Central obesity, type 2 diabetes and insulin: exploring a pathway full of thorns

Georgios S. Papaetis[1,2], Panagiotis Papakyriakou[3], Themistoklis N. Panagiotou[2]

[1]Diabetes Clinic, Paphos, Cyprus
[2]Diabetes Clinic, 3rd Department of Medicine, University of Athens Medical School, 'Sotiria' General Hospital, Athens, Greece
[3]Department of Internal Medicine and Hepatology, Paphos General Hospital, Paphos, Cyprus

Submitted: 19 May 2013
Accepted: 4 July 2013

Arch Med Sci 2015; 11, 3: 463–482
DOI: 10.5114/aoms.2015.52350

Corresponding author:
Georgios S. Papaetis MD, PhD
Diabetes Clinic
Eleftherios Venizelos
Avenue 62
Paphos, Cyprus
Phone: 0035799273573
Fax: 0035726220451
E-mail: gpapaetis@yahoo.gr

Abstract

The prevalence of type 2 diabetes (T2D) is rapidly increasing. This is strongly related to the contemporary lifestyle changes that have resulted in increased rates of overweight individuals and obesity. Central (intra-abdominal) obesity is observed in the majority of patients with T2D. It is associated with insulin resistance, mainly at the level of skeletal muscle, adipose tissue and liver. The discovery of macrophage infiltration in the abdominal adipose tissue and the unbalanced production of adipocyte cytokines (adipokines) was an essential step towards novel research perspectives for a better understanding of the molecular mechanisms governing the development of insulin resistance. Furthermore, in an obese state, the increased cellular uptake of non-esterified fatty acids is exacerbated without any subsequent β-oxidation. This in turn contributes to the accumulation of intermediate lipid metabolites that cause defects in the insulin signaling pathway. This paper examines the possible cellular mechanisms that connect central obesity with defects in the insulin pathway. It discusses the discrepancies observed from studies organized in cell cultures, animal models and humans. Finally, it emphasizes the need for therapeutic strategies in order to achieve weight reduction in overweight and obese patients with T2D.

Key words: adipokines, central obesity, cardiovascular disease, insulin resistance, non-esterified fatty acids, type 2 diabetes.

Introduction

The prevalence of type 2 diabetes (T2D) is evolving globally at an alarming rate [1]. It is estimated that by the year 2030 approximately 366 million people will have diabetes and more than 90% of them T2D [2]. The increased prevalence of T2D is strongly related to the contemporary global lifestyle "modernization" (overnutrition, changes in the food environment and a sedentary lifestyle) that has resulted in increased rates of overweight individuals and obesity [3]. The prevalence of T2D is three to seven times higher in obese adults compared to normal-weight ones. Specifically, adults with body mass index (BMI) > 35 kg/m^2 are 20 times as likely to develop T2D compared to those with a BMI between 18.5 kg/m^2 and 24.9 kg/m^2 [4]. It is also estimated that for every 1 kg increase in body weight there is a 4.5% higher risk of developing T2D [5]. Fur-

thermore, obesity-related T2D is increasingly diagnosed in the third decade of life, while in some countries and ethnic populations children and adolescents develop T2D [6].

Both obesity and physical inactivity underlie the development of insulin resistance. Insulin resistance is observed in approximately 90% of patients with T2D and in 66% of individuals with impaired glucose tolerance (IGT). Insulin resistance together with β-cell dysfunction and apoptosis are the two fundamental mechanisms for the development of T2D [7, 8]. Insulin resistance per se doubles the risk for cardiovascular disease, which is the ultimate cause of death in about 80% of patients with T2D. This fact suggests that an important part of the higher cardiovascular risk observed is due to its proatherogenic effects [9, 10]. It has also been correlated with a higher rate of cerebrovascular disease, coronary artery disease (CAD) and peripheral arterial disease (PAD) [11–13].

Central (intra-abdominal) obesity is observed in the majority of patients with T2D. It is associated with insulin resistance, mainly at the level of adipose tissue, liver and skeletal muscle [4, 7]. The discovery of macrophage infiltration in the abdominal adipose tissue and the unbalanced production of adipocyte protein factors and hormones (adipokines) was a major step towards novel research perspectives, allowing for a better understanding of the mechanisms governing the development of insulin resistance. Furthermore, the increased cellular uptake of non-esterified fatty acids (NEFAs) is exacerbated in an obese state without any subsequent β-oxidation. This in turn contributes to the accumulation of intermediate lipid metabolites and causes defects in the insulin signaling pathway [4, 7].

This paper examines the possible cellular mechanisms that connect central obesity with defects in the insulin pathway. It discusses the discrepancies observed from studies organized in cell cultures, animal models and humans. It also emphasizes the need for therapeutic approaches in order to achieve weight reduction in overweight and obese patients with T2D.

Search of literature

We performed the literature search through PubMed, Scope and Google (January 1980–May 2013) and identified the relevant studies to be included in the review. The search terms we used were central obesity, adipokines, insulin resistance, non-esterified fatty acids and T2D.

The insulin pathway in a sensitive and resistant state

Insulin receptor (IR) is a transmembrane protein that is composed of four subunits: two α and two β. In nonvascular cells insulin exerts its activity after binding to the α-subunit at the extracellular surface of the sarcolemmal membrane (Figure 1) [14]. This in turn causes autophosphorylation of the intracellular domain of the IR β-subunit that has tyrosine kinase (TK) activity and a subsequent tyrosine phosphorylation of intracellular adapter proteins, such as IR substrates 1 and 2 (IRS-1 and IRS-2) and Shc [15, 16]. In hepatic cells IRS-2 phosphorylation mediates insulin activity after binding to IR, while in muscle cells IRS-1 serves as the main docking protein for the insulin pathway [15]. Tyrosine-phosphorylated IRS-1 and IRS-2 bind to the src-homology 2 (SH2) domain of intracellular proteins. One of these proteins is the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI 3-kinase). The interaction between the p85 subunit and the phosphorylated IRS promotes activation of the p110 catalytic subunit of PI 3-kinase. The final result of this cascade is activation of the PI 3-kinase pathway by insulin. This pathway is linked to three fundamental metabolic functions of insulin: (i) Glucose transport, through stimulation of sarcolemmal glucose transporter 4 (GLUT-4) to the cell surface. Glucose transport is the rate controlling step for insulin-stimulated muscle glycogen production in patients with T2D. Insulin mediated translocation of GLUT-4 is reduced by 50% in skeletal muscle cells of patients with T2D. This reduction is correlated with the severity of T2D. (ii) Glucose phosphorylation through hexokinase-II stimulation. (iii) Glycogen synthesis via glycogen synthase expression. Hexokinase-II and glycogen synthase activity are reduced when the PI 3-kinase pathway is inhibited [16, 17]. The association of p85 protein with IRS-1 is also highly suppressed in patients with T2D and obese individuals without diabetes compared to lean healthy ones [18].

Activation of the PI 3-kinase pathway has also been linked to the production of nitric oxide (NO), partially due to an increased rate of endothelial nitric oxide synthase (eNOS) gene expression [19]. In vascular cells PI 3-kinase downregulation may suppress the vasodilator effect of insulin via NO production; this in turn causes endothelial dysfunction and promotes the development of hypertension. Furthermore, it stimulates several pro-atherogenic mechanisms. These are mainly vascular smooth muscle cell (VSMC) proliferation and migration, leukocyte adhesion to endothelial cells and platelet aggregation.

Tyrosine-phosphorylated Shc and IRS proteins can also bind to the SH2 domain of GRB2, leading to activation of the extracellular regulated kinase (ERK)/mitogen-activated protein (MAP) kinase signaling pathway [20]. In patients with severe insulin resistance, the cellular events that lead to

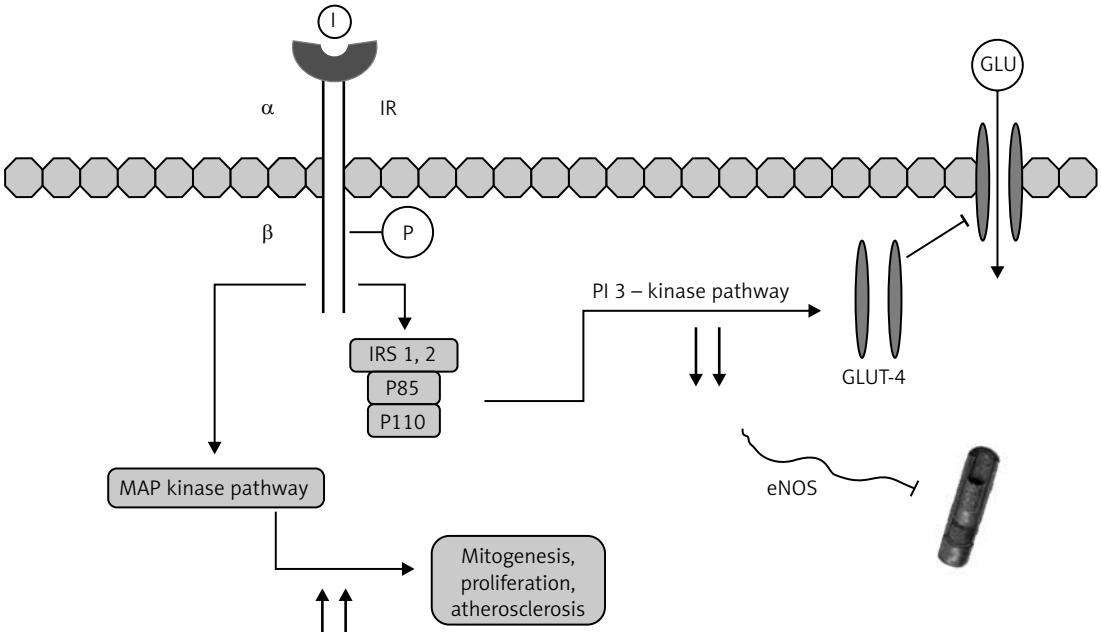

Figure 1. The insulin pathway in a sensitive and an insulin resistant state. Insulin exerts its activity after binding to the α-subunit of the insulin receptor (IR) at the extracellular surface of the sarcolemmal membrane. This, in turn, causes the autophosphorylation of the IR β-subunit, which has tyrosine kinase (TK) activity, and subsequent tyrosine phosphorylation of intracellular adapter proteins, such as IR substrates 1 and 2 (IRS-1 and IRS-2). Tyrosine-phosphorylated IRS-1 or IRS-2 bind to the src-homology 2 (SH2) domain of intracellular proteins. One of these proteins is the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI 3-kinase). The interaction between the p85 subunit and the phosphorylated IRS promotes activation of the p110 catalytic subunit of PI 3-kinase. The final result of this cascade is activation of the PI 3-kinase pathway by insulin, which promotes glucose transport through the stimulation of glucose transporter 4 (GLUT-4) to the cell surface. Activation of the PI 3-kinase pathway has also been linked to nitric oxide (NO) production partially due to an increase in the endothelial nitric oxide synthase (eNOS) gene expression. PI 3-kinase downregulation may lessen the vasodilator effect of insulin via NO production and promote endothelial dysfunction. Tyrosine-phosphorylated Shc and IRS proteins can also lead to activation of the mitogen-activated protein (MAP) kinase signaling pathway. Increased insulin activity through the MAP kinase pathway plays an accelerating role in the development of diabetes-related complications, such as inflammation, proliferation and atherosclerosis

activation of the PI-3 kinase pathway are inhibited. Increased insulin secretion stimulates the MAP kinase pathway since it is normally sensitive to insulin. Hence, it is excessively hyperactive. MAP kinase pathway activation promotes the growth effects of insulin. It is connected to VSMC proliferation, increased collagen formation, expression of extracellular matrix proteins and the activation of multiple inflammatory pathways. The major pathways involved are the inhibitor κB (IκB)/nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK) [20]. Increased insulin activity through the MAP kinase pathway has an accelerating role in the development of diabetes related complications such as inflammation, proliferation and atherosclerosis. The VSMCs and endothelial cells also express IRs. The IR phosphorylation causes tyrosine phosphorylation of IRS-1, IRS-2 and Shc. In this way both PI 3-kinase and ERK/MAP kinase pathways are activated in a sensitive insulin state.

3-phosphoinositide-dependent protein kinase-1 (PDK-1) is a serine/threonine kinase, ubiquitously expressed in human tissues; it plays a pivotal role in mediating signal transduction downstream of the PI 3-kinase pathway in response to mitogen stimulation [21]. This effect is achieved after the phosphorylation of several kinases that are downstream effectors of PI 3-kinase. The best characterized substrate of PDK-1 is protein kinase B (PKB). At the cellular level, PDK-1 regulates key insulin effects such as GLUT-4 membrane translocation, glycogen synthesis, protein synthesis, and cell survival [22]. Mammalian target of rapamycin (mToR) is a member of the PI kinase-related kinase pathway. It has an essential role in insulin induced subcellular redistribution of IRS-1. It can also control negatively insulin-stimulated glucose transport, mainly through the redistribution of IRS-1, while the subsequent degradation of IRS-1 downregulates insulin-stimulated activation of Akt [23].

Several genetic mechanisms have been associated with the development of insulin resistance: (i) Polymorphisms of insulin, IRs, IRS-1 and other post-receptor molecules. (ii) Primary target cell defects. (iii) Auto-antibodies against insulin and/or IR. (iv) Accelerated insulin degradation. (v) Mitochondrial dysfunction [23–25]. Acquired effects

that block insulin activity are mainly age, obesity, lipotoxicity and glucotoxicity [26]. Accumulating evidence suggests an essential role of central obesity in the development of insulin resistance and progressively T2D (Tables I, II).

Adipokines and other molecules that may induce insulin resistance

White adipose tissue (WAT) is mainly white/yellow in color and it is concentrated in the subcutaneous regions and the abdomen [27]. Brown adipose tissue (BAT) is relatively sparse and has its main location in small "pockets" in the thorax [28]. The WAT has a major role in the regulation of fatty acid homeostasis. When calorie abundance is the case, it stores NEFAs in the form of triglycerides (TG). In times of energy lack it releases them back into the circulation [27, 29, 30]. Subcutaneous WAT is by far the largest adipose depot within the human body. Visceral WAT (mainly composed by omental, mesenteric, retroperitoneal and epicardial fat) constitutes about 15% of total fat in obese individuals [27, 31].

The preferable accumulation of macrophages in visceral compared to subcutaneous fat is associated with the secretion of a plethora of bioactive signaling proteins, the adipokines. Adipokines and other molecules predominantly secreted from visceral fat can play an essential role in the development, exacerbation and maintenance of an insulin resistant state. Adipokines have an important contribution to the regulation of local metabolic processes. They mainly have an autocrine and paracrine function. Some of them also regulate systemic processes displaying typical endocrine properties; in this way they establish adipose tissue as an evolving endocrine organ, critical in the regulation of cellular insulin activity [27]. Most of these molecules are associated with the downregulation of IR and mainly with a postreceptor failure to activate its TK activity. The latter is achieved through serine/threonine phosphorylation of IRS-1 and/or IRS-2 and has major impact on the development of insulin resistance (Figure 2) [32].

Tumor necrosis factor-α

The TNF-α is suggested to play a significant role in the development of insulin resistance at the level of adipocyte, hepatocyte and skeletal muscle cell. It is mainly produced from macrophages infiltrating adipose tissue and less so from adipocytes [33]. It decreases the expression of IR and suppresses its autophosphorylation [34]. It also decreases the expression of IRS-1 and GLUT-4, while it promotes a JNK pathway mediated serine phosphorylation of IRS-1 [35]. The TNF-α has a stimulatory effect on lipolysis after downregulation of the lipid droplet-associated protein perilipin, which suppresses the activity of hormone-sensitive lipase [36]. It also causes reduced oxidation of NEFAs in skeletal muscle and hepatocyte cells through suppression of 5' AMP-activated protein kinase (AMPK) and the induction of protein phosphatase 2C [37]. The reduced rate of NEFA oxidation results in increased accumulation of intermediate bioactive lipid metabolites, which in turn inhibit IRS activity [38]. Deletion of TNF-α or TNF-α receptors resulted in an enhanced insulin sensitivity status both in leptin-deficient ob/ob mice and in diet-induced obese mice [34]. Treatment with the TNF-α inhibitor marimastat was also found to improve insulin sensitivity and reverse hepatic steatosis in mouse models of diet-induced obesity and leptin deficiency [39].

TNF-α levels in humans were associated with insulin resistance after adjustment for adiposity and metabolic syndrome status; weight loss was found to decrease TNF-α levels [40]. The extended use of anti-TNF-α therapies in inflammatory diseases, such as ankylosing spondylitis and rheumatoid arthritis (RA), suggested a potential role of TNF-α inhibition in the improvement of insulin sensitivity in non-diabetic rheumatic patients [41]. In a recent large observational study, in which 121,280 patients with RA or psoriasis were enrolled, the adjusted risk for developing T2D was lower for individuals starting a TNF inhibitor or hydroxychloroquine compared to the initiation of other nonbiologic disease-modifying antirheumatic drugs [42]. However, the administration of anti-TNF-α drugs in individuals with obesity and metabolic syndrome and patients with T2D was not found to improve insulin sensitivity, and consequently there is lingering uncertainty about the biological importance of this pathway in human insulin resistant states [43–45]. This lack of association could be the result of the autocrine/paracrine action of TNF-α in the adipose tissue, the small sample size of these studies, the dosing duration of anti-TNF-α therapy and the possible presence of more powerful determinants of insulin sensitivity in humans [43].

Interleukin 6

Adipose tissue produces approximately 15–35% of total human circulating levels of IL-6. Visceral adipose tissue produces three to four times more IL-6 than subcutaneous adipose tissue. IL-6 is mainly secreted from stromal vascular cells [46]. Elevated IL-6 plasma levels have been described in patients with T2D and especially in those who had features of insulin resistance [47]. These levels declined in parallel with weight loss in obese individuals undergoing bariatric surgery [48].

Table I. Major molecules secreted from visceral fat that may induce insulin resistance: main results from preclinical and clinical studies

Molecule	Preclinical and clinical studies (references)		Main results
	Cell cultures/ animal models	Humans	
TNF-α	34, 35, 39	40, 43–45	Preclinical studies suggested that TNF-α decreased the expression of IR, IRS-1 and GLUT-4, promoting a serine phosphorylation of IRS-1. Its levels were associated with insulin resistance in humans. However, the administration of anti-TNF-α drugs in individuals with obesity, metabolic syndrome and patients with T2D was not found to improve insulin sensitivity
IL-6	49, 50	47, 48	IL-6 decreased the expression of IR, IRS-1 and GLUT-4, and inhibited the phosphorylation of IRS-1 in preclinical studies. Elevated IL-6 plasma levels have been described in patients with T2D, and especially in those with features of insulin resistance. Increased IL-6 levels were found during exercise
IL-18	61	62	IL-18 has an inhibitory effect on the insulin-induced Akt phosphorylation in human adipocytes. Studies in patients with T2D suggested that it was negatively related to fasting glucose levels and insulin activity
Leptin	64, 65, 66, 72, 69	68, 70	Leptin may enhance insulin sensitivity in several preclinical studies, while in some cell models it can promote an insulin resistant state. Leptin levels were also found to be higher in insulin-resistant than in insulin-sensitive subjects, serving as an endogenous response to an ambient insulin resistant state
Resistin	75–80, 83	84, 85	Resistin was found to cause insulin resistance in preclinical studies. However, several preclinical and clinical studies did not demonstrate any positive association
RBP-4	88, 93	89, 90, 95–98	RBP-4 plasma levels have been positively associated with the grade of insulin resistance in several preclinical and clinical studies. However, several studies in humans suggest that RBP-4 is not an independent determinant of insulin resistance
MCP-1	100	101	Preclinical and clinical studies suggested that MCP-1 stimulated insulin resistance in the liver and skeletal muscle, promoting an insulin resistant state
PAI-1	104, 105	106, 107, 109	PAI-1 promoted an insulin resistant state in preclinical studies. Increased levels were found in patients with T2D. It was suggested that PAI-1 was associated with increased cardiovascular morbidity and mortality
A-SAA	113, 114, 117	115	A-SAA promoted the down-regulation of phosphotyrosine IRS-1 and GLUT-4 expression in preclinical studies. Circulating levels of A-SAA were found to be increased in subjects with obesity and patients with T2D in clinical studies
ET-1	119, 120,121	123, 124	ET-1 suppressed the intracellular activity of insulin by blocking the insulin-mediated phosphorylation of IRS-1 and IRS-2 in preclinical studies. ET-1 concentrations were found to be significantly elevated in patients with T2D and obese individuals with or without IGT
AG-II	134, 135, 138	136	Preclinical studies suggested that AG-II levels directly interfered with insulin signaling at the postreceptor level, modulating IRS protein phosphorylation and/or PI 3-kinase activity
MIF	146	147	Preclinical studies demonstrated that MIF reduced the tyrosine phosphorylation IRS-1 and its association with the p85 unit of the insulin pathway. Increased MIF levels were reported in obese and IGT subjects as well as in patients with T2D compared to controls

TNF-α – Tumor necrosis factor-α, NEFAs – non-esterified fatty acids, T2D – type 2 diabetes, IGT – impaired glucose tolerance, IR – insulin receptor, IRS – insulin receptor substrate, GLUT-4 – glucose transporter 4, IL-6 – interleukin-6, IL-18 – interleukin-18, RBP-4 – retinol binding protein, MCP-1 – monocyte chemotactic protein-1, PAI-1 – plasminogen activator inhibitor-1, A-SAA – acute-phase serum amyloid A, ET-1 – endothelin-1, AG-II – angiotensin-II, MIF – macrophage migration inhibitory factor.

Table II. Adiponectin, novel adipokines and insulin resistance: main results from preclinical and clinical studies

Molecule	Cell cultures[X]/ animal models	Humans[Y]	Main results
AD	158, 159, 160, 161	163–167	Preclinical studies suggested that adiponectin exerted its insulin-sensitizing effects through: (i) AMPK activation. (ii) PPAR-α stimulation. (iii) Inhibition of the NF-κB pathway. (iv) A reduced rate of IRS-1 inhibitory serine phosphorylation and higher expression of GLUT-4. (v) Downregulation of the expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase thus a reduction of neoglucogenesis. Clinical studies suggested that reduced adiponectin levels may predict the development of atherosclerosis and were associated with the development of insulin resistance, IGT and T2D
Visfatin	170, 171	172–174, 178, 179, 180	Preclinical studies suggested an insulin-like activity which was the result of IRS-1 and IRS-2 tyrosine phosphorylation and the subsequent activation of the PI 3-kinase pathway. Studies in humans suggested either an association of its levels with visceral fat and T2D or absence of an association
Vaspin	182, 183	184, 185	Vaspin improved insulin sensitivity in preclinical models by inducing GLUT-4 expression. However, studies in humans suggested that its levels were associated with obesity and impaired insulin sensitivity
Omentin	187	190, 192	It improves insulin sensitivity in preclinical models by inducing GLUT-4 expression and Akt phosphorylation. Omentin-1 levels were inversely associated with waist circumference, BMI and insulin resistance in humans. Absence of an association between circulating omentin levels and postprandial blood glucose levels was found in slim adults
Apelin	193	194	Apelin enhanced glucose utilization in preclinical models. However, in patients with T2D both increased and decreased plasma apelin levels were observed compared to healthy controls
Chemerin	199, 200	198	Preclinical studies suggested improved insulin-stimulated glucose uptake through IRS-1 phosphorylation. Its levels did not differ significantly between T2D patients and normal individuals

AD – Adiponectin, AMPK – 5' AMP-activated protein kinase, PPAR-α – peroxisome proliferator-activated receptor α, NF-κB – nuclear factor-κB, IRS – insulin receptor substrate, GLUT-4 – glucose transporter 4, T2D – type 2 diabetes, IGT – impaired glucose tolerance, PI 3-kinase – phosphatidylinositol 3-kinase, BMI – body mass index, [X]preclinical studies organized in cell cultures and animal/models (references), [Y]clinical studies (references).

IL-6 may have a role in the development of insulin resistance in adipocytes and hepatocytes after decreasing the expression of IR, IRS-1 and GLUT-4. This phenomenon could be attributed to the upregulation of the suppressor of cytokine signaling-3 (SOCS-3) protein that inhibits the expression of IRs, promoting the proteosomal degradation of IRS proteins [49]. IL-6 can also inhibit the phosphorylation of IRS-1 partly through SOCS-3 up-regulation [50]. Plasma IL-6 concentrations were also associated with an increase of NEFAs and C-reactive protein (CRP) levels [48].

However, IL-6 may promote glucose uptake and oxidation of NEFAs in animal and human skeletal muscle cells through the activation of AMPK [51]. Intracerebroventricular administration of IL-6 decreased body fat in rats, while increased IL-6 levels were also found during exercise [52, 53]. These data suggest that IL-6 can act both centrally and on peripheral tissues so as to influence glucose homeostasis and body weight in different ways [54]. Chronic increases of this cytokine in states of persistent inflammation may enhance insulin resistance, whereas acute transient increases may contribute to normal glucose homeostasis [50, 54].

Interleukins 1, 18 and 8

Among the first cytokines reported to exert pro-inflammatory functions is IL-1 (α and β) [55]. IL-1β can impair insulin activity by reducing IRS-1 expression in adipocytes; it may have a potential role in the loss of pancreatic β-cell mass in T2D [55, 56]. IL-18 is another pro-inflammatory cytokine that plays an important role in joint inflammation and inflammatory bowel diseases [57]. Studies in IL-18$^{-/-}$ and IL-18R$^{-/-}$ mice suggested increased body weight accompanied by an insulin resistant state compared to wild type mice [58]. Increased IL-18 levels were found in patients with T2D [59].

IL-8 production in human adipocytes is stimulated by IL-1 and TNF-α, and has an important role in the recruitment of neutrophils, lymphocytes and monocytes [60]. It was recently shown to have an inhibitory effect on the insulin-induced Akt phosphorylation in human adipocytes [61]. IL-8 is mainly produced from visceral adipose tissue and

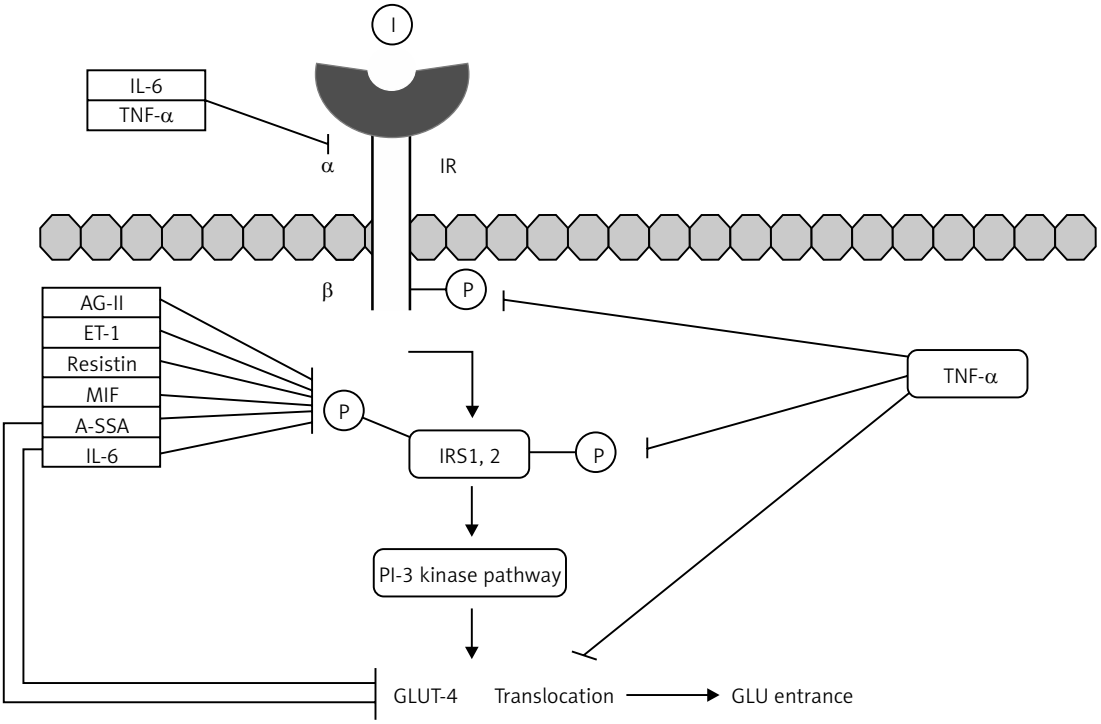

Figure 2. Adipokines, other molecules secreted from visceral fat and the insulin pathway: possible cellular mechanisms of resistance in cell cultures and animal models

I – Insulin, IR – insulin receptor, α and β – α and β subunit of the insulin receptor, IRS-1 and IRS-2 – insulin receptor substrates 1 and 2, PI 3-kinase pathway – phosphatidylinositol 3-kinase pathway, GLUT-4 – glucose transporter 4, GLU – glucose, TNF-α – tumor necrosis factor-α, IL-6 – interleukin-6, AG-II – angiotensin-II, A-SAA – acute-phase serum amyloid A, ET-1 – endothelin-1, MIF – macrophage migration inhibitory factor.

is negatively related to fasting glucose levels and insulin activity in patients with T2D [62].

Leptin

Leptin, a 16-kDa protein, is solely secreted in a pulsatile fashion by adipose tissue and mainly by subcutaneous fat. It exerts its activities after binding to specific leptin receptors that are expressed in the brain and peripheral tissues. Leptin acts as a satiety signal on the hypothalamus and causes suppression of food intake, stimulating energy expenditure [63]. It may enhance insulin sensitivity in muscle cells through AMPK activation, inhibition of acetyl-coenzyme A carboxylase, and subsequent stimulation of fatty acid oxidation, through reduced malonyl CoA levels, leading to the reduction of intra-myocellular lipid levels [64]. It can also decrease visceral adiposity and intracellular hepatic triacylglycerol levels and prevent the lipotoxic effects of obesity on pancreatic β-cells [63, 64]. Several studies have reported that leptin can increase insulin sensitivity in normal and diabetic preclinical models; it may also correct the diabetic phenotype of *ob/ob* mice. These effects were achieved through the following: (i) An insulin-independent mechanism. (ii) An insulin-sensitizing mechanism. (iii) By reducing food intake and body weight [65, 66]. However, it must be noted that several other studies did not establish an association of leptin with enhanced cellular insulin activity [67].

The vast majority of obese individuals have high circulating leptin plasma levels, which are associated with increased concentration of inflammatory markers. Leptin levels were also found to be higher in insulin-resistant than in insulin-sensitive men regardless of the adiposity status; they may serve as an endogenous response to an ambient insulin resistant state [68, 69]. Obesity-related leptin resistance or tolerance and subsequent hyperleptinemia has also been described in pancreatic β-cells, leading to deregulation of the adipocyte-insulin axis. In turn, this was associated with higher insulin levels and stimulated adipogenesis, leading to a further increase in insulin secretion and β-cell dysfunction. Deleterious effects on various peripheral tissues including liver, vasculature and myocardium have also been suggested [70, 71]. Studies in obese mice suggested that leptin's anorexic effects were attenuated, while its effect of increasing cardiovascular and renal sympathetic actions remained intact. This fact suggested the presence of selective leptin resistance that can partially explain the adverse cardiovascular actions of leptin in obesity and the promotion of an insulin resistant state [69].

At the cellular level leptin can promote insulin resistance through the serine phosphorylation of IRS-1 residues and the downregulation of IRS-2-associated PI3-kinase activity in various cell models [72]. It may also induce expression of the SOCS-3 protein, which has a negative impact on IR and IRS protein function. Interestingly, SOCS-3 can induce leptin resistance in the hypothalamus, creating a vicious circle [73]. Currently the precise role of leptin in the pathogenesis of insulin resistance and T2D remains complex and challenging [67].

Resistin

Resistin is primarily secreted from mature adipocytes in rodents. In humans it is expressed primarily from adipose infiltrating macrophages [74]. Higher resistin mRNA expression was described in abdominal fat compared to thigh fat [75]. Resistin was found to decrease glucose transport in adipocyte cultures, causing severe hepatic insulin resistance in rodents [75, 76]. Absence of the resistin gene in rodents can activate the AMPK pathway and reduce the expression of genes that encode enzymes essential for hepatic gluconeogenesis. Furthermore, *in vivo* findings suggest that resistin can suppress muscle and liver AMPK activation [77, 78]. In rodents, circulating levels of resistin were positively associated with obesity and T2D.

Resistin promotes insulin resistance through up-regulation of the SOCS-3 pathway, the induction of Ser-636 phosphorylation of IRS-1 and the suppression of PI 3-kinase activation [79, 80]. It can stimulate the expression of TNF-α and IL-6 both in human and murine macrophages. It may also activate endothelial cells by promoting endothelin (ET-1) production and upregulating monocyte chemotactic protein (MCP)-1 secretion [81]. Impaired glucose transport in isolated cardiomyocytes was also suggested [82].

Lack of association among circulating resistin levels, BMI, insulin sensitivity and/or other metabolic parameters has been reported from several trials organized in adipocyte cells and mainly in humans; other groups suggested a positive association of resistin with obesity and T2D. This fact creates uncertainty on whether the role of adipocyte-derived rodent resistin in glucose metabolism can be translated to the biology of human resistin produced by macrophages [83–85]. However, a recent study organized in a humanized resistin mouse mode has shown that human resistin is produced in response to inflammation and modulates glucose homeostasis, promoting an insulin resistant state [86].

Retinol binding protein (RBP)-4

RBP-4 was described as a rodent adipokine several years ago. It is mainly produced by hepatocytes and adipocyte cells [87]. Its possible role in humans was recently reported [87]. Insulin resistance was stimulated by the following (i) Enhanced expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase. (ii) By promoting downregulation of the insulin signaling pathway in skeletal muscle cells [88]. RBP-4 plasma levels have been positively associated with the grade of insulin resistance in specific GLUT-4 knockout mice, obese and IGT individuals and patients with T2D [88, 89]. Circulating levels of RBP-4 were associated with visceral fat and/or waist-to-hip ratio rather than BMI [89]. Increased levels of RBP-4 have also been associated with increased liver fat accumulation and hepatic insulin resistance [90]. Physical activity, life-style modification and gastric banding surgery were found to reduce RBP-4 levels and improved insulin sensitivity [91, 92]. A possible negative effect of RBP-4 in β-cell function, directly or by preventing the binding of transthyretin to its receptor, has been suggested [93]. However, it was recently postulated that the correlation of RBP-4 with insulin resistance could be attributed to the presence of renal insufficiency [94]. Recent evidence also suggests that RBP-4 is not an independent determinant of insulin resistance [95–98]. Hence, its role in human glucose metabolism is not well clarified [98].

Monocyte chemotactic protein (MCP)-1

Chemokine (C-C motif) ligand 2 (CCL2), known as MCP-1, is a potent chemoattractant molecule playing an essential role in the recruitment of monocytes/macrophages into the adipose tissue. It is produced from visceral fat in higher amounts compared to subcutaneous fat and mainly from vascular-stromal cells and hypertrophic adipocytes [99]. 3T3-L1 differentiated adipocytes treated with MCP-1 showed decreased insulin-stimulated glucose uptake and reduced expression of a variety of adipogenic genes [100]. Moreover, polymorphisms of the MCP-1 gene were positively associated with plasma MCP-1 levels and promoted an insulin resistant state [101]. MCP-1 was also shown to stimulate insulin resistance in the liver and skeletal muscle, suggesting a role as an endocrine hormone. However, MCP-1 may not be the only critical adipokine for macrophage recruitment in adipose tissue. Absence of MCP-1 in mice did not limit the obesity-associated infiltration of macrophages into the adipose tissue [102].

Plasminogen activator inhibitor-1 (PAI-1)

PAI-1 is an inhibitor of the fibrinolytic system. Visceral adipose tissue expresses more PAI-1 than subcutaneous fat. It is mainly produced from stromal-vascular cells rather than adipocytes [103]. Increased levels of PAI-1 have been associated

both with a pro-thrombotic and an insulin resistant state. PAI-1 levels decreased substantially after weight reduction [104, 105]. PAI-1 also affects WAT growth as it impairs pre-adipocyte migration and attachments to vibronectin [106].

The administration of a synthetic PAI-1 inhibitor in high-fat diet mice improved insulin sensitivity [107]. It was demonstrated that patients with T2D had three times higher PAI-1 plasma levels compared to controls [108]. PAI-1 could be a very early risk factor for the development of insulin resistance and eventually T2D [107]. It may also predict the formation of an atheromatic plaque [109]. It was found to be associated with increased cardiovascular morbidity and mortality [110].

Acute-phase serum amyloid A (A-SAA)

A-SAA is secreted from several body tissues including mature adipocytes [111]. Although the liver is considered to be the most important organ for A-SAA secretion, its expression was found to be higher in adipocytes than in hepatocytes under an insulin resistant state [112]. Down-regulation of phosphotyrosine IRS-1 and GLUT-4 expression by A-SAA was suggested to be responsible for the insulin resistant state observed in 3T3-L1 adipocytes [113]. Chemotaxis, infiltration of adipose tissue by macrophages, cytokine induction and the secretion of extracellular-matrix degrading proteases can also be induced by A-SAA [114, 115]. Circulating levels of A-SAA were found to be increased in obesity and T2D; they were correlated with the degree of insulin resistance [116, 117].

Endothelin-1 (ET-1)

ET-1 is currently considered as the most powerful natural vasoconstrictor. It is mainly produced from endothelial cells. It is also secreted from macrophages and VSMCs that surround adipocytes [118]. It mainly exerts its activity in a paracrine and autocrine fashion [119]. ET-1 may suppress the intracellular activity of insulin by blocking the insulin-mediated phosphorylation of IRS-1 and IRS-2 and the subsequent activation of the PI3-K pathway in VSMCs. In this way it aggravates insulin resistance [119, 120]. *In vitro* studies suggest that long-term adipocyte treatment with ET-1 promotes insulin signaling desensitization and decreases glucose transport [121]. ET-1 also inhibits the differentiation of preadipocytes to adipocytes [122]. Plasma ET-1 concentrations were found to be significantly elevated in insulin resistant states (T2D patients, obese individuals with or without IGT) compared to controls [123–126]. In a study organized in an obese population it was found that ET-1, either produced from adipose tissue or derived from the circulation, may have a major contribution in the selective resistance of visceral adipose tissue to the antilipolytic effect of insulin. It could also provide a vascular link between visceral fat accumulation and reduced insulin activity [127].

Adipocyte renin-angiotensin system (RAS)

Angiotensinogen and/or angiotensin peptides have been identified as secretory products of visceral fat in a higher rate than subcutaneous fat, early in the dynamic adipocyte development [128]. Angiotensin-II type 1 receptor (AT1R) and angiotensin-II type 2 receptor (AT2R) are also expressed in adipocyte cells [128, 129]. *In vivo* studies have suggested that the effects of angiotensin-II (AG-II) are mediated primarily through the AT1R receptor. The final result of these effects was the expansion of adipose tissue primarily through adipocyte hypertrophy [130, 131]. AG-II promotes an increased WAT mass since it has a local trophic factor effect early in the evolution of new adipocytes [132]. It also increases local oxidative stress in the adipose tissue [133].

Increased adipose tissue AG-II levels directly interfere with insulin signaling at the postreceptor level, modulating IRS protein phosphorylation and/or PI 3-kinase activity [134]. The administration of an AT1R antagonist contributed to an improvement of insulin activity in T2D mice. It also reduced plasma glucose levels and increased peroxisome proliferator-activated receptor γ (PPAR-γ) expression [135]. Interestingly, the hyperinsulinemia observed in an obese state can double the ability of AG-II to transactivate NF-κB. In this way it can stimulate multiple inflammatory pathways that block the insulin pathway and are involved in atherogenesis [136, 137]. Recently it was also shown that overexpression of angiotensinogen from WAT can cause glucose intolerance and may induce a systemic insulin resistant state through the reduction in muscle glucose uptake [138].

Deregulated NO synthesis

Of vital importance in maintaining vascular homeostasis is the production of NO. NO promotes smooth muscle relaxation and vasodilation. It also has important anti-thrombogenic and anti-inflammatory activities [139]. It suppresses VSMC proliferation, leukocyte adhesion and migration as well as platelet activation and adhesion [139]. NO is generated from the metabolism of l-arginine by the enzyme NOS. There are three isoforms of this enzyme: neuronal (nNOS), eNOS, and the inducible type (iNOS) [140].

Both eNOS and to a lesser extent iNOS are expressed in adipose tissue. In obese individuals eNOS expression was found to be increased in

omental obesity compared to subcutaneous fat [141]. However, the asymmetric dimethylarginine (ADMA) produced in an obese state can act as an autocrine and/or paracrine inhibitor of eNOS. Hence it can deregulate a variety of adipose-related NO-mediated functions such as lipolysis, local blood flow, glucose metabolism and mitochondrial biogenesis [142]. ADMA has been associated with an insulin resistant state and CAD [142].

Recent evidence also suggested that the M1 macrophage phenotype found in visceral fat expresses iNOS, while the M2 macrophage phenotype seen in lean adipose tissue expresses the chitinase-like protein Ym1 arginase that inhibits iNOS activity [143]. M1 macrophage phenotype has been associated with a pro-inflammatory state compared to the "alternative activated" M2 macrophage phenotype [31]. Studies in mice demonstrated that visceral fat secreted increased amounts of iNOS, which resulted in increased nitrotyrosine levels in the liver, associated with insulin resistance [144]. It was also shown that chronic blockade of iNOS by N(G)-nitro-l-arginine methyl ester (L-NAME) in mice resulted in an improved high-fat diet-induced obesity state. This effect was accompanied by reduced adipocyte inflammation, enhanced insulin signaling in skeletal muscle cells and better glucose control [145].

Macrophage migration inhibitory factor (MIF), nerve growth factor (NGF), acylation stimulating protein (ASP)

MIF is expressed in a variety of tissues. It acts as a regulator of acute inflammatory and adaptive immune reactions [146]. MIF was shown to reduce the tyrosine phosphorylation of IRS-1 and its association with the p85 unit of the insulin pathway, while MIF deficiency improved insulin sensitivity and reduced macrophage infiltration into WAT [147]. Increased MIF levels were reported in obese and IGT subjects as well as patients with T2D compared to controls. However, it still remains unclear whether these abnormalities are causal factors or epiphenomena [147]. NGF is the best known neurotrophin [148]. Increased NGF levels were described in overweight and obese subjects as well as in subjects with metabolic syndrome; it is suggested that its levels are associated with increased levels of several proinflammatory cytokines [149]. ASP is produced from adipose tissue after the interaction of adipsin, complement C3 and factor B; these three precursor proteins increase with the differentiation of adipocytes [150]. The main function of ASP is the promotion of lipid storage in adipocytes through enhancement of fatty acid re-esterification, inhibition of lipolysis and stimulation of glucose uptake. A number of studies have shown positive associations between ASP levels and patients with T2D and subjects with metabolic syndrome with or without obesity [151]. However, it is not clear enough whether increased ASP levels reflect increased activity or resistance to ASP [150].

Adiponectin

Adiponectin is solely secreted from adipocytes and is a marker of adipocyte differentiation [152]. It is a 30-kDa adipocyte-derived vasoactive peptide that exerts its metabolic activities after binding to its cell-surface receptors (AdipoR1 and AdipoR2). AdipoR1 is expressed ubiquitously and mainly in skeletal muscle, while AdipoR2 is more abundant in hepatocytes. Full-length adiponectin synergizes with insulin in order to downregulate hepatic glucose production. The isolated globular domain of adiponectin also stimulates NEFA oxidation in skeletal muscle cells [153]. However, its strongest association is the improvement of hepatic insulin sensitivity and the suppression of hepatic glucose output [154, 155].

Circulating adiponectin is mainly found in three different isoforms: low molecular weight (LMW) trimers, middle molecular weight (MMW) multimers and high-molecular weight (HMW) (18mer) forms [156]. Most of its insulin-sensitizing effects are though to be mediated through the HMW isoform [156]. The HMW isoform was reported to be superior compared to total adiponectin in predicting an insulin resistant state. Enlarged hypertrophic fat cells secrete reduced amounts of adiponectin. Weight loss increases its levels [157].

Adiponectin exerts its insulin-sensitizing effects through the following: (i) AMPK activation. In this way an increased rate of NEFA β-oxidation is achieved while the rate of lipogenesis is reduced. (ii) PPAR-α stimulation. Knockout of AdipoR2 in mice caused downregulation of PPAR-α signaling pathways and insulin resistance; concurrent inhibition of both AdipoR1 and AdipoR2 resulted in increased glucose intolerance and insulin resistance. (iii) Inhibition of the NF-κB pathway. (iv) A reduced rate of IRS-1 inhibitory serine phosphorylation and higher expression of GLUT-4. (v) Downregulation of the expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, leading to a reduction of neoglucogenesis [158–161]. Adiponectin can also improve glucose metabolism after stimulation of pancreatic insulin secretion both *in vivo* and *in vitro* [162].

Reduced adiponectin levels have been demonstrated in individuals with central obesity. Lower levels are also found in visceral compared to subcutaneous fat. Downregulation of adiponectin receptors was also shown in an insulin resistant state. Low levels of adiponectin led to an increased rate of TNF-α production from adipose tissue,

while both IL-6 and TNF-α can reduce the secretion of adiponectin [163]. Reduced adiponectin levels may predict the development of atherosclerosis in both diabetic patients and non-diabetic individuals. They have also been correlated with the development of insulin resistance, IGT and T2D in different ethnic groups [164–167]. Adiponectin levels vary among different populations, reflecting the ethnic differences in development of an insulin resistant state [168].

Novel adipokines

Visfatin

Visfatin, also called pre-B cell colony-enhancing factor 1 (PBEF1), is produced by the liver, muscles, human bone marrow and preferentially from visceral fat [169]. *In vitro* studies have suggested enhanced glucose uptake by myocytes and adipocytes. Inhibition of hepatocyte glucose release was also reported. This insulin-like activity of visfatin was suggested to be the result of IRS-1 and IRS-2 tyrosine phosphorylation and the subsequent activation of the PI 3-kinase pathway. It was also reported that visfatin exerted its insulin-mimetic effects by binding to the IR at a site distinct from insulin [170]. A possible role of visfatin in the regulation of β-cell function, as a systemic nicotinamide adenine dinucleotide (NAD) biosynthetic enzyme, was also demonstrated [171].

However, several studies in humans have reported an association of its levels with visceral fat and T2D, suggesting that visfatin may play a role in the pathogenesis of insulin resistance [172–174]. Moreover, visfatin levels were found to be reduced after exercise in patients with early onset T2D [175]. Recent evidence also indicates that visfatin levels may be associated with the severity of proteinuria and an advanced carotid atherosclerosis state in patients with T2D [176, 177]. Absence of an association between visfatin and an insulin resistant state has been demonstrated in several studies performed both in rodents and humans [178–180].

Vaspin

Vaspin (visceral adipose tissue-derived serine protease inhibitor) is mainly produced from visceral adipose tissue [181]. It improves insulin sensitivity in preclinical models by inducing GLUT-4 expression [182]. When recombinant vaspin was tested in high fat and sucrose obese mice, improved glucose tolerance and insulin sensitivity were reported [183]. However, increased vaspin serum levels were associated with obesity and impaired insulin sensitivity; hyperglycemia was reported to amplify this association [184, 185]. It was also suggested that the upregulation of vaspin secretion from human adipose tissue could represent a compensatory mechanism in order to antagonize the actions of unknown proteases that are up-regulated in states of insulin resistance and/or IGT [183]. Metformin therapy in overweight women with polycystic ovary syndrome significantly decreased serum vaspin levels, possibly through the suppression of hepatic glucose production [186].

Omentin

Omentin is mainly produced from visceral fat compared to subcutaneous fat; stromal-vascular cells are the major source of its secretion. It improves insulin sensitivity in preclinical models by inducing GLUT-4 expression and Akt phosphorylation [187]. Omentin-1 is the major circulating form of omentin, while omentin-2 shares 83% amino acid identity with omentin/intelectin [188]. Omentin-1 was also identified in human epicardial fat [189]. Plasma levels of omentin-1 are inversely associated with waist circumference, BMI, leptin levels and insulin resistance. They are also positively correlated with adiponectin and high-density lipoprotein cholesterol (HDL-C) levels, and appear to improve insulin sensitivity in the adipocyte level [190]. Increased circulating omentin-1 levels were reported after weight loss; they were correlated with reduced BMI and improved insulin activity [191]. Absence of an association between circulating omentin levels and postprandial blood glucose levels was found [192].

Apelin

Apelin is produced similarly both in visceral and subcutaneous adipose tissue. Acute injection of apelin in normal mice resulted in a glucose-lowering effect that was correlated with improved glucose utilization in adipose tissue and skeletal muscle. In obese insulin resistant mice acute infusion of apelin also enhanced glucose utilization [193]. However, in patients with T2D both increased and decreased plasma apelin levels were observed compared to healthy controls [174, 194]. It was reported that apelin and apelin receptor expression both in mice and humans are regulated in a tissue-dependent manner and according to the severity of insulin resistance [195].

Chemerin

Chemerin is a chemoattractant protein expressed in the WAT, liver, pancreas and lung [196]. It has a described role in host defense, such as complement fibrinolysis, chemoattraction and coagulation. Chemerin and its receptor, chemokine-like receptor 1 (CMKLR1, or ChemR23), have an important role in adipocyte differentiation [197]. Chemerin regulates the expression of adipocyte

genes encoding for molecules involved in lipid and glucose homeostasis (fatty acid synthase, GLUT-4, adiponectin) [198]. Improved insulin-stimulated glucose uptake through IRS-1 phosphorylation was demonstrated in 3T3-L1 adipocytes [199]. However, enhanced cross talk between adipose tissue and skeletal muscle can be induced by this protein; this, in turn, promotes a reduction of insulin activity [200]. Chemerin expression was found to be significantly higher in adipose tissue of diabetic and IGT *Psammomys obesus* compared with normal glucose-tolerant sand rats. Its levels did not differ significantly between T2D patients and normal individuals; however, in subjects with normal glucose tolerance, its levels were positively associated with BMI, blood pressure and triglyceride levels [197, 198].

NEFAs and the insulin pathway

Plasma NEFAs are elevated in an insulin resistant state. Moreover, insulin resistance is improved when plasma NEFA levels are reduced [201]. Increased plasma NEFA levels are the result of three major pathophysiological events: (i) Insulin is a potent inhibitor of lipolysis by inhibiting the enzyme hormone-sensitive lipase. A reduction of insulin activity so as to suppress lipolysis from adipose tissue (mainly in hypertrophic adipocytes of visceral fat) in the post-prandial state is observed in the case of insulin resistance. Hence, it promotes the release of NEFAs to the circulation. (ii) NEFAs clearance is reduced. (iii) Elevated plasma NEFA levels further inhibit the anti-lipolytic effect of insulin; this in turn stimulates higher rates of NEFA release into the circulation [38, 202].

Fasting NEFA levels were inversely and independently associated with the risk of developing T2D [202]. Furthermore, T2D patients (lean and obese) are characterized by increased plasma NEFA levels during the whole day when compared with matched normoglycemic individuals, who are not normally suppressed after ingestion of a mixed meal or an oral glucose load [203]. It is also shown that the reported link between plasma NEFAs and T2D is strongest for the level of adiposity as opposed to insulin sensitivity and/or glycemic control, highlighting the close association of obesity with the development of T2D [204]. Increased cellular NEFA concentrations inhibit insulin secretion, stimulate gluconeogenesis and promote severe tissue insulin resistance [204, 205]. The main cellular mechanisms that underlie the effects of NEFAs on the insulin pathway are shown in Table III [38, 206–213].

Conclusions

During the last two decades a wealth of information has been accumulated regarding the structure and function of WAT. All this evidence has strongly improved our understanding of the pathophysiological mechanisms that underlie the development of insulin resistance and T2D [27, 31]. Continued accumulation of visceral fat contributes to adipocyte hypertrophy and increased infiltration with macrophages. Both adipocytes and macrophages secrete several adipokines, which create a complex network of factors, promoting, maintaining and exacerbating an insulin resistant state. Moreover, the increased cellular uptake of NEFAs without subsequent β-oxidation is exacerbated in an obese state, contributing to the accumulation of intermediate lipid metabolites; these metabolites cause defects in the insulin-signaling pathway [27, 214].

However, the following important observations must be taken into consideration: (i) There are still many discrepancies between data reported from rodents and humans regarding the roles of adipokines. This fact raises the possibility of species differences in the regulation of possible activities and cellular roles of adipokines. Data from studies in animal models may not be readily reflected in humans, possibly due to the variety and complexity of the cellular pathways involved. Hence, results from these studies must be interpreted with caution [34, 35, 39, 40–43, 54, 67, 75, 76, 83–85, 88, 89, 95–98, 172–174, 178–180, 183–185, 198]. (ii) The evidence for the systemic effects of some cytokines in humans is less well supported; some studies find no association, whereas others show an association between inflammatory cytokines and insulin activity. This discrepancy can be partially explained by the differences in the study populations and the methodological inter-study variation of the diagnostic methods used [215]. (iii) Questions also remain about the exact molecular mechanisms by which a number of these adipokines potentially exert their detrimental effects on insulin action. Numerous adipokines can act independently or in consonance. This complicated interplay along with their overlapping cellular activities creates uncertainties about their exact role and importance [216, 217]. (iv) Several adipokines are produced from non-adipose tissues, and thus it is not always straightforward to evaluate the specific contribution of WAT to the circulating levels of these factors. Whatever the case may be, it is absolutely essential that any potential relevance to human physiology is supported after a variety of distinct models report similar interpretations that are also described in humans; any inference on causality must be demonstrated in methodologically well-designed studies.

The integration of a lifestyle intervention program in order to improve glycemic control and cardiovascular risk factors is more than obligatory in

Table III. Main cellular mechanisms of NEFAs' inhibitory effect on the insulin pathway

N	Pathophysiological mechanisms	Ref.
(i)	Enhanced uptake of NEFAs without subsequent β-oxidation. This in turn promotes the accumulation of lipid metabolites within cells. Defects in the insulin signaling pathway from increased intracellular levels of triacylglycerol intermediate metabolites (mainly long-chain fatty acyl Co-A, DAG and ceramides) have been reported in several studies. Intermediate metabolites mainly inhibit the insulin signaling pathway by increasing IRS-1 and IRS-2 serine/threonine phosphorylation. These effects are mediated through activation of multiple pro-inflammatory signaling pathways, such as PKC, JNK, IKK, IkB kinase/NF-κB and mTOR. In muscle cells, IRS-1 serine/threonine phosphorylation suppresses GLUT-4 translocation and consequently insulin-mediated glucose uptake is reduced. In hepatic cells, IRS-2 serine/threonine phosphorylation reduces the insulin stimulation of glycogen synthase activation and decreases the phosphorylation of FOXO, leading to increased hepatic gluconeogenesis	38, 206–208
(ii)	TLR-4 in macrophages is activated by saturated NEFAs and stimulates intracellular pathways with major importance in the induction of insulin resistance, such as NF-κB and JNK. It also induces the production of adipokines in primary adipocytes or adipocyte cell lines, such as MCP-1. MCP-1 can further enhance macrophage infiltration into the adipose tissue	207
(iii)	Stimulation of MMPs activity from NEFAs has been described. MMPs cause extracellular matrix degradation. Extracellular matrix degradation and remodelling is a crucial cellular event in order to allow adipocyte cells to increase their size and their pro-inflammatory potential → reduced tissue sensitivity to insulin	209
(iv)	NEFAs promote endothelial dysfunction. The latter is associated with an accelerated insulin resistant state since it alters the transcapillary passage of insulin to its target tissues	209, 210
(v)	Elevated plasma levels of NEFAs as well as their intermediate metabolites in skeletal muscle cells promote reduced expression of nuclear genes that encode enzymes involved in mitochondrial oxidative metabolism, such as PPAR-γ coactivator (PGC-1) → mitochondrial lipotoxicity, which contributes to an increased intramyocellular fat content and exacerbates the insulin resistant state	211, 212
(vi)	NEFAs can reduce the insulin-stimulated glucose transport after modulating glucose GLUT-4 gene transcription and mRNA stability	213

N – Number, NEFAs – non-esterified fatty acids, DAG – diacylglycerol, IRS – insulin receptor substrate, IR – insulin receptor, GLUT-4 – glucose transporter 4, JNK – c-Jun NH2-terminal kinase, IKK – IK kinase, NF-κB – nuclear factor-κB, PKC – protein kinase C, mTOR – mammalian target of rapamycin, FOXO – forkhead box protein O, TLR – Toll-like receptors, MMPs – matrix metalloproteinases, PPAR-γ – peroxisome proliferator-activated receptor-γ, ATP – adenosine triphosphate, MCP-1 – monocyte chemotactic protein-1.

patients with T2D [218, 219]. A large meta-analysis, in which approximately 10,000 participants were treated with either orlistat or placebo for at least 1 year, showed a mean placebo-subtracted weight loss of 2.9 kg [220]. Metformin therapy may facilitate a small or modest weight loss, reduce insulin requirements and improve hepatic biochemistry [221, 222]. The thorough understanding of the incretin system in the pathogenesis of T2D led to the evolution of incretin-based therapies. GLP-1 receptor (GLP-1R) agonists, namely exenatide and liraglutide, have achieved significantly lower hemoglobin A_{1c} (HbA_{1c}) values that were associated with significant weight reduction in several clinical trials [223, 224]. GLP-1R agonists can be a very useful therapeutic tool in overweight or obese T2D patients with profound insulin resistance. A new class of drugs, the sodium glucose cotransporter 2 (SGLT-2) inhibitors, are under advanced investigation. These drugs exert their main activity in the proximal tubule by inhibiting glucose reabsorption, resulting in increased glucose excretion through the kidney; they have also been associated with significant weight loss [225]. Dapagliflozin has been approved by the European Medicines Agency (EMA) and canagliflozin by the US Food and Drug Administration (FDA) for the treatment of patients with T2D [226]. Intestinal bypass procedures, mainly Roux-en-Y gastric bypass and biliopancreatic diversion with duodenal switch, have resulted not only in significant weight reduction but also in the remission of T2D within days or weeks after the procedure; they can also reduce any possible future renal and cardiovascular complications as well as cardiovascular mortality [227–230]. Further research is also needed in order to investigate whether leptin sensitizers can induce weight loss in leptin resistant or tolerant obese states, and whether they play a role in weight loss maintenance [231].

The ultimate goal of any treatment strategy should be to maintain β-cell function, increase insulin sensitivity and achieve long sustained microvascular and macrovascular benefits in this population. In order to accomplish this goal, it is first essential to achieve a better understanding of the pathogenesis of T2D and elucidate the cellular mechanisms that cause defects in the insulin pathway. This will be helpful in order to devise novel treatment strategies that can correct the multiple pathophysiologic defects of T2D, as well as its common and closely associat-

ed comorbid conditions, overweight and obesity [31, 232, 233].

Conflict of interest

The authors declare no conflict of interest.

References

1. Meigs JB. Epidemiology of type 2 diabetes and cardiovascular disease: translation from population to prevention: the Kelly West award lecture 2009. Diabetes Care 2010; 33: 1865-71.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-1053.
3. Khunti K, Stone MA, Bankart J, et al. Physical activity and sedentary behaviours of South Asian and white European children in inner city secondary schools in the UK. Fam Pract 2007; 24: 237-44.
4. Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA 2003; 289: 76-9.
5. Ford ES, Williamson DF, Liu S. Weight change and diabetes incidence: findings from a national cohort of US adults. Am J Epidemiol 1997; 146: 214-222.
6. Fagot-Campagna A, Narayan K. Type 2 diabetes in children. BMJ 2001; 322: 377-87.
7. Urbanavičius V, Abalikšta T, Brimas G, Abraitienė A, Gogelienė L, Strupas K. Comparison of changes in blood glucose, insulin resistance indices, and adipokine levels in diabetic and non-diabetic subjects with morbid obesity after laparoscopic adjustable gastric banding. Medicina (Kaunas) 2013; 49: 9-14.
8. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003; 52: 102-10.
9. Hanley AJ, Williams K, Stern MP, Haffner SM. Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease: the San Antonio Heart Study. Diabetes Care 2002; 25: 1177-84.
10. Meigs JB, Rutter MK, Sullivan LM, Fox CS, D'Agostino RB Sr, Wilson BW. Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome. Diabetes Care 2007; 30: 1219-25.
11. Bavenholm P, Proudler A, Tornvall P, et al. Insulin, intact and split proinsulin, and coronary artery disease in young men. Circulation 1995; 92: 1422-9.
12. Ergul A, Kelly-Cobbs A, Abdalla M, Fagan SC. Cerebrovascular complications of diabetes: focus on stroke. Endocr Metab Immune Disord Drug Targets 2012; 12: 148-58.
13. Schaper NC, Nabuurs-Franssen MH, Huijberts MS. Peripheral vascular disease and type 2 diabetes mellitus. Diabetes Metab Res Rev 2000; 16 (Suppl 1): S11-5.
14. Kasuga MF, Karlsson A, Kahn CR. Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor. Science 1982; 215: 185-6.
15. Sun XJ, Rothenberg P, Kahn CR, et al. Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 1991; 352: 73-7.
16. Skolnik EY, Lee CH, Batzer A, et al. The SH2/SH3 domain-containing protein GRB2 interacts with tyrosine-phosphorylated IRS1 and Shc: implications for insulin control of ras signalling. EMBO J 1993; 12: 1929-36.
17. Bryant NJ, Govers R, James DE. Regulated transport of the glucose transporter GLUT4. Nat Rev Mol Cell Biol 2002; 3: 267-77.
18. Cusi K, Maezono K, Osman A, et al. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest 2000; 105: 311-20.
19. Zeng G, Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by Wortmannin. Direct measurement in vascular endothelial cells. J Clin Invest 1996; 98: 894-8.
20. Carel K, Kummer JL, Schubert C, Leitner W, Heidenreich KA, Draznin B. Insulin stimulates mitogen-activated protein kinase by a Ras-independent pathway in 3T3-L1 adipocytes. J Biol Chem 1996; 271: 30625-30.
21. Currie RA, Walker KS, Gray A, et al. Role of phosphatidylinositol 3,4,5-trisphosphate in regulating the activity and localization of 3-phosphoinositide-dependent protein kinase-1. Biochem J 1999; 337: 575-83.
22. Vanhaesebroeck B, Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. Biochem J 2000; 346: 561-76.
23. Takano A, Usui I, Haruta T, et al. Mammalian target of rapamycin pathway regulates insulin signaling via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. Mol Cell Biol 2001; 21: 5050-62.
24. McGettrick AJ, Feener EP, Kahn CR. Human insulin receptor substrate-1 (IRS-1) polymorphism G972R causes IRS-1 to associate with the insulin receptor and inhibit receptor autophosphorylation. J Biol Chem 2005; 280: 6441-6.
25. van de Weijer T, Sparks LM, Phielix E, et al. Relationships between mitochondrial function and metabolic flexibility in type 2 diabetes mellitus. PLoS One 2013; 8: e51648.
26. Bajaj M, Prentki M, Joly E, El-Assaad W, Roduit R. Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in beta-cell adaptation and failure in the etiology of diabetes. Diabetes 2002; 51 (Suppl 3): S405.
27. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci 2013; 9: 191-200.
28. Virtanen KA, Lidell ME, Orava J, et al. Functional brown adipose tissue in healthy adults. N Engl J Med 2009; 360: 1518-25.
29. Wagenknecht LE, Langefeld CD, Scherzinger AL, et al. Insulin sensitivity, insulin secretion, and abdominal fat: the Insulin Resistance Atherosclerosis Study (IRAS) family study. Diabetes 2003; 52: 2490-6.
30. Zierath JR, Livingston JN, Thorne A, et al. Regional difference in insulin inhibition of non-esterified fatty acid release from human adipocytes: relation to insulin receptor phosphorylation and intracellular signalling through the insulin receptor substrate-1 pathway. Diabetologia 1998; 41: 1343-54.
31. Papaetis GS, Orphanidou D, Panagiotou TN. Thiazolidinediones and type 2 diabetes: from cellular targets to cardiovascular benefit. Curr Drug Targets 2011; 12: 1498-512.
32. Morino K, Petersen KF, Dufour S, et al. Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. J Clin Invest 2005; 115: 3587-93.
33. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993; 259: 87-91.

34. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 1997; 389: 610-4.
35. Stephens JM, Lee J, Pilch PF. Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. J Biol Chem 1997; 272: 971-6.
36. Guo L, Tabrizchi R. Peroxisome proliferator-activated receptor gamma as a drug target in the pathogenesis of insulin resistance. Pharmacol Ther 2006; 111: 145-73.
37. Steinberg GR, Michell BJ, van Denderen BJ, et al. Tumor necrosis factor alpha-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. Cell Metab 2006; 4: 465-74.
38. Yu C, Chen Y, Cline GW, et al. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J Biol Chem 2002; 277: 50230-6.
39. de Meijer VE, Le HD, Meisel JA, Sharma AK, Popov Y, Puder M. Tumor necrosis factor α-converting enzyme inhibition reverses hepatic steatosis and improves insulin sensitivity markers and surgical outcome in mice. PLoS One 2011; 6: e25587.
40. Hivert MF, Sullivan LM, Shrader P, et al. The association of tumor necrosis factor alpha receptor 2 and tumor necrosis factor alpha with insulin resistance and the influence of adipose tissue biomarkers in humans. Metabolism 2010; 59: 540-6.
41. Ursini F, Naty S, Grembiale RD. Infliximab and insulin resistance. Autoimmun Rev 2010; 9: 536-9.
42. Solomon DH, Massarotti E, Garg R, Liu J, Canning C, Schneeweiss S. Association between disease-modifying antirheumatic drugs and diabetes risk in patients with rheumatoid arthritis and psoriasis. JAMA 2011; 305: 2525-31.
43. Bernstein LE, Berry J, Kim S, Canavan B, Grinspoon SK. Effects of etanercept in patients with the metabolic syndrome. Arch Intern Med 2006; 166: 902-8.
44. Paquot N, Castillo MJ, Lefebvre PJ, Scheen AJ. No increased insulin sensitivity after a single intravenous administration of a recombinant human tumor necrosis factor receptor: Fc fusion protein in obese insulin-resistant patients. J Clin Endocrinol Metab 2000; 85: 1316-9.
45. Dominguez H, Storgaard H, Rask-Madsen C, et al. Metabolic and vascular effects of tumor necrosis factor-alpha blockade with etanercept in obese patients with type 2 diabetes. J Vasc Res 2005; 42: 517-25.
46. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab 1998; 83: 847-50.
47. Hrnciar J, Avdicova M, Gabor D, et al. Prevalence of metabolic syndrome, insulin resistance, and microvascular angina pectoris in 500 consecutive patients referred to coronarography. Endocr Regul 2013; 47: 33-8.
48. Kopp HP, Kopp CW, Festa A, et al. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. Arterioscler Thromb Vasc Biol 2003; 23: 1042-7.
49. Senn JJ, Klover PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes 2002; 51: 3391-9.
50. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor alpha, overexpressed in human fat cells from insulin resistant subjects. J Biol Chem 2003; 278: 45777-84.
51. Al-Khalili L, Bouzakri K, Glund S, Lonnqvist F, Koistinen HA, Krook A. Signaling specificity of interleukin-6 action on glucose and lipid metabolism in skeletal muscle. Mol Endocrinol 2006; 20: 3364-75.
52. Pedersen BK, Akerstrom TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. J Appl Physiol 2007; 103: 1093-8.
53. Wallenius K, Wallenius V, Sunter D, Dickson SL, Jansson JO. Intracerebroventricular interleukin-6 treatment decreases body fat in rats. Biochem Biophys Res Commun 2002; 293: 560-5.
54. Bastard JP, Lagathu C, Caron M, Capeau J. Point-counterpoint: interleukin-6 does/does not have a beneficial role in insulin sensitivity and glucose homeostasis. J Appl Physiol 2007; 102: 821-2.
55. Jager J, Gremeaux T, Cormont M, Marchand-Brustel Y, Tanti JF. Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. Endocrinology 2007; 148: 241-51.
56. Dinarello CA, Donath MY, Mandrup-Poulsen T. Role of IL-1beta in type 2 diabetes. Curr Opin Endocrinol Diabetes Obes 2010; 17: 314-21.
57. Dinarello CA. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin Nephrol 2007; 27: 98-114.
58. Netea MG, Joosten LA, Lewis E, et al. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med 2006; 12: 650-6.
59. Fischer CP, Perstrup LB, Berntsen A, Eskildsen P, Pedersen BK. Elevated plasma interleukin-18 is a marker of insulin-resistance in type 2 diabetic and non-diabetic humans. Clin Immunol 2005; 117: 152-60.
60. Gerhardt CC, Romero IA, Cancello R, Camoin L, Strosberg AD. Chemokines control fat accumulation and leptin secretion by cultured human adipocytes. Mol Cell Endocrinol 2001; 175: 81-92.
61. Kobashi C, Asamizu S, Ishiki M, et al. Inhibitory effect of IL-8 on insulin action in human adipocytes via MAP kinase pathway. J Inflamm (Lond) 2009; 6: 25.
62. Samaras K, Botelho NK, Chisholm DJ, Lord RV. Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. Obesity (Silver Spring) 2010; 18: 884-9.
63. Paraskevas KI, Liapis CD, Mikhailidis DP. Leptin: a promising therapeutic target with pleiotropic action besides body weight regulation. Curr Drug Targets 2006; 7: 761-71.
64. Minokoshi Y, Kim YB, Peroni OD, Fryer LGD, Müller C, Carling D. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002; 415: 339-43.
65. Chinookoswong N, Wang JL, Shi ZQ. Leptin restores euglycemia and normalizes glucose turnover in insulin-deficient diabetes in the rat. Diabetes 1999; 48: 1487-92.
66. Muzzin P, Eisensmith RC, Copeland KC, Woo SL. Correction of obesity and diabetes in genetically obese mice by leptin gene therapy. Proc Natl Acad Sci USA 1996; 93: 14804-8.
67. Ceddia RB, Koistinen HA, Zierath JR, Sweeney G. Analysis of paradoxical observations on the association between leptin and insulin resistance. FASEB J 2002; 16: 1163-76.
68. Askari H, Tykodi G, Liu J, Dagogo-Jack S. Fasting plasma leptin level is a surrogate measure of insulin sensitivity. J Clin Endocrinol Metab 2010; 95: 3836-43.
69. Rahmouni K, Morgan DA, Morgan GM, Mark AL, Haynes WG. Role of selective leptin resistance in diet-induced obesity hypertension. Diabetes 2005; 54: 2012-8.

70. Frühbeck G, Salvador J. Relation between leptin and the regulation of glucose metabolism. Diabetologia 2000; 43: 3-12.
71. Martin SS, Qasim A, Reilly MP. Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. J Am Coll Cardiol 2008; 52: 1201-10.
72. Hennige AM, Stefan N, Kapp K, et al. Leptin down-regulates insulin action through phosphorylation of serine-318 in insulin receptor substrate 1. FASEB J 2006; 20: 1206-8.
73. Howard JK, Flier JS. Attenuation of leptin and insulin signaling by SOCS proteins. Trends Endocrinol Metab 2006; 17: 365-71.
74. Patel L, Buckels AC, Kinghorn IJ, et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun 2003; 300: 472-6.
75. McTernan CL, McTernan PG, Harte AL, Levick PL, Barnett AH, Kumar S. Resistin, central obesity, and type 2 diabetes. Lancet 2002; 359: 46-7.
76. Junkin KA, Dyck DJ, Mullen KL, Chabowski A, Thrush AB. Resistin acutely impairs insulin-stimulated glucose transport in rodent muscle in the presence, but not absence, of palmitate. Am J Physiol Regul Integr Comp Physiol 2009; 296: R944-5.
77. Banerjee RR, Rangwala SM, Shapiro JS, et al. Regulation of fasted blood glucose by resistin. Science 2004; 303: 1195-8.
78. Qi Y, Nie Z, Lee YS, et al. Loss of resistin improves glucose homeostasis in leptin deficiency. Diabetes 2006; 55: 3083-90.
79. Barnes KM, Miner JL. Role of resistin in insulin sensitivity in rodents and humans. Curr Protein Pept Sci 2009; 10: 96-107.
80. Tzatsos A, Kandror KV. Nutrients suppress phosphatidylinositol 3-kinase/Akt signaling via raptor-dependent mTOR-mediated insulin receptor substrate 1 phosphorylation. Mol Cell Biol 2006; 26: 63-76.
81. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. J Immunol 2005; 174: 5789-95.
82. Graveleau C, Zaha VG, Mohajer A, et al. Mouse and human resistins impair glucose transport in primary mouse cardiomyocytes, and oligomerization is required for this biological action. J Biol Chem 2005; 280: 31679-85.
83. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Resistin gene expression in human adipocytes is not related to insulin resistance. Obes Res 2002; 10: 1-5.
84. Lee JH, Chan JL, Yiannakouris N, et al. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. J Clin Endocrinol Metab 2003; 88: 4848-56.
85. Utzschneider KM, Carr DB, Tong J, et al. Resistin is not associated with insulin sensitivity or the metabolic syndrome in humans. Diabetologia 2005; 48: 2330-3.
86. Park HK, Qatanani M, Briggs ER, Ahima RS, Lazar MA. Inflammatory induction of human resistin causes insulin resistance in endotoxemic mice. Diabetes 2011; 60: 775-83.
87. Janke J, Engeli S, Boschmann M, et al. Retinol-binding protein 4 in human obesity. Diabetes 2006; 55: 2805-10.
88. Yang Q, Graham TE, Mody N, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005; 436: 356-62.
89. Graham TE, Yang Q, Bluher M, et al. Retinol binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006; 354: 2552-63.
90. Stefan N, Hennige AM, Staiger H, et al. High circulating retinol-binding protein 4 is associated with elevated liver fat, but not with total-, subcutaneous-, visceral-, or intramyocellular fat in humans. Diabetes Care 2007; 30: 1173-8.
91. Haider DG, Schindler K, Prager G, et al. Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. J Clin Endocrinol Metab 2007; 92: 1168-71.
92. Lim S, Choi SH, Jeong IK, et al. Insulin-sensitizing effects of exercise on adiponectin and retinol binding protein-4 concentrations in young and middle-aged women. J Clin Endocrinol Metab 2008; 93: 2263-8.
93. Refai E, Dekki N, Yang SN, et al. Transthyretin constitutes a functional component in pancreatic beta-cell stimulus-secretion coupling. Proc Natl Acad Sci USA 2005; 102: 17020-5.
94. Henze A, Frey SK, Raila J, et al. Evidence that kidney function but not type 2 diabetes determines retinol-binding protein 4 serum levels. Diabetes 2008; 57: 3323-6.
95. Ribel-Madsen R, Friedrichsen M, Vaag A, Poulsen P. Retinol-binding protein 4 in twins: regulatory mechanisms and impact of circulating and tissue expression levels on insulin secretion and action. Diabetes 2009; 58: 54-60.
96. Broch M, Vendrell J, Ricart W, Richart C, Fernández-Real JM. Circulating retinol-binding protein-4, insulin sensitivity, insulin secretion, and insulin disposition index in obese and nonobese subjects. Diabetes Care 2007; 30: 1802-6.
97. von Eynatten M, Lepper PM, Liu D, et al. Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. Diabetologia 2007; 50: 1930-7.
98. Yao-Borengasser A, Varma V, Bodles AM, et al. Retinol binding protein 4 expression in humans: relationship to insulin resistance, inflammation, and response to pioglitazone. J Clin Endocrinol Metab 2007; 92: 2590-7.
99. Bruun JM, Lihn AS, Pedersen SB, Richelsen B. Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT. J Clin Endocrinol Metab 2005; 90: 2282-9.
100. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci USA 2003; 100: 7265-70.
101. Kouyama K, Miyake K, Zenibayashi M, et al. Association of serum MCP-1 concentration and MCP-1 polymorphism with insulin resistance in Japanese individuals with obese type 2 diabetes. Kobe J Med Sci 2008; 53: 345-54.
102. Inouye KE, Shi H, Howard JK, et al. Absence of CC chemokine ligand 2 does not limit obesity-associated infiltration of macrophages into adipose tissue. Diabetes 2007; 56: 2242-50.
103. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth W. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 2004; 145: 2273-82.
104. Festa A, D'Agostino R Jr, Mykkänen L, et al. Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of

glucose tolerance. The Insulin Resistance Atherosclerosis Study (IRAS). Arterioscler Thromb Vasc Biol 1999; 19: 562-8.
105. Potter van Loon BJ, Kluft C, Radder JK, Blankenstein MA, Meinders AE. The cardiovascular risk factor plasminogen activator inhibitor type 1 is related to insulin resistance. Metabolism 1993; 42: 945-9.
106. Lopez-Alemany R, Redondo JM, Nagamine Y, Munoz-Canoves P. Plasminogen activator inhibitor type-1 inhibits insulin signaling by competing with alphavbeta3 integrin for vitronectin binding. Eur J Biochem 2003; 270: 814-21.
107. Lijnen HR, Alessi MC, Van Hoef B, Collen D, Juhan-Vague I. On the role of plasminogen activator inhibitor-1 in adipose tissue development and insulin resistance in mice. J Thromb Haemost 2005; 3: 1174-9.
108. Festa A, D'Agostino R Jr, Tracy RP, Haffner SM. Insulin Resistance Atherosclerosis Study. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes 2002; 51: 1131-7.
109. Thogersen AM, Jansson JH, Boman K, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first myocardial infarction in both men and women. Circulation 1998; 98: 2241-7.
110. Alessi MC, Juhan-Vague I. Contribution of PAI-1 in cardiovascular pathology. Arch Mal Coeur Vaiss 2004; 97: 673-8.
111. Yang RZ, Lee MJ, Hu H, et al. Acute-phase serum amyloid A: an inflammatory adipokine and potential link between obesity and its metabolic complications. PLoS Med 2006; 3: e287.
112. Sjöholm K, Palming J, Olofsson LE, et al. A microarray search for genes predominantly expressed in human omental adipocytes: adipose tissue as a major production site of serum amyloid A. J Clin Endocrinol Metab 2005; 90: 2233-9.
113. Ye XY, Xue YM, Sha JP, Li CZ, Zhen ZJ. Serum amyloid A attenuates cellular insulin sensitivity by increasing JNK activity in 3T3-L1 adipocytes. J Endocrinol Invest 2009; 32: 568-75.
114. Badolato R, Wang JM, Murphy WJ, et al. Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. J Exp Med 1994; 180: 203-9.
115. Patel H, Fellowes R, Coade S, Woo P. Human serum amyloid A has cytokine-like properties. Scand J Immunol 1998; 48: 410-418.
116. Jylhava J, Haarala A, Eklund C, et al. Serum amyloid A is independently associated with metabolic risk factors but not with early atherosclerosis: the Cardiovascular Risk in Young Finns Study. J Intern Med 2009; 266: 286-95.
117. Scheja L, Heese B, Zitzer H, et al. Acute-phase serum amyloid A as a marker of insulin resistance in mice. Exp Diabetes Res 2008; 2008: 230837.
118. Luscher TF, Barton M. Endothelins and endothelin receptor antagonists: therapeutic considerations for a novel class of cardiovascular drugs. Circulation 2000; 102: 2434-40.
119. Shih KC, Kwok CF, Ho LT. Combined use of insulin and endothelin-1 causes decrease of protein expression of beta-subunit of insulin receptor, insulin receptor substrate-1, and insulin-stimulated glucose uptake in rat adipocytes. J Cell Biochem 2000; 78: 231-40.
120. Jiang ZY, Zhou QL, Chatterjee A, et al. Endothelin-1 modulates insulin signaling through phosphatidylinositol 3-kinase pathway in vascular smooth muscle cells. Diabetes 1999; 48: 1120-30.
121. Ishibashi KI, Imamura T, Sharma PM, Huang J, Ugi S, Olefsky JM. Chronic endothelin-1 treatment leads to heterologous desensitization of insulin signaling in 3T3–L1 adipocytes. J Clin Invest 2001; 107: 1193-202.
122. Hauner H, Petruschke T, Gries FA. Endothelin-1 inhibits the adipose differentiation of cultured human adipocyte precursor cells. Metabolism 1994; 43: 227-32.
123. Mangiafico RA, Malatino LS, Santonocito M, Spada RS. Plasma endothelin-1 concentrations in non-insulin-dependent diabetes mellitus and nondiabetic patients with chronic arterial obstructive disease of the lower limbs. Int Angiol 1998; 17: 97-102.
124. Caballero AE, Arora S, Saouaf R, et al. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. Diabetes 1999; 48: 1856-62.
125. Sarafidis PA, Bakris GL. Insulin and endothelin: an interplay contributing to hypertension development? J Clin Endocrinol Metab 2007; 92: 379-85.
126. Piatti PM, Monti LD, Conti M, et al. Hypertriglyceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans. Diabetes 1996; 45: 316-21.
127. van Harmelen V, Eriksson A, Aström G, Wåhlén K, Näslund E, Karpe F. Vascular peptide endothelin-1 links fat accumulation with alterations of visceral adipocyte lipolysis. Diabetes 2008; 57: 378-86.
128. Mathai ML, Chen N, Cornall L, Weisinger RS. The role of angiotensin in obesity and metabolic disease. Endocr Metab Immune Disord Drug Targets 2011; 11: 198-205.
129. Giacchetti G, Faloia E, Mariniello B, et al. Overexpression of the renin-angiotensin system in human visceral adipose tissue in normal and overweight subjects. Am J Hypertens 2002; 15: 381-8.
130. Massiéra F, Bloch-Faure M, Ceiler D, et al. Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. FASEB J 2001; 15: 2727-9.
131. Tomono Y, Iwai M, Inaba S, Mogi M, Horiuchi M. Blockade of AT1 receptor improves adipocyte differentiation in atherosclerotic and diabetic models. Am J Hypertens 2008; 21: 206-12.
132. Saint-Marc P, Kozak LP, Ailhaud G, Darimont C, Negrel R. Angiotensin II as a trophic factor of white adipose tissue: stimulation of adipose cell formation. Endocrinology 2001; 142: 487-92.
133. Ferder L, Inserra F, Martínez-Maldonado M. Inflammation and the metabolic syndrome: role of angiotensin II and oxidative stress. Curr Hypertens Rep 2006; 8: 191-8.
134. Folli F, Kahn CR, Hansen H, Bouchie JL, Feener EP. Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. J Clin Invest 1997; 100: 2158-69.
135. Iwai M, Chen R, Imura Y, Horiuchi M. TAK-536, a new AT1 receptor blocker, improves glucose intolerance and adipocyte differentiation. Am J Hypertens 2007; 20: 579-86.
136. Golovchenko I, Goalstone ML, Watson P, Brownlee M, Draznin B. Hyperinsulnemia enhances transcriptional activity of nuclear factor-kappaB induced by angiotensin II, hyperglycemia, and advanced glycosylation

end products in vascular smooth muscle cells. Circ Res 2000; 87: 746-62.
137. Perkins JM, Davis SN. The renin-angiotensin-aldosterone system: a pivotal role in insulin sensitivity and glycemic control. Curr Opin Endocrinol Diabetes Obes 2008; 15: 147-52.
138. Kalupahana NS, Massiera F, Quignard-Boulange A, et al. Overproduction of angiotensinogen from adipose tissue induces adipose inflammation, glucose intolerance, and insulin resistance. Obesity (Silver Spring) 2012; 20: 48-56.
139. Napoli C, Paolisso G, Casamassimi A, et al. Effects of nitric oxide on cell proliferation: novel insights. J Am Coll Cardiol 2013; 62: 89-95.
140. Chatterjee A, Black SM, Catravas JD. Endothelial nitric oxide (NO) and its pathophysiologic regulation. Vascul Pharmacol 2008; 49: 134-40.
141. Ryden M, Elizalde M, van Harmelen V, et al. Increased expression of eNOS protein in omental versus subcutaneous adipose tissue in obese human subjects. Int J Obes Relat Metab Disord 2001; 25: 811-5.
142. Xue HM, Yu CM, Underwood MJ, Huang JH, Yang Q. AVE3085 protects coronary endothelium from the impairment of asymmetric dimethylarginine by activation and recoupling of eNOS. Cardiovasc Drugs Ther 2012; 26: 383-92.
143. Mills CD. M1 and M2 macrophages: oracles of health and disease. Crit Rev Immunol 2012; 32: 463-88.
144. Charbonneau A, Marette A. Inducible nitric oxide synthase induction underlies lipid-induced hepatic insulin resistance in mice: potential role of tyrosine nitration of insulin signaling proteins. Diabetes 2010; 59: 861-71.
145. Tsuchiya K, Sakai H, Suzuki N, et al. Chronic blockade of nitric oxide synthesis reduces adiposity and improves insulin resistance in high fat-induced obese mice. Endocrinology 2007; 148: 4548-56.
146. Zernecke A, Bernhagen J, Weber C. Macrophage migration inhibitory factor in cardiovascular disease. Circulation 2008; 117: 1594-602.
147. Kleemann R, Bucala R. Macrophage migration inhibitory factor: critical role in obesity, insulin resistance, and associated comorbidities. Mediators Inflamm 2010; 2010: 610479.
148. Hristova M, Aloe L. Metabolic syndrome-neurotrophic hypothesis. Med Hypotheses 2006; 66: 545-9.
149. Bullo M, Peeraully MR, Trayhurn P, Folch J, Salas-Salvadó J. Circulating nerve growth factor levels in relation to obesity and the metabolic syndrome in women. Eur J Endocrinol 2007: 157: 303-10.
150. Murray I, Kôhl J, Cianflone K. Acylation-stimulating protein (ASP): structure-function determinants of cell surface binding and triacylglycerol synthetic activity. Biochem J 1999; 342: 41-8.
151. MacLaren RE, Cui W, Lu HL, Simard S, Cianflone K. Association of adipocyte genes with ASP expression: a microarray analysis of subcutaneous and omental adipose tissue in morbidly obese subjects. BMC Med Genomics 2010; 3: 3.
152. Xita N, Tsatsoulis A. Adiponectin in diabetes mellitus. Curr Med Chem 2012; 19: 5451-8.
153. Athyros VG, Tziomalos K, Karagiannis A, Anagnosits P, Mikhailidis GP. Should adipokines be considered in the choice of the treatment of obesity-related health problems? Curr Drug Targets 2010; 11: 122-35.
154. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002; 8: 1288-95.
155. Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J Clin Invest 2001; 108: 1875-81.
156. Wang Y, Lam KS, Yau MH, Xu A. Post-translational modifications of adiponectin: mechanisms and functional implications. Biochem J 2008; 409: 623-33.
157. Yang WS, Lee WJ, Funahashi T, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 2001; 86: 3815-9.
158. Ajuwon KM, Spurlock ME. Adiponectin inhibits LPS-induced NF-kappaB activation and IL-6 production and increases PPARgamma2 expression in adipocytes. Am J Physiol Regul Integr Comp Physiol 2005; 288: R12200-25.
159. Wang C, Mao X, Wang L, et al. Adiponectin sensitizes insulin signaling by reducing p70 S6 kinase-mediated serine phosphorylation of IRS-1. J Biol Chem 2007; 282: 7991-6.
160. Yamauchi T, Nio Y, Maki T, et al. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med 2007; 13: 332-9.
161. Fu Y, Luo N, Klein RL, Garvey WT. Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation. J Lipid Res 2005; 46: 1369-79.
162. Okamoto M, Ohara-Imaizumi M, Kubota N, et al. Adiponectin induces insulin secretion in vitro and in vivo at a low glucose concentration. Diabetologia 2008; 51: 827-35.
163. Kumada M, Kihara S, Sumitsuji S, et al. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003; 23: 85-9.
164. Daimon M, Oizumi T, Saitoh T, et al. Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese population: the Funagata study. Diabetes Care 2003; 26: 2015-20.
165. Snehalatha C, Mukesh B, Simon M, Viswanathan V, Haffner SM, Ramachandran A. Plasma adiponectin is an independent predictor of type 2 diabetes in Asian Indians. Diabetes Care 2003; 26: 3226-9.
166. Snijder MB, Heine RJ, Seidell JC, et al. Associations of adiponectin levels with incident impaired glucose metabolism and type 2 diabetes in older men and women: the Hoorn study. Diabetes Care 2006; 29: 2498-503.
167. Mather KJ, Funahashi T, Matsuzawa Y, et al. Adiponectin, change in adiponectin, and progression to diabetes in the diabetes prevention program. Diabetes 2008; 57: 980-6.
168. Mente A, Razak F, Blankenberg S, et al. Ethnic variation in adiponectin and leptin levels and their association with adiposity and insulin resistance. Diabetes Care 2010; 33: 1629-34.
169. de Luis DA, Aller R, Gonzalez Sagrado M, Conde R, Izaola O, de la Fuente B. Serum visfatin levels and metabolic syndrome criteria in obese female subjects. Diabetes Metab Res Rev 2013; 29: 576-81.
170. Fukuhara A, Matsuda M, Nishizawa M, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 2005; 307: 426-30.
171. Revollo JR, Körner A, Mills KF, et al. Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. Cell Metab 2007; 6: 363-75.

172. Chen MP, Chung FM, Chang DM, et al. Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 2006; 91: 295-9.
173. Haider DG, Schindler K, Schaller G, et al. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. J Clin Endocrinol Metab 2006; 91: 1578-81.
174. Li L, Yang G, Li Q, et al. Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. Exp Clin Endocrinol Diabetes 2006; 11: 544-8.
175. Brema I, Hatunic M, Finucane F, et al. Plasma visfatin is reduced after aerobic exercise in early onset type 2 diabetes mellitus. Diabetes Obes Metab 2008; 10: 600-2.
176. Eyileten T, Sonmez A, Saglam M, et al. Effect of renin-angiotensin-aldosterone system (RAAS) blockade on visfatin levels in diabetic nephropathy. Nephrology (Carlton) 2010; 15: 225-9.
177. Kadoglou NP, Sailer N, Moumtzouoglou A, et al. Visfatin (nampt) and ghrelin as novel markers of carotid atherosclerosis in patients with type 2 diabetes. Exp Clin Endocrinol Diabetes 2010; 118: 75-80.
178. Berndt J, Klöting N, Kralisch S, et al. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. Diabetes 2005; 54: 2911-6.
179. Pagano C, Pilon C, Olivieri M, et al. Reduced plasma visfatin/pre-B cell colony-enhancing factor in obesity is not related to insulin resistance in humans. J Clin Endocrinol Metab 2006; 91: 3165-70.
180. Oki K, Yamane K, Kamei N, Nojima H, Kohno N. Circulating visfatin level is correlated with inflammation, but not with insulin resistance. Clin Endocrinol (Oxf) 2007; 67: 796-800.
181. Ko BJ, Lee M, Park HS, et al. Elevated vaspin and leptin levels are associated with obesity in prepubertal Korean children. Endocr J 2013; 60: 609-16.
182. Hida K, Wada J, Eguchi J, et al. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci USA 2005; 102: 10610-5.
183. Wada J. Vaspin: a novel serpin with insulin-sensitising effects. Expert Opin Investig Drugs 2008; 17: 327-33.
184. Youn BS, Klöting N, Kratzsch J, et al. Serum vaspin concentrations in human obesity and type 2 diabetes. Diabetes 2008; 57: 372-7.
185. Klöting N, Berndt J, Kralisch S, et al. Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. Biochem Biophys Res Commun 2006; 339: 430-6.
186. Tan BK, Heutling D, Chen J, et al. Metformin decreases the adipokine vaspin in overweight women with polycystic ovary syndrome concomitant with improvement in insulin sensitivity and a decrease in insulin resistance. Diabetes 2008; 57: 1501-7.
187. Yang R, Lee MJ, Hu H, et al. Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. Am J Physiol Endocrinol Metab 2006; 290: E1253-61.
188. Lee JK, Schnee J, Pang M, et al. Human homologs of the Xenopus oocyte cortical granule lectin XL35. Glycobiology 2001; 11: 65-73.
189. Fain JN, Sacks HS, Buehrer B, et al. Identification of omentin-1 mRNA in human epicardial adipose tissue: comparison to omentin-1 in subcutaneous, internal mammary artery periadventitial and visceral abdominal depots. Int J Obes (Lond) 2008; 32: 810-5.
190. de Souza Batista CM, Yang RZ, Lee MJ, et al. Omentin plasma levels and gene expression are decreased in obesity. Diabetes 2007; 56: 1655-61.
191. Moreno-Navarrete JM, Catalán V, Ortega F, et al. Circulating omentin concentration increases after weight loss. Nutr Metab (Lond) 2010; 7: 27.
192. Wurm S, Neumeier M, Weigert J, Schäffler A, Buechler C. Plasma levels of leptin, omentin, collagenous repeat-containing sequence of 26-kDa protein (CORS-26) and adiponectin before and after oral glucose uptake in slim adults. Cardiovasc Diabetol 2007; 6: 7-13.
193. Dray C, Knauf C, Daviaud D, et al. Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. Cell Metab 2008; 8: 437-45.
194. Erdem G, Dogru T, Tasci I, Sonmez A, Tapan S. Low plasma apelin levels in newly diagnosed type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2008; 116: 289-92.
195. Dray C, Debard C, Jager J, et al. Apelin and APJ regulation in adipose tissue and skeletal muscle of type 2 diabetic mice and humans. Am J Physiol Endocrinol Metab 2010; 298: E1161-9.
196. Wittamer V, Franssen JD, Vulcano M, et al. Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. J Exp Med 2003; 198: 977-85.
197. Bozaoglu K, Bolton K, McMillan J, et al. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinology 2007; 148: 4687-94.
198. Goralski KB, McCarthy TC, Hanniman EA, et al. Chemerin: a novel adipokine that regulates adipogenesis and adipocyte metabolism. J Biol Chem 2007; 282: 28175-88.
199. Takahashia M, Takahashi Y, Takahashi K, et al. Chemerin enhances insulin signaling and potentiates insulin-stimulated glucose uptake in 3T3-L1 adipocytes. FEBS Letters 2008; 582: 573-8.
200. Sell H, Laurencikiene J, Taube A, et al. Chemerin is a novel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. Diabetes 2009; 58: 2731-40.
201. Santomauro ATMG, Boden G, Silva M, et al. Overnight lowering of free fatty acids with acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. Diabetes 1999; 48: 1836-41.
202. Il'yasova D, Wang F, D'Agostino RB Jr, Hanley A, Wagenknecht LE. Prospective association between fasting NEFA and type 2 diabetes: impact of post-load glucose. Diabetologia 2010; 53: 866-74.
203. Abdul-Ghani MA, Ubhayasekera SJ, Staaf J, Forslund A, Bergsten P, Bergquist J. Free fatty acid determination in plasma by GC-MS after conversion to Weinreb amides. Anal Bioanal Chem 2013; 405: 1929-35.
204. Hansen D, Dendale P, Beelen M, et al. Plasma adipokine and inflammatory marker concentrations are altered in obese, as opposed to non-obese, type 2 diabetes patients. Eur J Appl Physiol 2010; 109: 397-404.
205. Kashyap S, Belfort R, Berria R, et al. Discordant effects of a chronic physiological increase in plasma FFA on insulin signaling in healthy subjects with or without a family history of type 2 diabetes. Am J Physiol Endocrinol Metab 2004; 287: E537-46.
206. Gao Z, Zhang X, Zuberi A, et al. Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3-L1 adipocytes. Mol Endocrinol 2004; 18: 2024-34.

207. Nguyen MT, Favelyukis S, Nguyen AK, et al. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J Biol Chem 2007; 282: 35279-92.
208. Summers SA. Ceramides in insulin resistance and lipotoxicity. Prog Lipid Res 2006; 45: 42-72.
209. Steinberg HO, Tarshoby M, Monestel R, et al. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. J Clin Invest 1997; 100: 1230-9.
210. Cerosismo E, DeFronzo RA. Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. Diab Metab Res Rev 2006; 22: 423-36.
211. Patti ME, Butte AJ, Crunkhorn S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. Proc Natl Acad Sci USA 2003; 100: 8466-71.
212. Abdul-Ghani MA, DeFronzo RA. Mitochondrial dysfunction, insulin resistance, and type 2 diabetes mellitus. Curr Diab Rep 2008; 8: 173-8.
213. Armoni M, Harel C, Bar-Yoseph F, Milo S, Karnieli E. Free fatty acids repress the Glut4 gene expression in cardiac muscle via novel response elements. J Biol Chem 2005; 280: 34786-95.
214. Ismail NA, Ragab S, El Dayem SM, et al. Fetuin-A levels in obesity: differences in relation to metabolic syndrome and correlation with clinical and laboratory variables. Arch Med Sci 2012; 8: 826-33.
215. Graham TE, Wason CJ, Blüher M, Kahn BB. Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. Diabetologia 2007; 50: 814-23.
216. El-Mesallamy HO, Kassem DH, El-Demerdash E, Amin AI. Vaspin and visfatin/Nampt are interesting interrelated adipokines playing a role in the pathogenesis of type 2 diabetes mellitus. Metabolism 2011; 60: 63-70.
217. Stępień M, Wlazeł RN, Paradowski M, et al. Serum concentrations of adiponectin, leptin, resistin, ghrelin and insulin and their association with obesity indices in obese normo- and hypertensive patients – pilot study. Arch Med Sci 2012; 8: 431-6.
218. American Diabetes Association. Standards of Medical Care in Diabetes-2009. Diabetes Care 2009; 32 (Suppl 1): S13-61.
219. Ertek S, Cicero A. Impact of physical activity on inflammation: effects on cardiovascular disease risk and other inflammatory conditions. Arch Med Sci 2012; 8: 794-804.
220. Rucker D, Padwal R, Li SK, Curioni C, Lau DC. Long term pharmacotherapy for obesity and overweight: updated meta-analysis. BMJ 2007; 335: 1194-9.
221. Golay A. Metformin and body weight. Int J Obes 2008; 32: 61-72.
222. Krakoff J, Clark JM, Crandall JP, et al. Effects of metformin and weight loss on serum alanine aminotransferase activity in the diabetes prevention program. Obesity (Silver Spring) 2010; 18: 1762-7.
223. Davidson JA. Advances in therapy for type 2 diabetes: GLP-1 receptor agonists and DPP-4 inhibitors. Cleve Clin J Med 2009; 76 (Suppl 5): S28-38.
224. Shyangdan DS, Royle PL, Clar C, Sharma P, Waugh NR. Glucagon-like peptide analogues for type 2 diabetes mellitus: systematic review and meta-analysis. BMC Endocr Disord 2010; 10: 20.
225. Abdul-Ghani MA, Norton L, Defronzo RA. Role of sodium-glucose cotransporter 2 (SGLT 2) inhibitors in the treatment of type 2 diabetes. Endocr Rev 2011; 32: 515-31.
226. Kim Y, Babu AR. Clinical potential of sodium-glucose cotransporter 2 inhibitors in the management of type 2 diabetes. Diabetes Metab Syndr Obes 2012; 5: 313-27.
227. Gill RS, Sharma AM, Al-Adra DP, Birch DW, Karmali S. The impact of bariatric surgery in patients with type-2 diabetes mellitus. Curr Diabetes Rev 2011; 7: 185-9.
228. Lautz D, Halperin F, Goebel-Fabbri A, Goldfine AB. The great debate: medicine or surgery: what is best for the patient with type 2 diabetes? Diabetes Care 2011; 34: 763-70.
229. Iaconelli A, Panunzi S, De Gaetano A, et al. Effects of bilio-pancreatic diversion on diabetes complications: a 10-year follow-up. Diabetes Care 2011; 34: 561-7.
230. Sjöström L, Peltonen M, Jacobson P, et al. Bariatric surgery and long-term cardiovascular events. JAMA 2012; 307: 56-65.
231. Dardeno TA, Chou SH, Moon HS, Chamberland JP, Fiorenza CG, Mantzoros CS. Leptin in human physiology and therapeutics. Front Neuroendocrinol 2010; 31: 377-93.
232. DeFronzo RA. Current issues in the treatment of type 2 diabetes. Overview of newer agents: where treatment is going. Am J Med 2010; 123 (3 Suppl): S38-48.
233. Stolar MW, Hoogwerf BJ, Gorshow SM, Boyle PJ, Wales DO. Managing type 2 diabetes: going beyond glycemic control. J Manag Care Pharm 2008; 14 (5 Suppl B): s2-19.