelial function and arterial stiffness in normotensive normoglycemic first-degree relatives of diabetic patients are independent of the metabolic syndrome. *Nutr Metab Cardiovasc Dis* 2008; **18**: 349-356 [PMID: 17935958 DOI: 10.1016/j.numecd.2007.03.008]

36 **Sakuragi S**, Abhayaratna K, Gravenmaker KJ, O'Reilly C, Srikusalanukul W, Budge MM, Telford RD, Abhayaratna WP. Influence of adiposity and physical activity on arterial stiffness in healthy children: the lifestyle of our kids study. *Hypertension* 2009; **53**: 611-616 [PMID: 19273744 DOI: 10.1161/HYPERTENSIONAHA.108.123364]

37 **Xi B**, Zhang L, Mi J. Reduced arterial compliance associated with metabolic syndrome in Chinese children and adolescents. *Biomed Environ Sci* 2010; **23**: 102-107 [PMID: 20514984 DOI: 10.1016/S0895-3988(10)60038-4]

38 **Gungor N**, Thompson T, Sutton-Tyrrell K, Janosky J, Arslanian S. Early signs of cardiovascular disease in youth with obesity and type 2 diabetes. *Diabetes Care* 2005; **28**: 1219-1221 [PMID: 15855596 DOI: 10.2337/diacare.28.5.1219]

39 **Iannuzzi A**, Licenziati MR, Acampora C, Salvatore V, Auriemma L, Romano ML, Panico S, Rubba P, Trevisan M. Increased carotid intima-media thickness and stiffness in obese children. *Diabetes Care* 2004; **27**: 2506-2508 [PMID: 15451928 DOI: 10.2337/diacare.27.10.2506]

40 **Iannuzzi A**, Licenziati MR, Acampora C, Salvatore V, De Marco D, Mayer MC, De Michele M, Russo V. Preclinical changes in the mechanical properties of abdominal aorta in obese children. *Metabolism* 2004; **53**: 1243-1246 [PMID: 15334391 DOI: 10.1016/j.metabol.2004.03.023]

41 **Scuteri A**, Najjar SS, Muller DC, Andres R, Hougaku H, Metter EJ, Lakatta EG. Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness. *J Am Coll Cardiol* 2004; **43**: 1388-1395 [PMID: 15093872 DOI: 10.1016/j.jacc.2003.10.061]

42 **Iannuzzi A**, Licenziati MR, Acampora C, Renis M, Agrusta M, Romano L, Valerio G, Panico S, Trevisan M. Carotid artery stiffness in obese children with the metabolic syndrome. *Am J Cardiol* 2006; **97**: 528-531 [PMID: 16461050 DOI: 10.1016/j.amjcard.2005.08.072]

43 **Hopkins KD**, Lehmann ED, Jones RL, Turay RC, Gosling RG. A family history of NIDDM is associated with decreased aortic distensibility in normal healthy young adult subjects. *Diabetes Care* 1996; **19**: 501-503 [PMID: 8732717 DOI: 10.2337/diacare.19.5.501]

44 **Juonala M**, Järvisalo MJ, Mäki-Torkko N, Kähönen M, Viikari JS, Raitakari OT. Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study. *Circulation* 2005; **112**: 1486-1493 [PMID: 16129802 DOI: 10.1161/CIRCULATIONAHA.104.502161]

45 **Zebekakis PE**, Nawrot T, Thijs L, Balkestein EJ, van der Heijden-Spek J, Van Bortel LM, Struijker-Boudier HA, Safar ME, Staessen JA. Obesity is associated with increased arterial stiffness from adolescence until old age. *J Hypertens* 2005; **23**: 1839-1846 [PMID: 16148607 DOI: 10.1097/01.hjh.0000179511.93889.e9]

46 **Maple-Brown LJ**, Piers LS, O'Rourke MF, Celermajer DS, O'Dea K. Central obesity is associated with reduced peripheral wave reflection in Indigenous Australians irrespective of diabetes status. *J Hypertens* 2005; **23**: 1403-1407 [PMID: 15942464 DOI: 10.1097/01.hjh.0000173524.80802.5a]

47 **Greenfield JR**, Samaras K, Campbell LV, Jenkins AB, Kelly PJ, Spector TD, Hayward CS. Physical activity reduces genetic susceptibility to increased central systolic pressure augmentation: a study of female twins. *J Am Coll Cardiol* 2003; **42**: 264-270 [PMID: 12875762 DOI: 10.1016/S0735-1097(03)00631-4]

48 **Jourdan C**, Wühl E, Litwin M, Fahr K, Trelewicz J, Jobs K, Schenk JP, Grenda R, Mehls O, Tröger J, Schaefer F. Normative values for intima-media thickness and distensibility of large arteries in healthy adolescents. *J Hypertens* 2005; **23**: 1707-1715 [PMID: 16093916 DOI: 10.1097/01.hjh.0000178834.26353.d5]

49 **Urbina EM**, Kimball TR, Khoury PR, Daniels SR, Dolan LM. Increased arterial stiffness is found in adolescents with obesity or obesity-related type 2 diabetes mellitus. *J Hypertens* 2010; **28**: 1692-1698 [PMID: 20647860 DOI: 10.1097/HJH.0b013e32833a6132]

50 **Kappus RM**, Fahs CA, Smith D, Horn GP, Agiovlasitis S, Rossow L, Jae SY, Heffernan KS, Fernhall B. Obesity and overweight associated with increased carotid diameter and decreased arterial function in young otherwise healthy men. *Am J Hypertens* 2014; **27**: 628-634 [PMID: 24048148 DOI: 10.1093/ajh/hpt152]

51 **Wildman RP**, Mackey RH, Bostom A, Thompson T, Sutton-Tyrrell K. Measures of obesity are associated with vascular stiffness in young and older adults. *Hypertension* 2003; **42**: 468-473 [PMID: 12953016 DOI: 10.1161/01.HYP.0000090360.78539.CD]

52 **Urbina EM**, Khoury PR, McCoy CE, Dolan LM, Daniels SR, Kimball TR. Triglyceride to HDL-C ratio and increased arterial stiffness in children, adolescents, and young adults. *Pediatrics* 2013; **131**: e1082-e1090 [PMID: 23460684 DOI: 10.1542/peds.2012-1726]

53 **Liao D**, Arnett DK, Tyroler HA, Riley WA, Chambless LE, Szklo M, Heiss G. Arterial stiffness and the development of hypertension. The ARIC study. *Hypertension* 1999; **34**: 201-206 [PMID: 10454441 DOI: 10.3109/07853890008995943]

54 **Peralta CA**, Adeney KL, Shlipak MG, Jacobs D, Duprez D, Bluemke D, Polak J, Psaty B, Kestenbaum BR. Structural and functional vascular alterations and incident hypertension in normotensive adults: the Multi-Ethnic Study of Atherosclerosis. *Am J Epidemiol* 2010; **171**: 63-71 [PMID: 19951938 DOI: 10.1093/aje/kwp319]

55 **van den Munckhof ICL**, Jones H, Hopman MTE, de Graaf J, Nyakayiru J, van Dijk B, Eijsvogels TMH, Thijssen DHJ. Relation between age and carotid artery intima-medial thickness: a systematic review. *Clin Cardiol* 2018; **41**: 698-704 [PMID: 29752816 DOI: 10.1002/clc.22934]

56 **Laakso M**, Sarlund H, Salonen R, Suhonen M, Pyörälä K, Salonen JT, Karhapää P. Asymptomatic atherosclerosis and insulin resistance. *Arterioscler Thromb* 1991; **11**: 1068-1076 [PMID: 2065028 DOI: 10.1161/01.ATV.11.4.1068]

57 **Agewall S**, Fagerberg B, Attvall S, Wendelhag I, Urbanavicius V, Wikstrand J. Carotid artery wall intima-media thickness is associated with insulin-mediated glucose disposal in men at high and low coronary risk. *Stroke* 1995; **26**: 956-960 [PMID: 7762045 DOI: 10.1161/01.STR.26.6.956]

58 **Howard G**, O'Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad MF, Savage P, Bergman R. Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Circulation* 1996; **93**: 1809-1817 [PMID: 8635260 DOI: 10.1161/01.CIR.93.10.1809]

59 **Bertoni AG**, Wong ND, Shea S, Ma S, Liu K, Preethi S, Jacobs DR, Wu C, Saad MF, Szklo M. Insulin resistance, metabolic syndrome, and subclinical atherosclerosis: the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care* 2007; **30**: 2951-2956 [PMID: 17704348 DOI: 10.2337/dc07-1042]

60 **Rajala U**, Laakso M, Päivänsalo M, Pelkonen O, Suramo I, Keinänen-Kiukaanniemi S. Low insulin sensitivity measured by both quantitative insulin sensitivity check index and homeostasis model assessment method as a risk factor of increased intima-media thickness of the carotid artery. *J Clin Endocrinol Metab* 2002; **87**: 5092-5097 [PMID: 12414877 DOI: 10.1210/jc.2002-020703]

61 **Ryder JR**, Dengel DR, Jacobs DR, Sinaiko AR, Kelly AS, Steinberger J. Relations among Adiposity and Insulin Resistance with Flow-Mediated Dilation, Carotid Intima-Media Thickness, and Arterial Stiffness in Children. *J Pediatr* 2016; **168**: 205-211 [PMID: 26427963 DOI: 10.1016/j.jpeds.2015.08.034]

62 **Arad Y**, Newstein D, Cadet F, Roth M, Guerci AD. Association of multiple risk factors and insulin resistance with increased prevalence of asymptomatic coronary artery disease by an electron-beam computed tomographic study. *Arterioscler Thromb Vasc Biol* 2001; **21**: 2051-2058 [PMID: 11742884 DOI: 10.1161/hq1201.100257]

63 **Ong KL**, McClelland RL, Rye KA, Cheung BM, Post WS, Vaidya D, Criqui MH, Cushman M, Barter PJ, Allison MA. The relationship between insulin resistance and vascular calcification in coronary arteries, and the thoracic and abdominal aorta: the Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis* 2014; **236**: 257-262 [PMID: 25108074 DOI: 10.1016/j.atherosclerosis.2014.07.015]

64 **Meigs JB**, Larson MG, D'Agostino RB, Levy D, Clouse ME, Nathan DM, Wilson PW, O'Donnell CJ. Coronary artery calcification in type 2 diabetes and insulin resistance: the framingham offspring study. *Diabetes Care* 2002; **25**: 1313-1319 [PMID: 12145227 DOI: 10.2337/diacare.25.8.1313]

65 **Dabelea D**, Kinney G, Snell-Bergeon JK, Hokanson JE, Eckel RH, Ehrlich J, Garg S, Hamman RF, Rewers M; Coronary Artery Calcification in Type 1 Diabetes Study. Effect of type 1 diabetes on the gender difference in coronary artery calcification: a role for insulin resistance? The Coronary Artery Calcification in Type 1 Diabetes (CACTI) Study. *Diabetes* 2003; **52**: 2833-2839 [PMID: 14578303 DOI: 10.2337/diabetes.52.11.2833]

66 **Reilly MP**, Wolfe ML, Rhodes T, Girman C, Mehta N, Rader DJ. Measures of insulin resistance add incremental value to the clinical diagnosis of metabolic syndrome in association with coronary atherosclerosis. *Circulation* 2004; **110**: 803-809 [PMID: 15289378 DOI: 10.1161/01.CIR.0000138740.84883.9C]

67 **Qasim A**, Mehta NN, Tadesse MG, Wolfe ML, Rhodes T, Girman C, Reilly MP. Adipokines, insulin resistance, and coronary artery calcification. *J Am Coll Cardiol* 2008; **52**: 231-236 [PMID: 18617073 DOI: 10.1016/j.jacc.2008.04.016]

68 **Young MH**, Jeng CY, Sheu WH, Shieh SM, Fuh MM, Chen YD, Reaven GM. Insulin resistance, glucose intolerance, hyperinsulinemia and dyslipidemia in patients with angiographically demonstrated coronary artery disease. *Am J Cardiol* 1993; **72**: 458-460 [PMID: 8352190 DOI: 10.1016/0002-9149(93)91141-4]

69 **Shinozaki K**, Suzuki M, Ikebuchi M, Hara Y, Harano Y. Demonstration of insulin resistance in coronary artery disease documented with angiography. *Diabetes Care* 1996; **19**: 1-7 [PMID: 8720524 DOI: 10.2337/diacare.19.1.1]

70 **Folsom AR**, Eckfeldt JH, Weitzman S, Ma J, Chambless LE, Barnes RW, Cram KB, Hutchinson RG. Relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Stroke* 1994; **25**: 66-73 [PMID: 8266385 DOI: 10.1161/01.STR.25.1.66]

71 **Iannuzzi A**, Licenziati MR, Acampora C, De Michele M, Iannuzzo G, Chiariello G, Covetti G, Bresciani A, Romano L, Panico S, Rubba P. Carotid artery wall hypertrophy in children with metabolic syndrome. *J*

Hum Hypertens 2008; **22**: 83-88 [PMID: 17928879 DOI: 10.1038/sj.jhh.1002289]

72 **Koskinen J**, Magnussen CG, Sinaiko A, Woo J, Urbina E, Jacobs DR, Steinberger J, Prineas R, Sabin MA, Burns T, Berenson G, Bazzano L, Venn A, Viikari JSA, Hutri-Kähönen N, Raitakari O, Dwyer T, Juonala M. Childhood Age and Associations Between Childhood Metabolic Syndrome and Adult Risk for Metabolic Syndrome, Type 2 Diabetes Mellitus and Carotid Intima Media Thickness: The International Childhood Cardiovascular Cohort Consortium. *J Am Heart Assoc* 2017; **6**: e005632 [PMID: 28862940 DOI: 10.1161/JAHA.117.005632]

73 **Berenson GS**, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med* 1998; **338**: 1650-1656 [PMID: 9614255 DOI: 10.1056/NEJM199806043382302]

74 **McGill HC**, Herderick EE, McMahan CA, Zieske AW, Malcolm GT, Tracy RE, Strong JP. Atherosclerosis in youth. *Minerva Pediatr* 2002; **54**: 437-447 [PMID: 12244281 DOI: 10.1016/S0021-9150(98)00326-8]

75 **Bonora E**, Tessari R, Micciolo R, Zenere M, Targher G, Padovani R, Falezza G, Muggeo M. Intimal-medial thickness of the carotid artery in nondiabetic and NIDDM patients. Relationship with insulin resistance. *Diabetes Care* 1997; **20**: 627-631 [PMID: 9096992 DOI: 10.2337/diacare.20.4.627]

76 **Menezes AMB**, da Silva CTB, Wehrmeister FC, Oliveira PD, Oliveira IO, Gonçalves H, Assunção MCF, de Castro Justo F, Barros FC. Adiposity during adolescence and carotid intima-media thickness in adulthood: Results from the 1993 Pelotas Birth Cohort. *Atherosclerosis* 2016; **255**: 25-30 [PMID: 27816805 DOI: 10.1016/j.atherosclerosis.2016.10.026]

77 **Femia R**, Kozakova M, Nannipieri M, Gonzales-Villalpando C, Stern MP, Haffner SM, Ferrannini E. Carotid intima-media thickness in confirmed prehypertensive subjects: predictors and progression. *Arterioscler Thromb Vasc Biol* 2007; **27**: 2244-2249 [PMID: 17656672 DOI: 10.1161/ATVBAHA.107.149641]

78 **Solberg LA**, Strong JP. Risk factors and atherosclerotic lesions. A review of autopsy studies. *Arteriosclerosis* 1983; **3**: 187-198 [PMID: 6342587 DOI: 10.1161/01.ATV.3.3.187]

79 **Holme I**, Solberg LA, Weissfeld L, Helgeland A, Hjermann I, Leren P, Strong JP, Williams OD. Coronary risk factors and their pathway of action through coronary raised lesions, coronary stenoses and coronary death. Multivariate statistical analysis of an autopsy series: the Oslo Study. *Am J Cardiol* 1985; **55**: 40-47 [PMID: 3966398 DOI: 10.1016/0002-9149(85)90296-6]

80 **Kullo IJ**, Malik AR, Bielak LF, Sheedy PF 2nd, Turner ST, Peyser PA. Brachial artery diameter and vasodilator response to nitroglycerine, but not flow-mediated dilatation, are associated with the presence and quantity of coronary artery calcium in asymptomatic adults. *Clin Sci (Lond)* 2007; **112**: 175-182 [PMID: 16987102 DOI: 10.1042/cs20060131]

81 **Schächinger V**, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000; **101**: 1899-1906 [PMID: 10779454 DOI: 10.1161/01.CIR.101.16.1899]

82 **Benetos A**, Safar M, Rudnichi A, Smulyan H, Richard JL, Ducimetieère P, Guize L. Pulse pressure: a predictor of long-term cardiovascular mortality in a French male population. *Hypertension* 1997; **30**: 1410-1415 [PMID: 9403561 DOI: 10.1016/S1078-5884(97)80134-7]

83 **Laurent S**, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; **37**: 1236-1241 [PMID: 11358934 DOI: 10.1161/hy0102.099031]

84 **Boutouyrie P**, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension* 2002; **39**: 10-15 [PMID: 11799071]

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

86 **Sawabe M**, Takahashi R, Matsushita S, Ozawa T, Arai T, Hamamatsu A, Nakahara K, Chida K, Yamanouchi H, Murayama S, Tanaka N. Aortic pulse wave velocity and the degree of atherosclerosis in the elderly: a pathological study based on 304 autopsy cases. *Atherosclerosis* 2005; **179**: 345-351 [PMID: 15777552 DOI: 10.1016/j.atherosclerosis.2004.09.023]

87 **Yamashina A**, Tomiyama H, Arai T, Hirose K, Koji Y, Hirayama Y, Yamamoto Y, Hori S. Brachial-ankle pulse wave velocity as a marker of atherosclerotic vascular damage and cardiovascular risk. *Hypertens Res* 2003; **26**: 615-622 [PMID: 14567500 DOI: 10.1291/hypres.26.615]

88 **van Popele NM**, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, Hoeks AP, van der Kuip DA, Hofman A, Witteman JC. Association between arterial stiffness and atherosclerosis: the Rotterdam Study. *Stroke* 2001; **32**: 454-460 [PMID: 11157182 DOI: 10.1161/01.STR.32.2.454]

89 **Lorenz MW**, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation* 2007; **115**: 459-467 [PMID: 17242284 DOI: 10.1161/CIRCULATIONAHA.106.628875]

90 **Schmidt C**, Bergström G. Carotid Artery Intima-Media Thickness Predicts Major Cardiovascular Events During 7-Year Follow-Up in 64-Year-Old Women Irrespective of Other Glucometabolic Factors. *Angiology* 2017; **68**: 553-558 [PMID: 27729556 DOI: 10.1177/0003319716672526]

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ORIGINAL ARTICLE

Retrospective Cohort Study

New results on the safety of laparoscopic sleeve gastrectomy bariatric procedure for type 2 diabetes patients

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Author contributions: Guetta O performed data collection, statistical analysis, and the writing of this article; Ovnat A and Vakhrushev A recruited the patients and performed all the operations as well as treated further complications and perioperative care; Sebbag G made critical revision of the article and final approval of the version to be published; Dukhno O contributed to this paper.

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Abstract

BACKGROUND

It has been established that bariatric surgery, including laparoscopic sleeve gastrectomy (LSG), has a positive impact on type 2 diabetes mellitus (T2DM). However, less frequently T2DM is reported as a risk factor for complications with this type of surgery.

AIM

To evaluate the safety of LSG in T2DM.

METHODS

A retrospective cohort study was conducted over patients admitted for LSG from January 2008 to May 2015. Data was collected through digitized records. Any deviation from normal postoperative care within the first 60 d was defined as an early complication, and further categorized into mild or severe.

RESULTS

Nine hundred eighty-four patients underwent LSG, among these 143 (14.5%) were diagnosed with T2DM. There were 19 complications in the T2DM group (13.3%) compared to 59 cases in the non-T2DM (7.0%). Out of 19 complications in the T2DM group, 12 were mild (8.4%) and 7 were severe (4.9%). Compared to the non-T2DM group, patients had a higher risk for mild complications (Odds-ratio 2.316, CI: 1.163-4.611, $P = 0.017$), but not for severe ones ($P = 0.615$). An increase of 1% in hemoglobin A1c levels was associated with a 40.7% increased risk for severe complications ($P = 0.013$, CI: 1.074-1.843) but not for mild ones.

CONCLUSION

Our data suggest that LSG is relatively safe for patients with T2DM. Whether pre-operative control of hemoglobin A1c level will lower the complications rate has to be prospectively studied.

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Core tip: Laparoscopic sleeve gastrectomy is the most popular bariatric procedure worldwide today. Its impact among diabetic patients has been beneficial regarding diabetes control. This study is the first to examine the safety of the procedure in this subgroup of the population. We found that the diagnosis of diabetes mellitus is associated with an increased rate of mild postoperative complications but not with severe ones. Elevated hemoglobin A1c is a good predictor for the risk of severe complications.

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INTRODUCTION

Bariatric surgery is the standard of care for obesity and related morbidity. In the past few years, the most popular procedure worldwide has become laparoscopic sleeve gastrectomy (LSG) and accounting for more than 50% of bariatric procedures in the United States since 2014[1,2]. In addition, bariatric surgery has proved to be the only modality that has the potential to achieve complete remission of type 2 diabetes mellitus (T2DM), and its advantage over conservative therapy is significant[3-6]. The Diabetes Surgery Summit of experts in Rome in 2007, the American Diabetes Association in 2009, and the International Diabetes Federation in 2011 published guidelines to consider laparoscopic bariatric surgery as a treatment for T2DM[4,7].

T2DM is a proven risk factor for postoperative complications in other fields of surgery[8-12]. There is likely no single mechanism to explain this increased risk but wound healing, re-epithelization, angiogenesis, inflammatory response, pain, and coagulopathy are all negatively affected by T2DM. In addition, the risk for renal, cardiovascular, and respiratory failure is increased in T2DM patients undergoing surgery[13-22]. In bariatric procedures involving stapling of the gastrointestinal tract, specifically gastric bypass, T2DM is a significant risk factor for re-admission and early complications in some reports but not in others[23-31].

Nevertheless, reports of the contributing effects of T2DM in post-LSG complications are far and few between. As a result, the quoted risk in LSG for the general population was adopted for the T2DM subgroup and described in diabetes literature and guidelines as up to 15% for mild complications, 2%-6% for severe complications, and 0.1%-0.5% for mortality[32]. Our hypothesis is that postoperative morbidity of LSG in T2DM patients is higher than that of patients without T2DM[33]. The aim of this study is to examine the prevalence of complications after LSG in T2DM patients in comparison to control non-T2DM patients.

MATERIALS AND METHODS

Settings

A retrospective cohort study including all patients admitted for LSG from January 2008 to May 2015 at the Soroka University Medical Center (SUMC) was conducted. SUMC is a regional academic tertiary 1044-bed medical center in southern Israel, providing healthcare to a diverse population of one million.

Data sources

SUMC has been using digitized records, including in-hospital reports and outpatient follow-up (diagnoses, chronic diseases, surgical reports, BMI measurements, laboratory, and imaging tests) since 2000. After approval from the local ethics committee, data regarding patients in our study were gathered using this database.

Definitions

Complication: Any deviation from normal postoperative course in the first 60 d was defined as an early complication. An admission longer than 5 d and readmissions or reoperations within 60 d after surgery were considered a complication and were reviewed. In such cases, the record was expeditiously inspected in order to classify the exact type (leak, bleeding, stricture, dysphagia, renal and respiratory failure, or other) and the grade of the complication. The complications were graded using the conventional Clavien-Dindo (CD) classification system for postoperative complications[34]. In this study, severe complications are defined as CD 3b or higher (requiring intervention under general anesthesia, ICU hospitalization, multiorgan failure, or death). Complications graded as CD 3a or below (any deviation from the normal postoperative course requiring any drug therapy, parenteral nutrition, blood transfusion, or intervention not under general anesthesia) are defined as mild.

Type 2 diabetes: Patients are said to have T2DM if one of the following conditions is fulfilled: (1) diagnosis made by the general practitioner or taken from the admission note; (2) a diagnosis of complication of T2DM (ICD-9 code 2504 for diabetes with nephrotic manifestations, 2505 for diabetes with ophthalmic manifestations, 2506 for diabetes with neurological manifestations, 2507 for diabetes with peripheral circulatory disorders) was previously performed; and (3) hemoglobin A1c (HbA1c) level above 6.5% (48 mmol/mol) drawn 2 years prior to the operation.

Preoperative evaluation

In the bariatric practice, every patient with a BMI > 40 kg/m^2 is eligible for bariatric surgery. Additionally, patients with T2DM are considered for bariatric surgery with a BMI as low as 35 kg/m^2 or above. In Israel, a patient who is planned for a bariatric procedure needs approval of an institutional multidisciplinary committee that is composed by a bariatric surgeon, anesthesiologist, internist, nutritionist, and social worker. The patient was admitted the day before operation for final evaluation and preparation, which includes blood tests, preoperative anticoagulation therapy, fasting, and IV fluid administration.

Surgical technique

LSG was performed in a standardized fashion. At induction of anesthesia, a first-generation cephalosporin was given for prophylaxis. After peritoneal CO_2 insufflation unto 15 mmHg, 4 to 5 trocars were inserted through the abdominal wall. The greater omentum was dissected away from the gastric greater curvature. A bougie was then introduced by the anesthesiologist and positioned along the lesser curvature of stomach, as a template for gastric resection that starts about 5 cm above the pylorus, up proximally to the gastro-esophageal junction. Resection was performed with laparoscopic staplers fired along the greater curvature close to the bougie. Upon surgeon decision, staple line reinforcement technique was performed (with suture, bio-material, or none). A closed suction drain was positioned along the stomach stump. The bougie that was inserted to the stomach by the anesthesiologist was withdrawn at the end of the operation.

Postoperative care and follow-up

In the first postoperative day the patient was encouraged to drink 300 mL of clear liquid, followed by unlimited drink in the second postoperative day. In the third postoperative day, the patient was instructed to ingest a liquid diet. On the third postoperative day, if vital signs were within normal limits, the patient was in well condition, did not complain of abdominal pain, maintained an acceptable liquid intake, and the suction drain was of serous content under 100 mL a day, he or she was discharged. All patients were advised by a dietitian about the recommended diet for the next month. Every patient was discharged with prophylaxis anticoagulation therapy for the first 30 postoperative days. Follow-up visits at the bariatric clinic for encounter with the surgeon were held at 1 wk, 4 mo, 8 mo, and 1 year post-op.

T2DM patients were routinely followed in the pre-, intra-, and post-operative periods for plasma glucose levels. Insulin therapy was administered if needed in order to control levels below 180 mg/dL.

Statistical analysis

All analyses were performed using IBM SPSS Statistics, version 24.0 (Armonk, NY, United States, IBM Corp). All tests were two-tailed and were considered significant at $P \leq 0.05$. Baseline clinical and demographic variables were compared between study groups by Chi-square for categorical variables or *t*-test for continuous variables. We used chi-square or binomial logistic regression to examine the association between total, mild, and severe early complications as dependent variables and the following

independent variables: diabetes type 2, fasting glucose level, and HbA1c. In addition, the following independent variables were examined as well: age, gender, BMI, previous bariatric surgery, concomitant removal of gastric banding, length of operation, surgeon identity, cumulative surgeon experience for each case, and comorbidities including hypertension, chronic ischemic heart disease, dyslipidemia, smoking status, asthma, sleep apnea, and fatty liver. In the next stage we used multivariate binomial logistic regression with stepwise method to assess the variables presenting statistical significance in the univariate study ($P < 0.05$), and the possible interactions were considered.

RESULTS

In a seven-year study period, 984 patients underwent LSG (66.2% were women). Mean age and BMI were 39.2 ± 12.2 years and 41.7 ± 5.9 kg/m^2, respectively. There were 143 patients (14.4%) with T2DM. Only one mortality (0.1%) was reported from the whole cohort and occurred at postoperative day 56 in the non-T2DM group. Unfortunately, we could not find any information about any illnesses before the death of the patient, and that raises the suspicion that it was not caused by a medical condition.

Patient characteristics differed among the study and control group in a few variables. The majority of the T2DM patients were male, older, and had a higher prevalence of essential hypertension, dyslipidemia, chronic ischemic heart disease, and sleep apnea.

Fasting plasma glucose (FPG) levels were available for 560 patients (56.9%) of the total study population, of which 112 were T2DM patients (78.3%). Mean FPG levels were 152 ± 51 mg/dL and 96 ± 13 mg/dL in the T2DM group and non-T2DM group, respectively ($P = 0.001$).

HbA1c levels were available for 286 patients (29.1%) of the total study population, of which 115 were T2DM patients (80.4%). Mean HbA1c levels were 7.6% ± 1.8% (60 ± 15 mmol/mol) and 5.6% ± 0.5% (38 ± 5 mmol/mol) in the T2DM group and non-T2DM group, respectively ($P = 0.001$).

Demographic, biometric, and morbidity characteristics are shown in Table 1. To note, chronic diseases such as anemia, smoking status, dyslipidemia, and chronic ischemic heart disease were reported by the general practitioner and collected from the patient's record.

Table 2 shows the complications by CD Classification. There were 78 early complications in this study (7.9%) with 44 (4.5%) of them categorized as mild and 34 (3.5%) of them categorized as severe. T2DM patients had significantly higher early complications compared to the non-T2DM patients (13.3% *vs* 7.0%, $P = 0.01$). When analyzing the subgroups of mild and severe complications, T2DM patients had significantly more mild complications (8.4% *vs* 3.8%, $P = 0.01$) but not severe complications (4.9% *vs* 3.2%, $P = 0.31$). In a multivariate binomial logistic regression for total early complications, we included T2DM, gender, age, dyslipidemia, hypertension, chronic ischemic heart disease, sleep apnea, and previous bariatric history. After a stepwise procedure, only T2DM was observed to be a significant factor for early complications (Odds ratio 2.031, CI: 1.171-3.522, $P = 0.012$). A similar result was observed for mild complications (Odds ratio 2.316, CI: 1.163-4.611, $P = 0.017$) but not for severe complications ($P = 0.615$).

When analyzing FPG level as an independent variable, it was not found to be significant for early complications ($P = 0.557$), mild complications ($P = 0.668$), or severe complications ($P = 0.701$). When setting a cut point of FPG level below 126 or equal and above 126, we did not find any significant differences in early complications ($P = 0.260$), mild complications ($P = 0.708$), or severe complications ($P = 0.230$).

When analyzing HbA1c as an independent variable, we found that for every elevation of 1% in HbA1c, there was an elevation of 1.314 in the risk for early complications ($P = 0.008$, CI: 1.074-1.609). A similar result of an elevation of 1.407 in risk was observed for severe complications ($P = 0.013$, CI: 1.074-1.843) but not for mild complications. Data on complication subtypes across study population are detailed in Table 3. Due to low event rates in these subgroups, only descriptive statistics are detailed.

DISCUSSION

Reports of early complications after bariatric surgery are abundant, specifically in

Table 1 Patient characteristics

	Non-type 2 diabetes group, *n* = 841, *n* (%) or mean (± SD)	Type 2 diabetes group, *n* = 143, *n* (%) or mean (± SD)	*P*-value
Female	573 (68.1)	78 (54.5)	0.02
Age, yr	38 (± 12.0)	48 (± 11.0)	< 0.01
BMI, kg/m^2	41.8 (± 5.9)	41.4 (± 6.1)	0.48
Operative time, min	55 (20.8)	57 (24.6)	0.57
Dyslipidemia	110 (13.1)	75 (52.4)	< 0.01
Essential hypertension	106 (12.6)	63 (44.1)	< 0.01
Chronic ischemic heart disease	10 (1.2)	10 (7.0)	< 0.01
Smoking status	89 (10.6)	22 (15.4)	0.09
Asthma	34 (4.0)	9 (6.3)	0.22
Sleep apnea	36 (4.3)	13 (9.1)	0.01
Anemia	15 (1.8)	4 (2.8)	0.41
Previous bariatric surgery	237 (29.0)	29 (20.3)	0.03
Hemoglobin A1c level % (± SD), mmol/mol (± SD)	5.6 (± 0.5), 38 (± 5.0)	7.6 (± 1.8), 60 (± 15.0)	< 0.01
Fasting plasma glucose mg/dL (± SD)	96 (± 13.0)	152 (± 51.0)	< 0.01

LSG. In large cohorts, the rate of early complications ranges from 5.4% to 7.3% and readmission rate within 30 d is 2.8%[35-38]. The rate of severe complication in LSG is reported to be 1.2% to 2.2%. Our results show a higher rate of complications (7.9%) when compared to other studies and not only in T2DM patients. This discrepancy could be explained by the difference in definition of complication in each study. In this study we focused on the impact of the complication on general patient health and on the healthcare system rather than on the type of complication. For example, a leak is considered a formidable surgical complication, although in some cases it only moderately affects the patient, whereas a simple postoperative non-surgical complication such as pneumonia can lead to respiratory failure and death. We believe that this holistic approach is more instrumental for a non-surgeon professional, such as a general practitioner, endocrinologist, internist, or dietitian, when considering a bariatric surgery with the patient. In addition, because SUMC is the only medical center in southern Israel, every complicated case and readmission after surgery are seen at SUMC. Therefore, almost every complication is reported in this study. In other studies, it may be difficult to track complications after discharge of the patient due to the patient seeking treatment at a different facility.

For T2DM patients who undergo LSG, we found a significant increased risk for early complications, but this is significant only in the mild group and not in the severe group. This result is aligned with a retrospective Spanish-Portuguese multicenter study by Sánchez-Santos *et al*[39]. Of 2882 patients, 29.2% of them were T2DM patients, and they found a significantly higher risk for early complications (Odds-ratio 1.48, CI: 1.12-1.95) in the T2DM group compared to the non-T2DM group. Mortality in the group of T2DM was increased as well in this study, but not in our study. In another study based on the American College of Surgeons-National Surgical Quality Improvement Program (ACS-NSQIP) database of 2012-2013, T2DM was associated with increased risk for re-admission during the first 30 postoperative days[40].

In a study by Creange *et al*[41] based on the American College of Surgeons-National Surgical Quality Improvement Program database of 2012, 941 out of 6062 LSG patients had T2DM (15.5%). As in our study, the T2DM patient group was more likely to be male and older. In contrast to our results, T2DM was not found to be associated with increased 30 d complication rate.

Aminian *et al*[42] published an LSG risk calculator based on the same American College of Surgeons-National Surgical Quality Improvement Program 2012 database. In this analysis, type 1 and type 2 diabetes was found as a significant risk factor for 30 d complications. Creange *et al*[41] state that this difference in results stems from the contribution of type 1 diabetes patients in the Aminian *et al*[42] report. In our population, all patients with diabetes were diagnosed with T2DM.

This study is the first to assess the effect of FPG levels and HbA1c levels on 30 d complications after LSG. FPG was not found to affect 30 d complication rate, but it was found that any increase of 1% in HbA1c is associated with a significant increase of 31% in the risk for early postoperative complications. This result was maintained

Table 2 Mild and severe early complications (by Clavien-Dindo classification)[1], *n* (%)

Grade	Non-type-2-diabetes group, *n* = 841	Type 2 diabetes group, *n* = 143	*P*-value
1	14 (1.7)	6 (4.2)	
2	15 (1.8)	6 (4.2)	
3a	3 (0.4)	0 (0.0)	
Mild (CD ≤ 3a)	32 (3.8)	12 (8.4)	0.01
3b	15 (1.8)	4 (2.8)	
4a	7 (0.8)	1 (0.7)	
4b	4 (0.5)	2 (1.4)	
5	1 (0.1)	0 (0.0)	
Severe (CD ≥ 3b)	27 (3.2)	7 (4.9)	0.31
Total	59 (7.0)	19 (13.3)	0.01

[1]This is a univariate analysis. CD: Clavien-Dindo.

when analyzing the subgroup of severe complications, but not for mild ones. The effect of elevated HbA1c upon early complications after surgery was reported in general surgery as a protective factor is some reports (probably due to heightened postoperative vigilance and lower threshold to treat hyperglycemia), or as risk factor in trauma surgery and several orthopedic procedures in other reports[43-46]. In our study, the discrepancy between the result that elevated HbA1c is associated with higher risk for early postoperative complications (mild and severe) and the fact the T2DM as a disease by itself is not a risk factor for early complications (mild and severe) could be explained by the fact that many of the T2DM patients have balanced glucose levels, and the more important parameter when evaluating a patient is HbA1c level.

This study has several limitations. First, it is composed of retrospective data. This fact may be tempered by the large number of patients and the wide range of background variables that were collected. Second, despite comprehensive data collection, most patient records were not reviewed individually. Moreover, some data, such as reoperations or readmissions in other medical centers (even if they are part of the same medical insurance organization as our center) were not retrieved. Third, the definition of T2DM is mostly based on patient and primary physician report and not on detailed biochemical evaluation of every patient. This may lead to distortion in the distribution between the two groups. Fourth, only 56.9% of patients had FPG levels drawn and even less had reported HbA1c levels drawn (29.1% of total study population and 80.4% of T2DM group). In addition, this study does not analyze long-term complications in LSG in T2DM patients. The strengths are large sample size and the meticulous assessment of complications. Both of which enabled us to carefully inform the patient of the spectrum of complications that they may face.

Our data show an increased risk only for mild complications of LSG in T2DM patients. Together with extensive data on the chance of T2DM remission[6], we believe there is good evidence that LSG is a relatively safe and effective option for these patients. In addition, increased HbA1c should be noted as a risk factor for severe complications and further studies are required in order to assess whether strict diabetic control prior to operation may lead to reduced postoperative complications.

Table 3 Early complications (by type), *n* (%)

Early complication by type	Non-type 2 diabetes group, *n* = 841	Type 2 diabetes group, *n* = 143
Staple line dehiscence and leak	16 (1.9)	2 (1.4)
Stricture and dysphagia	20 (2.4)	8 (5.6)
Bleeding	7 (0.8)	3 (2.1)
Acute renal failure	0 (0.0)	3 (2.1)
Respiratory failure	4 (0.5)	0 (0.0)
Other	11 (1.3)	3 (2.1)
Total	59 (6.9)	19 (13.2)

ARTICLE HIGHLIGHTS

Research background

Bariatric surgery has been advocated as an effective therapy for type 2 diabetes mellitus (T2DM) in an abundance of studies. Nevertheless, when considering a modality of treatment, its benefits should be weighed against its risks.

Research motivation

The risks that lie in bariatric surgery in the subgroup of T2DM have not been thoroughly investigated. Complications after other types of surgery within this subgroup of patients has led us to believe that post-bariatric surgery complication rates may be elevated in T2DM patients.

Research objectives

The main objectives of the study were to evaluate any kind of postoperative complications in the T2DM group *vs* non-T2DM patients within 60 d of surgery. Any deviation from the normal postoperative course was considered a complication. Further categorization into mild and severe complications was performed. This categorization was based upon Clavien-Dindo classification which is a common postoperative complications grading system.

Research methods

All patients who underwent laparoscopic sleeve gastrectomy performed by three surgeons in a single institute were included. Data was extracted from a digitized database through specific queries regarding length of stay, imaging, reoperations, and readmissions in the first 60 d after the operation. Mortality was extracted from that system as well. Any case of deviation from the average length of stay (more than 3 d after operation), further imaging (no imaging is routinely performed after operation), reoperation, or readmission was studied carefully in order to define the exact type of complication and categorize as mild or severe.

Research results

Nine hundred and eighty-four patients underwent laparoscopic sleeve gastrectomy, among these 143 (14.5%) were diagnosed with T2DM. There were 19 complications in the T2DM group (13.3%) compared to 59 cases in the non-T2DM (7.0%). Out of 19 complications in the T2DM group, 12 were mild (8.4%) and 7 were severe (4.9%). Compared to the non-T2DM group, patients had a higher risk for mild complications (Odds-ratio 2.316, CI: 1.163-4.611, $P = 0.017$), but not for severe ones ($P = 0.615$). Any increase of 1% in hemoglobin A1c levels was associated with a 40.7% increased risk for severe complications ($P = 0.013$, CI: 1.074-1.843).

Research conclusions

In this study, we find that the rate of mild complications is increased in T2DM patients. It means that these patients will suffer more from problems such as dysphagia, surgical site infection, dehydration, pneumonia, and bleeding. But these complications can be treated easily and conservatively without the need for interventions under general anesthesia, reoperations, or prolonged ICU admissions. Together with our knowledge of significant weight loss and reduction in glycemic burden after bariatric surgery, we believe that these complications should be well tolerated in face of the potential long-term benefit of this therapy in this subgroup of patients.

Research perspectives

Another result of our study, that any elevation of 1% in HbA1c levels is associated with a 40.7% increased risk for severe complications should commence a process of evaluating preoperative diabetes control. We believe that in a future study, patients with relatively high HbA1c level (above 9%) should have a short course of pre-operative tight glycemic control tested against patients who do not receive this preoperative intervention. This will also help us understand the pathophysiology of diabetes in surgical patients, and whether complications are driven purely from glycemic control or from chronic micro- and macro-vascular damage associated with diabetes.

REFERENCES

1 **Ponce J**, Nguyen NT, Hutter M, Sudan R, Morton JM. American Society for Metabolic and Bariatric Surgery estimation of bariatric surgery procedures in the United States, 2011-2014. *Surg Obes Relat Dis* 2015; **11**: 1199-1200 [PMID: 26476493 DOI: 10.1016/j.soard.2015.08.496]

2 **American Society for Metabolic and Bariatric Surgery**. Estimate of Bariatric Surgery Numbers 2011–2014. Available from: URL: http://asmbs.org/resources/estimate-of-bariatric-surgery-numbers

3 **Wu GZ**, Cai B, Yu F, Fang Z, Fu XL, Zhou HS, Zhang W, Tian ZQ. Meta-analysis of bariatric surgery versus non-surgical treatment for type 2 diabetes mellitus. *Oncotarget* 2016; **7**: 87511-87522 [PMID: 27626180 DOI: 10.18632/oncotarget.11961]

4 **Rubino F**, Kaplan LM, Schauer PR, Cummings DE; Diabetes Surgery Summit Delegates. The Diabetes Surgery Summit consensus conference: recommendations for the evaluation and use of gastrointestinal surgery to treat type 2 diabetes mellitus. *Ann Surg* 2010; **251**: 399-405 [PMID: 19934752 DOI: 10.1097/SLA.0b013e3181be34e7]

5 **Dixon JB**, le Roux CW, Rubino F, Zimmet P. Bariatric surgery for type 2 diabetes. *Lancet* 2012; **379**: 2300-2311 [PMID: 22683132 DOI: 10.1016/S0140-6736(12)60401-2]

6 **Yska JP**, van Roon EN, de Boer A, Leufkens HG, Wilffert B, de Heide LJ, de Vries F, Lalmohamed A. Remission of Type 2 Diabetes Mellitus in Patients After Different Types of Bariatric Surgery: A Population-Based Cohort Study in the United Kingdom. *JAMA Surg* 2015; **150**: 1126-1133 [PMID: 26422580 DOI: 10.1001/jamasurg.2015.2398]

7 **American Diabetes Association**. Standards of medical care in diabetes--2009. *Diabetes Care* 2009; **32** Suppl 1: S13-S61 [PMID: 19118286 DOI: 10.2337/dc09-S013]

8 **Buehler L**, Fayfman M, Alexopoulos AS, Zhao L, Farrokhi F, Weaver J, Smiley-Byrd D, Pasquel FJ, Vellanki P, Umpierrez GE. The impact of hyperglycemia and obesity on hospitalization costs and clinical outcome in general surgery patients. *J Diabetes Complications* 2015; **29**: 1177-1182 [PMID: 26355027 DOI: 10.1016/j.jdiacomp.2015.07.027]

9 **Isik O**, Kaya E, Sarkut P, Dundar HZ. Factors Affecting Surgical Site Infection Rates in Hepatobiliary Surgery. *Surg Infect (Larchmt)* 2015; **16**: 281-286 [PMID: 25830815 DOI: 10.1089/sur.2013.195]

10 **Ata A**, Lee J, Bestle SL, Desemone J, Stain SC. Postoperative hyperglycemia and surgical site infection in general surgery patients. *Arch Surg* 2010; **145**: 858-864 [PMID: 20855756 DOI: 10.1001/archsurg.2010.179]

11 **Yang Z**, Liu H, Xie X, Tan Z, Qin T, Kang P. The influence of diabetes mellitus on the post-operative outcome of elective primary total knee replacement: a systematic review and meta-analysis. *Bone Joint J* 2014; **96-B**: 1637-1643 [PMID: 25452366 DOI: 10.1302/0301-620X.96B12.34378]

12 **Underwood P**, Askari R, Hurwitz S, Chamarthi B, Garg R. Preoperative A1C and clinical outcomes in patients with diabetes undergoing major noncardiac surgical procedures. *Diabetes Care* 2014; **37**: 611-616 [PMID: 24170760 DOI: 10.2337/dc13-1929]

13 **Blakytny R**, Jude E. The molecular biology of chronic wounds and delayed healing in diabetes. *Diabet Med* 2006; **23**: 594-608 [PMID: 16759300 DOI: 10.1111/j.1464-5491.2006.01773.x]

14 **Alzoubi F**, Joseph B, Andersson L. Healing of soft tissue lacerations in diabetic-induced rats. *Dent Traumatol* 2017; **33**: 438-443 [PMID: 28888078 DOI: 10.1111/edt.12372]

15 **Graiani G**, Emanueli C, Desortes E, Van Linthout S, Pinna A, Figueroa CD, Manni L, Madeddu P. Nerve growth factor promotes reparative angiogenesis and inhibits endothelial apoptosis in cutaneous wounds of Type 1 diabetic mice. *Diabetologia* 2004; **47**: 1047-1054 [PMID: 15164170 DOI: 10.1007/s00125-004-1414-7]

16 **Esser N**, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res Clin Pract* 2014; **105**: 141-150 [PMID: 24798950 DOI: 10.1016/j.diabres.2014.04.006]

17 **Delamaire M**, Maugendre D, Moreno M, Le Goff MC, Allannic H, Genetet B. Impaired leucocyte functions in diabetic patients. *Diabet Med* 1997; **14**: 29-34 [PMID: 9017350 DOI: 10.1002/(SICI)1096-9136(199701)14:1<29::AID-DIA300>3.0.CO;2-V]

18 **Rajamäki TJ**, Jämsen E, Puolakka PA, Nevalainen PI, Moilanen T. Diabetes is associated with persistent pain after hip and knee replacement. *Acta Orthop* 2015; **86**: 586-593 [PMID: 25953426 DOI: 10.3109/17453674.2015.1044389]

19 **Pomero F**, Di Minno MN, Fenoglio L, Gianni M, Ageno W, Dentali F. Is diabetes a hypercoagulable state? A critical appraisal. *Acta Diabetol* 2015; **52**: 1007-1016 [PMID: 25850539 DOI: 10.1007/s00592-015-0746-8]

20 **James MT**, Grams ME, Woodward M, Elley CR, Green JA, Wheeler DC, de Jong P, Gansevoort RT, Levey AS, Warnock DG, Sarnak MJ; CKD Prognosis Consortium. A Meta-analysis of the Association of Estimated GFR, Albuminuria, Diabetes Mellitus, and Hypertension With Acute Kidney Injury. *Am J Kidney Dis* 2015; **66**: 602-612 [PMID: 25975964 DOI: 10.1053/j.ajkd.2015.02.338]

21 **Estep JD**, Aguilar D. Diabetes and heart failure in the post-myocardial infarction patient. *Curr Heart Fail Rep* 2006; **3**: 164-169 [PMID: 17129509 DOI: 10.1007/s11897-006-0017-7]

22 **Klekotka RB**, Mizgała E, Król W. The etiology of lower respiratory tract infections in people with diabetes. *Pneumonol Alergol Pol* 2015; **83**: 401-408 [PMID: 26379004 DOI: 10.5603/PiAP.2015.0065]

23 **Garg T**, Rosas U, Rivas H, Azagury D, Morton JM. National prevalence, causes, and risk factors for bariatric surgery readmissions. *Am J Surg* 2016; **212**: 76-80 [PMID: 27133197 DOI: 10.1016/j.amjsurg.2016.01.023]

24 **Sanni A**, Perez S, Medbery R, Urrego HD, McCready C, Toro JP, Patel AD, Lin E, Sweeney JF, Davis SS. Postoperative complications in bariatric surgery using age and BMI stratification: a study using ACS-NSQIP data. *Surg Endosc* 2014; **28**: 3302-3309 [PMID: 25115863 DOI: 10.1007/s00464-014-3606-7]

25 **Maciejewski ML**, Winegar DA, Farley JF, Wolfe BM, DeMaria EJ. Risk stratification of serious adverse events after gastric bypass in the Bariatric Outcomes Longitudinal Database. *Surg Obes Relat Dis* 2012; **8**: 671-677 [PMID: 23058451 DOI: 10.1016/j.soard.2012.07.020]

26 **Campos GM**, Ciovica R, Rogers SJ, Posselt AM, Vittinghoff E, Takata M, Cello JP. Spectrum and risk factors of complications after gastric bypass. *Arch Surg* 2007; **142**: 969-975; discussion 976 [PMID: 17938311 DOI: 10.1001/archsurg.142.10.969]

27 **Tao W**, Plecka-Östlund M, Lu Y, Mattsson F, Lagergren J. Causes and risk factors for mortality within 1 year after obesity surgery in a population-based cohort study. *Surg Obes Relat Dis* 2015; **11**: 399-405 [PMID: 25604834 DOI: 10.1016/j.soard.2014.08.015]

28 **Geubbels N**, de Brauw LM, Acherman YI, van de Laar AW, Bruin SC. Risk Stratification Models: How

Well do They Predict Adverse Outcomes in a Large Dutch Bariatric Cohort? *Obes Surg* 2015; **25**: 2290-2301 [PMID: 25937046 DOI: 10.1007/s11695-015-1699-2]
29 **Longitudinal Assessment of Bariatric Surgery (LABS) Consortium**; Flum DR, Belle SH, King WC, Wahed AS, Berk P, Chapman W, Pories W, Courcoulas A, McCloskey C, Mitchell J, Patterson E, Pomp A, Staten MA, Yanovski SZ, Thirlby R, Wolfe B. Perioperative safety in the longitudinal assessment of bariatric surgery. *N Engl J Med* 2009; **361**: 445-454 [PMID: 19641201 DOI: 10.1056/NEJMoa0901836]
30 **Turner PL**, Saager L, Dalton J, Abd-Elsayed A, Roberman D, Melara P, Kurz A, Turan A. A nomogram for predicting surgical complications in bariatric surgery patients. *Obes Surg* 2011; **21**: 655-662 [PMID: 21161606 DOI: 10.1007/s11695-010-0325-6]
31 **Rawlins L**, Rawlins MP, Brown CC, Schumacher DL. Effect of elevated hemoglobin A1c in diabetic patients on complication rates after Roux-en-Y gastric bypass. *Surg Obes Relat Dis* 2013; **9**: 749-752 [PMID: 22884300 DOI: 10.1016/j.soard.2012.06.011]
32 **American Diabetes Association**. 7. Obesity Management for the Treatment of Type 2 Diabetes. *Diabetes Care* 2017; **40**: S57-S63 [PMID: 27979894 DOI: 10.2337/dc17-S010]
33 **Wölnerhanssen B**, Peterli R. State of the art: sleeve gastrectomy. *Dig Surg* 2014; **31**: 40-47 [PMID: 24819496 DOI: 10.1159/000354320]
34 **Clavien PA**, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, Schulick RD, de Santibañes E, Pekolj J, Slankamenac K, Bassi C, Graf R, Vonlanthen R, Padbury R, Cameron JL, Makuuchi M. The Clavien-Dindo classification of surgical complications: five-year experience. *Ann Surg* 2009; **250**: 187-196 [PMID: 19638912 DOI: 10.1097/SLA.0b013e3181b13ca2]
35 **Finks JF**, Kole KL, Yenumula PR, English WJ, Krause KR, Carlin AM, Genaw JA, Banerjee M, Birkmeyer JD, Birkmeyer NJ; Michigan Bariatric Surgery Collaborative, from the Center for Healthcare Outcomes and Policy. Predicting risk for serious complications with bariatric surgery: results from the Michigan Bariatric Surgery Collaborative. *Ann Surg* 2011; **254**: 633-640 [PMID: 21897200 DOI: 10.1097/SLA.0b013e318230058c]
36 **Pradarelli JC**, Varban OA, Ghaferi AA, Weiner M, Carlin AM, Dimick JB. Hospital variation in perioperative complications for laparoscopic sleeve gastrectomy in Michigan. *Surgery* 2016; **159**: 1113-1120 [PMID: 26506567 DOI: 10.1016/j.surg.2015.08.044]
37 **Birkmeyer NJ**, Dimick JB, Share D, Hawasli A, English WJ, Genaw J, Finks JF, Carlin AM, Birkmeyer JD; Michigan Bariatric Surgery Collaborative. Hospital complication rates with bariatric surgery in Michigan. *JAMA* 2010; **304**: 435-442 [PMID: 20664044 DOI: 10.1001/jama.2010.1034]
38 **Berger ER**, Huffman KM, Fraker T, Petrick AT, Brethauer SA, Hall BL, Ko CY, Morton JM. Prevalence and Risk Factors for Bariatric Surgery Readmissions: Findings From 130,007 Admissions in the Metabolic and Bariatric Surgery Accreditation and Quality Improvement Program. *Ann Surg* 2018; **267**: 122-131 [PMID: 27849660 DOI: 10.1097/SLA.0000000000002079]
39 **Sánchez-Santos R**, Corcelles Codina R, Vilallonga Puy R, Delgado Rivilla S, Ferrer Valls JV, Foncillas Corvinos J, Masdevall Noguera C, Socas Macias M, Gomes P, Balague Ponz C, De Tomas Palacios J, Ortiz Sebastian S, Sanchez-Pernaute A, Puche Pla JJ, Del Castillo Dejardin D, Abasolo Vega J, Mans Muntwyler E, Garcia Navarro A, Duran Escribano C, Cassinello Fernández N, Perez Climent N, Gracia Solanas JA, Garcia-Moreno Nisa F, Hernández Matias A, Valentí Azcarate V, Perez Folques JE, Navarro Garcia I, Dominguez-Adame Lanuza E, Martinez Cortijo S, González Fernández J. Prognostic Factors for Morbimortality in Sleeve Gastrectomy. The Importance of the Learning Curve. A Spanish-Portuguese Multicenter Study. *Obes Surg* 2016; **26**: 2829-2836 [PMID: 27193106 DOI: 10.1007/s11695-016-2229-6]
40 **Khorgami Z**, Andalib A, Aminian A, Kroh MD, Schauer PR, Brethauer SA. Predictors of readmission after laparoscopic gastric bypass and sleeve gastrectomy: a comparative analysis of ACS-NSQIP database. *Surg Endosc* 2016; **30**: 2342-2350 [PMID: 26307598 DOI: 10.1007/s00464-015-4477-2]
41 **Creange C**, Sethi M, Fielding G, Ren-Fielding C. The safety of laparoscopic sleeve gastrectomy among diabetic patients. *Surg Endosc* 2017; **31**: 907-911 [PMID: 27501726 DOI: 10.1007/s00464-016-5053-0]
42 **Aminian A**, Brethauer SA, Sharafkhah M, Schauer PR. Development of a sleeve gastrectomy risk calculator. *Surg Obes Relat Dis* 2015; **11**: 758-764 [PMID: 26117166 DOI: 10.1016/j.soard.2014.12.012]
43 **Jones CE**, Graham LA, Morris MS, Richman JS, Hollis RH, Wahl TS, Copeland LA, Burns EA, Itani KMF, Hawn MT. Association Between Preoperative Hemoglobin A1c Levels, Postoperative Hyperglycemia, and Readmissions Following Gastrointestinal Surgery. *JAMA Surg* 2017; **152**: 1031-1038 [PMID: 28746706 DOI: 10.1001/jamasurg.2017.2350]
44 **Jehan F**, Khan M, Sakran JV, Khreiss M, O'Keeffe T, Chi A, Kulvatunyou N, Jain A, Zakaria ER, Joseph B. Perioperative glycemic control and postoperative complications in patients undergoing emergency general surgery: What is the role of Plasma Hemoglobin A1c? *J Trauma Acute Care Surg* 2018; **84**: 112-117 [PMID: 29040204 DOI: 10.1097/TA.0000000000001724]
45 **Hikata T**, Iwanami A, Hosogane N, Watanabe K, Ishii K, Nakamura M, Toyama Y, Matsumoto M, Kamata M. High preoperative hemoglobin A1c is a risk factor for surgical site infection after posterior thoracic and lumbar spinal instrumentation surgery. *J Orthop Sci* 2014; **19**: 223-228 [PMID: 24368606 DOI: 10.1007/s00776-013-0518-7]
46 **Goldstein DT**, Durinka JB, Martino N, Shilling JW. Effect of preoperative hemoglobin A(1c) level on acute postoperative complications of total joint arthroplasty. *Am J Orthop (Belle Mead NJ)* 2013; **42**: E88-E90 [PMID: 24278910]

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ORIGINAL ARTICLE

Observational Study

Quantities of comorbidities affects physical, but not mental health related quality of life in type 1 diabetes with confirmed polyneuropathy

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Author contributions: Brock C and Brock B conceptualised the study; Brock C, Brock B, Jakobsen PE and Drewes AM designed the study; Meldgaard T acquired the data; Wegeberg AML, Hyldahl S and Brock C interpreted data; Wegeberg AML drafted the manuscript and all author contributed intellectual property and approved the final version; the authors declare that there is no conflict of interest associated with this manuscript.

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Informed consent statement: All

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Abstract

BACKGROUND

A large number of adults with long-term type 1 diabetes are affected by symmetrical peripheral neuropathy. These complications increase socioeconomic expenses and diminish the individual quality of life. The 36-Item Short Form Health Survey (SF-36) is a generic patient reported questionnaire, measuring mental and physical health related quality of life. We hypothesized that diabetic neuropathy would decrease physical and mental quality of life measured with SF-36, and that clinical appearance may be associated with the decline.

AIM

To investigate if diabetic neuropathy would decrease physical and mental quality of life measured with SF-36, and if clinical appearance may be associated with the decline.

METHODS

Forty-eight adults [age 50 ± 9 years, 10 females, disease duration 32 (14-51) years] with verified diabetic symmetrical peripheral neuropathy and 21 healthy participants (age 51 ± 6 years, 6 females) underwent standardised nerve

subjects gave their informed consent prior to inclusion.

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conduction testing and completed the SF-36 questionnaire. Furthermore, disease duration, number of comorbidities, both diabetes related and nondiabetes related, vibration perception threshold, number of hypoglycaemic events, HbA1c and administration way of insulin was notified.

RESULTS

In comparison to healthy subjects, patients' mental composite score was not significantly diminished (51.9 ± 8.9 *vs* 53.1 ± 5.5, $P = 0.558$), while the physical composite score was (46.3 ± 11.7 *vs* 54.6 ± 3.3, $P = 0.002$). As expected, the overall physical health related symptoms in patients were associated to total number of comorbidities ($P < 0.0001$), comorbidities relation to diabetes ($P = 0.0002$) and HbA1c ($P = 0.005$) as well as comorbidities not related to diabetes ($P = 0.0006$).

CONCLUSION

The finding of this study emphasises the importance of focusing on quality of life in adults with diabetes and especially in those with multiple comorbidities as well as the possibility of HbA1c as a biomarker for severe complication.

Key words: Quality of life; 36-Item Short Form Health Survey (SF-36); Diabetes mellitus, Type 1; Diabetic neuropathies; Comorbidity

Core tip: In this study, we found a diminishment of physical domains more so than mental components from the 36-Item Short Form Health Survey (SF-36), in 48 people with type 1 diabetes and verified diabetic symmetrical peripheral neuropathy when compared to 21 healthy controls. Additionally, this physical diminishment was associated with increases in number of comorbidities and HbA1c. To our knowledge a study of health related quality of life exclusively in people with type 1 diabetes and diabetic symmetrical peripheral neuropathy compared to healthy age-matched controls has not previously been performed, therefore these results are interesting for health care professionals interested in the connection between neuropathy and patient experience.

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INTRODUCTION

Diabetic symmetrical peripheral neuropathy is a frequent complication to type 1 diabetes, although the prevalence varies between countries[1]. The pathogenesis of diabetic neuropathy is not fully understood; however, it is generally accepted to be a consequence of hyperglycaemic exposure leading to activation of metabolic, biochemical, inflammatory and immune mediated pathways. Clinically, neuropathy can present itself both with and without symptoms, including decreased sensation (numbness) or pain[2,3]. The severity of peripheral neuropathy is associated with incapacitating complications and a shorter life expectancy for the individual. On top of this, research within the last years has focused increasingly on the physical as well as mental burden of diabetes on quality of life.

It has become more and more acknowledged in clinical practice, to evaluate patient centred outcomes in the measurement of disease burden, progression and treatment outcome and how these impacts fundamental health related quality of life (HRQoL). In an effort to standardise and examine HRQoL the development and validation of instruments, such as the 36-Item Short Form Health Survey (SF-36), enables comparisons of different conditions between diseases, populations and countries[4]. The SF-36 has proved valid and useful in surveys of general and specific populations comparing the relative burdens of diseases, and in differentiating the health benefits produced by a wide range of treatments. Previous trans-sectional studies have investigated the HRQoL status in diabetes; however, most studies were carried out in

different types of diabetes and with mixed phenotypes. Thus, there is a knowledge gap in characterising the HRQoL in a population with type 1 diabetes and diabetic symmetrical peripheral neuropathy[5,6].

We hypothesized that the presence of diabetic symmetrical peripheral neuropathy would decrease the individual physical and mental quality of life. Thus, the aim of this study was to compare the HRQoL in adults with type 1 diabetes as compared with healthy age-matched controls using the SF-36 questionnaire. In addition, in patients we wanted to investigate associations between HRQoL and: (1) disease duration; (2) number of comorbidities, both diabetes related and non-diabetes related; (3) vibration thresholds; (4) nerve conduction velocity of the efferent median nerve; (5) nerve conduction velocity of the afferent sural nerve; (6) number of hypoglycaemic events; (7) glycaemic state; and (8) the use of insulin pen or pump.

MATERIALS AND METHODS

Subjects

The study comprised baseline observations of 48 people with long-term type 1 diabetes and verified diabetic symmetrical peripheral neuropathy recruited at the Department of Endocrinology, Aalborg University Hospital, Denmark from June 2014 to March 2016, as part of a clinical trial investigating the effect of liraglutide on neuropathy (TODINELI trial, (EUDRA CT 2013-004375-12). The local ethics committee approved the study protocols (N-20130077). Inclusion criteria were adults (> 18 years) with type 1 diabetes for a minimum duration of two years and diabetic sensory neuropathy verified by nerve conduction velocity testing. Additional criteria has been described elsewhere[7]. In comparison, 21 adult healthy, age-matched, participants were recruited for comparison. Informed consent was obtained from all individual participants included in the study.

Health quality and pain perception questionnaires

To assess HRQoL, participants completed the SF-36[8-10]. Even though it is not specific for diabetes, it has been validated and the components are relevant to assess the symptom burden experienced in diabetes[11]. The SF-36 was developed as a multipurpose eight-scale profile of functional health and well-being scores (physical functioning, role limitation due to physical problems, bodily pain, general health, vitality, social functioning, role limitation due to emotional problems, and mental health), and two summary scores (physical component summary and mental component summary), to explain variations in patient outcomes, covering 4 wk prior to the test[8,12]. Scores from the 36 items are transformed to a 0-100 scale with higher scores equals better quality of life[6,12]. A greater than five point change on this scale is considered clinically significant[6].

Protocol

Health care professionals obtained information about disease duration, comorbidities and way of insulin intake. Vibration perception threshold was measured using a biothesiometer (Bio-Medical Instruments, Newbury, OH, United States) on the distal plantar surface of the big toes. Peripheral nerve conduction testing of the efferent median and afferent sural nerves was evaluated at the elbow and the ankle, respectively, with plastic bar electrodes at skin temperatures above 32 °C. A blood sample was taken for measurement of HbA1c (IFCC) and number of hypoglycaemic events was registered in a patient diary two days prior to the study day.

Statistical analysis

The statistical methods of this study were reviewed by all the authors. Normally distributed data was reported as means and standard deviations, non-normally distributed data as median and interquartile range while categorical data is provided as a percentage. An independent-sample *t*-test was undertaken to determine differences in HRQoL between the two groups. For parametric data, a Pearson's correlation tests were performed to investigate associations between HRQoL and disease duration, HbA1c, average vibration thresholds and nerve conduction velocity for the efferent median and sural nerves. For nonparametric data, a Spearman's correlation tests were performed to investigate associations between HRQoL and number of hypoglycaemic events, total number of comorbidities, diabetes related and non diabetes related comorbidities. Diabetes related includes hypertension, retinopathy, pain, albuminuria, erectile dysfunction and cardiovascular diseases. Non-diabetes related includes hypercholesterolemia, thrombose prophylaxis, operations, reflux, arthritis and arthroses, asthma and allergies, metabolic diseases, vitamin deficiencies and more. Additionally, an independent samples *t*-test was run

to determine if there was a difference between people with diabetes who used insulin pens or pump.

RESULTS

A total of 48 people with type 1 diabetes and 21 adult heathy volunteers were included in and completed the study. The demographic distribution is shown in Table 1 and displays no notable difference in demographic characteristic between the two groups.

Comparison between type 1 diabetics and healthy controls

As seen in Figure 1, when diabetes was present, a numerical decline was observed in every SF-36 domain, compared to healthy subject. Significant differences were found on physical functioning (78.6 ± 27.7 *vs* 96.7 ± 6.2, $P = 0.005$), role limitation due to physical problems (82.4 ± 31.7 *vs* 100 ± 0, $P = 0.01$), general health (64.4 ± 24.5 *vs* 85.3 ± 13.1, $P < 0.001$), vitality (65.5 ± 23.9 *vs* 78.1 ± 13.9, $P = 0.03$), role limitations due to personal or emotional problems (87.0 ± 27.3 *vs* 100 ± 0, $P = 0.03$) and the physical composite score (46.3 ± 11.7 *vs* 54.6 ± 3.3, $P = 0.002$). However, no significance was found lookig at bodily pain (76.2 ± 24.34 *vs* 87.3 ± 17.7, $P = 0.07$), social functioning (91.9 ± 13.9 *vs* 95.2 ± 15.0, $P = 0.39$), mental health (81.2 ± 16.9 *vs* 86.7 ± 13.6, $P = 0.20$) and the mental composite score (51.9 ± 8.9 *vs* 53.1 ± 5.5, $P = 0.56$).

Associations

There was a negative association between the physical composite score of SF-36 and number of comorbidities (r = -0.62, $P < 0.001$), both diabetes (r = -0.53, $P = 0.018$) and non-diabetes related (r = -0.51, $P < 0.001$), and HbA1c level (r = -0.41, $P = 0.005$), as can be seen in Figure 2. However, one of these were associated with the mental composite score of SF-36 ($P > 0.05$).

Additionally, physical parameters of physical function, role limitation due to physical health, bodily aim and general health were all associated to and number of comorbidities ($P < 0.01$), both diabetes ($P < 0.03$) and non-diabetes related ($P < 0.02$), while only physical function and bodily pain were associated to HbA1c ($P < 0.02$). More detail can be found in Table 2.

Disease duration, vibration threshold, nerve conduction velocity of the efferent median nerve and the afferent sural nerve, and number of hypoglycaemic events were not associated with HRQoL scores. Additionally, there was no difference in symptoms between people using standard insulin pens and people using insulin pumps.

DISCUSSION

This study partly confirms our hypothesis, as in particular physical domains and not mental domains were negatively affected in people with diabetes and diabetic symmetrical peripheral neuropathy, potentially limiting the patients in their daily work and social activities. This emphasizes the importance of assessing HRQoL in long-term diabetes. Additionally, increased numbers of comorbidities and high levels of HbA1c, were associated with decreased HRQoL scores.

Decreased physical HRQoL

Decreased HRQoL is of great importance. It has been shown that a 1 point decrease in physical functioning and physical composite scores equals an 9% increase in mortality risk, a 4% increase in the risk of hospitalization within six months, and a 12% increase in the risk of being unable to work[13]. As preventive medicine may be initiated in order to delay or reverse the negative impact on self-assessed health, the importance of assessing HRQoL is essential in monitoring the self-assessed burden of diabetes. Decreased HRQoL in people with diabetes has previously been shown in studies from Croatia, Norway and Australia[5,6,14,15]. However, the previously investigated cohorts consisted of both type 1 and type 2 diabetes. As patients with type 2 diabetes often appear with other comorbidities and stereotypical life style, this can have negative impact on the combined HRQoL[14]. In contrast, a study by Jacobson *et al*[16] studied HRQoL in people with type 1 diabetes over an average of 23 years and found no decrease in HRQoL scores over time. The present study we looked into HRQoL in patients with type 1 diabetes compared to healthy and we found numerically decreased and clinically relevant declines in HRQoL for all sub-scores, except social functioning and a most significant decrease in the physical components. This finding,

Table 1 Demographics

	Type 1 diabetes (*n* = 48)	Healthy controls (*n* = 21)	*P* value
Gender, male *n* (%)	38 (79)	15 (71)	0.48
Age	50 ± 9	51 ± 6	0.53
Height	178.4 ± 1.2	179.9 ± 1.9	0.51
Weight	90.0 ± 2.3	87.3 ± 4.5	0.56
Right handed *n* (%)	41 (85)	17 (81)	0.64
Smoking *n* (%)	10 (21)	4 (19)	0.87
Disease duration	32 (14-51)		
HbA1c (IFCC), mmol/mol	65.5 ± 9.7		

Numerical data was compared with a *t*-test and categorical data with a χ^2-test.

related to the mixed cohorts, is possibly because people with type 1 diabetes are found in all social groups, and hence also in all social groups with larger psychological resources in comparison to people with type 2 diabetes. Contrary, compared with the study by Jacobson *et al*[16], we only had a cross-sectional look at HRQoL and therefore do not know the long-term ramifications for our patient group.

No decrease in mental HRQoL in people with severe diabetic neuropathy

Mental health has received increased recognition in recent years. Studies have shown that people with type 1 diabetes have a three-fold rate of depression in comparison to the general population. However, in the current study we did not find a significant decreased mental composite score, nor in the mental domains of social functioning and mental health.

Comorbidities decrease HRQoL

The presence of physical disabling diabetic complications such as cardiovascular events, gastrointestinal dysfunction and neuropathy with or without pain, have been shown to decrease HRQoL[6,14,17,18]. In a cohort of people with type 1 diabetes who were followed over 6 years, disease duration and the presence of complications convincingly decreased the physical composite scores[19], and the presence of neuropathy in type 1 diabetes negatively influenced the physical composite score[20], in line with the impact on the sensory and motor system. Additionally, a study over 17 years in people with type 1 diabetes showed that development of microvascular complications significantly decreased HRQoL[16]. We showed no association between the severity of neuropathy and the HRQoL scores, nor with disease severity and duration. These findings are plausibly biased by the fact that all patients were included based on severe polyneuropathy. In a study assessing HRQoL in chronic diseases with the presence of comorbidities, HRQoL was decreased due to the chronicity, but this was exaggerated as the number of comorbidities increased[21]. In particular, Bjorner *et al*[13] showed that number of comorbidities did not affect mental composite score. The current finding where HRQoL decreased with an increased number of comorbidities, needs to be considered when preventive medicine and adequate disease management is planned.

HbA1C decreases HRQoL

Even though HbA1c only indicates the preceding 3-mo' glycaemic status, it provides patients and clinicians with an objective measure. Mellerio *et al*[22] studied the association between HbA1C and HRQoL in adult people with childhood onset type 1 diabetes. They concluded that no metabolic parameters, including HbA1c, was predictive of HRQoL, thereby indicating that social impact was more important than glycaemic control for the well-being[22]. In contrast, the mean disease duration in this cohort was 32 years and we showed that HRQoL decreased as HbA1c increased, which may reflect lack of life-long tight glucose control. On the other hand, this could also reflect the impact of neuropathy and its effect on the ability to tightly control glucose, thus pointing to HbA1c as a potential marker of complications and not solely of glucose control. The data on the association between HRQoL and hypoglycaemia is conflicting. Hypoglycaemic events affects negatively on the individual health, and quality of life[20]. However, in a larger cohorts of people with type 1 diabetes no association between hypoglycaemia and HRQoL was shown[20,23]. In contrast, in younger individuals an association between role limitations due to physical problems and hypoglycaemic events was reported[18]. Unfortunately, in the current study,

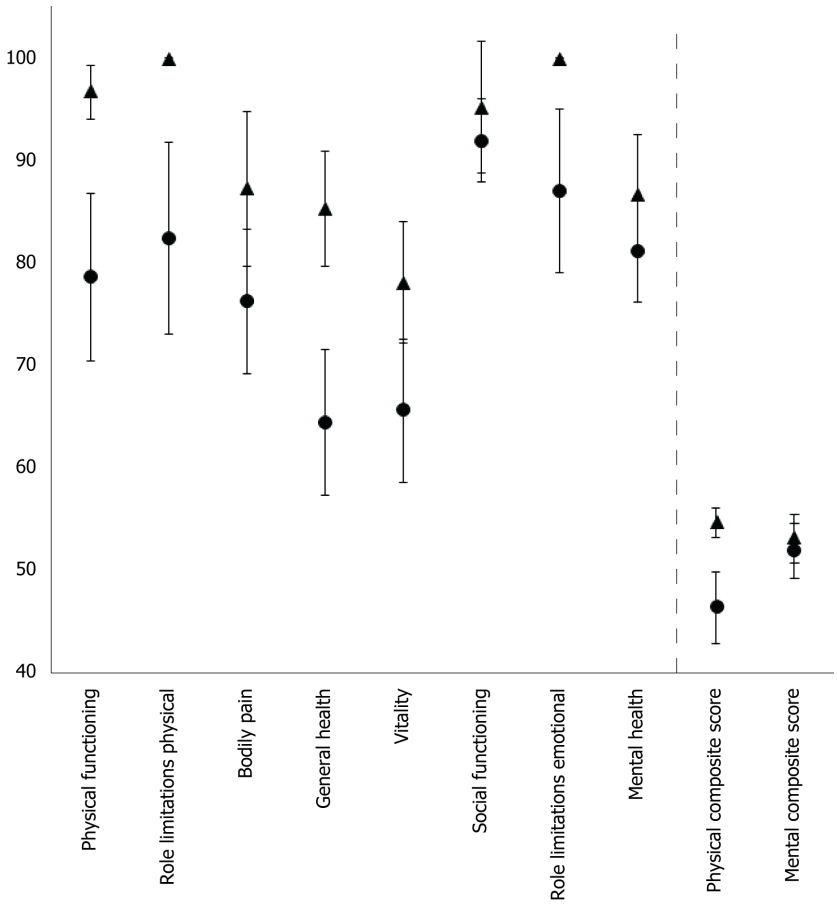

Figure 1 Comparison of mean SF-36 scores with confidence intervals for people with type 1 diabetes (●) and healthy participants (▲).

hypoglycaemic episodes were only sparsely recorded (48 h), and therefore it was not surprisingly, that we did not find any association between the number of hypoglycaemic events and HRQoL.

Insulin treatment is the core of glycaemic control and management of type 1 diabetes and with the rise of new technologies such as continuous glucose measurements combined with improved treatment the last couple of years, this has become easier for the patients. Surprisingly, Hart *et al*[17] showed that continuous insulin treatment decreased the mental composite score, due to the stress of regular blood glucose monitoring. Such findings were not supported by this study, as no differences in HRQoL were shown between people using insulin pens or pump.

Limitations

This study was not without limitations. Firstly, this study was conducted in a well-defined, middle-aged cohort with verified severe diabetic symmetrical peripheral neuropathy and thus the results cannot be directly generalised to other patient groups. Additionally, these were compared with healthy individuals, and therefore the effects measured may be skewed due to the effect of diabetes alone on SF-36. Secondly, we used the SF-36 to measure HRQoL assessments. Future studies may use the diabetes specific quality of life questionnaires and potentially add more insight into diabetes HRQoL. Lastly, it would have been interesting to study if hyperglycaemic events were associated to HRQoL in this cohort.

Conclusion

In summary, as hypothesised this study showed a decrease in the physical components of the HRQoL in a well-defined cohort of people with type 1 diabetes and severe diabetic symmetrical peripheral neuropathy. To our surprise, no associations were found in the mental components. Furthermore, decreased HRQoL was associated to number of co-morbidities and dysregulated glycaemic control, but not to the severity of neuropathy. This emphasises the importance of considering quality of life in people with diabetes, especially in those with multiple comorbidities. Furthermore, it is important to consider HbA1c as a biomarker for complication and

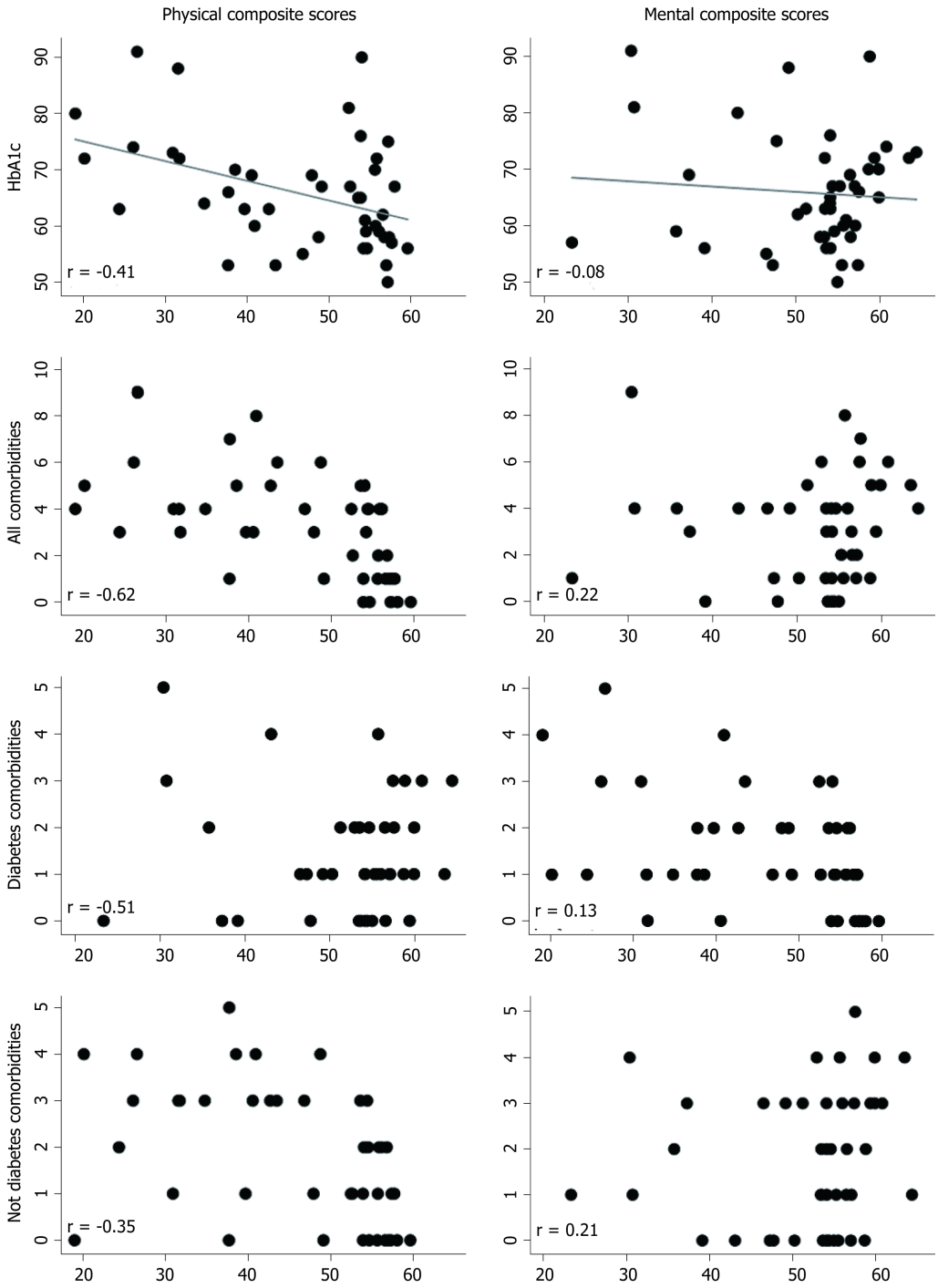

Figure 2 Correlations between physical and mental composite scores, HbA1c and comorbidities.

thereby indirectly for quality of life.

Table 2 Associations between 36-Item Short Form Health Survey and HbA1c, comorbidities, both diabetes and non-diabetes related in patients with type 1 diabetes

		HbA1c	All omorbidities	Diabetes elated	Non-diabetes related
Physical functioning	r	-0.51	-0.58	-0.42	-0.52
	p	< 0.01	< 0.01	< 0.01	< 0.01
Role limitation physical health	r		-0.44	-0.33	-0.38
	p		< 0.01	0.03	0.01
Bodily pain	r	-0.34	-0.48	-0.43	-0.38
	p	0.02	< 0.01	< 0.01	0.01
General health	r		-0.41	-0.34	-0.36
	p		< 0.01	0.02	0.02

Diabetes related comorbidities includes hypertension, retinopathy, pain, albuminuria, erectile dysfunction and heart and vessel diseases. Non-diabetes related comorbidities includes hypercholesterolemia, thrombose prophylaxis, operations, reflux, arthritis and arthroses, asthma and allergies, metabolic diseases, vitamin deficiencies and more.

ARTICLE HIGHLIGHTS

Research background

Diabetic symmetrical peripheral neuropathy is a frequent complication to type 1 diabetes and is associated to incapacitating complication and decreased lifespan, possibly affecting health related quality of life (HRQoL). The 36-Item Short Form Health Survey (SF-36) is a generic patient reported questionnaire, which can be used to evaluate mental and physical HRQoL in patients with diabetes.

Research motivation

HRQoL is an increasingly acknowledged method in clinical practice, to evaluate patient centred outcomes in the measurement of disease burden, progression and treatment outcome.

Research objective

To investigate if diabetic neuropathy would decrease physical and mental quality of life measured with SF-36, and if clinical appearance may be associated with the decline.

Research methods

Baseline data of standardised nerve conduction and SF-36 questionnaire as well as information on disease duration, number of comorbidities, vibration perception threshold, number of hypoglycaemic events, HbA1c and administration way of insulin was collected from 48 adults with verified diabetic symmetrical peripheral neuropathy and 21 healthy participants as part of a clinical trial.

Research results

People with diabetic symmetrical peripheral neuropathy had a significantly decreased physical score, but not mental score compared with healthy. Furthermore, this decrease in physical score was associated with total number of comorbidities, comorbidities relation to diabetes and HbA1c as well as comorbidities not related to diabetes.

Research conclusions

HRQoL is an important tool for evaluate patient centred outcomes in people with diabetes and is decreased with diabetic symmetrical peripheral neuropathy but also with increase in symptoms and suboptimal long-term glucose measures.

Research perspectives

HRQoL is an informative measure for use in investigation of diabetes and related neuropathy or symptoms in the future.

REFERENCES

1 **Tesfaye S**, Stevens LK, Stephenson JM, Fuller JH, Plater M, Ionescu-Tirgoviste C, Nuber A, Pozza G, Ward JD. Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: the EURODIAB IDDM Complications Study. *Diabetologia* 1996; **39**: 1377-1384 [PMID: 8933008 DOI: 10.1007/s001250050586]

2 **Tesfaye S**, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P; Toronto Diabetic Neuropathy Expert Group. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care* 2010; **33**: 2285-2293 [PMID: 20876709 DOI: 10.2337/dc10-1303]

3 **Callaghan BC**, Little AA, Feldman EL, Hughes RA. Enhanced glucose control for preventing and treating diabetic neuropathy. *Cochrane Database Syst Rev* 2012; **6**: CD007543 [PMID: 22696371 DOI: 10.1002/14651858.CD007543.pub2]

4 **Frendl DM**, Ware JE. Patient-reported functional health and well-being outcomes with drug therapy: a systematic review of randomized trials using the SF-36 health survey. *Med Care* 2014; **52**: 439-445 [PMID: 24714581 DOI: 10.1097/MLR.0000000000000311]

5 **Dermanovic Dobrota V**, Hrabac P, Skegro D, Smiljanic R, Dobrota S, Prkacin I, Brkljacic N, Peros K, Tomic M, Lukinovic-Skudar V, Basic Kes V. The impact of neuropathic pain and other comorbidities on the quality of life in patients with diabetes. *Health Qual Life Outcomes* 2014; **12**: 171 [PMID: 25468384 DOI: 10.1186/s12955-014-0171-7]

6 **Poljicanin T**, Ajduković D, Sekerija M, Pibernik-Okanović M, Metelko Z, Vuletić Mavrinac G. Diabetes mellitus and hypertension have comparable adverse effects on health-related quality of life. *BMC Public Health* 2010; **10**: 12 [PMID: 20070882 DOI: 10.1186/1471-2458-10-12]

7 **Brock C**, Jessen N, Brock B, Jakobsen PE, Hansen TK, Rantanen JM, Riahi S, Dimitrova YK, Dons-Jensen A, Aziz Q, Drewes AM, Farmer AD. Cardiac vagal tone, a non-invasive measure of parasympathetic tone, is a clinically relevant tool in Type 1 diabetes mellitus. *Diabet Med* 2017; **34**: 1428-1434 [PMID: 28703868 DOI: 10.1111/dme.13421]

8 **Ware JE**, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992; **30**: 473-483 [PMID: 1593914 DOI: 10.1097/00005650-199206000-00002]

9 **McHorney CA**, Ware JE, Raczek AE. The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. *Med Care* 1993; **31**: 247-263 [PMID: 8450681 DOI: 10.1097/00005650-199303000-00006]

10 **McHorney CA**, Ware JE, Lu JF, Sherbourne CD. The MOS 36-item Short-Form Health Survey (SF-36): III. Tests of data quality, scaling assumptions, and reliability across diverse patient groups. *Med Care* 1994; **32**: 40-66 [PMID: 8277801 DOI: 10.1097/00005650-199401000-00004]

11 **Polonsky WH**. Emotional and quality-of-life aspects of diabetes management. *Curr Diab Rep* 2002; **2**: 153-159 [PMID: 12643134 DOI: 10.1007/s11892-002-0075-5]

12 **Quah JH**, Luo N, Ng WY, How CH, Tay EG. Health-related quality of life is associated with diabetic complications, but not with short-term diabetic control in primary care. *Ann Acad Med Singapore* 2011; **40**: 276-286 [PMID: 21779616 DOI: 10.1016/j.disamonth.2011.05.008]

13 **Bjorner JB**, Lyng Wolden M, Gundgaard J, Miller KA. Benchmarks for interpretation of score differences on the SF-36 health survey for patients with diabetes. *Value Health* 2013; **16**: 993-1000 [PMID: 24041349 DOI: 10.1016/j.jval.2013.06.022]

14 **Hasler WL**. Gastroparesis: symptoms, evaluation, and treatment. *Gastroenterol Clin North Am* 2007; **36**: 619-647, ix [PMID: 17950441 DOI: 10.1111/j.1572-0241.2001.03350.x]

15 **Søfteland E**, Brock C, Frøkjær JB, Brøgger J, Madácsy L, Gilja OH, Arendt-Nielsen L, Simrén M, Drewes AM, Dimcevski G. Association between visceral, cardiac and sensorimotor polyneuropathies in diabetes mellitus. *J Diabetes Complications* 2014; **28**: 370-377 [PMID: 24355661 DOI: 10.1016/j.jdiacomp.2013.10.009]

16 **Jacobson AM**, Braffett BH, Cleary PA, Gubitosi-Klug RA, Larkin ME; DCCT/EDIC Research Group. The long-term effects of type 1 diabetes treatment and complications on health-related quality of life: a 23-year follow-up of the Diabetes Control and Complications/Epidemiology of Diabetes Interventions and Complications cohort. *Diabetes Care* 2013; **36**: 3131-3138 [PMID: 23835693 DOI: 10.2337/dc12-2109]

17 **Hart HE**, Redekop WK, Bilo HJ, Berg M, Jong BM. Change in perceived health and functioning over time in patients with type I diabetes mellitus. *Qual Life Res* 2005; **14**: 1-10 [PMID: 15789936 DOI: 10.1007/s11136-004-0782-2]

18 **Klein BE**, Klein R, Moss SE. Self-rated health and diabetes of long duration. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Diabetes Care* 1998; **21**: 236-240 [PMID: 9539988 DOI: 10.2337/diacare.21.2.236]

19 **Hart HE**, Redekop WK, Berg M, Bilo HJ, Meyboom-de Jong B. Factors that predicted change in health-related quality of life were identified in a cohort of diabetes mellitus type 1 patients. *J Clin Epidemiol* 2005; **58**: 1158-1164 [PMID: 16223659 DOI: 10.1016/j.jclinepi.2005.02.021]

20 **Hirai FE**, Tielsch JM, Klein BE, Klein R. Ten-year change in self-rated quality of life in a type 1 diabetes population: Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Qual Life Res* 2013; **22**: 1245-1253 [PMID: 22872499 DOI: 10.1007/s11136-012-0245-0]

21 **Hutchinson AF**, Graco M, Rasekaba TM, Parikh S, Berlowitz DJ, Lim WK. Relationship between health-related quality of life, comorbidities and acute health care utilisation, in adults with chronic conditions. *Health Qual Life Outcomes* 2015; **13**: 69 [PMID: 26021834 DOI: 10.1186/s12955-015-0260-2]

22 **Mellerio H**, Guilmin-Crépon S, Jacquin P, Labéguerie M, Lévy-Marchal C, Alberti C. Long-term impact of childhood-onset type 1 diabetes on social life, quality of life and sexuality. *Diabetes Metab* 2015; **41**: 489-497 [PMID: 25869639 DOI: 10.1016/j.diabet.2014.12.006]

23 **Hart HE**, Bilo HJ, Redekop WK, Stolk RP, Assink JH, Meyboom-de Jong B. Quality of life of patients with type I diabetes mellitus. *Qual Life Res* 2003; **12**: 1089-1097 [PMID: 14651426 DOI: 10.1023/A:1026197119569]

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SYSTEMATIC REVIEWS

Effectiveness of royal jelly supplementation in glycemic regulation: A systematic review

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Author contributions: Omer K and Newton G conceptualized research question and created search strategy; Omer K and Gelkopf MJ conducted full text inclusions and risk of bias assessments; Omer K and Newton G conducted GRADE assessment; Newton G reviewed manuscript throughout writing stage; Omer K conducted database search, data extraction and analysis, and manuscript preparation.

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Abstract

BACKGROUND

Royal jelly (RJ) has been observed to have therapeutic properties in diabetic individuals, including the reduction of high blood sugar. This systematic review synthesized existing evidence to investigate the effectiveness of RJ supplementation in managing measures of blood glucose.

AIM

To determine the effectiveness of RJ supplementation on glycemic responses in healthy and non-insulin dependent diabetic adults, as well as animal models of diabetes.

METHODS

This was a systematic review employing the PRISMA strategy. Peer-reviewed, published articles were extracted from several databases using key words related to target population, intervention and outcome and hand-selected for inclusion. Included articles proceeded to data extraction phase, where information on target parameters and effectiveness of treatment was summarized. Following this, the risk of bias for each included study was evaluated. Then, the long-term and immediate effectiveness of RJ supplementation in glycemic control were assessed using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) tool, which rates the quality of evidence.

RESULTS

Of 168 articles extracted from database searching, eighteen were included for analysis in this systematic review. Across the studies, studied populations, intervention styles and outcome measures were largely heterogeneous. Despite this, the results in studies indicate a general trend of positive effect of RJ in glycemic regulation *in vitro* and *in vivo*. Additionally, some dose-dependent glycemic effects were observed, along with some large effect sizes. The risk of bias for human and animal studies is generally low-unclear risk, although lack of

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blinding is a serious concern in both categories. Overall, as per the GRADE tool, the quality of evidence is low, and very low for long-term and immediate effectiveness of RJ, respectively. A major limitation affecting evidence quality is the heterogeneity among included studies. Fasting blood glucose and glucose clearance appear to be most affected by RJ supplementation.

CONCLUSION
Quality of evidence suggesting that RJ is an effective modulator of glycemic regulation is low for long-term effects of RJ, and very low for immediate effects.

Key words: Royal jelly; Type 2 diabetes; Dietary supplement; Glycemic control; 10-hydroxy-trans-2-decenoic acid; Hyperglycemia; Adults; Animals

Core tip: Royal jelly (RJ) is a promising natural treatment to improve high blood glucose. Insulin sensitivity, fasting blood glucose levels, and rate of glucose clearance are among the glycemic parameters investigated in the current systematic review that are shown to approach normal levels due to regular RJ intake.

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INTRODUCTION

The incidence of type 2 diabetes (T2D) has drastically increased over the past thirty years, making it among the most taxing diseases for health agencies worldwide[1-3]. As of 2017, projections for global prevalence of diabetes in 2030 have already been surpassed[4,5]. By 2040, the prevalence of T2D is expected to reach 642 million worldwide[5]. The epidemic is believed to stem from rapid changes in society since the 1980s that promote long periods of inactivity, energy and nutrient overconsumption; however, further epigenetic and genetic interactions continue to be explored[1,2]. T2D is heavily associated with cardiovascular disease and obesity, which largely accounts for the morbidity and mortality in patients with the disease[6]. Thus, to minimize risk of complications in patients, food intake and concentrations of blood glucose must be tightly managed[6].

Numerous natural health products, including royal jelly (RJ), have been explored as potential hypoglycemic agents[7,8]. RJ is a yellow, milky substance secreted by worker honey bees (*Apis mellifera*) through cephalic exocrine glands, such as the mandibular and hypopharyngeal glands[9]. RJ functions to nourish larvae for the first three days after being reared, although larvae destined to be reproductive queen bees are fed the material throughout their entire larval and adult life[9]. 10-hydroxy-2-decenoic acid (10H2DA), a fatty acid unique to RJ, is a major compound of interest in blood glucose management for its observed hypoglycemic effects[7]. Takikawa *et al*[7] found that 10H2DA significantly increases non-insulin dependent phosphorylation of AMP kinase (AMPK) in skeletal muscle, increasing translocation of glucose transporter type 4 (GLUT4) to cell surface and consequently, glucose transport into the cell. An *in vivo* study examining life-extending effects of RJ found that 10H2DA induces upregulation of molecules involved in caloric restriction, reducing energy intake[10]. 10H2DA has demonstrated interactions with estrogen receptors leading to alterations in gene expression, potentially including those involved in glucose regulation[11]. The glucose modulating role of 10H2DA is the most well-investigated mechanism by which RJ might benefit patients with T2D, although other RJ components, such as sebacic acid, may also be important.

In addition to *in vitro* studies, RJ administration has demonstrated therapeutic potential in human and rodent diabetic models. In a randomized controlled trial, Khoshpey *et al*[12] found that daily ingestion of capsules containing 3000 mg RJ for eight weeks significantly decreased fasting blood glucose (FBG) in diabetic individuals compared to a placebo group. However, the effects of RJ administration

on glycemic control outcomes are inconsistent across studies, possibly due to considerable variation in studied population and intervention. For example, while Khoshpey *et al*[12] found no significant change in carbohydrate (CHO) intake in the RJ-treated group, Pourmoradian *et al*[13] found a significant decrease in CHO intake in diabetic individuals in response to daily ingestion of 1000 mg lyophilized RJ for eight weeks. Rodent studies have shown a more pronounced effect: Ghanbari *et al*[14] found that addition of 100 mg/kg RJ in drinking solution improved circulating insulin and FBG in diabetic mice to levels similar to the healthy control group. Zamami *et al*[15] observed a similar magnitude of effect in insulin-resistant rats following administration of 300 mg/kg enzymatically treated RJ. It is difficult to apply the results of rodent studies into a human context due to variation in physiologic processes, particularly absorption and distribution of nutrients such as 10H2DA[16]. Presently, there is a lack of synthesis and analysis of these human and animal studies investigating the therapeutic effects of RJ.

This systematic review will investigate the effectiveness of RJ as a therapeutic agent in individuals with T2D. Specifically, we will assess animal, human and *in vitro* studies examining the effects of administration of RJ and its constituents on various outcomes that relate to glycemic control, such as plasma glucose levels, plasma lipid levels and hemoglobin A1c (HbA1c) levels in healthy and diabetic individuals. Included studies will investigate outcomes following both acute and long-term administration of RJ on glycemic control. The synthesis and evaluation of existing trials provides individuals and health care professionals with a resource to make informed decisions regarding T2D therapy. To our knowledge, no systematic review investigating RJ as a treatment for diabetes exists.

MATERIALS AND METHODS

The guidelines of the 2009 PRISMA model strategy were followed throughout this review[17].

Search strategy

We conducted a systematic search of peer-reviewed articles relating to the impact of RJ on glycemic outcomes. A set of keywords were developed by Kamel Omer and Genevieve Newton to yield trials with study variables that are appropriate for the research question. These were subsequently searched in five databases: Cochrane Library, CINAHL Plus, PubMed (*via* NCBI), Web of Science, and ProQuest. Operator commands were used to yield studies containing at least one keyword for each variable within the title or abstract. A review protocol does not exist for this systematic review.

Selection of articles

Following extraction and compilation of articles from the database results, duplicate studies were electronically removed. Bibliographies of studies were manually scanned to capture relevant studies. Titles and abstracts were manually screened and articles not meeting inclusion criteria were removed. Full text screening was completed for the remaining articles, where studies not meeting the inclusion criteria were removed. This was conducted in duplicate by Kamel Omer and Maxwell J Gelkopf; final decisions were settled by discussion. The resultant articles proceeded to the quality appraisal stage.

Inclusion criteria

(1) Population: Healthy or diabetic human adults or animal models; (2) Intervention: Oral administration of RJ or its constituents; (3) Outcomes assessed: Direct measures of glycemic control or measures pertinent to glycemic control; (4) *In vitro* studies: Effects of RJ (or constituents) administration were investigated on an outcome directly related to glycemic control; (5) Methodology includes control group for comparison with treatment; and (6) Available in English.

Data extraction

Data on study design, subjects, treatment, and relevant outcomes and results were abstracted for each included study, where applicable, qualitative and quantitative details were added to each of these study variables. For subjects, the extracted number of participants was those that completed the study. Data summaries of each included study were then classified into one of three different tables, depending on study population and intervention style. The classifications are: (1) Acute administration (examining immediate RJ effects) of RJ in human trials; (2) Long-term administration (examining long-term RJ effects) of RJ in human trials; and (3) Long-term

administration of RJ in animal trials and *in vitro* trials.

Risk of bias

For each included human and animal study, the Cochrane Collaboration's risk of bias assessment tool was used to determine the risk of bias[18]. The tool covers five pre-specified areas of bias: Selection bias (allocation concealment and random sequence generation), performance bias (blinding of participants and researchers), detection bias (blinding of outcome assessment), attrition bias (incomplete outcome data), and reporting bias (selective reporting)[18]. The tool also includes an "other bias" category for other sources of bias that do not fall within these prespecified areas[18]. For each area of bias, the tool provides criteria to rate the study as low, high, or unclear risk of bias. A high risk of bias indicates a high possibility of bias that is likely to impact the study results, while a low risk suggests negligible risk[18]. An unclear risk of bias indicates that insufficient information is provided to determine if results are impacted by bias, but some doubt is raised[18].

Two authors, Kamel Omer and Maxwell J Gelkopf, assessed all included articles to reach a consensus on the risk of bias for each study. Higgins *et al*[18] provides a detailed guide on the ranking procedure, which was applied by reviewers throughout the risk of bias assessment. Although the tool is designed for human trials, O'Connor and Sargeant[19] developed a modified version for use in risk of bias assessment of animal trials. This modified version was used by the reviewers to guide ranking of risk of bias for the included animal studies. Justification was noted for each judgement, including paraphrases and direct quotes from the article when available.

Quality of evidence

Following determination of the risk of bias for each study, the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) tool was used to rate the overall quality evidence across all included studies for a single outcome[20]. In this review, GRADE was used to assess the quality of evidence for RJ's capacity to manage blood glucose levels following long-term supplementation of RJ as well as acute effects of RJ administration. The GRADE tool encompasses five domains to determine overall quality: risk of bias, indirectness to research question, imprecision of results, inconsistency between studies and publication bias[20].

To represent the GRADE rating, a summary of findings table was created. Ranking begins at high quality due to the majority of trials being randomized controlled trial but are gradually degraded if serious concern exists in any of the five domains[20]. Ranking can also be upgraded if there is no plausible confounding bias, or if magnitude of effect is large or dose-dependent[20]. The overall ranking is determined by number and magnitude of all downgrades and upgrades[20]. Each of the four possible rankings directly correspond to a certain overall quality of evidence (very low, low, moderate, high)[19]. Ryan and Hill[21] provide criteria for what constitutes a concern in these domains, which was used as a guide for reviewers when determining the GRADE ranking. To factor animal studies in, a modified version of GRADE for animal studies developed by Wei *et al*[22] was used. Kamel Omer and Genevieve Newton reached a consensus on the GRADE score based on criteria and justification was provided for each decision on the summary of findings table.

Summary measures

To determine effect size and the precision of the quantitative data of the study results, appropriate measures were manually calculated. Standardized mean difference (SMD, also known as Cohen's d) was calculated as described by Faraone[23] using a pooled standard deviation and sample means of the treatment and placebo group to estimate magnitude of treatment effect. A large effect size constitutes a SMD of > 0.8, while a small effect size is considered to have a SMD of < 0.2; all values in between are medium effect sizes[23]. A negative value indicates that the treatment reduces the parameter being investigated[23]. The SMD was calculated for values taken at the endpoint of the study. For studies with multiple treatment groups, the SMD was calculated for the group with highest dosage. Where numerical values were unavailable, values were interpolated from provided graphs. If values were reported as medians, these were used in place of the mean throughout the effect size calculation. As part of the GRADE evaluation, 95%CIs of SMDs for long-term, hypoglycemic outcomes were calculated to assess imprecision of the effect sizes. For simplicity of evaluation and comparison, SMDs were reported in this review for statistically significant outcomes only.

RESULTS

Study selection

The pre-specified search strategy was conducted on March 21st, 2018, yielding 168 results. Grey literature databases were searched, but no pertinent articles were found. One study was captured from scanning the bibliographies of collected studies. After removal of duplicates, the total number of unique studies was determined to be 83. These studies went on to the title and abstract screening. Fifty-seven studies were removed due to not meeting the inclusion criteria at this stage. The remaining 26 underwent a full-text screening to determine eligibility. Of these 26, eight were excluded: two because they were not written in English, two for assessing outcomes not related to glycemic control, one because it lacked an oral intervention in treatment groups, one because the study design did not have a control group, one because it lacked an intervention, and one because it did not target the desired population for this review. The resultant 18 articles were included in the systematic literature review for quality of evidence appraisal (Figure 1).

Study characteristics

Following the systematic search, study characteristics, including intervention style, length, participants and results were manually extracted and summarized into the Tables 1, 2 and 3[24-36].

Risk of bias within studies

For human trials, the pre-specified areas of bias are generally low risk. The biggest source of concern for bias stems from the lack of transparency of measures taken to prevent a given area of bias. This was particularly evident in the allocation concealment category, as evidenced by the high proportion of human trials at high or unclear risk in that category (Figure 2). Moreover, although many studies claimed that participants and personnel were blinded, description of the actual blinding methodology was rarely provided, suggesting that performance and detection bias are considerable concerns among included human studies. The next biggest source of concern for bias is apparent in the "other bias" section-this is largely due to confounding bias arising from the recruitment of participants from a single source (*e.g.*, common hospital). Overall, of the human trials, domains of bias at high risk are relatively few.

Across the animal studies, the risk of bias is a notably bigger concern. One hundred percent of the studies are considered high risk of detection bias (*i.e.*, group allocations known to outcome assessors), potentially due to blinding being uncommon in animal studies (Figure 3). Attrition bias is another serious concern across the animal studies; most included animal studies excluded some individual subjects from analysis without any explanation. Performance and selection bias, however, are well accounted for in the included animal studies, with nearly 100% of both domains at low risk. Like human trials, reporting bias is not a serious concern in the animal trials (Figure 4).

Overall quality of evidence

The quality of evidence was evaluated as per the GRADE criteria with results shown in Tables 4 and 5. This evaluation integrated study results with risk of bias across all studies included in this review.

Effectiveness

Direct measures of glycemic control (FBG, glucose clearance rate, insulin levels) appear to be appreciably impacted by RJ administration. Most of the included studies which examined FBG and rate of glucose clearance observed significant change from baseline due to oral supplementation of RJ. Of these results, the majority had large effect size estimates, substantiating the role of the intervention in the observations. Most of the studies investigating insulin levels also reported a beneficial effect. Furthermore, in studies with multiple experimental groups, a dose-response relationship was observed in plasma insulin levels and rate of glucose clearance, but not FBG levels. Abnormal regulation of these parameters give rise to other secondary conditions associated with T2D, such as high levels of HbA1c.

The beneficial effect of RJ supplementation was demonstrated by improved indirect measures of glycemic control. These indirect measures are precursors (*e.g.*, high circulating fat) or indicators (*e.g.*, HbA1c) of hyperglycemia. The effectiveness of RJ administration in improving these parameters was not as apparent, as there was no clear trend in outcome responses to RJ treatment. However, when significant changes were detected in the experimental group, these results mostly had large effect magnitude estimates. The inconsistency between observed effects may be due to heterogeneity among the included studies.

The wide range of supplementation forms included in the evidence may explain

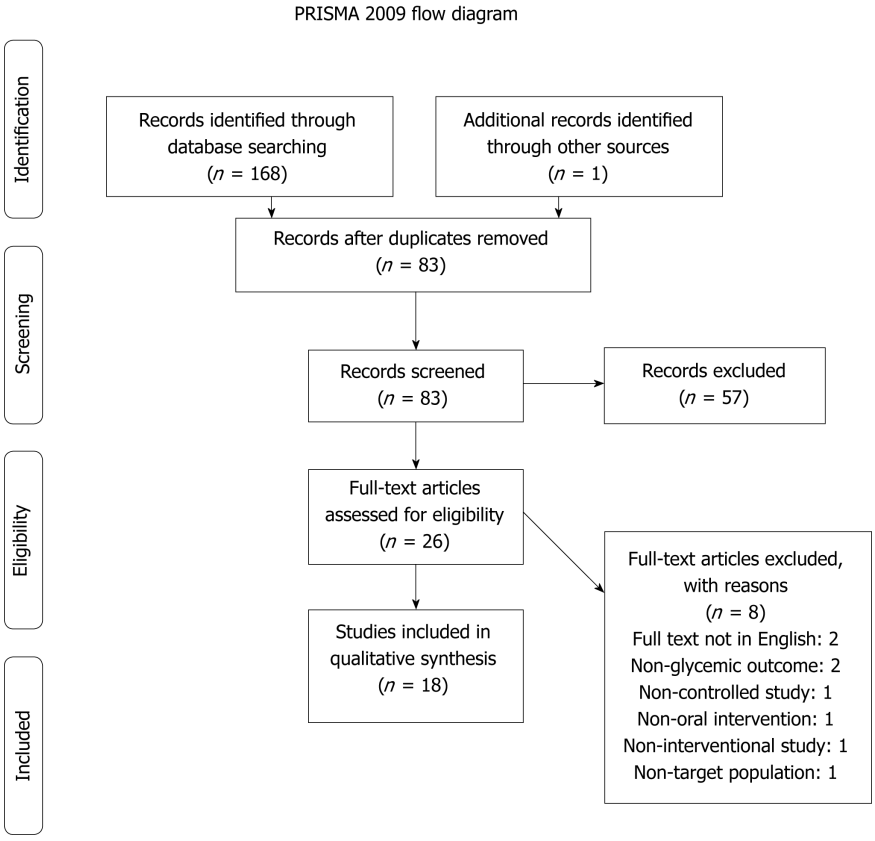

Figure 1 Flow diagram summarizing study selection process.

some of the observed inconsistency between results. For example, many studies used lyophilized RJ, which is known to be chemically different and less bioactive compared to fresh RJ[27,37]. Numerous clinical trials used enteric coated capsules to deliver RJ, which is known to alter pharmacodynamic properties of compounds[38]. Some animal and human studies added the supplementation to meals, which may affect effectiveness due to food-drug interactions[39]. When similar populations were studied, some outcomes showed reduced, negated or contradictory effects in response to RJ as compared to 10H2DA and vice versa, such as between Yoshida *et al*[36] and Watadani *et al*[35]. This circumstance indicates the possibility of RJ constituents having interactions amplifying or diminishing effectiveness of certain glycemic outcomes.

Due to the different investigation types and subjects across the included studies, it is important to contextualize the effectiveness for the different populations. The largest improvements to blood glucose were observed in diabetic models, both human and animals. Healthy (human) models had some improvements in blood-glucose parameters; however, for most normal blood-glucose parameters, there is a limit on the possible difference from baseline. Only one *in vitro* was included and was used in this review primarily to elucidate mechanisms and support the *in vivo* results. Thus, the effectiveness of RJ as a glycemic regulator was determined through the lens of diabetic human patients, with the other populations used to support the findings.

Mostly displayed in the rodent trials, there was a marked difference on effectiveness of RJ administration between studies that investigated the effects of RJ between genders, with greater efficacy seen in males. The divergent effects may potentially be due to the estrogenic activity of compounds derived from RJ, particularly 10H2DA and a sterol, 24-methylenecholesterol[11]. These have weak affinity for estrogen receptors that induce changes in gene expression[11]. Because these compounds would compete with endogenous estrogen for receptor binding, RJ may not be effective in individuals with elevated estrogen levels (*i.e.*, premenopausal females). Correspondingly, estrogen perfusion has previously been linked to improved hyperglycemic symptoms in postmenopausal females and males, which is consistent with the evidence in this review[40,41].

Although very few participants in the included study reported undesirable effects of RJ consumption, potential adverse effects in humans are an important factor in their feasibility as a glycemic regulator. One case report found an association between haemorrhagic colitis and daily RJ intake, which had never been documented

Table 1 Human trials examining acute effects of royal jelly treatment

Ref.	Study design	Subjects	Treatment	Outcome measures	Effectiveness
Iaconelli *et al*[24]	Crossover study	N = 10 + 10 healthy individuals and individuals with type 2 diabetes	Each subject went through three studies on different days: 0 g, 12 g, or 23 g of sebacic acid substituted fats in a meal	Glucose clearance: Postprandial	Significantly improved glucose clearance in diabetic subjects only in dose-dependent manner (d = -1.70)
				Insulin secretion/clearance rate	Significantly improved GLUT4 expression (d = 0.81) and glucose uptake in L6 cells (d = 0.67)
				GLUT4 expression in L6 myotube cells	Insulin secretion/clearance decreases significantly in similar fashion between healthy and diabetic patients. Dose-response relationship. For diabetics, d = -1.12
Mobasseri *et al*[25]	Randomized controlled trial	N = 20 + 20 adults with type 2 diabetes aged 30-65 in control and treatment groups	15 g of royal jelly ingested orally after overnight fasting	Hyperglycemia: Fasting blood glucose, glucose clearance after royal jelly consumption	No significant difference in outcome measures between two groups
				Hyperinsulinemia: Serum c-peptide and insulin	
Münstedt *et al*[26]	Controlled trial	N= 10 + 10 healthy males, split into experimental and control groups	20 g of fresh royal jelly ingested orally	Glucose clearance: Plasma samples during OGTT	Significantly increased rate of glucose clearance (insufficient information for effect size calculation)
				Insulin resistance: Serum insulin and c-peptide	No significant change in serum insulin profile
Münstedt *et al*[27]	Randomized controlled trial	N = 15 healthy male adults aged 20-34, unspecified distribution between treatment and control groups	0.55 g lyophilized royal jelly in enteric-coated capsule ingested orally	Hyperglycemia: Glucose clearance (OGTT)	Improved glucose clearance and decreased plasma insulin, unspecified statistical significance (insufficient information for effect size calculation)
				Insulin resistance: Serum insulin and c-peptide	

OGTT: Oral glucose tolerance test; GLUT4: Glucose transporter type 4.

previously[42]. Bronchospasm and anaphylaxis have also been noted in individual cases[43,44]. The aforementioned cases are possibly due to allergic reactions to RJ proteins[42,43,44]. Harmful RJ-drug interactions should be considered; consuming RJ while taking warfarin has been associated with hematuria[45].

DISCUSSION

The present systematic review suggests that RJ has a positive effect on both direct and indirect measures of glycemic control in diabetic and healthy individuals. This general trend was observed in both animal and adult human trials but was more pronounced in the former. In healthy individuals, supplementation of RJ may reduce risk of developing hyperglycemia and insulin resistance. With the evidence presented, RJ is likely more effective as a long-term dietary supplement rather than for acute treatment of hyperglycemia. Effective clinical doses appear to be as low as 1000 mg of fresh RJ daily for diabetic humans, but true values may vary between individuals and supplementation form.

Regulation of glycemic control by 10H2DA

In a normal state, intracellular protein-protein interactions arising from insulin binding to its surface receptor are critical to blood glucose regulation[46,47]. One major result of the signaling cascade induced by insulin on various tissue types is the translocation of the GLUT4 glucose transporter to the cell surface, which works to import glucose into the cell[46]. The insulin-dependent pathway also modifies gene

Table 2 Human trials examining effects of long-term royal jelly treatment

Ref.	Study design	Subjects	Treatment	Outcome measures	Effectiveness
Khoshpey *et al*[12]	Randomized double-blind controlled trial	N = 11 females + 12 males aged 20-65 with type 2 diabetes in control group (placebo)	3000 mg royal jelly oral capsules once per day for 8 wk. Control received placebo	Macronutrient intake	No significant change in macronutrient intake
		N = 13 females + 10 males aged 20-65 with type 2 diabetes in treatment group		Hyperglycemia: Fasting blood glucose	Fasting blood glucose significantly reduced in comparison to control group (d = -0.87)
Mobasseri *et al*[28]	Randomized controlled trial	N = 25 + 25 females with type 2 diabetes aged 30-65 in control and treatment groups	200 mg royal jelly powder prepared in gel form and served with breakfast for 8 wk. Control group received placebo	Plasma triglyceride	Significantly decreased plasma triglyceride in comparison to control (d = -0.476)
Morita *et al*[29]	Randomized double-blind controlled trial	N = 30 healthy adults 42-83 yr of age in control (placebo)	3000 mg royal jelly in 100 mL liquid daily for 6 mo. Control received placebo identical in appearance	Body weight: BMI	Significantly improved fasting blood glucose (d = -0.9596)
		N = 31 healthy adults 42-83 yr of age in treatment group		Insulin resistance: HOMA-IR	No significant changes in other outcomes of interest
				Hyperglycemia: HbA1c, fasting blood glucose	
				Plasma triglyceride	
Pourmoradian *et al*[13]	Human double-blinded randomized clinical trial	N = 23 females aged 30-65 with type 2 diabetes in treatment group	1000 mg lyophilized royal jelly in soft gel form served after breakfast for 8 wk. Control group received placebo soft gel	Body weight: weight scale before and after study period	Significantly decreased body weight within same group, before and after intervention (d = -0.3808)
		N = 22 females aged 30-65 with type 2 diabetes in control group		Macronutrient intake: 24-h recall food questionnaire for 3 d before and after study period	Significantly decreased energy intake within same group, before and after intervention (d = -9.52)
Pourmoradian *et al*[30]	Human double-blinded randomized controlled trial	N = 21 females aged 30-65 with type 2 diabetes in treatment group	1000 mg lyophilized royal jelly in soft gel form served after breakfast for 8 wk. Control group received placebo soft gel	Plasma insulin	Significantly decreased plasma insulin and HbA1c and insignificantly decreased fasting blood glucose compared to baseline within same group, before and after intervention.
		N = 20 females aged 30-65 with type 2 diabetes in control group		HbA1c	d = 0.016 (HbA1c)
				Hyperglycemia: Fasting blood glucose	d = -0.0785 (plasma insulin)
Shidfar *et al*[31]	Human double-blinded randomized controlled trial	N = 23 + 23 adults 25-65 yr old with type 2 diabetes in experimental and control (placebo) groups	1000 mg royal jelly in soft gelatin capsules 3 times daily for 8 wk. Control group received placebo identical in appearance to treatment	Fasting blood sugar	Significantly decreased fasting blood levels to more normal range (d = -0.3725)
				Macronutrient intake: 24-h recall diet questionnaire	Did not significantly alter macronutrient intake
				Insulin resistance: HOMA-IR	Significantly decreased HOMA-IR: improved insulin sensitivity (d = -0.79)

RJ: Royal jelly; HOMA-IR: Homeostatic model assessment of insulin resistance; BMI: Body mass index; HbA1c: Hemoglobin A1c.

expression and protein activity such as those involved in glycogen breakdown[46]. Insulin receptor substrate (IRS) proteins are key intermediates in the pathway[47]. With elevated levels of circulating fatty acids as in diabetic individuals, phosphorylation of IRS proteins is inhibited *via* activation of protein kinase C (PKC)[47,48]. As a result of decreased sensitivity to insulin, the cellular responses involved in regulating blood

Table 3 Animal and *in vitro* trials examining effects of long-term royal jelly treatment

Ref.	Study design	Subjects	Treatment	Outcome measures	Effectiveness
Ghanbari *et al*[14]	Randomized controlled trial	N = 8 healthy male Wistar rats aged 10-12 wk (control) N = 8 diabetic male Wistar rats aged 10-12 wk N = 8 healthy male Wistar rats aged 10-12 wk receiving treatment N = 8 diabetic male Wistar rats aged 10-12 wk receiving treatment	100 mg/kg BW royal jelly dissolved in 1 mL of water daily for 6 wk	Hyperinsulinemia: ELISA test on plasma sample Hyperglycemia: Fasting plasma glucose	Treatment significantly improved insulin levels (d = 1.67) and hyperglycemic fasting blood glucose (d = -2.72) levels to levels similar to healthy control group
Fujii *et al*[32]	Controlled trial	N = 80 male streptozotocin-diabetic rats aged 5 wk equally split into three experimental groups and one control group	Each experimental group had one of 1, 10, and 100 mg/kg body weight royal jelly administered orally by force for 4 wk. Control group received purified water	Hyperglycemia: Blood glucose (unknown whether fasting) Body weight	Royal jelly administration overall slightly decreased blood glucose levels in non-dose dependent manner (no information on statistical significance) No significant change in body weight between groups
Membrez *et al*[33]	Randomized controlled trial	N = 15 male db/db mice aged 6-8 wk in control group N = 30 male db/db mice aged 6-8 wk equally split in two experimental groups	1 g/kg body weight of sebacic acid was added to chow food in one experimental group, and 10 g/kg body weight SA to second experimental group's chow for 6 wk	Hyperglycemia: OGTT and fasting (plasma samples) HbA1c: Plasma samples Liver gene expression: RNA extracted from liver samples Food intake: Chow consumed	In more heavily supplemented group: Hyperglycemia significantly improved (d = -1.86) and improved glucose clearance (d = -3.20), HbA1c significantly decreased (d = -1.89), ketone bodies significantly increased (d = 1.16), dose response relationship observed, gluconeogenic and lipogenic enzyme expression significantly decreased (insufficient information for SMD estimation), food intake was significantly decreased (d = -1.82).
Takikawa *et al*[7]	*In vitro*	L6 myotubes grown in cell culture and collected from healthy male mice 7 wk of age	Cell cultured myotubes treated with 10H2DA Mice fed 1.6 mmol/kg 10H2DA	Glucose clearance: GLUT4 translocation to plasma membrane	Significantly improved GLUT4 translocation to plasma membrane in skeletal muscle cells compared to non-treated myotube cells (d = 0.4698)
Yoneshiro *et al*[34]	Controlled trial	N = 8 3-wk old healthy male mice (control) N = 11 3-wk old healthy male mice fed HFD N = 11 3-wk old healthy male mice fed high fat diet with treatment	High fat diet with 5% lyophilized royal jelly powder for 17 wk	Body weight gain Hyperlipidemia: Plasma sample Hyperglycemia: Plasma sample Insulin resistance: HOMA-IR	Body weight gain due to white adipose tissue significantly reduced compared to HFD group (d = -2.82) Significantly decreased levels of NEFA compared to HFD (d = -1.6072) Significantly improved hyperglycemia compared to HFD group (d = -2.04) HOMA-IR significantly decreased compared to HFD group, not significantly different from control group (d = -1.23)

Zamami *et al*[15]	Controlled trial	N = 6 6-wk old healthy male Wistar rats (control, received water)	Two experimental groups: One fed 100 mg/kg and the other 300 mg/kg of dilute enzymatically treated royal jelly supplementation daily for 8 wk	Insulin resistance: HOMA-IR	High fructose diet induced insulin resistance in rats
		N = 5 6-wk old healthy male Wistar rats as vehicle-treated group (received high fructose consumption)		Food intake	Plasma insulin levels and HOMA-IR similar between healthy control group and fructose drinking rats supplemented with 300 mg/kg royal jelly. Dose dependent relationship observed d = -0.7063 (effect size of 300 mg/kg royal jelly on fructose drinking rats)
		N = 6 + 6 6-wk old healthy male Wistar rats (received high fructose consumption) in two treatment groups		Body weight	No significant difference in body weight and FBG between groups
				Plasma triglycerides	Plasma triglycerides significantly decreased compared to control dose-dependently (d = -1.62)
Watadani *et al*[35]	Controlled trial	N = 7 female KK-Ay mice 5 wk of age in control group	3 mg/kg 10H2DA for 4 wk	Hyperglycemia: Plasma glucose samples collected in intervals after OGTT	Significantly improved glucose clearance (d = -1.33) and fasting blood glucose (d = -1.23)
		N = 8 female KK-Ay mice 5 wk of age in treatment group		Body weight: Adiposity index of abdominal, mesenteric and retroperitoneal fat tissue	Body weight did not differ between groups
				Insulin resistance: HOMA-IR	Significantly improved insulin sensitivity (d = -4.44)
				Glucose regulatory proteins: AMPK, G6Pase, Pck1 levels, GLUT4, GS/GSK in tissue homogenates	Significantly increased levels of G6Pase (d = 1.22) and Pck1(d = 0.77) mRNA in liver cells. Significantly increased levels of pAMPK in muscle (d = 3.13), but no change in liver. Insignificant increase in GLUT4 in muscle cells. No change in GS/GSK levels between groups
Yoshida *et al*[36]	Controlled trial	16 female KK-Ay mice split into control and experimental groups	10 mg/kg royal jelly in 1/15M phosphate buffer 5 d/wk for 4 wk		Significantly improved rates of glucose clearance (d = -1.25)
					Insignificantly decreased body weight
					Significantly increased pAMPK levels in liver (d = 2.39) and skeletal muscle (d = 1.73). Significantly decreased G6Pase mRNA levels in liver (d = -1.65), but no change in Pck mRNA levels. Insignificantly increased GLUT4 levels in skeletal muscle
					Significantly decreased plasma NEFA (d = -1.42). No change in plasma TG
					No significant change in plasma insulin

GLUT4: Glucose transporter type-4; 10H2DA: 10-hydroxydecanonic acid; OGTT: Oral glucose tolerance test; HOMA-IR: Homeostatic model assessment of insulin resistance; GS(K): Glycogen synthase (kinase); AMPK: AMP-dependent kinase; NEFA: Non-esterified fatty acids; HFD: High fat diet.

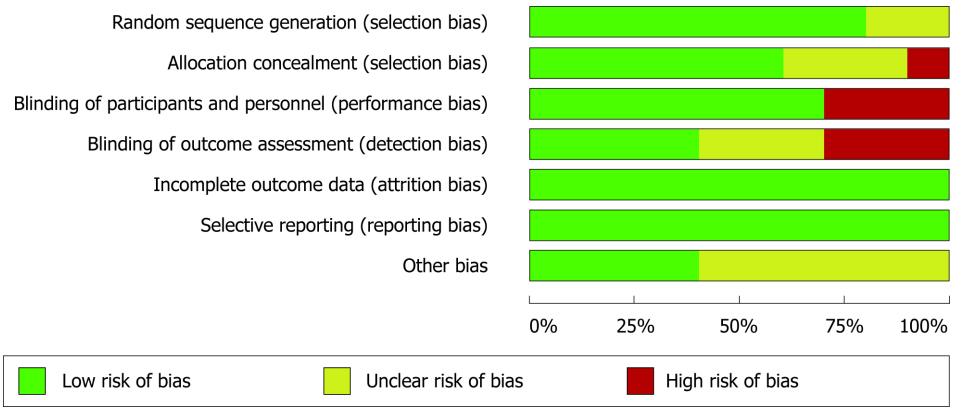

Figure 2 Risk of bias graph showing proportion of bias risk ratings across all included human studies.

glucose are impaired, leading to hyperglycemia[48].

Intracellular AMPK, which induces similar glycemic responses to insulin in a non-insulin dependent pathway, has been observed to be activated by 10H2DA, the fatty acid derived from RJ that is thought to underlie its glycemic effects[7,49]. Takikawa *et al*[7] found AMPK to be activated by 10H2DA *via* activation of CaMKKβ (calcium/calmodulin-dependent protein kinase kinase beta). In skeletal muscle, pathways mediated by AMPK have been observed to increase GLUT4 gene expression and translocation[7,50]. In addition to this, AMPK-mediated regulation of GLUT4 has been observed to improve insulin-stimulated GLUT4 regulation, thus potentially improving response (sensitivity) of insulin receptors bound to ligand[51]. Moreover, activated AMPK in adipocytes and skeletal muscle are also responsible for enhancing enzymes involved in fatty acid oxidation, thus potentially leading to a decrease in body weight and circulating fatty acids[36]. Finally, in Yoshida *et al*[36], increased levels of AMPK following oral administration in mice appeared to stunt activity of glucose-6-phosphatase in hepatocytes, thus suggesting that the kinase can also regulate gluconeogenesis (and therefore glucose export) independently of insulin. By activating an enzyme that works to increase cellular energy levels in the cell, 10H2DA from RJ may mediate the desired hypoglycemic effects in diabetic subjects.

In the hypothalamus, AMPK plays an important regulatory role in food intake[52]. When activated, a signaling cascade that leads to increased energy intake is initiated[53]. Downstream of this cascade, mammalian target of rapamycin (mTOR) signaling is known to play a direct and important role[52]. However, in the presence of 10H2DA, mTOR activity has been observed to be decreased *in vitro*, resulting in decreased energy intake in hypothalamic cells[10,53]. The beneficial effect of RJ on food intake and body weight is observed in Pourmoradian *et al*[13], where both parameters were significantly decreased in diabetic female subjects. Following a decrease in macronutrient intake, there is a decrease in fatty acid synthesis and circulating lipids, leading to a decrease in activation of PKC. Thus, RJ's possible action to decrease body weight and food intake is a mechanism that adds to its beneficial effects on glycemic regulation.

As previously mentioned, rodent studies showed a difference between effectiveness of RJ as administered to males compared to females. Fatty acids and sterols in RJ putatively have weak affinity for estrogen receptors, which when activated principally affect gene expression[11,54]. In addition to affecting transcriptional and translational activity that may regulate glycemic activity, estrogen receptors are able to induce activation of AMPK intracellularly[54]. Moreover, activated estrogen receptors in skeletal muscle have been shown to amplify GLUT4 translocation[54]. In the hypothalamus, estrogen suppresses energy intake through mechanisms not fully understood[54]. Thus, in accord with the evidence collected in this systematic review, activation of estrogen receptors in the body work to increase energy expenditure and decrease energy intake[54].

Study limitations

Generally, the principal limitation within the included evidence is the wide range of intervention methodology between the included studies. Although this heterogeneity provides data on the various ways to supplement RJ orally (*e.g.*, prandial), there may not be sufficient evidence for one particular intervention method, including duration and dosage of supplementation, to be adopted by health care providers or researchers. As previously described, this variation in intervention methodology also

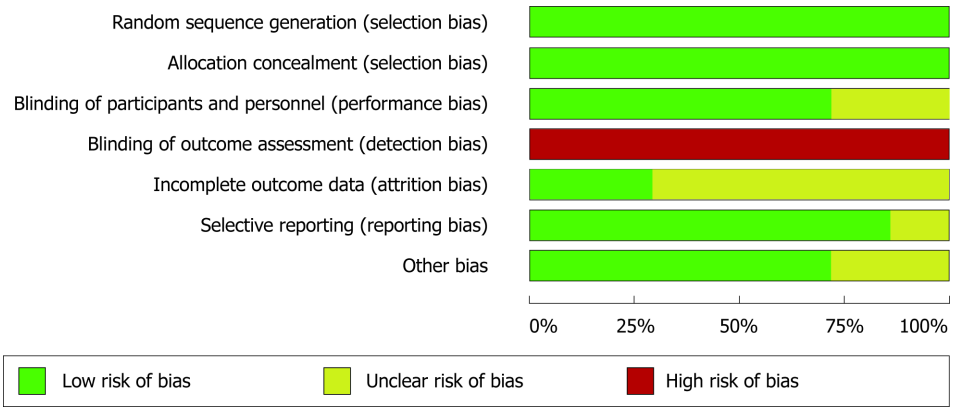

Figure 3 Risk of bias graph showing proportion of bias risk ratings across all included animal studies.

likely contributes to the inconsistency across study results. Moreover, outcomes associated with different interventions at times contradict each other-Watadani *et al*[35] observed significantly increased expression of G6Pase in liver cells following 10H2DA administration, while Yoshida *et al*[36] observed significantly decreased expression of G6Pase in liver cells following RJ administration. The source of RJ also differs between studies, which is a potential confounder of the evidence, as the chemical composition of RJ is known to differ between time of year, honeybee age, and geographic location[55].Thus, the inconsistency across intervention methods and resultant outcomes is a notable limitation of the overall evidence.

Across human studies, limitations exist largely due to the study populations. Although most of the clinical trials included a balance of male and females, few studies examined exclusively females, and none examined males exclusively. Considering RJ's potential estrogenic activity, a comparison of effects on male and female populations might have provided clearer evidence on the effectiveness of RJ as treatment for diabetes. Notably, the animal studies, which examined exclusively either male or female rodents, displayed differential responses to similar treatment between sexes. Similarly, the age ranges of included subjects in the included trials were relatively wide; inclusion criteria based on tighter ranges (*e.g.*, younger adults, menopausal women) might have determined if certain groups are more affected by the treatment than others, once again potentially due to RJ's activation of estrogen receptors. Moreover, the exclusion and inclusion criteria of the clinical trials affect the generalizability of RJ as treatment for diabetes: For example, many studies excluded individuals who had not taken glucose-lowering medications, or those who had diabetes for a certain length of time. Information on quality of participant dietary patterns and management of diabetes were rarely provided in the included studies but have a large impact on the effect of a supplement such as RJ. These factors are all important when considering the external validity of the synthesized evidence for clinical application.

The main limitation associated with animal studies is the physiological variability between animals and humans. Rodent studies, which comprise a considerable portion of the evidence, are good models of T2D in humans, but lack key pathologies in the disease, including pathologies found in pancreatic islets, the secretion site of insulin[8]. Moreover, when investigating the effects of an oral agent such as RJ, bioavailability is critical: absorption, distribution and metabolism of nutrients such as fatty acids found in RJ may vary between rodents and humans[16]. Our preliminary research indicates that bioavailability and metabolism of RJ in human models has not yet been established, so the enhanced response of RJ observed in animal trials may not be applicable to human patients.

Another limitation stems from the similarity between aspects of some studies. A considerable number of the included studies share the same authors, such as Pourmoradian *et al*[13], Pourmoradian *et al*[30], Mobasseri *et al*[28], and Mobasseri *et al*[25]. As a result, there is noteworthy overlap in the methodology of these studies, although different outcomes are assessed in each. In maintaining very similar study population and methodology, there is the potential for similar undetected bias to affect the results of these studies. Because it presents the risk of the overall evidence misrepresenting RJ's true effect, overlapping methodology between studies is an important factor to consider when interpreting the evidence.

Review limitations

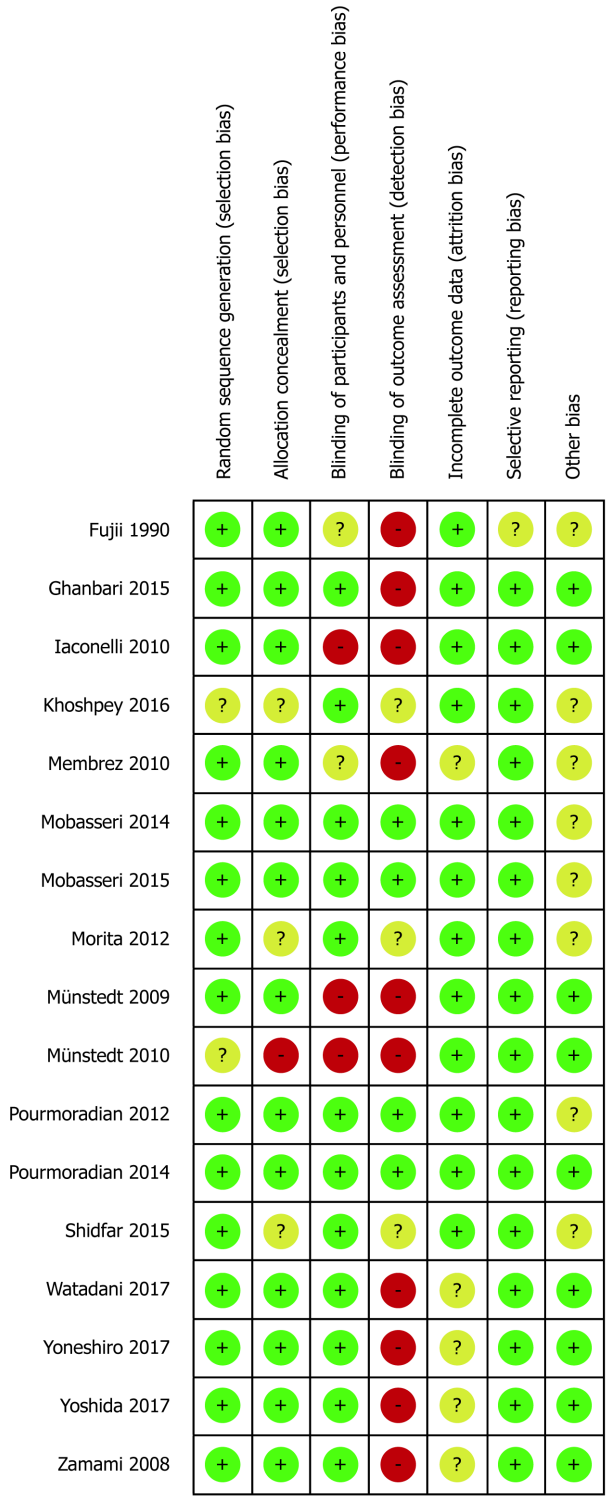

Figure 4 Risk of bias summary: Judgements about each risk of bias item for all included *in vivo* studies.

The evidence synthesized in this review relied completely on the included studies, which may be unrepresentative of RJ's true effect on the probed outcomes. The exclusion of non-English studies may have removed a considerable number of studies; this is a concern particularly for this topic because almost all the included studies have non-English speaking origins. Also, despite having known insulin-like properties and thus potentially a role in glycemic regulation, no studies on RJ proteins were included in this review[56]. Lastly, while this review focused on RJ as a dietary supplement, other forms of administration (*e.g.*, topical) may improve effectiveness or bioavailability.

In conclusion, RJ supplementation presents promising potential for treatment of glycemic T2D symptoms. The evidence synthesized in this review complements

Table 4 GRADE assessment for long-term effectiveness of RJ treatment on glycemic control

GRADE criteria	Rating	Support for judgement	Overall quality of evidence
Outcome: Long term glycemic control (n = 14 studies)			
RoB (assessed on Cochrane RoB Collaboration Tool)	No Serious (-1)[1] Very serious (-2)	Only one study had low RoB for all categories. Most studies had at least one item at high or unclear RoB	High
Inconsistency	No Serious (-1) [1] Very serious (-2)	Generally, positive clinical effects demonstrated but some studies indicate null effects. There are also large variations in magnitude of effect. Heterogeneity is notable between the studies (in population, intervention and outcome assessment)	Moderate
Indirectness	No[1] Serious (-1) Very serious (-2)	Evidence synthesized from studies addresses review question with respect to population, interventions and outcome	Low[1]
Imprecision	No Serious (-1) Very serious (-2) [1]	All studies have groups with small sample sizes (≤ 20), with no indication that they meet required sample sizes to detect difference in outcome; observable but statistically insignificant measures in many studies suggest sample sizes were too small to detect difference. 95% confidence intervals of effect size estimates mostly suggest an appreciable benefit for treatment, but there are several that suggest possibility of no meaningful effect	Very low
Publication bias	Undetected[1] Strongly suspected (-1)	There is chance of publication bias considering the review is entirely "small-scale" trials; this area of research is not well-established and there is potential for publication bias, but none was overtly detected	
Other	Large effect (+1[1] or +2) Dose response (+1[1] or +2) No plausible confounding (+1 or +2)	Standardized mean difference of studies (effect size) indicates large magnitude of effect of treatment. Dose-response relationships observed	

[1]Indicates decision. RoB: Risk of Bias. Adapted from Ryan and Hill[21] and Wei *et al*[22].

existing research that demonstrates other therapeutic effects of RJ administration in T2D symptoms, such as oxidative stress, impaired wound-healing and inflammation[32,57,58]. Future studies should examine the pharmacodynamic properties of RJ, particularly with respect to dosage forms, effectiveness and bioavailability in different populations to further elucidate the effectiveness of RJ as a therapeutic agent of hyperglycemia.

Table 5 GRADE assessment for acute effects of RJ administration on glycemic control

GRADE criteria	Rating	Support for judgement	Overall quality of evidence
Outcomes: Acute glycemic control outcomes (n = 4 studies)			
RoB (assessed on Cochrane RoB Collaboration Tool)	No Serious (-1) Very serious (-2)[1]	Majority of studies had overall high RoB, likely affecting the study results	High
Inconsistency	No Serious (-1) [1] Very serious (-2)	Outcome effects are somewhat consistent, and studied population are similar enough to not be considered detrimental to evidence quality. Intervention, however, was heterogeneous across all relevant studies	Moderate
Indirectness	No[1] Serious (-1) Very serious (-2)	Research question is addressed by majority of the animal studies	Low
Imprecision	No Serious (-1) Very serious (-2) [1]	All studies have groups with small sample sizes (≤ 20), with no indication that they meet required sample sizes to detect difference in outcome. For those with calculable effect sizes, the confidence intervals suggest potential for no appreciable benefit	Very low[1]
Publication bias	Undetected[1] Strongly suspected (-1)	There is chance of publication bias considering the review is entirely "small-scale" trials; this area of research is not well-established and there is potential for publication bias, but none was overtly detected	
Other	Large effect (+1 or +2) Dose response (+1 or +2) No plausible confounding (+1 or +2)	Some dose response relationships observed, however not enough studies to confirm this relationship. Insufficient effect size estimates to determine if effect is large or not	

[1]Indicates decision. RoB: Risk of Bias. Adapted from Ryan and Hill[21] and Wei *et al*[22].

ARTICLE HIGHLIGHTS

Research background

Existing evidence suggests that royal jelly (RJ) is a promising therapeutic option in hyperglycemic cases. Few studies have specifically examined the clinical viability of RJ as treatment, and no study has critically analyzed the existing evidence. Knowledge of the factors that influence effectiveness of RJ intake provides an alternative treatment for hyperglycemia, which is often associated with diabetes.

Research motivation

This systematic review demonstrated that the intervention style (*e.g.*, length of supplementation, ingestion form) as well as pre-existing patient characteristics may be important factors in its effectiveness, and future research should further investigate these factors to inform patients and health care providers.

Research objectives

This review sought to examine whether there is support for RJ as a glycemic regulator in models of type 2 diabetes as well as healthy individuals. Our analysis found that the existing evidence suggests that RJ is a promising therapeutic option in hyperglycemic cases, with effective doses as low as 1000 mg of fresh RJ daily for diabetic patients.

Research methods

This was a systematic review employing the PRISMA strategy. Five databases were searched using keywords pertinent to the research objectives. Two reviewers conducted full-text screening to select included articles that met eligibility criteria. Relevant information (*i.e.*, intervention style, results, participant characteristics) was extracted from the included articles. Risk of bias was assessed by two reviewers. GRADE, a novel tool developed by Cochrane used to assess overall quality of evidence, was also determined by two reviewers.

Research results
Effective doses of RJ may be as low as 1000 mg of fresh RJ for a diabetic patient. Overall, the quality of evidence for RJ as a treatment is low for long-term effectiveness, and very low for acute effects of RJ consumption.

Research conclusions
Synthesis and analysis of existing studies shows that RJ may be viable as part of a treatment plan in lowering blood sugar. Due to the heterogeneity in studied population and intervention, RJ may have more pronounced effects in certain dosage forms (*e.g.*, fresh RJ) and in certain populations (*e.g.*, postmenopausal females). This information may be useful for individuals and health care practitioners wishing to explore hyperglycemia treatment options.

Research perspectives
Future clinical trials should consider the potential effects of intervention form and length, as well as the effect of participant characteristics to clarify which patient populations or conditions would benefit most from RJ supplementation.

REFERENCES

1 **Chen L**, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus--present and future perspectives. *Nat Rev Endocrinol* 2011; **8**: 228-236 [PMID: 22064493 DOI: 10.1038/nrendo.2011.183]
2 **Zimmet P**, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; **414**: 782-787 [PMID: 11742409 DOI: 10.1038/414782a]
3 **Vijan S**. In the clinic. Type 2 diabetes. *Ann Intern Med* 2010; **152**: ITC31-15; quiz ITC316 [PMID: 20194231 DOI: 10.7326/0003-4819-152-5-201003020-01003]
4 **Wild S**, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047-1053 [PMID: 15111519 DOI: 10.2337/diacare.27.5.1047]
5 **Reusch JE**, Manson JE. Management of Type 2 Diabetes in 2017: Getting to Goal. *JAMA* 2017; **317**: 1015-1016 [PMID: 28249081 DOI: 10.1001/jama.2017.0241]
6 **Khunti K**, Bodicoat DH, Davies MJ. Type 2 diabetes: lifetime risk of advancing from prediabetes. *Lancet Diabetes Endocrinol* 2016; **4**: 5-6 [PMID: 26575607 DOI: 10.1016/S2213-8587(15)00394-0]
7 **Takikawa M**, Kumagai A, Hirata H, Soga M, Yamashita Y, Ueda M, Ashida H, Tsuda T. 10-Hydroxy-2-decenoic acid, a unique medium-chain fatty acid, activates 5'-AMP-activated protein kinase in L6 myotubes and mice. *Mol Nutr Food Res* 2013; **57**: 1794-1802 [PMID: 23754629 DOI: 10.1002/mnfr.201300041]
8 **Tundis R**, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. *Mini Rev Med Chem* 2010; **10**: 315-331 [PMID: 20470247 DOI: 10.2174/138955710791331007]
9 **Chen C**, Chen SY. Changes in protein components and storage stability of royal jelly under various conditions. *Food Chem* 1995; **54**: 195-200 [DOI: 10.1016/0308-8146(95)00031-D]
10 **Honda Y**, Fujita Y, Maruyama H, Araki Y, Ichihara K, Sato A, Kojima T, Tanaka M, Nozawa Y, Ito M, Honda S. Lifespan-extending effects of royal jelly and its related substances on the nematode Caenorhabditis elegans. *PLoS One* 2011; **6**: e23527 [PMID: 21858156 DOI: 10.1371/journal.pone.0023527]
11 **Suzuki KM**, Isohama Y, Maruyama H, Yamada Y, Narita Y, Ohta S, Araki Y, Miyata T, Mishima S. Estrogenic activities of Fatty acids and a sterol isolated from royal jelly. *Evid Based Complement Alternat Med* 2008; **5**: 295-302 [PMID: 18830443 DOI: 10.1093/ecam/nem036]
12 **Khoshpey B**, Djazayeri S, Amiri F, Malek M, Hosseini AF, Hosseini S, Shidfar S, Shidfar F. Effect of Royal Jelly Intake on Serum Glucose, Apolipoprotein A-I (ApoA-I), Apolipoprotein B (ApoB) and ApoB/ApoA-I Ratios in Patients with Type 2 Diabetes: A Randomized, Double-Blind Clinical Trial Study. *Can J Diabetes* 2016; **40**: 324-328 [PMID: 27026221 DOI: 10.1016/j.jcjd.2016.01.003]
13 **Pourmoradian S**, Mahdavi R, Mobasseri M, Faramarzi E, Mobasseri M. Effects of royal jelly supplementation on body weight and dietary intake in type 2 diabetic females. *Health Promot Perspect* 2012; **2**: 231-235 [PMID: 24688939 DOI: 10.5681/hpp.2012.028]
14 **Ghanbari E**, Nejati V, Khazaei M. Improvement in Serum Biochemical Alterations and Oxidative Stress of Liver and Pancreas following Use of Royal Jelly in Streptozotocin-Induced Diabetic Rats. *Cell J* 2016; **18**: 362-370 [PMID: 27602318 DOI: 10.22074/cellj.2016.4564]
15 **Zamami Y**, Takatori S, Goda M, Koyama T, Iwatani Y, Jin X, Takai-Doi S, Kawasaki H. Royal jelly ameliorates insulin resistance in fructose-drinking rats. *Biol Pharm Bull* 2008; **31**: 2103-2107 [PMID: 18981581 DOI: 10.1248/bpb.31.2103]
16 **Baker DH**. Animal models in nutrition research. *J Nutr* 2008; **138**: 391-396 [PMID: 18203909 DOI: 10.1093/jn/138.2.391]
17 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**: e1000097 [PMID: 19621072 DOI: 10.1371/journal.pmed.1000097]
18 **Higgins JP**, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA; Cochrane Bias Methods Group; Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011; **343**: d5928 [PMID: 22008217 DOI: 10.1136/bmj.d5928]
19 **O'Connor AM**, Sargeant JM. Critical appraisal of studies using laboratory animal models. *ILAR J* 2014; **55**: 405-417 [PMID: 25541543 DOI: 10.1093/ilar/ilu038]
20 **Schünemann HJ**, Oxman AD, Brozek J, Glasziou P, Jaeschke R, Vist GE, Williams JW, Kunz R, Craig J, Montori VM, Bossuyt P, Guyatt GH; GRADE Working Group. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. *BMJ* 2008; **336**: 1106-1110 [PMID: 18483053 DOI: 10.1136/bmj.39500.677199.AE]

21 **Ryan R**, Hill S; Cochrane consumers and communication group. How to GRADE the quality of the evidence. Cochrane consumers and communication group. 2016; Available from: URL: https://cc.cochrane.org/sites/cc.cochrane.org/files/public/uploads/how_to_grade.pdf

22 **Wei D**, Tang K, Wang Q, Estill J, Yao L, Wang X, Chen Y, Yang K. The use of GRADE approach in systematic reviews of animal studies. *J Evid Based Med* 2016; **9**: 98-104 [PMID: 26997212 DOI: 10.1111/jebm.12198]

23 **Faraone SV**. Interpreting estimates of treatment effects: implications for managed care. *P T* 2008; **33**: 700-711 [PMID: 19750051]

24 **Iaconelli A**, Gastaldelli A, Chiellini C, Gniuli D, Favuzzi A, Binnert C, Macé K, Mingrone G. Effect of oral sebacic Acid on postprandial glycemia, insulinemia, and glucose rate of appearance in type 2 diabetes. *Diabetes Care* 2010; **33**: 2327-2332 [PMID: 20724647 DOI: 10.2337/dc10-0663]

25 **Mobasseri M**, Ghiyasvand S, Ostadrahimi A, Ghojazadeh M, Noshad H, Pourmoradian S. Effect of Fresh Royal Jelly Ingestion on Glycemic Response in Patients With Type 2 Diabetes. *Iran Red Crescent Med J* 2015; **17**: e20074 [PMID: 26473074 DOI: 10.5812/ircmj.20074]

26 **Münstedt K**, Bargello M, Hauenschild A. Royal jelly reduces the serum glucose levels in healthy subjects. *J Med Food* 2009; **12**: 1170-1172 [PMID: 19857086 DOI: 10.1089/jmf.2008.0289]

27 **Münstedt K**, Böhme M, Hrgovic I, Hauenschild A. An approach to the application of Royal Jelly: Encapsulation of lyophilized Royal Jelly and its effect on glucose metabolism in humans. *J Api Product Api Med Sci* 2010; **2**: 29-30 [DOI: 10.3896/IBRA.4.02.1.03]

28 **Mobasseri M**, Pourmoradian S, Mahdavi R, Faramarzi E. Effects of royal jelly supplementation on lipid profile and high-sensitivity c-reactive protein levels in type-2 diabetic women: A pilot study. *Curr Top Nutraceutical Res* 2014; **12**: 101-105

29 **Morita H**, Ikeda T, Kajita K, Fujioka K, Mori I, Okada H, Uno Y, Ishizuka T. Effect of royal jelly ingestion for six months on healthy volunteers. *Nutr J* 2012; **11**: 77 [PMID: 22995464 DOI: 10.1186/1475-2891-11-77]

30 **Pourmoradian S**, Mahdavi R, Mobasseri M, Faramarzi E, Mobasseri M. Effects of royal jelly supplementation on glycemic control and oxidative stress factors in type 2 diabetic female: a randomized clinical trial. *Chin J Integr Med* 2014; **20**: 347-352 [PMID: 24610413 DOI: 10.1007/s11655-014-1804-8]

31 **Shidfar F**, Jazayeri S, Mousavi SN, Malek M, Hosseini AF, Khoshpey B. Does Supplementation with Royal Jelly Improve Oxidative Stress and Insulin Resistance in Type 2 Diabetic Patients? *Iran J Public Health* 2015; **44**: 797-803 [PMID: 26258092 DOI: 10.1177/2047487312472079]

32 **Fujii A**, Kobayashi S, Kuboyama N, Furukawa Y, Kaneko Y, Ishihama S, Yamamoto H, Tamura T. Augmentation of wound healing by royal jelly (RJ) in streptozotocin-diabetic rats. *Jpn J Pharmacol* 1990; **53**: 331-337 [PMID: 2391765 DOI: 10.1254/jjp.53.331]

33 **Membrez M**, Chou CJ, Raymond F, Mansourian R, Moser M, Monnard I, Ammon-Zufferey C, Mace K, Mingrone G, Binnert C. Six weeks' sebacic acid supplementation improves fasting plasma glucose, HbA1c and glucose tolerance in db/db mice. *Diabetes Obes Metab* 2010; **12**: 1120-1126 [PMID: 20977585 DOI: 10.1111/j.1463-1326.2010.01308.x]

34 **Yoneshiro T**, Kaede R, Nagaya K, Aoyama J, Saito M, Okamatsu-Ogura Y, Kimura K, Terao A. Royal jelly ameliorates diet-induced obesity and glucose intolerance by promoting brown adipose tissue thermogenesis in mice. *Obes Res Clin Pract* 2018; **12**: 127-137 [PMID: 28089395 DOI: 10.1016/j.orcp.2016.12.006]

35 **Watadani R**, Kotoh J, Sasaki D, Someya A, Matsumoto K, Maeda A. 10-Hydroxy-2-decenoic acid, a natural product, improves hyperglycemia and insulin resistance in obese/diabetic KK-Ay mice, but does not prevent obesity. *J Vet Med Sci* 2017; **79**: 1596-1602 [PMID: 28740028 DOI: 10.1292/jvms.17-0348]

36 **Yoshida M**, Hayashi K, Watadani R, Okano Y, Tanimura K, Kotoh J, Sasaki D, Matsumoto K, Maeda A. Royal jelly improves hyperglycemia in obese/diabetic KK-Ay mice. *J Vet Med Sci* 2017; **79**: 299-307 [PMID: 27890887 DOI: 10.1292/jvms.16-0458]

37 **Messia MC**, Caboni MF, Marconi E. Storage stability assessment of freeze-dried royal jelly by furosine determination. *J Agric Food Chem* 2005; **53**: 4440-4443 [PMID: 15913307 DOI: 10.1021/jf0404647]

38 **Maroni A**, Moutaharrik S, Zema L, Gazzaniga A. Enteric coatings for colonic drug delivery: state of the art. *Expert Opin Drug Deliv* 2017; **14**: 1027-1029 [PMID: 28749188 DOI: 10.1080/17425247.2017.1360864]

39 **Bushra R**, Aslam N, Khan AY. Food-drug interactions. *Oman Med J* 2011; **26**: 77-83 [PMID: 22043389 DOI: 10.5001/omj.2011.21]

40 **Louet JF**, LeMay C, Mauvais-Jarvis F. Antidiabetic actions of estrogen: insight from human and genetic mouse models. *Curr Atheroscler Rep* 2004; **6**: 180-185 [PMID: 15068742 DOI: 10.1007/s11883-004-0030-9]

41 **Ahmed MA**, Hassanein KM. Effects of estrogen on hyperglycemia and liver dysfunction in diabetic male rats. *Int J Physiol Pathophysiol Pharmacol* 2012; **4**: 156-166 [PMID: 23071873]

42 **Yonei Y**, Shibagaki K, Tsukada N, Nagasu N, Inagaki Y, Miyamoto K, Suzuki O, Kiryu Y. Case report: haemorrhagic colitis associated with royal jelly intake. *J Gastroenterol Hepatol* 1997; **12**: 495-499 [PMID: 9257239 DOI: 10.1111/j.1440-1746.1997.tb00472.x]

43 **Laporte JR**, Ibáñez L, Vendrell L, Ballarín E. Bronchospasm induced by royal jelly. *Allergy* 1996; **51**: 440 [PMID: 8837671 DOI: 10.1111/j.1398-9995.1996.tb04645.x]

44 **Katayama M**, Aoki M, Kawana S. Case of anaphylaxis caused by ingestion of royal jelly. *J Dermatol* 2008; **35**: 222-224 [PMID: 18419679 DOI: 10.1111/j.1346-8138.2008.00448.x]

45 **Lee NJ**, Fermo JD. Warfarin and royal jelly interaction. *Pharmacotherapy* 2006; **26**: 583-586 [PMID: 16553520 DOI: 10.1592/phco.26.4.583]

46 **Virkamäki A**, Ueki K, Kahn CR. Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. *J Clin Invest* 1999; **103**: 931-943 [PMID: 10194465 DOI: 10.1172/JCI6609]

47 **Morino K**, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes* 2006; **55** Suppl 2: S9-S15 [PMID: 17130651 DOI: 10.2337/db06-S002]

48 **Boden G**, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur J Clin Invest* 2002; **32** Suppl 3: 14-23 [PMID: 12028371 DOI: 10.1046/j.1365-2362.32.s3.3.x]

49 **Mihaylova MM**, Shaw RJ. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol* 2011; **13**: 1016-1023 [PMID: 21892142 DOI: 10.1038/ncb2329]

50 **Ojuka EO**, Jones TE, Nolte LA, Chen M, Wamhoff BR, Sturek M, Holloszy JO. Regulation of GLUT4

biogenesis in muscle: evidence for involvement of AMPK and Ca(2+). *Am J Physiol Endocrinol Metab* 2002; **282**: E1008-E1013 [PMID: 11934664 DOI: 10.1152/ajpendo.00512.2001]
51 **Habegger KM**, Hoffman NJ, Ridenour CM, Brozinick JT, Elmendorf JS. AMPK enhances insulin-stimulated GLUT4 regulation via lowering membrane cholesterol. *Endocrinology* 2012; **153**: 2130-2141 [PMID: 22434076 DOI: 10.1210/en.2011-2099]
52 **Minokoshi Y**, Shiuchi T, Lee S, Suzuki A, Okamoto S. Role of hypothalamic AMP-kinase in food intake regulation. *Nutrition* 2008; **24**: 786-790 [PMID: 18725075 DOI: 10.1016/j.nut.2008.06.002]
53 **Anderson KA**, Ribar TJ, Lin F, Noeldner PK, Green MF, Muehlbauer MJ, Witters LA, Kemp BE, Means AR. Hypothalamic CaMKK2 contributes to the regulation of energy balance. *Cell Metab* 2008; **7**: 377-388 [PMID: 18460329 DOI: 10.1016/j.cmet.2008.02.011]
54 **Mauvais-Jarvis F**, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev* 2013; **34**: 309-338 [PMID: 23460719 DOI: 10.1210/er.2012-1055]
55 **Zheng HQ**, Hu FL, Dietemann V. Changes in composition of royal jelly harvested at different times: consequences for quality standards. *Apidologie* 2011; **42**: 39-47 [DOI: 10.1051/apido/2010033]
56 **Kramer KJ**, Childs CN, Spiers RD, Jacobs RM. Purification of insulin-like peptides from insect haemolymph and royal jelly. *Insect Biochem* 1982; **12**: 91-98 [DOI: 10.1016/0020-1790(82)90075-0]
57 **El-Nekeety AA**, El-Kholy W, Abbas NF, Ebaid A, Amra HA, Abdel-Wahhab MA. Efficacy of royal jelly against the oxidative stress of fumonisin in rats. *Toxicon* 2007; **50**: 256-269 [PMID: 17490698 DOI: 10.1016/j.toxicon.2007.03.017]
58 **Kohno K**, Okamoto I, Sano O, Arai N, Iwaki K, Ikeda M, Kurimoto M. Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. *Biosci Biotechnol Biochem* 2004; **68**: 138-145 [PMID: 14745176 DOI: 10.1271/bbb.68.138]

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SYSTEMATIC REVIEWS

SGLT-2 inhibitors in non-alcoholic fatty liver disease patients with type 2 diabetes mellitus: A systematic review

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a common comorbidity with type 2 diabetes. The existing therapeutic options for NAFLD are not adequate. Hypocaloric diet and exercise is the cornerstone of therapy in NAFLD. Pioglitazone is the only drug recommended in diabetes patients with biopsy proven non-alcoholic steatohepatitis. The frequent coexistence of NAFLD and type 2 diabetes with their combined adverse health consequences and inadequate therapeutic options makes it necessary to search for newer alternatives.

AIM

To assess the effect of sodium glucose cotransporter-2 (SGLT-2) inhibitors on liver enzymes in type 2 diabetes patients with NAFLD.

METHODS

We searched PubMed/MEDLINE, Cochrane library, Google scholar, and Clinicaltrials.gov for the relevant articles to be included in this systematic review. Human studies done in type 2 diabetes patients with NAFLD treated with SGLT-2 inhibitors for at least 12 wk were included. Data from eight studies (four randomised controlled trials and four observational studies) were extracted and a narrative synthesis was done. A total of 214 patients were treated with SGLT-2 inhibitors in these studies (94 in randomised controlled trials and 120 in observational studies).

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RESULTS

The primary outcome measure was change in serum alanine aminotransferase level. Out of eight studies, seven studies showed a significant decrease in serum alanine aminotransferase level. Most of the studies revealed reduction in serum level of other liver enzymes like aspartate aminotransferase and gamma glutamyl transferase. Five studies that reported a change in hepatic fat exhibited a significant reduction in hepatic fat content in those treated with SGLT-2 inhibitors. Likewise, among the three studies that evaluated a change in indices of hepatic fibrosis, two studies revealed a significant improvement in liver fibrosis. Moreover, there was an improvement in obesity, insulin resistance, glycaemia, and lipid parameters in those subjects taking SGLT-2 inhibitors. The studies disclosed that about 17% (30/176) of the subjects taking SGLT-2 inhibitors developed adverse events and more than 40% (10/23) of them had genitourinary tract infections.

CONCLUSION

Based on low to moderate quality of evidence, SGLT-2 inhibitors improve the serum level of liver enzymes, decrease liver fat, and fibrosis with additional beneficial effects on various metabolic parameters in type 2 diabetes patients with NAFLD.

Key words: Alanine aminotransferase; Hepatic fat; Hepatic fibrosis; Non-alcoholic fatty liver disease; Sodium-glucose cotransporter-2 inhibitor; Type 2 diabetes mellitus

Core tip: The frequent coexistence of non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes, their adverse health consequences, and lack of adequate therapeutic options makes it necessary to search for newer alternatives. Currently, pioglitazone and vitamin E are recommended in addition to lifestyle modifications for the management of NAFLD. Animal studies have shown that sodium glucose cotransporter-2 inhibitors might be beneficial in NAFLD present in diabetes patients. The current systematic review shows that sodium glucose cotransporter-2 inhibitors improve the serum level of liver enzymes, liver fat, and liver fibrosis with additional beneficial effects on various metabolic parameters in type 2 diabetes patients with NAFLD.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is an emerging public health issue worldwide. The prevalence of NAFLD in type 2 diabetes mellitus patients is three times greater as compared to the general population. Its prevalence in diabetic subjects ranges from 69%-87% depending upon the imaging modality used[1]. The spectrum of NAFLD includes simple steatosis, steatohepatitis, and cirrhosis[2]. Besides NAFLD is a risk factor for extrahepatic complications like cardiovascular disease, chronic kidney disease, and type 2 diabetes. In addition, the prevalence of both microvascular and macrovascular complications is increased in patients with NAFLD and type 2 diabetes[3].

The existing therapeutic options for NAFLD are not adequate. Hypocaloric diet and exercise is the cornerstone of therapy in NAFLD. Pioglitazone and vitamin E are recommended only in biopsy-proven non-alcoholic steatohepatitis (NASH), but vitamin E is not recommended in diabetic patients due to inadequate evidence[4]. The frequent coexistence of NAFLD and type 2 diabetes with their combined adverse health consequences and inadequate therapeutic options makes it necessary to search for newer alternatives. Based on the information from animal studies, sodium glucose

cotransporter-2 (SGLT-2) inhibitors appear promising in the management of NAFLD[5-7]. This systematic review is an effort to review the available literature on the effect of SGLT-2 inhibitors on NAFLD in type 2 diabetes patients.

MATERIALS AND METHODS

Protocol and registration

This systematic review was performed according to the predefined protocol registered in PROSPERO (Registration ID: CRD42018104572). The protocol can be accessed at the website address https://www.crd.york.ac.uk/prospero. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2009 guidelines for reporting this systematic review[8]. Ethics committee approval was not required for this systematic review because it was done using published data found in the public domain.

Eligibility criteria

All observational and randomised controlled trials (RCTs) done using SGLT-2 inhibitors among type 2 diabetes patients with NAFLD having both baseline and post-treatment serum alanine aminotransferase (ALT) level data with a minimum follow-up duration of 12 wk were included in this systematic review. The studies with concomitant pharmacological therapy like pioglitazone or α-tocopherol (vitamin E) for treating NAFLD were excluded to avoid the confounding effects of these drugs on liver function tests. Only those studies that were done in humans and published in English were considered for inclusion. We excluded abstract-only articles, case reports, conference presentations, editorials, reviews, expert opinions, and studies with five participants and less.

Primary and secondary outcomes

The primary outcome was the change in serum ALT levels in type 2 diabetes patients with NAFLD treated with SGLT-2 inhibitors. The secondary outcomes were change in serum aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) levels, hepatic fat, hepatic fibrosis, metabolic profile, anthropometric parameters, and the adverse effects of SGLT-2 inhibitors.

Information sources

PubMed/MEDLINE, Cochrane library, Google scholar, and Clinicaltrials.gov were searched from their date of inception until 31st August, 2018.

Literature search and study selection

The search terms/MeSH terms used were "NAFLD", "Nonalcoholic fatty liver disease", "Non-alcoholic fatty liver disease", "Non alcoholic fatty liver disease", "NASH", "Non-alcoholic steatohepatitis", "Nonalcoholic steatohepatitis", "Non alcoholic steatohepatitis", "Fatty liver", "Type 2 diabetes mellitus", "Type 2 diabetes", "Diabetes mellitus type 2", "Diabetes type 2", "SGLT-2 inhibitors", "Sodium glucose cotransporter-2 inhibitors", "SGLT-2", "SGLT2", "SGLT 2", "Canagliflozin", "Dapagliflozin", "Empagliflozin", "Ipragliflozin", "Luseogliflozin", "Tofogliflozin", "Sotagliflozin", "Remogliflozin", "Ertugliflozin", and "Sergliflozin"(Table 1). The references of the search articles were scrutinised for relevant articles.

Data collection process

The titles and/or abstracts of studies were retrieved using the search strategy and those from additional sources were scrutinised independently by two review authors (HR and JPS) to identify studies that potentially met the inclusion criteria as outlined above. The full texts of these potentially eligible studies were retrieved and independently assessed for eligibility by three review team members (HD, SS, and RP). Any disagreements between the reviewers over the eligibility of particular studies were resolved through discussion with a fourth senior reviewer (SKK). A standardised, pre-formatted excel form was used to extract data from the included studies for the assessment of study quality.

Data items and synthesis of results

The extracted data included the author of the study with year, the study methodology, the recruitment and study completion rates, the types of population, the exposure/intervention (dose of SGLT-2 inhibitor, duration), the results (outcome measures like change in serum ALT, AST, GGT, hepatic fat, markers of liver fibrosis, fasting plasma glucose (FPG), glycosylated haemoglobin (HbA1c), lipid profile, homeostasis model assessment-estimated insulin resistance (HOMA-IR), body mass

Table 1 Literature search strategy

S. No	Search terms
1	NAFLD
2	Nonalcoholic fatty liver disease
3	Non-alcoholic fatty liver disease
4	Non alcoholic fatty liver disease
5	NASH
6	Non-alcoholic steatohepatitis
7	Nonalcoholic steatohepatitis
8	Non alcoholic steatohepatitis
9	Fatty liver
10	1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9
11	Type 2 diabetes mellitus
12	Type 2 diabetes
13	Diabetes mellitus type 2
14	Diabetes type 2
15	11 OR 12 OR 13 OR 14
16	SGLT-2 inhibitors
17	Sodium glucose cotransporter-2 inhibitors
18	SGLT-2
19	SGLT2
20	SGLT 2
21	Canagliflozin
22	Dapagliflozin
23	Empagliflozin
24	Ipragliflozin
25	Luseogliflozin
26	Tofogliflozin
27	Sotagliflozin
28	Remogliflozin
29	Ertugliflozin
30	Sergliflozin
31	16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30
32	10 AND 15 AND 31

NAFLD: Non-alcoholic fatty liver disease; NASH: Non alcoholic steatohepatitis; SGLT-2: Sodium glucose cotransporter-2.

index (BMI), any adverse effects, information for the assessment of the risk of bias, and sources of funding/support.

The statistical review of the study was performed by a biomedical statistician (SSK). A narrative synthesis of the results of individual studies was done. The change in the difference in means and difference in proportions and the respective *P* values as mentioned in the original manuscripts were tabulated and explained in our study.

Risk of study bias

The risk of bias of the RCTs was done using Cochrane risk of bias tool[9]. The studies were graded as "good quality" or "fair quality" or "poor quality" according to the level of risk. Methodological Index for Non-Randomized Studies (MINORS) scale was used to assess the risk of bias of observational studies[10]. A study was considered to be an ideal study if the score was 16 for single arm and 24 for comparative studies.

RESULTS

Study selection

Our literature search from all the aforementioned databases yielded 73 articles (including references of the relevant articles). After eliminating duplicate articles, 55

articles were screened. Eight articles met all of the inclusion criteria (total 214 patients were on SGLT-2 inhibitors) (Figure 1).

Study characteristics

The summary of all studies included in this systematic review is given in Tables 2 and 3. Out of the eight studies, four are RCTs[11-14] and four are observational[15-18]. Five studies were conducted amongst the Japanese population. Ipragliflozin was used in three studies whereas canagliflozin and luseogliflozin were used in two studies each, but dapagliflozin and empagliflozin were used in one study each. All studies used one type of SGLT-2 inhibitor except the one authored by Seko *et al*[16], where both canagliflozin and ipragliflozin were used. The change in serum ALT was a secondary outcome while the effect of SGLT- 2 inhibitors on liver fat was the primary outcome in all RCTs.

Risk of bias within studies

The risk of bias of RCTs was assessed using the Cochrane risk of bias tool. Among the four RCTs, the studies done by Kuchay *et al*[11] and Eriksson *et al*[14] were of good quality however those done by Ito *et al*[12] and Shibuya *et al*[13] were of fair quality (Table 4). The risk of bias of observational studies was assessed using the MINORS scale. All the observational studies were of less than ideal quality (Table 5).

Primary outcome

Change in serum ALT levels: In all of the studies, there was a decrease in serum ALT levels from the baseline in those treated with SGLT-2 inhibitors (Table 6) but in the study done by Shibuya *et al*[13] it did not reach statistical significance.

Kuchay *et al*[11] found a significant decrease in serum ALT levels in the empagliflozin arm compared to the control arm at the end of the study (difference between the two arms was -10.9 IU/L, $P = 0.005$). In the study done by Ito *et al*[12] ALT levels decreased equally in both the groups [Change from baseline in ipragliflozin group: -17.5 (4) and pioglitazone group: -20 (3.4), $P = 0.642$]. Similar results were found in the study by Shibuya *et al*[13] [ΔALT in luseogliflozin arm was 9 (-20, 1) and in metformin arm was 4.5 (-5, 9), $P = 0.064$]. Eriksson *et al*[14] found that the ALT reduction in the dapagliflozin arm was more compared to placebo [ΔALT in dapagliflozin arm was -8.24 (8.24) and in the placebo arm was -0.18 (8.82), $P < 0.05$]. Seko *et al*[16] demonstrated that the serum ALT levels in SGLT-2 inhibitor arm was lower compared to the sitagliptin arm at the end of the study [48.8 (5.5) *vs* 71.1 (10), $P = 0.039$]

Secondary outcomes

Change in serum AST levels: Seven of the included studies had data regarding change in serum AST levels (Table 7). The study done by Shibuya *et al*[13] did not have data on AST levels. All the studies showed a significant reduction in serum AST levels in those treated with SGLT-2 inhibitors. The decrease in AST with empagliflozin and ipragliflozin was similar compared to placebo and pioglitazone respectively whereas dapagliflozin was better than placebo.

Change in serum GGT levels: Seven studies had data regarding GGT levels. Six studies reported a significant decrease in serum GGT levels in those treated with SGLT-2 inhibitors (Table 8). In the study done by Seko *et al*[16], there was an insignificant decrease in both the SGLT-2 inhibitor and DPP-4 inhibitor groups. The decrease in GGT with empagliflozin and ipragliflozin was similar compared to placebo and pioglitazone correspondingly while dapagliflozin was better than placebo.

Change in hepatic fat: Kuchay *et al*[11] and Eriksson *et al*[14] evaluated hepatic fat using magnetic resonance imaging- derived proton density fat fraction (Table 9). It was found that there was a significant reduction in hepatic fat in the empagliflozin arm compared to the control arm in the study done by Kuchay *et al*[11]. In the study done by Eriksson *et al*[14], dapagliflozin or omega-3 carboxylic acid when administered alone or in combination reduced hepatic fat fraction significantly. When compared with placebo, only the combination of both drugs reduced hepatic fat fraction significantly. Sumida *et al*[18] showed that luseogliflozin significantly reduced hepatic fat fraction using magnetic resonance imaging-hepatic fat fraction. Ito *et al*[12] and Shibuya *et al*[13] used liver/spleen attenuation ratio for measuring hepatic fat. They found that ipragliflozin was equivalent to pioglitazone in improving liver/spleen attenuation ratio while luseogliflozin was found to be superior to metformin in the same aspect.

Effect on liver fibrosis indices

Ito *et al*[12] and Ohki *et al*[15] evaluated liver fibrosis using the FIB-4 index (Table 10).

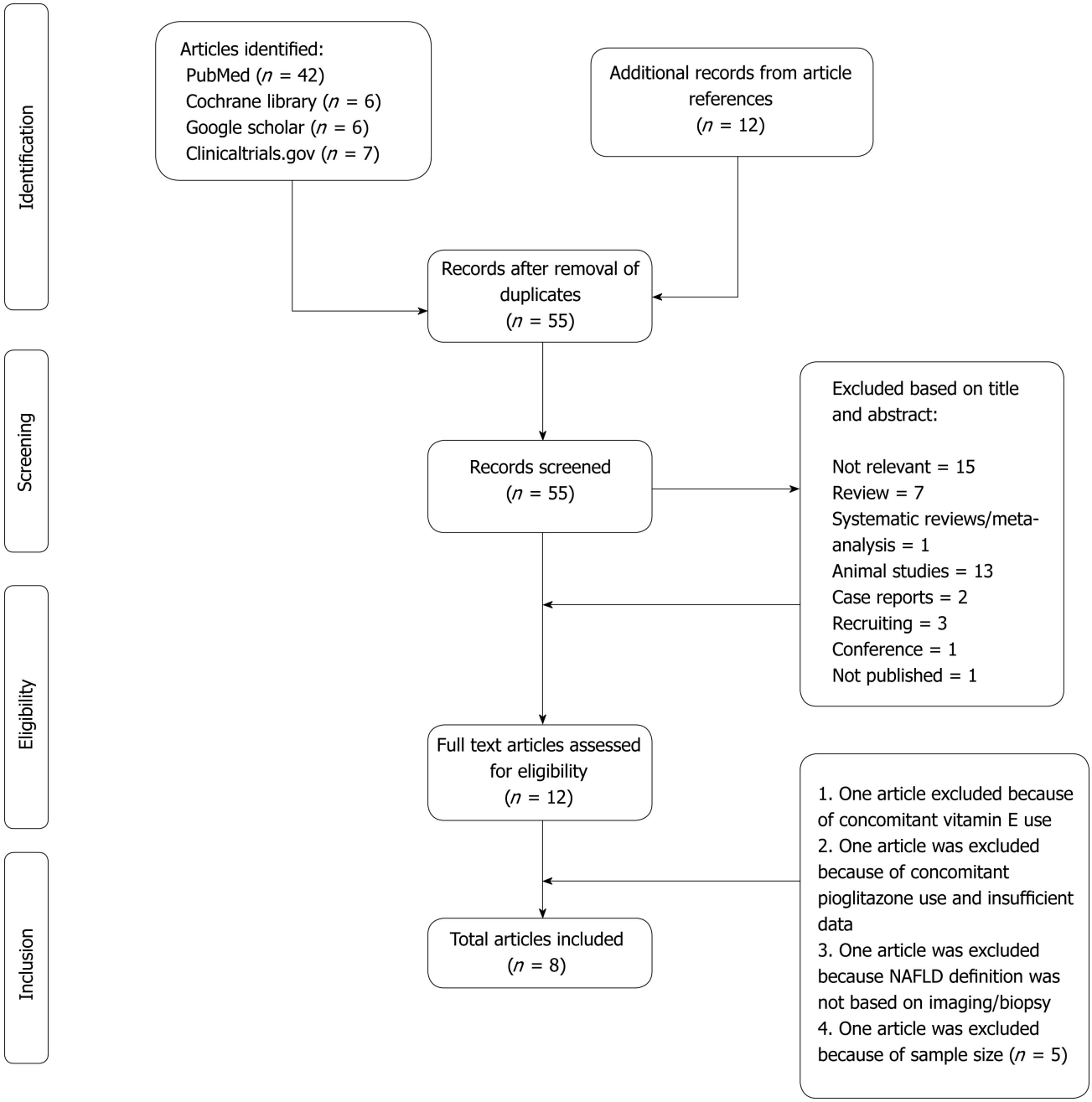

Figure 1 Literature search and study selection.

There was a significant decrease in the FIB-4 index in the ipragliflozin arms compared to baseline. Ipragliflozin was similar to pioglitazone in decreasing the FIB-4 index. Sumida *et al*[18] used both the FIB-4 index and NAFLD fibrosis score. There was no significant change in either indices.

Change in metabolic and anthropometric parameters

Seven studies reported changes in FPG and HbA1c (Tables 11 and 12). The majority of the studies showed a decrease in FPG and HbA1c.

In the study done by Ito *et al*[12] there was no difference in the change in HOMA-IR in those treated with either ipragliflozin or pioglitazone (P = 0.401) (Table 13). There was a significant decrease in HOMA-IR in those treated with dapagliflozin compared to placebo in the study done by Eriksson *et al*[14]. Surprisingly there was an insignificant increase in HOMA-IR in those treated with either a SGLT-2 inhibitor or a gliptin in the study done by Seko *et al*[16].

Six studies included data on the changes in lipid profile (Tables 14, 15, and 16). There was a significant decrease in serum triglycerides in two studies (Kuchay *et al*[11] and Ito *et al*[12]). Three studies exhibited an increase in high-density lipoprotein cholesterol levels (Ito *et al*[12], Ohki *et al*[15], and Seko *et al*[16]). Most of the studies (Ito *et*

Table 2 Randomised controlled trials

S. No	Ref.	Inclusion criteria	Age (yr)	Male gender	Intervention arm	Control arm	Follow-up duration	Primary outcome
1	Kuchay *et al*[11], 2018	Age > 20 yr, hepatic steatosis (MRI-PDFF > 6%), HbA1c > 7.0% to < 10.0%	Intervention arm: 50.7 (12.8) Control arm: 49.1 (10.3)	Intervention arm: 16 (64%) Control arm: 17 (68%)	Standard treatment + Empagliflozin 10 mg daily (*n* = 25)	Standard treatment (*n* = 25)	20 wk	Change in liver fat content by MRI-PDFF
2	Ito *et al*[12], 2017	Age 20-75 yr, HbA1c 7.0–11.0%, BMI < 45 kg/m^2, On diet and exercise therapy alone or with oral hypoglycaemic agents other than SGLT-2 inhibitors and thiazolidinediones and/or insulin, NAFLD, findings suggesting hepatic steatosis and hepatic dysfunction on clinical laboratory tests or on imaging studies (*e.g.*, computed tomography or ultrasound)	Pioglitazone arm: 59.1 (9.8) Ipragliflozin arm: 57.3 (12.1)	Pioglitazone arm: 18 (53%) Ipragliflozin arm: 14 (44%)	Ipragliflozin 50 mg daily (*n* = 32)	Pioglitazone 15-30 mg daily (*n* = 34)	24 wk	Change in L/S attenuation ratio
3	Shibuya *et al*[13], 2018	Fatty liver diagnosed on the basis of computed tomography or abdominal sonography, HbA1c 6.0%–10.0%, age 20–70 yr	Luseogliflozin arm: 51 (47-62) Metformin arm: 60 (53-66)	Luseogliflozin arm: 10 (62.5%) Metformin arm: 8 (50%)	Luseogliflozin 2.5 mg daily (*n* = 16)	Metformin 1.5 g daily (*n* = 16)	24 wk	Change in L/S attenuation ratio
4	Eriksson *et al*[14], 2018	Age 40–75 yr, treated with a stable dose of metformin or sulfonylurea alone or in combination for at least 3 mo, MRI-PDFF > 5.5%, BMI 25–40 kg/m^2	Dapagliflozin arm: 65 (6.5) Omega 3-carboxylic acid arm: 66.2 (5.9) O + D arm: 65(5.4) Placebo arm: 65.6 (6.1)	Dapagliflozin arm: 16 (76.2%) Omega 3-carboxylic acid arm: 11 (55%) O + D arm: 15 (68.2%) Placebo arm: 17 (81%)	Dapagliflozin 10 mg daily (*n* = 21) or Omega 3-carboxylic acid 4 g daily (*n* = 20) or Combination (*n* = 22)	Placebo (*n* = 21)	12 wk	Change in liver fat content by MRI-PDFF

MRI-PDFF: Magnetic resonance imaging-derived proton density fat fraction; L/S: Liver/spleen; O + D: Omega 3-carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2; NAFLD: Non-alcoholic fatty liver disease.

al[12], Eriksson *et al*[14], Ohki *et al*[15], Seko *et al*[16], and Sumida *et al*[18]) showed no change in serum LDL levels.

Five studies included BMI change (Table 17). There was a reduction in BMI in the SGLT-2 inhibitor arms in all the studies. Empagliflozin was similar to placebo in reducing BMI whereas luseogliflozin was superior to metformin in reducing BMI.

Adverse effects of SGLT-2 inhibitors

Out of the eight studies, six studies reported the adverse effects of SGLT-2 inhibitors. There were a total of 30 reported adverse events in 176 patients taking SGLT-2

Table 3 Observational studies

S. No	Ref.	Design	Inclusion criteria	Age (yr)	Male gender	Sample size	SGLT-2 inhibitor	Follow-up duration
1	Ohki *et al*[15], 2016	Prospective study	Type 2 diabetes with NAFLD treated with GLP-1 analogues or DPP-4 inhibitors and failed to normalise serum ALT levels	54.2 (49.3-60.1)	19 (79.2%)	24	Ipragliflozin 25-50 mg daily	320 d (302-329)
2	Seko *et al*[16], 2016	Retrospective cohort study	Type 2 diabetes with NAFLD	SGLT-2 inhibitor arm: 60.3 (1.8) Sitagliptin arm: 59.4 (3.7)	SGLT-2 inhibitor arm: 9 (37.5%) Sitagliptin arm: 8 (38.1%)	24 (SGLT-2 inhibitor); 21 (Sitagliptin)	Canagliflozin 100 mg (n = 18) or Ipragliflozin 50 mg daily (n = 6)	24 wk
3	Gautam *et al*[17], 2018	Prospective study	Type 2 diabetes with NAFLD	-	-	32	Canagliflozin 100 mg daily	24 wk
4	Sumida *et al*[18], 2018	Prospective study	Age > 20 yr, HbA1c > 6.5% to < 8.5%, NAFLD	55.4 (13.6)	28 (70%)	40	Luseogliflozin 2.5 mg daily	24 wk

NAFLD: Non-alcoholic fatty liver disease; SGLT-2: Sodium glucose cotransporter-2; GLP-1: Glucagon like peptide-1; DPP-4: Dipeptidyl peptidase-4.

inhibitors (Table 18). The most common adverse event was genitourinary tract infection (10 events).

DISCUSSION

Type 2 diabetes is commonly associated with NAFLD. Serum ALT levels are commonly above the upper limit of normal with AST levels lesser than ALT levels[19]. Animal studies have shown that SGLT-2 inhibitors decrease liver enzymes (ALT, AST), liver weight, and hepatic steatosis[20-23]. There are several mechanisms for improvement in serum liver enzymes in the patients taking SGLT-2 inhibitors. These drugs cause hyperglucagonemia by increasing glucagon secretion from the pancreatic α cells. Glucagon stimulates gluconeogenesis and β-oxidation of fatty acids in the liver via stimulation of peroxisome proliferator-activated receptor alpha and carnitine palmitoyl transferase-1[13]. Thus SGLT-2 inhibitors help to reduce hepatic fat. They reduce collagen deposition and inflammatory cytokine expression in liver[5,22]. They decrease liver enzymes by additionally improving glycaemic parameters and insulin resistance. Out of eight studies, seven showed a decrease in serum ALT and AST levels in our systematic review. Shibuya *et al*[13] observed a decrease in ALT that almost reached statistical significance, however data regarding AST was unavailable[13]. Out of seven studies, six illustrated a significant decrease in GGT levels while in the study by Seko *et al*[16] the change in serum GGT level almost reached statistical significance.

Liver enzymes are surrogate markers of liver histological response, but an improvement in liver histology is not always associated with a decrease in serum liver enzymes[11]. The five studies that evaluated changes in hepatic fat showed a decrease in hepatic fat. There was no correlation of a change in ALT with a change in hepatic fat in the study by Shibuya *et al*[13], however there was a correlation between these two parameters in the study by Sumida *et al*[18]. The decrease in hepatic fat in the SGLT-2 inhibitor arm was comparable to pioglitazone, which is an approved drug for treatment of NAFLD irrespective of the presence of diabetes. Eriksson *et al*[14] observed that although the hepatic fat content decreased in the dapagliflozin arm it did not reach statistical significance compared to placebo. The lesser duration of this study (12 wk) compared to other studies may have contributed to this difference.

The progression of NAFLD to cirrhosis is determined to a large extent by the liver histology. Studies with up to 20 years follow-up have shown that the risk of progression to cirrhosis for simple steatosis, NASH, and NASH with fibrosis are 0%-4%, 25%, and 38%, respectively[24]. The FIB-4 index is a non-invasive tool to assess liver

Table 4 Assessment of study quality of randomised controlled trials

Study	Criteria	Risk of bias	Study quality
Kuchay *et al*[11]	Random sequence generation	Low risk	Good quality
	Allocation concealment	Low risk	
	Selective reporting	Low risk	
	Other bias	Low risk	
	Blinding of participants and personnel	Low risk	
	Blinding of outcome assessment	Low risk	
	Incomplete outcome data	Low risk	
Ito *et al*[12]	Random sequence generation	Low risk	Fair quality
	Allocation concealment	Unclear risk	
	Selective reporting	Low risk	
	Other bias	Low risk	
	Blinding of participants and personnel	Low risk	
	Blinding of outcome assessment	Low risk	
	Incomplete outcome data	Low risk	
Shibuya *et al*[13]	Random sequence generation	Unclear risk	Fair quality
	Allocation concealment	Unclear risk	
	Selective reporting	Low risk	
	Other bias	Low risk	
	Blinding of participants and personnel	Low risk	
	Blinding of outcome assessment	Low risk	
	Incomplete outcome data	Low risk	
Eriksson *et al*[14]	Random sequence generation	Low risk	Good quality
	Allocation concealment	Low risk	
	Selective reporting	Low risk	
	Other bias	Low risk	
	Blinding of participants and personnel	Low risk	
	Blinding of outcome assessment	Low risk	
	Incomplete outcome data	Low risk	

fibrosis[25]. It is calculated from the patient's age, platelet count, ALT levels, and AST levels. The FIB-4 index was decreased with SGLT-2 inhibitor therapy in two out of three studies. Sumida *et al*[18] used the NAFLD fibrosis score in addition to the FIB-4 index to assess liver fibrosis. The NAFLD fibrosis score is a composite score of six variables (age, BMI, hyperglycaemia, platelet count, albumin, and AST/ALT ratio)[26]. There was no significant change in either indices in this study.

It has been shown that NAFLD is more common in those with poor glycaemic control than those with good glycaemic control[27]. SGLT-2 inhibitors promote glycosuria by inhibiting SGLT-2 in the proximal convoluted tubule. Therefore their action is dependent on blood glucose levels but insulin independent[28]. They cause a significant reduction in FPG[29]. A meta-analysis of RCTs has concluded that the average HbA1c reduction at 52 wk of SGLT-2 inhibitor therapy to be 0.6%[30]. Another meta-analysis has shown that SGLT-2 inhibitor monotherapy is equivalent to metformin monotherapy in reducing HbA1c levels[31]. However, the decrease in HbA1c was more in the luseogliflozin arm compared to the metformin arm in the study by Shibuya *et al*[13]. Four out of seven studies and six out of seven studies showed a decrease in FPG and HbA1c, respectively, in the SGLT-2 inhibitor arm. Thus, the improved glycaemic status is one of the mechanisms by which SGLT-2 inhibitors ameliorate NAFLD.

SGLT-2 inhibitors ameliorate insulin resistance in numerous ways. SGLT-2 inhibitors improve obesity associated insulin resistance by regulating macrophage

Table 5 Assessment of study quality of observational studies

S. No	Criteria	Ohki *et al*[15]	Seko *et al*[16]	Gautam *et al*[17]	Sumida *et al*[18]
1	A clearly stated aim	2	2	2	2
2	Inclusion of consecutive patients	0	2	2	1
3	Prospective collection of data	2	0	2	2
4	Endpoints appropriate to the aim of the study	2	2	2	2
5	Unbiased assessment of the study endpoint	0	0	0	0
6	Follow-up period appropriate to the aim of the study	2	2	2	2
7	Loss to follow up less than 5%	2	2	2	2
8	Prospective calculation of the study size	0	0	0	0
9	An adequate control group	NA	0	NA	NA
10	Contemporary groups	NA	2	NA	NA
11	Baseline equivalence of groups	NA	2	NA	NA
12	Adequate statistical analyses	NA	2	NA	NA
13	Total score	10/16	16/24	12/16	11/16

recruitment and altering the proportion of pro-inflammatory and anti-inflammatory macrophages. They enhance fat utilization by promoting β-oxidation of fatty acids and browning of white adipose tissue by inducing the expression of thermogenin leading to an improvement in the lipid profile. Similar to other antidiabetic drugs, SGLT-2 inhibitors reduce insulin resistance by decreasing glucotoxicity. Dapagliflozin has been shown to improve insulin sensitivity by increasing adiponectin and zinc-A2-glycoprotein levels[32]. Only dapagliflozin was shown to decrease insulin resistance in the study by Eriksson *et al*[14].

SGLT-2 inhibitors cause weight reduction. The major mechanism that causes weight reduction is the decrease in fat mass. The decrease in fat mass is due to the shift in substrate utilization to lipids instead of carbohydrates[33,34]. Ito *et al*[12] and Shibuya *et al*[13] demonstrated that SGLT-2 inhibitors caused a significant reduction in abdominal visceral and subcutaneous fat area as measured by computed tomography scan. Similarly, Eriksson *et al*[14] showed that dapagliflozin significantly reduced abdominal visceral and subcutaneous adipose tissue volume as assessed by magnetic resonance imaging. The other mechanisms of weight loss are the urinary glucose loss which amounts to approximately 200 Kcal/d and osmotic diuresis[33,35]. Unlike the other weight-reducing effects of SGLT-2 inhibitors, which are potentially beneficial, osmotic diuresis is clearly an adverse effect. Seko *et al*[16] showed that ipragliflozin and canagliflozin significantly reduced total body water in addition to body fat mass as measured by bioelectrical impedance analysis. Five studies showed a significant decrease in BMI in patients on SGLT-2 inhibitor therapy. Thus, the major beneficial effects of SGLT-2 inhibitors on NAFLD are exerted via reduction in hepatic fat and fibrosis, improved glycaemic control, decrease in insulin resistance, and weight loss.

The most common adverse effects of SGLT-2 inhibitors are genitourinary tract infections. In addition, they may cause diabetic ketoacidosis, dizziness, acute kidney injury, lower limb amputations, and bone fractures[36,37]. A meta-analysis concluded that there was no difference between placebo and SGLT-2 inhibitors for serious adverse events[38]. Among the 30 adverse events reported in all the studies, the most common was genitourinary tract infections (10 out of 23 characterised events).

The major strength of this systematic review was that the effect of five SGLT-2 inhibitors on NAFLD in patients with type 2 diabetes was evaluated in both RCTs and observational studies. Moreover, liver fat, liver fibrosis, metabolic, and anthropometric parameters in addition to liver enzymes were assessed as outcome variables following SGLT-2 inhibitor therapy. Yet this systematic review has a few limitations. First, most of the studies were done amongst the Japanese population. As a result, the study findings may not be applicable to patients from other ethnicities. Second, the sample size was considerably small and the duration of follow-up was of limited period in most of the studies. Third, the confounding effect of concomitant anti-diabetes drugs like metformin, DPP- 4 inhibitors, and glucagon like peptide-1 analogues on NAFLD cannot be ruled out, particularly in observational studies. Fourth, two studies (Eriksson *et al*[14] and Sumida *et al*[18]) were funded by pharmaceutical companies, which is a source of potential conflicts of interest.

Summary and conclusion

In conclusion based on the available evidence, SGLT-2 inhibitors were found to

Table 6 Change in serum alanine aminotransferase levels in individual studies

Study	Serum ALT level (IU/L)			*P* value	*P* value between groups
	Group	Baseline	Study completion		
Kuchay *et al*[11]	Empagliflozin	64.3 (20.2)	49.7 (25.8)	0.001	0.005
	Control	65.3 (40.3)	61.6 (38.4)	0.422	
Ito *et al*[12]	Ipragliflozin	57.4 (27.3)	38.2 (20.5)	< 0.05	0.642
	Pioglitazone	53.1 (26.6)	36.8 (15.1)	< 0.05	
Shibuya *et al*[13]	Luseogliflozin	49.5 (31.0, 70.0)	31 (26.0, 55.0)	0.057	0.064
	Metformin	39 (23.0, 56.0)	39 (27.0, 51.0)	0.518	
Eriksson *et al*[14]	Placebo	33.53 (12.4)	-0.2 (8.8)[1]	-	-
	Omega-3 CA	37.65 (14.7)	+5.9 (16.5)[1]	-	Non-significant[2]
	Dapagliflozin	39.41 (14.7)	-8.2 (8.2)[1]	-	< 0.05[2]
	O + D	35.88 (17.1)	+0.1 (12.9)[1]	-	Non-significant[2]
Ohki *et al*[15]	Ipragliflozin	62 (43.0-75.0)	38.0 (31.0-65.0)	0.01	-
Seko *et al*[16]	SGLT-2 inhibitor	70.8 (8.1)	48.8 (5.5)	0.002	0.039
	Sitagliptin	92.4 (11.2)	71.1 (10.0)	0.012	
Gautam *et al*[17]	Canagliflozin	96 (18.7)	60.0 (17.6)	< 0.00001	-
Sumida *et al*[18]	Luseogliflozin	54.7 (28.2)	42.4 (26.5)	< 0.001	-

[1]Change from baseline.
[2]Compared to placebo.
ALT: Alanine aminotransferase; CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

improve serum levels of liver enzymes, liver fibrosis indices, and liver fat without significant side effects in type 2 diabetes patients with NAFLD. They showed additional beneficial effects on obesity, glycaemic parameters, insulin resistance, and dyslipidaemia in these subjects. However, the quality of evidence was low to moderate. Prospective studies, preferably RCTs, comparing different SGLT-2 inhibitors with standard treatments of NAFLD in multi-ethnic populations with a longer follow-up period are needed in the future.

Table 7 Change in serum aspartate aminotransferase levels in individual studies

Study	Serum AST levels (IU/L)			*P* value	*P* value between groups
	Group	Baseline	Study completion		
Kuchay *et al*[11]	Empagliflozin	44.6 (23.5)	36.2 (9.0)	0.04	0.212
	Control	45.3 (24.3)	44.6 (23.8)	0.931	
Ito *et al*[12]	Ipragliflozin	39.7 (16.7)	27.3 (8.9)	< 0.05	0.802
	Pioglitazone	43.3 (20.5)	32.4 (15.4)	< 0.05	
Eriksson *et al*[14]	Placebo	29.4 (13.2)	-1.2 (7.2)[1]	-	-
	Omega-3 CA	30.6 (10.2)	+4.8 (9.0)[1]	-	Non-significant[2]
	Dapagliflozin	31.2 (11.4)	-4.2 (5.4)[1]	-	< 0.05[2]
	O + D	30 (10.2)	+1.2 (5.4)[1]	-	Non-significant[2]
Ohki *et al*[15]	Ipragliflozin	37 (29.0-52.0)	28 (23.0-31.0)	0.03	-
Seko *et al*[16]	SGLT-2 inhibitor	54.4 (5.6)	38 (3.1)	0.001	-
	Sitagliptin	67 (7.7)	52.5 (7.7)	0.016	
Gautam *et al*[17]	Canagliflozin	72 (16.7)	53 (10.3)	< 0.00001	-
Sumida *et al*[18]	Luseogliflozin	40.7 (22.2)	31.9 (18.2)	< 0.001	-

[1]Change from baseline.
[2]Compared to placebo.
AST: Aspartate aminotransferase; CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 8 Change in serum gamma-glutamyl transferase levels in individual studies

Study	Serum GGT (IU/L)			*P* value	*P* value between groups
	Group	Baseline	Study completion		
Kuchay *et al*[11]	Empagliflozin	65.8 (36.1)	50.9 (24.6)	0.002	0.057
	Control	63.9 (45.3)	60.0 (39.0)	0.421	
Ito *et al*[12]	Ipragliflozin	62.8 (58.3)	44.0 (38.3)	< 0.05	0.642
	Pioglitazone	71.6 (54.1)	48.8 (61.2)	< 0.05	
Eriksson *et al*[14]	Placebo	32.4 (17.4)	+2.4 (9.6)[1]	-	-
	Omega-3 CA	54.0 (57.6)	+2.4 (12.0)[1]	-	Non-significant[2]
	Dapagliflozin	58.2 (43.2)	-4.8 (13.8)[1]	-	< 0.05[2]
	O + D	40.2 (14.4)	-0.6 (13.8)[1]	-	Non-significant[2]
Ohki *et al*[15]	Ipragliflozin	75.0 (47.0-105.0)	60.0 (40.0-101.0)	0.03	-
Seko *et al*[16]	SGLT-2 inhibitor	61.7 (9.1)	58.7 (11.5)	0.051	-
	Sitagliptin	89.2 (11.8)	82.4 (11.9)	0.36	
Gautam *et al*[17]	Canagliflozin	75.1 (31.8)	69.2 (26.2)	0.003	-
Sumida *et al*[18]	Luseogliflozin	62.4 (77.1)	48.2 (56.3)	0.003	-

[1]Change from baseline.
[2]Compared to placebo.
CA: Carboxylic acid; GGT: Gamma-glutamyl transferase; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 9 Change in hepatic fat in individual studies

Study	Parameter	Group	Baseline	Study completion	*P* value	*P* value between groups
Kuchay *et al*[11]	MRI-PDFF	Empagliflozin	16.2 (7)	11.3 (5.3)	< 0.0001	< 0.0001
		Control	16.4 (7.3)	15.5 (6.7)	0.054	
Ito *et al*[12]	L/S ratio	Ipragliflozin	0.8 (0.2)	1.0 (0.2)	< 0.05	0.90
		Pioglitazone	0.8 (0.3)	1.0 (0.2)	< 0.05	
Shibuya *et al*[13]	L/S ratio	Luseogliflozin	0.9 (0.6-1.0)	1.0 (0.8-1.2)	0.0008	0.00002
		Metformin	1.0 (0.8-1.1)	0.9 (0.7-1.0)	0.017	
Eriksson *et al*[14]	MRI-PDFF	Placebo	15.1 (6.5)	-0.6 (1.9)[1]	-	-

		Omega-3 CA	22.2 (11.0)	-3.2 (2.9)[1]	-	Non-significant[2]
		Dapagliflozin	17.3 (9.1)	-2.2 (3.3)[1]	-	Non-significant[2]
		O + D	17.8 (9.2)	-3.2 (3.5)[1]	-	< 0.05[2]
Sumida *et al*[18]	MRI-HFF	Luseogliflozin	21.5 (7.2)	15.7 (6.8)	< 0.001	-

[1]Change from baseline.
[2] Compared to placebo.
MRI-PDFF: Magnetic resonance imaging-derived proton density fat fraction; L/S ratio: Liver/spleen attenuation ratio; MRI-HFF: Magnetic resonance imaging-hepatic fat fraction; CA: Carboxylic acid; O + D: Omega-3 CA + Dapagliflozin.

Table 10 Assessment of liver fibrosis in individual studies

Study	Parameter	Group	Baseline	Study completion	*P* value	*P* value between groups
Ito *et al*[12]	FIB-4 index	Ipragliflozin	1.44 (0.64)	1.22 (0.55)	< 0.05	0.596
		Pioglitazone	1.84 (1.13)	1.71 (1.19)	Non-significant	
Ohki *et al*[15]	FIB-4 index	Ipragliflozin	1.75 (0.82-1.93)	1.39 (0.77-1.99)	0.04	-
Sumida *et al*[18]	FIB-4 index	Luseogliflozin	1.63 (1.19)	1.52 (0.92)	0.17	-
	NAFLD fibrosis score	Luseogliflozin	1.61 (0.71)	1.62 (0.88)	0.86	-

FIB: Fibrosis 4; NAFLD: Non-alcoholic fatty liver disease.

Table 11 Change in fasting plasma glucose in individual studies

Study	Fasting plasma glucose (mg/dL)			*P* value	*P* value between groups
	Group	Baseline	Study completion		
Kuchay *et al*[11]	Empagliflozin	173.0 (44.0)	124.0 (17.0)	< 0.001	0.85
	Control	176.0 (57.0)	120.0 (19.0)	< 0.0001	
Ito *et al*[12]	Ipragliflozin	160.1 (38.7)	136.5 (26.7)	< 0.05	0.785
	Pioglitazone	169.4 (50.9)	139.0 (26.6)	< 0.05	
Shibuya *et al*[13]	Luseogliflozin	127.0 (116.0, 136.0)	125.0 (113.0, 138.0)	0.87	0.583
	Metformin	147.0 (126.0, 161.0)	134.0 (122.0, 145.0)	0.32	
Eriksson *et al*[14]	Placebo	169.2 (29.7)	+6.7 (14.8)[1]	-	-
	Omega-3 CA	162.4 (26.6)	+3.8 (19.3)[1]	-	Non-significant[2]
	Dapagliflozin	161.8 (33.3)	-17.6 (26.8)[1]	-	< 0.05[2]
	O + D	168.8 (35.5)	-16.4 (36.0)[1]	-	< 0.05[2]
Ohki *et al*[15]	Ipragliflozin	162.0 (135.0-189.0)	135.0 (120.0-166.0)	0.3	-
Seko *et al*[16]	SGLT-2 inhibitor	125.0 (6.0)	116.6 (4.2)	0.07	Non-significant
	Sitagliptin	114.6 (7.0)	134.0 (10.5)	0.067	
Sumida *et al*[18]	Luseogliflozin	142.0 (30.3)	135.4 (25.6)	0.04	-

[1]Change from baseline.
[2]Compared to placebo.
CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 12 Change in glycosylated haemoglobin in individual studies

Study	Glycosylated haemoglobin (%)			*P* value	*P* value between groups
	Group	Baseline	Study completion		
Kuchay *et al*[11]	Empagliflozin	9.0 (1.0)	7.2 (0.6)	< 0.001	0.88
	Control	9.1 (1.4)	7.1 (0.9)	< 0.0001	
Ito *et al*[12]	Ipragliflozin	8.5 (1.5)	7.6 (1.0)	< 0.05	0.522
	Pioglitazone	8.3 (1.4)	7.1 (0.9)	< 0.05	
Shibuya *et al*[13]	Luseogliflozin	7.8 (7.2, 7.9)	6.5 (6.4, 7.0)	0.002	0.023
	Metformin	7.4 (6.9, 7.7)	7.3 (6.7, 7.6)	0.362	

Eriksson *et al*[14]	Placebo	7.4 (0.8)	-0.1 (0.4)[1]	-	-
	Omega-3 CA	7.4 (0.7)	+0.1 (0.4)[1]	-	Non-significant[2]
	Dapagliflozin	7.4 (0.6)	-0.6 (0.7)[1]	-	< 0.05[2]
	O + D	7.5 (0.8)	-0.5 (0.5)[1]	-	Non-significant[2]
Ohki *et al*[15]	Ipragliflozin	8.4 (7.8-8.9)	7.6 (6.9-8.2)	< 0.01	-
Seko *et al*[16]	SGLT-2 inhibitor	6.7 (0.1)	6.5 (0.1)	0.055	Non-significant
	Sitagliptin	7.0 (0.3)	6.9 (0.3)	0.331	
Sumida *et al*[18]	Luseogliflozin	7.3 (0.7)	7.0 (0.7)	0.002	-

[1]Change from baseline.
[2]Compared to placebo.
CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 13 Change in homeostasis model assessment-estimated insulin resistance in individual studies

Study	HOMA-IR			*P* value	*P* value between groups
	Group	Baseline	Study completion		
Ito *et al*[12]	Ipragliflozin	5.2 (2.5)	4.8 (5.5)	Non-significant	0.401
	Pioglitazone	5.7 (3.4)	4.5 (2.7)	< 0.05	
Eriksson *et al*[14]	Placebo	4.2 (2.4)	-0.2 (1.4)[1]	-	-
	Omega 3-CA	5.4 (2.9)	+0.3 (2.4)[1]	-	Non-significant[2]
	Dapagliflozin	4.3 (1.9)	-1.1 (1.4)[1]	-	< 0.05[2]
	O + D	4.4 (1.7)	-0.9 (1.6)[1]	-	< 0.05[2]
Seko *et al*[16]	SGLT-2 inhibitor	4.5 (0.5)	7.9 (2.3)	0.955	-
	Sitagliptin	4.4 (0.5)	6.5 (0.8)	0.163	

[1]Change from baseline.
[2] Compared to placebo.
HOMA-IR: Homeostasis model assessment-estimated insulin resistance; CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 14 Change in serum triglycerides in individual studies

Study	Serum triglycerides (mg/dL)			*P* value	*P* value between groups
	Group	Baseline	Study completion		
Kuchay *et al*[11]	Empagliflozin	201.0 (124.0)	155.0 (52.0)	0.01	0.678
	Control	212.0 (115.0)	175.0 (43.0)	0.019	
Ito *et al*[12]	Ipragliflozin	166.9 (76.4)	143.4 (81.4)	< 0.05	0.938
	Pioglitazone	188.4 (148.8)	169.3 (131.3)	Non-significant	
Eriksson *et al*[14]	Placebo	169.2 (84.1)	-11.5 (45.6)[1]	-	-
	Omega-3 CA	186.9 (81.5)	-15.9 (47.4)[1]	-	Non-significant[2]
	Dapagliflozin	178.0 (103.6)	+14.2 (40.5)[1]	-	Non-significant[2]
	O + D	168.3 (72.6)	-25.7 (57.1)[1]	-	Non-significant[2]
Ohki *et al*[15]	Ipragliflozin	148.0 (107.0, 222.)	145.0 (114.0, 172.0)	0.75	-
Seko *et al*[16]	SGLT-2 inhibitor	153.8 (15.9)	137.8 (10.5)	0.236	-
	Sitagliptin	193.4 (25.2)	191.1 (23.8)	0.986	
Sumida *et al*[18]	Luseogliflozin	158.1 (110.5)	129.4 (59.5)	0.062	-

[1]Change from baseline.
[2]Compared to placebo.
CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 15 Change in serum low-density lipoprotein cholesterol in individual studies

Study	Serum low-density lipoprotein cholesterol (mg/dL)			*P* value	*P* value between groups
	Group	Baseline	Study completion		
Kuchay *et al*[11]	Empagliflozin	112.0 (35.0)	95.0 (22.0)	0.018	0.512
	Control	114.0 (30.0)	96.0 (17.0)	0.001	
Ito *et al*[12]	Ipragliflozin	108.3 (36.2)	110.7 (40.1)	Non-significant	0.057
	Pioglitazone	104.0 (27.9)	114.6 (29.5)	< 0.05	
Eriksson *et al*[14]	Placebo	98.2 (34.4)	+1.6 (15.5)[1]	-	-
	Omega-3 CA	111.8 (34.4)	+2.3 (17.4)[1]	-	Non-significant[2]
	Dapagliflozin	109.4 (34.8)	+7.7 (20.5)[1]	-	Non-significant[2]
	O + D	88.9 (23.2)	+5.8 (21.7)[1]	-	Non-significant[2]
Ohki *et al*[15]	Ipragliflozin	113.0 (89.0-142.0)	103.0 (92.0-122.0)	0.08	-
Seko *et al*[16]	SGLT-2 inhibitor	119.2 (5.8)	119.8 (5.7)	0.943	-
	Sitagliptin	112.9 (4.9)	127.1 (8.8)	0.063	
Sumida *et al*[18]	Luseogliflozin	101.0 (22.4)	105.0 (24.4)	0.11	-

[1]Change from baseline.
[2]Compared to placebo.
CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 16 Change in serum high-density lipoprotein cholesterol in individual studies

Study	Serum high-density lipoprotein cholesterol (mg/dL)			*P* value	*P* value between groups
	Group	Baseline	Study completion		
Kuchay *et al*[11]	Empagliflozin	42.0 (12.0)	45.0 (12.0)	0.087	0.752
	Control	45.0 (15.0)	47.0 (12.0)	0.097	
Ito *et al*[12]	Ipragliflozin	48.9 (9.3)	54.7 (10.4)	< 0.05	0.82
	Pioglitazone	47.4 (11.6)	52.7 (13.5)	< 0.05	
Eriksson *et al*[14]	Placebo	51.4 (14.9)	-0.4 (5.0)[1]	-	-
	Omega-3 CA	49.9 (14.1)	+0.4 (3.2)[1]	-	Non-significant[2]
	Dapagliflozin	49.9 (9.5)	+0.4 (4.8)[1]	-	Non-significant[2]
	O + D	51.4 (10.2)	+1.6 (5.0)[1]	-	Non-significant[2]
Ohki *et al*[15]	Ipragliflozin	42.0 (40.0-50.0)	44.0 (42.0-59.0)	0.01	-
Seko *et al*[16]	SGLT-2 inhibitor	53.9 (2.5)	55.4 (2.6)	0.043	-
	Sitagliptin	54.8 (3.3)	55.6 (2.3)	0.531	
Sumida *et al*[18]	Luseogliflozin	55.6 (11.7)	57.5 (13.4)	0.062	-

[1]Change from baseline.
[2]Compared to placebo.
CA: Carboxylic acid; O + D: Omega-3 carboxylic acid + Dapagliflozin; SGLT-2: Sodium glucose cotransporter-2.

Table 17 Change in body mass index in individual studies

Study	Body mass index (kg/m^2)			*P* value	*P* value between groups
	Group	Baseline	Study completion		
Kuchay *et al*[11]	Empagliflozin	30.0 (3.8)	28.7 (3.5)	0.001	0.124
	Control	29.4 (3.1)	28.8 (2.8)	0.019	
Shibuya *et al*[13]	Luseogliflozin	27.9 (26.2, 28.7)	27.0 (25.6, 28.3)	0.002	0.031
	Metformin	27.2 (24.8, 32.1)	27.3 (24.3, 31.6)	0.646	
Ohki *et al*[15]	Ipragliflozin	30.1 (26.1-31.4)	27.6 (25.3-30.2)	< 0.01	-
Seko *et al*[16]	SGLT-2 inhibitor	29.6 (0.7)	28.3 (0.7)	< 0.001	-
	Sitagliptin	29.2 (1.5)	28.9 (1.4)	0.295	

Sumida *et al*[18]	Luseogliflozin	27.8 (3.6)	27.2 (1.0)	< 0.001	-

SGLT-2: Sodium glucose cotransporter-2.

Table 18 Adverse effects of sodium glucose cotransporter-2 inhibitors in individual studies

Study	No. of adverse events	No. of patients	Types of adverse events
Kuchay *et al*[11]	3	25	Nonspecific fatigue: 1
			Arthralgia: 1
			Balanoposthitis: 1
Ito *et al*[12]	9	32	UTI: 3
			Increased appetite: 2
			Nausea: 1
			Headache: 1
			Diarrhoea: 1
			Vaginal candidiasis: 1
Eriksson *et al*[14]	7	21	-
Seko *et al*[16]	2	26	UTI: 2
Gautam *et al*[17]	1	32	Recurrent UTI with genital candidiasis: 1
Sumida *et al*[18]	8	40	Low blood pressure: 3
			Vaginal itching: 2
			Constipation: 1
			Vertigo: 1
			Dehydration: 1
Total	30	176	Most common adverse event: Genitourinary tract infections-10

UTI: Urinary tract infection.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is a common comorbidity with type 2 diabetes. The existing therapeutic options for NAFLD are not adequate. Hypocaloric diet and exercise is the cornerstone of therapy in NAFLD. Pioglitazone is the only drug recommended in diabetes patients with biopsy proven non-alcoholic steatohepatitis. The frequent coexistence of NAFLD and type 2 diabetes along with their combined adverse health consequences and inadequate therapeutic options makes it necessary to search for newer alternatives. This systematic review is an effort to review the available literature on the effect of sodium glucose cotransporter-2 (SGLT-2) inhibitors on NAFLD in type 2 diabetes patients.

Research motivation

Because the existing therapeutic options are not adequate for NAFLD patients, there is a need for finding newer alternatives. SGLT-2 inhibitors have shown promise in the management of NAFLD in animals. Hence, we reviewed the available literature on the effect of SGLT-2 inhibitors in NAFLD in type 2 diabetes patients. This will promote further high quality research on the effect of SGLT-2 inhibitors in NAFLD.

Research objectives

The primary outcome was the change in serum alanine aminotransferase levels in type 2 diabetes patients with NAFLD treated with SGLT-2 inhibitors. The secondary outcomes were change in serum aspartate aminotransferase and gamma-glutamyl transferase levels, hepatic fat, hepatic fibrosis, metabolic profile, anthropometric parameters, and the adverse effects of SGLT-2 inhibitors.

Research methods

This systematic review was registered in PROSPERO and performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines. We searched PubMed/MEDLINE, Cochrane library, Google scholar, and Clinicaltrials.gov for the relevant articles to be included in this systematic review. A narrative synthesis of the results of individual studies was done. The change in the difference in means and difference in proportions and the respective *P* values as mentioned in the original manuscripts were tabulated and explained. The quality of the randomised controlled trials and observational studies was analysed using the

Cochrane risk of bias tool and MINORS scale, respectively.

Research results
Eight articles (four randomised controlled trials and four observational studies) were included in this systematic review. A total of 214 patients were treated with SGLT-2 inhibitors. SGLT-2 inhibitors caused a significant improvement in liver enzymes, hepatic fat, hepatic fibrosis, glycaemia, insulin resistance, obesity, and lipid parameters with minimal adverse effects. However, the quality of evidence is low to moderate.

Research conclusions
We found that SGLT-2 inhibitors improved the serum levels of liver enzymes, liver fat, and liver fibrosis with additional beneficial effects on various metabolic and anthropometric parameters in type 2 diabetes patients with NAFLD. However, the number of patients treated with SGLT-2 inhibitors was small. The findings of this systematic review will have impact in choosing anti-diabetes medication like SGLT-2 inhibitors to treat NAFLD associated with type 2 diabetes.

Research perspectives
The studies included in this systematic review were heterogeneous with regard to study design and intervention drugs. Most of the studies were done amongst the Japanese population. Prospective studies, preferably randomised controlled trials, comparing different SGLT-2 inhibitors with standard treatments of NAFLD in multi-ethnic populations with a longer follow-up period are needed in the future.

REFERENCES

1 **Saponaro C**, Gaggini M, Gastaldelli A. Nonalcoholic fatty liver disease and type 2 diabetes: common pathophysiologic mechanisms. *Curr Diab Rep* 2015; **15**: 607 [PMID: 25894944 DOI: 10.1007/s11892-015-0607-4]

2 **Burt AD**, Lackner C, Tiniakos DG. Diagnosis and Assessment of NAFLD: Definitions and Histopathological Classification. *Semin Liver Dis* 2015; **35**: 207-220 [PMID: 26378639 DOI: 10.1055/s-0035-1562942]

3 **Williams KH**, Shackel NA, Gorrell MD, McLennan SV, Twigg SM. Diabetes and nonalcoholic Fatty liver disease: a pathogenic duo. *Endocr Rev* 2013; **34**: 84-129 [PMID: 23238855 DOI: 10.1210/er.2012-1009]

4 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]

5 **Qiang S**, Nakatsu Y, Seno Y, Fujishiro M, Sakoda H, Kushiyama A, Mori K, Matsunaga Y, Yamamotoya T, Kamata H, Asano T. Treatment with the SGLT2 inhibitor luseogliflozin improves nonalcoholic steatohepatitis in a rodent model with diabetes mellitus. *Diabetol Metab Syndr* 2015; **7**: 104 [PMID: 26594248 DOI: 10.1186/s13098-015-0102-8]

6 **Tahara A**, Kurosaki E, Yokono M, Yamajuku D, Kihara R, Hayashizaki Y, Takasu T, Imamura M, Li Q, Tomiyama H, Kobayashi Y, Noda A, Sasamata M, Shibasaki M. Effects of SGLT2 selective inhibitor ipragliflozin on hyperglycemia, hyperlipidemia, hepatic steatosis, oxidative stress, inflammation, and obesity in type 2 diabetic mice. *Eur J Pharmacol* 2013; **715**: 246-255 [PMID: 23707905 DOI: 10.1016/j.ejphar.2013.05.014]

7 **Yokono M**, Takasu T, Hayashizaki Y, Mitsuoka K, Kihara R, Muramatsu Y, Miyoshi S, Tahara A, Kurosaki E, Li Q, Tomiyama H, Sasamata M, Shibasaki M, Uchiyama Y. SGLT2 selective inhibitor ipragliflozin reduces body fat mass by increasing fatty acid oxidation in high-fat diet-induced obese rats. *Eur J Pharmacol* 2014; **727**: 66-74 [PMID: 24486393 DOI: 10.1016/j.ejphar.2014.01.040]

8 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med* 2009; **6**: e1000097 [PMID: 19621072 DOI: 10.1371/journal.pmed.1000097]

9 **Higgins JP**, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA; Cochrane Bias Methods Group; Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011; **343**: d5928 [PMID: 22008217 DOI: 10.1136/bmj.d5928]

10 **Slim K**, Nini E, Forestier D, Kwiatkowski F, Panis Y, Chipponi J. Methodological index for non-randomized studies (minors): development and validation of a new instrument. *ANZ J Surg* 2003; **73**: 712-716 [PMID: 12956787 DOI: 10.1046/j.1445-2197.2003.02748.x]

11 **Kuchay MS**, Krishan S, Mishra SK, Farooqui KJ, Singh MK, Wasir JS, Bansal B, Kaur P, Jevalikar G, Gill HK, Choudhary NS, Mithal A. Effect of Empagliflozin on Liver Fat in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease: A Randomized Controlled Trial (E-LIFT Trial). *Diabetes Care* 2018; **41**: 1801-1808 [PMID: 29895557 DOI: 10.2337/dc18-0165]

12 **Ito D**, Shimizu S, Inoue K, Saito D, Yanagisawa M, Inukai K, Akiyama Y, Morimoto Y, Noda M, Shimada A. Comparison of Ipragliflozin and Pioglitazone Effects on Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes: A Randomized, 24-Week, Open-Label, Active-Controlled Trial. *Diabetes Care* 2017; **40**: 1364-1372 [PMID: 28751548 DOI: 10.2337/dc17-0518]

13 **Shibuya T**, Fushimi N, Kawai M, Yoshida Y, Hachiya H, Ito S, Kawai H, Ohashi N, Mori A. Luseogliflozin improves liver fat deposition compared to metformin in type 2 diabetes patients with non-alcoholic fatty liver disease: A prospective randomized controlled pilot study. *Diabetes Obes Metab* 2018; **20**: 438-442 [PMID: 28719078 DOI: 10.1111/dom.13061]

14 **Eriksson JW**, Lundkvist P, Jansson PA, Johansson L, Kvarnström M, Moris L, Miliotis T, Forsberg GB, Risérus U, Lind L, Oscarsson J. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. *Diabetologia* 2018; **61**: 1923-1934 [PMID: 29971527 DOI: 10.1007/s00125-018-4675-2]

15 **Ohki T**, Isogawa A, Toda N, Tagawa K. Effectiveness of Ipragliflozin, a Sodium-Glucose Co-transporter

2 Inhibitor, as a Second-line Treatment for Non-Alcoholic Fatty Liver Disease Patients with Type 2 Diabetes Mellitus Who Do Not Respond to Incretin-Based Therapies Including Glucagon-like Peptide-1 Analogs and Dipeptidyl Peptidase-4 Inhibitors. *Clin Drug Investig* 2016; **36**: 313-319 [PMID: 26914659 DOI: 10.1007/s40261-016-0383-1]

16 **Seko Y**, Sumida Y, Tanaka S, Mori K, Taketani H, Ishiba H, Hara T, Okajima A, Umemura A, Nishikawa T, Yamaguchi K, Moriguchi M, Kanemasa K, Yasui K, Imai S, Shimada K, Itoh Y. Effect of sodium glucose cotransporter 2 inhibitor on liver function tests in Japanese patients with non-alcoholic fatty liver disease and type 2 diabetes mellitus. *Hepatol Res* 2017; **47**: 1072-1078 [PMID: 27925353 DOI: 10.1111/hepr.12834]

17 **Gautam A**, Agrawal PK, Doneria J, Nigam A. Effects of Canagliflozin on Abnormal Liver Function Tests in Patients of Type 2 Diabetes with Non-Alcoholic Fatty Liver Disease. *JAPI* 2018; **66**: 62-66

18 **Sumida Y**, Murotani K, Saito M, Tamasawa A, Osonoi Y, Yoneda M, Osonoi T. Effect of luseogliflozin on hepatic fat content in type 2 diabetes patients with non-alcoholic fatty liver disease: A prospective, single-arm trial (LEAD trial). *Hepatol Res* 2019; **49**: 64-71 [PMID: 30051943 DOI: 10.1111/hepr.13236]

19 **Sattar N**, Fitchett D, Hantel S, George JT, Zinman B. Empagliflozin is associated with improvements in liver enzymes potentially consistent with reductions in liver fat: results from randomised trials including the EMPA-REG OUTCOME® trial. *Diabetologia* 2018; **61**: 2155-2163 [PMID: 30066148 DOI: 10.1007/s00125-018-4702-3]

20 **Nakano S**, Katsuno K, Isaji M, Nagasawa T, Buehrer B, Walker S, Wilkison WO, Cheatham B. Remogliflozin Etabonate Improves Fatty Liver Disease in Diet-Induced Obese Male Mice. *J Clin Exp Hepatol* 2015; **5**: 190-198 [PMID: 26628836 DOI: 10.1016/j.jceh.2015.02.005]

21 **Komiya C**, Tsuchiya K, Shiba K, Miyachi Y, Furuke S, Shimazu N, Yamaguchi S, Kanno K, Ogawa Y. Ipragliflozin Improves Hepatic Steatosis in Obese Mice and Liver Dysfunction in Type 2 Diabetic Patients Irrespective of Body Weight Reduction. *PLoS One* 2016; **11**: e0151511 [PMID: 26977813 DOI: 10.1371/journal.pone.0151511]

22 **Jojima T**, Tomotsune T, Iijima T, Akimoto K, Suzuki K, Aso Y. Empagliflozin (an SGLT2 inhibitor), alone or in combination with linagliptin (a DPP-4 inhibitor), prevents steatohepatitis in a novel mouse model of non-alcoholic steatohepatitis and diabetes. *Diabetol Metab Syndr* 2016; **8**: 45 [PMID: 27462372 DOI: 10.1186/s13098-016-0169-x]

23 **Wang D**, Luo Y, Wang X, Orlicky DJ, Myakala K, Yang P, Levi M. The Sodium-Glucose Cotransporter 2 Inhibitor Dapagliflozin Prevents Renal and Liver Disease in Western Diet Induced Obesity Mice. *Int J Mol Sci* 2018; **19** [PMID: 29301371 DOI: 10.3390/ijms19010137]

24 **Calzadilla Bertot L**, Adams LA. The Natural Course of Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci* 2016; **17** [PMID: 27213358 DOI: 10.3390/ijms17050774]

25 **Shah AG**, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ; Nash Clinical Research Network. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1104-1112 [PMID: 19523535 DOI: 10.1016/j.cgh.2009.05.033]

26 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]

27 **Afolabi BI**, Ibitoye BO, Ikem RT, Omisore AD, Idowu BM, Soyoye DO. The Relationship Between Glycaemic Control and Non-Alcoholic Fatty Liver Disease in Nigerian Type 2 Diabetic Patients. *J Natl Med Assoc* 2018; **110**: 256-264 [PMID: 29778128 DOI: 10.1016/j.jnma.2017.06.001]

28 **Kalra S**. Sodium Glucose Co-Transporter-2 (SGLT2) Inhibitors: A Review of Their Basic and Clinical Pharmacology. *Diabetes Ther* 2014; **5**: 355-366 [PMID: 25424969 DOI: 10.1007/s13300-014-0089-4]

29 **Abdul-Ghani MA**, Norton L, Defronzo RA. Role of sodium-glucose cotransporter 2 (SGLT 2) inhibitors in the treatment of type 2 diabetes. *Endocr Rev* 2011; **32**: 515-531 [PMID: 21606218 DOI: 10.1210/er.2010-0029]

30 **Monami M**, Nardini C, Mannucci E. Efficacy and safety of sodium glucose co-transport-2 inhibitors in type 2 diabetes: a meta-analysis of randomized clinical trials. *Diabetes Obes Metab* 2014; **16**: 457-466 [PMID: 24320621 DOI: 10.1111/dom.12244]

31 **Palmer SC**, Mavridis D, Nicolucci A, Johnson DW, Tonelli M, Craig JC, Maggo J, Gray V, De Berardis G, Ruospo M, Natale P, Saglimbene V, Badve SV, Cho Y, Nadeau-Fredette AC, Burke M, Faruque L, Lloyd A, Ahmad N, Liu Y, Tiv S, Wiebe N, Strippoli GF. Comparison of Clinical Outcomes and Adverse Events Associated With Glucose-Lowering Drugs in Patients With Type 2 Diabetes: A Meta-analysis. *JAMA* 2016; **316**: 313-324 [PMID: 27434443 DOI: 10.1001/jama.2016.9400]

32 **Mohammad SH**, Fadhil NN, Mahmood MD. Effects of metformin and dapagliflozin on glycemic indices and HOMA-IR in type 2 diabetes mellitus patients. *Int J Pharm Biol Sci* 2018; **8**: 66-73

33 **Trujillo JM**, Nuffer WA. Impact of Sodium-Glucose Cotransporter 2 Inhibitors on Nonglycemic Outcomes in Patients with Type 2 Diabetes. *Pharmacotherapy* 2017; **37**: 481-491 [PMID: 28102030 DOI: 10.1002/phar.1903]

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

35 **Ferrannini G**, Hach T, Crowe S, Sanghvi A, Hall KD, Ferrannini E. Energy Balance After Sodium-Glucose Cotransporter 2 Inhibition. *Diabetes Care* 2015; **38**: 1730-1735 [PMID: 26180105 DOI: 10.2337/dc15-0355]

36 **Esteban-Jiménez O**, Navarro-Pemán C, Urieta-González L. Seguridad de los iSGLT-2. Revisión de las reacciones adversas notificadas a nivel nacional. *Med Fam SEMERGEN* 2018; **44**: 23–29 [DOI: 10.1016/j.semerg.2017.10.003]

37 **Blau JE**, Taylor SI. Adverse effects of SGLT2 inhibitors on bone health. *Nat Rev Nephrol* 2018; **14**: 473–474 [DOI: 10.1038/s41581-018-0028-0]

38 **Storgaard H**, Gluud LL, Bennett C, Grøndahl MF, Christensen MB, Knop FK, Vilsbøll T. Benefits and Harms of Sodium-Glucose Co-Transporter 2 Inhibitors in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis. *PLoS One* 2016; **11**: e0166125 [PMID: 27835680 DOI: 10.1371/journal.pone.0166125]

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CASE REPORT

Bilateral gangrene of fingers in a patient on empagliflozin: First case report

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Abstract

BACKGROUND

Sodium glucose cotransporter 2 (SGLT2) inhibitors use has been associated with toe amputations and non-healing ulcers and gangrene mostly of lower extremities. There are no case reports about association of Empagliflozin with finger ulcers or gangrene. This is the first case report of Empagliflozin (Jardiance) an SGLT2 inhibitor causing gangrene of fingers and second case in literature about any SGLT2 inhibitor causing gangrene of upper extremity.

CASE SUMMARY

A 76-year-old man with type 2 diabetes mellitus sustained minimal trauma to both middle fingers, which started healing. He was started on empagliflozin a week later for management of type 2 diabetes mellitus and started developing gangrene to both middle finger tips along with neuropathic pain which worsened over the course of next four months. Investigations were negative for vascular insufficiency, infection and vasculitis and imaging of hand was normal. Discontinuation of empagliflozin slowed progression of gangrene and caused symptomatic improvement with reduction in neuropathic pain.

CONCLUSION

This case report suggests possible association of empagliflozin and finger gangrene and recommends that more research and awareness among clinicians is needed in this area.

Key words: Empagliflozin; Finger gangrene; Non-healing ulcer; Type 2 diabetes mellitus; Sodium glucose cotransporter 2 inhibitor; Jardiance; Case report

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Core tip: Empagliflozin can cause finger gangrene in patients with type 2 diabetes mellitus. Empaglifozin has gained popularity recently as a newer anti diabetic agent with improved cardiovascular outcomes and better glycemic control in addition to lowering blood pressure and helping with weight loss. Lack of proper awareness about this condition can lead to progression of disease if not identified early on and can result in amputations. This medication should be used with caution in patients who have high risk of gangrene such as that on prednisone and in those with diabetic neuropathy.

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INTRODUCTION

This is the first ever case reported in literature about empaglifozin (Jardiance) as a possible cause of finger gangrene. Sodium glucose cotransporter 2 (SLGT2) inhibitors inhibit sodium and glucose cotransport at proximal renal tubules. SLGT2 inhibitors have been associated with an increased risk of genital infections secondary to increased glycosuria. According to the results of CANVAS trial, Dapagliflozin, another SGLT2 inhibitor of the same class as empagliflozin, has been shown to significantly reduce the risk of cardiovascular events by 14% but it doubled the risk of amputation in patients with type 2 diabetes mellitus[1]. In a similar study conducted on patients with type 2 diabetes mellitus at high risk for cardiovascular events, patients were given empaglifozin *vs* placebo and those on empagliflozin had lesser adverse cardiovascular events and lower all-cause mortality. Among patients receiving empagliflozin, there was an increased rate of genital infections but there was no increase in lower limb amputations[2]. In another study of over eight million case safety reports, increased risk of lower-limb amputations especially toe amputations were reported with empagliflozin[3].

A data analysis conducted based on data from US Food and Drug Administration adverse event Reporting System showed a total of 66 cases of SGLT2 inhibitor-associated amputations[3]. Among these, there was only one case of hand amputation which was from Dapagliflozin. All others were lower extremity gangrene and ulcers, most commonly of toes[4]. There are two case reports of empagliflozin related Fournier's gangrene in literature[5,6] which pointed the benefit of keeping a high index of suspicion and early cessation of SGLT2 inhibitors could potentially prevent the progression of these infections requiring surgical debridement later. Empagliflozin has also been associated with vulvovaginal candidiasis along with other SGLT2 inhibitors[7].

SGLT2 inhibitors are used in general, cautiously in patients with vascular insufficiency, neuropathy, risk of amputations and very high hemoglobin A1C over 11. However, there are no case reports to date about an empagliflozin as a possible cause of non-healing finger ulcers or gangrene. Ours is the first reported case of empagliflozin (a SGLT2 inhibitor) as likely cause of gangrene of fingers.

CASE PRESENTATION

Chief complaint

Gangrene both middle fingers.

History of present illness

A 76-year-old man with moderately controlled type 2 diabetes mellitus (hba1c of 8.6) sustained minor injury to the tip of both middle fingers while doing some mechanical work. He had no burns or exposure to heat. Initially, the fingers were healing well with minimal scarring. A week after the injury, he was started on empagliflozin 10 mg for better glycemic control in addition to his other medications. Three weeks after the injury (two weeks after being started on empagliflozin), he started noticing significant pain on tip of both middle fingers which also started changing color to brown and then to black (Figure 1).

Figure 1 Gangrene tip of fingers while on empaglifozin.

History of past illness
No history of previous vasculitis. He has history of polymyalgia rheumatica and was on prednisone 3 mg daily for the past few years. His other medications included aspirin, atorvastatin, metformin and saxagliptin. No history of diabetic neuropathy.

Personal and family history
He is a nonsmoker with no alcohol use. No family history of diabetes, gangrene or significant illnesses.

Physical examination upon admission
He was seen and evaluated in the emergency room twice in the following four months due to worsening symptoms and investigations were done. On exam during both times, he was afebrile, and physical exam was normal except for gangrenous changes tips of both middle fingers. There was no area of erythema around the region of gangrene on either side. Ankle brachial pressure index was normal and filling pressures were normal in both upper extremities.

Laboratory examinations
Blood counts, erythrocyte sedimentation rate, C reactive protein were within normal limits. Tests for vasculitis were negative including Anti-nuclear cytoplasmic antibody and anti-nuclear antibody.

Imaging examinations
Hand X-rays were normal. Echocardiogram showed no evidence of embolic sources.

FINAL DIAGNOSIS
Possible etiology was concluded to be from microvascular damage of unclear etiology.

TREATMENT
Plastic surgery, vascular surgery, dermatology and rheumatology referrals were completed. Biopsy was withheld as there was no surrounding erythema. Patient was seen in endocrinology outpatient for diabetes management and his endocrinologist suspected empagliflozin as a possible cause and discontinued the medication. He was switched to alternate medications for better glycemic control.

OUTCOME AND FOLLOW UP
After a week of stopping empagliflozin, patient started noticing improvement in his pain as well as slowing of blackish discoloration near tip of fingers.

DISCUSSION

Occurrence of finger gangrene or upper extremity gangrene in individuals with type 2 diabetes on treatment with empaglifozin has not been described previously in the literature. We suggest this adverse event could be under reported due to low index of suspicion.

Patient mentioned in this case presented with gangrene at the same site where he sustained minimal trauma initially, therefore the suspicion was more for vasculitis. But the patient had noticed that the sites were healing well initially. Starting of empagliflozin coincided with onset of symptoms of neuropathic pain and worsening of non-healing ulcers and development of gangrene tip of fingers and vasculitis markers were negative.

Even though this patient has polymyalgia rheumatica and was on prednisone at the time of symptoms, markers for vasculitis were negative and he was on consistent dose of low dose prednisone for few years before onset of symptoms. Addition of empagliflozin again was the only other contributing factor for development of symptoms.

The timing of empaglifozin and onset of symptoms as well as improvement after stopping empaglifozin point towards a likely association of the medication with finger gangrene.

CONCLUSION

This first case report of empaglifozin causing finger gangrene suggests the possibility that upper extremity gangrene with use of empaglifozin could go undiagnosed as occurred initially in this case. Prescribers need to be aware of this association and future studies are warranted to clarify if upper extremity ulcers or gangrene are associated with SGLT2 inhibitor use.

Increased awareness among primary care physicians and surgeons about this association could prevent progression of non-healing upper extremity ulcers, gangrene and resultant amputations.

REFERENCES

1 **Neal B**, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, Shaw W, Law G, Desai M, Matthews DR; CANVAS Program Collaborative Group. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N Engl J Med* 2017; **377**: 644-657 [PMID: 28605608 DOI: 10.1056/NEJMoa1611925]

2 **Zinman B**, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 2117-2128 [PMID: 26378978 DOI: 10.1056/NEJMoa1504720]

3 **Khouri C**, Cracowski JL, Roustit M. SGLT-2 inhibitors and the risk of lower-limb amputation: Is this a class effect? *Diabetes Obes Metab* 2018; **20**: 1531-1534 [PMID: 29430814 DOI: 10.1111/dom.13255]

4 **Fadini GP**, Bonora BM, Avogaro A. SGLT2 inhibitors and diabetic ketoacidosis: data from the FDA Adverse Event Reporting System. *Diabetologia* 2017; **60**: 1385-1389 [PMID: 28500396 DOI: 10.1007/s00125-017-4301-8]

5 **Kumar S**, Costello AJ, Colman PG. Fournier's gangrene in a man on empagliflozin for treatment of Type 2 diabetes. *Diabetic Medicine* 2017; 1646-1648 [DOI: 10.1111/dme.13508]

6 **Voelker R**. Rare Infection Linked With SGLT-2 Inhibitor Use. *JAMA* 2018; **320**: 1309 [PMID: 30285161 DOI: 10.1001/jama.2018.14128]

7 **Li D**, Wang T, Shen S, Fang Z, Dong Y, Tang H. Urinary tract and genital infections in patients with type 2 diabetes treated with sodium-glucose co-transporter 2 inhibitors: A meta-analysis of randomized controlled trials. *Diabetes Obes Metab* 2017; **19**: 348-355 [PMID: 27862830 DOI: 10.1111/dom.12825]

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