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REVIEW

Nutrition, insulin resistance and dysfunctional adipose tissue determine the different components of metabolic syndrome

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Abstract

Obesity is an excessive accumulation of body fat that may be harmful to health. Today, obesity is a major public health problem, affecting in greater or lesser proportion all demographic groups. Obesity is estimated by body mass index (BMI) in a clinical setting, but BMI reports neither body composition nor the location of excess body fat. Deaths from cardiovascular diseases, cancer and diabetes accounted for approximately 65% of all deaths, and adiposity and mainly abdominal adiposity are associated with all these disorders. Adipose tissue could expand to inflexibility levels. Then, adiposity is associated with a state of low-grade chronic inflammation, with increased tumor necrosis factor-α and interleukin-6 release, which interfere with adipose cell differentiation, and the action pattern of adiponectin and leptin until the adipose tissue begins to be dysfunctional. In this state the subject presents insulin resistance and hyperinsulinemia, probably the first step of a dysfunctional metabolic system. Subsequent to central obesity, insulin resistance, hyperglycemia, hypertriglyceridemia, hypoalphalipoproteinemia, hypertension and fatty liver are grouped in the so-called metabolic syndrome (MetS). In subjects with MetS an energy balance is critical to maintain a healthy body weight, mainly limiting the intake of high energy density foods (fat). However, high-carbohydrate rich (CHO) diets increase postprandial peaks of insulin and glucose. Triglyceride-rich lipoproteins are also increased, which interferes with reverse cholesterol transport lowering high-density lipoprotein cholesterol. In addition, CHO-rich diets could move fat from peripheral to central deposits and reduce adiponectin activity in peripheral adipose tissue. All these are improved with monounsaturated fatty acid-rich diets. Lastly, increased portions of ω-3 and ω-6 fatty acids also decrease triglyceride levels, and complement the healthy diet that is recommended in patients with MetS.

Key words: Obesity; Metabolic syndrome; Metabolism; Adipokines; Insulin resistance; Lipotoxicity and nutrition

Core tip: Central obesity, the insulin resistance, hyperglycemia, hypertriglyceridemia, hypoalphalipoproteinemia, hypertension and fatty liver are grouped in the so-called metabolic syndrome (MetS). In subjects with MetS an energy balance is critical to maintain a healthy body weight, mainly limiting the intake of high energy density foods. However, high-carbohydrate rich (CHO) diets increase postprandial peaks of insulin and glucose. Triglyceride-rich lipoproteins are also increased, which interferes with reverse cholesterol transport lowering high-density lipoprotein cholesterol. In addition, CHO-rich diets could move fat from peripheral to central deposits and reduce adiponectin activity in peripheral adipose tissue. All these are improved with monounsaturated fatty acid-rich diets. Lastly, increased portions of ω-3 and ω-6 fatty acids also decrease triglyceride levels, and complement the healthy diet that is recommended in patients with MetS.

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INTRODUCTION

Overweight and obesity are an excessive accumulation of body fat that may be harmful to health. Today, obesity is a major public health problem, affecting in greater or lesser proportion all demographic groups. Obesity is estimated by body mass index (BMI) in a clinical setting, but BMI reports neither body composition nor the location of excess body fat. People with normal weight but high body fat percentages could have a cardiovascular risk equal to that of people with obesity.

Deaths from cardiovascular diseases (CVD), cancer and diabetes accounted for approximately 65% of all deaths, and general adiposity and mainly abdominal adiposity are associated with increased risk of death for all these disorders. Adipose tissue could expand to levels of inflexibility. Then, adiposity is associated with a state of low-grade chronic inflammation, with increased tumor necrosis factor (TNF)-α and interleukin (IL)-6 release, which interfere with adipose cell differentiation, and the action pattern of adiponectin and leptin until the adipose tissue begins to be dysfunctional. In this state the subject presents insulin resistance (IR) and hyperinsulinemia, probably the first step of a dysfunctional metabolic system. Subsequent to central obesity, insulin resistance, hyperglycemia, hypertriglyceridemia, hypoalphalipoproteinemia, hypertension and fatty liver are grouped in the so-called metabolic syndrome (MetS).

In subjects with MetS an energy balance is critical to maintain a healthy body weight, mainly limiting high energy density foods. The first factor to be avoided in the prevention of MetS is obesity, and the percentage of fat in the diet has traditionally been associated with the development of obesity. However, it is well established that the type of fat consumed could be more decisive than the total amount of fat consumed when we only look at changes in body composition and distribution of adipose tissue. In addition, insulin resistance is a feature of MetS and is associated with other components of the syndrome. The beneficial impact of fat quality on insulin sensitivity (IS) was not seen in individuals with a high fat intake (> 37E%). Other dietary factors that can influence various components of MetS, like postprandial glycemic and insulin levels, triglycerides and high-density lipoprotein (HDL)-C levels, weight regulation and body composition, as well as fatty liver, are the glycemic load (GL) and the excess of fructose, and amount of dietary fiber content of food eaten. The increased levels of triglycerides associated with hypoalphalipoproteinemia are a feature of insulin resistance and MetS, and increase cardiovascular risk regardless of low-density lipoprotein (LDL) cholesterol levels.

High-carbohydrate rich (CHO) diets increase postprandial peaks of insulin and glucose. Triglyceride-rich lipoproteins are also increased, which interferes with reverse cholesterol transport lowering HDL cholesterol. In addition, CHO-rich diets could move fat from peripheral to central deposits and reduce adiponectin activity in peripheral adipose tissue. All these are improved with monounsaturated fatty acids (MUFA)-rich diets.

The American Diabetes Association (ADA) recommends an intake of dietary fiber of 20 to 35 g/d mainly because of the cholesterol-lowering and glucose-lowering effects of soluble fiber. However, more beneficial effects of a higher intake of dietary fiber, particularly of the soluble type, above the level recommended by the ADA, were reported to improve glycemic control, decreases hyperinsulinemia, and lower plasma lipid concentrations in patients with type 2 diabetes.

Lastly, the prevalence of enlarged waist circumference, hypertension and hypertriacylglycerolemia were reduced after the isoenergetic low fat high complex carbohydrates (LFHCC) supplemented with ω-3 diet. Thus, the prevalence of MetS fell by 20.5% after LFHCC ω-3 diet compared with the high saturated fatty acids (HSFA) (10.6%), high MUFA (HMUFA) (12%) or LFHCC (10.4%) diets. Therefore, increased fish intake instead of meat portions increases ω-3 fatty acids, and moderate portions of dried fruits (walnuts) increases ω-6, could complement the healthy diet that is recommended in patients with MetS.

In summary, an equilibrate calory diet, low in animal fat, sugar and fructose, high in MUFA and polyunsaturated fatty acids (PUFA), fresh vegetables high in fiber, and with moderate complex carbohydrates portions, could improve weight loss, lower postprandial glucose and insulin levels, and triglyceride levels could also decrease, and, eventually, increased HDL cholesterol levels are observed.

The maintenance of an ideal body weight, usually established between 18 and 25 years of age, requires achieving a life-long energy balance, where the amount of energy intake must equal the amount of energy expended. However, in the study of obesity in humans, if we look at only the imbalance between energy intake and energy expenditure, we have failed in its clinical application[1,2]. In humans, obesity depends on multiple factors apart from diet, like age and stage of development, genes and epigenetic factors, physical activity, environment, level of instruction and nutrition education, as well as several diseases that alter both physical and psychosocial interaction[3,4]. Therefore, the increase in overweight and obesity rates are classified as major public health issues, affecting in greater or lesser proportion all demographic groups, irrespective of age, sex, race, education or economic level[5]. World Health Organization (WHO) expects the 400 million obese adults worldwide registered in 2005[6] to double, and in the United States, obesity has been increasing in both adults and children in the last few years[7-9]. The age-standardized rate of death from any cause was generally lowest among subjects with an optimal BMI of 22.5 to 24.9 kg/m^2[10-12].

Recently, it has been observed that death attributed to factors related to high BMI is in fourth place behind deaths from high blood pressure, smoking, and unhealthy diets; and is ahead of deaths attributable to diabetes, physical inactivity, high salt intake, alcoholism and high blood cholesterol levels[13]. In addition, epidemiology studies have established associations between food and nutrient intake with specific diseases such as cancer, diabetes and CVD[14,15] as well as with obesity, body fat distribution, hypertension, insulin resistance and hyperglycemia[16-18].

Deaths from CVD, cancer and diabetes accounted for up to approximately 65% of all deaths, and general adiposity and central adiposity are related with increased risk of death for all these disorders, shortened life expectancy and causes disability in addition to high economic costs. Where levels of BMI are higher than 25 kg/m^2 a direct relationship with high mortality due to CVD is well established[3,19-23]. Cardiovascular disease accounts for approximately 38.5% of all deaths in EE.UU., although have declined substantially since the 1940s and 1960s[10]. This trend may be related with several primary prevention activities (for example, smoking cessation, sugar, trans fat and excess of saturated fat ingestion), improved treatment for ischemic acute phase and finally, improved secondary intervention (treatment of hypertension, hyperglycemia and hypercholesterolemia)[24,25]. The pattern of obesity may also influence this CVD risk and those with a waist-hip ratio higher to or equal than the average have in general an odds ratio of 3.0 (95%CI: 2.1-4.2) for ischemic cerebrovascular, even when BMI and other risk factors were adjusted[26]. Last, a weight loss of 10% maintained over time in obese subjects may decrease the expected events of coronary and stroke diseases[27].

On the other hand, concurrent with obesity rates during the 90s, there was an increase of diabetes to 61% in the United States (mainly approximately 90%-95% of type 2 diabetes, T2D)[28]. The mortality rate directly attributable to diabetes is about 3%, and diabetic patients have 2-4 times higher cardiovascular risk and many die of CVD[29]. Obesity and high body fat are related with diabetes in all ethnic groups. In the United States approximately the 70% of T2D prevalence could be attributed to overweight and obesity and, after 10 years, each kilogram gain from ideal body weight, raises the risk by 4.5%[10]. However, again "central obesity" is more strongly associated with metabolic complications linked to insulin resistance including diabetes[30,31]. For the prevention and treatment of T2D maintenance of a healthy body weight (BMI < 27-30 kg/m^2) plus physical activity, limit the intake of sugar and saturated fat, and increase the consumption of mono and PUFA, as well as whole grains and fiber[32-34], is recommended.

Finally, all cancers combined accounted for approximately 23% of the total number of deaths[10]. The relationship between BMI and a high mortality due to cancer in most specific sites[12,35] is well established. Obesity may account for up to 14% of cancer in men and up to 20% of cancer in women, and the risk of death from cancer in people with BMI $\geqslant$ 40 kg/m^2 increases up to 52% in men and 62% in women as compared with people with normal weight[36]. The underlying pathophysiological mechanisms that may be attributed to increase cancer rates are uncertain but can involve higher circulating levels of glucose, low-grade inflammatory state in many tissues, increased oxidative stress, as well as the bioavailability of hormones, mainly insulin, estrogens and androgens.

After obesity is developed most subjects present IR and hyperinsulinemia, probably the first step of a dysfunctional metabolic system. Subjects with more central obesity present a higher risk of IR, hyperglycemia, hypertriglyceridemia, hypoalphalipoproteinemia, hypertension and fatty liver, and different combination are grouped in so-called MetS. In subjects with MetS achieving an energy balance is critical to maintain a healthy body weight, limiting the consumption of food with high energy density (fat). However, high-carbohydrate rich (CHO) diets increase postprandial peaks of insulin and glucose, and triglyceride-rich lipoproteins are also increased, which interferes with reverse cholesterol transport lowering HDL cholesterol, and could deposit fat mainly in central deposits and reduce adiponectin activity in peripheral adipose tissue. However, all these were improved with MUFA-rich diets. In addition, food with high fiber content (vegetables and whole-grain) and food rich in ω-3 and ω-6 fatty acids could improve some components of this dysfunctional metabolic system.

The traditional Mediterranean diet is featured by a moderate to high ingestion of olive oil, a lower density of calories in the diet, legumes and vegetables, fruit, nuts, and whole cereals; a moderate to higher intake of poultry and fish; a moderate intake of dairy products, but more restrictive in higher caloric density foods such as red and processed meats, and sweets; finally, mainly red wine is drunk with meals[37]. Selected subjects at high cardiovascular risk, a Mediterranean style diet supplemented either with extra-virgin olive oil or nuts decrease the incidence of major cardiovascular events[38]. Finally, studies of healthy habits in the 50s[39] show that physical activity at work, walking and cycling as a means of transport all contributed to overall energy expenditure. However, these physical activities have decreased dramatically in societies today because of sedentary habits at work and in holiday life[40]. Thus, dietary habits, a major factor in controlling obesity, are made up of environmental, cultural, economic and technological aspects. These can be modified by agricultural policies that govern prices, extending the range and availability of food and regulating beneficial or harmful dietary components[41,42].

OBESITY ASSESSMENT

Obesity could be estimated only by measures of the body weight; however, relating body weight to height give us a more accurate measure of obesity[43]. The BMI or the Quetelet's index is the measure that is currently used in clinical setting to graduate from the normal weight to obesity in adults, and is estimated by the weight/height ratio squared, and expressed as kg/m^2. The approach taken by WHO is: (1) BMI between 18-25 kg/m^2 is considered normal weight; (2) BMI between 25-29.9 kg/m^2 is considered overweight; and (3) a BMI greater than or equal to 30 kg/m^2 is defined as obesity[44,45]. However, BMI does not gives us information about body composition and body fat distribution, neither about individual variations in terms of amounts of lean body mass (fat-free muscle mass), or the pattern of depot on body fat distribution. Thus, the percentage of body fat (BF%) is a better measure as it relates the ratio of total weight of fatty body weight. However, it is more difficult to measure BF% than single BMI, but several methods of varying accuracy and complexity exist[46]. In a clinical setting the most commonly used anthropometric indicator of body composition analysis involving two components (body fat and free-fat mass) are estimated from measurements of skinfold thicknesses, that should be measured in several regions, in order to obtain a clearer picture of fat composition[47]. In research, the percentage of body fat determined by hydrostatic weighing (body weight by immersion), is the gold standard[48]. In addition, the bioelectrical impedance analysis technique is also used to measure body composition, and using a four-terminal bioimpedance analyzer has a prediction error less or equal to the standard anthropometry for estimating body fat[49]. Therefore, it is possible to estimate the amount of body water and the proportion of fat-free mass and by subtracting body fat from total body weight[50]. Furthermore, a relatively simple technique to evaluate the total and regional adiposity in an individual involves a study of the whole body with a scan densitometer (dual energy X-ray absorptiometry, DEXA)[51,52].

People with normal weight but high body fat percentages could have a cardiovascular risk equal to that of people with obesity. The range of normal body fat is 2%-5% in men and 10%-13% in women, while the obesity range of body fat percentage is above of 25% in men and 32% in women[53,54]. Experimentally, it was observed that BMI = 30 kg/m^2 implies approximately 30% of BF% at 20 years of age but increase to 40% at 60 year in men, while in older women these values were to 40% and 50%, respectively. Therefore, body fat composition changes with age and sex. Body fat percentage for adults can be estimated from the BMI as follows: BF% = 1.2 × BMI + 0.23 × age - 5.4 - (10.8 × gender) (being 0 if gender is male and 1 if female; it differ for children). The correlation between BMI-BF% is r = 0.75 in male and r = 0.82 in females, for all ages[55].

On the other hand, BMI does not report on the location or distribution of excess body fat, it is to say about the distribution of body fat. Central obesity is characterized mainly by excess fat depot in the abdominal area and within the peritoneal cavity and lower expansion of peripheral adipose tissue. In a clinical setting, several parameters can be used to estimates central obesity; the most widely used being the perimeter of waist circumference (WC), hip ratio (HR) and waist-HR (WHR). Recently, the waist-to-height ratio, which relates waist circumference to height, has also been used to identify higher cardiometabolic risk in adults[56-58] and children[59,60]. This has advantages compared to the BMI, and even with WC and WHR, and a healthy individual should maintain a waist circumference to less than half their height[61]. All these parameters help to predict the risk of metabolic diseases such as T2D[62], and could be more effective in the case of CVD[63]. In addition, mortality due to any cause was increased with a BMI < 30 when the subjects have a large WC[64]. Thus, WC and WHR help to identify high-risk individuals regardless of their BMI[65]. The WC range that estimates mainly central adiposity varies with race and it is currently suggested that for individuals of the United States > 88 cm in women and > 102 cm in men; for the European Union ≥ 80 cm in women and ≥ 94 cm in men; for Chinese and South Asia > 90 cm and for Japanese > 85 cm for both women and men[66]. These assessments are used mainly in the clinic, but there are others more complex and more expensive techniques used in research, which are more accurate, such as DEXA, computed tomography (CT), and magnetic resonance imaging (MRI). Distribution of body fat is evaluated by DEXA by automatic scanning of default regions (arms, legs and trunk). The trunk is the area bounded by the horizontal line under the chin, side edges of the ribs and oblique lines through the femoral neck; and leg area includes the area under these oblique lines. This measure has a coefficient of variation of approximately 2%[51,52,67]. Central obesity is composed of abdominal subcutaneous fat and intraabdominal fat, as is seen by MRI and CT. In addition, intraabdominal adipose tissue is composed of visceral adipose tissue (VAT) as omental and mesenteric fat (intraperitoneal fat) and retroperitoneal fat mass[68]. Finally, single-voxel magnetic resonance spectroscopy is the gold-standard for ectopic fat quantification. Although very similar to MRI, it does not give anatomical information in image form, but gives information about the chemical composition as it is based on chemical shift. The water protons from (-OH) hydroxyl groups have a spectral peak at 4.7 ppm (parts-per-million). However, the triglycerides have the predominant protons from the $(-CH_2)n$ methylene groups[69,70]. Finally, ectopic fat is estimated with accurate methods that separate water and fat signals within each voxel (software such as jMRUI). Occasionally other techniques have been used in determining the ectopic fatty tissue including ultrasonography (US), with a highly significant correlation between CT and by US[71].

ADIPOCYTE AND ADIPOSITY DEVELOPMENT

Adipocyte differentiation

In humans there are two types of well-differentiated adipose tissue, which have different distribution and functions, and are referred to as white adipose tissue (WAT) and brown adipose tissue (BAT) (Figure 1A). The WAT is mainly related to the function of deposit of surplus energy as triacylglycerol (fat), which could be mobilized and offered through hormonal signaling and has a tremendous ability to expand; excess fat storage is associated with mechanical overload and slow to moderate increased risk of metabolic disorders. Mature WAT are characterized by the increased expression of transporters of glucose sensitive to insulin (GLUT4), and enzymes like fatty acid synthase (FAS) and glycerol-2-phosphate dehydrogenase[72,73]. By contrast, BAT is involved in thermogenesis functions and thus in energy expenditure and body weight regulation[74,75]. In mammals, BAT is the primary site of thermogenesis without accompanying muscle contraction. This function is stimulated by exposure to cold or after lipid-rich calorie food, and this process is called adaptive thermogenesis[76]. This thermogenic function of BAT is mediated by the activation of a specific mitochondrial uncoupling protein 1 (UCP1), which is ubiquitous in the inner mitochondrial membrane, uncoupling electron transport of mitochondrial respiration, where the saturation of the production of ATP is dissipated as heat (Figure 1B). The presence of functionally active BAT in rodents has been known for many years. In humans, the first evidence of BAT function was related to the control of body temperature after birth and in early childhood[77]. However, several data from adipose tissue samples together with evidence provided by positron emission tomography coupled with computed tomography have established the existence of functionally active brown adipose tissue in adult humans[78-81]. Furthermore, some of these studies have also related data between the size of activation of these sites with BAT and lower BMI, increased basal energy expenditure and decreased onset of diabetes[82]. Different amounts of BAT in adult humans can be found in the cervical and supraclavicular[83], and are known as canonical BAT. Although brown adipocytes are also observed infiltrating skeletal muscle and in different areas of WAT[84]. Therefore, a third fat cell or new functional adipose tissue is being defined[85,86].

Transcriptional signaling of adipocyte formation

Expansion of WAT in ideal weight or in obesity is not only the result of hypertrophy and/or hyperplasia of adipocytes, but supporting elements like vascular and mesenchymal stromal including immune cells, endothelial cells, and undifferentiated or adipocyte precursor cells (APs) must also be developed. Alterations in vascular tissue development and hypoxia is associated with adipocyte apoptosis and macrophage infiltration, and an appropriate induction of vascular endothelial growth factor A in adipose tissue is essential during expandability of adipose tissue (Figure 2)[87].

The hypertrophy of WAT only depends on its own renewal from APs which remain present during the entire life span and after suitable signaling can form different mature fat cells (Figure 1A)[88]. In WAT development several key transcription factors have been identified and among them the binding proteins CCAAT/enhancer (C/EBP) and peroxisome proliferator-activated receptor (PPAR) should be mentioned. Sterol regulatory element binding transcription factor 1 (SREBP1c) has been found as a pro-adipogenic basic helix-loop-helix transcription factor which activates peroxisome proliferator-activated receptor-γ (PPAR-γ) expression[89] and mediates the induction of lipid biosynthesis by insulin[90]. On the other hand, BAT derived from Myf5 + progenitors paraxial mesoderm layer shares a common origin with the development of skeletal myoblasts[91]. The development of BAT requires that PRDM16 interacts with either PPAR-γ coactivator (PGC-1α/β) or CtBPs to activate brown genes or the inhibition of several transcription factors that induce WAT, respectively[92,93]. In addition, it has been shown that bone morphogenetic protein 7 turn on a complete program of brown adipogenesis involving induction of early key regulatory transcription to brown cells as PRDM16 and PGC-1α, and increased expression of UCP-1 which is characteristic of brown cells[94]. Finally, Myf5 was found to drive the expression of classical BAT depots in retroperitoneal and anterior subcutaneous WATs, and the existence of Myf5 positive cells mixed in WAT has been confirmed[95]. The term "beige" has been used to describe those cells that are morphologically identical to white adipocytes, but may be inducible to cells expressing brown adipocytes definitive characteristics of UCP1 activity with β-adrenergic stimulation[96,97]. Adipose tissue located in the inguinal area is seen today as the largest and physiologically

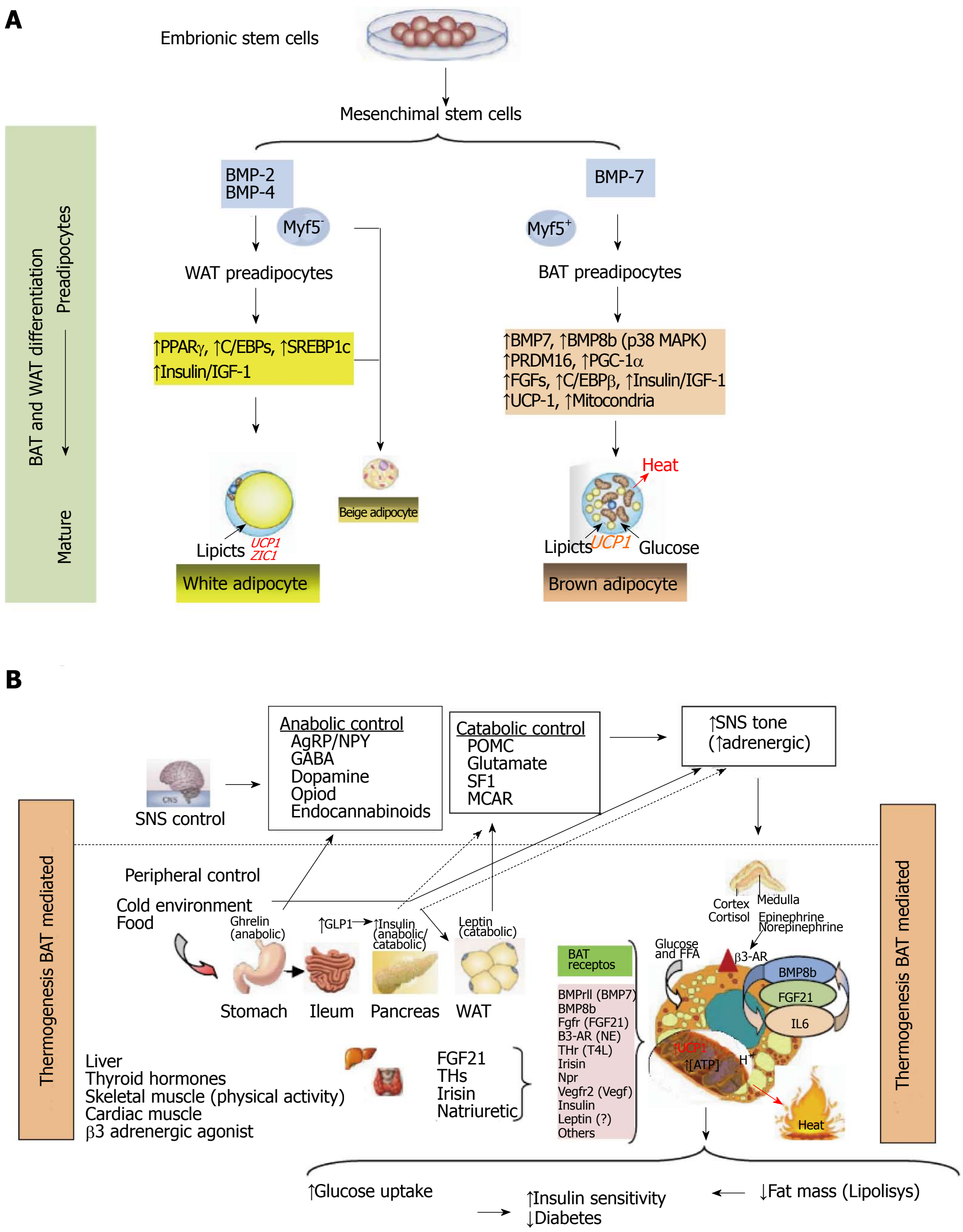

Figure 1 Thermogenesis brown adipose tissue (Bat) mediated. A: Adipocytes were developed because non adipocytes cells are unable to store calories as fat to meet fuel needs during long periods without eating. If the energy intake is more than energy expenditure, WAT is expanded and leads to obesity. However, a second type of adipose tissue, called BAT was developed especially for energy expenditure (thermogenesis). Today, research in identifying the main genes that control differentiation, development and activation of BAT is highly active, because, activation of BAT, in detriment of WAT, could have anti-obesity effects, which can be utilized to keep the system of fat deposit balanced. In this research, PRDM16, PPAR-γ and PGC-1α, have been identified as the key nodes in the regulation of inducible BAT; B: The thermogenic potential of BAT is controlled by the SNS, which densely innervates brown fat depots. In addition, BAT is activated in response to cold temperatures, hormones and possibly diet. BAT content and activation is highest in children and decreases with age. BAT activation is decreased in fatness, and BAT activity has been inversely correlated to BMI, body fat, and visceral obesity. In humans, BAT amount and activation is higher in women than in men. Of clinical relevance, BAT activation is very low in diabetic patients in comparison with non-diabetic subjects. Thyroid hormones play a main role in control of BAT activation, therefore the cold-induced enhancement of the enzyme 5'-deiodinase type II activity, which deiodinates thyroxine (T4) to T3. Catecholamines such as norepinephrine binds to β-ARs and induce PGC1α through p38 MAPK and finally triggers expression of UCP1. Whereas β1-AR is considered important for proliferation of classical brown adipocyte precursors in response to norepinephrine, β3-AR plays a major role in thermogenic function of mature brown adipocytes. Another signal, Irisin hormone which comes from muscle to fat tissue, is able to induce a robust browning programme, and mediates the beneficial effects of exercise and could reduce diet-induced obesity and insulin resistance. A more generalized program in the control of adipose tissue is conducted by FGF21 through regulating lipolysis in WAT as well as increasing substrate utilization by increasing fatty acid oxidation in the liver. Last, beige fat cell functions include either a like to "WAT" when energy balance is exceeded, or a like to "BAT" in response to many stimuli similar to BAT activation. WAT: White adipose tissue; BAT: Brown adipose tissue; PRDM16: PR domain containing 16; PPAR-γ: Peroxisome proliferator-activated receptor-γ; PGC-1α: Peroxisome proliferator-activated receptor γ coactivator 1α; SNS: Sympathetic nervous system; BMI: Body mass index; FGF21: Fibroblast growth factor 21.

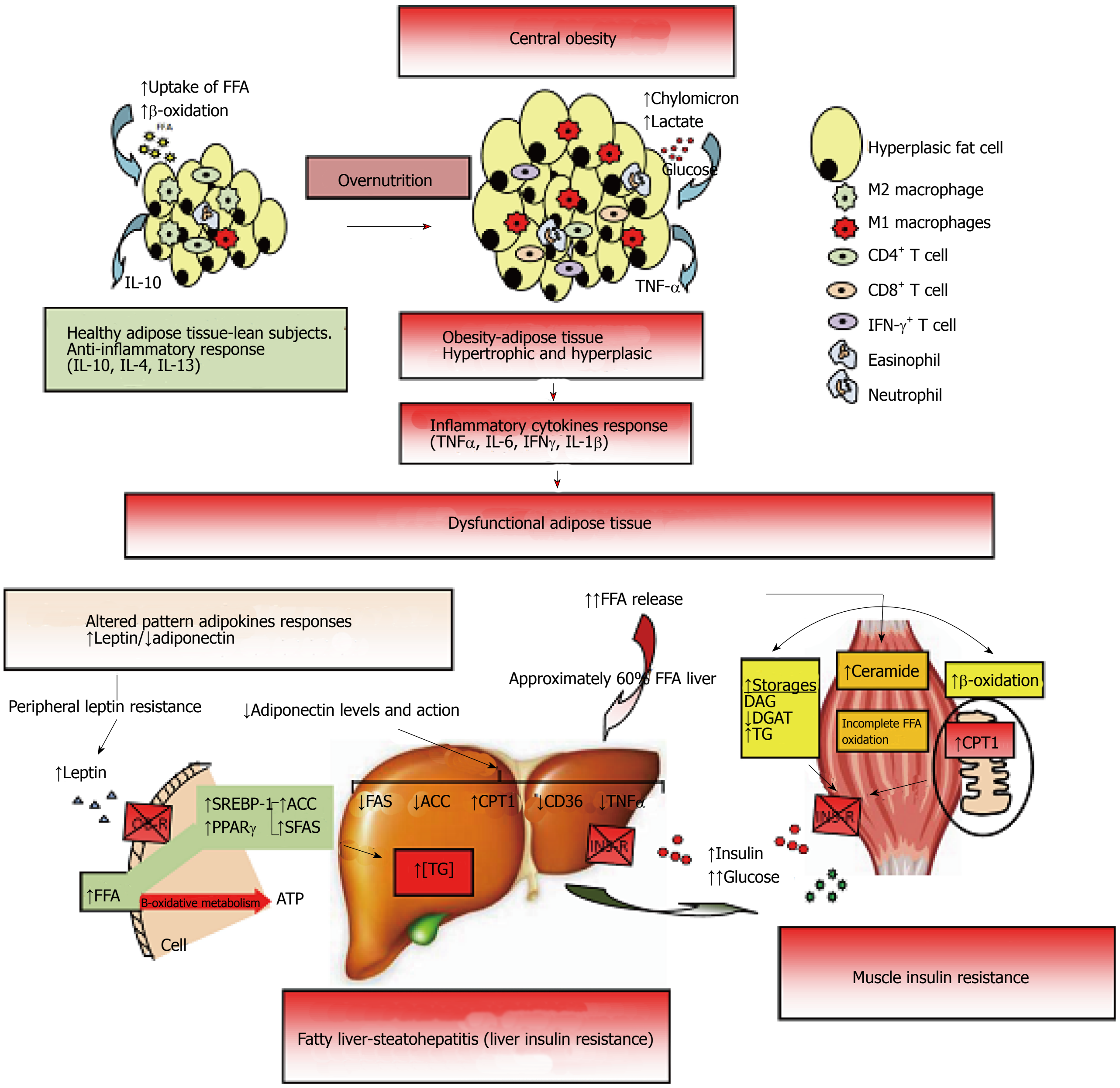

Figure 2 Dysfunctional adipose tissue. Early central obesity is associated with a low-grade chronic inflammatory state characterized by slow infiltration of macrophages which are an important source of inflammation of this adipose tissue[275,276]. Several macrophage subtypes can be found, and simply put, are divided in pro-inflammatory M1 or alternatively activated M2, although *in vivo* studies reveal a spectrum of macrophage phenotypes[277]. Adipocytes and immune cells such as T cells and macrophages participate in the activation and production of inflammatory cytokines[170,275,278,279]. The M1 macrophages mainly found in obesity, are induced from precursor M0 macrophages by stimulation of components of bacteria (lipopolysaccharide) and type 1 T-helper (Th1) inflammatory cytokines like IFN-γ and TNF-α. The M2 macrophages are activated by type 2 (Th2) cytokines such as IL-4 and IL-13. The M2 macrophages are abundant in adipose tissue of lean subjects and appear to be involved in remodeling, tissue repair, and maintenance of insulin sensitivity through the production and expression of IL-10, IL-1 receptor antagonist, and arginase-1. Whereas M1 macrophages use glucose for energy, M2 macrophages activate the β-oxidation of fatty acids[277,280]. Finally, M1 macrophages are the major source of inflammatory cytokines including TNF-α which inhibits adipose cell differentiation by activating Wnt signaling and suppressing expression of PPAR-γ transcription factor essential for the development and function of adipocyte, and reducing the effect on stored triglycerides[281,282]. The subcutaneous adipose tissue will continue to expand to an equilibrium point. When this capacity is exceeded, glucose and lipid uptake begins to decline and insulin levels are raised to maintain serum glucose in the normal range[215]. In addition, when WAT is unable to expand (inflexibility), associated with insulin resistance state, a continuous release of FFA to interstice begins, generating a systemic lipotoxic effect in muscle, liver, *etc.*, (lipotoxicity). The adipose tissue itself begins a slow process of low-level chronic inflammation (macrophages, lymphocytes, *etc.*) which increases local release of TNF-α and IL-6[166]. TNF-α and IL-6 levels are inversely related with peripheral and hepatic glucose-uptake which is insulin-mediated[283]. The liver keeps excess uptake of FFA in serum to capacity by joining with glycerol (TAG) and slowly fatty liver is developed (NAFLD). It has been shown that peripheral fatty acids contribute approximately 60% of total TAG stored in the liver, whereas the novo lipogenesis in the liver is approximately 26% and approximately 15% is from the diet[284]. On the other hand, leptin levels respond directly to adipose expansion, while adiponectin levels tend to decrease when metabolic syndrome is developed. The elevated leptin levels should increase lipolysis in non-adipose tissues, decreasing excess fatty acids in these cells. However, this action of leptin may be partially blocked by the anabolic effect established by hyperinsulinemia, settling down leptin system dysfunction (peripheral leptin resistance)[115]. In addition, the decreased adiponectin levels are inversely related to peripheral glucose uptake and directly related with progressive development of chronic liver disease by fat infiltration. Adiponectin exerts a protective action on liver fat accumulation, favoring lipolysis by promoting the action of CPT-1, while interfering with the action of FAS, ACO and TNF-α, and decreasing the expression and action of CD-36 protein that promotes the transport of fatty acids[129]. Finally, both leptin and adiponectin seem to regulate the deposition of fat in insulin-sensitive tissues by increasing fat oxidation. IFN-γ: Interferon-γ; TNF-α: Tumor necrosis factor-α; IL: Interleukin; PPAR-γ: Peroxisome proliferator-activated receptor-γ; WAT: White adipose tissue; FFA: Free fatty acids; NAFLD: Non alcoholic fatty liver disease; CPT-1: Carnitine palmitoyltransferase-1; FAS: Fatty acid synthase; ACO: Acyl CoA carboxylase.

most relevant fat depot capable of inducing brought beige adipocytes[96]. In addition, it has been observed that in this fat depot the beige mature adipocytes can be interconvert in adipocytes with characteristics typical of white and brown adipocytes, without the need for "*de novo*" cell differentiation from precursors[97]. Thus, physiologically this could mean that the rate of lipid storage or lipid oxidation could be adapted and adjusted in response to external stimuli such as a decrease or increase in temperature, but it still requires further investigation.

EFFECT OF HORMONES AND ADIPOKINES ON ADIPOGENESIS

The adipose tissue can be expanded and developed by many factors such as hormones, growth factors, factors produced by adipose tissue itself (adipokines) and specific effects induced by nutritional factors and some pharmacological components (Figure 1B).

Hormones and growth factors

Insulin: In "*in vitro*" studies, a mixture of dexamethasone, isobutylmethylxanthine and insulin is regularly used to generate well-differentiated adipose tissue, insulin being the most potent of the three factors. Insulin within the physiological range induces lipogenesis and insulin receptor is required for adipocyte differentiation[98]. Insulin regulates brown preadipocyte determination through a necdin-E2F4 interaction that represses PPAR-γ transcription *via* a cyclic AMP response element binding protein-dependent pathway[99]. Hyperinsulinemia either undergone exogenously (treatment) or endogenously (secretion), is clearly related with weight gain, which is a feature of the MetS. However, several molecules such as TNF-α, leptin, resistin, interact and block multiple steps of insulin signaling and antagonize its effects on adipocytes.

Growth hormone and insulin like growth factor 1: Growth hormone (GH) is not only involved in postnatal somatic growth to adulthood, but also has a role in the regulation of metabolic substrates in the control of body composition and body fat distribution, through the combination of lipolytic and anabolic effects[100]. In fact, patients with GH deficiency have a smaller number of adipocytes which also has less volume, and these are partially normalized with GH replacement therapy[101]. GH is involved in the conversion of preadipocytes into mature adipocytes, and subsequently plays a role in the maturation of adipocytes which makes them sensitive to insulin and IGF-I[102]. The effect of GH on adipogenesis seems mainly mediated *via* stimulating Stat5A/5B inducing the transcriptional activity of PPAR in cooperation with C/EBPb/δ[103].

Thyroid hormones: Thyroid hormones are involved in the growth and maturation of several organs and tissues during fetal and neonatal development[104]. Finally, in adult life, thyroid hormones regulate energy metabolism and function of organs such as the adipose tissue, liver, heart, skin tissue, muscle or adipose tissue. It has been observed that thyroid function in BAT is mediated by the C/EBPs signaling which induces the expression of thyroid hormone receptor and PGC1α (PPAR-γ coactivator) and deiodinase (D2) activity determines grade of thyroid function "*in situ*"[105,106].

Glucocorticoids and sexual hormones: In humans, infusion of hydrocortisone for 6 h increased levels of circulating FFA, and several mechanisms for the lipolysis of glucocorticoids have been observed[107,108]. In addition, dexamethasone is involved in the expression of PPAR-γ transcription factors and C/EBPδ, and decreases the expression of pref-1 which is a negative regulator of adipogenesis[109]. Therefore, the central obesity phenotype is associated mainly with the consumption of peripheral adipose tissue (lipolysis), and it is observed in human hypercortisolism situations as in Cushing's syndrome. The adrenal glands and gonads are the main primary source of serum levels of steroid hormones. However, adipose tissue has a full arsenal of enzymes that induce, interconvert, and inactivate peripheral steroid sex hormones[110]. The regulation of glucocorticoids levels is critical for the maintenance of homeostasis and the activity in some tissues of 11-β-hydroxysteroid dehydrogenase 1 and 2 (11 βHSD1 and 2) interconvert the active form of cortisol in other inactive product called cortisone and *vice versa*[111]. This enzyme is highly expressed in adipose tissue and an increase in its activity seems involved in an increased level of visceral adipose tissue[112,113]. Moreover, the distribution of body fat is characteristically different between men and women; while they are sexually active, resulting in so-called "android or apple" obesity with abdominal fat depot and "gynoid or pear" obesity where fat accumulates predominately in the buttock. However, the actions that sex steroids have on adipogenesis are poorly known. In addition, the main determinants of the action of sex steroids is given by free circulating levels of the hormone in question and the degree of expression in the target organ receptors. The prereceptor tissue-specific metabolism of steroid hormones is also involved in its function. Adipose tissue and preadipocytes have a great activity either cytochrome P450-dependent aromatase and 17βHSD enzymes. Aromatase regulate the rate of formation of androgens into estrogens: Androstenedione to estrone and testosterone to estradiol. Whereas, the 17βHSD is involved in the production of more active forms of testosterone and androstendiona from their weaker precursors, and the rate 17B-HSD/aromatasa in adipose tissue is correlated positively with central adiposity[110,114]. Finally, many men with insulin resistance, T2D or MetS present low testosterone concentrations with high or low gonadotropins (25% and 4%, respectively).

Adipokines: The developed and mature adipocyte acquires the ability to synthesize and release many proteins, known generally by Adipokines. These proteins and hormones are involved in energy homeostasis by regulating energy intake and basal metabolism. Therefore, adipose tissue is implicated in the metabolic control of energy substrates such as glucose and lipids, and interacts with several hormonal systems. The molecules produced by adipose tissue act remotely (endocrine) and locally as paracrine and autocrine on stroma and other components of the adipose tissue (blood vessels, inflammatory cells, *etc.*) and also other tissues such as muscle. All these actions will contribute in the regulation of the different adipose tissue depots, for expanding the size of peripheral adipose tissue or in fat redistribution to other depots.

In obese and insulin resistant patients increased levels of some adipokines (*e.g.*, leptin, resistin) are often observed while others such as adiponectin levels are typically decreased[115] (Figure 2).

Major adipokines

Leptin: Leptin is specifically secreted by fat cells whose primary function assigned was to establish an adiposity signal between the amount of developed adipose tissue and satiety centers in the brain completing a negative feedback loop[116,117]. People who lose weight following a low calorie diet usually decrease circulating leptin levels. This decrease in leptin appears to mediate reversible decrease in thyroid activity, sympathetic tone, and a decrease in basal energy expenditure[118]. Treating leptin deficiency with recombinant leptin reduces food intake and body weight[119]. Therefore, in subjects with very low levels of serum leptin, the recombinant leptin treatment also improved several abnormalities including infertility, lipodystrophy and impaired glucose metabolism and impaired immunity[120-123]. The expression and release of leptin is controlled by several hormones and factors. Therefore, appears to be stimulated by insulin, glucocorticoids, TNF-α, estrogens, and C/EBPA; by contrast, is decreased by androgens, β3-adrenergic activity, GH, free fatty acids, and PPAR-γ agonist[124]. The action of leptin is essential for energy metabolism, but is also involved in the mobilization of lipids from different fat depots and may be related to the protection of some tissues on lipotoxicity syndrome[125,126]. Thus, lipid oxidation in cells that have this capacity (mitochondria) could be increased through the signal of leptin and could reduce excessive fatty acids and protect against lipotoxicity in the liver, pancreas, heart, kidney, and muscle tissue (Figures 2 and 3).

Adiponectin: Adiponectin is produced specifically in mature adipocytes and RNA abundance is higher in peripheral adipose tissue compared with visceral adipose tissue[127]. Adiponectin receptors are G protein-coupled and have high expression in muscle and liver. Adiponectin is involved in lipid oxidation in skeletal muscle and in the liver, and moreover reduce hepatic production glucose load and postprandial hyperglycemic[128,129]. An inverse relationship has been found between plasma adiponectin levels and the development of obesity, insulin resistance and T2D[130]. However, conflicting data have been observed between adiponectin levels and the development of cardiovascular disease[131]. Adiponectin treatment decreases TNF-a plasma levels and its hepatic production. Adiponectin was able of improving hepatomegaly, steatosis, and alanine aminotransferase levels related with nonalcoholic obese subjects (Figure 2)[129]. Finally, adiponectin levels is early decreased in insulin resistance syndrome, even before the onset of obesity, and adiponectin administration improves IS[132].

TNF-α: TNF-α is a transmembrane protein released mostly by activated macrophages, and also by several other cell types including lymphoid cells, cardiac myocytes, endothelial cells, adipose tissue, *etc.*[133-135] (Figure 2). Therefore, TNF-α is regarded as an adipokine implicated in process of local and systemic inflammation and in proliferation and differentiation of the cells. TNF-α exerts its effects by binding two receptors, TNFR1 (TNF type 1 or CD120a) and TNFR2 (TNF type 2 or CD120b)[136]. Both *TNF-α* gene and its receptors are expressed and modulated in adipocytes and is expressed at higher levels in WAT[127]. Some metabolic effects induced by TNF-α implicates it in inhibiting differentiation to mature adipocyte. This in turn leads to insulin resistance, and finally an increase of free fatty acids could result[137,138]. In this way, TNF-α treatment decreased the expression of PPAR-γ and repressed genes involved in lipid and glucose uptake[138,139].

IL-6: IL-6 is secreted by T cells and macrophages involved in the immune response (Figure 2). Smooth muscle cells in blood vessels can also produce IL-6 as a pro-inflammatory cytokine. Finally, IL-6 is synthesized by adipocytes and appears to be associated with elevated levels of CRP and inflammatory states found in obese patients[140]. An important part of the total concentration of IL-6 (approximately 1/3) is produced in adipose tissue. However, the expression and release of IL-6 is two to three times higher in visceral adipose tissue compared to peripheral adipose tissue[127]. Finally, circulating levels of IL-6 have been found to be directly linked to both obesity and insulin resistance[141]. IL-6 inhibits the activity of lipoprotein lipase (LPL) and reduces the differentiation of human preadipocytes, both associated with adipogenesis[142].

Others main adipokines

Resistin: Resistin is a cytokine whose role is not well defined, although firstly was related to obesity, insulin resistance and development of T2D[143].

Visfatin: Visfatin is mainly synthesized in the abdominal adipose tissue of humans but not by peripheral adipose tissue, and the first role appeared to have insulin-mimetic actions[144,145]. However, the relevance of visfatin

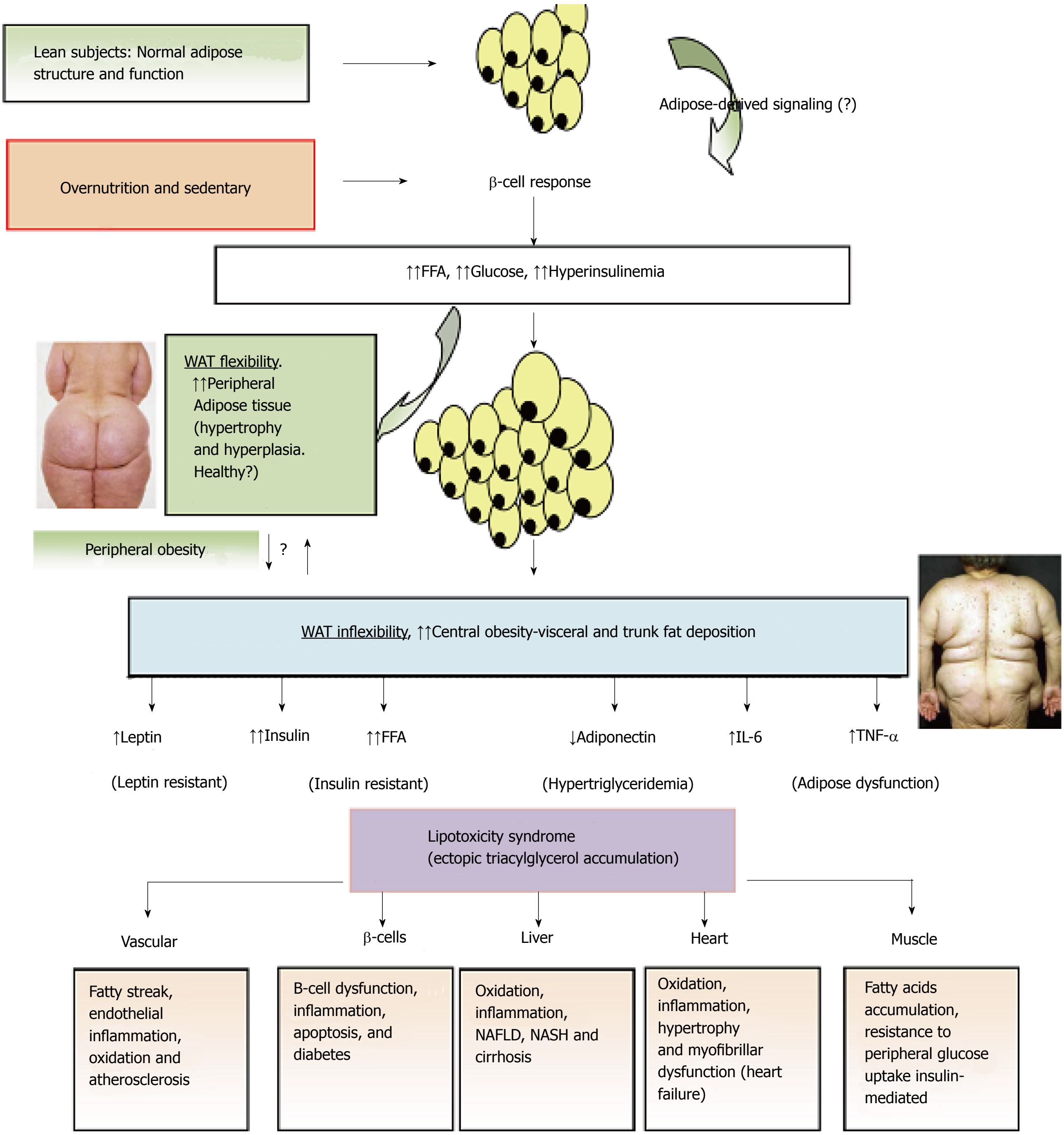

Figure 3 Adipose tissue expandability and metabolic syndrome. After a long period of overeating with positive energy balance, associated with increased hormones such as insulin, adipose tissue responds by increasing its storage capacity, which is determined by a number of factors. Individuals with a higher capacity for storing fat, mainly when peripheral WAT is expanded (WAT flexibility), most subjects will remain metabolically normal for a longer period, despite obesity developing. These subjects are observed to be metabolically healthy (MHO). Chronic inflammatory response leads to dysfunctional adipose tissue with increased local and endocrine secretion of acute phase reactants and inflammatory signaling pathways[285]. Abnormal cytokine and adipokines production is related to insulin resistance, hyperglycemia, altered lipid profile and cardiovascular diseases[115,286,287]. Insulin resistance slowly results from increased accumulation of lipids in other nonadipose tissues such as muscle (lipotoxicity) due to enhanced release of fatty acids from hypertrophic and hyperplasic adipocyte cells. In addition, when adipocytes achieve their maximal storage capacity, they begin to alter their adipokynes secretion profile. Therefore, a proinflammatory milieu with elevation in IL-6 and TNF-α and altered adipokines profile, with decreased adiponectin and increased leptin levels, with peripheral leptin resistance, in a dysfunctional adipose system is observed. This suggests that the limitation in storage capacity could be necessary and even precedes the development of metabolic factors. Ectopic lipid accumulation in non-adipocyte cells causes lipotoxicity in these organs and tissues, including inflammation and finally apoptosis. Thus, lipotoxicity in β-cell could decrease beta cell mass (dysfunction of β-cell secretion) and would cause diabetes. Increased fat in liver leads to hepatic steatosis (NAFLD) and steatohepatitis (NASH) and would cause hepatic dysfunction, in the heart would cause myocardiac dysfunction, in the endothelial fatty streak would be precursor of generalized arteriosclerosis, *etc*. At what point the adipose tissue begins to fail is likely to be determined by genetic and epigenetic factors. However, the question is: Can storage capacity in WAT be enhanced to meet an increased demand[288]? So far, in human trials, the PPAR-γ agonists (TZDs), that remove fat from central deposits toward more favorable peripheral deposits, have been shown to improve lipid profile, insulin-sensitivity, and reduce diabetes and NAFLD[269]. WAT: White adipose tissue; MHO: Metabolically healthy obese; IL: Interleukin; TNF-α: Tumor necrosis factor-α; NAFLD: Non alcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; PPAR-γ: Peroxisome proliferator-activated receptor-γ; TZD: Thiazolidinedione.

in the regulation of glucose metabolism is not clear[146].

Omentin 1: Plasma levels and omentin gene expression in visceral adipose are decreased in obesity[147]. Omentin 1 is decreased in obese women with polycystic ovary syndrome (PCOS), both glucose and insulin negatively regulate omentin-1 levels *ex vivo* and *in vivo*, and women with PCOS who were treated with metformin increased serum omentin levels[148,149].

Effect of fatty acid metabolism and enzymes on adipogenesis

Fatty acids (FFA) are energy-rich molecules that play a role in metabolism. The excess of calories ingested as fat, protein and carbohydrates, and unspent, are stored as triglycerides (TG; FFA plus glycerol) in mature white adipocytes. They are also an integral part of the cell membrane, conferring functions in fluidity and in the expression of receptors and transporters. In addition, FFA have hormone-like actions and can influence gene expression in preadipocytes, affecting adipogenesis through proliferation and differentiation[150]. In humans, food is an important source of FFAs, but biosynthesis could supply most of the fatty acids requirements[151]. However, humans are unable to synthesize certain PUFA. Therefore, some precursor in the diet are essentials for two series of PUFA, linoleic acid series (ω-6 series) and linolenic acid (ω-3 series), that are related with decreased CVD. Today, most diets in the world provide enough ω-6 and too little ω-3, with an increased ratio ω-6: ω-3. By contrast, diets with excess saturated fatty acids (and unsaturated trans) have been associated with a significantly increased risk of CVD.

In differentiation and maturation of adipocytes, insulin has a definitive influence increasing the expression and activity of LPL, which is needed for an effective FFA uptake and storage. Adipocytes release and express apo CII and apo CIII by regulating extracellular LPL activity[152]. In addition, fatty acid binding proteins (FABPs) are cytoplasmic proteins that carry out intracellular transport of FFA[153]. It appears that the expression of fatty acid binding protein-4 (FABP4) is involved in the balance between lipogenesis and lipolysis and in the process of differentiation of preadipocytes. Therefore, it is likely that FABPs serve as a critical link between lipid metabolism, hormone action and cellular function in adipocytes and other cells and thus contribute to systemic energy homeostasis involving glucose metabolism[154].

In humans, "*de novo*" synthesis of straight-chain fatty acids is formed predominantly in the liver where acetyl-CoA is formed from pyruvate, and to a lesser extent in adipose tissue. FFA can be endogenously synthesized from acetyl-CoA and malonyl-CoA precursors through two enzymatic steps, including acetyl-CoA carboxylase (ACC) and FAS. The ACC controls six recurring reactions until production of short fatty acids and then the fatty acids are elongated until 16-carbon palmitic acid is produced by the action of FAS (Citosol). Humans can synthesize nearly all fatty acids required from palmitic acid by combining several mechanisms of oxidation and elongation[155]. In mammals seven Elovl family enzymes (Elovl1-7) have been identified, and these enzymes are the limitations in control of production by fatty acid elongation[156]. The enzyme activity of Elovl3 is transcriptionally regulated by PPAR-γ, and in turn the levels of VLCFAs (C18: 1 and C20: 1) produced by the expression of Elov13 activate PPARγ. Therefore Elovl3-PPAR activity is implicated in the regulation of adipogenesis[157]. Saturated fatty acids are amply available from the food by humans, thus FAS enzyme has been shown to have less importance. However, the malonyl-CoA levels are determined by the rate of synthesis by ACC and FAS-mediated catabolic rate, and appear to be an important energy status sensor in the hypothalamus in the metabolic control of body weight[158]. Moreover, in the process of differentiation of preadipocytes to mature adipocytes a lower activity of FAS has effects reducing adipose tissue[159]. Finally, in the process of synthesis of triglycerides in adipose cells, several enzymes have been observed with an interest in adipogenesis[160]. The levels of mRNA and protein of diacylglycerol acyltransferase 1 (DGAT1) increase during the process of differentiation of preadipocytes. DGAT1-deficient mice are resistant to diet-induced obesity associated with a higher energy expenditure. While over-expression of DGAT1 resulting in increased adipose tissue without affecting IS, but increased the secretion of TNF, which interferes with insulin signaling[161].

OBESITY AND LIPOTOXICITY SYNDROME

After absorption in intestine and after synthesis in the liver triglycerides (TG) are packed in specialized lipoproteins [chylomicron and very like density lipoprotein (VLDL)]. They are transported in a network between different locations such as the digestive system, liver, adipose tissue and other tissues. The formation of TG can also be considered a cellular detoxification process by controlling the levels of diacylglycerol and the input and output flows of FFA and acyl-CoA[162]. In this regard, droplets containing TG were found in all investigated cells, and even brain tissue has this capacity to form TG. These fat droplets are surrounded by a monolayer of phospholipids hooked by a specific protein called Perilipin (ADRP) which appear to regulate, and are rate limiting factor in its formation, growth and dissolution[163].

Downloading and uptake of free fatty acids in non adipose tissues typically is coupled to its necessity. During periods of fasting and physical exercise should be increased the lipolysis, that is mediated by suppression of plasma insulin and elevation of contrainsulin hormones (glucagon, cortisol, *etc*.), generating a coupled fuel delivery. Thus, for an optimal mobilization and storage of lipid an efficient adipose tissue is required. By contrast, after a prolonged overfeeding state, fatty acid load offered may exceed the storage capacity of adipose tissue (inflexibility) (Figure 3). Nuclear receptor PPAR-γ is a key gene that regulates adipogenesis and lipid storage, but it appears that is also needed for the control of the lipolysis, dysregulation of which is a prominent characteristic of obesity-induced insulin resistance in humans[164]. In addition, the expression of leptin receptor is found in several tissues in the body involving leptin actions in many different sites, including as be a mediator of energy expenditure[124]. Leptin secretion rises in parallel with fat expansion in adipocytes and it has been proposed that this prevents lipotoxicity by minimizing ectopic accumulation of lipids into nonadipocytes because leptin induced β-oxidation increasing transcription of PPAR-α. Therefore, excess fatty acids will increase activation of PPAR-α which is a transcription factor of lipolytic enzymes such as carnitine palmitoyl transferase-1 and acyl CoA oxidase. Lipolysis is forced by increasing β-oxidation and uncoupling proteins activity, which corresponds with the observed increase in heat and finally would protect these tissues from the accumulation of fatty acids[165,166]. However, although insulin treatment acutely increases leptin levels, it has been observed that patients with insulin resistance syndrome have lower mRNA leptin abundance in adipocytes than IS patients[115,167]. In addition, a leptin resistance syndrome in humans for central hypothalamic action has also been found. Finally, this system of chronic increase of β-oxidation can already generate oxidative stress "*per se*" and an inflammatory condition, which can be harmful to these tissues. On the other hand, adiponectin have a key role like insulinsensitizing, anti-inflammatory, anti-apoptotic and pro-angiogenic properties increasing the metabolic flexibility of adipose tissue, *i.e.*, to make adipose tissue more efficient at discharging FFAs when are required and upgrade the rate of FFA re-esterification during the postprandial state[168]. Finally, in insulin resistant patients early lower serum adiponectin levels that could not adequately prevent all these processes are observed[115]. When these mechanisms are exceeded, an accumulation of fatty acids occurs, and its derived metabolites, which generate lipotoxicity and increased cell death in those tissue not prepared to accumulate this excess of lipids such as muscle, β-cells pancreatic, liver, heart, kidneys, *etc.*[126].

FROM INSULIN RESISTANCE TO OTHER CARDIOVASCULAR RISK FACTORS

In conditions of overnutrition the adipose tissue (AT) expands to levels of inflexibility (adiposity), and in this state the subject presents a longer postprandial state which leads to hyperinsulinemia, probably the first step in this altered dysfunctional metabolic system (Figure 4). Thus, a lower capacity of disposal and storage of fatty acids associated with an increased lipolysis by AT, and dysfunctional pattern of adipocytokine release (*e.g.*, decreased adiponectin, and increased leptin, TNF-α and IL-6), may result in inflexibility of AT and indirectly induce redistribution of fat towards undesired and toxic lipids ectopic accumulation. Therefore, when central obesity is slowly being developed, it is observed that hyperinsulinemia and hyperglycemia also progress slowly in postprandial state and later a global hyperglycemia (T2D), hypertriglyceridemia, hypoalphalipoproteinemia, hypertension and fatty liver (dysfunctional metabolism) are developed. When a combination of any of these factors cluster together in the same individual the concept of MetS is established[169].

The elevated levels of TG are directed toward white adipose tissue and changes occur in adipocyte size, which leads to changes in its function, and an increase in secretion of TNF-α and Leptin, which stimulates the secretion of monocyte chemotactic protein (MCP-1)[170]. This attracts more macrophages to the adipose tissue. Increasing leptin secretion also stimulates macrophage transport to adipose tissue[171] and macrophage adhesion to endothelial cells[172]. Whatever the stimulus for attracting these macrophages, once present and the recruitment is active, the cytokine production of these macrophages interfere with the normal function of adipocytes (adipose tissue dysfunction)[173]. When an inflammatory environment is established in the adipose tissue, the lipid metabolism is altered, initiating postprandial hypertriglyceridemia, because the liver overproduction of VLDL is not removed in time and remains for longer in plasma (postprandial hyperlipidemia). Further, because lipolysis from peripheral adipose tissue is extended, the interstitial content of free fatty acids increases, which can be taken up by the adjacent muscle cells (↓ IS) or again transferred into lipoproteins to the plasma and could be taken up by the liver (↑ VLDL production) and other organs (lipotoxicity). However, not all obese individuals necessarily develop metabolic complications, as some remain insulin sensitive and do not develop fatty liver[115]. On top of all these factors, the link between obesity and associated metabolic abnormalities seems to be better related to the topography, anatomical distribution and/or the functional peculiarities of the adipose tissue, a phenomenon which seems to be more relevant in patients with relatively normal weight (Figures 2 and 3).

In obese people elevated triglyceride levels, that are independently associated with an increased risk of cardiovascular disease, are often observed. The liver frees VLDL which are carriers of triglycerides, cholesterol esters and phospholipids, and the hydrolysis of VLDL-TG macromolecule provides cholesterol to peripheral tissues and triglycerides mainly to adipose tissue. The metabolism of triglycerides in adipose tissue is affected by adipokines (leptin and adiponectin) and other factors such as LPL and cholesterol ester transferase protein (CETP)[174]. Moreover, the LDL molecules remain longer in plasma, and slowly lose some cholesterol and become small and dense particles, which make these particles more susceptible to changes in oxidation and glycosilation

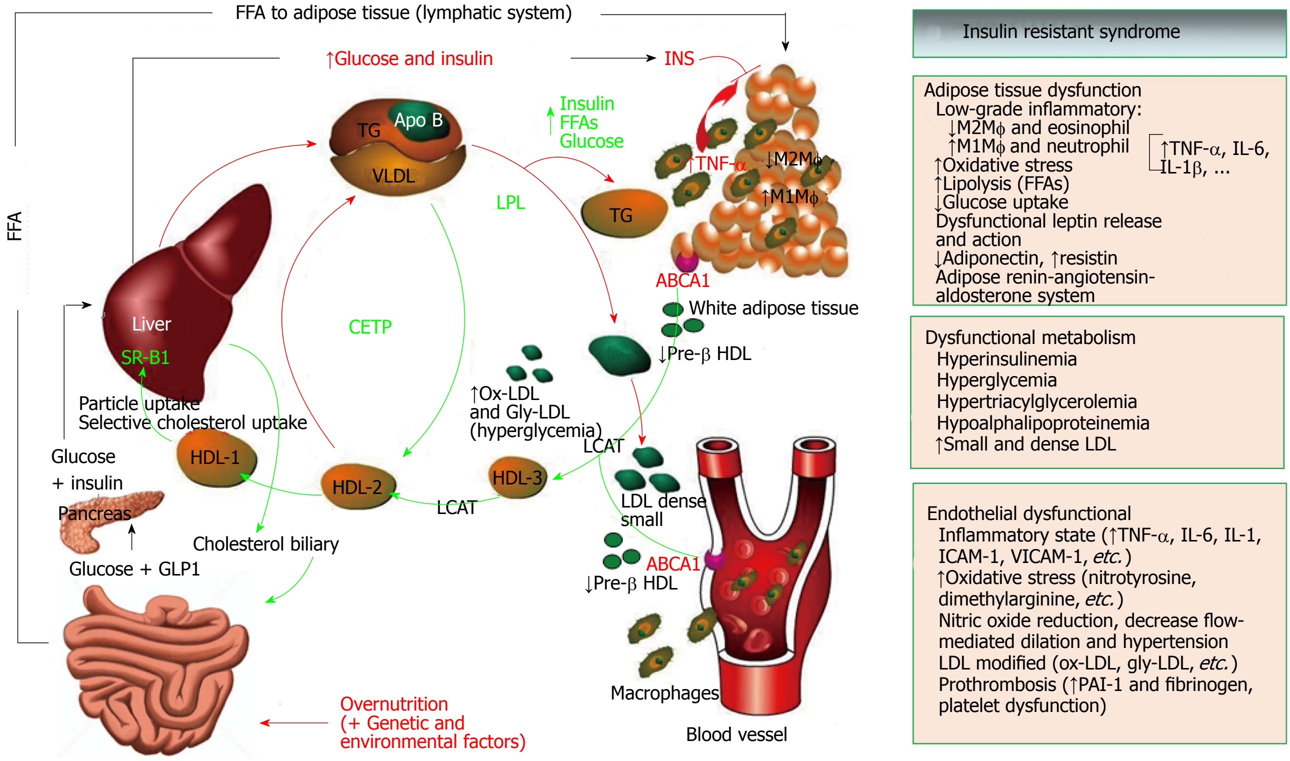

Figure 4 Insulin resistant syndrome and lipid metabolism. When obesity is developing, early abnormalities are observed at this time including hyperinsulinemia and low grade of proinflammatory state (↑ cytokines and PCR-hs), increase liberation of free fatty acids from adipose tissue (↑ lipolysis) and altered release of adipokines (↓ adiponectin, ↑leptin with leptin resistance). In some subjects, fatty liver develops later and consequently affects some functions of the liver. These include an early altered postprandial state (increasing glucose and triglyceride-rich VLDL particles), but finally these finding are observed in fasting state[289]. The VLDL particles undergo reduction by LPL and triglycerides are taken up by adipose tissue. The final result is the increase of cholesterol-rich small and dense LDL particles in serum. These LDL particles are highly susceptible to modifications like oxidation and glycation and the result is the increasing levels of ox-LDL, gly-LDL and the generation of antibodies to ox-LDL[190]. Finally, modified LDL are phagocytosed by macrophages in endothelial blood vessels and an inflammatory pattern that alters endothelial function initiating arteriosclerosis begins[177]. On the other hand, through ABC1 ligand the lipid efflux from peripheral cells to start the reverse transport of cholesterol is mediated. Mature HDL3 are generated from lipid-free apo A1 or lipid-poor pre-β1-HDL as the precursors, and LCAT-mediated sterification of cholesterol generates mature HDL3 and HDL2[189]. In T2D insulin-resistant patients, after adequate metabolic control the HDL3 cholesterol and APO A1 levels were increased. These findings were associated with a higher specific binding activity of HDL3 in those patients that showed improved insulin resistance[190]. Cholesterol efflux capacity has a strong inverse association with carotid intima-media thickness and was inversely associated with the incidence of cardiovascular events in a population-based cohort[188,290]. LCAT-mediated cholesterol esterification generates large spherical HDL2 particles, but large HDL2 can be converted in turn to small HDL3 upon CETP-mediated transfer of CE from HDL to apoB-containing lipoproteins, interfering with reverse cholesterol transport. Finally, SR-BI mediates the selective uptake of cholesteryl esters from HDL particles into mainly liver and steroidogenic organs[291]. VLDL: Very light density lipoprotein; LPL: Lipoprotein lipase; ox-LDL: Oxidized-LDL; gly-LDL: Glycated-LDL; ABC1: ATP-binding cassette transporter 1; LCAT: Lecithin cholesterol acyltransferase; CETP: Cholesteryl ester transfer protein; SR-BI: Scavenger receptor class-B, type I.

(ox-LDL, gly-LDL, *etc*). The removal and phagocytosis of oxidized and modified forms of LDL cholesterol (LDL-C) by macrophages located in blood vessel walls is a main event in the development of atherosclerosis[175]. Under these conditions, also possibly being affected by high insulin levels and increasing macrophage infiltration, which when activated produce proinflammatory cytokines and adhesion molecules (CRP, TNF-α, IL-6, VCAM, ICAM and MCP-1), blood vessels endothelial cells undergoes hypertrophy[176]. In early obese T2D patients, even serum ox-LDL levels are influenced by short-term serum glucose variations and flow-mediated endothelium-dependent dilation was decreased and inversely related with increments of circulating ox-LDL levels (endothelial dysfunction)[177]. Finally, HDL, which removes surplus cholesterol in peripheral tissues and moves it to the liver either to reuse or excretion, what is recognized as reverse cholesterol transport (RCT), are also lowered by effects at various points[178]. Therefore, elevated triglycerides and decreased HDL-C, also so-called atherosclerotic profile, are considered a risk factor for CVD, independent of LDL-C levels[174,179-183]. The RCT begins when small precursors of HDL (nascent Apo AI/HDL, pre-β HDL) accept the cholesterol and phospholipids through interaction with ATP-binding cassette (ABC) transporters ABCA1 and ABCG1[184]. ApoA-I is released mainly by the liver and small bowel as lipid-poor apoA-I and nascent phospholipid-rich cholesterol-poor HDL particles. In humans, various mutations in the ABCA1 gene outcome in lowered plasma HDL-C levels and great storage of cholesterol in macrophages located in lymph tissue, and they have an enhanced risk of atherosclerotic events. The liver X receptors LXRα (NR1H3) and LXRβ (NR1H2) have a key role in the control of cholesterol metabolism. Storage intracellular cholesterol levels results in increased cholesterol oxidized forms (oxysterol) which are endogenous ligands for LXRs; therefore, it is as sensors to keep cholesterol at suitable levels and to equilibrate it in all sites of body[185]. The LXR system could intervene in gene expression, controlling the efflux of cholesterol from peripheral cells (macrophages), the elimination of cholesterol from the liver, and the regulation of cholesterol absorption in the small bowel[186,187]. Although the efflux of cholesterol from macrophages is a small part of reverse cholesterol flux, it is the most significant component of atheroprotection. Thus, both plasma HDL cholesterol level and the ability to efflux are highly significant indicators of cardiovascular disease status[188]. In obesity HDL functions change dramatically during acute and chronic inflammation of adipose tissue, and changes in quality of HDL can contribute to the failure of atheroprotective capacity, and decreased efflux capacity in patients with MetS and diabetes have been shown[189]. In addition, after adequate metabolic control of diabetes in T2D insulin resistant patients, the HDL_3 cholesterol and APO A1 levels, directly associated with higher specific binding activity of HDL_3, were increased[190]. Moreover, LCAT (lecithin cholesterol acyl-transferase) enzymes bound to HDL particles play an important role in the change from nascent to mature HDL. LCAT converts free and unesterified cholesterol (form of efflux) in cholesteryl ester, a hydrophobic form of cholesterol (form of transport), that make particles of HDL more spherical and mature. The mature HDL_2 and HDL_3 particles in plasma are constantly remodeled by lipase and interact with other lipoproteins through lipid transfer. This can affect the normal reverse transport of HDL cholesterol to its routes of removal (mainly liver). Therefore, the CETP mediates exchange of HDL cholesteryl ester (CE) with VLDL-triglycerides lipoproteins, and this result in a CE reduction with higher amount of TG in HDL lipoproteins (Figure 4). Thus, in clinical situations of obesity, like insulin resistance and T2D, where VLDL particles are frequently increased (hypertriglyceridemia), HDL cholesterol levels are inversely lowered. In addition, HDL has a variety of anti-atherogenic properties apart from efflux of cholesterol and RCT. It improves endothelial function, inhibits thrombosis and has powerful antioxidant and anti-inflammatory effects.

Last, most patients with features of MetS have increased blood pressure. Several contributing factors such as hyperinsulinemia increases the reabsorption of Na^+ and also activates the sympathetic nervous system. In addition, releasing factors from adipose tissue could stimulate aldosterone secretion independently of angiotensin Ⅱ, K^+ or ACTH[191]. Furthermore, local source of angiotensin II in adipose tissue may also be raised in obese hypertensive subjects establishing the participation of adipose-tissue renin-angiotensin system in insulin resistant syndrome[192].

FROM OBESITY AND INSULIN RESISTANCE TO METS

MetS was referred to as a group of related metabolic disorders for the first time in 1920 by Kylin. Decades before of the introduction of measurements with specific methods for insulin, Himsworth (1936) suggests that diabetes could be found two types, what he termed "insulin-sensitive" and "insulin-insensitive" types. Later, Reaven[193] (1988) observed that several risk factors (dyslipidemia, hypertension, hyperglycemia) commonly cluster together in insulin resistant subjects (Figure 4). He described it and underscored their clinical importance in their Banting lecture, and he used the name "Syndrome X" but obesity was not including in their definition. Today it is known as "MetS" defined as a "set of metabolic disorders and cardiovascular risk factors, which foresee a high risk of developing diabetes and CVD". The more clinical definition was advanced by Grundy[194] in 1999, who described MetS as "a set of metabolic disorders, many of which promoted the development of atherosclerosis and increase the risk of CVD", and were established in the national cholesterol education program's adult treatment panel Ⅲ report (ATP Ⅲ) and later updated in 2004[195]. It avoids the implication that insulin resistance is the primary or only cause of associated risk factors. In addition, because the presence of abdominal obesity is more highly correlated with the metabolic risk factors, measurement of waist circumference was included as a clinical method to identify patients susceptible to MetS[196]. When it is > 102 cm in men and > 88 cm in women it is called abdominal obesity, which is a high risk factor of MetS[194]. Other clinical criteria that Grundy established for the diagnosis of MetS were a blood pressure ⩾ 135/85 mmHg[197], elevated fasting glucose levels ⩾ 110[198], triglycerides ⩾ 150 mg/dL[199] and HDL-C < 40 mg/dL for men and < 50 mg/dL for women (Atherogenic dislipemia). When any 3 of the 5 listed characteristics are present, a diagnosis of MetS must be made. A proinflammatory state, clinically observed by elevation of C-reactive protein (CRP-hs), and a prothrombotic state characterized by increased plasma levels of the inhibitor of plasminogen activator (PAI-1) and fibrinogen are also recognized in MetS.

At the same time (1999) the expert committee of the WHO described MetS as a cardiovascular disorder associated with insulin resistance. In order to diagnose MetS according to WHO criteria, insulin resistance should be identified, together with two or more risk factors, with minimal changes of the factors previously described, but including urinary albumin excretion rate ⩾ 20 μg/min or albumin: Creatinine ratio ⩾ 30 mg/g (microalbuminuria)[200,201].

Last, in order to unify both epidemiologic criteria as clinical, the International Diabetes Federation (IDF) established a set of criteria for diagnosing MetS[202]. While the pathogenesis of MetS and each of its components is complex, multifactorial and not well established, either central obesity and insulin resistance or both are recognized as the main causative requirements. Cardiometabolic risk is mainly associated with abdominal obesity because VAT triggers dyslipidemia, insulin resistance and hypertension[203,204]. This VAT could be assessed by CT, MRI and DEXA, costly measures and not for everyday use. However WC and WHR may be used as proxy measures of VAT, as they are correlated with it[205-207]. Waist circumference gives a closer approximation of abdominal obesity than BMI, the range being different between ethnic populations with respect to overall adiposity, abdominal obesity and visceral fat[208-210]. However, IDF dropped the WHO requirement for insulin resistance but made abdominal obesity necessary as 1 of 5 factors required in the diagnosis. IDF provides the following criteria to define MetS: Central (abdominal) obesity is readily measured using waist circumference and is particularly related with each of the other MetS components, singularly with insulin resistance, and "is a prerequisite risk factor". Abnormality in the distribution of body fat, associated with central obesity and ethnic specific values for waist circumference (BMI ≥ 30 kg/m^2; WC ≥ 94 and 80 cm and 102 and 84 cm, respectively for men and women in Europe and United States).

In addition, any two of the following four factors: The atherogenic dyslipidemia with: (1) high levels of triglycerides (≥ 150 mg/dL); (2) reduced cholesterol-HDL (< 40 mg/dL in men and < 50 mg/dL in women), and more precise analysis high level of apolipoprotein B (Apo B) and high number of small and thick LDL particles and small HDL particles[211]; (3) Treatment of previously diagnosed hypertension or high blood pressure (≥ 130 mmHg systolic and ≥ 85 mmHg diastolic); and (4) The hyperglycemia defined as impaired fasting glucose > 100 mg/dL or previously diagnosed T2D.

Other factors such as genetic profile, physical inactivity, aging, proinflammatory state and hormonal dysregulation could be considered[202].

Therefore, additional metabolic measurements are recommended. Lipodystrophic disorders, either genetic (*e.g.*, Dunnigan familial partial lipodystrophy, Berardinelli-Seip congenital lipodystrophy, *etc.*) or acquired are almost associated with MetS, and occasionally a genetic study could be considered. Most components of MetS are correlated with a sedentary lifestyle. MetS prevalence and each of its components is directly related with age in most people on the world. Assessment of body fat distribution (DEXA) or central obesity (CT/MRI) or fatty liver content (spectroscopy) could be advised. Proinflammatory state presents an increased levels of CRP, and adipocytes and macrophages release inflammatory cytokines (TNF-α, IL-6), and decrease antiinflammatory adiponectin and increased leptin levels are associated with adipose dysfunction[212,213]. Prothrombotic state with increased PAI-1 and fibrinogen[214]. Vascular dysregulation (apart of hypertension) could be estimated with endothelial function and presence of microalbuminuria. Insulin resistance with measurements of fasting insulin/proinsulin levels, HOMA-IR[215], by Bergman Minimal Model[216], during oral glucose tolerance test[217], and gold standard from M value from euglycemic-hyperinsulinemic clamp[218,219].

Finally, several organizations have attempted to harmonize criteria for the definition of MetS [International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute (NHLBI), American Heart Association (AHA), World Heart Federation; International Atherosclerosis Society, and International Association for the Study of Obesity]. They concluded that three abnormal findings out of five would be sufficient to diagnose a person as having MetS. The IDF and AHA/NHLBI agreed that central obesity may not be a prerequisite for diagnosing MetS but could be one of the 5 criteria[66].

EFFECTS OF NUTRITION ON METS COMPONENTS

The prevalence of MetS based on the ATP criteria rose from 28% in the Third National Health and Nutrition Examination and Survey (NHANES) 1988-1994, to 32% in NHANES 1999-2000. It is estimated that 11% of men and 18% of women between the age of 20-39 have MetS. But, rates increase to 40% in men and 46% in women older than 60 years of age, the frequencies being similar in many developed countries of the world[220]. However, at the moment epidemiological and clinical research has released complex and partial information to guide the development of finished nutrition prevention programs. The US Departments of Agriculture and Health and Human Services issued dietary recommendations in the Dietary Guidelines for Americans (DGA), to aid decrease the risk of CVD. This document was also recommended by the AHA (in 2005 and update in 2010) as a dietary proposal to decline the incidence of MetS[221,222]. The updated edition of the DGA accentuates about calory density of the nutrient, and recommends a reduced intake of saturated fat and a confined intake of trans fats, but a greater intake of whole grain, variety of fruit and vegetables, and its adherences have been related with a improve in incidence and prevalence of MetS[223,224].

Recently, Scientific Report of the 2015 Dietary Guidelines Advisory Committee (DGAC) also shows that the dietary standard of the majority of the United States people, as well as other developed countries, has a low intake of key food groups that are important sources of shortfall nutrients, including vegetables, fruits, whole grains, and dairy[225]. In addition, a higher intake of red and processed meats are shown as harmful compared with a lower intake, and higher ingestion of sugar-sweetened foods and beverages as well as derived of refined grains have been found damaging with moderate to strong evidence. Moreover, the DGAC also found that sodium and saturated fat are being over-consumed by Americans, and probably in many westernized countries as well. However, overweight and obesity rates have continued to increase despite actions to recommend decreasing the percentage of fat in food, suggesting that the actions on obesity are more complex. In addition, the healthy Mediterranean-style diet is one of three diets recommended by DGAC, because variations of this diet include many components associated with health benefits. Mediterranean diet is part of an ancient culture of nutrition and is being adopted by different peoples and countries. Previously, an elegant study identified the subjects with MetS as a target for dietary therapies to reduce several components of this syndrome. Patients with MetS, received elaborate advice on how to raise daily ingestion of whole grains, vegetables, fruits, nuts, and olive oil; whereas patients in the control group followed a prudent diet. After 2 years, patients that follow the Mediterranean diet had an intake higher in monounsaturated fat, as well as polyunsaturated fat, and fiber and had a decrease ratio of ω-6 to ω-3 fatty acids. At 2 years of follow-up, patients consuming the Mediterranean diet had significantly reduced serum concentrations of hs-CRP, interleukins, as well as IS, and endothelial function score were improved. Moreover, the Mediterranean diet prevented MetS compared with the control group[226]. Last, its beneficial effects have recently been reported among persons at high cardiovascular risk. A Mediterranean diet supplemented with extra-virgin olive oil or nuts reduced the incidence of major cardiovascular events and prevalence of MetS[38,227].

In the prevention and treatment of MetS it has been found that it is not one specific diet, but rather various changes of nutrients in the diet that should be recommended to treat or prevent the onset of each different component of the syndrome.

Effects of nutrition on obesity

The first factor to be avoided in the prevention of MetS is obesity, and the percentage of fat in the diet has traditionally been associated with the development of obesity. There is evidence to show that metabolic stressors including energy-dense high-fat diets develop obesity, and probably insulin resistance and MetS[228-230]. In overweight subjects, selected on the basis of impaired glucose tolerance, the prevalence of overweight and MetS decreased after two and four years of an extensive life-style intervention which mainly included a reduction of energy and SFA intake and an increase in physical activity[231,232]. However, other strong epidemiological evidence has reported contradictory results at this point. An important epidemiological analysis from the European Prospective Investigation into Cancer and Nutrition, which included 519978 participants, found no significant relationship between the amount and type of fat consumed and annual weight gain. Recently, in this cohort it has also been observed that higher SFA consumption was not related with higher ischemic heart disease risk[230,233]. But, residual confounding factors, such as cholesterol-lowering therapy and trans fat intake or limited variation in SFA and PUFA intake, may explain these findings. Moreover, in well-conducted intervention studies, in extremely obese subjects with a raised prevalence either diabetes or MetS, a higher weight loss was showed after six months on a carbohydrate-restricted diet than on a fat-restricted diet, with a relative upgrade in IS and triglyceride levels, even after control for the amount of weight lost[234]. Additionally, in a randomized controlled trial to observe weight loss in overweight premenopausal women, where four diets containing a gradual and inverse fat and carbohydrate content were compared, the diet with less carbohydrate content (Atkins) achieved greater weight loss and metabolic success[235]. It has also been found that high-protein and low glycemic index (GI) diets are better tolerated than low-protein with high GI. In addition, the low protein with high GI diet was associated with subsequent significant weight regain[236]. Further, higher weight loss with low-carbohydrate diets may be associated to the satiating effects of fat and protein content. We have previously found that following the intake of a standard breakfast, the glucagon like peptide-1 (GLP-1) postprandial release was significantly raised in those patients who had eaten an isocaloric olive oil-enriched meal compared to when they had a CHO-rich meal, further supporting the idea that monounsaturated (MUFA) fatty acids may act as secretagogues of GLP-1 (Figure 5)[237]. The biological effects of GLP-1 well know include stimulation of glucose-dependent insulin secretion which is lowered until a normal blood glucose level, delay gastric emptying and inhibition of food intake, increases the β-cell proliferation and inhibition of their cell death[237]. Finally, epidemiological studies have established an inverse relationship between the consumption of dietary fiber and body weight and waist perimeter[238,239]. Therefore, several controlled intervention studies demonstrated that dietary fiber content in the diet is negatively associated with weight gain, and may have a satiating effect and decreases the amount of calories ingested[240,241]. Thus, weight loss can be difficult to attain and maintain long-term with interventions of more or less experimental diets. Therefore, important data to reduce and maintain body weight should include the total amount of energy consumed, others characteristics and combinations of the nutrients ingested and the amount and type of physical exercise performed daily. The main interest of research today is to define the potential therapeutic effects of replacing SFA with MUFA or with a low-fat diet on regression of MetS or the effect on the different components of the syndrome.

Effects of nutrition on central fat distribution

It is well established that the type of fat consumed could be more decisive than the total amount of fat consumed when we only look at changes in body composition and distribution of adipose tissue[242,243]. It has been proposed that high adiposity and central fat deposit is related to diets with a high ratio of saturated to unsaturated fatty acids[115]. In this regard, SFA refers mainly to Myristic (C14), Palmitic (C16) and Stearic (C18) acids; MUFA refers mainly to oleic acid (C18:1n-9) in Western and Mediterranean countries; PUFA refers mainly to linoleic acid (C18:2n-6), a less ratio of alpha-linolenic acid (C18:3n-3) and, in relation of seafood ingested, a

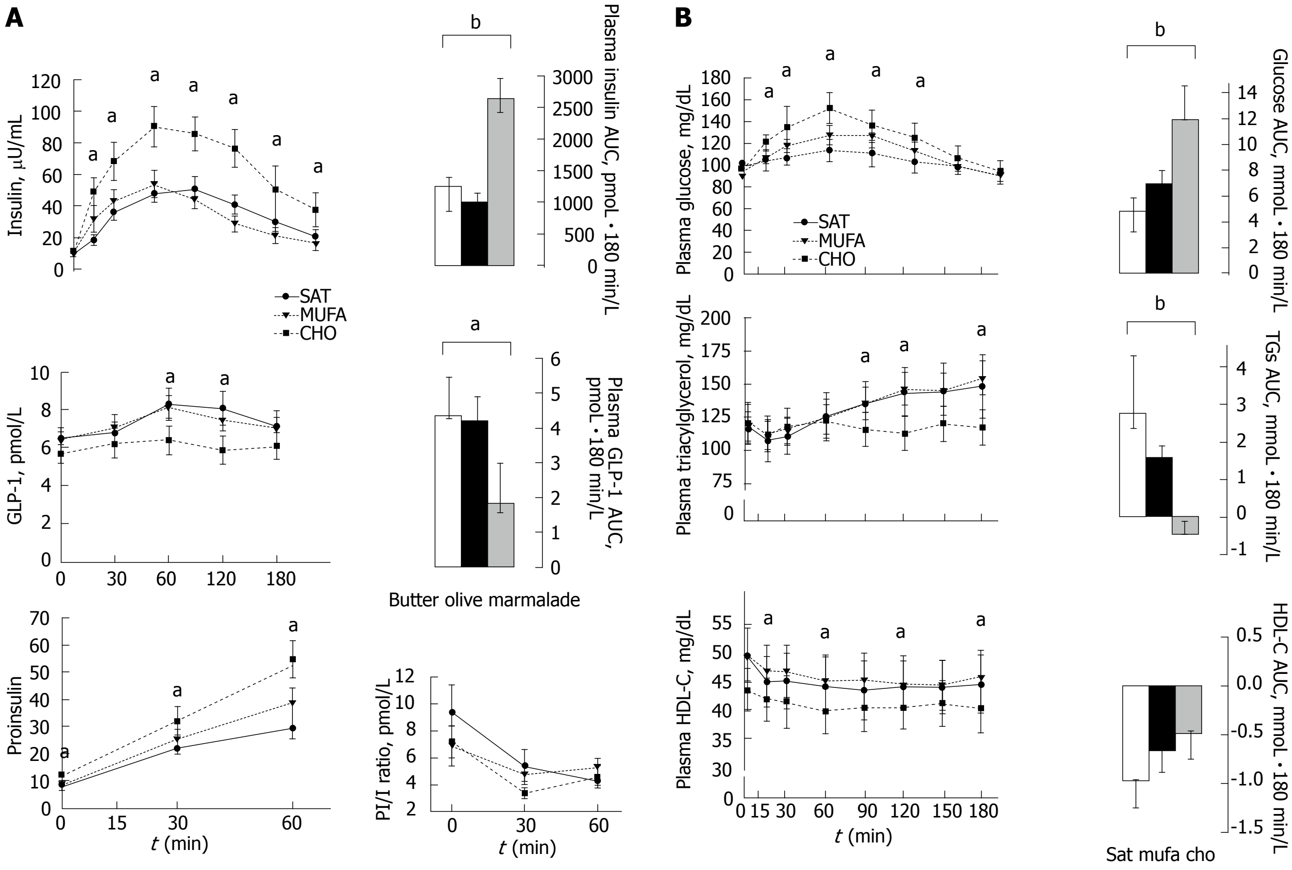

Figure 5 Mean (± SE) postprandial responses of insulin, proinsulin and glucagon-like peptide-1 levels (A), and glucose, triacylglycerol and high-density lipoprotein cholesterol levels (B), in 11 insulin-resistant subjects to three isocaloric (443 kcal) standard breakfasts. A breakfast rich in carbohydrates, a Mediterranean breakfast enriched with extra-virgin olive oil and standard breakfast high in saturated fat. The incremental AUC was calculated by the formula based on the trapezoid rule with adjustment for baseline concentrations. Repeated measures ANOVA and Tukey's test. $^{a}P < 0.05$; $^{b}P < 0.01$[237]. CHO: High-carbohydrate; MUFA: Monounsaturated fatty acids; AUC: Area under the curve; ANOVA: Analysis of variance.

changeable but lower rate of long chain PUFA such as Arachidonic, eicosapentaenoic (EPA), docosapentaenoic and docosahexaenoic (DHA) acids; and finally TFA remits to the main trans fatty acids which are isomers of 18:1 trans and are not found in nature and are the result of human processing (*e.g.*, hydrogenation). In this issue, the Nurses' Health Study showed just a weak direct association between whole fat intake and overweight. However, when the proportion of SFA and TFA was higher it showed a strong relationship to obesity, but the consumption of MUFA and PUFA were not associated[242]. In addition, MUFA and PUFA fat intake have been associated with healthy effects on body fat distribution and improved some other metabolic disorders, as compared with SFA and TFA intake, while maintaining stable body weight. Therefore, in subjects selected with central obesity, after a short intervention with a low-fat carbohydrate-rich diet, patients grouped according to insulin-resistant state (Matsuda < 4) showed a redistribution of their body fat from peripheral adipose tissue toward central body deposits as compared with isocaloric MUFA-rich diet (Table 1)[52]. Moreover, the substitution in the diet of saturated with unsaturated fat, mainly MUFA, resulted in little but consistent loss of body weight, decreased body fat content in limbs and trunk, while maintaining a high and isocaloric fat content (approximately 40%)[244]. Furthermore, the intake of n-3 PUFA, EPA and DHA have been linked to an effect on body weight and body composition. Therefore, higher plasma levels of total n-3 PUFA are related to a decreased BMI and waist and hip circumferences[245]. In addition, central fat distribution was negatively related with n-6 PUFA and MUFA in adipose tissue that correlated closely with fatty acids intake in obese patients from a Mediterranean area[246]. Recently, the long-term consumption of a LFHCC diet increased fasting FABP4 expression in adipose tissue, while it was reduced by the consumption of LFHCC supplemented with n-3 diet[247]. Finally, it was found that conjugated linoleic acids (CLA) produces a reduction on adiposity whereas the lean body mass was not altered or increased, and the waist-hip ratio decreased significantly compared with placebo in adults[248,249]. In another study it was found that the rate of body fat lowered in the CLA-treated group, whereas body weight, BMI, and central abdominal diameter were unmodified[250].

Effects of nutrition on insulin resistance

Insulin resistance is a main characteristic of MetS and is related with other components of the syndrome. The

Table 1 Composition and body fat distribution after three dietary interventions in insulin-resistant subjects

	Baseline	High-SAT	High-MUFA	High-CHO	*P*
EE, (kJ/min)	5.36 ± 0.40	5.49 ± 3.90	5.23 ± 0.37	5.02 ± 0.36	0.3
Anthropometry					
Weight, kg	84.4 ± 5.7	83.2 ± 5.7	83.6 ± 5.8	81.8 ± 6.03	0.3
Total body fat, kg	36.8 ± 4.1	35.0 ± 4.0	35.6 ± 4.0	34.9 ± 4.3	0.1
Lean body mass, kg	47.5 ± 2.5	48.1 ± 2.5	48.9 ± 2.6	46.8 ± 2.1	0.2
Waist to hip ratio	0.99 ± 0.01	0.99 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.9
DEXA analysis					
Total body trunk, g	-	37101 ± 2026	38154 ± 1911	39134 ± 2104	0.3
Fatty body trunk, g	-	14313 ± 1362	14842 ± 1437	16459 ± 1653	< 0.05
Total body limb, g	-	36420 ± 3886	36239 ± 3862	32887 ± 3825	0.7
Fat in arm, g	-	7097 ± 1528	7652 ± 1339	7225 ± 1830	0.4
Fat in leg, g	-	8517 ± 1588	8036 ± 1398	7358 ± 1253	< 0.05
Fat trunk:fat leg ratio	-	1.9 ± 0.3	2.1 ± 0.2	2.50 ± 0.2	< 0.05

Data are mean ± SE. *P* value is analysis of variance for repeated variables. Copyright 2007 American Diabetes Association. *Diabetes Care* 2007; 30: 1717-1723. Reprinted with permission from the American Diabetes Association. EE: Energy expenditure; SAT: Saturated fat; MUFA: Monounsaturated fat; CHO: Carbohydrates rich diets; DEXA: Dual energy X-ray absorptiometry.

KANWU study treated 162 healthy subjects selected at aleatory to eat a controlled, isoenergetic diet for 3 mo containing either a major rate of saturated (SAFA diet) or monounsaturated (MUFA diet) fatty acids. After 3 mo, subjects lowering saturated fatty acid and increasing monounsaturated fatty acid, enhanced IS but had no action on insulin secretion. This favorable effect of different proportion and fat quality on IS was not found in subjects with a fat proportion ingested higher than > 37% of energy eaten[251]. In addition, in healthy subjects, it has been shown that isoenergetic substitution of SFA for MUFA or complex carbohydrates (CCHO) improved IS, and other components of MetS such as blood pressure[252,253]. In selected subjects with central obesity and insulin-resistance on weight maintenance, a MUFA-rich diet improved IS (HOMA-IR) and fasting proinsulin levels as compared to the CHO-rich diet[237]. Finally, in subjects with early diagnosed non alcoholic fatty liver disease (NAFLD), those with more adiposity, higher trunk fat:leg fat ratio (by DEXA) and lower IS, had a higher ratio SAT:MUFA fat intake than insulin sensitive (IS) subjects[115]. By contrast, the LIPGENE was the largest human intervention study, pan-European and multicentre, developed to observe the effects and efficacy of changing the type and proportion of dietary fat eaten on IS and other metabolic components that integrate the MetS. This intervention was isoenergetic to avoid the effects of weight modification. At the time, it is partially known the metabolic consequence of adhering to low-SFA diets enriched in MUFA or to LFHCC diets, and whether LC n-3 PUFA can improve the negative effects of a low-fat high-carbohydrate diet in MetS. In conclusion, LC n-3 PUFA supplementation significantly lowered TG and FFA levels in men with MetS. The reduction of dietary SFA had no action on IS, blood pressure, LDL cholesterol levels and factors of inflammation. The LIPGENE study observed that the previous dietary consumed and environment may determine responsiveness to dietary fat modification with respect to IS. More specific dietary fat modifications may be necessary to significantly improve IS and other components of MetS; perhaps in combination with dietary restriction and weight loss[254]. There is evidence that a proportion of fat in the diet in excess of 40% worsens IS, especially when ingested fat is saturated[251]. However, recently in this same study those MetS subjects when were selected from the upper HOMA-IR were improved IR, with lowered insulin and HOMA-IR levels after ingestion of the HMUFA and LFHCC n-3 diets. Therefore, specifically insulin-resistant MetS subjects with more metabolic components make a response differently to dietary fat change, being more sensitive to a healthy effect from the exchange of the high SFAs diet by the HMUFA and LFHCC n−3 diets[255].

Effects of nutrition on glucose metabolism

Other dietary factors that can influence various components of MetS, like postprandial glycemic and insulin levels, triglycerides and HDL-C levels, weight regulation and body composition, as well as fatty liver, are the glycemic load (GL) and the excess of fructose and dietary fiber content of food eaten. On the glycemic index (GI) of a food we identify the area under the curve of blood glucose levels two hours after ingestion of a set amount of CHO where glucose is set to equal 100%. So a low GI food will cause a small rise ($\leqslant$ 55), while a high GI food will trigger a dramatic spike ($\geqslant$ 70)[256]. Diets higher in fat and a lower content of CHO necessarily have a lower GL and lower GI. Therefore, the beneficial effect of an olive oil enriched diet avoiding simple carbohydrates, *e.g.*, a typical Mediterranean breakfast with wheat bread and olive oil instead of white bread and marmalade, is also found during the postprandial state where lower glucose and insulin AUCs are observed, as compared with CHO-rich diets (Figure 5)[237]. By contrast, during an isocaloric low carbohydrate high fat (better MUFA) diet, after absorption the free fatty acids are transported *via* the lymphatic system without stimulating the secretion of insulin, so the fatty acids are carried directly to the peripheral adipose tissue; thus, postprandial insulin peak and hyperglycemia are reduced[237]. These higher postprandial levels of glucose and insulin after eating foods with a high GI or GL may mediate changes on adiposity and central fat redistribution observed in selected insulin-resistant subjects (Table 1)[52]. Following intestinal absorption of excess carbohydrates these are transported *via* portal and, after signaling insulin secretion in the pancreas, are deposited in the liver. However, in obese subjects, when the storage limit is exceeded, and through several metabolic pathways, that mainly include the transcription protein carbohydrate response element binding protein, which is activated by a high-carbohydrate diet, the glucose can be used to synthesize fatty acids which are released into plasma as VLDL rich in triglyceride[257]. Thus, triglycerides can be captured more widely and again can reach the central depot (Figure 4). Once the function of liver buffer is lost, a state of concomitant hyperglycemia, hyperinsulinemia and hypertriglyceridemia and fatty liver results, due to the consumption of diets high in carbohydrates and high GI. However, conflicting data have been published addressing this concept. It is possible that the type of CHO eaten as well as other macronutrients accompanying these diets could modify and partially explain these discrepant results. Therefore, intervention studies looking at the effects of GI and GL have not had clarifying results. The comparisons of four diets of varying GL on weight loss and cardiovascular risk reduction in a randomized controlled trial was made in 129 overweight and obese young adults[258]. The authors concluded that either high-protein or low-GI regimes could have effect on body fat loss, but effects on cardiovascular risk factors are improved by a high-carbohydrate but low-GI diet.

Effects of nutrition on atherogenic dyslipidemia

The increased levels of triglycerides associated with hypoalphalipoproteinemia, are a feature of insulin resistance and MetS, and increase cardiovascular risk regardless of LDL cholesterol levels. The high insulin levels in MetS constantly target the peripheral adipose tissue and stimulates its hypertrophy, which initiates an aberrant inflammatory condition (↑M1 Ø) with elevated levels of TNF-α and IL-6 resulting in adipose dysfunction. Therefore the activity of lipoprotein lipase is reduced in AT and the triglyceride clearance is decreased. Adiponectin levels are reduced and the β-oxidation can be lowered by muscles and liver as well as lowering the sensitivity to insulin (Figure 2)[52,259,260]. Furthermore, the increase of VLDL (↑TG) can interact with reverse cholesterol transport by exchanging triglycerides for cholesterol of HDL-C molecules, which eventually can be reduced in plasma. In fact, low HDL-C levels can be considered as one of the earliest signs of a state of insulin resistance. The consumption of a extra-virgin olive-oil-based breakfast by central-obese insulin-resistant subjects lowered postprandial glucose and insulin postprandial excursions, and increased GLP-1 levels as compared with a isocaloric standard CHO-rich breakfast (Figure 5)[237]. In addition, the effects of these dietary interventions on the plasma lipid profile in these insulin-resistant subjects independently of weight loss were also investigated. Serum total cholesterol and Apo B levels tended to decrease after the CHO diets, but a potentially harmful result lowering HDL-C concentrations (approximately 11%) was also observed. By contrast, the consumption of a hight MUFA diet was associated with significantly higher HDL-C levels. However, fasting serum triacylglycerol concentrations were not altered by any of the three diets (SAT, MUFA and CHO). These effects could be associated to the fact that body weight was maintained unchanged during the three dietary periods, suggesting that triglycerides levels are mainly related with total body fat[237]. By contrast, in the LIPGENE human dietary intervention study, MetS subjects (n = 472) from 8 European countries were randomly assigned 4 diets: A HSFA; a HMUFA diet; a LFHCC diet supplemented with long-chain n–3 polyunsaturated fatty acids (1.2 g/d); or a LFHCC diet supplemented with placebo for 12 wk (control). The LFHCC n-3 PUFA diet reduced plasma TG and FFA concentrations, particularly in men[254]. Finally, in this study, was made a post hoc analysis, selecting only those patients who had been diagnosed of MetS syndrome (according to NCEP MetS criteria updated by the joint scientific statement harmonizing the MetS criteria) to observe the effect after 12 wk of an isoenergetic dietary fat exchange on final incidence of each component of MetS. In addition, final regression of MetS and each component of MetS post-intervention were also investigated. This study concluded that an isoenergetic LFHCC diet supplemented with LC n-3 PUFA reduced some features of MetS compared with high-fat (HSFA and HMUFA) diets and low-fat diet without LC n-3 PUFA. The prevalence of enlarged waist circumference, hypertension and hypertriacylglycerolemia were reduced after the isoenergetic LFHCC n-3 diet. Thus, the prevalence of MetS fell by 20.5% after LFHCC n-3 diet compared with the HSFA (10.6%), HMUFA (12%) or LFHCC (10.4%) diets (Figure 6)[261]. Interestingly, the prevalence of hypertension was reduced after consumption of LFHCC diet supplemented with VLC n-3 PUFA. In a population-based study on food n-3 PUFA intake, an independent inverse relation of total n-3 PUFA intake to systolic and diastolic pressure has previously been shown[262]. In addition, the capacity of PUFAs to target the signaling on gene expression of SREBP-dependent, which controls genes implicated in cholesterol metabolism, gives an evidence of the potential effects of fatty acids on gene expression, beyond of purely nutritional[263]. Further, it has been observed that n-3 fatty acids but not SAT fatty acids are important activators of PPAR-α implicated in triglycerides reduction. Therefore, because of their capacity to repress inflammatory pathways and control the expression of a great quantity of genes associated to lipid metabolism and adipose tissue, n-3 fatty acids are being using as therapeutic agents in lipids, T2D,

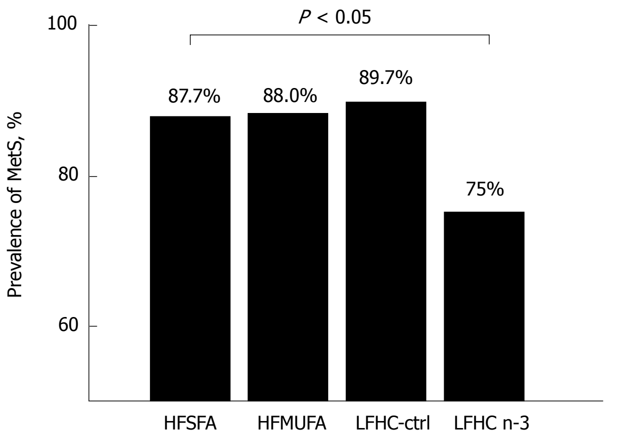

Figure 6 Prevalence of metabolic syndrome after 12-wk of diet assignment. HFSFA is a high fat diet rich in saturated fat and HFMUFA is a high fat diet rich in monounsaturated fat. LFHC-control is a low-fat, high complex carbohydrate diet and LFHC n-3 is a low-fat, high complex carbohydrate diet supplemented with 1.24 g/d of very long chain n-3 polyunsaturated fatty acid (VLC n-3 PUFA). χ^2 test, $P < 0.05$)[261]. MetS: Metabolic syndrome; HFSFA: High fat diets rich in saturated fat; HFMUFA: High fat diets rich in monounsaturated fat; PUFA: Polyunsaturated fatty acids.

steatohepatitis and MetS.

Effect of fiber on glucose and lipid metabolism

Different fiber content of the diet can influence several components of MetS. The ADA recommends an consumption of dietary fiber of 20 to 35 g per day mainly since of the cholesterol-lowering and glucose-lowering results of soluble fiber. However, more beneficial actions of a higher ingestion of dietary fiber, specially of the soluble form, over the amount advised by the ADA, were reported to get better glycemic control, lowers hyperinsulinemia, and decreases plasma lipid levels in type 2 diabetic patients[264]. This should warn us that the intake of complex carbohydrates with high fiber content (*e.g.*, whole bread) have healthier effects compared with refined CHO food popular in modern nutrition. Therefore, maintaining a diet that includes a high intake of fruits, vegetables, and whole grains, a rich sources of dietary fiber, such as a Mediterranean diet, should be strongly emphasized.

Effect of nutrition on adipokines

We have recently analyzed the repertoire of adipokines in patients diagnosed with fatty liver, a human model of central obesity, much of them with MetS. We confirmed that IR patients had lower serum adiponectin level than IS patients, and a positive correlation between IS index (ISi) and serum adiponectin levels was observed[115]. It has been shown that hypoadiponectinemia may play a pathophysiological role in the progression from NAFLD to NASH. Adiponectin exerts a endocrine protective action on liver fat accumulation favoring lipolysis (Figure 2)[129]. In addition, we have previously documented a differential postprandial regulation of adiponectin gene expression on peripheral adipose tissue in response to differences in the isocaloric macronutrient composition of diets. Therefore, after a CHO-rich breakfast a lowered adiponectin mRNA expression levels were found as compared when a MUFA-rich breakfast were eaten[52]. The paracrine effects of adiponectin can increase insulin sensitivity by increasing fat β-oxidation and energy expenditure on skeletal muscle[265]. Therefore, these actions and a direct adiponectin effect on the ability of adipose tissue to expand it seems play a key role for the regulation in differences in insulin sensitivity and the prevention of central-obesity in responses to different macronutrient composition of diets, in the context of isoenergetic diets and energy balance[52,115]. Finally, a recent review on the effects of diet on adiponectin levels summarizes that daily consumption of sea foods or omega-3 supplementation could increase adiponectin concentrations by 14%-60%. In addition, weight loss performed with a low-calorie diet more physical activity raised adiponectin concentrations by 18%-48%. Last, with fiber supplementation were improved adiponectin levels until a 60%-115%[266].

Adiponectin and leptin seem to regulate the deposition of fat in insulin-sensitive tissues by increasing fat oxidation. However, whereas leptin acts on peripheral target and through CNS, adiponectin seems to act mainly on peripheral tissue and liver. Therefore, deposition of fat in the trunk but not in the legs was directly related with increased liver enzyme levels. Fatty liver patients with IR show lower leptin (LEP) mRNA expression in peripheral adipose tissue in comparison with IS patients[115]. In these patients we failed to show differences in LEP serum levels between IR and IS patients. Nevertheless, since IR patients were more obese and had higher energy intake in comparison with IS subjects, we speculate that IR patients should have exhibited relatively higher plasma leptin concentrations. This may indicate a dysfunction of adipose tissue in maintaining appropriate levels of leptin to overcome the state of leptin resistance observed in obese subjects particularly where insulin resistance is developed[115]. With respect to the response to diets with different macronutrient composition, there is evidence that long-term change in diet (approximately 1 year), including decreased intake of SATs and increased PUFA reduced plasma LEP concentration regardless of changes in fat mass[267]. The results of a study conducted in our laboratory, are interesting. The study was on the acute effects of different isocaloric diets during postprandial state and after an insulin treatment on molecular markers characteristically involved in the process of WAT expansion. All patients were previously diagnosed with fatty liver and IR (n = 15) and were stabilized for 2 wk with an isocaloric standard diet (National Cholesterol Education Program step 1) for fasting peripheral adipose biopsy. They then randomly eat each one of three isocaloric-specific diets for 4 wk, finally undergoing a postprandial biopsy of adipose tissue after three specific meal test meals; a high saturated fat (SAS), high monounsaturated (MUFA) and low-FAT high-carbohydrates (CHO) (Table 2). The gene-expression array profiles in IR patients showed that acute response after an isocaloric-specific diet (180 min) (MUFA, SAT and CHO) presented similar postprandial transcripts. Our gene-expression arrays further confirmed that an anabolic stimulus induced by insulin treatment can acutely increase LEP and PPARG gene expression in WAT *per se*. It has been observed that PPARG and insulin are involved in the nutritional regulation of the *fsp27* gene in WAT, which is needed for the most favorable energy storage and performs a key role regulating whole-body energy equilibrium[268]. Additionally, this is important because PPARG pharmacologic ligands, such as the thiazolidinediones, increase peripheral AT capacity and decreases liver fat deposition, resulting in a healthier insulin action and liver enzymatic profile[269]. However, as with glitazone treatment, insulin therapy is often associated with weight gain due to its lipogenic effects. Therefore, this approach has the potential of initiating a vicious cycle ultimately leading to further obesity and metabolic stress, and eventually to more IR.

Effect of nutrition on oxidative stress

Finally, we have gathered information about the energy balance of these patients. Our results indicate that in early stages of the disease, changes in REE, RQ, and CHO, fat and protein oxidation could not be differentiated between IR and IS patients. However, the higher waist-to-hip ratio early correlated negatively with CHO oxidation and directly with fat-oxidation, suggesting that central adipose fat distribution could decrease glucose utilization as fuel. In addition, the increase in energy intake in IR patients seemed to be primarily related to their apparent preference for higher saturated fat and refined cereal, sugar and soft drink intake. Several mechanisms have implicated high SAT and sugar diets with the development of fatty liver. This includes its association with higher insulin resistance, as well as an increase in markers associated with endoplasmic reticulum stress, excessive production of reactive oxygen species, leading to inflammatory and proapoptotic responses.

The ability of the adipose tissue to keep in reserve fats in obesity is associated with different cellular actions. Thus, a key function in this issue is improving performance of the endoplasmic reticulum (ER) in the adipocytes. ER is a main organelle that regulates nutrient storage, and the surplus of nutrients increases the amount of altered proteins synthesis witch accumulate in the ER. ER stress has been related to many effects of disability cellular that include activation of inflammation and stress networks, closely linked to turn on with by oxidative stress and insulin resistance. The actions of different dietary fat compositions in functions of ER stress on adipose tissue has been investigated in patients with obesity and MetS. In a substudy accomplished within the LIPGENE study, 39 MetS patients were assigned to one of four isocaloric diets: High-SFA (38% E from fat, 16% as SFA), high MUFA (38% E from fat, 20% MUFA), and two low-fat, high-complex carbohydrate (28% E from fat) diets supplemented with 1.24 g/d of long-chain n-3 PUFA or placebo for 12 wk each. This study observed that during the postprandial state, several genes linked to ER stress, such as sXBP-1 and BiP, independent of the fat consumed, in peripheral adipose tissue of patients with MetS, are activated. In addition, after the 12 wk of HSFA diet the expression of *PDIA3* gene was twice higher than after 12 wk of LFHCC n-3 diet. Overall, these data indicate that increase of ER stress in adipose tissue, by amount and different types of fat intake, could play a key role for regulating the capacity of glucose and TG clearance. Thus, ER capacity of AT may modulate metabolic flexibility, initially during postprandial state, accelerating remove of glucose and lipid[270].

Moreover, a high oxidative stress is found in MetS patients, which is showed by a raised activity of NADPH-oxidase and a reduced expression of antioxidant enzymes in the adipose tissue. In patients from the LIPGENE study it was observed that MUFA fat intake decreases oxidative stress as compared with high SAT fat diet by increasing postprandial antioxidant reaction in adipose tissue. Therefore, changing a proportion of SFA by MUFA in the diets could have any beneficial effect to decrease the oxidative stress in MetS patients[271]. Last, MetS patients normally present higher inflammatory state in AT, which is increased during postprandial response, which was seen with independence of the fat eaten. We have found that *p65*, *IκBα*, *MCP-1* and *IL-1β* gene transcripts were induced during the postprandial response, also with independence of fat intake. Of note, IL-6 expression was only identify after the postprandial responses[272].

In summary, in patients at risk, achieving and maintaining an ideal body weight, adjusting energy balance between calorie intake and daily regular exercise is essential in preventing the development of MetS, regardless of the distribution of macronutrient energy. However, the composition of macronutrients can have beneficial or harmful effects on several factors of the metabolic profile, and this can be very important in the dietary counseling of patients with MetS.

Therefore, in subjects with early central obesity associated with other components of MetS, the first recommendation would be to reduce calorie intake and ensure daily physical exercise in order to achieve an ideal weight. Secondly, avoid the intake of trans fat, mainly cakes, biscuits, pastries, *etc.*, and moderate the intake of saturated fat, mainly red meat, processed meats and meat sauces. Thirdly, avoid eating simple carbohydrates, such as sugar, soft drinks, and fruit and juices in excess. This will prevent insulin spike, an increment in triglycerides levels, and also improve the reverse transport cholesterol, and probably fatty liver and central obesity. It is preferable to increase the intake of complex carbohydrates with a lower glycemic index such as wholemeal bread and legumes. Moderate intake of white pasta, potatoes, white rice, *etc.*, is permitted, but these should be eaten with plenty of vegetables, thus increasing fiber content will decrease its GI. The fourth recommendation, is to moderate protein intake

Table 2 Gene expression ARRAYS of peripheral-white adipose tissue in fasting state and its responses to postprandial specific diets and insulin stimulus

	Baseline (n = 8)	$P1$	High-MUFA (n = 9)	High-SAT (n = 9)	High-CHO (n = 9)	$P2$	Postinsulin (n = 9)	$P3$	$P4$
INSR	6.74 ± 0.11	0.69	6.94 ± 0.06	6.95 ± 0.01	6.98 ± 0.04	0.95	6.99 ± 0.04	0.93	0.10
GCGR	6.25 ± 0.13	0.03	5.92 ± 0.06	5.90 ± 0.07	5.89 ± 0.06	0.95	5.75 ± 0.07	0.93	0.04
BMP7	4.80 ± 0.08	0.69	4.49 ± 0.07	4.45 ± 0.09	4.53 ± 0.11	0.95	4.55 ± 0.09	0.93	0.33
BMP2	6.21 ± 0.10	0.62	5.89 ± 0.09	5.96 ± 0.06	5.92 ± 0.09	0.95	5.89 ± 0.07	0.93	0.12
PPARG	9.06 ± 0.28	0.11	9.64 ± 0.08	9.44 ± 0.10	9.57 ± 0.09	0.95	9.97 ± 0.13	0.14	0.04
ADIPOQ	10.89 ± 0.4	0.69	11.65 ± 0.06	11.33 ± 0.22	11.46 ± 0.11	0.95	11.53 ± 0.16	0.93	0.10
LEP	9.37 ± 0.34	0.60	9.87 ± 0.16	9.64 ± 0.22	9.91 ± 0.18	0.95	10.11 ± 0.14	0.93	0.04
ADRβ3	5.23 ± 0.12	0.69	5.06 ± 0.12	5.01 ± 0.10	5.05 ± 0.17	0.95	5.22 ± 0.11	0.93	0.82
RETN	6.19 ± 0.05	0.02	5.59 ± 0.10	5.63 ± 0.07	5.72 ± 0.09	0.95	5.68 ± 0.08	0.93	0.10
IL-6	4.33 ± 0.12	0.69	4.24 ± 0.10	4.27 ± 0.08	4.43 ± 0.16	0.95	4.26 ± 0.11	0.93	0.60
IL6R	6.50 ± 0.08	0.11	6.83 ± 0.06	6.86 ± 0.06	6.86 ± 0.07	0.95	6.85 ± 0.05	0.93	0.10
TNF-α	5.25 ± 0.05	0.69	5.11 ± 0.07	5.13 ± 0.06	5.26 ± 0.08	0.95	5.08 ± 0.07	0.93	0.10
TNFRSF1A	7.49 ± 0.11	0.69	7.68 ± 0.05	7.79 ± 0.07	7.74 ± 0.06	0.95	7.70 ± 0.06	0.93	0.38
TNFRSF1B	8.19 ± 0.09	0.20	8.51 ± 0.04	8.52 ± 0.04	8.56 ± 0.03	0.95	8.52 ± 0.04	0.93	0.10

Gene-expression profiles of the same patient with NAFLD (n = 9) from human peripheral (white) adipose tissue were generated with the use of the Affymetrix U133 Plus 2.0 platform. Gene-chip normalization avoids the use of unstable single housekeeping genes and thus can be technically superior for clinical biomarker studies. Data are mean ± SE. $P1$ value compare baseline in fasting state and postprandial MUFA, SAT and CHO dietary periods with ANOVA repeated measured (n = 8). $P2$ value compares postprandial MUFA, SAT and CHO dietary periods with ANOVA repeated measured (n = 9). $P3$ value compares post-insulin infusion state (180′) and postprandial MUFA, SAT and CHO dietary periods with ANOVA repeated measured (n = 9). $P4$ value compares baseline in fasting state and post-insulin infusion state (180′) with paired t-test analysis (n = 8). P-values for multiple comparisons were adjusted by Hommel's test. P < 0.05 was considered significant. INSR: Insulin receptor; GCGR: Glucagon receptor; BMP: Bone morphogenetic protein; PPARG: Peroxisome proliferator-activated receptor gamma; ADIPOQ: Adiponectin; LEP: Leptin; ADRβ3: Adrenergic β-3 receptor; RETN: Resistin; IL-6: Interleukin-6; IL-6R: Interleukin-6 receptor; TNF-α: Tumor necrosis factor-α; TNFRSF1A: Tumor necrosis factor receptor superfamily, member 1A; TNFRSF1B: Tumor necrosis factor receptor superfamily, member 1B; MUFA: High monounsaurated fatty acid diet; SAT: High saturated fatty acid diet; CHO: Low fat-high carbohydrate diet.

of high biological value associated with polyunsaturated fatty acids ω-3, which can be achieved by replacing portions of meat with seafood. Lastly, take abundant and varied vegetables daily in the two main dishes, fresh and steamed, seasoned with moderate portions of extra virgin olive oil and small portions of dried fruits. This will not only ensure vitamin and mineral requirements are met, but will also give the meal a high fiber volume, flatten postprandial blood glucose of carbohydrates eaten, and the dried fruits will ensure that ω-6 polyunsaturated needs are met. In addition, olive oil should be used in moderate amounts, not more than 20 cc (approximately 180 kcal) per 1000 calories consumed, thus avoiding their overuse, which can lead to obesity. Olive oil is a healthy fat with obvious improvements in atherogenic lipid profile, and contains polyphenols as well as some fat-soluble vitamins like vitamin E which are natural antioxidants[273]. A modest reduction in salt consumption causes significant decreases in blood pressure either hypertensive or normotensive individuals. Thus, the current guidance to decrease salt ingestion to 5-6 g/d should be advised, but a further reduction lower 3 g/d could be required in MetS[16]. In addition, moderate ingestion of red wine is related with a inferior prevalence of MetS, as well as with beneficial effects on central adiposity, lipid profile and fasting insulin levels[274]. Finally, until more conclusive data, it is essential that 2-3 servings per day of semi-skimmed milk and derivatives, and at least 2-3 eggs per week should be included in the diet. Both nutrients provide proteins of high biological value, provide some needs in essential minerals, and are reasonably low in fat.

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Abstract

Synchrony of biological processes with environmental cues developed over millennia to match growth, reproduction and senescence. This entails a complex interplay of genetic, metabolic, chemical, light, hormonal and hedonistic factors across life forms. Sleep is one of the most prominent rhythms where such a match is established. Over the past 100 years or so, it has been possible to disturb the synchrony between sleep-wake cycle and environmental cues. Development of electric lights, shift work and continual accessibility of the internet has disrupted this match. As a result, many non-communicable diseases such as obesity, insulin resistance, type 2 diabetes, coronary artery disease and malignancies have been attributed in part to such disruption. In this presentation a review is made of the origin and evolution of sleep studies, the pathogenic mediators for such asynchrony, clinical evidence and relevance and suggested management options to deal with the disturbances.

Key words: Insulin resistance; Chronotype; Obesity; Evolution; Clock; Shift work

Core tip: Humans evolved to match external environment with internal metabolism. Day-night cycle is an important rhythm to achieve synchrony. A central clock interacts with peripheral clocks in various parts of the body. Reduced sleep, shift work and inappropriate exposure to light during sleep hours disturb this rhythm leading to abnormalities such as obesity, insulin resistance and type 2 diabetes. Understanding the complex interactions of the various factors involved in this system can help in the prevention and in treatment of such adverse effects.

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BACKGROUND

Sleep is the most pronounced human rhythmic activity in humans. Rhythmicity of biological systems developed over the course of evolution so that adaptation occurred to changes of environment with the physiology of organisms[1,2]. Such alignment ensured their survival, and is a powerful evolutionary pressure. While it was recognized that altered core circadian clock genes alters sleep architecture and duration, targeted deletion of *BMAL1/Mop3* gene, which is a partner to CLOCK resulted in disturbances in generation of sleep and wakefulness. These were in addition to wakefulness and the timing of vigilance[3]. Besides, the CLOCK transcription factor is a key component of the circadian clock in the hypothalamic suprachiasmatic nucleus, that leads to attenuation in feeding rhythm leading to hyperphagia, obesity and metabolic syndrome in mice having mutant homozygous CLOCK genes[4]. The interaction between genes of the circadian clock and of metabolic genes is mediated by the remodeling of histone proteins[5].

Despite human beings now having the ability to alter the light-dark cycle, the strong role of circadian clock is still evident on the social and metabolic effects. From the first human experimental work of Jurgen Ashoff emerging studies suggest the role of lunar cycles could also be involved, operating through changes in physical activity[6,7].

Such asynchrony of social and biological clocks leads to obesity, diabetes, cardiovascular disease and cancer[6]. Disturbed daily rhythms reflect in expression of different gene groups as well, suggesting a close relation between rhythmicity and biological well-being[8].

The relation of CLOCK transcription factor and various metabolic abnormalities has been reported in the past few years. Gene variants of the CLOCK transcription factor was shown to be associated with nonalcoholic fatty liver disease (NAFLD), a condition linked to insulin resistance[9]. Among 136 subjects with NAFLD and 64 controls, rs11932595 and rs6843722 showed a significant association with NAFLD. This suggests a potential relation between CLOCK polymorphisms and NAFLD. A more recent study showed that variants of the CLOCK gene could have a role in the expression of obesity and other metabolic traits. Unrelated subjects who were lean (*n*: 715) and obese (*n*: 391) were recruited from a cross sectional population based cohort. SNPs with minor allele frequency were genotyped. Four tag SNP genotype frequencies (rs1554483, rs6843722, rs6850524 and rs4864548 showed associations with overweight or obesity[10]. The fine-tuning of the body's clock evolved to conserve energy and to improve efficiency. Such synchronization allows one to anticipate and respond to environmental alterations[11].

Obesity and type 2 diabetes have become leading causes of disease and death world-wide. Part of the reason for the epidemic appears to be desynchrony over the last 100 years between the body's endogenous clock located in the anterior hypothalamic suprachiastic nuclei, which responds to the dark-light cycle and the iatrogenic disturbance of such rhythmicity. The central clock is aided by similar clocks in the periphery at the liver, fat tissue and gastrointestinal tract, which together, regulate energy metabolism *via* enzymatic activation or suppression[12]. The integration of clock mechanism with metabolism occurs through hormones, nutrients and meal timings.

Recent evidence has shown that variation in genes related to circadian rhythm is associated with extreme obesity, which can be modified by variants in *CLOCK* genes. Mutations of genes in hypothalamus, a key regulator of energy intake, result in early life obesity. To identify gene variants in the background of obesity, a selected phenotype with extreme obesity was taken. One hundred and sixty-six genes functionally related to the hypothalamus, were subjected to complete exome sequencing in 30 extremely obese subjects, for novel rare indel, nonsense and missense variants. The authors identified six novel rare deleterious missense variants (in genes for *BAIAP3*, *NBEA*, *PRRC2A*, *RYR1*, *SIM1* and *TRH*; a novel indel variant was found in *LEPR*). Both rare and common variants of genes thus regulate circadian food intake and hypothalamic signal process are involved in extreme obesity[13].

Similarly there was an association of habitual sleep duration, BMI, nutrient intake and CLOCK variants. In an "inverse-variance weighted, fixed-effect meta-analysis of adjusted associations of sleep duration and BMI and macronutrient intake as percentages of total energy" interactions were studied with CLOCK variants[14]. Data were obtained from nine cohort subjects (*n*: 14896). Interestingly there was a significant association of lower intake of saturated fatty acids and sleep duration among younger adults, and with a lower intake of carbohydrates, higher total fats, higher PUFA intake in older women. In addition interactions were seen between sleep duration and rs12649507 on PUFA intake and with sleep duration and rs6858749 on protein intake. The results imply suggest that longer duration of sleep can attenuate genetic predisposition to obesity acting through intake of appropriate diet[14].

Along the same lines, associations of circadian clock and SIRTUIIN1 (SIRT1) dependent functions may lead to evening preference of food intake and resistance to weight loss. SIRT1 (rs1467568) and CLOCK (3111T > C, rs1801260) were genotyped in a large cohort of subjects who were overweight or obese (*n*: 1465). On follow up for weight loss *via* behavior therapy, those with minor alleles of SIRT1 and CLOCK loci had higher resistance to weight loss compared to homozygotes. Subjects carrying the R genotype had elevated levels of plasma ghrelin, which could modulate the gene variants in the resistance to weight loss[15].

In addition to their putative role in sleep timing, depression and obesity, variant *CLOCK* genes could also influence the duration of sleep. From a sample of 77000 subjects administered Munich ChronoType questionnaire, a subsample on follow up was evaluated by a two-stage design, linkage disequilibrium based association study with short sleep (< 7 h) and long (> 8.5 h) sleep. In the discovery sample (*n*: 283) 194 SNPs were genotyped covering 19 candidate clock genes. In the confirmation sample, two of the best association signals as analyzed by linear regression model were examined[16]. Associations ere found in a *CLOCK* gene intronic region (rs12649507 and rs11932595). Significance persisted for the multiple-marker association signal of rs1264905/rs11932595 haplotype GGAA with long sleep. The authors surmised that an association exists between human *CLOCK* gene variants and sleep duration.

SLEEP IN HUNTER-GATHERERS

One can hypothesize that before the advent of the electric bulb and the concept of shift-work, humans slept at sunset and awoke at sunrise, but evidence is hard to come by. A recent study on societies from Tanzania, Nambia and Boivia, who are hunter-gathers/horticulturalists has provided information on their sleep pattern. These communities do not have access to electric light, television internet, nor do they use caffeine beverages. The principal findings are that their sleep duration averaged 6.9-8.5 h, with variation occurring due to changes in going to sleep, rather than their wake up time. Interestingly they slept on an average, 3.3 h after sunset, but generally woke up before sunrise[17]. Environmental temperature played a major part in regulating sleep, with falling temperatures associated with sleep. It is intriguing to consider whether temperature control in industrialized societies could be contributing, at least partly, to the disturbances of the sleep cycle.

In addition comparative analyses across species is possible by studying the genomic changes in the visual and olfactory ability of the kiwis[18]. Sequencing of the kiwi genome provided information about evolutionary changes in genomic sequences that allowed it to adopt to a nocturnal lifestyle.

BURDEN OF DISEASE

Studying the global burden of acute and chronic diseases between 1990 and 2013 from 188 countries, non-communicable diseases were responsible for leading chronic sequelae[19]. Long working hours (defined as working more than 55 h/wk) were associated with increased risk of cerebrovascular disease[20]. The association with coronary artery disease was weaker; the strength of association with cerebrovascular disease was greater.

Type 2 diabetes mellitus, obesity and metabolic syndrome are known predisposing factors to vascular disease, both cardiovascular and cerebrovascular. Longer working hours entail both exposure to greater stress and a potential abbreviation of sleep duration and quality.

SLEEP STUDIES: ORIGIN AND PROGRESS

Sleep has evolved from being considered a single uniform state[21]. However, epidemiological studies of sleep disturbances appeared from the 1980's. Interest arose initially from sleep problems being associated with accidents and errors of human performance; in addition they were common, likely to increase in number, and recognition that sleep problems had immediate and long term consequences such as risk of premature death, cardiovascular disease, hypertension, inflammation, insulin resistance, type 2 diabetes and psychiatric disorders[22].

While short sleep duration and long sleep duration had greater risk of developing type 2 diabetes, the Whitehall study evaluated whether a change in duration of sleep altered the risk of incident diabetes mellitus. Computation of sleep duration was made at four cycles of 5-years each: 1985-1988 to 1991-1994, 1991-1994 to 1997-1999, 1997-1997-1999 to 2002-2004 and 2002-2004 to 2007-2009. When compared to those who persistently slept 7 h, an increase of sleep of 2 h or more per night was associated with increasing risk of diabetes; similar increased risk was also observed in those who had persistent short duration of sleep. This is new evidence that individuals whose duration of sleep increased over time could be at risk of type 2 diabetes mellitus, which may be related in part, to weight gain[23]. The concept arises that sleep duration and disease risk must be interpreted in light of potential confounding factors such as physical debility. What is evident is that otherwise healthy adults do not habitually extend their sleep duration beyond optimal levels[24].

Meanwhile a meta-analysis of sleep duration and risk of type 2 diabetes mellitus showed a U-shaped relation between duration of sleep and the risk of developing T2DM[25]. Among 482502 subjects who were followed up for periods between 2.5 and 16 years, there were 18483 who developed incident diabetes. Lowest risk of diabetes was found among those who slept 7-8 h a day. In comparison pooled relative risk for T2DM was 1.09 for each 1-h shorter sleep duration among those who slept less than 7 h/d; it was 1.14 for each 1-h increase of sleep duration among those who slept longer. This underscores the fact that optimal sleep duration, *viz* neither less nor more, is important in delaying or even preventing the onset of type 2 diabetes mellitus[25].

MEDIATORS OF ADVERSE CONSEQUENCES

A coupled relation exists between circadian and metabolic systems[26], known mechanisms postulated include hormonal and hedonic causes, alteration in cardiovascular autonomic reactivity, exposure to ambient light, and shift work[17]. The basic concordance of the internal physiological system with external environment results from a natural selection process. Recent evidence from a rodent model suggested that those with 24-h "resonant" rhythms lived longer and produced more litter than those whose rhythms were shortened by a mutation of circadian $Ck1_{\varepsilon}$ allele[27]. This could have important consequences in abnormal work or lighting schedules.

Shift work is a more common cause of rhythmic misalignment in modern society, which is associated with adverse health consequences. It is associated with a misalignment of behavioural and environmental cycles relative to endogenous circadian system. Short-term misalignment of circadian rhythm led to adverse cardiovascular risk factors in healthy adults[28]. The mediators involved increased blood pressure during sleep, decreased cardiac vagal modulation, increased serum levels of interleukin-6, C-reactive protein, resistin and tumour necrosis factor-alpha[28]. A putative link between shift work and hypertension, inflammation and cardiovascular risk may exist.

The concept of a "sleep connectome" can help understand how transition among the various stages of sleep occurs: Vigilance, non-REM sleep and REM sleep. A population of neuronal populations in medial cells which expressed Atoh1 in embryonic life may be important for switching between sleep stages non-REM and REM[29].

CLINICAL RELEVANCE

How do all these genomic and biochemical alterations translate into human disease? A variety of sleep disturbances have been shown to parallel an increasing prevalence of non communicable diseases, particularly obesity and type 2 diabetes. The interaction may occur through changes in hormones that mediate appetite, altered responses to metabolic signals by peripheral tissues as well as to changes in energy intake and expenditure[30]. Increased prevalence of sleep disturbances in type 2 diabetes has been recognized which can impair metabolic control, and must be corrected[31,32].

UNDERLYING MECHANISMS OF THE CIRCADIAN CLOCK INTERACTIONS

Recent evidence has thrown light on the underlying mechanism of circadian clock disturbances (Figure 1). An interesting observation links the coordination of a peripheral clock gene with pancreatic islet function and the etiology of T2DM[33]. Glucose induced secretion of insulin follows a circadian pattern, with transcriptional control over insulin secretory pathway[34]. A specific circadian clock which is found in the β cell of pancreas releases insulin which is dependent on the time of the day[35].

The hepatic glucose output is also similarly regulated by a circadian rhythm[36]. An "inverse-variance weighted, fixed-effect meta-analysis of results of adjusted associations and interactions between dietary intake/sleep duration..." and variants on cardiometabolic traits was carried out from 15 cohort studies. Of the clock genes, known *MTNR1B* associations were seen with higher fasting glucose. Nominally significant interactions occurred with carbohydrate ingestion and *MTNR1B-rs1387153* for fasting glucose. Of practical interest, lower carbohydrate ingestion and normal sleep were suggested to reduce adverse cardiometabolic traits resulting from circadian-related variants of the gene[37].

OTHER MECHANISMS, AND CLOCK DYSREGULATION IN OTHER REGIONS

As already mentioned, shift work rather than primary sleep loss, is the more prevalent sleep disturbance in modern societies. An experimental study mimicking shift work was carried out to evaluate changes of clock genes in the peripheral tissues at the epigenetic and transcriptional level[38]. A randomized 2-period, 2-condition, crossover clinical study was performed in 15 healthy men. With acute sleep deprivation, adipose tissue showed greater methylation in the promoter region of *CRY1* and in two promoter-interacting enhancer regions of *PER1*. In the skeletal muscle, there was a reduction in gene expression of *BMAL1* and of *CRY1.* Thus shift workers may have tissue specific alteration of clock genes which may mediate adverse health effects[38].

Sub-chronic sleep restriction alters insulin sensitivity at the liver, the peripheral tissues and of substrate utilization. Fourteen subjects were recruited to a randomized crossover study. As expected, sub-chronic sleep restriction was associated with decreased whole body insulin sensitivity, and of peripheral insulin sensitivity[39]. There was a modest increase of stress hormones (cortisol, metanephrine and normetanephrine), along with fasting non esterified fatty acids (NEFAs) and β-hdroxy butyrate. This suggests that there was peripheral insulin resistance following sub-chronic sleep restriction, with contributions from elevated NEFAs, cortisol and metanephrines[39]; the latter increase lipolysis and NEFA levels, leading to insulin resistance.

Sleep can influence the sympathetic nervous system, which in turn affects not only the cardiovascular system, but also the β cells of the pancreas[40]. Tasali *et al*[41] reported that even three nights of disrupted slow wave sleep impaired glucose clearance after a glucose load due to sympathetic dominance. Both environmental and genetic polymorphisms can result in disturbances in sympathetic activity and slow wave sleep.

It is well known that sleep homeostasis is undisturbed in young women during their menstrual cycles. Because adverse metabolic effects begin in the peri-menopausal women, EEG patterns of women in mid-life were assessed in the laboratory (20 women in the early menopausal transition) and were compared with 11 women having insomnia. The study was performed in the follicular and luteal phase of the menstrual cycle. Both groups had more awakenings and a low percentage of slow wave sleep[42]. Midlife women, whether or not they were insomniac, had greater sleep disruption in the luteal phase, attributed to the effect of progesterone affecting the sleep regulatory circuits.

Another interesting mechanism for artificial light

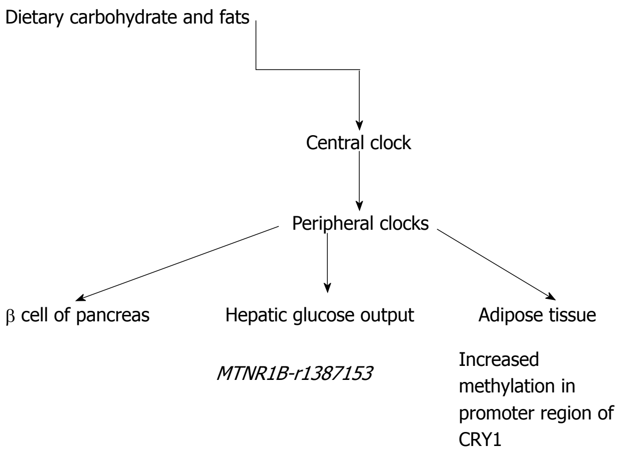

Figure 1 Newer players in circadian clock interactions.

induced obesity has been proposed: Disruption of the central clock mechanism can induce obesity by decreasing the energy expenditure. By increasing the number of hours exposed to light, attenuated brown adipose tissue activity increased body fat[43]. Prolonged light exposure reduces the sympathetic stimulation of brown adipose tissue and the ß3-adrenergic intracellular signal. These lower the uptake of fatty acids from triglyceride-rich lipoproteins, and of plasma glucose by brown adipose tissue[43].

How do all these translate clinically? At baseline (year 2000), the Nurses' Health Study, recruited 59031 women without diabetes. On follow up until 2012, decreases in duration of sleep was associated with adverse changes in physical activity and quality of diet[44]. Therefore lifestyle measures must also be prescribed in preventing obesity and diabetes. An animal study showed that circadian disruption synergizes with diet-induced obesity leading to pancreatic β-cell failure. Wild type Sprague Dawley rats and *Period-1* luciferase reported transgenic rats were studied for 10 wk. Circadian disruption by continuous exposure to constant light acted together with diet-induced obesity to β-cell failure; the proposed mechanism was impaired function of the pancreatic islet clock function *via* impaired amplitude phase and inter-islet synchrony of clock transcriptional oscillation[45].

In women of perimenopausal age, reproductive hormones influence physiological sleep. Thirty three perimenopausal women underwent a cross-sectional lab study for assessing interaction between sleep and reproductive hormones. Seventeen reported no sleep complaints while 16 had clinical insomnia. In the group without sleep complaints, follicular stimulating hormone (FSH) was positively associated with wakefulness after sleep onset and number of awakenings and arousals; the latter were defined using polysomnography[46]. On the contrary among those with known insomnia, sleep was correlated with anxiety and depression, but not with FSH level.

Iron may be another dietary regulator of circadian hepatic glucose metabolism. Little information is available about the specific dietary agents that can influence hepatic glucose output. In an experimental method to assess the effect of iron in diet on circadian gluconeogenesis, dietary iron affected circadian glucose metabolism[47]. Iron modulates peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 alpha), which affects hepatic heme through transcriptional activator of aminolevulinic acid synthase 1. Iron has a pivotal role in circadian rhythmicity through being bound to many circadian transcription factors. The levels of hepatic iron were kept within the physiological limits to avoid the known adverse effects of pathological hepatic overload as in hemochromatosis. Higher (physiological range) intake of iron altered the circadian rhythm of glucose and gluconeogenesis mediated through oxidative stress[48].

In addition to iron, dietary fat and carbohydrate content also influence human clock genes. To clarify whether common dietary components can influence circadian rhythms, diurnal patterns of clone and other genes were studied in 29 non-obese healthy subjects. A baseline and one and six week switch of diets was studied (high carbohydrate-low fat diet and low carbohydrate-high fat isocaloric diet)[49]. Salivary cortisol showed a phase delay one and six weeks after dietary switch. Alterations were found in core clock genes by this switch (*PER1*, *PER2, PER3* and *TEF*) along with inflammatory genes (*CD14, CD180, NFKBIA*, *IL-1B*)[49]. Non-oscillating genes involved in energy and fat metabolism were also altered (*SIRT1, ACOX3, IDH3A*). Dietary carbohydrate and fat were thus shown to alter clock and other genes involved in energy metabolism (Table 1).

SHIFT WORK

In the modern context, shift work is by far the most common cause for disturbed sleep and the consequent adverse health consequences. The effects may not be reversible, with persistent adverse cardiovascular outcomes documented on follow up[25]. As alluded to earlier, light-dark asynchronization accelerates weight gain in both animal models and in humans. The mechanistic explanations involved alteration in eating behavior, changes in hormones, alterations of melatonin, stress response due to lack of proper sleep. In addition recent evidence suggests that dysregulation of human transcriptome and metabolome could also contribute to adverse outcomes in shift workers[25].

Another possible target in treatment strategies is the serotonin and serotonin transporter gene variant. Platelet 5-HT, 5-hydroxyindoleacetic acid (*5-HIAA*) and functional polymorphism of serotonin transporter gene (*SLC64A*) promoter were studied in rotating shift workers (*n*: 246) and in controls (*n*: 437 workers in day shift). There was a difference in platelet 5-HT between the two groups. 5-HIAA was higher in day workers[50]. Similar differences in genotype distribution were found in SLCA4 promoter. It is possible to design drugs that can act at the serotonin pathway to manage adverse effects of shift work.

Table 1 Other pathways influencing sleep

Stress hormones
Cortisol, metanephrine, normetanephrine
Sympathetic nervous system
Menstrual cycle
Decreased energy expenditure by artificial light
Reproductive hormones in women
Dietary iron

SLEEP CHRONOTYPE

The concept of chronotype has been applied in humans to the onset of sleep. It is defined as a "construct that captures an individual's preference for being a 'morning' or 'evening' person"[51]. A recent study from Korea showed that at the level of population, an evening chronotype was associated with metabolic syndrome and diabetes, independent of other factors[52]. This was attributed to disturbed circadian rhythm impacting on metabolic regulation.

Improved work efficiency comes with the cost of adverse health outcomes, which must therefore be carefully balanced so that the risks do not outbalance the advantages[25].

SLEEP HYGIENE

Considering the overriding importance of adequate quality and quantity of sleep, a variety of ways have been devised to tackle this problem. They essentially involve avoiding stimulants at bedtime, proper sleep environment and in attempting to keep a regular sleep time[25]. In addition exercise if performed later in the day must be at least two hours before bedtime. Sleeping environment must be undisturbed, quiet, dark and comfortable.

CONCLUSION

Sleep has multifactorial "macro" dimensions involving work and sleep hours, socioeconomic and health habits in addition to health[53]. Such cross disciplinary studies extend to interesting observation in black bears during hibernation, which conserve energy and bone mass. A reciprocal balance between bone resorption and formation during hibernation of bears was suggested to contribute to conservation of energy[54]. From a macro perspective, multilevel analyses in genomics have been proposed to study circadian rhythms in relation to mood[55]. Ultimately the concept of homeostasis has evolved from being a constant steady-state to a "constant steady rhythm", linked by a network of mechanisms involving molecular clocks spanning gene transcription, metabolism, reproduction and behavior[55]. Establishment of this steady rhythm by balancing health *vs* productivity requires search further research. Currently it is a work in progress.

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ORIGINAL ARTICLE

Basic Study

Implanting 1.1B4 human β-cell pseudoislets improves glycaemic control in diabetic severe combined immune deficient mice

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Abstract

AIM
To investigate the potential of implanting pseudoislets formed from human insulin-releasing β-cell lines as an alternative to islet transplantation.

METHODS
In this study, the anti-diabetic potential of novel human insulin releasing 1.1B4 β-cells was evaluated by implanting the cells, either as free cell suspensions, or as three-dimensional pseudoislets, into the subscapular region of severe combined immune deficient mice rendered diabetic by single high-dose administration of streptozotocin. Metabolic parameters including food and fluid intake, bodyweight and blood glucose were monitored throughout the study. At the end of the study animals were given an intraperitoneal glucose

tolerance test. Animals were then culled and blood and tissues were collected for analysis. Insulin and glucagon contents of plasma and tissues were measured by insulin radioimmunoassay and chemiluminescent enzyme-linked immunosorbance assay respectively. Histological analyses of pancreatic islets were carried out by quantitative fluorescence immunohistochemistry staining.

RESULTS

Both pseudoislet and cell suspension implants yielded well vascularised β-cell masses of similar insulin content. This was associated with progressive amelioration of hyperphagia ($P < 0.05$), polydipsia ($P < 0.05$), body weight loss ($P < 0.05$), hypoinsulinaemia ($P < 0.05$), hyperglycaemia ($P < 0.05$ - $P < 0.001$) and glucose tolerance ($P < 0.01$). Islet morphology was also significantly improved in both groups of transplanted mice, with increased β-cell ($P < 0.05$ - $P < 0.001$) and decreased alpha cell ($P < 0.05$ - $P < 0.001$) areas. Whereas mice receiving 1.1B4 cell suspensions eventually exhibited hypoglycaemic complications, pseudoislet recipients displayed a more gradual amelioration of diabetes, and achieved stable blood glucose control similar to non-diabetic mice at the end of the study.

CONCLUSION

Although further work is needed to address safety issues, these results provide proof of concept for possible therapeutic applicability of human β-cell line pseudoislets in diabetes.

Key words: Human β-cell line; 1.1B4; Cell therapy; Insulin; Pseudoislets

Core tip: Human insulin-releasing 1.1B4 β-cell suspensions and psuedoislets were implanted in streptozotocin-diabetic severe combined immune deficient mice to assess their antidiabetic potential. Both cell configurations yielded vascularised, insulin positive β-cell masses. These were associated with beneficial effects on hyperphagia, polydipsia, body weight, hypoinsulinaemia, hyperglycaemia and glucose tolerance. Both treatments were also associated with significant improvements in islet morphology and increased β:α-cell ratio. Pseudoislet recipients displayed gradual glucose normalization, while cell suspension recipients ultimately presented with hypoglycaemic complications. These results provide proof of concept for possible clinical artificial human β-cell psuedoislets, although further work is needed to address the tumourigenicity of clonal cell-lines.

Green AD, Vasu S, McClenaghan NH, Flatt PR. Implanting 1.1B4 human β-cell pseudoislets improves glycaemic control in diabetic severe combined immune deficient mice. *World J Diabetes* 2016; 7(19): 523-533 Available from: URL: http://www.wjgnet.com/1948-9358/full/v7/i19/523.htm DOI: http://dx.doi.org/10.4239/wjd.v7.i19.523

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is caused by autoimmune mediated destruction of insulin producing β-cells in the pancreatic islets[1]. Uncontrolled hyperglycaemia leads to debilitating and in some cases life-limiting complications including retinopathy, nephropathy, neuropathy and metabolic ketoacidosis[2-5]. Protection against these ailments by insulin injections requires frequent monitoring of blood glucose to prevent over - or under-dosage. Hypoglycaemic episodes are not uncommon especially in brittle diabetes where patients often exhibit hypoglycaemia unawareness resulting in dangerous iatrogenic hypoglycaemia[6]. Cellular delivery of insulin achieved by replacement of pancreatic β-cells can help manage diabetes and in some cases eliminate the need for exogenous insulin therapy[7].

At present, the two methods employed to replace lost β-cells in T1DM are pancreatic transplantation (PTx) and islet transplantation (ITx)[8]. PTx involves an invasive procedure performed in combination with kidney transplantation and necessitates chronic immunosuppression to prevent graft rejection[9,10]. In contrast, ITx represents a less invasive alternative to PTx where islets are isolated by enzymatic digestion of donor pancreata and then administered to the recipient by percutaneous infusion into the liver *via* the portal vein[8]. While less risky than whole organ transplantation, ITx is limited by the requirement for immunosuppression to prevent rejection and promote long-term islet graft functionality but the majority of patients still revert to insulin use within five years of treatment[11,12]. Nevertheless, ITx can provide temporary insulin independence and even partial graft function can prevent dangerous hypoglycaemic events[8,13,14]. Unfortunately, pancreatic donors are scarce and current practices often require use of islets from two or more separate donors. This practice is not practical on a large scale and so there is a great impetus to find alternative solutions especially given that implant function also frequently fails with time[8].

One approach to providing a sustainable supply of insulin releasing tissue for transplantation is to generate insulin-producing cells from stem cells or to engineer cell-lines which mimic the functional response of normal human pancreatic β-cells[15-18]. Over the years, many rodent β-cell lines have been created by methods such as exposure of primary rodent β-cells to radiation or transfection with oncogenic viral vectors such as SV40[19-24]. While such cell-lines have proven invaluable in basic islet research their xenogeneic properties limit their therapeutic utility. Consequently, more recent endeavours have been focused on the creation of insulin-releasing cell-lines from human β-cells[25,26]. Unfortunately, this has proven to be extremely difficult as human β-cells tend to proliferate poorly and undergo rapid dedifferentiation when cultured *in vitro*. The majority of attempts to develop stable human β-cell lines have yielded cells with limited glucose sensitivity or insufficient insulin content[27-32].

Extensive functional studies using the novel human β-cell line 1.1B4 created by the electrofusion of freshly isolated human β-cells with immortal PANC1 epithelial partner cells have demonstrated that 1.1B4 cells possess intact cellular mechanisms for insulin production and secretion, and that they are responsive to glucose and other modulators of insulin secretion[25]. The cells also appear to possess similar cytoprotective mechanisms to primary β-cells[33-35].

Like many β-cell-lines, 1.1B4 cells spontaneously form three dimensional pseudoislets after 5 to 7 d when grown in suspension culture. These pseudoislets are morphologically similar to isolated primary islets and show increased expression of cell-cell communication genes together with remarkable potentiation of insulin secretory responses to glucose and other secretagogues *in vitro*[25,36]. Moreover, 1.1B4 cells showed significantly enhanced resistance to cytotoxicity when configured as pseudoislets compared to monolayers[37]. Transplantation of cells configured as pseudoislets may represent an attractive model to improve graft survival, function and resistance to hyperglycaemia. In the present study the ability of human insulin secreting 1.1B4 cells, administered as single cell suspensions or pseudoislets, to rescue diabetes and restore blood glucose control was studied using severe combined immunodeficient (SCID) mice rendered diabetic by administration of streptozotocin (STZ). These immunodeficient mice were used to prevent rejection of human 1.1B4 cell implants.

MATERIALS AND METHODS

Cell culture and pseudoislet formation

The generation and characterisation of the human 1.1B4 β-cell line has been described previously[25]. The cells were maintained at 37 ℃ with 5% CO_2 in RPMI-1640 media (Gibco® Invitrogen, Paisley, United Kingdom) containing 11.1 mol/L glucose and 2.0 mol/L L-glutamine supplemented with 10% (v/v) foetal calf serum (Gibco ® Invitrogen, Paisley, United Kingdom) and antibiotics (100 U/mL penicillin and 0.1 g/L streptomycin) (Gibco® Invitrogen, Paisley, United Kingdom). Cells were given fresh media every 2-3 d as necessary and were routinely used from passage 25-35. The cell line is available to purchase from Sigma-Aldrich (Dorset, United Kingdom). To form pseudoislets, 1.1B4 cells were seeded at a density of 1 × 10^5 cells/well into ultra-low-attachment, six-well, flat-bottomed plates (Corning Inc., NY, United States) with 5-mL/well culture medium. Cells typically formed three-dimensional pseudoislet clusters, each comprising 5000-6000 cells, within 5-7 d of seeding[37].

Animal and surgical procedures

Adult female SCID mice (15-20 wk) were bred and maintained under specific pathogen-free conditions in the Biomedical and Behavioral Research Unit (BBRU) at Ulster University, Coleraine. Food and water were provided *ad libitum* unless specified otherwise. Diabetes was induced by intraperitoneal administration of streptozotocin (165 mg/kg) after an 8 h fast. Hyperglycaemia was controlled with intensive insulin therapy (15 mg/kg body weight intraperitoneal bovine insulin every 8 h) prior to and during the early engraftment period as indicated in the Figures. Suspensions of 1.1B4 cells (1 × 10^7 cells/mL) were administered in 500 μL serum-free Roswell park memorial institute (RPMI) medium subscapularly into adipose tissue deposit at back of the neck using a 25-G needle. For pseudoislet implantation, harvested pseudoislets were resuspended at a density of 2000 pseudoislets per ml and 500 μL was injected to the same location using an 18-G needle. Control mice received vehicle only. Food intake, water intake and body weight were monitored daily while blood glucose was measured once every 3 d using Ascensia contour glucose strips (Bayar, Uxbridge, United Kingdom). At the end of the study, glucose tolerance was determined by measuring blood glucose and plasma insulin levels after glucose administration (18 mmol/kg *bw i.p.*) at 0 and 15, 30, 60, 90 and 120 min. Finally, terminal blood samples were collected and implants and pancreata were collected for both histology and hormone content assessment. Timeline of the procedures is depicted in Figure 1. All animal procedures were performed in adherence to the United Kingdom home office regulations (United Kingdom Animal Scientific Procedures Act 1986) and "Principles of laboratory animal care" (NIH Publication no 86-23, revised 1985).

Biochemical assays

Lysates of excised cell masses and pancreata were prepared by overnight extraction at 4 ℃ with acid ethanol (ethanol 75% v/v, water 23.5% v/v and concentrated HCl 1.5% v/v). Protein contents were determined by Bradford assay. Insulin was determined by radioimmunoassay as described previously[38]. Glucagon was determined using glucagon chemiluminescent assay (EZGLU-30K, Millipore, MA, United States) following manufacturer's instructions. Glucose in plasma samples was determined using an Analox GM9 glucose analyzer (Analox, London, United Kingdom).

Immunohistochemistry

For peroxidase immunostaining, de-waxed and rehydrated sections were blocked in 0.3% (v/v) H_2O_2 in 50% (v/v) methanol for 30 min to quench endogenous peroxidase activity, before incubation at 95 ℃ in citrate buffer (pH 6.0) for antigen retrieval. After cooling, sections were incubated at 4 ℃ with mouse anti insulin antibody (1:1000, Abcam, United Kingdom) overnight, and then incubated with ImmPRESS HRP anti mouse IgG (peroxidase) reagent (Vector labs, United Kingdom) and developed with 3, 3'-Diaminobenzidine substrate (Vector labs, United Kingdom). Lastly, sections were counterstained with haematoxylin at 60 ℃ for 5 min, and slides were cleared with Histo-clear Ⅱ and mounted with Histomount

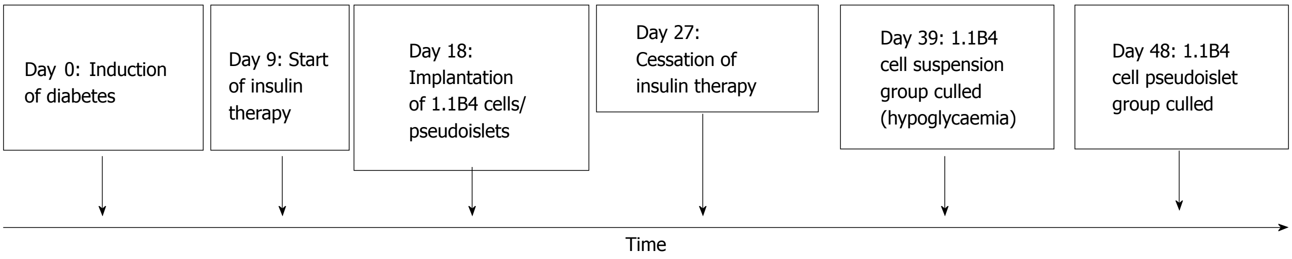


Figure 1 Timeline of experiment.

mounting medium. Slides were viewed using Olympus IX51 inverted microscope and photographed using the SPOT RT-Ke camera (Diagnostic Instruments Inc., Sterling Heights, MI, United States).

For fluorescence immunostaining, following dewaxing, rehydration, antigen retrieval with citrate buffer and blocking with BSA solution, sections were incubated at 4 ℃ overnight with primary antibodies (mouse anti insulin antibody, ab6995, 1:1000, Abcam; guinea pig anti glucagon antibody, PCA2/4, raised in house; rabbit anti Ki67 antibody, ab15580, 1:100, Abcam) prior to incubation at 37 ℃ for 45 min with secondary antibody (Alexa Fluor 488/594)[35,39]. Finally, slides were mounted with anti-fade mounting medium and viewed under FITC filter (488 nm) or TRITC filter using a fluorescent microscope (Olympus, model BX51) and photographed using a connected DP70 camera adapter system.

Image analysis

Closed polygon tool in Cell-F image analysis software (Olympus Soft Imaging Solutions, GmbH) was used to analyze islet parameters including islet, α cell and β cell areas. Number of islets was counted in a blinded fashion and expressed as number per mm^2 of pancreas. For analysis of islet size distribution, islets smaller than 10000 μm^2 were considered small, those larger than 10000 μm^2 but smaller than 25000 μm^2 were considered medium and those larger than 25000 μm^2 were considered large. Cells expressing both insulin and either Ki67 or TUNEL were counted and values were expressed as a percentage of the total number of insulin positive cells observed. Approximately 1000 β-cells were analyzed per replicate.

Statistical analysis

Results are expressed as mean ± SEM. Groups of data were compared using Student's unpaired *t*-test with two-tailed *P*-values. Groups were considered significant where $P < 0.05$.

RESULTS

Effects on food and fluid intake, body weight and blood glucose

Streptozotocin diabetes caused significant increases in food and fluid intake when compared to non-diabetic controls ($P < 0.05$, $P < 0.01$, $P < 0.001$, Figure 2A and B). Implantation of 1.1B4 cell suspensions or pseudoislets had small inhibitory effects on daily and cumulative food intake (Figure 2A). 1.1B4 pseudoislet transplantation significantly ($P < 0.05$) decreased fluid intake from day 18 post-implantation compared to the marked polydipsia exhibited by diabetic controls (Figure 2B). Fluid intake of cell suspension recipients did not significantly differ from control diabetic mice, indicating less effective amelioration of blood glucose control.

Streptozotocin diabetes resulted in significant and progressive body weight loss compared to non-diabetic controls ($P < 0.05$, $P < 0.01$, Figure 2C). Transplantation of 1.1B4 cells resulted in significantly increased body weight compared to diabetic controls 15 d post transplantation ($P < 0.05$, Figure 2C), while pseudoislets evoked a more gradual increase with values differing significantly from diabetic controls from 24 d post transplantation ($P < 0.05$, Figure 2C).

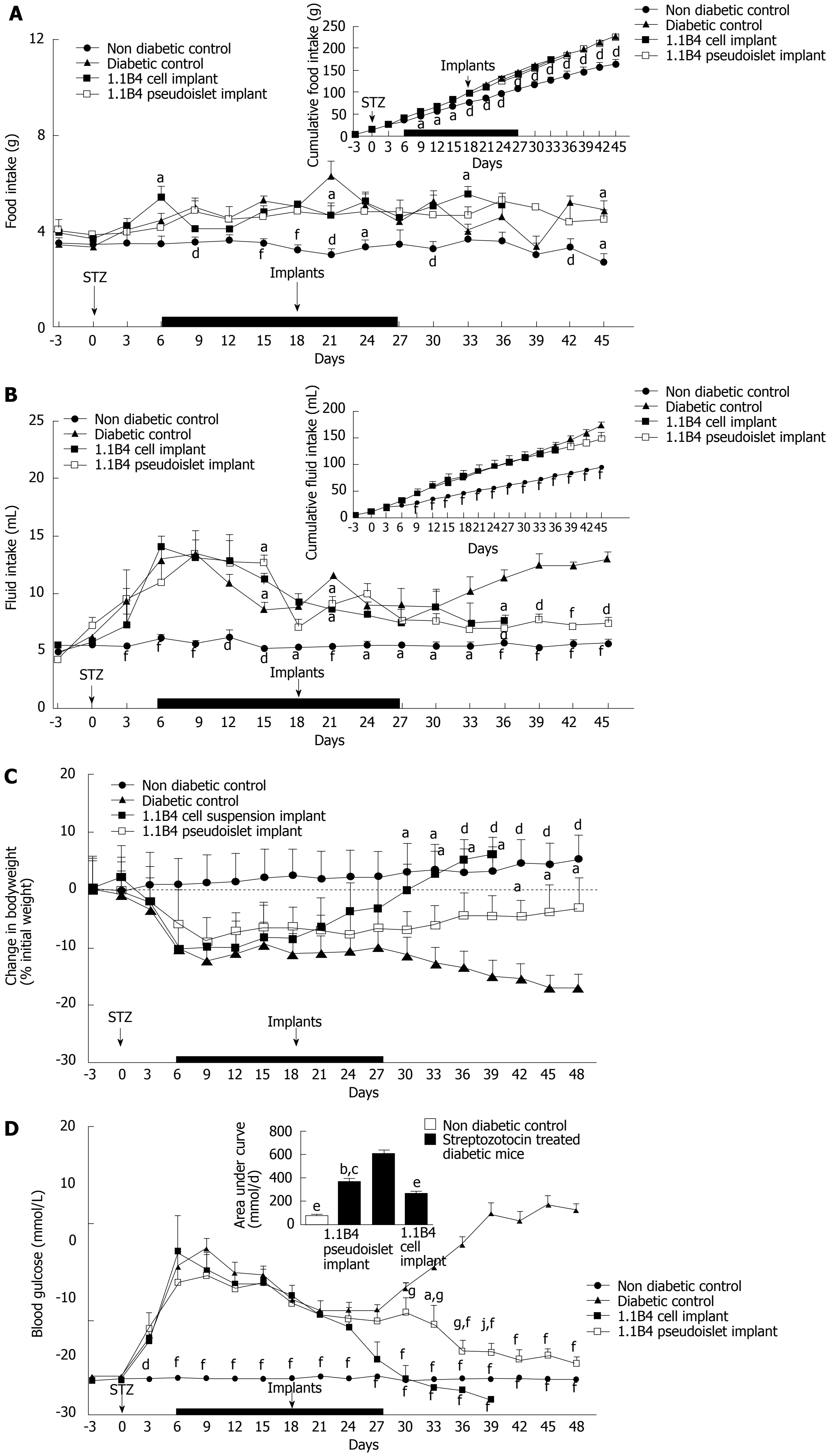
Streptozotocin diabetes significantly increased blood glucose levels within 3 d compared to non-diabetic controls ($P < 0.001$, Figure 2D). The hyperglycaemia was moderated during the period of insulin treatment but rebounded to very high levels thereafter. Blood glucose was significantly decreased at 12 and 15 d after implantation of 1.1B4 cells ($P < 0.001$, Figure 2D) or pseudoislets ($P < 0.05$, Figure 2D) respectively. From day 12 onwards, a much more moderate fall of blood glucose was observed in the pseudoislet recipient group ($P < 0.05$, $P < 0.01$, Figure 2D). Indeed, whereas mice receiving 1.1B4 cells were culled at 21 d post-transplantation to avoid severe hypoglycaemia, pseudoislet recipients exhibited normoglycaemia when the study was terminated at 30 d.

Effects on glucose tolerance

Following an 8 h fast and intraperitoneal glucose administration, blood glucose levels of both 1.1B4 cell suspension and pseudoislet recipients were significantly lower than diabetic control animals at all time-points observed ($P < 0.01$, Figure 2E). Furthermore, 1.1B4 cell suspension implants yielded significantly ($P < 0.05$) lower blood glucose levels than pseudoislet implants or normal control mice ($P < 0.05$, $P < 0.01$, Figure 2E). Pseudoislet recipients exhibited normal glucose tolerance.

Effects on plasma and pancreatic hormone content

Insulin content of cell suspension and pseudoislet implant


A
Food intake (g)
Cumulative food intake (g)
Non diabetic control
Diabetic control
1.1B4 cell implant
1.1B4 pseudoislet implant
STZ
Implants
Days
B
Fluid intake (mL)
Cumulative fluid intake (mL)
Non diabetic control
Diabetic control
1.1B4 cell implant
1.1B4 pseudoislet implant
STZ
Implants
Days
C
Change in bodyweight (% initial weight)
Non diabetic control
Diabetic control
1.1B4 cell suspension implant
1.1B4 pseudoislet implant
STZ
Implants
Days
D
Blood gulcose (mmol/L)
Area under curve (mmol/d)
Non diabetic control
Streptozotocin treated diabetic mice
1.1B4 pseudoislet implant
1.1B4 cell implant
Non diabetic control
Diabetic control
1.1B4 cell implant
1.1B4 pseudoislet implant
STZ
Implants
Days

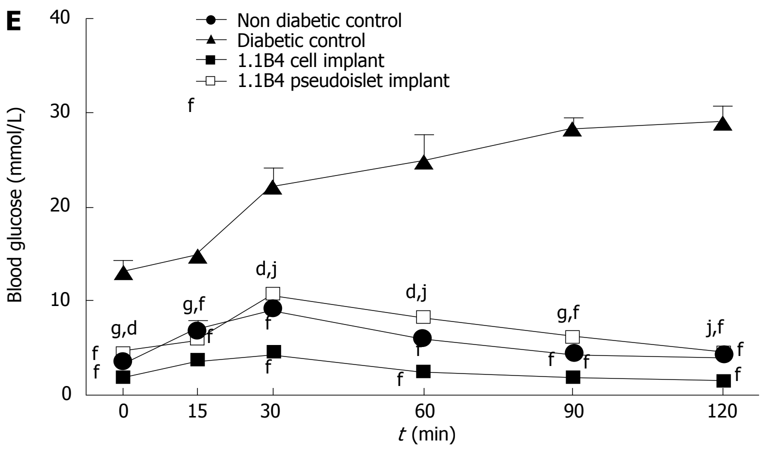


Figure 2 Effects on food and fluid intake, body weight and blood glucose of streptozotocin diabetic severe combined immunodeficient mice implanted with 1.1B4 cells/ pseudoislets. A: Food intake; B: Fluid intake; C: Change in body weight; D: Blood glucose. From day 6-27, all diabetic mice were injected with insulin (15 U/kg bw) every 8 h (Indicated by black bar). At the end of the study, glucose tolerance (E) was determined over a time course of 120 min. Values are mean ± SEM (n = 4). ^aP < 0.05, ^dP < 0.01 and ^fP < 0.001 *vs* diabetic control animals; ^gP < 0.05, ^jP < 0.01 *vs* 1.1B4 cell suspension recipients.

did not differ significantly (Figure 3A). Streptozotocin diabetes significantly decreased plasma insulin compared to non-diabetic mice (P < 0.001). Insulin concentrations were significantly raised in mice receiving 1.1B4 cell suspension and pseudoislet implants (10.8 and 7.9 fold increases respectively, P < 0.05, P < 0.01, Figure 3B). Streptozotocin diabetes also significantly decreased pancreatic insulin content (P < 0.05, Figure 3C) which was not altered by transplantation (Figure 3C). Plasma and pancreatic glucagon levels of diabetic mice were significantly increased compared to non-diabetic controls (P < 0.05, P < 0.01, Figure 3D and E) and this was partly normalized by cell transplantation (P < 0.05, Figure 3D and E).

Effects on pancreatic islets

Representative images showing insulin and glucagon staining in islets of non-diabetic, diabetic and cell/ pseudoislet implanted diabetic mice are shown in Figure 4A. Histological analysis of the islets showed that streptozotocin markedly diminished islet area, β cell area, β to α cell ratio and number of islets while increasing alpha cell area (P < 0.05, P < 0.01, P < 0.001, Figure 4B-F). Islet areas of 1.1B4 cell suspension recipients were marginally decreased compared to diabetic controls (P < 0.05, Figure 4B). However, α-cell areas were decreased and both β-cell and β- to α-cell ratios were significantly increased in 1.1B4 cell suspension and pseudoislet recipients (P < 0.05, Figure 4C-E). Percentage of smaller islets increased in diabetic mice which was not normalised by cell or pseudoislet transplantation (Figure 4G).

Representative images showing Ki67/insulin and TUNEL/insulin staining in islets of non-diabetic, diabetic and cell/pseudoislet implanted diabetic mice are shown in Figure 5A. Diabetes induction was associated with significant decreases in β-cell Ki67 to TUNEL ratio indicating an increase in the frequency of β-apoptosis and a decrease in β-cell proliferation (P < 0.05, Figure 5B-D). Implants did not significantly affect β-cell Ki67 or TUNEL expression.

DISCUSSION

The therapeutic potential of novel 1.1B4 human insulin-releasing β-cells configured as cell suspensions or pseudoislets was assessed by implantation into diabetic SCID mice. 1.1B4 cells exhibit marked decreases in secretory function and viability following prolonged exposure to high levels of glucose[33,37]. As a result, mice with chemically-induced diabetes were given insulin therapy for 9-27 d after STZ to moderate blood glucose levels during the engraftment of implanted 1.1B4 cell suspensions and pseudoislets. As expected, control STZ-treated mice characteristically exhibited hyperphagia, polydipsia, weight loss and marked hyperglycaemia which were temporarily moderated during the period of insulin treatment.

Implantation of 1.1B4 cell suspensions or pseudoislets yielded vascularised cell masses (data not included) which restored plasma insulin concentrations and reversed the hyperglycaemic state. We did not have the opportunity to measure human C-peptide for confirmation but we assume that this insulin was derived from extra-pancreatic source because analysis of pancreatic tissue at end of study revealed severe loss of islet beta cells and cellular insulin in both 1.1B4 cell implanted groups similar to untreated diabetic controls. Furthermore, human insulin and C-peptide were readily detectable in 1.1B4 cells[25]. This was associated with significant beneficial effects on glucose tolerance, body weight and both, food and fluid intakes, but plasma glucagon remained elevated. These results have parallels with previous studies where primary islets were implanted into insulin controlled diabetic animals[40-42]. However, recipients of 1.1B4 cell suspensions progressed to low blood glucose levels such that these mice were terminated at 21 d after transplantation. In

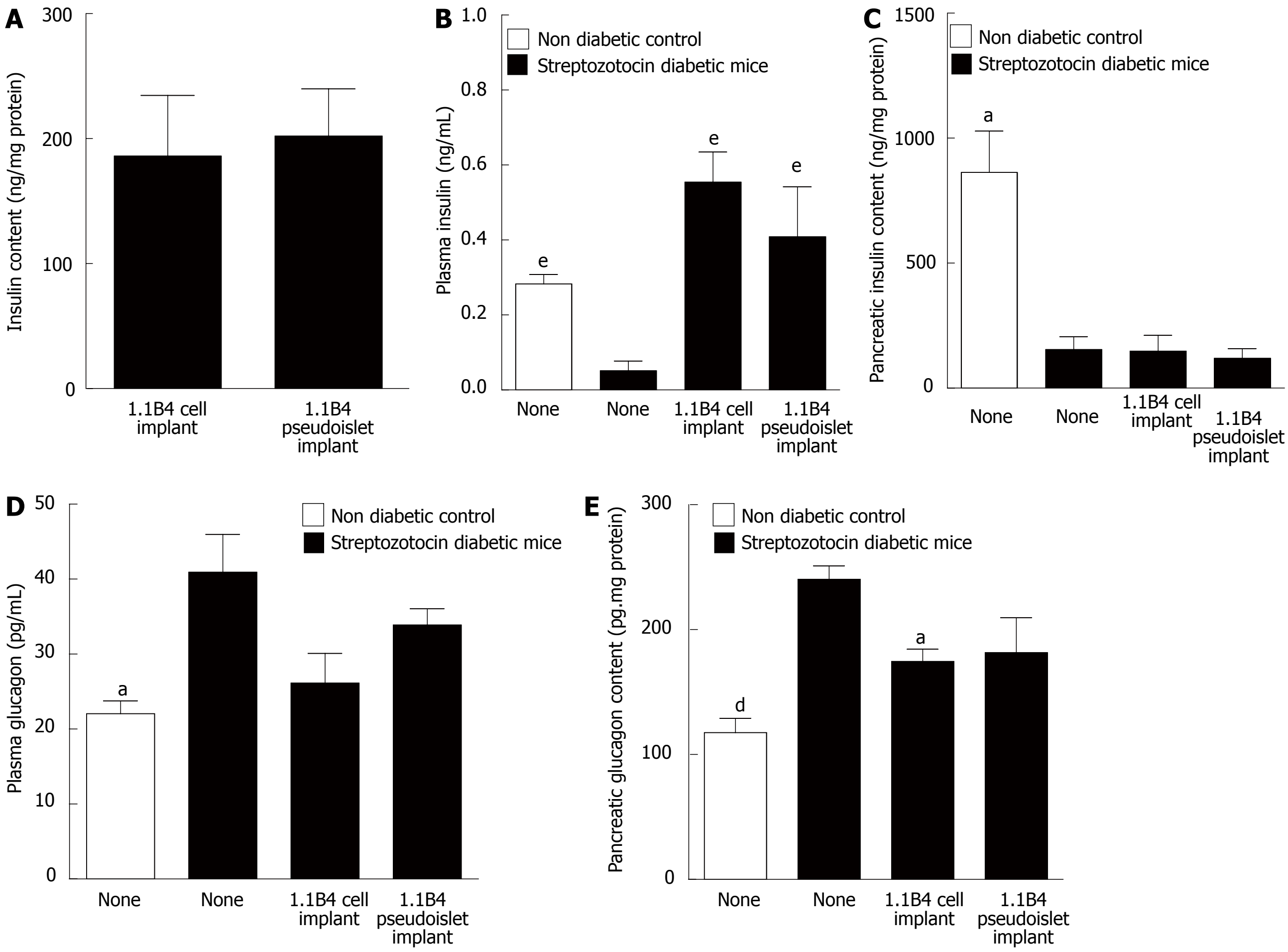


Figure 3 Insulin content (A) of excised 1.1B4 cell/pseudoislet cell masses and the effects of implantation on plasma insulin (B), pancreatic insulin content (C), plasma glucagon (D) and pancreatic glucagon content (E) of normal and streptozotocin diabetic severe combined immunodeficient mice. Values are mean ± SEM (n = 4). $^aP < 0.05$, $^dP < 0.01$, $^eP < 0.001$ *vs* diabetic control animals.

contrast, the anti-hyperglycaemic effects of pseudoislet implants manifested more slowly, achieving stable normoglycaemia without hypoglycaemic complications. Furthermore, energy and fluid balance, body weight, blood glucose and glucose tolerance improved gradually in these mice. This difference is most likely due to improved insulin secretory function in 1.1B4 pseudoislets compared to single isolated cells as described previously *in vitro*[25,36,37,43]. This better regulated insulin release is supported by similar insulin contents of the two types of resected β-cell masses. Nevertheless, part of the difference may also reflect the slower cellular proliferation following pseudoislet implantation.

Administration of STZ to SCID mice was associated with significant decreases in islet number, size and β-cell number together with significant α-cell hyperplasia. These observations accompanied by depletion of pancreatic insulin and enhancement of pancreatic glucagon, mirror previous studies of animal models of diabetes induced by STZ[35,39,44-49]. Implantation of 1.1B4 cell suspensions did not affect hormone contents but was associated with decreases in α-cell and islet areas but an increase in β-cell area and the β-cell to α-cell ratio. There were no significant changes in β-cell proliferation or apoptosis, so alterations of these processes in islet α-cells merits further study. However, both pancreatic insulin and glucagon were unchanged in transplanted mice. Given the present interest in changes of α cell populations in diabetes[35,47,49], this observation merits further investigation. The effects on pancreatic hormones and islets were similar in pseudoislet recipients but as with the metabolic effects, they were moderate compared with cell suspension recipients.

Both cell suspensions and, to a lesser extent, 1.1B4 pseudoislets developed into cell masses following transplantation. While no obvious signs of metastasis were apparent in either group following post-mortem examination, the tumorigenic nature of the cells remains an obstacle to therapeutic use. 1.1B4 cells configured as pseudoislets exhibited significantly decreased proliferation rates and are self-limiting in size *in vitro*[36]. This might be a consequence of cell-cell contacts playing a role in modulation of proliferation and apoptosis rates. However, it seems likely that an additional factor limiting pseudoislet growth *in vitro* is hypoxia, a common consequence of culturing cell spheroids in static cultures. This view is supported by the ability of MIN6 mouse β-cell pseudoislets cultured in bioreactor with continuous

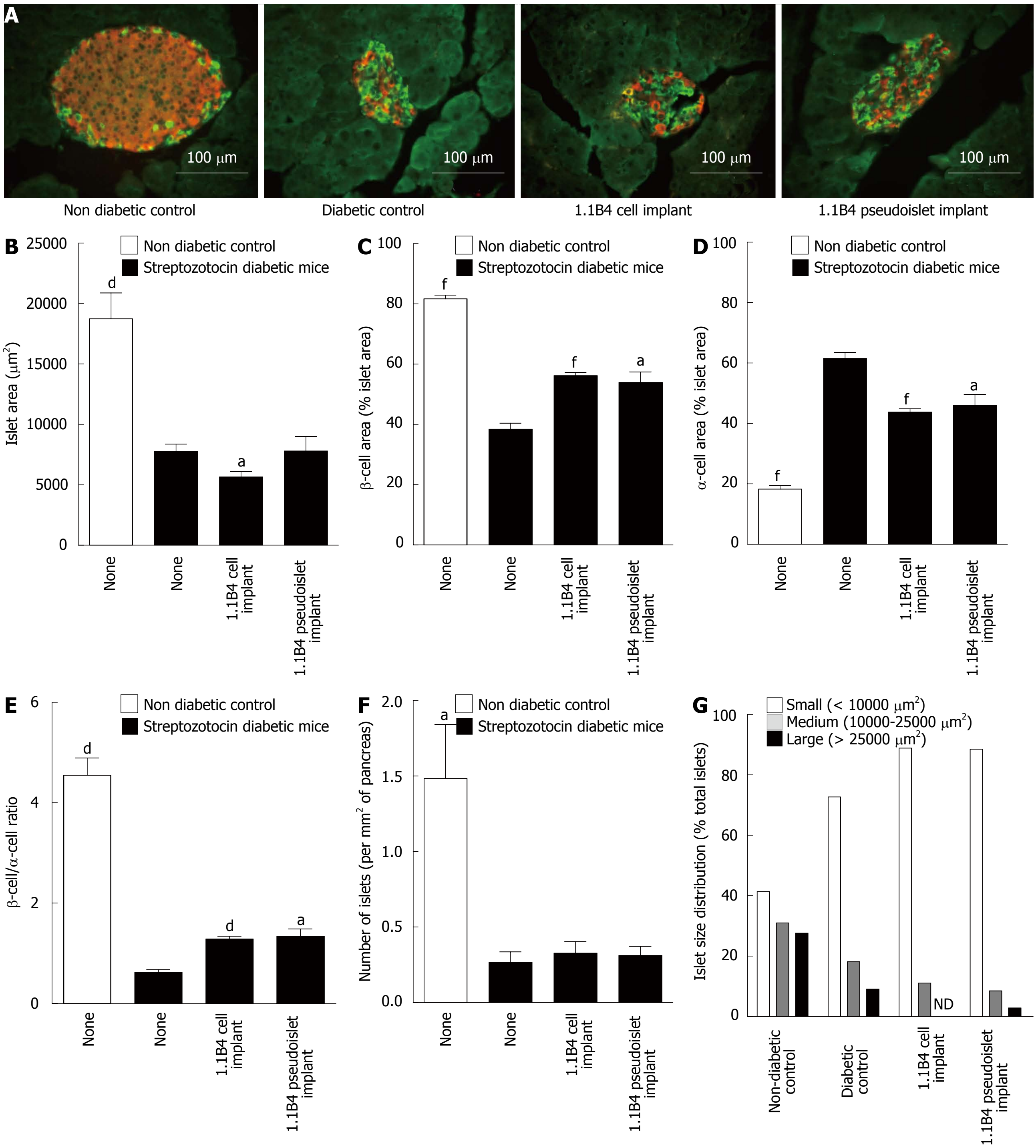


Figure 4 Result of insulin (red) and glucagon (green) staining in islets of non-diabetic and diabetic severe combined immunodeficient mice with or without cell/pseudoislet transplantation. Representative images are shown in A. Islet area (B), β cell area (C), α cell area (D), β to α cell ratio (E), number of islets (F), and islet size distribution (G) were all determined by quantitative histological analysis using cell^F software. Values are mean ± SEM (n = 5). $^aP < 0.05$, $^dP < 0.01$ and $^fP < 0.001$ *vs* diabetic control.

stirring to grow continuously for two wk without exhibiting any signs of hypoxia, reduced functionality, or growth arrest[50]. *In vivo* 1.1B4 cells pseudoislets were able to quickly muster a blood supply which allowed proliferation of the cells. This contrasts with the limited ability of human islets to establish effective vascularisation which is a major hindrance to clinical islet outcomes[51].

A number of groups have investigated potential ways of getting around the issue of tumorigenicity of engineered β-cells which need to be generated in large numbers in culture. The most popular approach is the use of tailored viral vectors which allows the inactivation or excision of oncogenes from the cell-lines genomes to reverse the immortal status of the cells once enough have been generated for use[17,26,32]. If such an approach could potentially be tailored to reverse the tumorigenic status of 1.1B4 cells, the therapeutic qualities observed

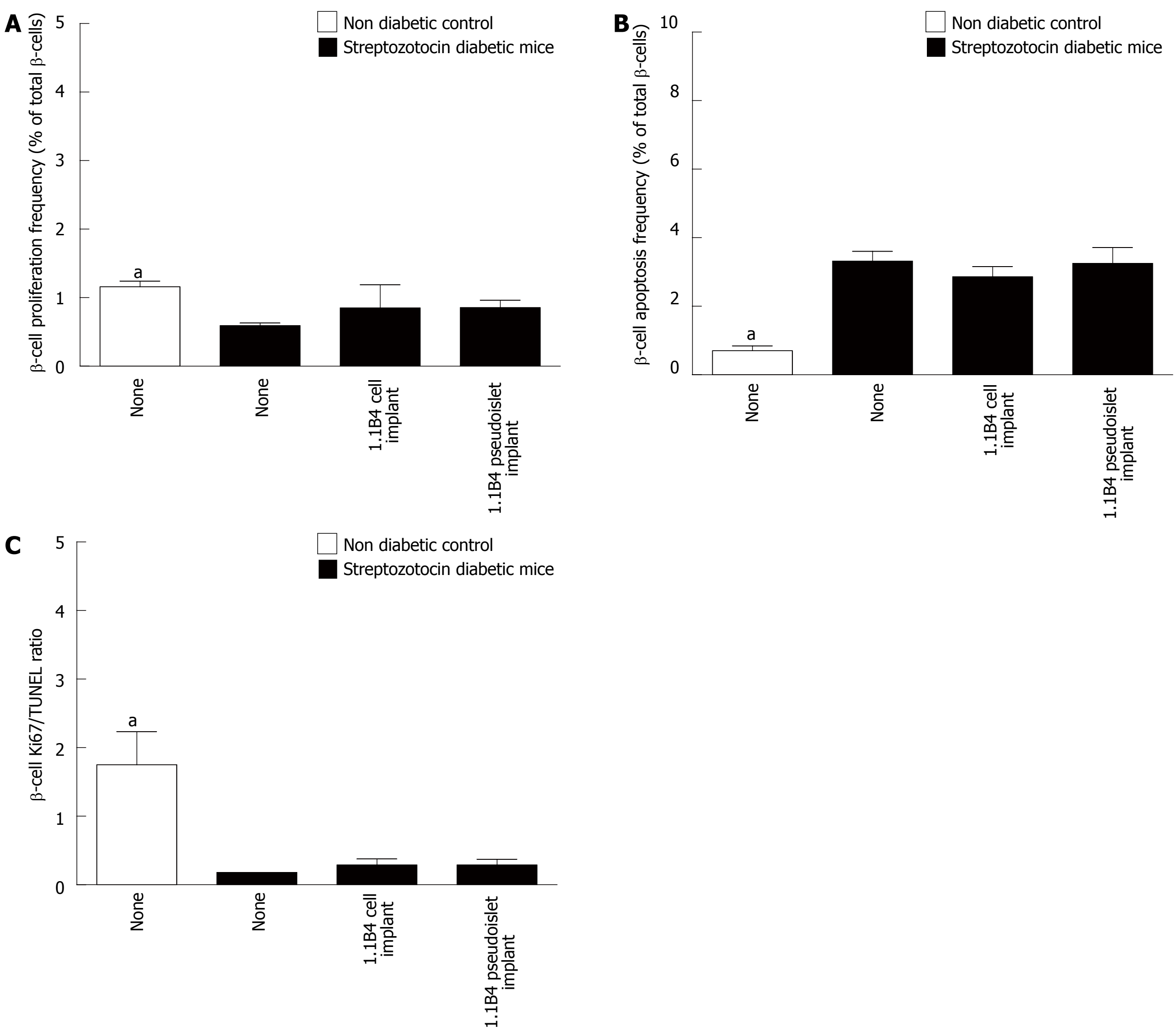


Figure 5 Frequency of β-cell proliferation (A) and apoptosis (B) and ratio of Ki67 to TUNEL positive β-cells (C) were determined by histological analysis. Values are mean ± SEM (*n* = 4). Approximately 1000 β-cells were counted per replicate. $^aP < 0.05$ *vs* diabetic controls.

in this study could be more usefully exploited for the treatment of T1DM. An additional or alternative approach involves the use of implantation devices that are currently under development[7,52]. These devices, such as TheraCyte™ macroencapsulation system and nanofiber-enabled encapsulation devices support cell function by providing good oxygen tension and protection from autoimmune attack, whilst providing against unwanted growth and spread of implanted cells[7,52,53].

To conclude, implantation of human 1.1B4 cells configured as pseudoislets rescued diabetes and significantly improved glucose tolerance, providing stable blood glucose control. Although the results provide proof-of-concept for possible therapeutic use of genetically engineered human β-cells configured as pseudoislets, further work to circumvent the tumorigenic properties of the cells, by genetic manipulation using viral vectors or implantation devices, will be required before such an approach can be realised in a clinical setting.

COMMENTS

Background

The clinical practicality of anti-diabetic islet transplantation therapy is hampered by poor long-term graft survival and the limited availability of donor pancreata. Implanting bioengineered human insulin releasing β-cell lines could potentially provide unlimited cells for such therapy.

Research frontiers

The electrofusion derived 1.1B4 human β-cell line has previously shown promise as a candidate for such therapy. Furthermore, *in vitro* studies of these cells have shown marked enhancements in functionality and survival when the cells were configured as psuedoislets rather than isolated cells.

Innovations and breakthroughs

This is the first study to show that the implantation of 1.1B4 pseudoislets can reverse diabetes in an animal model and to demonstrate additional beneficial effects of such treatment on the endocrine pancreas.

Applications

These results provide proof-of-concept for possible therapeutic use of genetically engineered human β-cells configured as psuedoislets as an alternative to the unsustainable practice of implanting primary human islets.

Peer-review

In this study, the authors investigated insulin secreting 1.1B4 cells as an option to rescue diabetes in severe combined immunodeficient mice. The manuscript is interesting, but several concerns need to be addressed before publication.

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ORIGINAL ARTICLE

Basic Study

Linagliptin alleviates fatty liver disease in diabetic *db/db* mice

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Institutional review board statement: The protocol was approved by the Ethics Committee of Institute of Clinical and Experimental Lymphology (Protocol Number 1/2, April 1, 2014) and Inter-Institutional Animal Ethics Committee based on the Institute of Cytology and Genetics SB RAS (Permission Number: 21, April 1, 2014).

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Russian National Center of Genetic Resources of Laboratory Animals based on the SPF Vivarium of the Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia (Permission Number: 246, April 8, 2014).

Conflict-of-interest statement: Klimontov VV received speaker honoraria from Boehringer Ingelheim. All other authors declare no conflicts of interests.

Data sharing statement: Statistical codes and the dataset are available from the corresponding author: klimontov@mail.ru.

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Abstract

AIM

To study the effects of linagliptin on the structural signs of non-alcoholic fatty liver disease (NAFLD) in *db/db* mice.

METHODS

Male diabetic *db/db* mice (BKS.Cg-Dock7^{m+}/$^{+}$Lepr^{db}/J) aged 10 wk received the dipeptidyl peptidase 4 (DPP4) inhibitor linagliptin (10 mg/kg) or saline as a placebo once per day by gavage for 8 wk. Intact *db/db* mice served as controls. Structural changes in the liver were analyzed from light and electron microscopic images of sections from intact, placebo-treated and linagliptin-treated animals. We estimated the changes in hepatocytes, sinusoidal cells, liver microvasculature and lymphatic roots. Hepatic staining for lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) was assessed by immunohistochemistry.

RESULTS

In 18-wk-old diabetic mice, liver steatosis (predominantly microvesicular and mediovesicular steatosis) was accompanied by dilation of the roots of the lymphatic system, interlobular blood vessels and bile canaliculi. Compared to saline-treated mice, linagliptin-treated mice exhibited a reduction in the mean numeral densities of hepatocytes with lipid droplets (92.4% ± 1.7% *vs* 64.9% ± 5.8% per field of view, *P* = 0.0002) and a lower proportion of hepatocytes with a high density of lipid droplets (20.7% ± 3.6% *vs* 50.4% ± 3.1%, *P* = 0.0007). We observed heterogeneous hepatocytes and relatively preserved cell structures in the linagliptin group. Dilation of blood and lymphatic vessels, as well as ultrastructural changes in the hepatocyte endoplasmic reticulum and mitochondria, were alleviated by linagliptin treatment. In intact and placebo-treated mice, immunohistochemical staining for LYVE-1 was observed in the endothelial cells of interlobular lymphatic vessels and on the membranes of some endothelial sinusoidal cells. We observed an enlarged LYVE-1 reaction area in linagliptin-treated mice compared to intact and placebo-treated mice. The improvement in the structural parameters of the liver in linagliptin-treated mice was independent to changes in the plasma glucose levels.

CONCLUSION

The DPP4 inhibitor linagliptin alleviates liver steatosis and structural changes in the hepatic microvasculature and lymphatic roots in a model of NAFLD in diabetic *db/db* mice.

Key words: Diabetes; Obesity; Non-alcoholic fatty liver disease; Dipeptidyl peptidase 4; Linagliptin

Core tip: Dipeptidyl peptidase 4 (DPP4) inhibitors are a relatively new class of hypoglycemic agents with multiple pleiotropic effects. In this study, we demonstrated that the DPP4 inhibitor linagliptin alleviates liver steatosis and diminishes structural changes in hepatic non-parenchymal compartments in *db/db* diabetic mice. The mechanism of the beneficial effect of linagliptin seems to be glucose-independent as no obvious hypoglycemic activity of the agent was observed in this model. The results of the study provide further evidence that linagliptin could be a promising agent for the treatment of non-alcoholic fatty liver disease in subjects with type 2 diabetes.

Michurina SV, Ishenko IJ, Klimontov VV, Archipov SA, Myakina NE, Cherepanova MA, Zavjalov EL, Koncevaya GV, Konenkov VI. Linagliptin alleviates fatty liver disease in diabetic *db/db* mice. *World J Diabetes* 2016; 7(19): 534-546 Available from: URL: http://www.wjgnet.com/1948-9358/full/v7/i19/534.htm DOI: http://dx.doi.org/10.4239/wjd.v7.i19.534

INTRODUCTION

Diabetes is associated with a spectrum of liver diseases, including non-alcoholic fatty liver disease (NAFLD) and steatohepatitis[1]. The current treatment for NAFLD primarily focuses on alleviating metabolic syndrome components *via* lifestyle modifications. However, the lack of success in their implementation and sustainment results in the need for effective pharmacological agents for the treatment of fatty liver[2]. Dipeptidyl peptidase 4 (DPP4) inhibitors are considered a new treatment option for NAFLD in patients with diabetes[3-5]. DPP4 inhibition reduces hepatic fat in experimental models of NAFLD[6-9], but the underlying mechanisms remain to be clarified. Several clinical trials are exploring the efficacy of DPP4 inhibitors for the treatment of NAFLD[5,10-12]. DPP4 inhibitors might have a beneficial effect on hepatic steatosis and serum transaminase activity, but the data regarding the effects of DPP4 inhibitors on liver histology are scarce.

Although DPP4 inhibitors have the same mode of action, they differ by some important pharmacokinetic and pharmacodynamic properties that may be clinically relevant. Linagliptin is a highly specific, potent inhibitor of DPP4 that is currently indicated for the treatment of type 2 diabetes (T2D). In clinical studies, linagliptin effectively reduced glycated hemoglobin (HbA1c) levels in patients with T2D and exhibited a placebo-like safety and tolerability profile[13]. Linagliptin has an interesting pharmacokinetic profile in terms of its predominantly non-renal elimination. Fecal excretion is the dominant excretion pathway of linagliptin[14]. This DPP4 inhibitor is mainly excreted unchanged *via* bile, but is also excreted directly into the gut independent of biliary excretion[15]. Linagliptin also accumulates in hepatic tissue and exhibits both anti-inflammatory and anti-steatotic activity in a model of non-alcoholic steatohepatitis in streptozotocin-treated neonatal mice on a high-fat diet[8]. Long-term linagliptin treatment reduces liver fat content in mice with diet-induced hepatic steatosis and insulin resistance[6].

Histopathological changes that occur with NAFLD are not limited by changes in the hepatic parenchyma. Involvement of other cell types (sinusoidal endothelial cells, Kupffer cells, and stellate cells) and the recruitment of inflammatory cells and platelets lead to abnormal microcirculation and impaired intrahepatic fluid transport[16,17]. Despite the accumulating data on the favorable influence of DPP4 inhibitors on liver steatosis, the effects of these agents on non-parenchymal cells, bile transport, microcirculation and lymphatic drainage in the liver remain unknown. Therefore, we studied the long-term effects of the DPP4 inhibitor linagliptin on structural changes in hepatocytes, endothelial sinusoidal cells, and the interstitial compartments of the liver in *db/db* mice with obesity and T2D.

MATERIALS AND METHODS

Animal experiments

Twenty-four specific pathogen free (SPF) male *db/db* mice (BKS.Cg-*Dock7*^{m}+/+*Lepr*^{db}/J) were utilized for the experiments. Mice homozygous for the diabetes spontaneous mutation (Lepr^{db}) became identifiably polyphagic and obese at approximately 3 to 4 wk of age and exhibited elevated blood glucose from 4-8 wk. The animals were acclimatized to laboratory conditions for two weeks prior to experimentation. The mice were housed in individually ventilated cages (Animal Care Systems, Colorado, United States) in groups of one to four animals per cage with ad libitum food (Ssniff, Soest, Germany) and water. The mice were housed in a room within an SPF animal facility with a regular 14/10 h light/dark cycle (lights on 02:00 AM), a constant room temperature of 24 ℃ ± 2 ℃, and a relative humidity of approximately 45% ± 10%.

After randomization, the experimental group of animals (n = 8) received linagliptin *(*Boeringer Inghelheim*)* at a dose of 10 mg/kg of body weight diluted in 200 μL of saline. Mice randomized to the "placebo" treatment (n = 8) received 200 μL of saline under the same scheme. Linagliptin or placebo was administered by gavage once per day for 56 d from the 10th to 18th week of age. Intragastric gavage administration was performed with conscious animals using straight gavage needles appropriate for the animal size. The control group was comprised of intact *db/db* male mice (n = 8).

At the 18th week, all mice were sacrificed by cervical dislocation under anesthesia. Liver samples were obtained for histological assessments, ultrastructural examinations and immunohistochemistry.

Outcomes

All mice were weighed weekly during the experiment using electronic scales. Blood samples were obtained from the retro-orbital sinus of linagliptin-treated and placebo-treated mice at the 10th, 14th and 18th weeks. No stress-inducible procedures, including blood sample collections, were performed in intact animals. Blood samples were centrifuged to obtain plasma that was stored at -20 ℃ until analysis. The levels of glucose, triglycerides, total cholesterol, alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT) in the blood plasma were measured using automatic clinical chemistry system (Dade Behring Inc, United States) and commercially available cartridges according to the manufacturer's instructions (Dimension Clinical kit, Siemens, United States).

Liver samples for the light-optical studies were fixed in 10% formalin (pH = 7.4), dehydrated in alcohol at increasing concentrations and embedded in Histomix material (BioVitrum, Russia). Sections 3-4 microns thick were prepared on a microtome LEICA RM2155 (Germany, Switzerland) and were stained with Mayer's hematoxylin and eosin (H and E). Liver samples for electron microscopy were fixed in a 4% solution of paraformaldegid with 0.1 mol/L phosphate buffer (PB, pH = 7.4) followed by 1% OsO_4. The samples were then dehydrated and embedded in Epon-812. Using the LEICA TM UC7 ultratom (Germany), semi-thin sections (1 micron thick) were prepared and stained with toluidine blue. Liver sections 35-45 nm thick were contrasted with aqueous uranyl acetate solution and lead citrate and were studied with the JEOL JEM-1400 electron microscope (Japan).

A morphometric analysis of computed digital images of semi-thin sections from the livers of placebo-treated and linagliptin-treated mice was used to evaluate liver steatosis. Specifically, we calculated the proportion of hepatocytes containing lipid droplets and the distribution of hepatocytes with different lipid droplet densities. Hepatocytes were attributed to a cell population with a high density of lipid inclusions if more than 15 lipid droplets were revealed in the cytoplasm. Low lipid accumulation density was defined as hepatocytes containing less than five droplets. Microvesicular steatosis was defined by the presence of small cytoplasmic lipid droplets around a centrally positioned nucleus. Steatosis was considered mediovesicular when several medium-sized lipid vacuoles were present in the cytoplasm of the hepatocytes[18]. Macrovesicular steatosis was recorded when the diameter of the lipid droplets exceeded half of the hepatocyte nucleus diameter. We also calculated the numeral density of hepatocytes with different sized lipid droplets and estimated the proportions of cells with micro-sized, middle-sized and macro-sized lipid droplets in the cytoplasm. For cases in which the lipid droplets were of different sizes, each cell was taken into account twice or thrice.

Immunohistochemical detection of the lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) marker was performed on 3-mm thick sections from the livers of intact, placebo-treated and linagliptin-treated mice using an indirect avidin-biotin ABC-peroxidase method with the VECTASTAIN Universal Quick Kit (Vector Laboratories, United States). Blocking of endogenous peroxidase was performed by incubating the sections in a 0.3% H_2O_2 solution for 10 min with a subsequent incubation in normal horse non-immune blocking serum for 20 min. Next, the sections were incubated for one

Table 1 Body weight and plasma biochemical parameters of the placebo-treated and linagliptin-treated *db/db* mice at the 10th, 14th and 18th week of age

Parameters	Placebo group (n = 8)			Linagliptin group (n = 8)		
	10 wk	14 wk	18 wk	10 wk	14 wk	18 wk
Body weight, g	35.1 (25.6-47.7)	33.2 (24.6-50.9)	37.3 (27.3-51.6)	37.6 (34.5-44.2)	39.9 (30.0-42.3)	41.7 (31.5-45.0)
Glucose, mg/dL	637 (549-678)	579 (551-671)	610 (506-683)	651 (631-693)	588 (520-640)	625 (601-646)
Triglycerides, mg/dL	415 (209-510)	324 (209-336)	316 (149-555)	385 (262-637)	279 (251-315)	391 (238-480)
Total cholesterol, mg/dL	129 (94-156)	100 (39-140)	131 (22-156)	112 (28-132)	104 (24-124)	71 (18-120)
ALT, U/L	132 (105-375)	126 (72-369)	170 (69-306)	146 (84-255)	185 (118-225)	203 (80-294)
GGT, U/L	16.7 (8.2-28.1)	13 (8.5-25.1)	14.6 (10.5-16.5)	14.4 (8.9-18.7)	12.5 (7.1-22)	13.6 (10.5-22.7)

Data are shown as the medians, minimal and maximal values. No significant differences in the variables in both groups at week 10 and 18 (Wilcoxon signed rank test, all $P > 0.05$). The differences between groups are not significant at week 10, 14 and 18 (U-test, all $P > 0.05$). ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transpeptidase.

hour at room temperature with anti-LYVE-1 (Isotype: Rabbit polyclonal, bs-1311R; Bioss) at a final dilution of 5 mg/mL; washed in 3 changes of phosphate buffer for 3 min; and further incubated for 30 min at room temperature with a biotinylated second antibody followed by washing in 3 changes of phosphate buffer for 5 min. Incubation with the ABC-peroxidase complex was performed for 30 min at room temperature followed by washing in 3 changes of phosphate buffer for 5 min. Immunohistochemical staining of the sections was performed with a chromogenic substrate (ImmPACT DAB, Vector Laboratories, United States). To quantify the LYVE-1 staining, computed morphometric analysis of the digital images was performed using the "VideoTest Morpho 3.2" program.

Ethical issues

All animal experiments were performed in compliance with the protocols and recommendations for the proper use and care of laboratory animals (ECC Directive 86/609/EEC). The protocol was approved by the Ethics Committee of Institute of Clinical and Experimental Lymphology (Protocol Number 1/2, April 1, 2014), and by the Inter-Institutional Animal Ethics Committee based on the Institute of Cytology and Genetics SB RAS (Permission Number: 21, April 1, 2014). All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Russian National Center of Genetic Resources of Laboratory Animals based on the SPF Vivarium of Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia (Permit Number: 246, April 8, 2014). All efforts were made to minimize the number of animals used and their pain or discomfort.

Statistical analysis

Statistical processing of the results was performed using the STATISTICA software package 10 (StatSoft Inc., United States). A statistical review of the study was performed by a biomedical statistician. The Shapiro-Wilk test was used for testing normality. For the analysis of normally distributed quantitative data, the mean (M) and standard error of the mean (SEM) were calculated. The significance of differences between the groups was assessed by Student's t-test. Non-normally distributed data (body weights and biochemical parameters) are presented as medians with minimum and maximum values; the significance of differences was determined using the non-parametric Mann-Whitney U-test or Wilcoxon signed rank test for repeated measurements. The differences were considered significant at $P < 0.05$.

RESULTS

Body weight and biochemical parameters

As expected, *db/db* mice became obese by week 10. The weight of the animals remained stable throughout experiment (Table 1). All animals had severe hyperglycemia at the 10th week with plasma glucose levels of 506 mg/dL (28.1 mmol/L) or more. The glucose levels remained elevated throughout the experiment in both the linagliptin and placebo groups. No significant differences in the levels of glucose, triglycerides, total cholesterol, ALT and GGT were observed between the groups at week 10, 14 or 18.

Liver histology

We observed diffuse lipid accumulation in the livers of all 18-wk-old *db/db* diabetic mice. Lipid droplets were found in 92.4% ± 1.7% of hepatocytes per field of view. Microvesicular and mediovesicular steatosis was the principal morphological finding, although sporadic large lipid droplets were also observed (Figure 1). Vacuolar degeneration was found in the pericentral and intermediate zones of predominantly hepatic lobuli. In some cells, glycogenized nuclei were noticed. The dilation of interlobular arteries and veins, central and sublobular veins, lymphatic vessels and bile canaliculi was present in most of the histological preparations (Figure 2). These changes were accompanied by edema in the connective tissue layers. The sludge of erythrocytes was found in intralobular sinusoidal capillaries. We detected no signs of inflammatory infiltration or interstitial fibrosis.

The liver histology in placebo-treated mice was very similar to intact animals (Figures 3 and 4). We observed heterogeneous hepatocytes in mice treated with linagliptin. Although lipid infiltration was present in

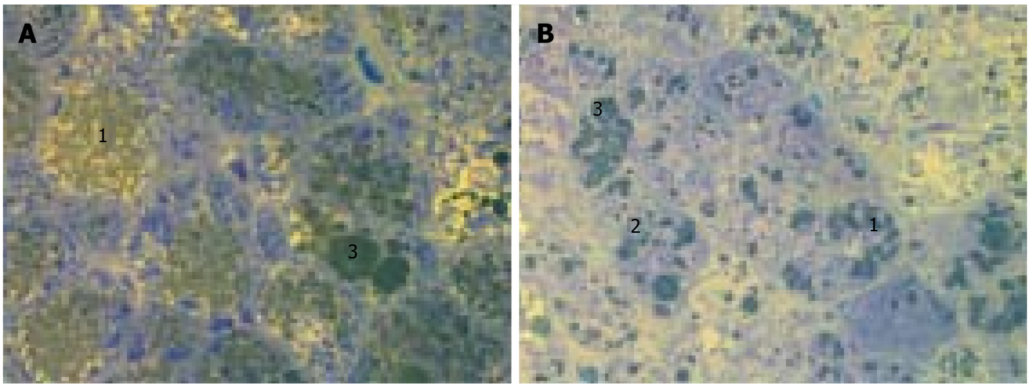

Figure 1 Liver histology in intact *db/db* mice. A, B: Microvesicular (1) and mediovesicular (2) lipid accumulation, sporadic large lipid droplets in hepatocytes (3). Light microscopy with yellow filter of semi-thin sections stained with toluidine blue; magnification × 1000.

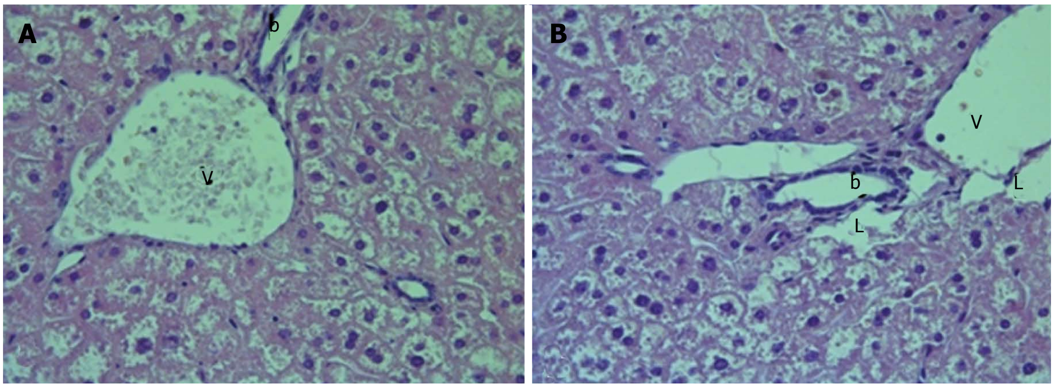

Figure 2 Liver histology in intact *db/db* mice. A, B: The dilatation of intelobular arteries and veins (v), lymphatic vessels (L) and bile canaliculi (b) was present in most of histological preparations. H and E; magnification × 400.

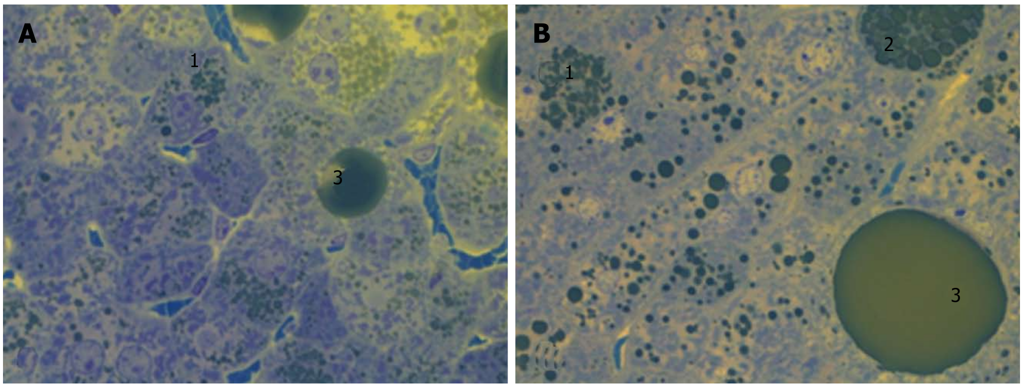

Figure 3 Liver histology in placebo-treated *db/db* diabetic mice. A, B: Microvesicular (1) and mediovesicular (2) lipid accumulation, sporadic large lipid droplets in hepatocytes (3). Light microscopy with yellow filter of semi-thin sections stained with toluidine blue; magnification × 1000.

some hepatocytes, other cells demonstrated preserved morphology (Figure 5). In the periportal zones, numerous diplocariocytes were found, which may be interpreted as a regenerative sign. In linagliptin-treated mice, compared to intact or placebo-treated mice, the dilation of blood and lymphatic vessels of the portal tracts, sublobular and central veins was less profound, and edema of the perisinusoidal lymphatic spaces was diminished (Figure 6). The severity of liver steatosis in the linagliptin group was alleviated. Specifically, the proportion of hepatocytes with a high numeral density of lipid droplets (> 15 per cell) was reduced significantly in the linagliptin group compared to the placebo group (20.7% ± 3.6% and 50.4% ± 3.1%, respectively, P = 0.0007; Figure 7). The mean percent of hepatocytes with lipid droplets per field of view was also decreased (linagliptin: 64.9% ± 5.8%, placebo: 92.4% ± 1.7%, P = 0.0002), mostly due to the reduction of microvesicular

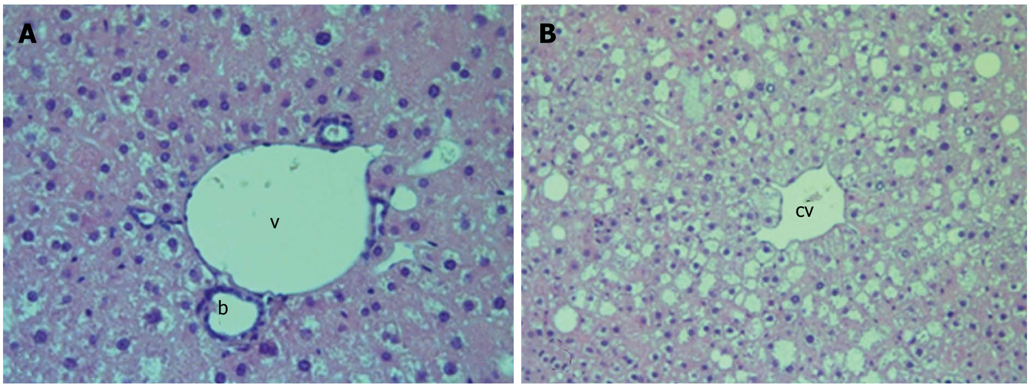

Figure 4 Liver histology in placebo-treated *db/db* mice. A: Dilatation of the interlobular veins (v), lymphatic vessels and bile ducts (b). H and E; magnification × 400; B: Extension of the central vein (cv), vacuolar degeneration of hepatocytes. H and E; magnification × 200.

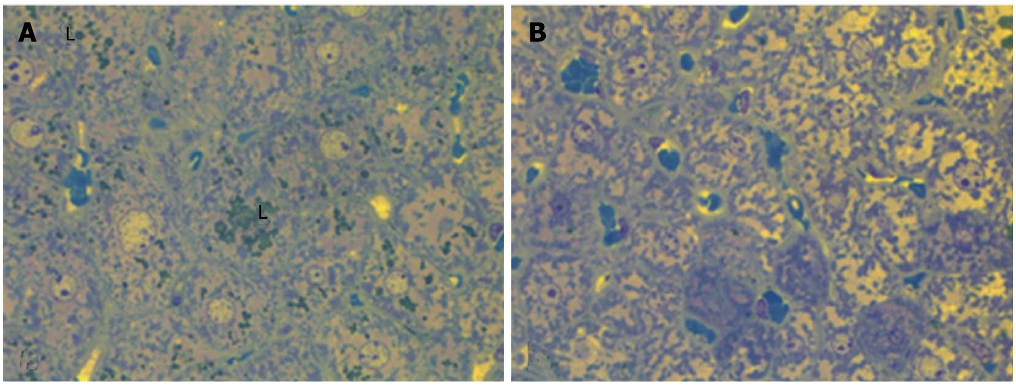

Figure 5 Liver histology in linagliptin-treated *db/db* diabetic mice. Heterogeneity of the changes of hepatocytes: A: Microvesicular lipid accumulation (L); B: No lipid accumulation. Light microscopy with yellow filter of semi-thin sections stained with toluidine blue; magnification × 1000.

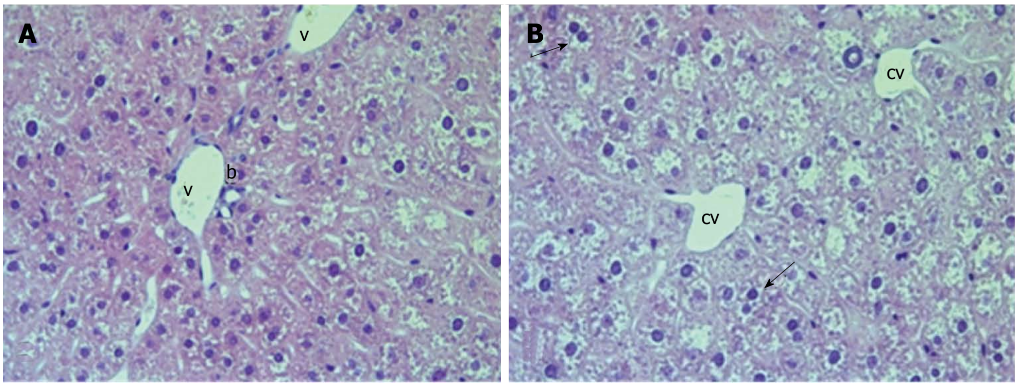

Figure 6 The liver of linagliptin-treated *db/db* diabetic mouse. The dilatation of blood and lymphatic vessels of portal tracts, central veins was less profound. Numerous diplocariocytes were present (arrows). V: The vein of portal tract; b: Bile duct of portal tract; cv: Central vein. H and E; magnification × 400.

and mediovesicular lipid accumulation (Figure 8).

Ultrastructural changes in the liver

In the hepatocytes of 18-wk-old intact mice, areas of hyperplasia of the smooth endoplasmic reticulum (ER) and lipid inclusions, predominantly small ones, were found *via* electron microscopy. We observed intense exocytosis of lipids into the Disse space and interstitial areas between hepatocytes. The hyperplasia of the microvilli on the vascular poles of hepatocytes was in concordance with enhanced lipid transport from the cells. The mitochondria were concentrated on the bile poles of hepatocytes and appeared condensed, with increased matrix density and indistinct cristae. Compartmentalization of the complexes of mitochondria and rough ER was found in many cells. We observed 1-2 active Golgi complexes, residual bodies and auto-phagosomes in addition to bile capillaries (Figure 9).

Ultrastructural changes in the placebo-treated mice were similar to those in intact animals. We observed

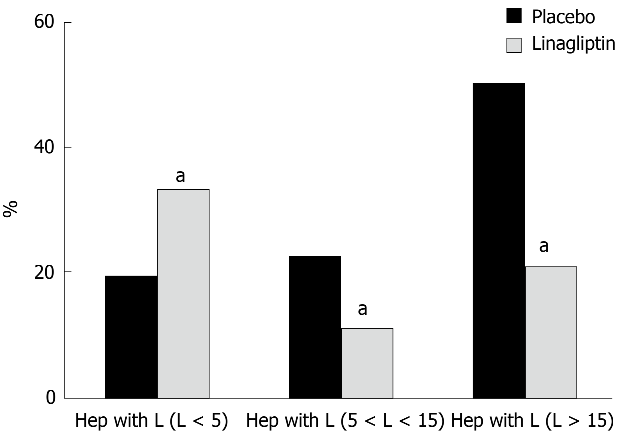

Figure 7 The mean proportions of hepatocytes with different densities of lipid droplets in linagliptin-treated and placebo-treated *db/db* mice. The percent of hepatocytes with high density of lipid droplets (more than 15 droplets per cell) is reduced in linagliptin-treated mice compared to placebo-treated mice (hep, hepatocyte, L < 5, less than 5 lipid droplets per cell, L > 15, more than 15 lipid droplets per cell, $^aP < 0.05$).

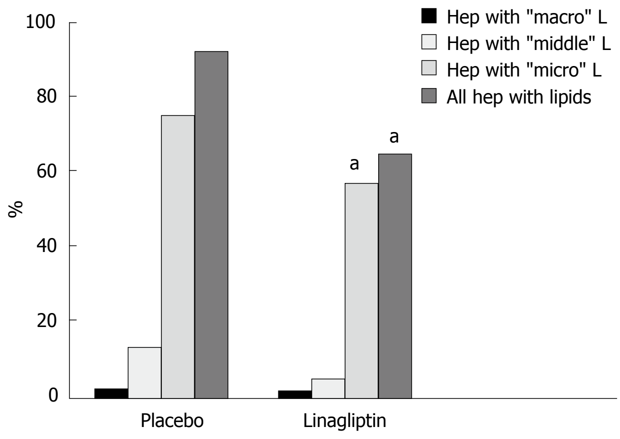

Figure 8 The distribution of hepatocytes with lipid inclusions depending on the size of lipid droplets in linagliptin-treated and placebo-treated *db/db* mice. The reduction in the numeral density of hepatocytes with microsized, mediosized and macrosized droplets in linagliptin-treated mice (hep, hepatocyte, $^aP < 0.05$ *vs* placebo group).

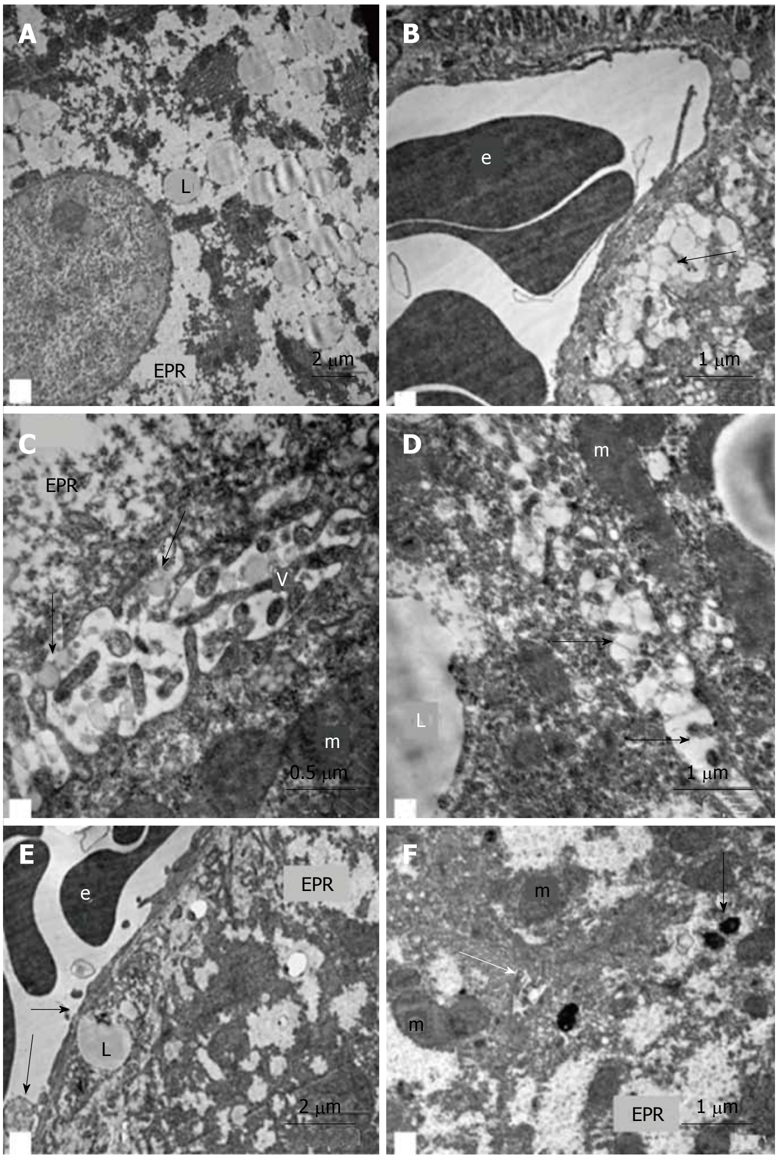

Figure 9 Ultrastructural changes in the hepatocytes of intact *db/db* mice. A: Fields of "foamy" hyperplastic smooth ER and fields of glycogen, lipid inclusions in the cytoplasm of hepatocytes; B and E: Pronounced exocytosis of vacuoles with lipid content into the Disse space (arrows); C and D: Pronounced exocytosis of vacuoles with lipid content into gaps between hepatocytes (arrows); F: The bile capillary (white arrow) and compartments of the mito-ER-complexes (complexes from ER and mitochondria), active Golgi complexes, residual bodies and autophagosomes (black arrow) at the biliary poles of hepatocytes. V: Microvilli on the lateral surface of hepatocytes; L: Lipid inclusions; m: Mitochondria; e: Erythrocyte; EPR: Endoplasmic reticulum.

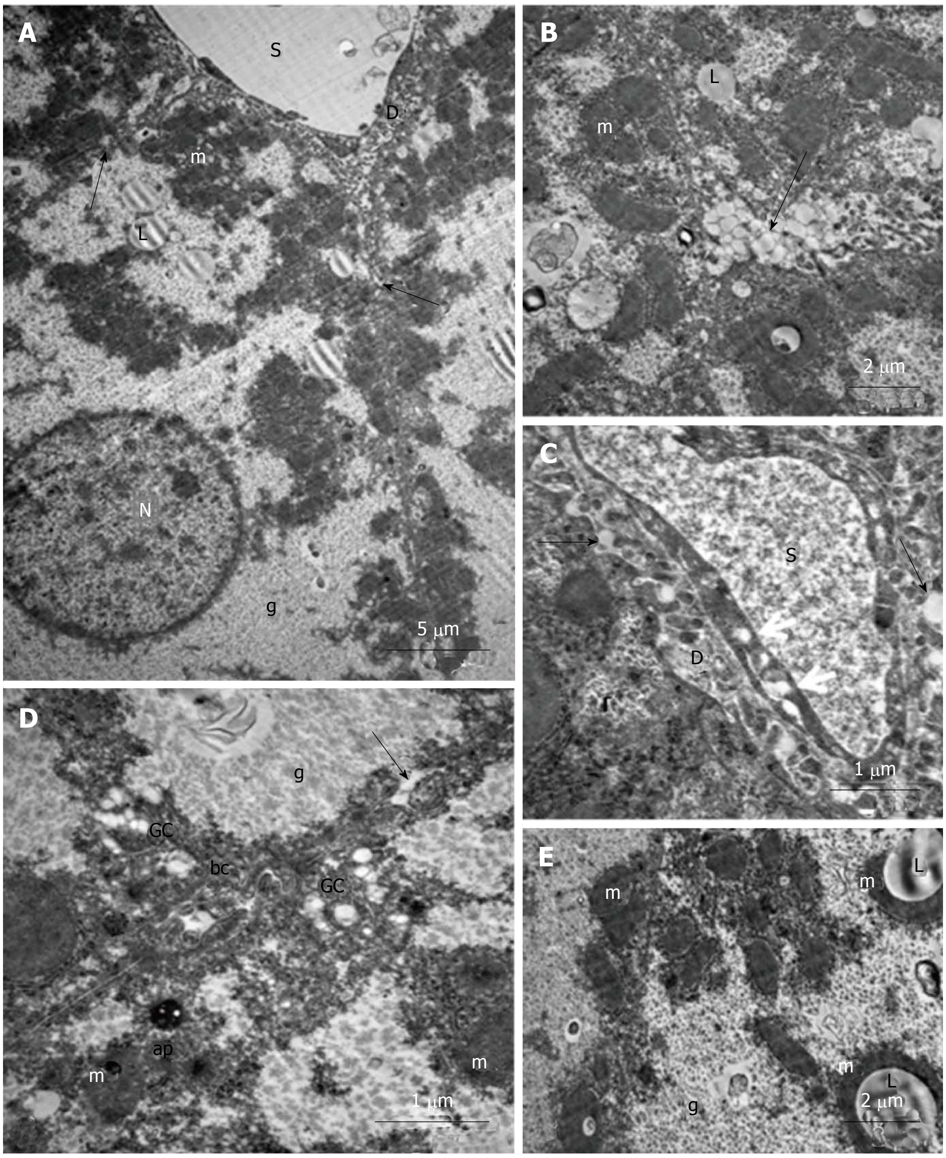

Figure 10 Ultrastructural changes in the hepatocytes of placebo-treated db/db mice. A: Fatty degeneration, numerous compartments of mito-ER-complexes, free ribosomes and polysomes, pronounced hyperplasia of the microvilli on the vascular poles and lateral sites of parenchymal cells, enlarged Disse spaces; the arrows indicate the extension between the lateral surfaces of adjacent hepatocytes; B: Hyperplasia of microvilli on the lateral parts of the hepatocytes and transport of lipid inclusions (arrow) into spaces between hepatocytes; C: The transport of lipid inclusions into Disse spaces (arrows), transport vacuoles into the cytoplasm of endothelial sinusoidal cells; D: Active Golgi complexes, autophagosomes with dark content and ribosomes in peribiliary areas of hepatocytes; the arrow shows the transport of lipid inclusions into the gap between hepatocytes; E: Structural complexes of lipid inclusions with mitochondria. ap: Autophagosome; g: Glycogen granules; D: The Disse space; bc: Bile capillary; GC: Golgi complex; L: Lipid inclusion; m: Mitochondria; s: Lumen of the sinusoid; e: Erythrocyte; N: The nucleus.

microvesicular and mediovesicular lipid inclusions, numerous compartments of mitochondria-ER complexes, as well as marked hyperplasia of the microvilli on the vascular and lateral poles of hepatocytes. The Disse space and gaps between hepatocytes were enlarged (Figure 10A). Intense exocytosis of small lipid droplets into the gaps between hepatocytes was observed (Figure 10B). Additionally, we found the exocytosis of lipid-containing vacuoles into the enlarged Disse space (Figure 10C). In the peribiliar areas of some hepatocytes, we observed 1-3 active Golgi complexes and autophagosomes with dense content and ribosomes (Figure 10D). Mitochondria complexes with lipid inclusions were also present (Figure 10E).

In the livers of linagliptin-treated mice, we observed heterogeneous ultrastructural changes. There were parenchymal cells with lipid accumulation and hyperplasia of the smooth ER (Figure 11A and B). Some hepatocytes demonstrated preserved (almost normal) cellular organization. In the cytoplasm of other cells, we observed zones of destructive ER membranes and quantities of free ribosomes and polyribosomes (Figure 11C and D). Aggregates from mitochondria, rough ER and lipids were present in some images (Figure 12A). In the peribiliary zones of some hepatocytes, we found myelin structures, vacuoles of Golgi complex and autophagosomes (Figure 12B). Hepatocytes with no ER hyperplasia and a homogenous distribution of mitochondria were observed in the livers from linagliptin-treated mice. The presence of large vacuoles with lipid content in the cytoplasm of endothelial sinusoidal cells was another structural feature of this group (Figure 12D).

Staining for LYVE-1

In 18-wk-old intact or placebo-treated diabetic mice, we detected immunohistochemical staining for LYVE-1 in the endothelial cells of interlobular lymphatic vessels and on the membranes of some endothelial sinusoidal cells. The LYVE-1 staining was intensified in linagliptin-treated animals compared to intact or saline-treated animals (Figure 13). An enlarged LYVE-1 reaction area was observed in the linagliptin group as revealed by

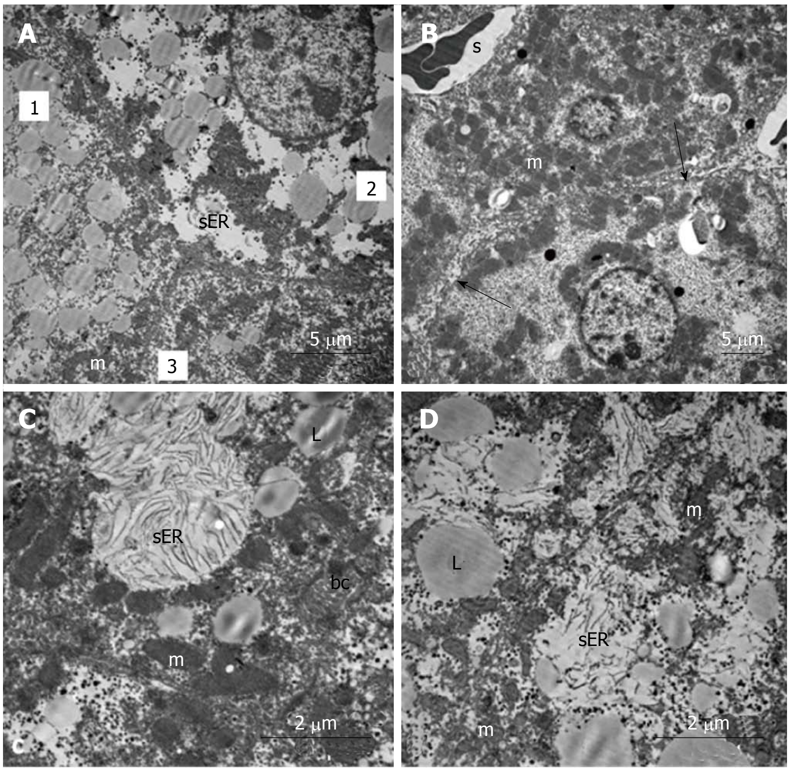

Figure 11 Ultrastructural changes in the hepatocytes of linagliptin-treated *db/db* mice. A: Heterogeneity of the hepatocytes: Cells with numerous lipid inclusions (1), cells with areas of hyperplasia of smooth ER and lipid vacuoles (2), cells with a relatively uniform distribution of organelles and rare lipid inclusions (3); B: Cells without hyperplasia of the smooth ER with a relatively homogenous distribution of organelles; distinct microvilli on vascular poles of the hepatocytes and on the lateral sides of parenchymal cells; the extension of spaces between hepatocytes (arrows); C and D: Plots of clusters of smooth ER membranes. L: Lipid inclusion; m: Mitochondria; s: Lumen of the sinusoid; SER: Smooth endoplasmic reticulum; bc: Bile capillary.

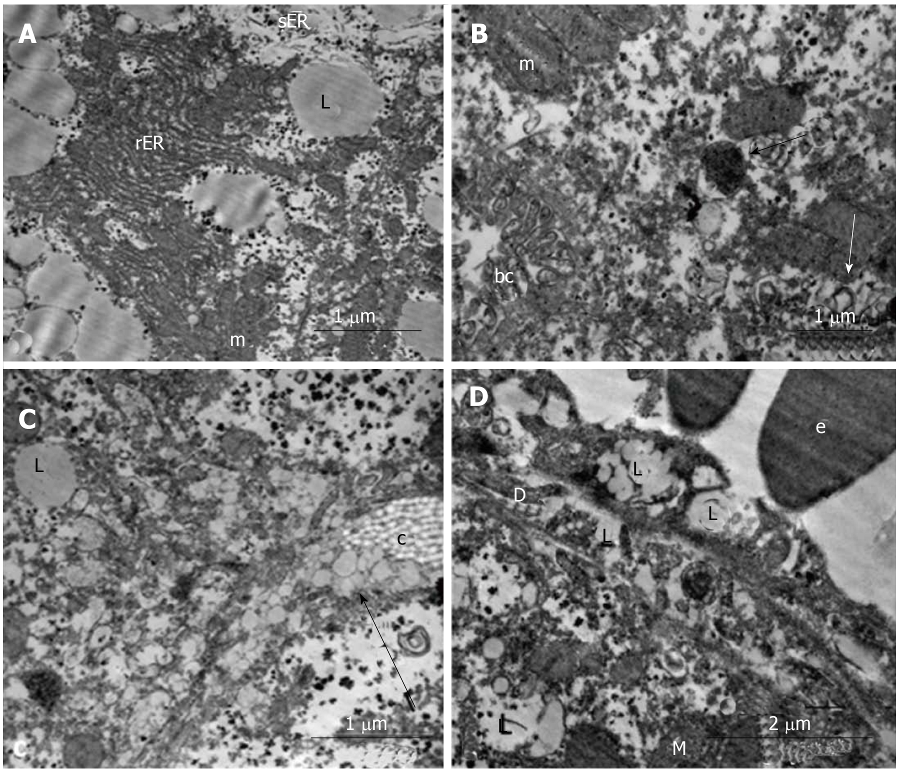

Figure 12 Ultrastructural changes in the hepatocytes of linagliptin-treated db/db mice. A: The complexes from the mitochondria, rough ER and lipid droplets; B: Mitochondria with separate granular ER profiles, myelin structures (white arrow), autophagosomes with electrondark content and ribosomes (black arrow) nearby the bile capillaries with pronounced microvilli; C: The transport of lipids into the gaps between hepatocytes (arrow); D: Large vacuoles in the cytoplasm of endothelial cells in the sinusoids. rER: Rough endoplasmic reticulum; sER: Smooth endoplasmic reticulum; bc: Bile capillary; D: The Disse space; c: A tuft of collagen; L: Lipid inclusion; m: Mitochondria; e: Erythrocyte.

morphometric analysis (Figure 14).

DISCUSSION

DPP4 inhibitors are a relatively new class of hypoglycemic agents that have a broad application for the treatment of diabetes worldwide. A growing body of evidence indicates that DPP4 inhibitors could produce multiple pleiotropic effects independent of lowering glucose levels[19,20]. In this study, we demonstrated the beneficial effects of the DPP4 inhibitor linagliptin on both parenchymal and non-parenchymal hepatic cells in T2D *db/db* mice (BKS. Cg-*Dockm*^{M}+/+*Lepr*^{db}/J). Our results demonstrate the protective effects of linagliptin on hepatocytes, sinusoidal cells and the roots of the hepatic lymphatic system in a T2D model.

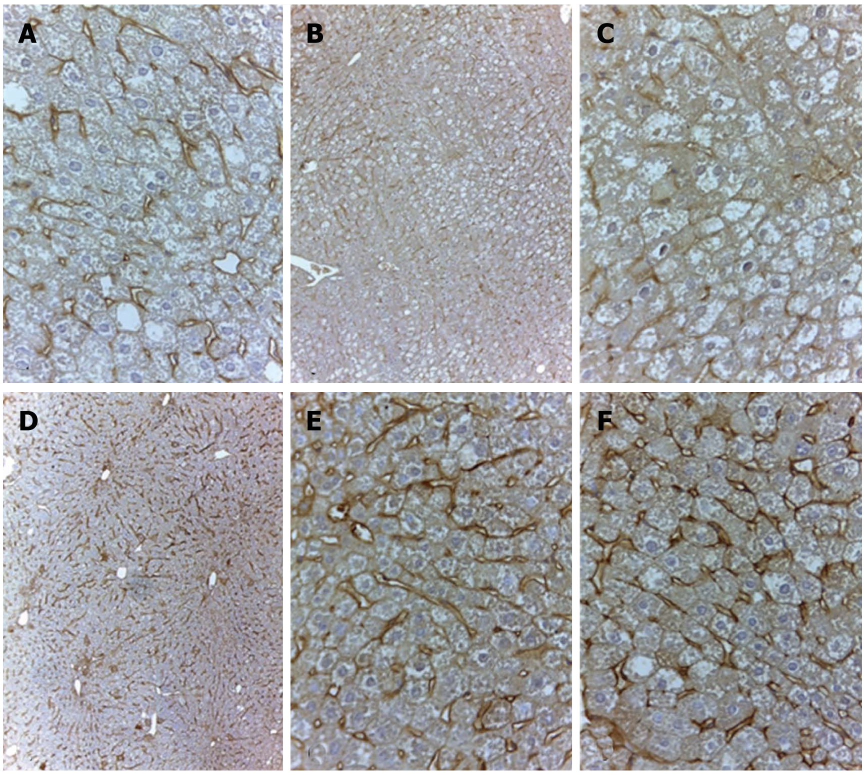

Figure 13 Immunohistochemical staining for lymphatic vessel endothelial hyaluronan receptor-1 in the liver of intact (A and B), placebo-treated (C) and linagliptin-treated (D, E and F) *db/db* mice. Staining by anti-LYVE-1 antibodies, indirect streptavidin-biotin method; A, C, E and F: × 400; B and D: × 100. LYVE-1: Lymphatic vessel endothelial hyaluronan receptor-1.

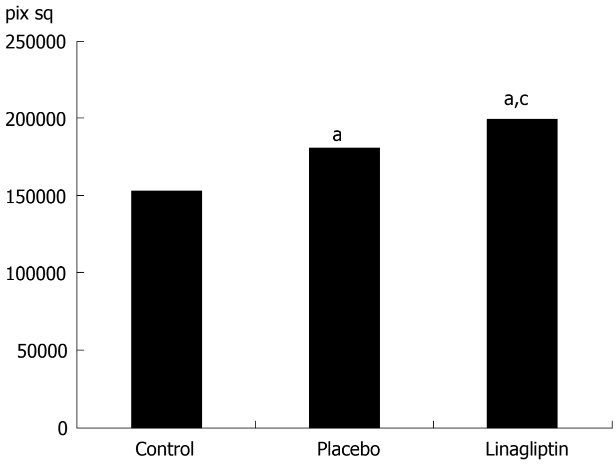

Figure 14 The area of immunohistochemical staining for lymphatic vessel endothelial hyaluronan receptor-1 in the liver of intact, placebo-treated and linagliptin-treated db/db mice. $^aP < 0.05$ *vs* control group (intact animals); $^cP < 0.05$ *vs* placebo group. pix sq: Square pixel.

Expectedly, lipid accumulation in the liver was the principal morphological finding characterizing the development of NAFLD in *db/db* mice. Specifically, micro-vesicular and mediovesicular steatosis were prevalent. However, we observed no evident signs of inflammation or fibrosis.

Structural changes in the ER and mitochondria were found in hepatocytes by electron microscopy. In particular, we observed compartmentalization of the complexes of mitochondria and rough ER. Although the ER and mitochondria play distinct cellular roles, these organelles also form physical interactions with each other at sites defined as mitochondria-associated ER membranes, which are essential for calcium, lipid and metabolite exchange. In the liver, obesity leads to a marked reorganization of mitochondria-associated ER membranes resulting in mitochondrial calcium overload, compromised mitochondrial oxidative capacity and augmented oxidative stress[21]. Mitochondrial dysfunction and ER stress or the unfolded protein response contribute to hepatocyte cell death during alterations of lipid and fatty acid metabolism[22]. An association between microvesicular steatosis and apoptosis was demonstrated recently in an NAFLD diabetic model[18].

Consistent with the findings of another research group[6,8], we documented the amelioration of liver steatosis in linagliptin-treated animals. The phenomenon of hepatocyte heterogeneity with the emergence of a relatively preserved cell structure was observed in the linagliptin group. Additionally, the ultrastructural changes in hepatocyte ER and mitochondria were alleviated by linagliptin treatment. Because we observed a preserving effect of the DPP4 inhibitor on ER and mitochondria structure, we anticipate improvement of hepatocyte synthetic function and energy expenditure. Modulation of mitochondrial function upon DPP4 inhibition has been recently described. In a model of Western-diet induced liver steatosis, DPP4 inhibitor MK0626 significantly reduced mitochondrial incomplete palmitate oxidation and increased the indices of pyruvate dehydrogenase activity[9].

As far as we know, we provide here the first detailed description of the morphological changes in the hepatic interstitium of *db/db* mice. The data indicate deviations in the structure of the interlobular blood vessels, hematolymphatic barrier and intrahepatic lymphatic collectors. Dilation of the roots of the lymphatic system, venous collectors and bile ducts provide morphological evidence of the impairment of lymphatic drainage and bile collection in this model of NAFLD. We also observed morphological signs of enhanced lipid transport into the interstitial tissue between hepatocytes and into the Disse space in *db/db* mice. Because hepatocyte homeostasis is intimately associated with blood microcirculation and lymphatic drainage, the changes in parenchymal cells and non-parenchymal compartments of the liver in subjects with diabetes could be mutually deteriorated.

We observed imminohistochemical staining for LYVE-1 in the endothelial cells of interlobular lymphatic vessels and on the membranes of endothelial sinusoidal cells in intact and saline-treated *db/db* mice. The LYVE-1 molecule is considered the primary immunohistochemical marker of lymphatic endothelial cells[23]. Nevertheless, LYVE-1 can be expressed by other cell types, including sinusoidal cells in the liver[24,25]. As a transmembrane receptor, LYVE-1 is involved in the transport and turnover of hyaluronan and may play a role in lymphangiogenesis[26]. The reduced expression of LYVE-1 in sinusoidal cells was reported previously in human chronic hepatitis and liver cirrhosis. A loss of fenestrae in the sinusoidal endothelium was observed in the damaged areas with low LYVE-1 expression. Interestingly, LYVE-1 attenuation in the sinusoidal endothelium is one of the manifestations of capillarization and is associated with hepatic disease progression[25]. We report here that linagliptin potently enhances the expression of LYVE-1 in the endothelial cells of interlobular lymphatic vessels and on the membranes of endothelial sinusoidal cells. Considering the previously mentioned data, we speculate that this phenomenon is associated with the activation of transendothelial transport and lymphatic drainage.

Importantly, the liver histology in linagliptin-treated mice improved significantly despite the absence of an obvious effect on hyperglycemia. Other authors also observed no significant effects of linagliptin on the glucose metabolism parameters of diabetic *db/db* mice[27]. Nevertheless, it has been documented that a protective effect of linagliptin on the kidneys could be achieved independent of the hypoglycemic action in this model of diabetes[27,28]. Although some of the effects of DPP4 inhibitors could be due to an overall improvement in the metabolic parameters, no data support improvements independent of weight loss or *via* direct effects on hepatocytes *in vitro*. In experimental and clinical diabetes, DPP4 activity in the blood serum and liver does not correlate with mean glucose or glycated hemoglobin A1c levels, which are both related to hepatic lipogenesis and liver damage[29]. The glucose-independent action of linagliptin in NAFLD could be mediated, at least partially, *via* the prolongation of the GLP-1 half-life and the extending GLP-1 effects in the liver. Multiple hepatocyte signal transduction pathways appear to be activated by GLP-1 and its analogues, and both cAMP-activated protein kinase and Akt are proposed key players in improving hepatic steatosis[3,30].

DPP4 itself might be an important target molecule in NAFLD. The liver expresses high levels of DPP4, and recent accumulating data suggest that DPP4 is involved in the development of various chronic liver diseases, such as NAFLD, hepatitis C virus infection, and hepatocellular carcinoma. In addition to its peptidase activity, DPP4 is associated with immune stimulation, binding to and the degradation of the extracellular matrix, resistance to anti-cancer agents, and lipid accumulation. Furthermore, DPP4 is expressed in hepatic stem cells and plays a crucial role in hepatic regeneration[29]. Normal and high fat diet fed DPP4-deficient rats exhibited reduced hepatic triglycerides, accompanied by the down-regulation of lipogenesis enzymes and the parallel up-regulation of carnitine palmitoyltransferase-1, a key enzyme in fatty acid β-oxidation[30]. Rats with DPP4 deficiency have improved bile secretory function in a high fat diet-induced steatosis model[7]. In patients with T2D and/or morbid obesity, circulating DPP4 activity is associated with current apoptosis and liver fibrosis[31].

Thus, it is highly plausible that the observed improvement in liver histology following linagliptin treatment could be mediated by both the prolongation of GLP-1 effects and the inhibition of hepatic DPP4 activity *per se.*

The results demonstrate the favorable effect of long-term linagliptin treatment on the liver structure of obese *db/db* mice with T2D. In this model of NAFLD, linagliptin alleviates structural signs of steatosis, and disturbances in microcirculation and lymphatic drainage. The improvement in the structural parameters of the liver in linagliptin-treated mice was independent to changes in the plasma glucose levels.

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COMMENTS

Background

Dipeptidyl peptidase 4 (DPP4) inhibitors are a relatively new class of hypoglycemic agents with multiple pleiotropic effects. The ability of DPP4 inhibitors to modify the development of diabetic complications remains unclear. It was recently demonstrated that some DPP4 inhibitors result in hepatic fat reduction in experimental models of non-alcoholic fatty liver disease (NAFLD). Preliminary data indicate that DPP4 inhibitors might have a beneficial effect on hepatic steatosis and serum transaminase activity, but the data on their effects on liver histology are limited.

Research frontiers

Despite the accumulating data on the favorable influence of DPP4 inhibitors on liver steatosis, the effects of these agents on non-parenchymal cells, bile transport, microcirculation and lymphatic draining in the liver remain unknown.

Innovations and breakthroughs

In this study, the authors demonstrated for the first time that the DPP4 inhibitor linagliptin not only alleviates liver steatosis but also diminishes structural changes in hepatic non-parenchymal compartments in *db/db* diabetic mice. Incremental changes in the lymphatic vessel endothelial hyaluronan receptor-1 expression in the endothelial cells of interlobular lymphatic vessels and on the membranes of some endothelial sinusoidal cells under linagliptin treatment may improve impaired lymphatic drainage and sinusoid function in NAFLD. The mechanism of the beneficial effect of linagliptin seems to be glucose-independent as no obvious hypoglycemic effect of the agent was observed in this model.

Applications

The results of this study provide further evidence that linagliptin could be a promising agent for the treatment of NAFLD in subjects with type 2 diabetes. Further studies regarding the effects of DPP4 inhibitors on liver structure and function in diabetes are urgently needed.

Terminology

Sinusoidal cells, a non-parenchymal cell population in the liver that includes sinusoidal endothelial cells, Kupffer cells, Ito cells and Pit cells. Lymphatic vessel endothelial hyaluronan receptor-1, a transmembrane receptor for the extracellular matrix glycosaminoglycan hyaluronan.

Peer-review

The investigation by Michurina *et al* aimed to study the effects of Linagliptin on the structural signs of non-alcoholic fatty liver disease in *db/db* mice. This is an interesting work form a basic science point of view, that may have clinical practice consequences.

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ORIGINAL ARTICLE

Observational Study

Effect of pioglitazone on nerve conduction velocity of the median nerve in the carpal tunnel in type 2 diabetes patients

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Abstract

AIM
To evaluate the impact of pioglitazone pharmacotherapy in median nerve electrophysiology in the carpal tunnel among type 2 diabetes patients.

METHODS
The study was executed in patients with type 2 diabetes, treated with oral drugs, categorized under pioglitazone or non-pioglitazone group (14 in each group), and who received electrophysiological evaluation by nerve conduction velocity at baseline and 3 mo.

RESULTS
At 3 mo, pioglitazone-category had inferior amplitude in sensory median nerve [8.5 interquartile range (IQR) = 6.5 to 11.5) *vs* non-pioglitazone 14.5 (IQR 10.5 to 18.75)] (P = 0.002). Non-pioglitazone category displayed amelioration in amplitude in the sensory median nerve [baseline 13 (IQR = 9 to 16.25) *vs* 3 mo 8.5 (IQR = 6.5 to 11.5)] (P = 0.01) and amplitude in motor median nerve [baseline 9 (IQR = 4.75 to 11) *vs* 3 mo 6.75 (IQR = 4.75 to 10.25)] (P = 0.049); and deterioration of terminal latency of in motor ulnar nerve [baseline 2.07 (IQR = 1.92 to 2.25) *vs* 3 mo 2.16 (IQR = 1.97 to 2.325)] (P = 0.043). There was amelioration of terminal latency in sensory ulnar nerve [baseline 2.45 (IQR = 2.315 to 2.88) *vs* 3 mo 2.37 (IQR = 2.275 to 2.445) for pioglitazone group (P = 0.038).

CONCLUSION
Treatment with pioglitazone accentuates probability of compressive neuropathy. In spite of comparable glycemic control over 3 mo, patients treated with pioglitazone showed superior electrophysiological parameters for the ulnar nerve. Pioglitazone has favourable outcome in nerve electrophysiology which was repealed when the nerve was subjected to compressive neuropathy.

Key words: Pioglitazone; Adipocytes; Diabetes mellitus; Neuropathy; Carpal tunnel

Core tip: Significant findings of the study: (1) Non-pioglitazone group showed favourable outcome in amplitude in the sensory and motor median nerve, and aggravation of terminal latency of motor ulnar nerve; and (2) Pioglitazone group showed favourable outcome of terminal latency in sensory ulnar nerve. What this study adds: (1) Pioglitazone has beneficial effect on nerve electrophysiology; and (2) The beneficial effect is nullified by the higher risk of compressive neuropathy conferred.

Chatterjee S, Sanyal D, Das Choudhury S, Bandyopadhyay M, Chakraborty S, Mukherjee A. Effect of pioglitazone on nerve conduction velocity of the median nerve in the carpal tunnel in type 2 diabetes patients. *World J Diabetes* 2016; 7(19): 547-553 Available from: URL: http://www.wjgnet.com/1948-9358/full/v7/i19/547.htm DOI: http://dx.doi.org/10.4239/wjd.v7.i19.547

INTRODUCTION

The carpal tunnel is a fibro-osseous space in the wrist, bound anteriorly by the transverse carpal ligament and posteriorly by the pisiform and tubercle of scaphoid in the proximal part; and the tubercle of trapezoid and hook of hamate in the distal part[1]. Nine digital flexor tendons and the motor and sensory divisions of the median nerve pass through it, which also contains small amounts of adipose tissue. Pioglitazone, a peroxisome proliferator activator receptor gamma (PPAR-γ) agonist, is an oral antidiabetic agent. In recent years, however, the use of pioglitazone is somewhat decreasing in patients with type 2 diabetes due to its adverse effects including edema, heart failure, bone fractures and the possible risk for bladder cancer. Animal studies have demonstrated the conversion of pre-adipocytes to adipocytes under the influence of pioglitazone, although the mechanisms continue to remain elusive[2,3]. In confined spaces like the orbit, this action has been known to cause compressive symptoms in a subgroup of patients. The incidence is higher when there is associated thyroid disease[4]. The algorithms available for clinical and electro-diagnostic evaluation of carpal tunnel syndrome (CTS) continue to evolve. After assessment by standard tests (*viz*. "distal median motor latency", "antidromic sensory recording from median nerve"), CTS can be diagnosed and classified by severity from "extreme" to "mild"[5]. For the "distal median motor latency" test, "onset motor latency > 4.2 milliseconds is abnormal", so also is a "compound muscle action potential (CMAP) amplitude < 5 mV"[6]. Extreme CTS cases are further evaluated by motor comparison study. In positive cases, needle electromyography is imperative[5].

Accordingly, CTS can be electro-diagnostically grouped into 5 grades, as follows: "Grade 1 - Very mild CTS - normal standard tests and abnormal comparative tests; Grade 2 - Mild CTS - abnormal sensory with a normal motor response; Grade 3 - Moderate CTS - abnormal median sensory and motor response; Grade 4 - Severe CTS -absence of sensory response and abnormal distal motor latency; Grade 5 - Extreme CTS - absence of median motor and sensory responses"[7,8].

We felt that as the carpal tunnel was a closed space with the presence of fatty tissue, it was possible that treatment with pioglitazone could decelerate nerve conduction of the median nerve. In order to generate this hypothesis we measured "terminal latency" and "amplitude" of the motor and sensory divisions of the median nerve over a fixed distance spanning the wrist and covering the carpel tunnel in patients of type 2 diabetes. This was done at baseline and after 3 mo in two matched groups of type 2 diabetes patients, one on treatment with pioglitazone and the other without.

MATERIALS AND METHODS

A single centre, prospective, comparative case-series was studied between June 2012 and September 2012 at a tertiary care institute in Kolkata. The study subjects comprised of patients with type 2 diabetes mellitus aged between 18 and 65 years attending the diabetic clinic, treated with oral anti-diabetic agents and complying to undergo electrophysiological testing by nerve conduction velocity (NCV) study at two time points, once at the baseline and later at a gap of 3 mo. Female patients were eligible to participate if they were non-pregnant and willing to adopt standard contraceptive methods over the next 6 mo. The exclusion criteria were clinical evidence of neuropathy or nephropathy, poor control of diabetes as defined by a glycated hemoglobin (HbA_{1c}) over 9% (75 mmol/mol); current treatment with insulin or likelihood of insulin treatment over the next 6 mo; electrophysiologically evident CTS, contraindication to pioglitazone use; myocardial infarction in the last 6 mo; and presence of other causes of CTS like rheumatoid arthritis, untreated hypothyroidism and pregnancy. For the median nerve, distal motor latency of Abductor pollicis brevis was measured by stimulating 3 cm above distal wrist crease. For the ulnar nerve Distal motor latency of Abductor digiti minimi was measured by stimulating 3 cm above distal wrist crease with elbow flexed at 90°. NCV evaluation was performed at baseline and 3 mo. The authors feel that NCV evaluation at 3 mo increases the sensitivity of diagnosing early and asymptomatic CTS.

The electro-diagnostic criteria for CTS used in our study were as follows: (1) Distal median motor latency > 4.2 ms; (2) Difference between distal motor latency of median and ulnar nerve > 1.1 ms; (3) Difference between distal sensory latency of median nerve and ulnar nerve > 0.2 ms; (4) Difference between median and ulnar sensory latency on stimulating fourth digit and recording from wrist at equal distance > 0.2 ms; (5) Difference between median and ulnar sensory latency on stimulating thumb and recording from wrist at equal distance > 0.4 ms; and (6) Palm wrist conduction: Difference between median and ulnar sensory latency across 8 cm > 0.4 ms.

After a run in period of 1 mo, the HbA_{1c} was reassessed. Those with HbA_{1c} over 7.5% (58 mmol/mol) were excluded from further study. The patients had their diabetes controlled on oral agents and belonged to either pioglitazone (Group 1) or non-pioglitazone group (Group 2) depending on whether they were receiving the drug as a part of their current therapy. Patients with electrophysiological evidence of CTS on NCV were excluded from further study (n = 34) and were labeled as Group 3. The remaining patients, 14 each in Groups 1 and 2, were requested to continue their usual diabetes treatment and were seen in the clinic every 6 wk, when fasting and 2 h post prandial blood sugar (FBS and PPBS) were checked and a clinical evaluation performed. At the end of 3 mo, HbA_{1c} level was re-estimated. The NCV study was repeated at the end of 3 mo. All the electrophysiology studies were done by the same observer who was not aware of the treatment status, and the parameters studied were terminal latency and amplitude in the motor component of left median nerve between the elbow and the wrist (L-M-motor-ew-TL and L-M-motor-ew-Amp), and also the sensory component of the same (L-M-sensory-TL and L-M-sensory-Amp); the terminal latency and amplitude in the motor component of left ulnar nerve across the wrist (L-U-motor-aw-TL and L-U-motor-aw-Amp), and also the sensory component of the same (L-U-sensory-TL and L-U-sensory-Amp).

Data have been summarized by routine descriptive statistics, and key proportions expressed with their 95%CI. Since the number of patients in each group was 14, non-parametric tests have been used for both inter-group and intra-group comparisons of all parameters studied. Numerical variables were compared between groups by Mann-Whitney *U* test. Categorical variables were compared between groups by Fisher's exact test. χ^2 test for trend analysis was used where applicable. Median values [with interquartile range (IQR)] of age, all parameters of electrophysiological assessment in NCV and HbA_{1c} over time were analyzed for statistically significant change by Wilcoxon matched pairs signed rank sum test. Median FBS and PPBS values over time were assessed for statistically significant change by Friedman's analysis of variance (ANOVA) with "Dunn's multiple comparison test" as post hoc test. All analyses were two-tailed and P < 0.05 was considered statistically significant. Statistical Version 6 (Tulsa, Oklahoma: StatSoft Inc., 2001) and GraphPad Prism version 4 (San Diego, California: GraphPad Software Inc., 2005) software were used for analysis. The statistical review of the study was performed by a biomedical statistician.

RESULTS

Data of all the 28 patients without electrophysiological evidence of CTS on NCV were analyzed. As illustrated in Table 1, demography, duration of diabetes and baseline characteristics was comparable in the two groups[9].

At the end of 3 mo, Group 1 patients had higher median amplitude in the sensory component of left median nerve [Group 2 8.5 (IQR = 6.5 to 11.5) *vs* Group 1 14.5 (IQR 10.5 to 18.75)] (P = 0.002) (Figure 1). There was improvement in median amplitude in the sensory component of left median nerve [Baseline 13 (IQR = 9 to 16.25) *vs* 3 mo 8.5 (IQR = 6.5 to 11.5)] for Group 2 patients) (Figure 1). In the same group, there was improvement in median amplitude in the motor component of left median nerve [baseline 9 (IQR = 4.75 to 11) *vs* 3 mo 6.75 (IQR = 4.75 to 10.25)] (P = 0.049) (Figure 2). Higher amplitude indicated greater delay in nerve conduction[9].

The HbA_{1c} values at the end of 3 mo were comparable between groups (P = 0.809), but the pioglitazone group showed improvement {baseline value: 7.1% (54 mmol/mol) [IQR = 6.2% (44 mmol/mol) - 7.8 % (62 mmol/mol)] to 3 mo value: 6.3% (45 mmol/mol) [IQR = 6% (42 mmol/mol) - 6.8% (51 mmol/mol)]} (P = 0.002). The FBS and PPBS values were comparable between Groups 1 and 2 at all time-points (data on file, not shown). There was worsening of median terminal latency of the motor component of left ulnar nerve [baseline 2.07 (IQR = 1.92 to 2.25) *vs* 3 mo 2.16 (IQR = 1.97 to 2.325) for Non pioglitazone group] (P = 0.043) (Figure 3). There was improvement of median terminal latency in the sensory component of left ulnar nerve [baseline 2.45 (IQR = 2.315 to 2.88) *vs* 3 mo 2.37 (IQR = 2.275 to 2.445) for pioglitazone

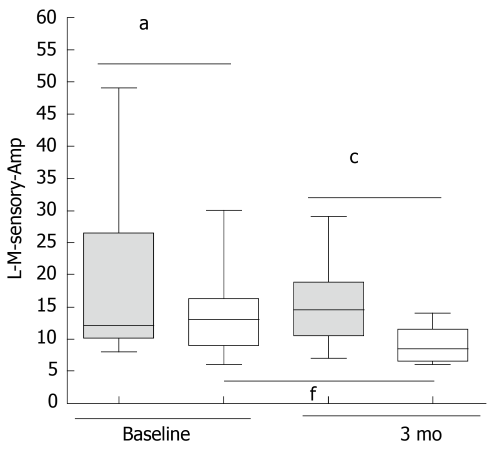

Figure 1 Amplitude in sensory component of Left Median nerve at baseline and 3 mo (Shaded bar: Pioglitazone arm; White bar: Non pioglitazone arm). ^{a}P = 0.496, ^{c}P = 0.002 (Mann-Whitney *U* Test); ^{f}P = 0.01 (Wilcoxon matched pairs signed rank sum test). L-M-sensory-Amp: Amplitude in sensory component of Left Median nerve.

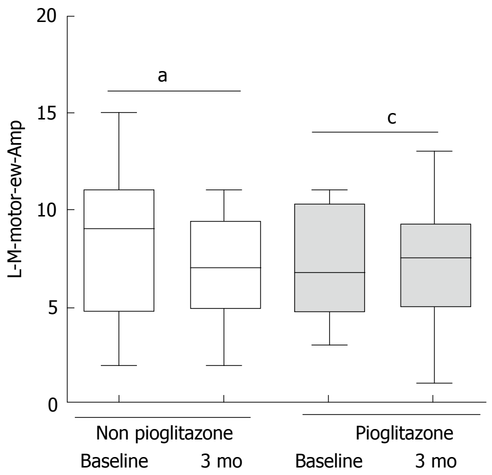

Figure 2 Amplitude in motor component of Left Median nerve in the segment between elbow and wrist at baseline and 3 mo (Shaded bar: Pioglitazone; White bar: Non pioglitazone arm). ^{a}P = 0.049, ^{c}P = 0.964 (Wilcoxon matched pairs signed rank sum test). L-M-motor-ew-Amp: Amplitude in motor component of Left Median nerve in the segment between elbow and wrist.

group] (P = 0.038) (Figure 4). Higher terminal latency indicates greater delay in nerve conduction. None of the patients developed symptoms of CTS at the end of 3 mo[9].

DISCUSSION

Pioglitazone is widely used in the pharmacotherapy of type 2 diabetes mellitus. Luciferase reporter assay has confirmed that pioglitazone stimulates preadipocyte multiplication by augmenting S and G(2)/M cell-cycle entry by amplifying the effect of PPARγ on cyclin-dependent kinase inhibitors by engaging 3T3-L1 preadipocytes, especially with p16(Ink4a) (p16) centered[2]. Preclinical studies show that pioglitazone produces an increase in subcutaneous adipocyte surface and whole body adipocity[10,11]. Although mature visceral adipocytes have a greater propensity to proliferate than subcutaneous adipocytes, it is the latter that proliferates following pioglitazone treatment[12,13].

Preadipocyte cell lines like 3T3-L1 and 3T3 F442A manifest miniscule quantum of PPAR-γ, but markers of late differentiation, such as aP2, PEPCK, and CAAT/

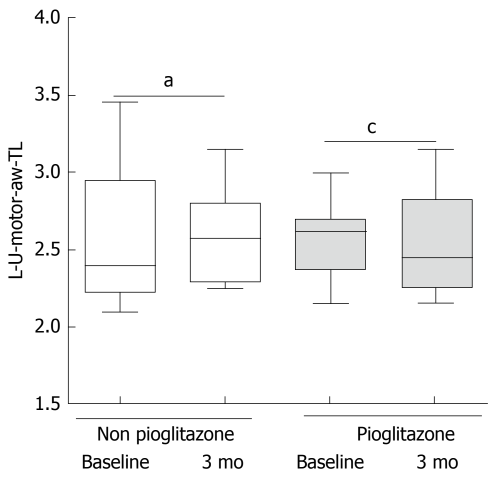

Figure 3 Terminal Latency in motor component of Left Ulnar nerve across wrist at baseline and 3 mo (Shaded bar: Pioglitazone; White bar: Non pioglitazone arm). ^{a}P = 0.043, ^{c}P = 0.055 (Wilcoxon matched pairs signed rank sum test). L-U-motor-aw-TL: Terminal Latency in motor component of Left Ulnar nerve across wrist.

Table 1 Baseline demographic and clinical summary of the study subjects

	Pioglitazone (*n* = 14)	Non-pioglitazone (*n* = 14)	*P* value
Gender (male:female)	7 (50%):7 (50%)	6 (42.86%):8 (57.14%)	1
Age (yr)	42 (35.5-52.5)	46 (42.75-51.75)	0.333
Diabetes duration (yr)	2 (1-5)	5.5 (2.75-10.25)	0.072
L-M-motor-ew-TL	3.5 (3-4)	3 (3-4)	0.756
L-M-motor-ew-Amp	6.5 (4.75-10.25)	9 (4.75-11)	0.431
L-M-sensory-TL	2 (2-3)	3 (2-3)	0.575
L-M-sensory-Amp	12 (9.75-26.5)	13 (9-16.5)	0.496
L-U-motor-aw-TL	3 (2-3)	2 (2-3)	0.264
L-U-motor-aw-Amp	5 (4-5.5)	5 (4-7)	0.796
L-U-sensory-TL	2 (2-2)	2 (2-2)	0.317
L-U-sensory-Amp	13.5 (9-19)	14 (7.75-17.25)	0.679
HbA1c	7.1 (6.2-7.8)	6.6 (6.25-7.25)	0.654

Values are stated as median (interquartile range). Counts are provided for gender distribution. *P* values in the last column are from intergroup comparison by Fisher's exact test (for gender), Mann-Whitney *U* test (for other variables). L-M-motor-ew-TL: Terminal latency in the motor component of left median nerve between the elbow and the wrist; L-M-motor-ew-Amp: Amplitude in the motor component of left median nerve between the elbow and the wrist; L-M-sensory-TL: Terminal latency in the sensory component of left median nerve between the elbow and the wrist; L-M-sensory-Amp: Amplitude in the sensory component of left median nerve between the elbow and the wrist; L-U-motor-aw-TL: Terminal latency in the motor component of left ulnar nerve across the wrist; L-U-motor-aw-Amp: Amplitude in the motor component of left ulnar nerve across the wrist; L-U-sensory-TL: Terminal latency and amplitude in the motor component of left ulnar nerve across the wrist; L-U-sensory-Amp: Amplitude in the motor component of left ulnar nerve across the wrist; HbA1c: Glycated hemoglobin.

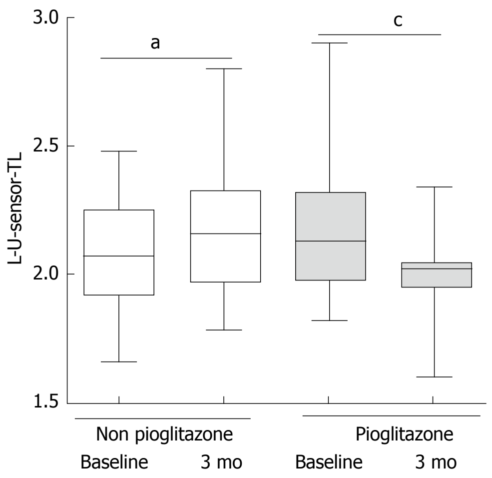

Figure 4 Terminal Latency in sensory component of Left Ulnar nerve at baseline and 3 mo (Shaded bar: Pioglitazone; White bar: Non Pioglitazone arm). ^{a}P = 0.161, ^{c}P = 0.038 (Wilcoxon matched pairs signed rank sum test). L-U-sensory-TL: Terminal Latency in sensory component of Left Ulnar nerve.

enhancer binding protein (C/EBP a) is preceded by PPAR-γ[14,15]. Thiozolidinediones (TZD), Wy-14643 and ETYA assist the transformation of preadipocytes into adipocytes[16-19]. Lipid-laden fibroblasts show high PPAR-γ expression in diverse fibroblastic lineage (*e.g.*, NIH-3T3, BALB/c-3T3, Swiss-3T3)[20]. Adipocyte deposition is a well established pathology in certain metabolic disorders like obesity and it has been shown in pre-clinical studies that PPAR activators promote differentiation of G8 myoblastic cells or transfected C2C12 myoblasts into adipocytes[21-23]. Similarly, TZD can differentiate bone marrow stromal cells into adipocytes, analogous to inappropriate adipogenesis that can occur in canine bone marrow[24,25]. The c-Cbl-associated protein (CAP) potentiates the phosphorylation of cCbl protooncogene in mature adipocytes, and its expression is accentuated by TZD[26,27].

A multitude of mechanisms have been put forward as the foundation of diabetic polyneuropathy, and therapeutic trials have evaluated the polyol pathway, the advanced glycation end product, protein kinase C, poly ADP-ribose polymerase, and aldose reductase[28,29]. The main pathophysiology is an escalation in hyperglycemia induced oxidative stresses and the impairment of anti-oxidative mechanisms in diabetic polyneuropathy[30]. TZDs can attenuate oxidative stresses and inflammatory responses[31]. Based on these effects, the neuroprotective potential of TZD treatment was investigated in an animal model. These reports explain the neuroprotective effect of TZD by diverse effects of PPAR c agonist like TNF-α inhibition and IL-6, suppressed protein kinase C (PKC) activity with diminished PKC-alpha in addition to insulin sensitization[32-35].

In spite of these available data, the clinical impact of this effect of pioglitazone in human subjects has not been studied in detail. Anecdotal data exist about compressive symptoms produced by pioglitazone in the orbit[4]. A search undertaken by us in "PubMed" using keywords *viz.* "pioglitazone", "carpal tunnel", "compressive neuropathy" yielded no published studies on the effect of pioglitazone therapy on the carpal tunnel. Our study was conceived to address this lacuna in medical literature.

In this case series we evaluated the electrophysiological changes in the left median nerve in the carpal tunnel, in two groups, one receiving pioglitazone and the other not. Both groups received other oral antidiabetic agents, had similar baseline characteristics and achieved similar glycemic control. The ulnar nerve passes superficial to the tunnel in the Guyon's canal, so the left ulnar nerve was also evaluated to assess, the effect of metabolic changes on neural electrophysiology outside the carpal tunnel. It is well known that there is significant association between electrophysiological parameters and metabolic control in diabetes[36]. The FBS, PPBS and HbA_{1c} were also studied in both the groups to assess whether the changes in diabetes control had an impact on the electrophysiological results in the groups.

We found that a majority, 34 out of 62 (54.84%) of the patients with type 2 diabetes, who underwent NCV testing, although asymptomatic, had electrophysiologically proven CTS. This was in conformity to earlier studies, that demonstrated similarly high prevalence of asymptomatic CTS among patients with diabetes[12].

There was improvement in the amplitude in both motor and sensory components of the median nerve in the non-pioglitazone group at 3 mo. The latter also had electrophysiologically better amplitude in the sensory component of the median nerve compared to pioglitazone group. In the non-pioglitazone group, there was worsening of terminal latency in the motor component of the ulnar nerve, and improvement in the terminal latency of the sensory component in the pioglitazone group. Pioglitazone has favourable effect on nerve electrophysiology which was repealed when the nerve was exposed to compressive neuropathy.

This study had its share of limitations. The sample size is 28 and the observation period was limited to 3 mo in this open label study. However it does generate the hypothesis that patients on pioglitazone are at risk of compressive neuropathy, the pathogenesis of which is established. We were intrigued by the finding that in spite of comparable glycemic control over 3 mo, patients treated with pioglitazone showed superior electrophysiological parameters for the ulnar nerve. The high prevalence of asymptomatic CTS in Indian patients, as found by us, is a novel finding. We are yet to encounter a similar result in published literature. Further studies, ideally randomized controlled trials, are needed to establish the role of pioglitazone in diabetic neuropathy and test our hypothesis.

COMMENTS

Background

The carpal tunnel is a fibro-osseous space in the wrist, which also contains small amounts of adipose tissue. In preclinical studies, pioglitazone, a peroxisome proliferator activator receptor gamma agonist, has been shown to convert pre-adipocytes to adipocytes, although the mechanisms continue to remain elusive. This action has been known to cause compressive symptoms in confined spaces like the orbit in a subgroup of patients. As the carpal tunnel was a closed space with the presence of fatty tissue, it is possible that treatment with pioglitazone could cause delay in the nerve conduction of the median nerve. In order to generate this hypothesis the authors measured terminal latency and amplitude of the motor and sensory components of the median nerve over a fixed distance spanning the wrist and covering the carpel tunnel in patients of type 2 diabetes, at baseline and after 3 mo, in two matched groups of type 2 diabetes patients, one on treatment with pioglitazone and the other without.

Research frontiers

Pioglitazone has been shown to augment pre-adipocyte proliferation, possibly as a result of cell cycle promoting effect through downregulation of p16(Ink4a) *via* PPAR. Pioglitazone has also been shown to produce an increase in subcutaneous adipocyte surface. Preclinical studies in rodents have demonstrated that pioglitazone increases whole body adipocity. Although mature visceral adipocytes have a greater propensity to proliferate than subcutaneous adipocytes, it is the latter that proliferates following pioglitazone treatment. In spite of these available data, the clinical impact of this effect of pioglitazone in human subjects has not been studied in detail. Anecdotal data exist about compressive symptoms produced by pioglitazone in the orbit. A search undertaken by us in "PubMed" using keywords *viz.* "pioglitazone", "carpal tunnel", "compressive neuropathy" yielded no published studies on the effect of pioglitazone therapy on the carpal tunnel. The study was conceived to address this lacuna in medical literature.

Innovations and breakthroughs

A majority, 34 out of 62 (54.84%) of the patients with type 2 diabetes, who underwent NCV testing, although asymptomatic, had electrophysiologically proven carpal tunnel syndrome. This was in conformity to earlier studies, that demonstrated similarly high prevalence of asymptomatic CTS among patients with diabetes. There was improvement in the amplitude in both motor and sensory components of the median nerve in the non-pioglitazone group at 3 mo. The latter also had electrophysiologically better amplitude in the sensory component of the median nerve compared to the pioglitazone group. In the non-pioglitazone group, there was worsening of terminal latency in the motor component of the ulnar nerve, and improvement in the terminal latency of the sensory component in the pioglitazone group. Pioglitazone thus appeared to have a beneficial effect on nerve electrophysiology which was nullified when the nerve was exposed to entrapment neuropathy. However it does generate the hypothesis that patients on pioglitazone are at risk of compressive neuropathy, the pathogenesis of which is established. The authors were intrigued by the finding that the ulnar nerve showed better electrophysiological parameters in patients who received pioglitazone, although the glycemic control of these patients was similar to those not on pioglitazone. The high prevalence of asymptomatic CTS in Indian patients, as found by the authors, is a novel finding. The authors are yet to encounter a similar result in published literature. Further studies, ideally randomized controlled trials, are needed to establish the role of pioglitazone in diabetic neuropathy and test the authors' hypothesis.

Applications

The study generates the hypothesis that patients on pioglitazone are at risk of compressive neuropathy, the pathogenesis of which is established. The high prevalence of asymptomatic CTS in Indian patients, as found by the authors, is a novel finding. Further studies, ideally randomized controlled trials, are needed to establish the role of pioglitazone in diabetic neuropathy and test the authors' hypothesis.

Terminology

L-M-motor-ew-TL: Terminal latency in the motor component of left median nerve between the elbow; L-M-motor-ew-Amp: Amplitude in the motor component of left median nerve between the elbow and the wrist; L-M-sensory-TL: Terminal latency in the sensory component of left median nerve between the elbow and the wrist; L-M-sensory-Amp: Amplitude in the sensory component of left median nerve between the elbow and the wrist; L-U-motor-aw-TL: Terminal latency in the motor component of left ulnar nerve across the wrist; L-U-motor-aw-Amp: Amplitude in the motor component of left ulnar nerve across the wrist; L-U-sensory-TL: Terminal latency in the sensory component of left ulnar nerve across the wrist; L-U-sensory-Amp: Amplitude in the sensory component of left ulnar nerve across the wrist.

Peer-review

This is an interesting and well-performed study that reports novel findings regarding the effects of pioglitazone on peripheral nerves and on carpal tunnel syndrome pathogenesis in patients with type 2 diabetes mellitus. The methods are appropriate and the results are clearly presented.

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SYSTEMATIC REVIEWS

Relationship between depression and diabetes in pregnancy: A systematic review

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Abstract

AIM

To systematically review the literature on women with both diabetes in pregnancy (DIP) and depression during or after pregnancy.

METHODS

In this systematic literature review, PubMed/MEDLINE and EMBASE were searched (13 November 2015) using terms for diabetes (type 1, type 2, or gestational), depression, and pregnancy (no language or date restrictions). Publications that reported on women who had both DIP (any type) and depression or depressive symptoms before, during, or within one year after pregnancy were considered for inclusion. All study types were eligible for inclusion; conference abstracts, narrative reviews, nonclinical letters, editorials, and commentaries were excluded, unless they provided treatment guidance.

RESULTS

Of 1189 articles identified, 48 articles describing women with both DIP and depression were included (sample sizes 36 to > 32 million). Overall study quality was poor; most studies were observational, and only 12 studies (mostly retrospective database studies) required clinical depression diagnosis. The prevalence of concurrent DIP (any type) and depression in general populations of pregnant women ranged from 0% to 1.6% (median 0.61%; 12 studies). The prevalence of depression among women with gestational diabetes ranged from 4.1% to 80% (median 14.7%; 16 studies). Many studies examined whether DIP was a risk factor for depression or depression was a risk factor for DIP. However, there was no clear consensus for either relationship. Importantly, we found limited guidance on the management of women with both DIP and depression.

CONCLUSION

Given the increasing prevalence of diabetes and depression, high-quality research and specific guidance for management of pregnant women with both conditions are warranted.

Key words: Depression; Diabetes; Postpartum depression; Depressive disorder; Gestational; Diabetes mellitus; Perinatal care; Postnatal care; Pregnancy

Core tip: Depression in women with diabetes in pregnancy (DIP) may be increasingly common. We identified 48 studies of depression and DIP, of variable and often poor quality. The prevalence of concurrent DIP and depression ranged from 0% to 1.6% (median 0.61%; 12 studies). Among women with gestational diabetes, the prevalence of depression ranged from 4.1% to 80% (median 14.7%; 16 studies). There was no clear consensus on whether DIP was a risk factor for depression. Given the increasing prevalence of diabetes and depression, high-quality research and specific guidance for management of pregnant women with both conditions are warranted.

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INTRODUCTION

Diabetes affects an increasing number of pregnancies worldwide. In 2015, almost 21 million births (16.2%) were affected by hyperglycemia during pregnancy[1,2]. Approximately 10% to 15% of these births involved mothers with pre-existing or newly detected type 1 or type 2 diabetes, with the remaining 85% to 90% being women with gestational diabetes mellitus (GDM)[1,2]. As the prevalence of both type 1 and type 2 diabetes in the general population is increasing[1], the number of women affected by diabetes in pregnancy (DIP) is also rising. Indeed, between 2000 and 2010, the age-standardized prevalence of pregnancies in the United States affected by type 1 or type 2 diabetes increased by 37%[3] and the prevalence of GDM increased by 56%[4]. Diabetes in pregnancy can have adverse effects on both the mother and child, including increased risk of miscarriage, stillbirth, preterm delivery, pre-eclampsia, cesarean section delivery, postpartum development of type 2 diabetes in women with GDM, congenital malformations, fetal macrosomia, neonatal hypoglycemia, neonatal respiratory distress, and obesity and insulin resistance in childhood, followed by impaired glucose tolerance and type 2 diabetes later in life[1,5,6].

Depression during pregnancy or postpartum also adversely affects women and their children. Depression during pregnancy is associated with poorer maternal health, increased likelihood of obstetric complications, preterm birth, and neonatal complications[5,6]. Postpartum depression is associated with difficulties with maternal-child bonding, inadequate care of the child, and lower rates of breastfeeding[7].

Recent evidence suggests a bidirectional relationship between diabetes and depression among non-pregnant patients. Several meta-analyses of longitudinal studies suggest that diabetes is a risk factor for the development of depression[8-10]. Conversely, depression has been suggested as a risk factor for the development of type 2 diabetes[11,12]. In addition, the prevalence of comorbid diabetes and depression is higher than expected, leading to speculation that diabetes and depression may share underlying biological mechanisms[10,13]. However, evidence for a link between DIP and depression during pregnancy or postpartum is limited[5]. Pregnancy represents a potentially stressful event, which could make women with pre-existing diabetes more vulnerable to depression. Similarly, a diagnosis of GDM could contribute to depressive symptoms, particularly during pregnancy. Importantly, depression is associated with poor diabetes self-care[14], which may be more challenging during pregnancy and postpartum when diabetes management and glycemic control are especially complex[15]. Indeed, women with DIP and depression may struggle to cope with the physical and psychological demands of pregnancy and early motherhood. Given the increasing prevalence of both diabetes and depression among women of childbearing years, the co-occurrence of both conditions during pregnancy or postpartum is likely to become more common. Despite this increase, and the impression among many clinicians that depression in pregnant or postpartum women with diabetes is common, current major guidelines for the treatment and management of DIP[15-17] or depression[18,19] do not provide adequate advice regarding care of these patients.

The aim of this systematic literature review was to assess the current knowledge regarding the prevalence, treatment, and management of women who have both DIP and depression before, during, or after pregnancy.

MATERIALS AND METHODS

Literature search strategy

We searched MEDLINE (PubMed) and EMBASE on 13 November 2015, using Medical Subject Heading (MeSH), EMTREE, or free-text terms: (pregnancy OR postpartum period OR pregnant OR postnatal OR post-natal OR antenatal) AND (depression OR depressive disorder, major OR major depression OR depression, postpartum OR puerperal depression OR major depressive disorder OR MDD OR postnatal depression) AND (diabetes mellitus OR diabetes mellitus, type 1 OR diabetes mellitus, type 2 OR diabetes, gestational OR insulin dependent diabetes OR non insulin dependent diabetes OR pregnancy diabetes mellitus OR diabetic OR juvenile diabetes OR type 1 diabetes OR type Ⅰ diabetes OR insulin-dependent diabetes OR type 2 diabetes OR type Ⅱ diabetes OR non-insulin dependent diabetes OR NIDDM OR gestational diabetes). Searches were tailored to each database and restricted to human studies. There were no restrictions on publication date, publication type, or language.

Eligibility criteria

Publications that reported on women who had both DIP (type 1, type 2, or GDM) and depression or depressive symptoms before, during, or within one year after pregnancy were considered for inclusion. All study types were eligible for inclusion, including meta-analyses, systematic reviews, randomized and nonrandomized clinical trials, observational studies (prospective and retrospective), case reports, clinical practice guidelines, and other publications providing guidance on diagnosis, treatment, or management.

Publications were excluded if they described studies not conducted in humans, studies in which data for women with DIP and depression were pooled with data for women with other conditions, studies that reported depressive symptoms based on measures of anxiety or bipolar disorder, or studies that only reported fetal or newborn outcomes (*i.e.*, no maternal outcomes or prevalence data). Conference abstracts, narrative reviews, systematic reviews that did not report original data, nonclinical letters, editorials, and commentaries were excluded, unless they provided treatment guidance.

Study selection and data extraction

One person (medical writer contracted by Eli Lilly and Company) conducted the literature search and screened the titles and abstracts of retrieved publications using the predefined eligibility criteria. The full text of publications identified for potential inclusion were rescreened using the same criteria. Reference lists of reviews and other relevant publications were screened to identify additional publications. All authors reviewed and approved the publications identified for inclusion.

The medical writer extracted all relevant data, including publication type and year, study design, study objectives, country of origin, sample size, patient characteristics, diabetes type(s), definition or measures of depression, and main outcomes, from the included publications. The risk of bias was assessed by study quality components (study design, sample size, outcomes) and by the depression and diabetes definitions used in each study. Because information on this topic is lacking, all levels of evidence were included in the review.

Outcome measures included: Incidence/prevalence of DIP and depression among pregnant or postpartum women; relationship between DIP and depression; relative risk of developing depression during or after pregnancy among women with DIP *vs* pregnant women without diabetes; relative risk of developing GDM among women with depression *vs* women without depression; clinical or demographic factors related to increased risk of having both DIP and depression during or after pregnancy; methods of diagnosis or measurement of depression; and treatment/management strategies.

RESULTS

Literature search results

A total of 1463 publications were retrieved from MEDLINE and EMBASE; after removal of duplicates, 1189 publications were screened (Figure 1). Of these, 46 publications were selected for inclusion[20-65]. Manual screening of bibliographies identified two additional relevant studies[66,67]. Overall, 48 publications were included in this review (Figure 1, Tables 1-3, Supplementary Table 1). Of these, 30 described prospective observational studies[20,21,23,24,26,27,29-31,34,35,37,38,41,43-45,47-49,51,54,57,58,60-62,65-67], 15 described retrospective observational studies[22,25,28,32,39,40,42,46,50,53,55,56,59,63,64], and three described

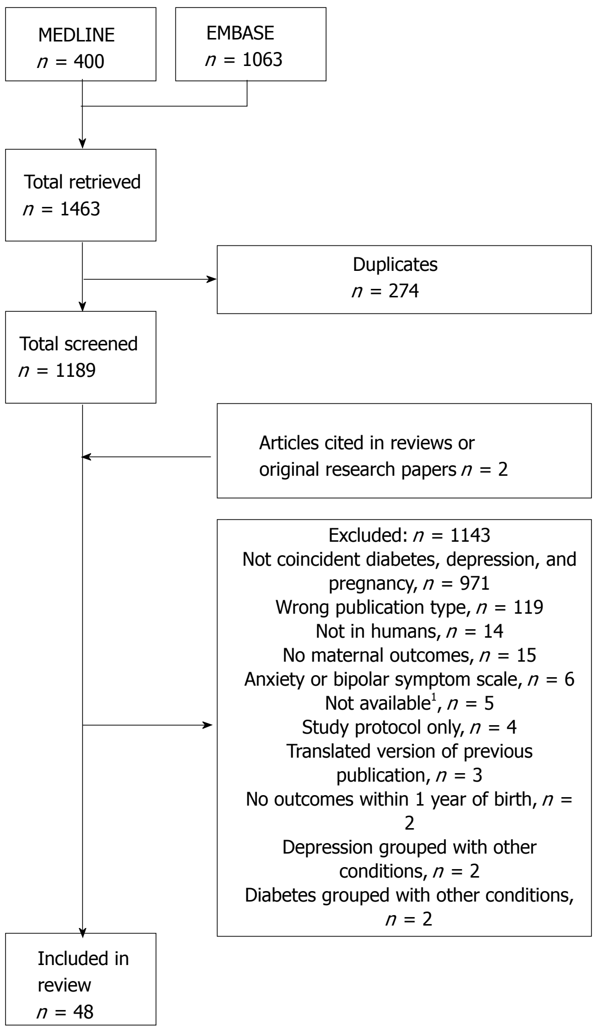

Figure 1 Publication flow diagram. [1]Unavailable articles were unlikely to be relevant based on the title and/or abstract.

randomized controlled trials (RCTs)[33,36,52], two of which reported only baseline data[36,52]. Two publications described the same study, but reported different subgroup analyses[23,29].

Overview of study characteristics

A total of 28 studies included only women with GDM[20,23,26-29,31,33,35-38,40,43-45,47-49,51-53,57,58,60,61,63,64], 14 included women with either GDM or pre-existing diabetes (although the type was not always reported)[22,25,39,41,42,46,50,54-56,59,62,65,66], one included women with either GDM or type 1 diabetes[34], one included only women with type 1 diabetes[67], one included only women with pre-existing diabetes (type not reported)[30] and three did not report the type of diabetes[21,24,32] (Tables 1-3, Supplementary Table 1). Sample sizes ranged from 36[65] to more than 32 million in a retrospective analysis of a nationwide hospital database[22] (Tables 1-3, Supplementary Table 1).

Study quality

Overall study quality was poor. Most studies were prospective observational studies (Tables 1-3, Supplementary Table 1), which were subject to limitations such as small sample size and selection bias. Further, most studies defined depression using measures of depressive symptoms rather than more rigorous clinical diagnosis tools. Among those that did use clinical diagnosis tools, most were retrospective, including six national, state/provincial, or veterans' health database studies[32,40,50,55,56,64], two claims registry studies[39,46], and three hospital records review studies[22,28,63]. Although these studies were large, their retrospective nature was an inherent limitation. Unlike the health database studies, the claims registry and hospital records review studies were subject to potential selection bias. Importantly, the primary objective of many of the studies was not relevant to this systematic review (Supplementary Table 1), and the results we collected were often secondary or incidental findings.

The small number of RCTs identified may reflect ethical concerns regarding enrolment of pregnant women in interventional studies. The one completed RCT was the highest quality study included in this review[33], having appropriate allocation sequence generation and concealment, as well as attempts to maintain blinding; however, Edinburgh Postnatal Depression Scale (EPDS) data at 3 mo postpartum were available for fewer than 60% of patients, indicating potential attrition bias.

Definition of depression

The definition of depression varied widely across the studies (Tables 1-3), and only a quarter of the studies (almost all retrospective) classified participants as having depression based on a formal clinical diagnosis. Only one prospective study defined depression using the Structural Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-Ⅳ)[60]. This study examined responses to oral glucose challenge tests among women with or without current or past diagnosis of a psychiatric disorder, including major depressive disorder. However, only 3 of 186 women were subsequently diagnosed with GDM, and the publication did not report whether these women had depression, another psychiatric disorder, or no psychiatric disorder. Eleven retrospective studies used International Classification of Diseases (ICD) codes[68] in medical records to classify participants as having current or a history of depression[22,28,32,39,40,46,50,55,56,63,64]. One of these retrospective studies also included diagnoses based on the DSM-Ⅳ[50]. One retrospective study[59] and one prospective study[62] relied on participant self-reporting of depression diagnosis. Aside from these studies, all other studies used measures of depressive symptoms, most commonly the EPDS; however, the cut-off score for clinically significant depression varied from 9 to 15. Other depressive symptom scales included the Beck Depression Inventory, the Centre for Epidemiologic Studies Depression scale, the Montgomery-Åsberg Depression Rating Scale, and the Patient Health Questionnaire.

In general, the large retrospective studies that used ICD codes reported a significant association between

Table 1 Outcomes of included studies involving women with gestational diabetes

First author, study design	Definition/ measures of depression	Timing of depression measures	Overall, *n* subgroups, *n*	Main outcomes/findings
Abdollahi[20] Prospective, cohort	EPDS ≥ 12	Within 12 wk after delivery	*n* = 1449	Women with GDM had greater risk of postpartum depression than women without GDM [adjusted OR (95%CI): 2.93 (1.46-5.88), *P* = 0.002]
[1]Bener[23] Prospective, cross-sectional	EPDS ≥ 12	Within 6 mo after delivery	*n* = 1379 With depression, *n* = 243; Without depression, *n* = 1136	Prevalence of GDM was numerically, but not statistically, higher in women with depression (9.9%) *vs* women without depression (6.2%) (*P* = 0.051)
Berger[25] Retrospective	EPDS ≥ 13 or did not answer "No" to self-harm question	Within 4 d after delivery	Unselected, *n* = 322 History of mental illness, *n* = 215	In the unselected group, prevalence of GDM was higher in women with postpartum depression (27.3%) *vs* women without depression (9.0%) (*P* = 0.04); there was no difference in the group with previous mental illness (19.4% *vs* 10.2%, *P* = 0.14) In the unselected group, GDM was associated with postpartum depression [OR (95%CI): 12.1 (1.9-77.8)] In the unselected group, overall prevalence of depression and GDM was 0.9% (3 of 322)
Bisson[26] Prospective, case-control	EPDS ≥ 10	Approx. 30 wk gestation	*n* = 52 GDM, *n* = 26; No GDM, *n* = 26	Women with GDM had a greater prevalence of depressive symptoms *vs* women without GDM (23% *vs* 0%, *P* = 0.023) Mean (SD) EPDS score was 6.8 (4.0) for women with GDM and 4.2 (2.6) for women without GDM (*P* < 0.05)
Blom[27] Prospective	EPDS > 12	2 mo after delivery	*n* = 4941 With depression, *n* = 396; Without depression, *n* = 4545	No significant difference in the proportion of women with GDM between those who did (4/396; 1.0%) and did not (28/4545; 0.6%) have depression (*P* ≥ 0.05) Calculated prevalence of women with both GDM and depression = 0.08% (4/4941)
Bowers[28] Retrospective	ICD9 codes 296.2, 296.3, and 311	Coded on medical history or hospital discharge record	*n* = 128295 With depression, *n* = 5815 (medical history, *n* = 5350); Without depression, *n* = 122480	Women with history of depression were more likely to have GDM than women without history of depression (5.4% *vs* 4.3%; *P* value NR) Depression was associated with significantly increased risk of GDM [OR (95%CI): adjusted for age, race/ethnicity, study site, insurance, and parity: 1.42 (1.26-1.60)]; similar results when restricted to women with history of pre-pregnancy depression [adjusted OR (95%CI): 1.36 (1.20-1.54)] Calculated prevalence of coincident GDM and depression was 313 of 128295 (0.24%)
[1]Burgut[29] Prospective, cross-sectional	EPDS ≥ 12	Within 6 mo of delivery	*n* = 1379 Qatari women, *n* = 837 Other Arab women, *n* = 542 With depression, *n* = 243 With history of diabetes, *n* = 310	GDM increased risk of depression in Qatari women [adjusted OR (95%CI): 1.65 (1.02-2.69)], but not in other Arab women [1.09 (0.63-1.91)]
Chazotte[31] Prospective	CES-D ≥ 16	Weeks 34-36 of gestation	*n* = 90 GDM, *n* = 30; High risk of preterm birth, *n* = 30 Low risk of preterm birth, *n* = 30	56.7% of women with GDM had CES-D ≥ 16; this was not significantly different *vs* women at low (33.3%) or at high (70%) risk of preterm birth (*P* ≥ 0.05) Mean (SD) CES-D score was 17.0 (9.1) for women with GDM, 20.9 (9.4) for women at high risk of preterm birth, and 13.7 (7.5) for women at low risk of preterm birth (*P* ≥ 0.05)
Crowther[33] RCT	EPDS ≥ 12	3 mo after delivery	*n* = 1000 Intervention[2], *n* = 490; Routine care, *n* = 510	Significantly lower proportion of women in the intervention group (8%; 23/278 respondents) had EPDS indicative of depression *vs* women in the routine care group (17%; 50/295 respondents) (*P* = 0.001)
Dalfra[34] Prospective	CES-D ≥ 16	3rd trimester and 8 wk after delivery	*n* = 245 GDM, *n* = 176 (treated with diet, *n* = 109; treated with insulin, *n* = 68); No DM, *n* = 39	Mean (SD) CES-D scores at 3rd trimester were 17.0 (8.6) among women with GDM and 18.0 (8.7) among women without DM (*P* = 0.52) Mean (SD) CES-D scores at 3rd trimester were 16.6 (8.1) among women with GDM treated with diet and 17.7 (9.4) among women with GDM treated with insulin (*P* = 0.58) The severity of depressive symptoms increased from the 3rd trimester to after delivery in women with GDM [estimated mean difference in CES-D score (95%CI): 5.7 (4.2-7.3)], but decreased in women without DM [-2.7 (-5.9-0.5); *P* < 0.0001 between groups]
Daniells[35] Prospective, longitudinal	MHI-5 ≥ 16	Weeks 30 and 36 of gestation, and 6 wk after delivery	*n* = 100 GDM, *n* = 50; No GDM, *n* = 50	Significantly higher proportion of women with GDM (30%) were depressed at Week 30 *vs* women who did not have GDM (12%) [OR (95%CI): 3.14 (1.1-8.94), *P* = 0.03]; however, there was no difference at Week 36 or after delivery (*P* ≥ 0.05) Mean (SD) MHI-5 scores: Week 30: GDM, 13.9 (4.8); no GDM, 11.4 (3.8), *P* = 0.004; Week 36: GDM, 10.9 (3.8); no GDM, 11.7 (4.0), *P* = 0.31; postpartum: GDM, 11.5 (4.5); no GDM, 11.7 (4.0), *P* = 0.79 No significant difference in MHI-5 scores in women who were being treated with insulin (*n* = 7) compared with those being managed with diet only (*P* = 0.06; MHI-5 scores NR)

de Wit[36] Analysis of baseline RCT data	WHO-5 < 50	Early pregnancy (< 20 wk)	*n* = 98 obese women Depressed, *n* = 26	Prevalence of GDM was 13.5% of total sample of obese women and 19.2% of the subgroup with depression (NS; *P* value NR)
Ertel[37] Prospective, cohort	EPDS ≥ 15	Early pregnancy (< 20 wk)	*n* = 934	No significant association between depressive symptoms in early pregnancy and GDM measures at mid-pregnancy [adjusted OR (95%CI): for abnormal glucose tolerance associated with depression: 1.34 (0.81-2.23); for impaired glucose tolerance associated with depression: 1.53 (0.73-3.22)]
Huang[38] Prospective, cohort	EPDS ≥ 13	Mid-pregnancy (median 27.9 wk) and 6 mo (median 6.5 mo) after delivery	Prenatal, *n* = 2112 Postpartum, *n* = 1686	Prevalence of GDM was 8% among women with prenatal depression, 6% among women without prenatal depression, 7% among women with postpartum depression, and 5% among women without postpartum depression Compared with women with normal glucose tolerance, the odds of prenatal depression were significantly higher in women with isolated hyperglycemia [adjusted OR (95%CI): 1.80 (1.08-3.00)], but not in women with impaired glucose tolerance [1.43 (0.59-3.46)] or GDM [1.45 (0.72-2.91)] There was a 25% higher odds of prenatal depression per SD increase (27 mg/dL) in glucose levels [OR (95%CI): 1.25 (1.07-1.48)] Pregnancy hyperglycemia was not associated with significantly higher odds of postpartum depression
Jovanovic[39] Retrospective, claims database	ICD-9 codes 311, 296.2, 296.3, 300.4, 301.12, 309.1	Not specified, but data spanned from 21 mo before to 3 mo after delivery	*n* = 839792 GDM, *n* = 52848 No DM, *n* = 773751	Prevalence of depression among women with GDM was 5.3% Relative risk (95%CI): of depression in women with GDM *vs* women with no DM was 1.17 (1.12-1.21) Prevalence of concurrent GDM and depression was 0.4%
Katon 2011[41] Cross-sectional analysis of prospective cohort	PHQ-9	3rd trimester	*n* = 2398 GDM, *n* = 425; No DM, *n* = 1747	Prevalence (95%CI): of probable major depression among women with GDM was 4.5% (2.5%-6.4%) by PHQ-9 score, 5.7% (3.5%-7.9%) by antidepressant use, and 8.7% (6.0%-11.4%) by either PHQ-9 or antidepressant use, compared with the prevalence among women without DM [PHQ-9: 4.1% (3.2%-5.1%); antidepressants: 6.2% (5.1%-7.3%); PHQ-9 and antidepressants: 9.6% (8.2%-11.0%)] After adjusting for demographic characteristics, chronic medical conditions, and pregnancy variables, GDM was not associated with major [OR (95%CI): 0.90 (0.61-1.32)] or any [OR (95%CI): 0.95 (0.68-1.33)] antenatal depression
Katon 2014 (VA)[40] Retrospective, VA database	ICD-9 codes 296.2-296.39	Up to date of delivery	*n* = 2288 GDM, *n* = 118 No GDM or hypertensive disorder, *n* = 1966	Prevalence of depression was 9.3% in women with GDM and 8.8% in women without DM (no statistical analysis)
Katon 2014 (PPD)[42] Retrospective, hospital database	PHQ-9	2nd or 3rd trimester and 6 wk after delivery	*n* = 1423	Prevalence of GDM did not differ between women with postpartum depression (19.3%) and women without postpartum depression (20.7%) (*P* = 0.89) GDM was not a risk factor for postpartum depression [OR (95%CI): 0.68 (0.40-1.13), *P* = 0.13] Prevalence of concurrent GDM and depression was 1.12%
Keskin[43] Prospective, cohort	BDI ≥ 17	24-28 wk gestation	*n* = 89 GDM, *n* = 44 No GDM, *n* = 45	Prevalence of depression did not differ between women with GDM (80%) and women without GDM (83%) (*P* = 0.4)
Kim[44] Prospective, longitudinal	CES-D (cut-off NR)	Week 12-20 of gestation and 8-12 wk after delivery	*n* = 1445 GDM, *n* = 64; No GDM, *n* = 1233	No difference in the proportion of women with depressive symptoms in the GDM (14.1%) *vs* no GDM (13.5%) group (*P* > 0.05) After adjustment, GDM was not associated with an increase in depressive symptoms between pregnancy and postpartum [adjusted OR (95%CI): 1.22 (0.54-2.77)] Calculated prevalence of both GDM and depression = 0.62%
Ko[45] (Korean) Prospective, cohort	Postpartum depression model (dissertation by JI Bae, Ewha Womans University)	Weeks 24 and 28 of gestation	*n* = 68 Coaching program group, *n* = 34 Control group, *n* = 34	Women with GDM who participated in a 4-wk educational coaching program had a greater decrease in depression scores [mean (SD) change from baseline: -3.77 (6.50)] than women with GDM who did not participate in the program [mean (SD) change from baseline: 1.23 (6.76)] (*P* = 0.043)
Kozhimannil[46] Retrospective, cohort	ICD9 codes 296.2, 296.3, 300.4, 301.12, 309.1, and 311	During the 6 mo before and up to 1 yr after delivery	*n* = 11024 With GDM, *n* = 346 (taking insulin, *n* = 163); No DM, *n* = 10367	Prevalence of depression in women with GDM taking insulin was 16.0% *vs* 13.7% among women with GDM not taking insulin (*P* value not reported) Relative to women without diabetes, risk of depression was higher in both women with GDM taking insulin [adjusted OR (95%CI): 1.85 (1.19-2.87)] and in women with GDM not taking insulin [adjusted OR (95%CI): 1.69 (1.09-2.62)]

Levy-Shiff[66] Prospective	BDI	2^{nd} trimester	$n = 153$ GDM, $n = 51$ No DM, $n = 49$	No significant difference in depression during 2^{nd} trimester between GDM [mean (SD) BDI score 6.70 (4.46)] and controls [6.59 (5.88), $P \geqslant 0.05$] For sample as a whole, higher levels of cognitive assessment of pregnancy as a challenge was associated with lower depression ($P < 0.05$)
Liu[47] Prospective	Survey asking if diagnosed or discussed with HCP	Postpartum (mean 9.7 mo)	$n = 3748$ White, $n = 1043$ Asian/Pacific Islander, $n = 425$ Hispanic, $n = 1253$ Black, $n = 1027$	Prevalence of GDM was 7.6% in white ($P < 0.05$ *vs* all other ethnic groups), 14.9% in Asian/Pacific Islander ($P < 0.05$ *vs* other ethnic groups), 10.1% in Hispanic ($P < 0.05$ *vs* white and Asian/Pacific Islander groups), and 10.1% in black ($P < 0.05$ *vs* white and Asian/Pacific Islander groups) populations Prevalence of pre-existing depression was 2.8% in white ($P < 0.05$ *vs* all other ethnic groups), 12.4% in Asian/Pacific Islander ($P < 0.05$ *vs* all other ethnic groups), 7.6% in Hispanic ($P < 0.05$ *vs* all other ethnic groups), and 5.5% in black ($P < 0.05$ *vs* all other ethnic groups) populations No association between GDM and PPD; African Americans with GDM had decreased likelihood of PPD compared with those without GDM [OR (95%CI): 0.1 (0.0-0.5)] Weighted percentage of women with PPD with or without GDM was 10% *vs* 7.5% in white women ($P < 0.05$), 18.6% *vs* 14.4% in Asian/Pacific Islander ($P \geqslant 0.1$), 13.8% *vs* 9.8% in Hispanic ($P \geqslant 0.1$), and 1.1% *vs* 10.4% in black women ($P \geqslant 0.1$)
Manoudi[48] Prospective, cross-sectional	MINI; HAM-D	NR	$n = 187$ GDM 2.7%	Proportion of patients with major depressive episode who also had GDM was 2.6% (same as overall population, which was 2.7%)
Mautner[49] Prospective	EPDS	24^{th}-37^{th} week of gestation; 2-5 d postpartum; 3-4 mo postpartum	$n = 40$ GDM, $n = 11$ No GDM, $n = 29$	Mean (SD) EPDS scores in late pregnancy [7.55 (5.48)], immediately postpartum [7.00 (3.74)], and 3-4 mo postpartum [6.36 (5.63)] were not different in women with GDM compared with women without pregnancy complications [mean (SD) EPDS scores 6.41 (4.37), 4.69 (4.43), and 5.48 (4.88) in late pregnancy, immediately postpartum, and 3-4 mo postpartum, respectively] ($P \geqslant 0.05$)
Mei-Dan[50] Retrospective, health administration database	ICD-9, ICD-10CA, and/or DSM-IV (ICD codes NR)	Within 5 yr before pregnancy	$n = 437941$ With pre-pregnancy depression, $n = 3724$ No known mental illness, $n = 432358$	Prevalence of GDM during the index pregnancy was 3.4% in women with pre-pregnancy depression and 4.7% in women with no known mental illness (no statistical analysis) Prevalence of GDM and pre-pregnancy depression was 0.029%
Natasha[51] Prospective, case-control	MADRS $\geqslant$ 13	Approx. 25 wk gestation	$n = 748$ GDM, $n = 382$ No GDM, $n = 366$	Prevalence of depression was higher in women with GDM (25.92%) than in women without GDM (10.38%) (P value NR) There were significant associations between depression and current GDM ($P < 0.001$) and between depression and a history of GDM ($P < 0.018$) Mean (variance) MADRS scores were significantly higher in women with GDM [8.33 (7.23)] than women without GDM [4.42 (5.89)] (P value NR) Relative to women without GDM, women with GDM were more likely to have mild (MADRS score 13-19; adjusted OR: 3.07 or 4.06)[3] or moderate (MADRS score 20-34; adjusted OR: 3.94) depression (P < 0.001)
Nicklas[52] Baseline description of RCT cohort	EPDS > 9	Mean (SD) 7.0 (1.7) wk postpartum (range, 4-15 wk)	$n = 71$	24 (34%) women with GDM had EPDS > 9 at postpartum visit [mean (SD) score 11.4 (2.2)]; cesarean delivery ($P = 0.005$) and greater gestational weight gain ($P = 0.035$), but not history of depression ($P = 0.97$), were associated with PPD
O'Brien[53] Retrospective, records review	EPDS $\geqslant$ 10	Mean (SD) 13.6 (8.2) wk gestation	$n = 362$ With depression, $n = 256$ Without depression, $n = 106$	No difference in prevalence of GDM between women with EPDS < 10 (14.6%) and those with EPDS $\geqslant$ 10 (15.0%) ($P \geqslant 0.05$)
Ragland[54] Prospective, cross-sectional	BDI > 13	During pregnancy	$n = 50$ GDM, $n = 22$	Mean BDI score among women with GDM was 13.7 9 (41%) women with GDM had BDI > 13
[4]Räisänen 2013[56] Retrospective, registry review	ICD10 codes F31.3, F31.5, F32-34	Up to 6 wk postpartum or a history of depression	$n = 511422$	Prevalence of GDM: 11.2% of women without any depression ($n = 492103$), 13.8% of women with history of depression but not PPD ($n = 17881$), 17.4% of women with PPD but no history of depression ($n = 431$), and 17.6% of women with both history of depression and PPD ($n = 1007$) ($P \leqslant 0.001$) Among women with history of depression, increased prevalence of PPD was associated with GDM [OR (95%CI): = 1.62 (1.23-2.14)]
[4]Räisänen 2014[55] Retrospective, registry review	ICD10 codes F31.3, F31.5, F32-34	Up to hospital discharge after delivery	$n = 511938$	Prevalence of GDM: 11.2% of women without any depression ($n = 493037$), 13.4% of women with history of depression but not during pregnancy ($n = 14781$), 14.5% of women with depression during pregnancy but no history of depression ($n = 2189$), and 17.6% of women with both depression during pregnancy and history of depression ($n = 1931$) ($P \leq 0.001$) An increased prevalence of depression during pregnancy was associated with GDM [adjusted OR (95%CI): 1.29 (1.11-1.50)]

Rumbold[57] Prospective	EPDS ⩾ 12	Late pregnancy (for GDM)	*n* = 212 GDM (or glucose intolerance of pregnancy), *n* = 25 Negative OGCT, *n* = 95 Positive OGCT/negative OGTT, *n* = 29	No difference in proportion of women with EPDS score ⩾ 12 in the GDM group (19%) compared with other groups (*P* ⩾ 0.05)
Silveira[58] Prospective, cohort	EPDS ⩾ 13	Early (mean 12.4 wk gestation) and mid (mean 21.3 wk) pregnancy	*n* = 1115 GDM, *n* = 52 No glucose abnormality, *n* = 953	Prevalence of GDM did not differ between women with at least minor depression (EPDS ⩾ 13) and women without depression (4.6% *vs* 5.6%) (*P* = 0.58) Prevalence of GDM did not differ between women with probably major depression (EPDS ⩾ 15) and women without major depression (4.1% *vs* 5.6%) (*P* = 0.51)
Singh[59] Retrospective	BDI ⩾ 10; self-reported medical history	During pregnancy	*n* = 152 History of depression, *n* = 39 No history of depression, *n* = 113	Of 39 women with history of depression, 15 (38%) had GDM Of 113 women with no history of depression, 67 (59%) had GDM (*P* value not reported)
Sit[60] Prospective	DSM-IV (SCID)	Past or current diagnosis	*n* = 186 Past MDD, *n* = 41 Current MDD, *n* = 39 Bipolar disorder, *n* = 45 No psychiatric disorder, *n* = 61	Mean (SD) glucose concentration after OGCT was 100 (25.0) mg/dL and did not differ among groups (*P* = 0.564) Rate of abnormal OGCT was 7% (13 of 186) and did not differ among the groups (*P* = 1.000) Only 3 women with abnormal OGCT were confirmed as having GDM (group not specified)
Song[61] (Chinese) Prospective	Self-rating Depression Scale ⩾ 41	During pregnancy	*n* = 104 GDM, *n* = 50 No GDM, *n* = 54	Incidence of depression was 22% in women with GDM, significantly higher than in women without GDM (7.4%) (*P* < 0.05) Among women with GDM, mean (SD) insulin concentration 1 h after OGTT was significantly lower in women with depression [58.3 (32.4) mIU/mL, *n* = 11] than in those without depression [102.1 (65.2) mIU/mL, *n* = 39] (*P* < 0.05)
Sundaram[62] Prospective, exploratory	Survey of PPD diagnosis; survey of symptoms based on PHQ-2	Postpartum	Up to 61733 pregnancies	In analysis of data from 22 states, GDM was not a significant predictor of PPD symptoms [OR (95%CI): 1.13 (0.93-1.30), *n* = 45642, *P* = 0.14) or diagnosis [OR (95%CI): 0.96 (0.64-1.52), *n* = 5919, *P* = 0.89)
Walmer[63] Retrospective, electronic medical records	ICD-9 codes 296.2, 296.3, 309.0, 309.1, 311, 300.4	Postpartum	*n* = 18888 pregnancies (14988 women) GDM, *n* = 696 pregnancies (659 women)	After adjusting for age, pre-eclampsia, and preterm birth, GDM was significantly associated with increased risk of PPD [adjusted OR (95%CI): 1.46 (1.16-1.83), *P* = 0.001]; however, the association was not significant after adjusting for other clinical and demographic characteristics [adjusted OR (95%CI): 1.29 (0.98-1.70), *P* = 0.064] In subanalyses of ethnic/racial groups, GDM was significantly associated with PPD in black and white women, but not Hispanic women, after adjusting for age, pre-eclampsia, and preterm birth; the associations were not significant after full adjustment GDM was significantly predictive of mental health disorder (including depression, anxiety, and others) within 3 mo postpartum [adjusted OR (95%CI): 1.38 (1.04-1.85), *P* = 0.028]
Whiteman[64] Retrospective, maternal and infant database	ICD-9-CM codes 293.83, 296.2, 296.3, 300.4, 301.12, 309.0, 309.1, 311	Up to hospital discharge after delivery	*n* = 1057647	GDM was significantly associated with increased risk of depression [adjusted OR (95%CI): 1.44 (1.26-1.65)] (*P* value NR) Obesity was also associated with increased risk of depression, but there was no significant, additive interaction between GDM and obesity

[1]The Bener *et al* and Burgut *et al* publications describe the same study, although different subgroups analyses are reported; [2]Intervention comprised dietary advice, blood glucose monitoring, insulin therapy as needed, and usual care; [3]Note that the adjusted OR for mild depression is variously reported as 3.065062 or 4.06 in the publication; [4]The Räisänen *et al* 2013 and 2014 publications use the same database within the same time period (2002-2010) and, therefore, the study populations are almost identical. BDI: Beck Depression Inventory; CES-D: Center for Epidemiologic Studies Depression scale; CI: Confidence interval; DM: Diabetes mellitus; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; EPDS: Edinburgh Postnatal Depression Scale; GDM: Gestational diabetes mellitus; HAM-D: Hamilton Depression Scale; HCP: Healthcare professional; ICD: International Classification of Diseases; ICD-9-CM: International Classification of Diseases, 9th revision, Clinical Modification; ICD-10CA: Enhanced version of International Classification of Diseases, 10th revision, for use in Canada; MADRS: Montgomery-Åsberg Depression Rating Scale; MDD: Major depressive disorder; MHI 5: Mental Health Inventory-5; MINI: Mini International Neuropsychiatric Interview; NR: Not reported; NS: Not significant; OGCT: Oral glucose challenge test; OR: Odd ratio; PHQ: Patient Health Questionnaire; PPD: Postpartum depression; RCT: Randomized controlled trial; SCID: Structured Clinical Interview for DSM-IV; SD: Standard deviation; VA: Veterans affairs; WHO-5: World Health Organization Well-Being Index.

depression and DIP, especially GDM. Two claims registry studies (*n* = 11024[46]; *n* = 839792[39]) reported that women with DIP (except type 1 diabetes) were at increased risk of developing depression during or after

Table 2 Outcomes of included studies involving women with pre-existing type 1 or type 2 diabetes

First author study design	Definition/measures of depression	Timing of depression measures	Overall *n* Subgroups, *n*	Main outcomes/findings
Berger[25] Retrospective	EPDS ⩾ 13 or did not answer "No" to self-harm question	Within 4 d after delivery	Unselected, *n* = 322 History of mental illness, *n* = 215	Prevalence of pre-existing DM did not differ between women with or without postpartum depression in either the unselected group or the group with history of mental illness Of 5 women with pre-existing DM, none had depression
Callesen[30] Prospective, cohort	HADS ⩾ 8	8 wk gestation	*n* = 148 Type 1, *n* = 118 Type 2, *n* = 30	Women with DM and depression were more likely to have preterm delivery (54% *vs* 16%, *P* = 0.003) and less likely to be nulliparous (23% *vs* 54%, *P* = 0.03) than women with DM without depression
Dalfra[34] Prospective	CES-D ⩾ 16	3rd trimester and 8 wk after delivery	*n* = 245 Type 1, *n* = 30; No DM, *n* = 39	Mean (SD) CES-D scores at 3rd trimester were 19.1 (9.6) among women with Type 1 DM and 18.0 (8.7) among women without DM (*P* = 0.67) The severity of depressive symptoms increased from the 3rd trimester to after delivery in women with Type 1 DM [estimated mean difference in CES-D score (95%CI): 6.6 (2.9-10.2)], but decreased in women without DM [-2.7 (-5.9-0.5), *P* < 0.0001 between groups]
Jovanovic[39] Retrospective, claims database	ICD-9 codes 311, 296.2, 296.3, 300.4, 301.12, 309.1	During pregnancy and/or within 3 mo after delivery	*n* = 839792 Type 1, *n* = 1125 Type 2, *n* = 10136 No DM, *n* = 773751	Prevalence of depression was 5.2% and 8.3% among women with type 1 and type 2 DM, respectively Prevalence of concurrent type 1 DM and depression was 0.006% Prevalence of concurrent type 2 DM and depression was 0.086% Relative risk (95%CI): of depression in women with type 1 DM *vs* women with no DM was 1.16 (0.86-1.56) Relative risk (95%CI): of depression in women with type 2 DM *vs* women with no DM was 1.84 (1.70-2.00)
Katon 2011[41] Cross-sectional analysis of prospective cohort	PHQ-9	3rd trimester	*n* = 2398 Pre-existing DM (type NR), *n* = 226; No DM, *n* = 1747	Prevalence (95%CI): of probable major depression among women with pre-existing DM was 5.8% (2.7%-8.8%) by PHQ-9 score, 8.9% (5.1%-12.6%) by antidepressant use, and 13.3% (8.8%-17.7%) by either PHQ-9 or antidepressant use, compared with the prevalence among women without DM [PHQ-9: 4.1% (3.2%-5.1%); antidepressants: 6.2% (5.1%-7.3%); PHQ-9 and antidepressants: 9.6% (8.2%-11.0%)] After adjusting for demographic characteristics, chronic medical conditions, and pregnancy variables, pre-existing DM was not associated with major or any antenatal depression (*P* value not reported)
Katon 2014 (PPD)[42] Retrospective, hospital database	PHQ-9	2nd or 3rd trimester and 6 wk after delivery	*n* = 1423	Prevalence of pre-existing DM was higher in women with PPD (14.5%) than in women without PPD (6.9%) (*P* = 0.02) Of 104 women with pre-existing DM, 12 (11.5%) had PPD Pre-existing DM was a risk factor for postpartum depression [OR (95%CI): 1.98 (1.12-3.52)] (*P* = 0.02) Prevalence of concurrent pre-existing DM and depression was 0.84%
Kozhimannil[46] Retrospective cohort	ICD9 codes 296.2, 296.3, 300.4, 301.12, 309.1, and 311	During the 6 mo before and up to 1 yr after delivery	*n* = 11024 With pre-existing DM (type NR), *n* = 311 (taking insulin, *n* = 57); no DM, *n* = 10367	Prevalence of depression in women with pre-existing DM taking insulin was 14.0% *vs* 16.1% among women with pre-existing DM not taking insulin (*P* value not reported)
Levy-Shiff[66] Prospective	BDI	2nd trimester	*n* = 153 Pre-existing DM, *n* = 53 (type NR) No DM, *n* = 49	No significant difference in depression during 2nd trimester between pre-existing DM [mean (SD) BDI score 6.17 (5.16)] and controls [6.59 (5.88)] (*P* ⩾ 0.05) For sample as a whole, higher levels of cognitive assessment of pregnancy as a challenge was associated with lower depression (*P* < 0.05) Among women with pre-existing DM, higher levels of medical support were associated with lower levels of depression (*P* < 0.01)
Mei-Dan[50] Retrospective, health administration database	ICD-9, ICD-10CA, and/or DSM-IV (ICD codes NR)	Within 5 yr before pregnancy	*n* = 437941 With pre-pregnancy depression, *n* = 3724 No known mental illness, *n* = 432358	Prevalence of DM (type NR) within 1 year before the index pregnancy was significantly higher in women with pre-pregnancy depression (3.4%) than in women with no known mental illness (1.2%) (*P* value NR) Prevalence of pre-existing DM and pre-pregnancy depression was 0.029%
Moore[67] Prospective	Depression Adjective Checklist; Perceived Stress Scale	3rd trimester	*n* = 131 Pre-existing insulin-dependent DM, *n* = 73 High risk of preterm birth, *n* = 48 Low risk of preterm birth, *n* = 25	White women with DM who were tested at a private clinic had higher Depression Adjective Checklist and Perceived Stress Scale scores than any other group (variables of white *vs* black, private *vs* public medical centre, DM *vs* low or high risk of preterm birth) (*P* value not reported)
Ragland[54] Prospective, cross-sectional	BDI > 13	During pregnancy	*n* = 50 Type 1 DM, *n* = 8 Type 2 DM, *n* = 20	Mean BDI score was 10.0 among women with type 1 DM and 17.1 among women with type 2 DM No women with type 1 DM and 12 (60%) women with type 2 DM had BDI > 13

[1]Räisänen 2013[56] Retrospective, registry review	ICD10 codes F31.3, F31.5, F32-34	Up to 6 wk postpartum or a history of depression	*n* = 511422	Prevalence of pre-existing DM: 8.4% of women without any depression (n = 492103), 11.1% of women with history of depression but not PPD (*n* = 17881), 14.6% of women with PPD but no history of depression (*n* = 431), and 13.3% of women with both history of depression and PPD (*n* = 1007) (*P* ≤ 0.001)
[1]Räisänen 2014[55] Retrospective, registry review	ICD10 codes F31.3, F31.5, F32-34	At hospital discharge after delivery	*n* = 511938	Prevalence of pre-existing DM (type NR): 8.4% of women without any depression (*n* = 493037), 10.9% of women with history of depression but not during pregnancy (*n* = 14781), 11.6% of women with depression during pregnancy but no history of depression (*n* = 2189), and 13.6% of women with both depression during pregnancy and history of depression (*n* = 1931) (*P* ≤ 0.001) Depression during pregnancy was not associated with pre-existing DM [adjusted OR (95%CI): = 1.10 (0.93-1.31)]
Singh[59] Retrospective	BDI ≥ 10; self-reported medical history	During pregnancy	*n* = 152 History of depression, *n* = 39 No history of depression, *n* = 113	Type 2 DM was significantly more common in women with history of depression than in women with no history of depression (*P* < 0.05) Of 39 women with history of depression, 5 (13%) had type 1 DM, and 19 (49%) had type 2 DM Of 113 women with no history of depression, 18 (16%) had type 1 DM, and 28 (25%) had type 2 DM
Sundaram[62] Prospective, exploratory	Survey of PPD diagnosis; survey of symptoms based on PHQ-2	Postpartum	Up to 61733 pregnancies	In analysis of data from 22 states, pre-existing DM was not a significant predictor of PPD symptoms [OR (95%CI): 1.16 (0.78-1.59), *n* = 45669, *P* = 0.39) or diagnosis [OR (95%CI): 1.31 (0.45-3.06), *n* = 5924, *P* = 0.56)] In analysis of data from 2 states that included both PPD symptoms and diagnosis on the survey, pre-existing DM was a significant predictor of PPD diagnosis [OR (95%CI): 5.65 (1.72-15.37), *n* = 2136, *P* < 0.01)]

[1]The Räisänen *et al* 2013 and 2014 publications use the same database within the same time period (2002-2010) and, therefore, the study populations are almost identical. BDI: Beck Depression Inventory; CES-D: Center for Epidemiologic Studies Depression scale; CI: Confidence interval; DM: Diabetes mellitus; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; EPDS: Edinburgh Postnatal Depression Scale; HADS: Hospital Anxiety and Depression Scale; ICD: International Classification of Disease; ICD-10CA: enhanced version of International Classification of Diseases, 10th revision, for use in Canada; NR: Not reported; PHQ: Patient Health Questionnaire; PPD: Postpartum depression; SD: Standard deviation.

Table 3 Outcomes of included studies involving women with any type of diabetes (not specified or data grouped)

First author study design	Definition/ measures of depression	Timing of depression measures	Overall *n* Subgroups, *n*	Main outcomes/findings
Ahmed[21] Prospective, cross-sectional	EPDS ⩾ 10	6-8 wk postpartum	*n* = 1000 With DM (type NR), *n* = 31 No DM, *n* = 969	The proportion of women with DM who had PPD (51.6%) was significantly higher than the proportion of women without DM who had PPD (27.7%) (*P* = 0.004) Calculated prevalence of women with both DM and PPD was 1.6% (16 of 1000)
Bansil[22] Retrospective	ICD9 codes 296.2, 296.3, 300.4, 311, 298.0, 309.0, 309.1	At the time of delivery	*n* = 32156438 With depression, *n* = 244939; With DM (type 1, type 2, or GDM), *n* = 1536514 With DM and depression, *n* = 18245	Rate of concurrent DM at the time of delivery higher in women with depression (74.5 per 1000 deliveries) *vs* women without depression (47.6 per 1000 deliveries; OR (95%CI): 1.52 (1.47-1.58)] Calculated prevalence of DM and depression = 0.06% (18245 of 32156438 deliveries)
Benute[24] Prospective	PRIME-MD	During prenatal outpatient visits/ hospital-isation	*n* = 326 With DM, *n* = 84 With MDD, *n* = 29	Prevalence of DM in women with MDD was 7.1% Calculated prevalence of DM and MDD = 0.61% (7.1% of 29 = 2; 2/326 = 0.61%)
Berger[25] Retrospective	EPDS ⩾ 13 or did not answer "No" to self-harm question	Within 4 d after delivery	Unselected, *n* = 322 History of mental illness, *n* = 215	Prevalence of any DM did not differ between women with or without postpartum depression in either the unselected group or the group with history of mental illness
Chen[32] Retrospective	ICD9 codes 296.2, 296.3, 300.4, and 311	History of depression within 2 years before delivery	*n* = 5283 With DM (type NR), *n* = 319	Calculated prevalence of DM among women with depression was 6.0%
Kozhimannil[46] Retrospective cohort	ICD9 codes 296.2, 296.3, 300.4, 301.12, 309.1, and 311	During the 6 mo before and up to 1 year after delivery	*n* = 11024 With DM (pre-existing or GDM), *n* = 657; No DM, *n* = 10367	Overall calculated prevalence of women with both DM (any type) and depression was 1.1% Prevalence of depression among women with any DM was 15.2% *vs* 8.5% among women without DM (*P* value not reported)
Ragland[54] Prospective, cross-sectional	BDI > 13	During pregnancy	*n* = 50 Type 1 DM, *n* = 8 Type 2 DM, *n* = 20 GDM, *n* = 22	Women with any DM had an increased odds of experiencing depression during or after pregnancy [OR (95%CI): 1.85 (1.45-2.36)] *vs* women without DM Women with any DM and no prenatal depression (9.6%) had increased odds of experiencing PPD or taking an antidepressant in the year after delivery [OR (95%CI): 1.69 (1.27-2.23)] *vs* women without DM

				Mean (SD) BDI score was 14.1 (9.9), range 3-43 Number (%) women with DM and severe (BDI ≥ 29), moderate (BDI 20-28), mild (BDI 14-19), and minimal (BDI 0-13) depression was 5 (10%), 8 (16%), 8 (16%), and 29 (58%) 42% of women with DM had BDI scores > 13, indicating clinical depression Among patients with clinical depression, only 19% were receiving treatment for depression Number of pregnancies showed a positive correlation with BDI score (*P* = 0.0078) Least mean squares of HbA1c level was higher, but not significantly, in women with depression [7.3% (56 mmol/mol)] than in those without [6.9% (52 mmol/mol)] (*P* ≥ 0.05)
Räisänen 2013[56] Retrospective, registry review	ICD10 codes F31.3, F31.5, and F32-34	Up to 6 wk postpartum or a history of depression	*n* = 511422	Calculated prevalence of DM (any type) and depression in pregnant women = 0.06%
Singh[59] Retrospective	BDI ≥ 10; self-reported medical history	During pregnancy	*n* = 152 History of depression, *n* = 39 No history of depression, *n* = 113	Current BDI scores were higher in women with DM and history of depression [mean (SD) 17.2 (11.5)] than in women with DM and no history of depression [7.8 (7.4), *P* < 0.0001] Percentage of women with BDI ≥ 10 significantly greater in women with DM and history of depression (72%) than in women with DM and no history of depression (28%, *P* < 0.0001)
York[65] Prospective	Multiple Adjective Check List	36 wk gestation, and 2 d, 1 wk, 4 wk, and 8 wk postpartum	*n* = 36 Pre-existing DM, *n* = 6 GDM, *n* = 30	Most women did not report high levels of depression Among all women with DM, depression scores decreased significantly (*P* < 0.001) over time [mean (SD) scores of 9.2 (6.6), 10.1 (8.3), 6.7 (8.2), 5.6 (7.0), and 3.8 (4.2) at 36 wk gestation, 2 d postpartum, 1 wk postpartum, 4 wk postpartum, and 8 wk postpartum, respectively] There were no differences between women with GDM and women with pre-existing DM in depression scores during pregnancy (*P* = 0.17) or postpartum (P value not reported)

BDI: Beck Depression Inventory; CI: Confidence interval; DM: Diabetes mellitus; EPDS: Edinburgh Postnatal Depression Scale; GDM: Gestational diabetes mellitus; ICD: International Classification of Disease; MDD: Major depressive disorder; NR: Not reported; PPD: Postpartum depression; PRIME-MD: Primary Care Evaluation of Mental Disorders classification system; SD: Standard deviation.

pregnancy relative to pregnant women without diabetes [any DIP: OR (95%CI): 1.85 (1.45-2.36)[46]; GDM: Relative risk (95%CI): 1.17 (1.12-1.21)[39]; type 2 diabetes: Relative risk (95%CI): 1.84 (1.70-2.00)[39]; type 1 diabetes: Relative risk (95%CI): 1.16 (0.86-1.56)[39]]. Similarly, a maternal and infant database study (*n* = 1057647) reported that GDM was significantly associated with increased risk of depression at the time of hospital discharge after delivery [adjusted OR (95%CI): 1.44 (1.26-1.65)][64]. A hospital records review (*n* = 18192 pregnancies) reported that GDM was significantly associated with increased risk of postpartum depression after adjustment for age, pre-eclampsia, and preterm birth [OR (95%CI): 1.46 (1.16-1.83); *P* = 0.001], but not after adjustment for other clinical and socioeconomic factors [OR (95%CI): 1.29 (0.98-1.70); *P* = 0.064)[63]. Conversely, another hospital records review (*n* = 128295) reported that a history of depression was a risk factor for the development of GDM [OR (95%CI): 1.42 (1.26-1.60)][28]. A national health database study (n > 32 million) reported that women with depression at delivery were more likely to also have diabetes (type not specified) than women without depression [OR (95%CI): 1.52 (1.47-1.58)][22]. In another national health database study that examined the relationship between reproductive risk factors and postpartum depression (*n* = 511422), the prevalence of DIP (pre-existing or gestational) was greater among women with a history of depression or with postpartum depression than among those without any depression[56]. This study also reported that in women with a history of depression, the risk of postpartum depression is increased in those who also have GDM [OR (95%CI): 1.62 (1.23-2.14)]. A related study using the same database reported that an increased prevalence of depression during pregnancy was associated with GDM [adjusted OR (95%CI): 1.29 (1.11-1.50)], but not with pre-existing diabetes [adjusted OR (95%CI): 1.10 (0.93-1.31)][55]. The remaining health database studies that used ICD codes only reported prevalence data[32,40,50].

The timing of depression assessment also varied (Tables 1-3). There were 22 studies that measured depression only during pregnancy[22,24,26,30,31,36,37,40,41,43,45,51,53-55,57-59,61,64,66,67]. Conversely, 11 studies focussed on postpartum depression, most commonly measured within the first 3 mo[20,21,23,25,27,29,33,47,52,62,63]. There were nine studies that measured depression during both pregnancy and postpartum[34,35,38,39,42,44,46,49,65] and five studies that classified participants based on a history of pre-pregnancy depression[28,32,50,56,60].

Prevalence of concurrent DIP and depression during or after pregnancy

The prevalence of concurrent DIP and depression in a general population sample of pregnant or post-

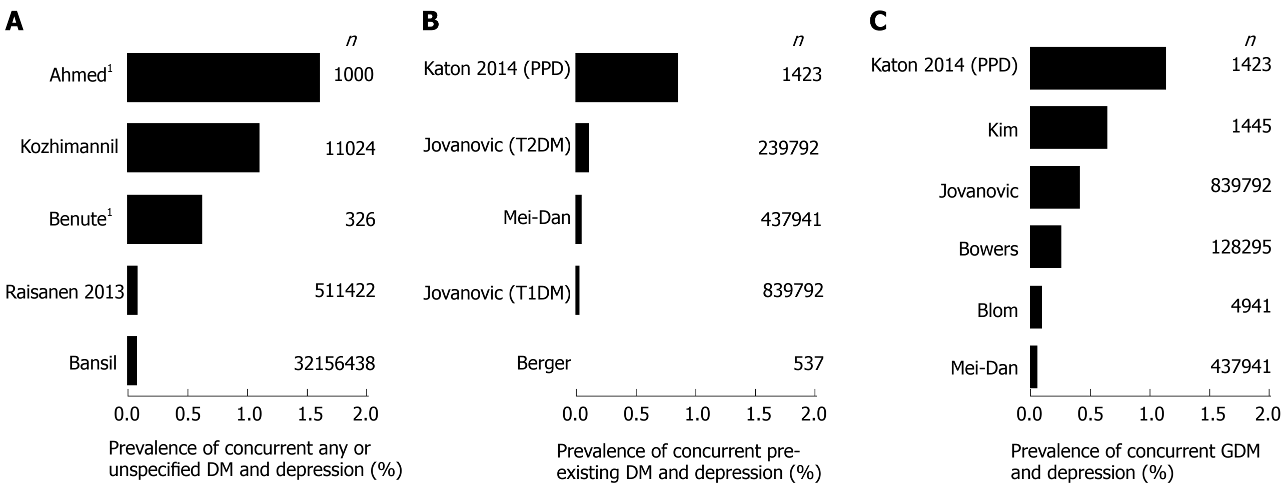

Figure 2 Prevalence of concurrent diabetes and depression reported in studies included in this review. The *n* for each study represents the overall sample size. A: Prevalence of concurrent diabetes (types combined or not specified[1]) and depression; B: Prevalence of concurrent pre-existing diabetes and depression; C: Prevalence of concurrent gestational diabetes and depression. DM: Diabetes mellitus; GDM: Gestational diabetes mellitus; PPD: Postpartum depression; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus.

partum women was reported or could be calculated from data in 12 retrospective or cross-sectional studies[21,22,24,25,27,28,39,42,44,46,50,56] and ranged from 0% to 1.6% (median 0.61%) (Figure 2). The prevalence of depression during or after pregnancy concurrent with any or unspecified diabetes ranged from 0.06% to 1.6% (5 studies; median 0.61%) (Figure 2A). The prevalence of concurrent pre-existing diabetes and depression during or after pregnancy ranged from 0.006% (type 1 diabetes only) to 1.1% (4 studies, median 0.03%) (Figure 2B). The prevalence of concurrent GDM and depression during or after pregnancy ranged from 0.029% to 1.12% (6 studies, median 0.32%) (Figure 2C).

Gestational diabetes

Among women with GDM (Table 1, Figure 3), the reported prevalence of depression during or after pregnancy ranged widely, from 4.1% to 80% (16 studies[26,31,33,35,39-41,43,44,46,51,52,54,57,58,61], median 14.7%). Heterogeneity in sample size, the definition of depression, and the timing of its assessment is likely to have contributed to this wide range of prevalence rates.

The prevalence of GDM among women with a history of depression, reported in seven studies[23,27,28,48,50,55,56], ranged from 1.0%[27] to 17.6% (women with both history of depression and postpartum depression[55,56]) (Table 1).

Pre-existing diabetes

Among women with pre-existing diabetes (Table 2), the prevalence of depression during or after pregnancy ranged from 0% to 60% (6 studies, median 8.3%), similar to the broad range reported for women with GDM. The prevalence of depression during or after pregnancy in women with pre-existing diabetes was 0% (in a small sample of five women with pre-existing diabetes)[25], 0% (in a small sample of eight women with type 1 diabetes)[54], 5.2% (type 1 diabetes)[39], 5.8%[41], 8.3% (type 2 diabetes)[39], 11.5%[42], 14.0% (women taking insulin)[46], 16.1% (women not taking insulin)[46], and 60% (women with type 2 diabetes)[54].

Diabetes as a risk factor for depression during or after pregnancy

Many of the studies examined whether DIP was a risk factor for depression during or after pregnancy, or compared the prevalence of depression between women with DIP and pregnant women without diabetes. Overall, there was no consensus regarding whether women with DIP were more likely to have depression than pregnant women without diabetes.

Gestational diabetes

In 11 studies[20,25,26,29,35,39,51,55,56,61,64], women with GDM had a significantly greater prevalence or risk of depression during or after pregnancy than pregnant women without diabetes (Table 1). In two of these studies, a significant effect of GDM was observed only for one subgroup of women (Qatari women, but not other Arab women[29]; women with a history of depression, but not women without a history of depression[56]). In one study[35], the prevalence of depression among women with GDM was significantly greater than pregnant women without diabetes at 30 wk gestation, but not at 36 wk gestation or postpartum. In contrast, 16 studies reported no significant effect of GDM on the prevalence or risk of depression[23,27,31,34,38,41-44,47,49,57,58,62,63,66].

Pre-existing diabetes

Four studies reported no significant difference in depression between pregnant women with pre-existing diabetes and those without diabetes[25,41,55,66] (Table 2). One exploratory study was inconclusive, reporting that pre-existing diabetes was a significant predictor of postpartum depression diagnosis in a subset of data from two states of the United States, but not in the

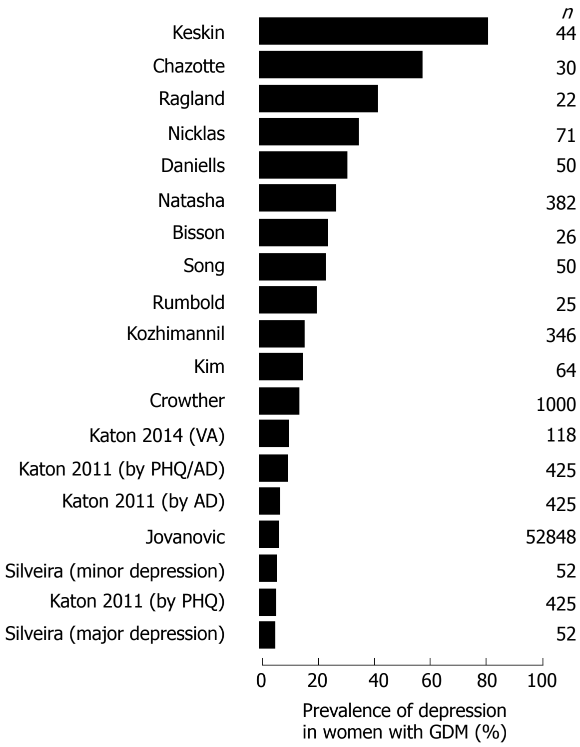

Figure 3 Prevalence of depression reported among women with gestational diabetes in studies included in this review. The *n* for each study represents the sample size of women with gestational diabetes. AD: Antidepressant medication; GDM: Gestational diabetes mellitus; PHQ: Patient Health Questionnaire; VA: Veterans Affairs.

nationwide analysis set[62]. In one retrospective study, pre-existing type 2 diabetes, but not type 1 diabetes, was associated with an increased risk of depression during or after pregnancy[39]. In another retrospective study, pre-existing diabetes was identified as a risk factor for postpartum depression[42].

Any type of diabetes
Two studies reported a greater prevalence[21] or risk (OR)[46] of depression among women with any type of DIP compared with pregnant women without diabetes (Table 3). One study reported a significant increase in the severity of depressive symptoms between the third trimester and postpartum among women with GDM or type 1 diabetes, but not among pregnant women without diabetes[34]. Another study reported no difference in the prevalence of any diabetes between women with postpartum depression and those without postpartum depression[25].

Depression as a risk factor for gestational diabetes
Several studies examined whether depression was a risk factor for the development of GDM, but again, there was no consensus (Table 1). Two studies of the same national database reported a greater prevalence[55,56] and one study reported a greater risk (OR[28]) of GDM among women with a pre-pregnancy history of depression. In contrast, two studies reported no difference in the prevalence of GDM (or abnormal glucose levels) among women with depression early in pregnancy compared with women without depression[37,53], and a third study reported similar prevalence rates of GDM in women with and without pre-pregnancy depression[50].

Treatment and management
Our literature search did not identify any specific guidelines on the treatment or management of women with both DIP and depression during or after pregnancy. Very few studies reported on the effects of treatment of either diabetes or depression on outcomes. In the completed RCT[33], a significantly lower proportion of women with GDM who received dietary advice, performed blood glucose monitoring, and were treated with insulin therapy as needed had postpartum depression compared with women with GDM who received usual obstetric care (8% *vs* 17%; $P = 0.001$). In the prospective study by Dalfrà *et al*[34], mean depressive symptom scores during the third trimester did not differ between women with GDM who were managed with diet only and women with GDM who were treated with insulin ($P = 0.58$). In the retrospective study by Kozhimannil *et al*[46], the prevalence of depression during or after pregnancy among women with GDM who were treated with insulin was slightly higher than in women who were not treated with insulin (16.0% *vs* 13.7%; *P* value not reported). In the same study, the prevalence of depression among women with pre-existing diabetes was slightly lower in those who were treated with insulin than in those who were not (14.0% *vs* 16.1%; *P* value not reported). In the prospective study by Levy-Shiff *et al*[66], higher levels of patient-reported support from medical staff were associated with lower levels of depression in women with pre-existing diabetes ($P < 0.01$). Similarly, in the prospective study by Ko *et al*[45], women with GDM who participated in a 4-week educational coaching program had a greater decrease in depression scores than those who did not participate. In the prospective study by Ragland *et al*[54], only 19% of women with concurrent DIP (any type) and depression (Beck Depression Inventory score > 13) were receiving treatment for depression. In the same study, the HbA1c level was numerically higher, but not significantly higher, in women with both DIP and depression compared with pregnant women without depression [7.3% (56 mmol/mol) *vs* 6.9% (52 mmol/mol); $P \geqslant 0.05$].

DISCUSSION
This is the first systematic literature review assessing what is known about women who have both DIP and depression during pregnancy or postpartum. Despite the number of studies identified, there was no clear consensus on whether women with DIP are more likely to develop depression than pregnant women without diabetes, or whether women with depression were more likely to develop GDM. Heterogeneity in the definition of depression, the scales used to measure depressive symptoms, the timing of measures, and the types of diabetes examined, together with the poor quality and

observational nature of most of the studies, are likely to have contributed to the lack of consensus. Further, the primary objective of many studies was not directly relevant to this review and the results we report were often secondary or incidental findings. Importantly, we did not identify any guidelines for the management of women with both DIP and depression. Given that 0.006% to 1.6% (median 0.61%) of pregnant women are reported to have both diabetes and depression, and that this prevalence is likely to rise, guidance on managing these women would be valuable to healthcare professionals.

Although many of the studies in this review examined the relationship between DIP and depression, there was no consensus on whether women with DIP are at greater risk of depression than pregnant women without diabetes. The reasons for the disparate results among the studies may in part be due to different definitions of depression and the timing of its measurement, as well as differences in study population, outcomes, and objectives. Only a quarter of the studies used a diagnosis of depression instead of symptoms, which may have made it more difficult to establish if there was a link. For example, in a meta-analysis of studies involving non-pregnant patients, diabetes was identified as a significant risk factor for depression as defined by diagnosis or prescription of antidepressants, but not when depression was defined by symptoms using questionnaires[9]. However, almost all the large, retrospective database studies that used ICD codes to define depression were suggestive of an increased prevalence or risk of depression among women with DIP, especially those with GDM[22,39,46,55,56,64].

Although the exact mechanisms that link diabetes and depression are not known, especially in pregnant or postpartum women, current hypotheses in non-pregnant patients focus on both psychological and biological factors[13]. For example, the higher prevalence of depression in patients with diabetes may be related to the burden of coping with a chronic disease[69]. Conversely, depression is often associated with lifestyle choices, such as poor diet and lack of exercise, which may increase the risk of developing type 2 diabetes. However, these behavioral factors do not account for all of the increased risk of diabetes in patients with depression[70,71]. Depression and diabetes may also share some biological pathologies, such as altered activity of the hypothalamic-pituitary-adrenal axis, sympathetic nervous system, and inflammatory processes[72]. Regardless of the underlying mechanisms, there is now considerable evidence that diabetes and depression are closely linked and that patients with either disease are at increased risk of developing the other[8-12]. Whether the same mechanisms are involved in linking depression with diabetes in pregnancy remains unclear, and studies designed to investigate these mechanisms are required.

Few studies examined the potential role of treatment or glycemic control on depression in women with DIP. Among these, the RCT by Crowther *et al*[33] reported that women with GDM who received active intervention (dietary advice, glucose monitoring, and insulin therapy, if needed) were significantly less likely to develop postpartum depression than women receiving routine obstetric care. Unfortunately, measures of glycemic control and their relationship to postpartum depression were not reported. A previous meta-analysis has indicated that depression among non-pregnant patients with diabetes was significantly associated with poorer glycemic control[73]. However, there is no similar evidence for a relationship between glycemic control and depression among pregnant women.

There was also no consensus among the few studies that examined whether pre-pregnancy depression increased the risk of GDM. Given that depression is linked to obesity and insulin resistance[13], women with depression who become pregnant should be carefully monitored for impaired glucose tolerance. In addition, certain antidepressant and centrally acting antipsychotic medications may increase the risk of type 2 diabetes[74]. This relationship is attributable to several mechanisms, both associated with and independent of weight gain[74], and a similar relationship may exist for GDM.

This review is strengthened by the systematic methods used to identify publications and by the absence of restrictions on publication date or language. In addition, the inclusion of studies involving all types of diabetes and definitions of depression increased the number of publications reviewed. However, the resulting heterogeneity, especially in the definition of depression, is likely to have contributed to the lack of consensus. Indeed, our original intent was to only include studies that used a formal clinical diagnosis of depression. However, preliminary searches revealed that few such studies exist and most of those that do are retrospective. For this reason, we expanded our inclusion criteria to also capture studies that used measures of depressive symptoms, allowing us to assess the wider body of evidence on this topic.

Our review is also limited by the observational nature of almost all the studies and because many of the studies were not designed to examine the relationship between depression and DIP. Observational studies are subject to a range of potential biases, including selection bias, information bias, recall bias, and attrition bias. In addition, many of the articles included in the review were poorly reported, making assessment of the true quality of individual studies difficult. Most studies did not report outcomes of specific interest to us, such as the effect of treatment for depression or diabetes on maternal outcomes, risk factors that contribute to co-occurrence of depression and DIP, and prevalence rates, many of which we calculated from reported data. However, RCTs involving pregnant women are uncommon because of ethical considerations, and observational studies may be the only way to examine the relationship between depression and DIP.

Importantly, we did not identify any specific guidelines for the management of women with both DIP and depression during or after pregnancy. Unfortunately, major clinical treatment guidelines for diabetes and depression do not address these patients. The American Diabetes Association (ADA) Standards of Medical Care recommend routine screening for depression in patients with diabetes, but any special care for pregnant women is not addressed[15]. Similarly, the American College of Obstetricians and Gynecologists Practice Bulletin on GDM does not address mental health issues[16]. Although the American Psychiatric Association Practice Guideline for major depressive disorder provides guidance for patients who also have diabetes or are pregnant, it does not provide guidance for women who have DIP[18]. However, limited management guidance for women with DIP and depression is provided by some country-specific guidelines (*e.g.*, Germany[75] and India[76]). In addition, a consensus statement published by the ADA in 2008 recommends screening for depression before and during pregnancy in women with pre-existing diabetes[77]. Although the consensus statement indicates that the management plan should be adjusted in women with DIP and depression, the only recommendation provided is to use structured psychotherapy as first-line treatment for mild depression[77]. Given the expected increase in the number of women with DIP and depression, together with the particular challenges these women face in caring for themselves and their children, healthcare professionals need more specific guidance on management strategies for these patients. A collaborative care approach involving primary care physicians and specialists improves outcomes in non-pregnant patients with both diabetes and depression[78], and a similar model may be effective for the management of pregnant and postpartum women. Such guidance, however, should be based on sound research evidence, which, as our review demonstrates, is currently lacking. In agreement with the results of our systematic review, two narrative reviews[5,6] and a systematic review focussing on the transition to motherhood in women with type 1 diabetes[79] have recognized that rigorous research into DIP and depression (and other psychosocial issues) is much needed. In addition, greater awareness of depression is needed among clinicians who treat women with diabetes, which will allow for better planning and management of pregnancy.

In conclusion, this systematic review highlights the need for additional, high-quality research into the relationship between DIP and depression. Such research is needed to inform the development of evidence-based guidelines that will help clinicians care for women with both DIP and depression.

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COMMENTS

Background

Diabetes in pregnancy (DIP) has adverse effects on women and their children, as does depression during pregnancy or postpartum. Both DIP and depression are increasingly common, and it is likely that the number of women with both conditions is also growing. However, major diabetes and mental health guidelines do not provide adequate advice regarding care of patients with both DIP and depression.

Research frontiers

At present, the prevalence of women with concurrent DIP and depression has not been established. In addition, recent evidence suggests a bidirectional relationship between diabetes and depression among non-pregnant patients, but it is not known if a similar link exists in pregnant or postpartum patients.

Innovations and breakthroughs

This is the first systematic literature review assessing what is known about women who have both DIP and depression during pregnancy or postpartum. Despite the number of studies identified (n = 48), there was no clear consensus on whether women with DIP are more likely to develop depression than pregnant women without diabetes, or whether women with depression were more likely to develop gestational diabetes. Importantly, they did not identify any guidelines for the management of women with both DIP and depression.

Applications

This systematic review highlights the need for additional, high-quality research into the relationship between DIP and depression. Such research is needed to inform the development of evidence-based guidelines that will help clinicians care for women with both DIP and depression.

Terminology

Women with DIP include those who had pre-existing type 1 or type 2 diabetes mellitus before becoming pregnant and those who developed gestational diabetes mellitus during pregnancy. Gestational diabetes mellitus is characterized by elevated blood glucose levels that develop during mid-pregnancy and that usually resolve after childbirth.

Peer-review

This manuscript is a systematic review of the literature about the relationship between depression (postpartum depression in particular) and diabetes in pregnancy. The assessment of the articles indicated overall poor study quality as many studies were observational and often lacked stringent, objective criteria to support a diagnosis of clinical depression. The main conclusion of the authors is that high quality research with stringent criteria and assessable parameters is needed to establish specific guidelines for management of pregnant women with depression and diabetes.

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