

Dietary fat and insulin resistance: a connection through leptin and PPAR γ activation

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ABSTRACT

Insulin resistance refers to reduced insulin action in peripheral tissues and impaired suppression of endogenous glucose production, a state which is critical for maintaining normal glucose homeostasis. Insulin resistance is partly explained by genetic factors and is strongly influenced by the individual's habitual lifestyle. Investigating factors that may influence the development of insulin resistance and their mechanisms of action is highly significant; one of these factors include dietary fat. Both quantitative and qualitative terms of dietary fat have been known to play an important role in the development of insulin resistance, although the mechanism underlying this effect is not fully understood. In this regard, the classical view has been that dietary fat quality mainly affects cell membrane fatty acid composition and consequently the membrane function. Recently, the relationship between dietary fat and insulin resistance has entered an advanced level due to the discovery that different fatty acids can regulate gene expression, transcriptional activity and adipocytokines secretion. In essence, this provides new mechanisms by which fatty acids exert their cellular effects. The present review critically assesses the effect of dietary fat quality on the development of insulin resistance in relation to the adipocytokine, leptin and the activation of the transcription factor, peroxisome proliferator-activated receptor gamma (PPAR γ). It is evident that fat quality influences the development of insulin resistance and has a more important role than quantity. Leptin and PPAR γ prove to be potential candidates linking dietary fat with insulin resistance. However, the exact role or mechanism of action of various types of dietary fat in the development of insulin resistance is still uncertain. Further well-controlled studies in humans are necessary to establish better evidence-based dietary fat recommendations for diabetes prevention and its clinical management.

Keywords: dietary fat, insulin resistance, diabetes, PPAR γ activation, leptin.

BACKGROUND

Diabetes type 2 is recognized as one of the fastest growing epidemics worldwide. It has been estimated that between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries [1]. Individuals with insulin resistance, glucose intolerance and hyperinsulinemia are at higher risk for developing diabetes type 2. It has been estimated that individuals with impaired fasting glucose (IFG) have a 20–30% chance of developing diabetes over the next 5–10 years [2]. Therefore, preventing the development of such disorders may help in diabetes prevention, thereby reducing public burden.

Recent evidence has shown success for lifestyle intervention, including increasing physical activity and dietary modification in preventing or delaying the development of diabetes [2-5]. There are several aspects of the diet that can help in diabetes prevention as well as treatment. The role of dietary fat type in the development of insulin resistance, which usually precedes the onset of diabetes type 2, has been of considerable interest. Many studies have been undertaken to examine the underlying mechanisms linking dietary fat type to insulin resistance [6-8]. Recently, more attention has been given to the ability of dietary fat to directly affect gene expression, transcriptional activity and adipocytokines secretion.

Peroxisome proliferator-activated receptor gamma (PPAR γ), a member of the nuclear receptor superfamily, is suggested to play a role in insulin resistance and diabetes. The activation of this receptor can either increase or decrease the transcription of target genes. PPAR γ activation represses the gene expression of tumor necrosis factor alpha (TNF α), resistin and leptin, all of which have been implicated in insulin resistance [9]. On the other hand, activation of PPAR γ induces gene expression of insulin-sensitizing proteins such as adiponectin and fatty acid transport protein [9]. The high affinity of thiazolidinediones (TZDs), a class of insulin-sensitizing agents, for PPAR γ confirms the important role of PPAR γ in controlling glucose homeostasis and partially explains TZDs mode of action in enhancing insulin sensitivity [10]. Knowing that fatty acids and their derivatives are natural ligands of PPAR γ [11] may provide a link between dietary fat and insulin resistance, in addition to diabetes.

Activation of PPAR γ represses the gene expression of leptin, a product of the ob gene that is mainly released by the adipose tissue [9]. This may indicate that leptin plays a role in the development of insulin resistance. Data concerning the role of leptin in glucose metabolism, insulin resistance and diabetes are not clear, with some suggesting a biphasic impact that seems to be concentration dependent [12]. Dietary fat may affect leptin gene expression and function differently [13]; these possible differential effects remain to be clarified.

Insulin resistance, diabetes type 2 and obesity

Insulin resistance is defined as a state of reduced responsiveness to the normal concentrations of circulating insulin [14]. It is associated with aging, sedentary lifestyle, as well as genetic predisposition [15]. Additionally, insulin resistance is usually present with atherosclerosis, central obesity, dyslipidemia and hypertension [9]. Insulin resistance can progress to diabetes type 2, which is characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production [14].

Insulin resistance usually precedes the onset of diabetes type 2. There is increasing evidence supporting the fact that by the time glucose tolerance or fasting glucose levels become impaired,

appreciable pancreatic β -cell destruction may have already occurred [16]. Moreover, despite the fact that treatment may prevent some of the diabetes devastating complications, it does not usually restore normoglycemia or eliminate all the adverse consequences of diabetes [17]. Therefore, it appears that attempts to prevent diabetes type 2 will be more successful if intervention is initiated when blood glucose levels are still in the normal range. Hence, a simple test for identifying insulin-resistant individuals is essential to start an early lifestyle intervention that may help in preventing or delaying the onset of diabetes. Indeed, for prediabetic individuals, lifestyle modification can be considered as the cornerstone of diabetes prevention with evidence of a 40%–70% relative risk reduction [18].

There is now substantive evidence implying that diabetes type 2 is associated with obesity, especially the accumulation of fat in the visceral abdominal part of the body [19]. Visceral abdominal fat leads to labile fatty acid release, inflammatory cell accumulation, disruption in adipokines secretion, including reduction in adiponectin levels, and decreased PPAR γ activity, [20] all of which can affect glucose and lipid metabolism contributing to obesity-related insulin resistance and diabetes. Additionally, obesity is characterized by a failure of adipose tissues to store excess energy appropriately leading to ectopic lipid deposition [21]. Ectopic lipid accumulation can contribute to organ injury and dysfunction which impair insulin signaling leading to insulin resistance [22, 23].

When fatty acid beta-oxidation in the mitochondria of nonadipose tissues such as the liver, skeletal muscle, pancreas, and heart cannot keep up with the increased supply of redirected free fatty acids (FFAs) from adipose tissue due to increase in its lipolytic activity, together with the impaired ability of taking up FFAs, accumulation of lipid intermediates like diacylglycerols (DAGs) and ceramides occurs in these organs leading to activation of serine/threonine kinases that phosphorylate insulin-receptor substrates (IRSs) molecules on serine residues [22]. Serine phosphorylated IRSs do not function properly, hence insulin signaling is impaired, and normal metabolic processes are disrupted [22].

Likewise, it has been found that insulin resistance is significantly and positively associated with body mass index (BMI), body fat percentage and waist circumference [24]. In a study by Grundy et al. [25], both body fat content and distribution correlated positively with insulin resistance in both men and women. Additionally, the risk of diabetes is reportedly increased by 7.3% upon each kilogram of weight gain [26]. Data from a prospective study indicated that as little as 4% weight loss is associated with a reduction in the risk of diabetes type 2 [27]. In fact, it has been shown that substantial weight loss mobilizes ectopic fat stores which are associated with an improvement in organs function such as the liver and skeletal muscles [22]. Consequently, a reduction in hepatic triglyceride (TG) and intramyocellular lipid contents was accompanied by a decline in fasting endogenous glucose production and an improved insulin-stimulated glucose disposal, respectively [22]. Thus, increasing evidence supports the view that body weight control improves insulin sensitivity and reduces the risk of diabetes type 2. Altogether, it appears that insulin resistance is displayed before and during diabetes type 2, and the development of diabetes type 2 is closely associated with obesity.

Despite the strong association between obesity and several metabolic abnormalities including insulin resistance and diabetes type 2, recent research is exploring a novel and interesting area revealing the concept of "metabolically healthy obesity". Individuals who are

described as "metabolically healthy obese" are protected from the metabolic abnormalities associated with obesity, such as diabetes and cardiovascular diseases [28]. These "metabolically healthy obese" show some unique features that set them apart. A hypothesis that some "metabolically healthy obese" will show increase in adipogenesis producing smaller insulin-sensitive adipocytes based on functional allelic variants of PPAR γ or other components of the adipogenic transcriptional program has been proposed. Additionally, other features that may explain the absence of the metabolic abnormalities associated with obesity in these individuals are reviewed by Denis and Obin [28].

Genetic determinants of insulin resistance

Both genes and the environment play a role in insulin resistance. In skeletal muscle, insulin resistance leads to lower rates of glucose uptake [29], whereas in the liver, it leads to higher rates of hepatic glucose production mainly as a result of increased gluconeogenesis [29]. Insulin resistance in adipose tissue leads to increased release of FFAs, which are used by the liver for triglyceride (TG) synthesis, and the resulting glycerol for gluconeogenesis [29]. High concentrations of FFAs and their metabolites (fatty acyl-CoA, DAGs and ceramides) can disrupt insulin signaling, block glucose oxidation, impair glucose transport and lead to impaired glucose metabolism; this cascade of events is probably influenced by variants in genes regulating insulin action in different tissues [23,30].

Recent studies in patients with insulin resistance and diabetes have demonstrated that the risk of diabetes type 2 and insulin resistance can be influenced by polymorphisms in a number of genes [30]. These include the genes for PPAR γ , a nuclear receptor involved in insulin action, the hepatocyte nuclear factor 4 α , a transcription factor that regulates pancreatic β -cell function, and others [30]. For example, a number of genetic variants in the PPAR γ gene have been found, ranging from very rare to highly prevalent gain-of-function or loss-of-function of the receptor [31]. Individuals with these genetic variants show decreased or increased lipid accumulation in adipose tissue, enhanced insulin sensitivity or insulin resistance, dyslipidemia, diabetes, and hypertension [32]. The most widely studied variant is the Pro12Ala polymorphism. This polymorphism is generally associated with reduced risk of diabetes type 2, increased insulin sensitivity and lower BMI [32]. These associations seem to be highly influenced by various gene-gene and gene-environment interactions which may explain the conflict results found in the literature.

Additionally, candidate gene association studies in diabetes type 2 and insulin resistance indicate a role for a number of genes involved in insulin action as well as pancreatic β -cell function, including insulin receptor, phosphatidyl inositol (PI) 3-kinases, and glucose transporter (GLUT)-2 [9]. Furthermore, several genes involved in adipocyte metabolism are involved in insulin resistance; these include leptin, leptin receptor, adiponectin, adiponectin receptor, resistin, and uncoupling protein 2 [30].

Dietary fat determinants of insulin resistance

Many aspects of diet composition have been considered to be important in the modulation of insulin resistance. During the past few years, more attention has been paid to the ability of the type of dietary fat, independent of the total amount, to influence insulin sensitivity and thus, the

risk of diabetes type 2. It is acceptable that saturated fatty acids (SFA) are associated with increased risk of insulin resistance. The ω -3 polyunsaturated fatty acids (PUFA) may be beneficial in animals but not in humans [33, 34]. The effect of ω -6 fatty acids on insulin resistance is more controversial. In addition, the association between monounsaturated fatty acids (MUFA) intake and the risk of insulin resistance and diabetes type 2 is less well understood.

Through reading the literature, it is observed that many studies failed to produce consistent results concerning the role of each type of dietary fat in insulin resistance and the development of diabetes type 2 [35-38]. These discrepancies may be due to differences and/or insufficient sample size, divers' clinical characteristics of the sample, the nature of the dietary modification, measures of insulin sensitivity and the study design.

Role of monounsaturated fatty acids

Several studies which have been made using a wide difference in total fat content (15–25% of energy) generally found a beneficial effect of MUFA diets on glycemic control and serum lipids [39]. Energy-controlled high MUFA diets do not promote weight gain and are more acceptable than low fat diets for weight loss in obese subjects [39]. Therefore, MUFA diets can be a good alternative to low fat diets for people with diabetes.

In the KANWU study, 162 healthy subjects received a controlled isoenergetic diets for 3 months containing high proportion of either SFA or MUFA. SFA-based diet significantly impaired insulin sensitivity while the MUFA-based diet did not show this effect. It is worth noting that both diets had no influence on body weight and the supplementation with ω -3 fatty acids did not influence insulin sensitivity [36]. In another study involving patients with diabetes type 2, changing from a PUFA-based diet to a MUFA-based diet resulted in a reduction in insulin resistance with an increase in the insulin-stimulated glucose transport [40].

In a population-based study that has examined the relationship of dietary fatty acids, especially MUFA, with insulin secretion and insulin resistance, suggested a favorable relationship of MUFA with pancreatic β -cell insulin secretion [41]. Additionally, an improvement in insulin sensitivity in patients with diabetes type 2 and in insulin-resistant subjects when diet is changed from high carbohydrate to an isocaloric high MUFA diet has been demonstrated [40]. In another study, an isocaloric MUFA-rich diet prevented central fat redistribution, the decrease in adiponectin gene expression and insulin resistance induced by a carbohydrate-rich diet in insulin-resistant subjects [8].

However not all studies show a positive effect of MUFA on insulin sensitivity. Some epidemiological studies have generally found no association between MUFA intake and the risk of diabetes type 2 [35, 38]. Furthermore, some studies have even found an inverse association between oleic acid, which makes up 92% of MUFA present in food, and insulin sensitivity [42].

Role of polyunsaturated fatty acids

In a randomized trial by Summers et al [43], a diet rich in ω -6 PUFA improved insulin sensitivity with a reduction in the abdominal subcutaneous fat area when compared with a SFA-rich diet after only 5 weeks. The results of another study [7], comparing the effect of SFA and ω -6 PUFA on insulin resistance indicated that dietary SFA induced insulin resistance while the diet rich in

ω -6 PUFA prevented this effect in rats fed those diets for 8 weeks. Moreover, ω -6 PUFA demonstrated a higher membrane fluidity when compared to SFA in rats fed diets rich in beef tallow or safflower oil for 8 weeks which suggests a positive impact on glucose transport, thus insulin sensitivity [44]. In a study by Ide et al [45], the insulin-dependent increase in glucose oxidation in response to insulin stimuli in isolated rat adipocytes was higher in rats fed γ -linolenic acid and linoleic acid compared to palmitic acid. However, the extent of insulin-dependent increase in glucose oxidation was much greater in rats fed γ -linolenic acid than in those fed linoleic acid.

With regard to ω -3 PUFA, some studies have found a protective effect against insulin resistant and diabetes type 2. In one study [46], the intake of α -linolenic acid alone and/or with its higher metabolites, eicosapentaenoic acid and docosahexaenoic acid was evaluated in a nonobese, hypertriglyceridemic and insulin-resistant rat model. The ω -3 experimental diets prevented changes in the fatty acid patterns in insulin-sensitive tissues, insulin resistance, and vascular dysfunction. This beneficial effect was large with an intake of long chain ω -3 PUFA (α -linolenic acid + eicosapentaenoic acid + docosahexaenoic acid) and to a lesser extent with dietary α -linolenic acid alone. In another study by Guelzim et al [47], ω -3 fatty acids improved body composition and insulin sensitivity during energy restriction in rats. In this study, rats were fed diets rich in lipids and sucrose for 10 weeks then rats were energy restricted and fed diets rich in 18:1 ω -9 (oleic acid), 18:3 ω -3 (α -linolenic acid) or ω -3 (long chain, >18carbons) PUFA for 4 weeks. The long chain-PUFA diet resulted in a higher weight loss, without negative impact on the muscle weight. Additionally, hepatic phosphorylation of insulin receptor and insulin receptor substrate (IRS)-1 was the highest in the long chain-PUFA group. The authors concluded that during energy restriction, a diet rich in ω -3 long chain-PUFA reinforces the effect of weight loss on insulin sensitivity and enhances the activation of the early steps of insulin signaling in the liver. This enhancement in insulin sensitivity is perhaps associated with the enrichment of cell membranes in ω -3 long chain-PUFA. In a recent study by Liu et al [48], 32 rats were randomly divided into four groups receiving either normal chow, high SFA, high ω -3/ ω -6 PUFA ratio (1:1, PUFA^{1:1}), or low ω -3/ ω -6 PUFA ratio (1:4, PUFA^{1:4}) for 16 weeks. Compared to SFA diet-fed rats, PUFA^{1:1} diet-fed rats exhibited decreased body and visceral fat weight, lowered blood lipids, and improved glucose tolerance and insulin sensitivity with decreased expression levels of circulating pro-inflammatory cytokines. However, PUFA^{1:4} diet-fed rats failed to exhibit these changes. Therefore, the authors concluded that a high ratio of dietary ω -3/ ω -6 PUFA improves obesity-linked inflammation and insulin resistance. In this regard, reviewing the literature implies that this positive effect of ω -3 PUFA regarding insulin sensitivity and glucose homeostasis seems to be pronounced in animals but not in humans [49-52]. However, in uncontrolled hyperglycemia in diabetic rats, the type of dietary fat, whether ω -6 PUFA, ω -3 PUFA, ω -9 MUFA or SFA has been shown to exert little or no influence on plasma glucose and lipids and lipoproteins, and body weight [53]. Fructose-induced diabetic rats consuming MUFA-rich olive oil have been reported to show significantly higher HOMA-IR and insulin concentration compared to normal maize starch-fed rats; and the latter exhibited altered insulin resistance as a result of feeding SFA-rich sheep tallow, MUFA-rich olive oil or PUFA-rich maize oil [54].

Role of saturated fatty acids

The bulk of data suggest potential health benefits of substituting SFA with unsaturated fatty acids [37, 43, 55], as several studies discovered impairment of insulin sensitivity with diets rich in SFA relative to MUFA and PUFA. In a study [6] designed to assess the possible effects of ω -3 PUFA as fish oil, MUFA as olive oil and SFA as butter oil on glucose tolerance and insulin sensitivity, a significant impairment in glucose tolerance and insulin insensitivity were observed in rats supplemented with SFA diet as compared to all other dietary groups.

In another study by Kim et al. [56], the effects of dietary fatty acid composition on the insulin signaling pathway was examined by measuring the gene expression of the earliest steps in the insulin action pathway in skeletal muscle of rats fed a safflower oil diet or a beef tallow diet. Feeding of a high-fat diet with SFA induced a decrease in IRS1 and PI 3-kinase mRNA and protein levels, causing insulin resistance in skeletal muscle. Moreover, in overweight and obese, non-diabetic humans, an oral ingestion of fats with different degrees of saturation resulted in different effects on insulin secretion and action with induction of insulin resistance observed by SFA ingestion [57].

In contrast, other studies failed to link SFA intake with the risk of insulin resistance and diabetes type 2. A study by Salmeron et al [35], suggested that total fat, SFA and MUFA intake are not associated with the risk of diabetes type 2 in women. Furthermore, a recent study [38] has found that substitution of unsaturated fats for SFA had no acute benefits on postprandial glycemia, insulin demand or short-term satiety in young men.

Insulin resistance: mechanism of action of dietary fat

The mechanisms linking dietary fat quality to insulin resistance are not fully understood. However, the effects of dietary fatty acids are believed to be mediated, at least partially, through the fatty acid composition of cell membranes. A specific cell membrane fatty acids profile might influence insulin action via several potential mechanisms, including altering insulin receptor binding affinity and influencing cell signaling [30]. More experimental data also point towards other mechanisms which involve direct regulatory effects on gene expression, transcriptional activity and adipocytokines secretion [58, 59]. Here the ligand-activated transcription factor, PPAR γ , and the adipocytokine, leptin, will be discussed as they show a direct regulatory effect of fatty acids.

PPAR γ , insulin resistance and diabetes type 2

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily. So far, three major types have been identified, namely; peroxisome proliferator-activated receptor alpha (PPAR α), peroxisome proliferator-activated receptor beta (PPAR β) and PPAR γ . The PPARs are differentially expressed in a wide range of tissues and thus participate in a variety of cellular functions in a number of tissues and organs [9]. The PPAR γ is predominantly detected in the adipose tissue and the large intestine, intermediately in the kidney, liver and small intestine and with very limited extent in the muscles [9].

While PPAR γ participates in a variety of normal physiological functions such as adipocyte differentiation and insulin sensitization, it is also associated with several pathological conditions

[9]. The role of PPAR γ in insulin sensitivity and diabetes type 2 have been the focus of several studies since the discovery that TZDs, one of the most extensively employed insulin-sensitizing drugs, possess a high affinity for PPAR γ [10]. Many studies have found that TZDs can ameliorate insulin resistance [49-52, 55-62] and the activation of PPAR γ by these chemicals mediates the anti-diabetic effects in the insulin resistance state mainly by altering the transcription of several genes involved in glucose and lipid metabolism. A study with PPAR γ deficient mice has confirmed the important role of PPAR γ , given that PPAR γ deficient mice have shown impairment in insulin sensitivity [63].

Insulin resistance: mechanism of action of PPAR γ

The PPAR γ is the master regulator of adipogenesis, thereby stimulating the production of small insulin-sensitive adipocytes [64]. The induction of adipogenesis associated with capability for fatty acid trapping has been shown to be an important contributor to the maintenance of systemic insulin sensitivity. Adipose PPAR γ protects nonadipose tissue against excessive lipid overload and maintains normal organ function (liver and skeletal muscle) [53]. Upon activation, PPAR γ heterodimerizes with the retinoid X receptor and binds to specific peroxisome proliferator response element (PPRE) of DNA to promote transcription of numerous target genes which are involved in glucose and lipid metabolism [31], thereby improving insulin sensitivity. For example, PPAR γ activation has been shown to upregulate the adipocyte fatty acid-binding protein, acyl-CoA synthase, and lipoprotein lipase [65]; all of these proteins are actively involved in lipid metabolism.

Additionally, PPAR γ represses the gene expression of TNF α , leptin, resistin and interleukin (IL)-6; all of which have been implicated in insulin resistance [66]. On the other hand, activation of PPAR γ induces gene expression of insulin-sensitizing proteins such as adiponectin, fatty acid transport protein and IRS-2 [66]. Collectively, the activation of PPAR γ improves lipid metabolism and mitigates insulin resistance. Nonetheless, the role of PPAR γ in mediating glucose and insulin homeostasis in other tissues is still debatable [63].

Dietary fatty acids: the natural ligands for PPAR γ

Fatty acids and fatty acid derivatives are natural ligands for PPARs including PPAR γ [11]. Certain MUFA and PUFA can directly bind PPARs [11]. These fatty acids bind all three PPARs, with PPAR α exhibiting the highest affinity at concentrations that are in agreement with their circulating blood levels [67]. In contrast, the very long chain fatty acid, erucic acid, which is a weak ligand, appears more selective for PPAR β than for PPAR α and PPAR γ [67]. Compared with the unsaturated fatty acids, SFA are poor PPARs ligands in general [67]. Furthermore, it is likely that the amount of intracellular fatty acid binding proteins within the cells and their binding of the FFAs are important for the actual PPARs activity [68].

One study [59] has examined the effect of fatty acids on PPAR γ response element activity in human adipocytes. Of the SFA, the short chain lauric acid was without any effect, but the long chain palmitic and stearic acids increased PPAR γ response element activity. The three long chain MUFA (palmitoleic, oleic and petroselinic acids) increased PPAR γ response element activity. Whereas a range of PUFA, except for linoleic acid, had no effect, the ω -3 fatty acids, linolenic acid and eicosapentaenoic acid, but not docosahexaenoic acid also stimulated PPAR γ response

element activity. In another study which used the rat pancreatic cell line INS-1 as a cell biological model [69], there was an increase in PPAR γ response element binding with oleic acid treatment. Additionally, in 3T3-L1 cells, treatment with both oleic and linoleic acids-containing media evoked higher levels of PPAR γ than observed in controls [70]; also, GLUT-4 protein has increased in response to treatment with both oleic and linoleic acids-containing media [70].

It is likely that most fatty acids are weak activators of PPAR γ [57]. In fact, most reported “natural” PPAR γ ligands are intermediates of lipid metabolism and oxidation that bind with low affinity at concentrations orders of magnitude greater than physiological conditions [71]. In contrast, nitro derivatives of unsaturated fatty acids (NO₂-FA) are endogenous products of nitric oxide (NO) and nitrite (NO₂⁻)-mediated redox reactions that represent a class of lipid-derived, receptor-dependent signaling mediators that affect downstream gene expression and the metabolic and inflammatory responses under their regulation [72]. For example, NO₂-FA inhibit pro-inflammatory cytokine, adhesion protein and enzyme expression by adduction of the nuclear factor-kappa B (NF- κ B) p65 subunit and inhibition of DNA binding by p65 [72]. Additionally, NO₂-FA are partial agonists of PPAR γ , which NO₂-FA activate via hydrogen bonding interactions and covalent adduction of the ligand binding domain Cys285 [71,72].

The presence of nitroalkane derivatives of many unsaturated fatty acids has been reported in human blood and urine [71,73]. Nitrated palmitoleic, oleic, linoleic, linolenic, arachidonic and eicosapentaenoic acids were detected in concert with their nitrohydroxy derivatives [73]. In a study by Baker et al. [73], nitrated oleic acid (OA-NO₂) has been found to be a potent ligand for different PPARs (PPAR γ , PPAR α and PPAR δ) at physiological concentrations. PPAR γ showed the greatest response, with significant activation at 100 nM in CV-1 cells. In addition, Nitrated oleic acid (OA-NO₂) also induced PPAR γ -dependent adipogenesis and deoxyglucose uptake in 3T3-L1 preadipocytes at a potency exceeding nitrolinoleic acid and rivaling synthetic TZD rosiglitazone. In another study by Schopfer et al. [71], NO₂-FA have been found to act as partial agonists of PPAR γ and covalently bind PPAR γ at Cys285 via Michael addition. NO₂-FA have also showed selective PPAR γ modulator characteristics by inducing coregulator protein interactions, PPAR γ -dependent expression of key target genes and lipid accumulation is distinctively different from responses induced by the TZD rosiglitazone. In addition, the administration of this class of signaling mediators to ob/ob mice revealed that NO₂-FA lower insulin and glucose levels without inducing adverse side effects such as the increased weight gain induced by TZDs [71].

Collectively and importantly, the nitration of some fatty acids in vivo yielding a wide spectrum of nitroalkane derivatives greatly increases the potency of these molecules as true PPAR γ activators as they can exert their effects at physiologically nanomolar concentrations compared to their native fatty acids that are required in very high and non-physiological concentrations to produce a significant effect. Moreover, it has been suggested that NO₂-FA might induce physiological responses that differ from TZDs which favorably modulate adipogenesis and circumvent the accelerated weight gain associated with TZDs administration [71]. This can be of huge importance since the adverse side effects of TZDs administration have limited the use of these drugs as will be discussed later. Thus, the potent and unique nature of PPAR γ binding by NO₂-FA encourages further clinical investigation.

PPAR γ -directed therapeutics: current evidence and future perspectives

It has been mentioned earlier that most fatty acids are probably weak PPAR γ activators [59]. This relatively weak agonist activity had limited their importance from clinical point of view. Other PPAR γ ligands such as TZDs are considered to be strong activators as they are full agonists of PPAR γ [9,20,59]. Because the binding affinity of TZDs to PPAR γ seems closely correlated with the potency of their insulin-sensitizing actions [9], TZDs have been widely used as a treatment option for people with type 2 diabetes. However, these strong synthetic ligands of PPAR γ often induce adverse side effects including peripheral edema, dilutional anemia, weight gain, reduction in bone mineral density, bladder cancer and even overt heart failure [9,20,71]. Therefore, TZDs unique benefits as antidiabetic drugs are shadowed by these side effects which restricted their clinical use. This raises the question as to whether it is possible to develop new agents that are safer, evoking fewer side effects, while preserving or even improving insulin-sensitivity potential.

PPAR γ signaling is not completely understood. A better understanding of PPAR γ signaling is crucial to develop a safer and more effective PPAR γ -directed therapeutics. Recent efforts have focused on PPAR γ ligands that block extracellular signal-regulated kinase (ERK)/cyclin-dependent kinase 5 (Cdk5)-mediated phosphorylation of PPAR γ at S273 [20]. It is suggested that phosphorylation of PPAR γ at S273 may not only correlate positively with the development of insulin resistance but may also be causal to this state [21]. One study [21] has discovered that the use of mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK) inhibitors cause a decrease in PPAR γ phosphorylation at S112 and S273 in ob/ob mice treated with MEK inhibitors compared to control animals. Mice receiving MEK inhibitors showed an improvement in glucose tolerance accompanied by decreased insulin levels and increased levels of the insulin-sensitizing hormone adiponectin without affecting body weight. These data identify an insulin-sensitizing role for MEK inhibitors as non-agonist PPAR γ ligands that specifically block PPAR γ phosphorylation at S273. In the latter study, the authors stated that the MEK inhibitory compounds that they have used are safe and are tolerated well enough to permit studies of metabolism in rodents and perhaps in humans suggesting a therapeutic window for improving insulin sensitivity via PPAR γ , using a safe, low-dose treatment of a MEK inhibitor.

G Protein-coupled Receptor 40 (GPR40) is a FFAs and TZDs cell membrane receptor associated with FFAs- and glucose- induced insulin secretion [74]. GPR40 has been also identified as another potential target for new type 2 diabetes therapeutics. Recently it has been demonstrated that GPR40 and PPAR γ can function as an integrated two-receptor signal transduction pathway in human endothelium [74]. In human endothelium, rosiglitazone and pioglitazone (TZDs drugs) bind to and activate both GPR40 and PPAR γ , which function together through p38 mitogen-activated protein kinase (MAPK) to optimally propagate PPAR γ genetic response [74]. However, GPR40 activation by these drugs also turns on ERK1/2, stress kinases pathway that suppresses PPAR γ signaling and promotes inflammation [20, 74].

Many FFAs and their derivatives, including lauric acid, myristic acid, palmitic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid and 9-hydroxyoctadecadienoic acid (9-HODE) have been shown to be endogenous ligands of both GPR40 and PPAR γ [20]. TZDs, including ciglitazone, troglitazone, rosiglitazone and pioglitazone, all bind to and activate GPR40 with subsequent signal transduction that includes

stress kinase pathways [75]. PPAR γ agonists that do not bind to or activate GPR40 have not yet been characterized in a systematic fashion. The natural PPAR γ ligand 15-deoxy-D12, 14-prostaglandin J2 (15d-PGJ2) has been reported to selectively activate PPAR γ , but not GPR40 in human bronchial epithelial cells [76]. However, 15d-PGJ2 does appear to activate stress kinases in other cell types, which could be a signature for unrecognized GPR40 activation.

Therefore, it is useful to design agents that activate PPAR γ independently of GPR40 or selectively activate GPR40/p38 MAPK while circumventing GPR40/ERK activation. Activating p38 MAPK would enhance downstream PPAR γ signaling through effects on PPAR γ co-activator-1 α (PGC-1 α) and E1A binding protein p300 (EP300), while bypassing ERK1/2 would avoid the inactivation of PPAR γ and the deleterious impact on insulin resistance and inflammation and thus have better risk/benefit profile in patients.

These findings hold implications for rational next generation drugs development that would require further investigation. Although these mechanisms have been largely viewed in a pharmaceutical context, they may also have implications for dietary fatty acids and naturally occurring PPAR γ ligands.

Leptin, insulin resistance and diabetes type 2

Leptin, a product of the *ob* gene is thought to regulate appetite and metabolic rate by reducing food intake and increasing energy expenditure [9, 77]. Leptin is produced by the adipose tissue and its serum levels are correlated with body fat in animals and humans [78,79]. In addition to its role in regulating appetite and metabolic rate, leptin might have a role in insulin resistance and diabetes. Resolving the exact role of leptin in the pathophysiology of insulin resistance and diabetes will be complex because of the paradoxical observations regarding the effects of leptin on glucose metabolism, insulin secretion and insulin sensitivity [25]. It has been found that both high and low levels of leptin are associated with insulin resistance [9], which emphasizes the importance of maintaining normal leptin levels.

Some animal studies have suggested a protective role of leptin concerning diabetes [79, 80], thus the elevated leptin levels observed in obesity indicate a state of leptin resistance [80], hence providing a link between obesity, insulin resistance and diabetes. Furthermore, leptin deficiency is associated with insulin resistance and diabetes that are observed in lipotrophic individuals or rodent models of lipotrophy [12]. Some results suggest that leptin therapy is effective in ameliorating the metabolic abnormalities in lipodystrophy including insulin resistance [81, 82].

It has been suggested that leptin improves insulin sensitivity by diverting FFA into adipose tissue for storage [9], also leptin seems to induce depletion of the TG content in skeletal muscles [83] and liver [82] therefore causing an improvement in insulin sensitivity. Moreover, a close association between plasma leptin and insulin has been reported and the presence of leptin receptors on pancreatic β -cells suggests a role of leptin in regulating insulin secretion [12]. Leptin appears to play an important role in the maintenance of normal glucose-stimulated insulin secretion (GSIS) through the prevention of TG accumulation in pancreatic β -cells, which prevents lipotoxicity and pancreatic β -cells apoptosis [12]. A failure of leptin to inhibit insulin secretion in pancreatic β -cells of human overweight subjects may result in chronic hyperinsulinemia and contribute to the pathogenesis of diabetes type 2 [84].

However, leptin has also been found to desensitize the activity of insulin most notably in the white adipose tissue [12]. Higher concentrations of leptin have been demonstrated to impair the activities of insulin-stimulated MAPK as well as tyrosine phosphorylation of the insulin receptor [9]. Furthermore, some studies have shown that high leptin levels are associated with insulin resistance independently of obesity [85, 86]. A study among Japanese Americans showed that an increase in baseline leptin concentration is associated with increased risk of developing diabetes in men independent of total fat [87]. Another study by Welsh et al [88] has found that baseline leptin levels can predict diabetes but not cardiovascular diseases. Furthermore, it has been suggested that adiponectin/leptin ratio might be more useful than homeostasis model assessment of insulin resistance (HOMA-IR) to assess insulin resistance in subjects without hyperglycemia [89] and in type 2 diabetic patients [90]. The fact that PPAR γ activation suppress the gene expression of leptin [9] may indicate that high leptin levels are associated with insulin resistance and type 2 diabetes; this can be supported by the results of some [91-93] but not all studies [94, 95] that examined the effect of TZDs on insulin sensitivity in diabetic patients and found that an improvement in insulin sensitivity was associated with a reduction in leptin levels.

Despite this, previous studies have reported various results regarding leptin levels in diabetic subjects. Levels were decreased [96], increased [97], or unchanged [98]. This can be explained by the fact that leptin levels are confounded by factors that affect insulin sensitivity and insulin secretion.

Dietary fat and leptin regulation

The regulation of plasma leptin levels is not completely understood. Plasma leptin levels and ob gene expression are correlated with adipose tissue mass in humans and animals [99, 100], suggesting that adipose tissue size is a major regulator of leptin production. However, because there are large variations in leptin concentrations among individuals with similar body compositions, it is likely that factors other than adipose tissue mass influence plasma leptin levels [101]. Potential modifiers of leptin levels are energy-yielding nutrients, such as fatty acids, carbohydrates and proteins and alcohol. Most studies published so far indicate that fasting and refeeding may change plasma leptin levels; under these conditions, leptin levels are down and up-regulated, respectively [102]. Physical activity is central for long-term regulation of body weight. Reseland et al [101] discovered that an increase in physical activity reduces plasma leptin levels in men with metabolic syndrome even after adjusting for BMI or fat mass. However, results of the effects of exercise on plasma leptin levels, independent of fat mass, are conflicting [103, 104].

The effect of increased dietary fat on circulating leptin has been assessed in several models. A high fat diet increased plasma leptin levels and body fat mass in male Sprague Dawley rats [54, 105]. However, the effects of different dietary fat types on leptin are unknown. A cross-sectional study by Rojo-Martinez et al. [106] examined the relation between serum leptin and nutrient intake. The results suggest that in non-experimental conditions, the levels of serum leptin in men with type 1 diabetes mellitus and, to a lesser extent, those in women, may be influenced by the composition of the habitual diet, especially the type of dietary fat. In the men, serum leptin concentration correlated significantly with the intake of saturated fat. In these patients, leptin also correlated positively with stearic acid of the plasma phospholipids and

negatively with eicosapentaenoic acid. In women, serum leptin levels correlated positively with linoleic acid from the serum phospholipids and negatively with arachidonic acid. Separate regression analyses for the men and the women showed that the intake of palmitic acid accounted for 42% of the variance in serum leptin in the men, while, the waist to hip ratio and the intake of docosahexaenoic acid accounted for 42% of the variance in leptin in the women.

Another study [101] examined the effect of long-term changes in diet and exercise on leptin levels in 186 men with metabolic syndrome. The results demonstrated that long-term changes in lifestyle, consisting of decreased intake of dietary fat and increased physical activity reduced plasma leptin concentration beyond the reduction expected as a result of changes in fat mass. Furthermore, in healthy nonobese men and women, the impact of dietary fat composition on serum leptin concentration was examined by giving three dietary treatments which contained refined olive oil, rapeseed oil or sunflower oil as the principle source of fat for 4 weeks [107]. Both of olive oil and sunflower oil did not affect leptin levels whereas leptin levels were differently affected in men and women on the rapeseed oil diet.

In animals, conflicting results concerning the effect of different types of dietary fat on leptin levels are reported. In a study by Rodriguez et al. [58], leptin levels were not affected by dietary fat type (olive oil, sunflower oil or beef tallow) whereas ob mRNA was higher in olive oil and sunflower oil than in beef tallow. Neither PPAR γ activation nor leptin concentration has been reported to be affected by the dietary fat type including MUFA-rich olive oil, PUFA-rich maize oil and SFA-rich sheep tallow [54]. On the other hand, a study by Cha and Jones [77] indicated that dietary fatty acid composition independent of adipose tissue mass, is an important determinant of circulating leptin levels in diet-induced obesity given that plasma leptin levels were 60% higher in the rats fed fish oil and safflower oil compared with rats fed beef tallow. In contrast to the previous study, it has been found that 3 weeks of ω -3 PUFA-enriched diet, as compared with 3 weeks of lard-enriched diet, induced lower leptin levels and reduced leptin mRNA expression in rats' epididymal adipose tissue [108].

CONCLUSIONS

There is no optimal mix of macronutrients for people who are trying to lose weight or for people with diabetes. Moreover, evidence is lacking for an ideal amount of fat. Therefore, personal diets should be individualized. In regards to fat, the American Diabetes Association [109] in its position statement stated that "fat quality appears to be far more important than quantity". Indeed, there is evidence in both humans and animals that dietary fat type independent of its quantity can influence several metabolic processes inside the body, either leading to or preventing the development of insulin resistance and thus influence the risk of diabetes. In general, it is recommended to substitute SFA with PUFA and MUFA. The ideal ratio between ω -3 and ω -6 PUFA has not been determined. Taken together, the role of dietary fat quality in insulin resistance should be further studied, using well controlled experimental designs, in an attempt to avoid multiple flaws that limited the validity of several studies. Furthermore, functionality of dietary fat is primarily based on its being whole, conventional, natural or processed and not pills, capsules, extracts or supplements [110], a matter that has not been the focus of most studies.

The evidence that different fatty acids have different regulatory actions inside our bodies in terms of gene expression, transcriptional activity and adipocytokines secretion is crucial from preventive and treatmental point of view. Additional investigation is needed to further understand the specific role of PPAR γ and its fatty acid-mediated activation, which will open a therapeutic window for improving insulin sensitivity via PPAR γ . Moreover, the potential influence of dietary fatty acids on ob gene expression and leptin secretion remains to be elucidated given the fact that leptin is complexly regulated by multiple factors. All of this increases the need for more properly designed studies to help us fully understand the exact role of fat, thus, establishing better evidence-based recommendations for prevention and clinical management.

List of abbreviations:

Body mass index, BMI; cyclin-dependent kinase 5, Cdk5; diacylglycerols, DAGs; 15-deoxy-D12,14-prostaglandin J2, 15d-PGJ2; E1A binding protein p300, EP300; extracellular signal-regulated kinase, ERK; free fatty acids, FFA; glucose transporter, GLUT; G Protein-coupled Receptor 40, GPR40; glucose stimulated insulin secretion, GSIS; 9-hydroxyoctadecadienoic acid, 9-HODE; homeostasis model assessment of insulin resistance, HOMA-IR; impaired fasting glucose, IFG; impaired glucose tolerance, IGT; interleukin, IL; insulin receptor substrate, IRS; mitogen-activated protein kinase, MAPK; mitogen-activated protein/extracellular signal-regulated kinase kinase, MEK; monounsaturated fatty acids, MUFA; nuclear factor-kappa B, NF-kB; nitric oxide, NO; nitrite, NO $_2^-$; nitro derivatives of unsaturated fatty acids, NO $_2$ -FA; nitrated oleic acid, OA-NO $_2$; PPAR γ co-activator-1alpha, PGC-1 α ; phosphatidyl inositol, PI; peroxisome proliferator-activated receptor alpha, PPAR α ; Peroxisome proliferator-activated receptors, PPARs; peroxisome proliferator-activated receptor beta, PPAR β ; peroxisome proliferator-activated receptor delta, PPAR δ ; peroxisome proliferator-activated receptor gamma, PPAR γ ; peroxisome proliferator response element, PPRE; polyunsaturated fatty acids, PUFA; saturated fatty acids, SFA; triglyceride, TG; tumor necrosis factor alpha, TNF α ; thiazolidinediones, TZDs.

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REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ: Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010, 87:4-14. doi: 10.1016/j.diabres.2009.10.007.
2. Bock G, Dalla Man C, Campioni M, Chittilapilly E, Basu R, Toffolo G, Cobelli C, Rizza R: Pathogenesis of pre-diabetes: mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance. *Diabetes* 2006, 55:3536-3549. PMID: 17130502.

3. Li G, Zhang P, Wang J, Gregg EW, Yang W, Gong Q, Li H, Li H, Jiang Y, An Y, Shuai Y, Zhang B, Zhang J, Thompson TJ, Gerzoff RB, Roglic G, Hu Y, Bennett PH: The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention Study: a 20-year follow-up study. *Lancet* 2008, 371:1783-1789. doi: 10.1016/S0140-6736(08)60766-7.
4. Diabetes Prevention Program Research Group: 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. *Lancet* 2009, 374:1677-1686. doi: [http://dx.doi.org/10.1016/S0140-6736\(09\)61457-4](http://dx.doi.org/10.1016/S0140-6736(09)61457-4).
5. Lindstrom J, Peltonen M, Eriksson JG, Ilanne-Parikka P, Aunola S, Keinänen-Kiukaanniemi S, Uusitupa M, Tuomilehto J, Finnish Diabetes Prevention S: Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). *Diabetologia* 2013, 56:284-293. doi: 10.1007/s00125-012-2752-5.
6. Alsaif MA: Effect of dietary fats on glucose tolerance, insulin sensitivity and membrane free fatty acid in rats. *Pak J Nutr* 2004, 3:56-63. doi: 10.3923/pjn.2004.56.63.
7. Lee JS, Pinnamaneni SK, Eo SJ, Cho IH, Pyo JH, Kim CK, Sinclair AJ, Febbraio MA, Watt MJ: Saturated, but not n-6 polyunsaturated, fatty acids induce insulin resistance: role of intramuscular accumulation of lipid metabolites. *J Appl Physiol* 2006, 100:1467-1474. PMID: 16357064.
8. Paniagua JA, Gallego de la Sacristana A, Romero I, Vidal-Puig A, Latre JM, Sanchez E, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F: Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. *Diabetes Care* 2007, 30:1717-1723. PMID: 17384344
9. Guo L, Tabrizchi R: Peroxisome proliferator-activated receptor gamma as a drug target in the pathogenesis of insulin resistance. *Pharmacol Ther* 2006, 111:145-173. PMID: 16305809.
10. Kim H, Haluzik M, Gavrilova O, Yakar S, Portas J, Sun H, Pajvani UB, Scherer PE, LeRoith D: Thiazolidinediones improve insulin sensitivity in adipose tissue and reduce the hyperlipidaemia without affecting the hyperglycaemia in a transgenic model of type 2 diabetes. *Diabetologia* 2004, 47:2215-2225. PMID: 15662559.
11. Capobianco E, White V, Higa R, Martinez N, Jawerbaum A: Effects of natural ligands of PPARgamma on lipid metabolism in placental tissues from healthy and diabetic rats. *Mol Hum Reprod* 2008, 14:491-499. doi: 10.1093/molehr/gan039.
12. Ceddia RB, Koistinen HA, Zierath JR, Sweeney G: Analysis of paradoxical observations on the association between leptin and insulin resistance. *FASEB J* 2002, 16:1163-1176. PMID: 12153984.
13. Heshka JT, Jones PJ: A role for dietary fat in leptin receptor, OB-Rb, function. *Life Sci* 2001, 69:987-1003. PMID: 11508653.
14. Rao SS, Disraeli P, McGregor T: Impaired glucose tolerance and impaired fasting glucose. *Am Fam Physician* 2004, 69:1961-1968. PMID: 15117017.

15. Saltiel AR: Series Introduction: The molecular and physiological basis of insulin resistance: emerging implications for metabolic and cardiovascular diseases. *J Clin Invest* 2000, 106:163-164. PMID: 10903328.
16. Kumar A, Tewari P, Sahoo SS, Srivastava AK: Prevalence of insulin resistance in first degree relatives of type-2 diabetes mellitus patients: A prospective study in north Indian population. *Indian J Clin Biochem* 2005, 20:10-17. doi: 10.1007/BF02867394.
17. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002, 346:393-403. PMID: 11832527.
18. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M: Prediabetes: A high-risk state for developing diabetes. *Lancet* 2012, 379:2279-2290. doi: 10.1016/S0140-6736(12)60283-9.
19. Bays H, Mandarin L, DeFronzo RA: Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. *J Clin Endocrinol Metab* 2004, 89:463-478. MID: 14764748.
20. Wang S, Dougherty E J, Danner RL: PPAR γ signaling and emerging opportunities for improved therapeutics. *Pharmacol Res* 2016, 111: 76–85.
<http://dx.doi.org/10.1016/j.phrs.2016.02.028>.
21. Banks AS, McAllister, FE, Camporez JPG, Zushin, PJH, Jurczak MJ, Laznik-Bogoslavski D, Shulman GI, Gygi SP, Spiegelman BM: An ERK/Cdk5 axis controls the diabetogenic actions of PPAR γ . *Nature*, 2015, 517(7534): 391-395. doi:10.1038/nature13887.
22. Snel M, Jonker JT, Schoones J, Lamb H, de Roos A, Pijl H, Smit JW, Meinders AE, Jazet IM:
Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. *Intern J Endocrinol* 2012, 2012:983814. doi: 10.1155/2012/983814.
23. Guebre-Egziabher F, Alix PM, Koppe L, Pelletier CC, Kalbacher E, Fouque D, Soulage CO: Ectopic lipid accumulation: a potential cause for metabolic disturbances and a contributor to the alteration of kidney function. *Biochimie* 2013, 95(11):1971-1979. doi: 10.1016/j.biochi.2013.07.017.
24. Do HD, Lohsoonthorn V, Jiamjarasrangsi W, Lertmaharit S, Williams MA: Prevalence of insulin resistance and its relationship with cardiovascular disease risk factors among Thai adults over 35 years old. *Diabetes Res Clin Pract* 2010, 89:303-308. doi: 10.1016/j.diabres. 2010.04.013.
25. Grundy SM, Adams-Huet B, Vega GL: Variable contributions of fat content and distribution to metabolic syndrome risk factors. *Metab Syndr Relat Disord* 2008, 6:281-288. doi: 10.1089/ met.2008.0026.
26. Koh-Banerjee P, Wang Y, Hu FB, Spiegelman D, Willett WC, Rimm EB: Changes in body weight and body fat distribution as risk factors for clinical diabetes in US men. *Am J Epidemiol* 2004, 159:1150-1159. PMID: 15191932.
27. Wannamethee SG, Shaper AG: Weight change and duration of overweight and obesity in the incidence of type 2 diabetes. *Diabetes Care* 1999, 22:1266-1272. PMID: 10480769.

28. Denis GV, Obin MS: 'Metabolically healthy obesity': origins and implications. *Mol Aspects Med* 2013, 34:59-70. doi: 10.1016/j.mam.2012.10.004.
29. Kovacs P, Stumvoll M: Fatty acids and insulin resistance in muscle and liver. *Best Pract Res Clin Endocrinol Metab* 2005, 19:625-635. PMID: 16311221.
30. Lopez-Miranda J, Perez-Martinez P, Marin C, Fuentes F, Delgado J, Perez-Jimenez F: Dietary fat, genes and insulin sensitivity. *J Mol Med (Berl)* 2007, 85:213-226. PMID: 17171387.
31. Stumvoll M, Häring H: The peroxisome proliferator-activated receptor- γ 2 Pro12Ala polymorphism. *Diabetes* 2002, 51:2341-2347. PMID: 12145143.
32. He W: PPAR γ 2(Pro12Ala) Polymorphism and human health. *PPAR Research* 2009, 2009:849538. PMID: 19390629.
33. Tierney AC, Roche HM: The potential role of olive oil-derived MUFA in insulin sensitivity. *Mol Nutr Food Res* 2007, 51:1235-1248. PMID: 17912722.
34. Riserus U, Willett WC, Hu FB: Dietary fats and prevention of type 2 diabetes. *Prog Lipid Res* 2009, 48:44-51. doi: 10.1016/j.plipres.2008.10.002.
35. Salmeron J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willett WC: Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr* 2001, 73:1019-1026. PMID: 11382654.
36. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nansen C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen E, Gustafsson IB, Storlien LH, Study K: Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* 2001, 44:312-319. PMID: 11317662.
37. Kennedy A, Martinez K, Chuang CC, LaPoint K, McIntosh M: Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. *J Nutr* 2009, 139:1-4. doi: 10.3945/jn.108.098269.
38. MacIntosh CG, Holt SH, Brand-Miller JC: The degree of fat saturation does not alter glycemic, insulinemic or satiety responses to a starchy staple in healthy men. *J Nutr* 2003, 133:2577-2580. PMID: 12888640.
39. Ros E: Dietary cis-monounsaturated fatty acids and metabolic control in type 2 diabetes. *Am J Clin Nutr* 2003, 78:617S-625S. PMID: 12936956.
40. Ryan M, McInerney D, Owens D, Collins P, Johnson A, Tomkin GH: Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. *QJM* 2000, 93:85-91. PMID: 10700478.
41. Rojo-Martinez G, Esteva I, Ruiz de Adana MS, Garcia-Almeida JM, Tinahones F, Cardona F, Morcillo S, Garcia-Escobar E, Garcia-Fuentes E, Soriguer F: Dietary fatty acids and insulin secretion: a population-based study. *Eur J Clin Nutr* 2006, 60:1195-1200. PMID: 16639414.
42. Mayer-Davis EJ, Monaco JH, Hoen HM, Carmichael S, Vitolins MZ, Rewers MJ, Haffner SM, Ayad MF, Bergman RN, Karter AJ: Dietary fat and insulin sensitivity in a triethnic population: the role of obesity. The Insulin Resistance Atherosclerosis Study (IRAS). *Am J Clin Nutr* 1997, 65:79-87. PMID: 8988917.

43. Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML, Moore NR, Frayn KN: Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. *Diabetologia* 2002, 45:369-377. PMID: 11914742.
44. Matsuo T, Sumida H, Suzuki M: Beef tallow diet decreases beta-adrenergic receptor binding and lipolytic activities in different adipose tissues of rat. *Metabolism* 1995, 44:1271-1277. PMID: 7476283.
45. Ide T, Kushi M, Takahashi Y: Dietary mold oil rich in gamma linolenic acid increases insulin-dependent glucose utilization in isolated rat adipocytes. *Comp Biochem Physiol B Biochem Mol Biol* 2001, 130:401-409. PMID: 11567903.
46. Robbez Masson V, Lucas A, Gueugneau AM, Macaire JP, Paul JL, Grynberg A, Rousseau D: Long-chain (n-3) polyunsaturated fatty acids prevent metabolic and vascular disorders in fructose-fed rats. *J Nutr* 2008, 138:1915-1922. PMID: 18806101.
47. Guelzim N, Huneau JF, Mathe V, Tesseraud S, Mourot J, Simon N, Hermier D: N-3 fatty acids improve body composition and insulin sensitivity during energy restriction in the rat. *Prostaglandins Leukot Essent Fatty Acids* 2014, 91:203-211. doi: 10.1016/j.plefa.2014.07.007.
48. Liu HQ, Qiu Y, Mu Y, Zhang XJ, Liu L, Hou XH, Zhang L, Xu XN, Ji AL, Cao R, Yang RH, Wang F: A high ratio of dietary n-3/n-6 polyunsaturated fatty acids improves obesity-linked inflammation and insulin resistance through suppressing activation of TLR4 in SD rats. *Nutr Res* 2013, 33:849-858. doi: 10.1016/j.nutres.2013.07.004.
49. Weickert MO: Nutritional modulation of insulin resistance. *Scientifica (Cairo)* 2012:424780, 2012. doi: 10.6064/2012/424780.
50. Flachs P, Rossmeisl M, Kopecky J: The effect of n-3 fatty acids on glucose homeostasis and insulin sensitivity. *Physiol Res* 2014, 63 Suppl 1:S93-118. PMID: 24564669.
51. Oh PC, Koh KK, Sakuma I, Lim S, Lee Y, Lee S, Lee K, Han SH, Shin EK: Omega-3 fatty acid therapy dose-dependently and significantly decreased triglycerides and improved flow-mediated dilation, however, did not significantly improve insulin sensitivity in patients with hypertriglyceridemia. *Int J Cardiol* 2014, 176:696-702. doi: 10.1016/j.ijcard.2014.07.075.
52. Lalia AZ, Johnson ML, Jensen MD, Hames KC, Port JD, Lanza IR: Effects of dietary n-3 fatty acids on hepatic and peripheral insulin sensitivity in insulin resistant humans. *Diabetes Care* 2015, 38(7):1228-1237. doi: 10.2337/dc14-3101.
53. Ahmad MN, Khatib FA: Effects of dietary saturated, monounsaturated and polyunsaturated fats on plasma lipids and lipoproteins in diabetic rats. *Ecol Food Nutr* 1990, 24: 141-148. doi: 10.1080/03670244.1990.9991132.
54. Al-Jada DN, Ahmad MN: Effect of dietary fats on the development of insulin resistance in relation to PPAR γ activation and leptin concentration in rats. *J Am Sci* 2014, 10(8):59-66. doi: 10.7537/marsjas100814.08.
55. Hodson L, Skeaff CM, Chisholm WA: The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. *Eur J Clin Nutr* 2001, 55:908-915. PMID: 11593354.

56. Kim YB, Nakajima R, Matsuo T, Inoue T, Sekine T, Komuro M, Tamura T, Tokuyama K, Suzuki M: Gene expression of insulin signal-transduction pathway intermediates is lower in rats fed a beef tallow diet than in rats fed a safflower oil diet. *Metabolism* 1996, 45:1080-1088. PMID: 8781294.
57. Xiao C, Giacca A, Carpentier A, Lewis GF: Differential effects of monounsaturated, polyunsaturated and saturated fat ingestion on glucose-stimulated insulin secretion, sensitivity and clearance in overweight and obese, non-diabetic humans. *Diabetologia* 2006, 49:1371-1379. PMID: 16596361.
58. Rodriguez VM, Pico C, Portillo MP, Teresa Macarulla M, Palou A: Dietary fat source regulates ob gene expression in white adipose tissue of rats under hyperphagic feeding. *Br J Nutr* 2002, 87:427-434. PMID: 12010582.
59. Sauma L, Stenkula KG, Kjolhede P, Stralfors P, Soderstrom M, Nystrom FH: PPAR-gamma response element activity in intact primary human adipocytes: effects of fatty acids. *Nutrition* 2006, 22:60-68. PMID: 16226011.
60. Hevener AL, Reichart D, Janez A, Olefsky J: Thiazolidinedione treatment prevents free fatty acid-induced insulin resistance in male wistar rats. *Diabetes* 2001, 50:2316-2322. PMID: 11574414.
61. Sidell RJ, Cole MA, Draper NJ, Desrois M, Buckingham RE, Clarke K: Thiazolidinedione treatment normalizes insulin resistance and ischemic injury in the Zucker Fatty rat heart. *Diabetes* 2002, 51:1110-1117. PMID: 11916933.
62. He J, Xu C, Kuang J, Liu Q, Jiang H, Mo L, Geng B, Xu G: Thiazolidinediones attenuate lipolysis and ameliorate dexamethasone-induced insulin resistance. *Metabolism* 2015, 64:826-836. doi: 10.1016/j.metabol.2015.02.005.
63. Kintscher U, Law RE: PPARgamma-mediated insulin sensitization: the importance of fat versus muscle. *Am J Physiol Endocrinol Metab* 2005, 288:E287-291. PMID: 15637349.
64. Koh YJ, Park BH, Park JH, Han J, Lee IK, Park JW, Koh GY: Activation of PPAR gamma induces profound multilocularization of adipocytes in adult mouse white adipose tissues. *Exp Mol Med* 2009, 41:880-895. doi: 10.3858/emmm.2009.41.12.094.
65. Berger J, Moller DE: The mechanisms of action of PPARs. *Annu Rev Med* 2002, 53:409-435. PMID: 11818483.
66. Rangwala SM, Lazar MA: Peroxisome proliferator-activated receptor gamma in diabetes and metabolism. *Trends Pharmacol Sci* 2004, 25:331-336. PMID: 15165749.
67. Desvergne B, Wahli W: Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 1999, 20:649-688. PMID: 10529898.
68. Larsen TM, Toubro S, Astrup A: PPARgamma agonists in the treatment of type II diabetes: is increased fatness commensurate with long-term efficacy? *Int J Obes Relat Metab Disord* 2003, 27:147-161. PMID: 12586994.
69. Vassiliou EK, Gonzalez A, Garcia C, Tadros JH, Chakraborty G, Toney JH: Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNF-alpha both in vitro and in vivo systems. *Lipids Health Dis* 2009, 8:25. doi: 10.1186/1476-511X-8-25.
70. Kokta TA, Strat AL, Papasani MR, Szasz JI, Dodson MV, Hill RA: Regulation of lipid accumulation in 3T3-L1 cells: insulin-independent and combined effects of

71. Schopfer FJ, Cole MP, Groeger AL, Chen CS, Khoo NK, Woodcock SR, Golin-Bisello F, Motanya UN, Li Y, Zhang J, Garcia-Barrio MT, Rudolph TK, Rudolph V, Bonacci G, Baker PR, Xu HE, Batthyany CI, Chen YE, Hallis TM, Freeman BA: Covalent peroxisome proliferator-activated receptor gamma adduction by nitro-fatty acids: selective ligand activity and anti-diabetic signaling actions. *J Biol Chem* 2010, 285(16):12321-12333. doi: 10.1074/jbc.M109.091512.
72. Fazzari M, Trostchansky A, Schopfer FJ, Salvatore SR, Sánchez-Calvo B, Vittur D, Valderrama R, Barroso JB, Radi R, Freeman BA, Rubbo H: Olives and olive oil are sources of electrophilic fatty acid nitroalkenes. *PloS one* 2014, 9(1): e84884. doi: 10.1371/journal.pone.0084884
73. Baker PRS, Lin Y, Schopfer FJ, Woodcock SR, Groeger AL, Batthyany C, Marshal SS, Long H, Iles KE, Baker LMS, Branchaud BP, Chen YE, Freeman BA: Fatty acid transduction of nitric oxide signaling: multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands. *J Biol Chem* 2005, 280(51): 42464-42475. PMID: 16227625.
74. Wang S, Awad KS, Elinoff JM, Dougherty EJ, Ferreyra GA, Wang JY, Cai R, Sun J, Ptasinska A, Danner RL: G protein-coupled receptor 40 (GPR40) and peroxisome proliferator-activated receptor γ (PPAR γ): an integrated two-receptor signaling pathway. *J Biol Chem* 2015, 290(32): 19544-19557. doi: 10.1074/jbc.M115.638924.
75. Smith NJ, Stoddart LA, Devine NM, Jenkins L, Milligan G: (2009). The action and mode of binding of thiazolidinediones ligands at free fatty acid receptor 1. *J Biol Chem* 2009, 284(26): 17527-17539. doi: 10.1074/jbc.M109.012849.
76. Gras D, Chanez P, Urbach V, Vachier I, Godard P, Bonnans C: Thiazolidinediones induce proliferation of human bronchial epithelial cells through the GPR40 receptor. *Am J Physiol Lung Cell Mol Physiol* 2009, 296(6), L970-L978. doi: 10.1152/ajplung.90219.2008.
77. Cha MC, Jones PJ: Dietary fat type and energy restriction interactively influence plasma leptin concentration in rats. *J Lipid Res* 1998, 39:1655-1660. PMID: 9717726.
78. Hynes GR, Heshka J, Chadee K, Jones PJ: Effects of dietary fat type and energy restriction on adipose tissue fatty acid composition and leptin production in rats. *J Lipid Res* 2003, 44:893-901. PMID:12562868.
79. Sivitz WI, Wayson SM, Bayless ML, Larson LF, Sinkey C, Bar RS, Haynes WG: Leptin and body fat in type 2 diabetes and monodrug therapy. *J Clin Endocrinol Metab* 2003, 88:1543-1553. PMID: 12679437.
80. Schmidt MI, Duncan BB, Vigo A, Pankow JS, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G, Investigators A: Leptin and incident type 2 diabetes: risk or protection? *Diabetologia* 2006, 49:2086-2096. PMID: 16850292.
81. Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, Wagner AJ, DePaoli AM, Reitman ML, Taylor SI, Gorden P, Garg A: Leptin-replacement therapy for lipodystrophy. *N Engl J Med* 2002, 346:570-578. PMID: 11856796.
82. Nagao K, Inoue N, Ujino Y, Higa K, Shirouchi B, Wang YM, Yanagita T: Effect of leptin infusion on insulin sensitivity and lipid metabolism in diet-induced lipodystrophy model mice. *Lipids Health Dis* 2008, 7:8. doi: 10.1186/1476-511X-7-8.

83. Doh KO, Park JO, Kim YW, Park SY, Jeong JH, Jeon JR, Lee SK, Kim JY: Effect of leptin on insulin resistance of muscle--direct or indirect? *Physiol Res* 2006, 55:413-419. PMID: 16238463.
84. Seufert J: Leptin effects on pancreatic beta-cell gene expression and function. *Diabetes* 2004, 53 Suppl 1:S152-158. PMID: 14749281.
85. Fischer S, Hanefeld M, Haffner SM, Fusch C, Schwanebeck U, Kohler C, Fucker K, Julius U: Insulin-resistant patients with type 2 diabetes mellitus have higher serum leptin levels independently of body fat mass. *Acta Diabetol* 2002, 39:105-110. PMID: 12357293.
86. Koebnick C, Roberts CK, Shaibi GQ, Kelly LA, Lane CJ, Toledo-Corral CM, Davis JN, Ventura EE, Alexander K, Weigensberg MJ, Goran MI: Adiponectin and leptin are independently associated with insulin sensitivity, but not with insulin secretion or beta-cell function in overweight Hispanic adolescents. *Horm Metab Res* 2008, 40:708-712. doi: 10.1055 /s-2008-1077097.
87. McNeely MJ, Boyko EJ, Weigle DS, Shofer JB, Chessler SD, Leonnetti DL, Fujimoto WY: Association between baseline plasma leptin levels and subsequent development of diabetes in Japanese Americans. *Diabetes Care* 1999, 22:65-70. PMID: 10333905.
88. Welsh P, Murray HM, Buckley BM, De Craen AJ, Ford I, Jukema JW, Macfarlane PW, Packard CJ, Stott DJ, Westendorp RG: Leptin predicts diabetes but not cardiovascular disease results from a large prospective study in an elderly population. *Diabetes care* 2009, 32:308-310. doi: 10.2337/dc08-1458.
89. Inoue M, Yano M, Yamakado M, Maehata E, Suzuki S: Relationship between the adiponectin-leptin ratio and parameters of insulin resistance in subjects without hyperglycemia. *Metabolism* 2006, 55:1248-1254. PMID: 16919546.
90. Inoue M, Maehata E, Yano M, Taniyama M, Suzuki S: Correlation between the adiponectin-leptin ratio and parameters of insulin resistance in patients with type 2 diabetes. *Metabolism* 2005, 54:281-286. PMID: 15736103.
91. Watanabe S, Takeuchi Y, Fukumoto S, Fujita H, Nakano T, Fujita T: Decrease in serum leptin by troglitazone is associated with preventing bone loss in type 2 diabetic patients. *J Bone Miner Metab* 2003, 21:166-171. PMID: 12720051.
92. Wu J, Lei MX, Chen HL, Sun ZX: [Effects of rosiglitazone on serum leptin and insulin resistance in patients with Type 2 diabetes]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2004, 29:623-626. PMID: 16114542.
93. Boden G, Cheung P, Mozzoli M, Fried SK: Effect of thiazolidinediones on glucose and fatty acid metabolism in patients with type 2 diabetes. *Metabolism - Clinical and Experimental* 2003, 52:753-759. PMID: 12800103.
94. Kim HJ, Kim SK, Shim WS, Lee JH, Hur KY, Kang ES, Ahn CW, Lim SK, Lee HC, Cha BS: Rosiglitazone improves insulin sensitivity with increased serum leptin levels in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2008, 81:42-49. doi: 10.1016/j.diabres.2008.02.001.
95. Satoh N, Ogawa Y, Usui T, Tagami T, Kono S, Uesugi H, Sugiyama H, Sugawara A, Yamada K, Shimatsu A, Kuzuya H, Nakao K: Antiatherogenic effect of pioglitazone in

- type 2 diabetic patients irrespective of the responsiveness to its antidiabetic effect. *Diabetes Care* 2003, 26:2493-2499. PMID: 12941708.
96. Marita AR, Sarkar JA, Rane S: Type 2 diabetes in non-obese Indian subjects is associated with reduced leptin levels: study from Mumbai, Western India. *Mol Cell Biochem* 2005, 275:143-151. PMID: 16335794.
 97. Al-Daghri N, Al-Rubean K, Bartlett WA, Al-Attas O, Jones AF, Kumar S: Serum leptin is elevated in Saudi Arabian patients with metabolic syndrome and coronary artery disease. *Diabet Med* 2003, 20:832-837. PMID: 14510865.
 98. Haffner SM, Stern MP, Miettinen H, Wei M, Gingerich RL: Leptin concentrations in diabetic and nondiabetic Mexican-Americans. *Diabetes* 1996, 45:822-824. PMID: 8635660.
 99. Lonnqvist F, Nordfors L, Jansson M, Thorne A, Schalling M, Arner P: Leptin secretion from adipose tissue in women. Relationship to plasma levels and gene expression. *J Clin Invest* 1997, 99:2398-2404. PMID: 9153282.
 100. Peyron-Caso E, Taverna M, Guerre-Millo M, Veronese A, Pacher N, Slama G, Rizkalla SW: Dietary (n-3) polyunsaturated fatty acids up-regulate plasma leptin in insulin-resistant rats. *J Nutr* 2002, 132:2235-2240. PMID: 12163668.
 101. Reseland JE, Anderssen SA, Solvoll K, Hjermann I, Urdal P, Holme I, Drevon CA: Effect of long-term changes in diet and exercise on plasma leptin concentrations. *Am J Clin Nutr* 2001, 73:240-245. PMID: 11157319.
 102. Monteleone P, Bortolotti F, Fabrazzo M, La Rocca A, Fuschino A, Maj M: Plasma leptin response to acute fasting and refeeding in untreated women with bulimia nervosa. *J Clin Endocrinol Metab* 2000, 85:2499-2503. PMID: 10902800.
 103. Kohrt WM, Landt M, Birge SJ, Jr.: Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. *J Clin Endocrinol Metab* 1996, 81:3980-3985. PMID: 8923847.
 104. Franks PW, Farooqi IS, Luan J, Wong MY, Halsall I, O'Rahilly S, Wareham NJ: Does physical activity energy expenditure explain the between-individual variation in plasma leptin concentrations after adjusting for differences in body composition? *J Clin Endocrinol Metab* 2003, 88:3258-3263. PMID: 12843173.
 105. Handjieva-Darlenska T, Boyadjieva N: The effect of high-fat diet on plasma ghrelin and leptin levels in rats. *J Physiol Biochem* 2009, 65:157-164. PMID: 1988639.
 106. Rojo-Martinez G, Soriguer FJ, Gonzalez-Romero S, Tinahones F, Moreno F, de Adana SR, Garriga MJ, Esteva I, Garcia-Arnes J, Gomez-Zumaquero JM, Garcia-Almeida JM: Serum leptin and habitual fatty acid dietary intake in patients with type 1 diabetes mellitus. *Eur J Endocrinol* 2000, 142:263-268. doi: 10.1530/eje.0.1420263.
 107. Kratz M, von Eckardstein A, Fobker M, Buyken A, Posny N, Schulte H, Assmann G, Wahrburg U: The impact of dietary fat composition on serum leptin concentrations in healthy nonobese men and women. *J Clin Endocrinol Metab* 2002, 87:5008-5014. PMID: 12414865.
 108. Reseland JE, Haugen F, Hollung K, Solvoll K, Halvorsen B, Brude IR, Nenseter MS, Christiansen EN, Drevon CA: Reduction of leptin gene expression by dietary polyunsaturated fatty acids. *J Lipid Res* 2001, 42:743-750. PMID: 11352981.

109. American Diabetes Association: 4. Foundations of care: education, nutrition, physical activity, smoking cessation, psychosocial care, and immunization. *Diabetes Care* 2015, 38:S20-S30. doi: 10.2337/dc15-S007.
110. Martirosyan DM, Singh J: A new definition of functional food by FFC: what makes a new definition unique? *Funct Foods Heal Dis* 2015, 5(6):209-223.