Vascular Pharmacology 57 (2012) 91–97

Contents lists available at SciVerse ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Review

From excess adiposity to insulin resistance: The role of free fatty acids

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ARTICLE INFO

Article history:
Received 6 March 2012
Received in revised form 1 May 2012
Accepted 8 May 2012

Keywords:
Insulin resistance
FFA
Metabolic syndrome
Obesity
Diabetes
Cytokines

ABSTRACT

With a positive caloric balance, adipocytes undergo excessive hypertrophy, which causes adipocyte dysfunction, as well as adipose tissue endocrine and immune responses. A preferential site of fat accumulation is the abdominal-perivisceral region, due to peculiar factors of the adipose tissue in such sites, namely an excess of glucocorticoid activity, which promotes the accumulation of fat; and the greater metabolic activity and sensitivity to lipolysis, due to increased number and activity of β3-adrenoceptors and, partly, to reduced activity of α2-adrenoceptors. As a consequence, more free fatty acids (FFA) are released into the portal system. Hypertrophic adipocytes begin to secrete low levels of TNF-α, which stimulate preadipocytes and endothelial cells to produce MCP-1, in turn responsible for attracting macrophages to the adipose tissue, thus developing a state of chronic low-grade inflammation which is causally linked to insulin resistance.
Excess of circulating FFA, TNF-α and other factors induces insulin resistance. FFA cause insulin resistance by inhibiting insulin signaling through the activation of serin-kinases, i.e. protein kinase C-Θ, and the kinases JNK and IKK, which promote a mechanism of serine phosphorylation of Insulin Receptor Substrates (IRS), leading to interruption of the downstream insulin receptor (IR) signaling. TNF-α, secreted by hypertrophic adipocytes and adipose tissue macrophages, also inhibits IR signaling by a double mechanism of serine-phosphorylation and tyrosine-dephosphorylation of IRS-1, causing inactivation and degradation of IRS-1 and a consequent stop of IR signaling.
Such mechanisms explain the transition from excess adiposity to insulin resistance, key to the further development of type 2 diabetes.

Contents

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doi:10.1016/j.vph.2012.05.003

1. Introduction

Obesity is an increasing health problem worldwide, and appears to be closely associated with several metabolic diseases. In the United States alone, it is estimated that approximately 66% of all adults are overweight and approximately 32% are obese (Ogden et al., 2006). In Western countries, obesity is strongly interrelated with common acquired factors such as a sedentary life style and aging (Mokdad et al., 2003; Reaven, 2005; Hamilton et al., 2007).

The most frequent pathologic condition associated with excess body fat and particularly with visceral obesity is known as the "Metabolic Syndrome" (MetS) (Avogaro et al., 1967; Kim and Reaven, 2004; Eckel et al., 2005; Reaven, 2005; Cornier et al., 2008; Giorgino, 2009), a constellation of symptoms, signs and pathophysiological conditions including visceral obesity, insulin resistance, impaired glucose metabolism and type 2 diabetes mellitus (DM), as well as atherogenic dyslipidemia, elevated blood pressure, and other comorbidities including a prothrombotic and proinflammatory state and nonalcoholic fatty liver disease. All these conditions independently increase the risk of atherosclerotic diseases, such as ischemic heart disease and stroke (DeFronzo et al., 1983; Ferrannini et al., 1983; Kelley et al., 1993; Berg and Scherer, 2005; Bays and Ballantyne, 2006; Schenk et al., 2008; Madonna and De Caterina, 2012).

Among metabolic derangements induced by excess fat accumulation, insulin resistance appears to be the most important metabolic consequence of fat accumulation, leading in most cases to type 2 DM. Insulin resistance (IR) is defined as an inadequate response by insulin target tissues – such as skeletal muscle, liver, and adipose tissue – to the physiologic effects of circulating insulin. The hallmarks of impaired insulin sensitivity in these three tissues are decreased insulin-stimulated glucose uptake into the skeletal muscle, impaired insulin-mediated inhibition of hepatic glucose production in the liver, and a reduced ability of insulin to inhibit lipolysis in the adipose tissue.

The primary cause of insulin resistance and DM is the increase in fat mass and obesity, particularly the intra-abdominal fat accumulation (Fujioka et al., 1987). We will here (a) discuss the mechanisms of adipogenesis and fat accumulation; and (b) the consequences of increased lipolysis and excess of circulating free fatty acids (FFA) on insulin receptor (IR) activity.

2. Adipogenesis and fat accumulation

The adipose tissue is composed primarily of adipocytes, which represent the majority of the adipose tissue cellular content. Adipocytes are surrounded by fibrous connective tissue, collagen, nerves and blood vessels. The framework that supports the adipose tissue contains the "stromal vascular fraction" cells, which include mesenchymal stem cells, fibroblasts, preadipocytes, endothelial precursor cells, smooth muscle cells, blood cells and immune cells (Poirier et al., 2006; Madonna and De Caterina, 2008; Bays, 2011). The adipose tissue mesenchymal stem cells, which derive from the mesoderm, can differentiate into skeletal myoblast, osteoblast, chondroblasts, tenoblasts, marrow stromal cells, neuron-like cells, and importantly, into cardiomyocytes, angiocytes, and adipocytes. Due to the common embryonic origin, visceral fat located around the vessels and in the epicardial pericardial region shares the same metabolic and inflammatory characteristics of the intra-abdominal fat (Zuk et al., 2002; Kode et al., 2009; Madonna and De Caterina, 2010). Contrary to the previous belief that adipogenesis ceases early in the life, with a fixed number of adipocytes after birth, fat cells experience a dynamic turnover, by which mesenchymal cells undergo lineage commitment, pre-adipocyte proliferation and terminal differentiation into mature adipocytes (Bays, 2011). Approximately 10% of fat cells are renewed annually at the adult stage and at all levels of body mass index (Spalding et al., 2008).

During a positive caloric balance, adipocytes normally undergo initial hypertrophy, which elicits cellular signaling for the recruitment, proliferation and differentiation of new fat cells. If new adipogenesis from preadipocytes is impaired, as is the case in the MetS, the lack of excess energy storage may cause existing fat cells to undergo excessive hypertrophy, causing adipocyte dysfunction, the production of pathogenic adipocytes, and adipose tissue endocrine and immune responses (Bays et al., 2008). Therefore, a failure of subcutaneous fat cell proliferation or differentiation results in excessive hypertrophy and dysfunction of fat cells, and in a consequent ectopic fat storage, i.e., intra-abdominal, perimuscular, perivascular, pericardial, and periosteal fat accumulation (Danforth, 2000; Ravussin and Smith, 2002; Pasarica et al., 2009). Pericardial and perivascular adipose tissue may have direct pathogenic effects on the myocardium, coronary arteries and peripheral vessels, via dysregulated local secretion of vasoactive and inflammatory factors that may contribute to atheroma instability and other cardiovascular disease.

3. Visceral fat accumulation

By definition, the MetS is primarily characterized by the accumulation of adipose tissue in the abdominal region. The visceral adipose tissue (VAT) is localized primarily as intra-abdominal depots around the intestine, the mesentery, the omentum and peri-renal areas, and drains directly to the liver through the portal circulation. Although the complex pathophysiology of VAT has not been completely elucidated, it is known that VAT adipocytes are more metabolically active, more sensitive to lipolysis and more insulin-resistant than the subcutaneous adipose tissue (SCAT). Conversely, SCAT is more avid in the absorption of circulating free fatty acids (FFA), in triglyceride synthesis and in the storage of lipids in fat cells (Martin and Jensen, 1991; Ibrahim, 2010). VAT metabolic activity is regulated by its peculiar physiochemical components, i.e., the presence of a greater number of glucocorticoid receptors and β-adrenoceptors, and a lower number of insulin receptors (IR).

3.1. Glucocorticoid receptors

Adipose tissue accumulation is controlled by steroid hormones. Glucocorticoids promote the accumulation of adipose tissue in the intra-abdominal depots (Pedersen, 2005). Accordingly, VAT was demonstrated to undergo relative and absolute accumulation due to a fourfold increase in glucocorticoid receptors in VAT compared with SCAT (Pedersen, 2005). Progesterone acts as a glucocorticoid receptor antagonist, and blocks the effects of glucocorticoids in the adipose tissue. These data suggests that progesterone in premenopausal women might protect against cortisol-induced intra-abdominal fat accumulation. Thus, men and post-menopausal women, who normally have low progesterone level, might experience the full-blown cortisol effect on intra-abdominal fat accumulation, and therefore tend to accumulate a larger proportion of their fat intra-abdominally. The increase in VAT is also attributable to the glucocorticoid-induced increase in appetite, as well as to a glucocorticoid-dependent increase in adipocyte differentiation and decrease in adipocyte proliferation, both of which promote adipocyte hypertrophy (Tomlinson et al., 2004). Due to these characteristics, VAT mass appears to be prone to very large increase, and VAT adipocytes to possibly become very hypertrophic (Rebuffe-Scrive et al., 1988).

3.2. β_3 and α_2-adrenoceptors

The process of fat mobilization from adipocytes consists of the hydrolysis of triacyl-glycerol stored in the adipocyte, to release non-esterified fatty acids (NEFA or FFA) into the circulation. The key

enzyme is here an intracellular lipase, the hormone sensitive lipase (HSL), sensitive to catecholamines. Catecholamines have dual effects on the lipolysis rate, both accelerating — through β-adrenoceptors — and retarding lipolysis — through α_2-adrenoceptors. In men and women, the lipolytic response to noradrenaline, which acts through α_2- and β-adrenoceptors, is more marked in the visceral than in the gluteal or femoral fat (Krotkiewski et al., 1983). Lonnqvist et al. (1995) found that the visceral fat cells from obese subjects were highly responsive to noradrenaline stimulation, which strongly enhances lipolytic response. The main finding was the markedly augmented β_3-adrenoceptor sensitivity and coupling efficiency in visceral adipocytes. The authors suggested that this enhanced β_3-adrenoreceptor activity of VAT was due to an increased receptor number in obese subjects. Therefore, the elevated rate of lipolysis in visceral fat cells appears to be largely due to increased number and activity of β_3-adrenoceptors and, partly, to a reduced activity of α_2-adrenoceptors. As a consequence, more FFA are released into the portal system in obesity.

3.3. Insulin receptors

The density of IR in VAT is lower than in SCAT, and this makes the abdominal visceral adipose tissue more sensitive to lipolytic stimuli and less sensitive to the inhibitory action of insulin than SCAT (Kopelman and Albon, 1997). Insulin inhibits lipolysis preferentially in the more insulin sensitive subcutaneous adipocytes, thus leaving visceral fat more exposed to the action of catecholamines (Rebuffe-Scrive et al., 1989). Other factors, however, intervene in the metabolic dysfunction of VAT IR, e.g., the chronic elevation of FFA and inflammatory adipocytokines, particularly TNF-α.

An increase of FFA level in VAT, primarily due to excess of lipolysis promoted by the enhanced β_3-adrenoreceptors activity, appears to play a crucial role in the initial phases of insulin receptor dysfunction and insulin resistance primarily in VAT. We will now describe first the adipose tissue accumulation and its low grade inflammation, then the IR structure and function and the effects of FFA and TNF-α on IR activity.

4. Lipid storage and low-grade inflammation of adipose tissue

By definition, the obese adipose tissue is characterized by inflammation and progressive infiltration by macrophages as obesity develops.

Besides its main function of lipid storage, the white adipose tissue has a major endocrine function, secreting several hormones, notably leptin, adiponectin and monocyte chemoattractant protein-1 (MCP-1); and a diverse range of other protein factors, which have been called adipocytokines or adipokines. The adipokinome includes proteins involved in lipid metabolism, insulin sensitivity, the complement system, blood pressure and angiogenesis, and a number of proteins involved in inflammation (TNFα, interleukin (IL)-1β, IL-6, IL-8, IL-10, Transforming Growth Factor-β, Nerve Growth Factor) and the acute phase response (plasminogen activator inhibitor (PAI)-1, haptoglobin, serum amyloid A) (Trayhurn and Wood, 2004; Bays, 2011). The increased production and raised circulating levels of these proteins in obesity have led to the view that obese individuals are characterized by a state of chronic low-grade inflammation, and that this is causally linked to insulin resistance, hyperlipidemia and the MetS (Yudkin et al., 1999; Das, 2001; Festa et al., 2001; Hotamisligil, 2003; Yudkin, 2003; Trayhurn and Wood, 2004). The unbalanced production of pro- and anti-inflammatory adipocytokines seen in visceral fat obesity critically contributes to the development of many aspects of the MetS (Berg and Scherer, 2005; Hotamisligil, 2006; Schenk et al., 2008).

A very interesting feature of this inflammatory profile is that it appears to be triggered, and reside predominantly, in the adipose tissue (Wellen and Hotamisligil, 2003; Hirosumi et al., 2002). While the role of adipocytes in metabolic pathways is clear, little is still understood about their role in inflammation. There is considerable evidence that the obese adipose tissue is markedly infiltrated by macrophages, which actively participate in the inflammatory pathways that are activated in the adipose tissue (Weisberg et al., 2003; Xu et al., 2003; Clement et al., 2004). It is noteworthy that macrophage infiltration and inflammation-related gene expression in the adipose tissue precede the development of insulin resistance in animal models (Weisberg et al., 2003; Xu et al., 2003), suggesting that infiltrated macrophages are an important source of inflammation in the adipose tissue. Most macrophage in adipose tissue are derived from the bone marrow and recruited by the adipose tissue (Weisberg et al., 2003) (Fig. 1).

According to a proposed timing of adipose tissue inflammatory steps in obesity, hypertrophic adipocytes begin to secrete low levels of TNF-α, which stimulate preadipocytes to produce MCP-1 (Xu et al., 2003). Endothelial cells also secrete MCP-1 in response to cytokines. Thus, preadipocytes, endothelial cells, or both appear to be responsible for attracting macrophages into the adipose tissue through the active secretion of MCP-1. Increased secretion of leptin (and/or decreased production of adiponectin) by adipocytes may also contribute to macrophage accumulation by stimulating transport of macrophages to the adipose tissue (Sierra-Honigmann et al., 1998) and promoting the adhesion of macrophages to endothelial cells (Maeda et al., 2002). Also hyperinsulinemia promotes monocyte adhesion and recruitment by increasing the expression of vascular cell adhesion molecule (VCAM)-1 in endothelial cells (Madonna et al., 2004; Madonna and De Caterina, 2012). It is conceivable that physical damage to the endothelium, caused either by sheer size changes and crowding, or by oxidative damage resulting from an increasingly lipolytic environment, can also play a role in macrophage recruitment, similar to what seen in atherosclerosis. Additionally, the increased adipose tissue expression of chemotactic factors such as MCP-1 and its cognate receptor chemokine (C-C motif) receptor (CCR)-2 has been implicated in the control of monocyte recruitment to the adipose tissue (Suganami and Ogawa, 2010). Finally, recent evidence suggests that increased metabolic stresses such as endoplasmic reticulum (ER) stress, hypoxia, and oxidative stress, as well as the down-regulation of mitogen activated protein (MAP) kinase phosphatase (MKP)-1 are involved in the induction of inflammatory changes in adipocytes during the course of adipocyte hypertrophy (Suganami and Ogawa, 2010). Whatever the initial stimulus, once macrophages are present and active into the adipose tissue, they, along with adipocytes and other cell types, perpetuate a vicious cycle of macrophage recruitment, production of inflammatory cytokines, and impaired adipocyte function.

5. The insulin receptor activity

To understand the role of FFA and other factors in the onset of insulin resistance one has to consider the IR structure and metabolic activity (Fig. 2).

The IR, which belongs to the large class of tyrosine kinase receptors, is a transmembrane receptor that is activated by insulin (Ward and Lawrence, 2009). The IR is a heterotetrameric protein consisting of two extracellular α-subunits and two transmembrane β-subunits, connected by disulfide bridges. Tyrosine kinase receptors, including the IR, mediate their activity by causing the addition of a phosphate group to particular tyrosines on certain proteins within the cells. With the binding of insulin to the IR, a number of endogenous substrates are phosphorylated on tyrosine residue (Greene et al., 2003). The first substrate to be tyrosine-phosphorylated by the insulin signaling is the transmembrane beta-subunit of the IR itself, with a mechanism of autophosphorylation. The preliminary tyrosine autophosphorylation of the IR tyrosine residues provides docking

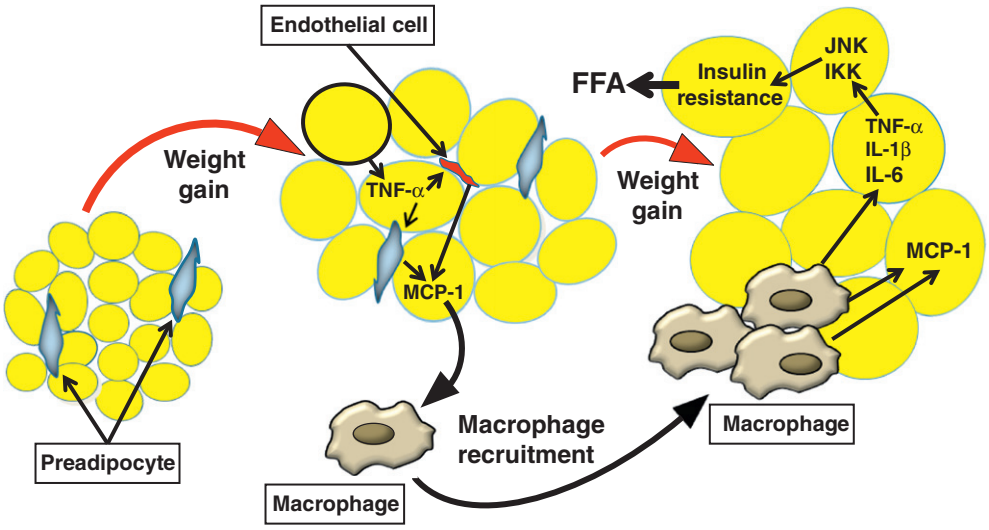

Fig. 1. The obese adipose tissue is characterized by inflammation and progressive infiltration by macrophages, as obesity develops. There is considerable evidence that the obese adipose tissue is markedly infiltrated by macrophages, which actively participate in the inflammatory pathways here activated. According to a proposed timing of adipose tissue inflammatory steps in obesity, hypertrophic adipocytes initially begin to secrete low levels of TNF-α, which stimulate preadipocytes to produce monocyte chemoattractant protein-1 (MCP-1) (Xu et al., 2003). Endothelial cells also secrete MCP-1 in response to cytokines. Thus, preadipocytes, endothelial cells, or both appear to be responsible for attracting macrophages into the adipose tissue through the active secretion of MCP-1. Once macrophages are present and active in the adipose tissue, they, along with adipocytes and other cell types, perpetuate a vicious cycle of macrophage recruitment, production of inflammatory cytokines, and impairment of adipocyte function. The inflammatory cytokines IL-6, IL-1β, and TNF-α activate JNK and IKK serine-kinases, which promote IRS-1 $\text{serine}_{307-312}$ phosphorylation. This serine phosphorylation is responsible for a reduction in IRS protein and a consequent inhibition of insulin receptor signaling by interrupting IRS/insulin receptor interaction. This molecular mechanism promotes insulin resistance in adipocytes and likely starts in the visceral adipose tissue (see also Figs. 2 and 3).

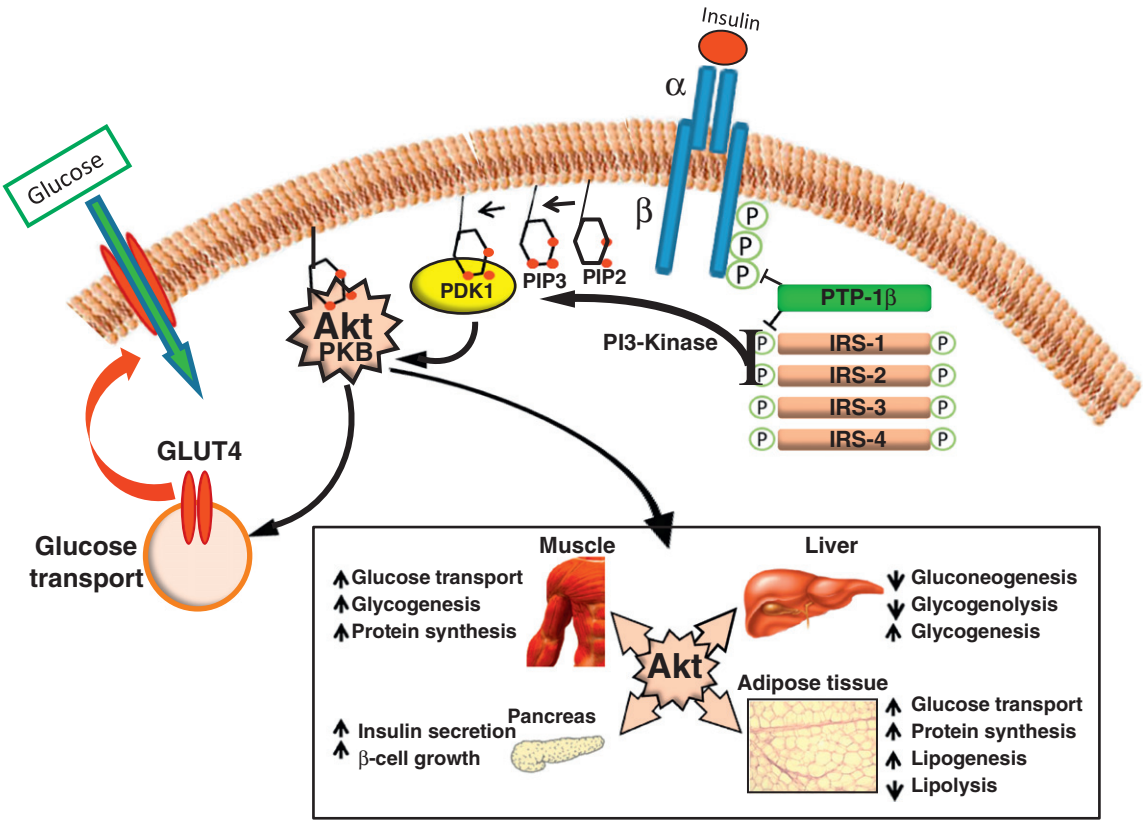

Fig. 2. The binding of insulin to the α-subunit of the insulin receptor activates autophosphorylation reactions whereby the intracellular part of the insulin receptor (β-subunit) becomes tyrosine-phosphorylated by the protein kinase activity of these same receptors. A phosphorylation cascade follows, initiating a protein phosphorylation cascade. The phosphorylation of IRS-1 and -2 leads to binding and activation of phosphatidylinositol 3-kinase (PI3K), which converts phosphatidylinositol 3,4 bisphosphate [PI(3,4)P2] to phosphatidylinositol 3,4,5 trisphosphate [PI(3,4,5)P3]. These nucleotides act as anchors, binding protein kinases to the plasma membrane and activating them. [PI(3,4,5)P3] bound to the plasma membrane associates with phosphoinositol-dependent kinase-1 (PDK-1), and this leads to phosphorylation and activation of protein kinase B, otherwise known as Akt. Activated Akt is thought to initiate many of the metabolic actions of insulin in the adipose tissue, the muscle, the liver and the pancreas.

sites for the recruitment of a number of proteins, each capable of initiating a distinct signaling pathway, starting up a protein phosphorylation cascade. First among such proteins are a set of proteins known as IR substrates (IRS)-1–4. The extracellular insulin binding induces the intra-cytoplasmic binding of IRS-1 to the receptor, through its *src homology 2* (SH2) domains. Multiple tyrosine residues of IRS-1 itself are then phosphorylated by the receptor. Tyrosine-phosphorylated IRS-1 and IRS-2 serve as the major docking proteins for numerous proteins containing SH2 domain. This enables IRS-1 to activate several additional protein kinase signal systems. It has been suggested that the most dominant one is the signaling of phosphatidylinositol-3-kinase (PI3K) (Fig. 2), which converts phosphatidylinositol 3,4 bisphosphate [PI(3,4)P2] to phosphatidylinositol 3,4,5 trisphosphate [PI(3,4,5)P3]. These nucleotides act as anchors, binding downstream protein kinases to the plasma membrane and activating them, particularly the kinase Akt. It has been suggested that Akt (also known as protein kinase B, PKB) is the central element in the actions of insulin, such as GLUT4 translocation and glucose transport, glycogen synthesis, protein synthesis and anti-lipolysis (Cho et al., 2001; Summers et al., 1999; Kitamura et al., 1999; Proud et al., 2001; Shepherd, 2005) (Fig. 2). The increase in the high affinity glucose transporter GLUT4 molecules on the outer membrane of insulin-responsive tissues, including the muscular and the adipose tissue, leads to increased uptake of glucose from blood into these tissues. In other words, the glucose transporter GLUT4 is transported from cellular vesicles to the cell surface, where it can then mediate glucose transport into the cell (Saltiel and Kahn, 2001). IRS-1 plays a key role in transmitting signals from the IR to intracellular pathways of PI3K/Akt.

The degradation of IRS-1 by the proteasome degradation system acts as a feedback mechanism to turn-off insulin signals. Insulin itself stimulates IRS-1 degradation, thus itself inhibiting insulin signaling (Greene et al., 2003). After activation of IR signaling pathway, insulin induces membrane association of a protein kinase, the isoform theta (Θ) of PKC (PKC-Θ), which is known to be negatively associated with insulin sensitivity in the cells (Standaert et al., 1999; Leitges et al., 2002). Upon activation, PKC-Θ is phosphorylated by a mechanism of autophosphorylation. IRS-1 binds to activated (phosphorylated) PKC-Θ and becomes phosphorylated in IRS-1protein serine$_{307}$ in rodent s and serine$_{312}$ in humans (Stephens et al., 1997; Sun et al., 1999). Serine-phosphorylated IRS uncouples from the IR and is degraded by the proteasome system (Greene et al., 2003). Other mechanisms of negative feedback have been described. Upon insulin stimulation, IRS-1 is tyrosine-phosphorylated by the IR, resulting in the activation of PI3-kinase, that mediates serine$_{307}$ phosphorylation of IRS-1, in turn inhibiting the ability of IRS-1 to be further tyrosine-phosphorylated by the IR and propagating insulin signaling (Rui et al., 2001). Despite the complexity of insulin signaling, there is agreement that serine/threonine phosphorylation of IRS-1 inhibits IR-catalyzed IRS-1 tyrosine phosphorylation and the subsequent downstream signaling actions of insulin (Greene et al., 2003).

6. The interruption of insulin receptor signaling

As obesity develops, the fine and complex mechanisms that regulate insulin receptors activity become seriously perturbed by some factors that initiate “turn-off” reactions. Among the numerous factors described, excess of circulating FFA and TNF-α appears to be crucial in quenching IR function (Fig. 3).

7. FFA and insulin receptor inactivation

It has been long known that FFA can induce insulin resistance (Boden et al., 1991 Boden, 1997). It was suggested that FFA cause insulin resistance through inhibition of insulin signaling, which occurs through activation of a serine-kinase cascade (Griffin et al., 1999).

Obesity results in an increased flux of FFA into the circulation and subsequent uptake by the myocyte, hepatocyte or adipose tissue. Activated fatty acids (i.e., fatty acyl-CoA) are metabolized primarily via one of two pathways, oxidation and storage (Fig. 3). When fatty acid flux exceeds the ability of these pathways to dispose of fatty acyl-CoA, fatty acids and intermediates of fatty acid metabolism (e.g., linoleic acid, diacyl glycerol (DAG), phosphatidic acid (PA), lysophosphatidic acid (LPA), ceramide) accumulate. In turn, these fatty acid intermediates can activate a number of different serine kinases that negatively affect insulin action. Recently, some studies have better elucidated the role of FFA in the IR inactivation and degradation (Gao et al., 2004; Schenk et al., 2008). FFA directly (e.g., linoleic acid) or through the intermediates DAG, PA, LPA or ceramide, activate the serine-kinase PKC-Θ, which becomes phosphorylated at threonine$_{538}$ residue. Phosphorylated PKCΘ starts a downstream activation of other two serine-kinase, the c-JUN NH_2-terminal kinase (JNK) and the inhibitor kB kinase (IKK). JNK and IKK associate with IRS-1, promoting its serine-phosphorylation on serine$_{312}$ in humans (Greene et al., 2003) and serine$_{307}$ in rodents (Gual et al., 2005) (Fig. 3). The serine phosphorylation is responsible for IRS-1 blocking and the occurrence of insulin resistance through interruption of IR/IRS interaction (Aguirre et al., 2002) and promotion of IRS-1 protein degradation (Greene et al., 2003). The inactivated IR is then internalized into the cell and catabolized by lysosomes. This molecular pathway operates in many cell types including adipocytes, myocytes, and hepatocytes (Gao et al., 2004).

8. TNF- α and insulin signaling

TNF-α, an inflammatory cytokine expressed mainly by macrophages of the adipose tissue (Fig. 1), inhibits insulin signaling and induces insulin resistance in human adipocytes by affecting IRS proteins (Hotamisligil et al., 1995; Skolnik and Marcusohn, 1996). In adipocytes, TNF-α inhibits insulin-stimulated tyrosine phosphorylation of both IR and IRS-1, and downregulates the insulin-sensitive glucose transporter GLUT-4. At the level of IRS-1, TNF-α acts by a double mechanism that involves (a) serine phosphorylation by IKK and by p38 MAP kinase (p38MAPK) at the serine$_{307}$ residue in rodents and serine$_{312}$ in humans; and (b) tyrosine dephosphorylation by protein-tyrosine phosphatase 1B (PTP1B). Inhibition of IKK activation with salicylate restores insulin sensitivity also in the presence of TNF-α (Kim et al., 2001). The mechanisms affecting IRS reduction involve proteasome-mediated degradation, phosphatase-mediated dephosphorylation, and serine-phosphorylation of IRS-1, which converts IRS-1 to a form that inhibits IR tyrosine kinase activity (White, 2003; Pirola et al., 2004). Obese individuals express 2.5-fold more TNF-α mRNA and protein in fat tissue relative to lean subjects, but circulating TNF-α levels are extremely low or undetectable. Therefore, rather than acting systemically, TNF-α seems to act locally at the site of the adipose tissue, through autocrine or paracrine mechanisms or both, having effects on insulin resistance and inducing IL-6 secretion (Arner, 2003). In conclusion, the inhibitory effect of TNF-α on insulin signaling is mediated by a double mechanism of serine-phosphorylation and tyrosine-dephosphorylation of IRS-1, leading to IRS-1 inactivation and degradation (Greene et al., 2003).

9. Conclusions

IRS proteins appear to be the keystone of insulin signaling, through a mechanism of phosphorylation. IRS-1 may be phosphorylated at the level of tyrosine or serine residues, with opposite effects. Tyrosine residue phosphorylation of IRS-1 and consequent docking of IRS-1 to the transmembrane β-subunits of IR activate the insulin signaling cascade in the muscle, the liver and the adipose tissue. Serine residue phosphorylation of IRS-1, on the contrary, functions as a

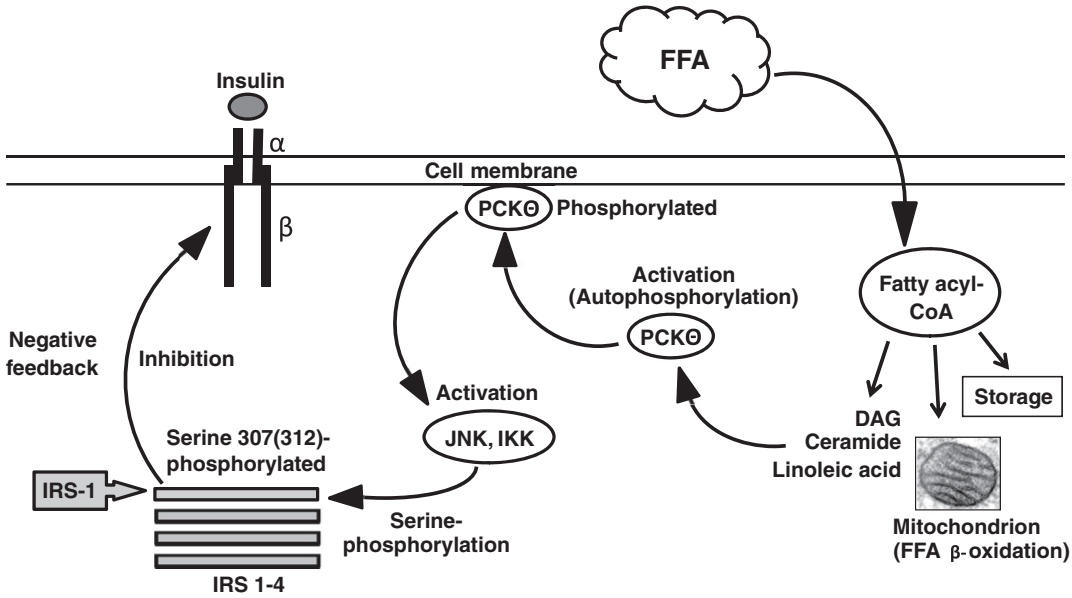

Fig. 3. Fatty acids, in their activated form (fatty acyl-CoAs), are metabolized primarily via one of two pathways, oxidation or storage. When fatty acid flux exceeds the capacity of these pathways, as it occurs in obesity, fatty acids and intermediates of fatty acid metabolism [linoleic acid, diacylglycerol (DAG), phosphatidic acid (PA), lysophosphatidic acid (LPA), ceramide] accumulate and activate phosphokinase C-theta (PKC-Θ), which becomes phosphorylated. Phosphorylated PKC-Θ starts a downstream activation of two serine-kinases, the c-JUN NH_2-terminal kinase (JNK), and the inhibitor kappaB kinase (IKK). JNK and IKK associate with IRS-1, promoting its serine-phosphorylation ($serine_{312}$ in humans, $serine_{307}$ in rodents). The serine phosphorylation is responsible for IRS-1 blocking and the occurrence of insulin resistance by interrupting insulin receptor/IRS interaction and promoting IRS-1 protein degradation.

stop signal for IR, with detachment of IRS-1 from IR and degradation in the proteasome system.

Insulin promotes first the autophosphorylation of tyrosine residues of the transmembrane β-subunits of IR and then the docking of IRS-1 to IR and the tyrosine-phosphorylation of IRS-1. Excess of circulating FFA plays an opposite role to that of insulin, quenching IR activity through a mechanism of serine phosphorylation of IRS-1 induced by the FFA-activated serine kinase PKC-Θ. Serine-phosphorylated IRS-1, up-anchoring it to IR, stops IR activity and prompts IR and IRS-1 degradation by the proteasome. The serine kinase PKC-Θ results to be chronically activated in obese individuals, and this is probably one important cause of insulin resistance in such conditions (Gao et al., 2004).

As far as the serine kinase activation is concerned, salicylate has been demonstrated to prevent the FFA-induced activation of the serine-kinase IKK-beta, preventing the serine phosphorylation and consequent inactivation of IRS-1 and IR (Kim et al., 2001). This capacity of salicylate to prevent fat-induced defects in insulin signaling makes it the prototype of a potentially novel class of therapeutic agents for type 2 diabetes and insulin resistance.

Finally, the inflammatory cytokine TNF-α, expressed mainly by macrophages resident in the "low-inflamed" adipose tissue of obese individuals, inhibits insulin signaling by a double mechanism that involves serine phosphorylation by IKK and tyrosine dephosphorylation by PTP1B of IRS-1. It is noteworthy that salicylate restores insulin sensitivity also in the presence of TNF-α (Kim et al., 2001).

Such mechanisms may explain the transition from excess adiposity to insulin resistance and – subsequently – type 2 diabetes.

Acknowledgment

This work was supported by Fondazione Dieta Mediterranea, Italy.

References

Aguirre, V., Werner, E.D., Giraud, J., Lee, Y.H., Shoelson, S.E., White, M.F., 2002. Phosphorylation of ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J. Biol. Chem. 277, 1531–1537.

Arner, P., 2003. The adipocyte in insulin resistance: key molecules and the impact of the thiazolidinediones. Trends Endocrinol. Metab. 14, 137–145.

Avogaro, P., Crepaldi, G., Enzi, G., Tiengo, A., 1967. Association of hyperlipidemia, diabetes mellitus and mild obesity. Acta Diabetol. Lat. 4, 572–590.

Bays, H.E., 2011. Adiposopathy. Is "sick fat" a cardiovascular disease? J. Am. Coll. Cardiol. 57, 2461–2473.

Bays, H., Ballantyne, C., 2006. Adiposopathy: why do adiposopathy and obesity cause metabolic disease? Future Lipidol. 1, 389–420.

Bays, H.E., González-Campoy, J.M., Bray, G.A., Kitabchi, A.E., Bergman, D.A., Schorr, A.B., Rodbard, H.W., Henry, R.R., 2008. Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. Expert Rev. Cardiovasc. Ther. 6, 343–368.

Berg, A.H., Scherer, P.E., 2005. Adipose tissue, inflammation, and cardiovascular disease. Circ. Res. 96, 939–949.

Boden, G., 1997. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46, 3–10.

Boden, G., Jadali, F., White, J., Liang, Y., Mozzoli, M., Chen, X., Coleman, E., Smith, C., 1991. Effects of fat on insulin stimulated carbohydrate metabolism in normal men. J. Clin. Invest. 88, 960–966.

Cho, H., Mu, J., Kim, J.K., Thorvaldsen, J.L., Chu, Q., Crenshaw III, E.B., Kaestner, K.H., Bartolomei, M.S., Shulman, G.I., Birnbaum, M.J., 2001. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 292, 1728–1731.

Clement, K., Viguerie, N., Poitou, C., Carette, C., Pelloux, V., Curat, C.A., Sicard, A., Rome, S., Benis, A., Zucker, J.D., Vidal, H., Laville, M., Barsh, G.S., Basdevant, A., Stich, V., Cancello, R., Langin, D., 2004. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J. 18, 1657–1669.

Cornier, M.A., Dabelea, D., Hernandez, T.L., Lindstrom, R.C., Steig, A.J., Stob, N.R., Van Pelt, R.E., Wang, H., Eckel, R.H., 2008. The metabolic syndrome. Endocr. Rev. 29, 777–822.

Danforth Jr., E., 2000. Failure of adipocyte differentiation causes type II diabetes mellitus? Nat. Genet. 26, 13.

Das, U.N., 2001. Is obesity an inflammatory condition? Nutrition 17, 953–966.

DeFronzo, R.A., Ferrannini, E., Koivisto, V., 1983. New concepts in the pathogenesis and treatment of noninsulin-dependent diabetes mellitus. Am. J. Med. 74, 52–81.

Eckel, R.H., Grundy, S.M., Zimmet, P.Z., 2005. The metabolic syndrome. Lancet 365, 1415–1428.

Ferrannini, E., Barrett, E.J., Bevilacqua, S., DeFronzo, R.A., 1983. Effect of fatty acids on glucose production and utilization in man. J. Clin. Invest. 72, 1737–1747.

Festa, A., D'Agostino Jr., R., Williams, K., Karter, A.J., Mayer-Davis, E.J., Tracy, R.P., Haffner, S.M., 2001. The relation of body fat mass and distribution to markers of chronic inflammation. Int. J. Obes. 25, 1407–1415.

Fujioka, S., Matsuzawa, Y., Tokunaga, K., Tarui, S., 1987. Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. Metabolism 36, 54–59.

Gao, Z., Zhang, X., Zuberi, A., Hwang, D., Quon, M.J., Lefevre, M., Ye, J., 2004. Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3–L1 adipocytes. Mol. Endocrinol. 18, 2024–2034.

Giorgino, F., 2009. Adipose tissue function and dysfunction: organ cross talk and metabolic risk. Am. J. Physiol. Endocrinol. Metab. 297, E975–E976.
Greene, M.W., Sakaue, H., Wang, L., Alessi, D.R., Roth, R.A., 2003. Modulation of insulin-stimulated degradation of human insulin receptor substrate-1 by Serine 312 phosphorylation. J. Biol. Chem. 278, 8199–8211 (Diabetes 48, 1270–1274).
Griffin, M.E., Marcucci, M.J., Cline, G.W., Bell, K., Barucci, N., Lee, D., Goodyear, L.J., Kraegen, E.W., White, M.F., Shulman, G.I., 1999. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. Diabetes 48, 1270–1274.
Gual, P., Le Marchand-Brustel, Y., Tanti, J.F., 2005. Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. Biochimie 87, 99–109.
Hamilton, M.T., Hamilton, D.G., Zderic, T.W., 2007. The role of low energy expenditure and sitting on obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. Diabetes 56, 2655–2667.
Hirosumi, J., Tuncman, G., Chang, L., Görgün, C.Z., Uysal, K.T., Maeda, K., Karin, M., Hotamisligil, G.S., 2002. A central role for JNK in obesity and insulin resistance. Nature 420, 333–336.
Hotamisligil, G.S., 2003. Inflammatory pathways and insulin action. Int. J. Obes. 27 (Suppl. 3), S53–S55.
Hotamisligil, G.S., 2006. Inflammation and metabolic disorders. Nature 444, 860–867.
Hotamisligil, G.S., Arner, P., Caro, J.F., Atkinson, R.L., Spiegelman, B.M., 1995. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J. Clin. Invest. 95, 2409–2415.
Ibrahim, M.M., 2010. Subcutaneous and visceral adipose tissue: structural and functional differences. Obes. Rev. 11, 11–18.
Kelley, D.E., Mokan, M., Simoneau, J.A., Mandarino, L.J., 1993. Interaction between glucose and free fatty acid metabolism in human skeletal muscle. J. Clin. Invest. 92, 91–98.
Kim, S.H., Reaven, G.M., 2004. The metabolic syndrome: one step forward, two steps back. Diab. Vasc. Dis. Res. 1, 68–75.
Kim, J.K., Kim, Y.J., Fillmore, J.J., Chen, Y., Moore, I., Lee, J., Yuan, M., Li, Z.W., Karin, M., Perret, P., Shoelson, S.E., Shulman, G.I., 2001. Prevention of fat-induced insulin resistance by salicylate. J. Clin. Invest. 108, 437–446.
Kitamura, T., Kitamura, Y., Kuroda, S., Hino, Y., Ando, M., Kotani, K., Konishi, H., Matsuzaki, H., Kikkawa, U., Ogawa, W., Kasuga, M., 1999. Insulin-induced phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B by the serine-threonine kinase Akt. Mol. Cell. Biol. 19, 6286–6296.
Kode, J.A., Mukherjee, S., Joglekar, M.V., Hardikar, A.A., 2009. Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. Cytotherapy 11, 377–391.
Kopelman, P.G., Albon, L., 1997. Obesity, non-insulin-dependent diabetes mellitus and the metabolic syndrome. Br. Med. J. 53, 322–340.
Krotkiewski, M., Bjorntorp, P., Sjostrom, L., Smith, U., 1983. Impact of obesity on metabolism in men and women: importance of regional adipose tissue distribution. J. Clin. Invest. 72, 1150–1162.
Leitges, M., Plomann, M., Standaert, M.L., Bandyopadhyay, G., Sajan, M.P., Kanoh, Y., Farese, R.V., 2002. Knockout of PKC enhances insulin signaling through PI3K. Mol. Endocrinol. 16, 847–858.
Lonnqvist, F., Thome, A., Nilsell, K., Hoffstedt, J., Arner, P., 1995. A pathogenic role of visceral fat P3-adrenoceptors in obesity. J. Clin. Invest. 95, 1109–1116.
Madonna, R., De Caterina, R., 2008. In vitro neovasculogenic potential of resident adipose tissue precursors. Am. J. Physiol. Cell Physiol. 295, C1271–C1280.
Madonna, R., De Caterina, R., 2010. Adipose tissue: a new source for cardiovascular repair. J. Cardiovasc. Med. (Hagerstown) 11, 71–80.
Madonna, R., De Caterina, R., 2012. Atherogenesis and diabetes: focus on insulin resistance and hyperinsulinema. Rev. Esp. Cardiol. 65, 309–313.
Madonna, R., Pandolfi, A., Massaro, M., Consoli, A., De Caterina, R., 2004. Insulin enhances vascular cell adhesion molecule-1 expression in human cultured endothelial cells through a pro-atherogenic pathway mediated by p38 mitogen-activated protein-kinase. Diabetologia 47, 532–536.
Maeda, N., Shimomura, I., Kishida, K., Nishizawa, H., Matsuda, M., Nagaretani, H., Furuyama, N., Kondo, H., Takahashi, M., Arita, Y., Komuro, R., Ouchi, N., Kihara, S., Tochino, Y., Okutomi, K., Horie, M., Takeda, S., Aoyama, T., Funahashi, T., Matsuzawa, Y., et al., 2002. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat. Med. 8, 731–737.
Martin, M.L., Jensen, M.D., 1991. Effects of body fat distribution on regional lipolysis in obesity. J. Clin. Invest. 88, 609–613.
Mokdad, A.H., Ford, E.S., Bowman, B.A., Dietz, W.H., Vinicor, F., Bales, V.S., Marks, J.S., 2003. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA 289, 76–79.
Ogden, C.L., Carroll, M.D., Curtin, L.R., McDowell, M.A., Tabak, C.J., Flegal, K.M., 2006. Prevalence of overweight and obesity in the United States, 1999–2004. JAMA 295, 1549–1555.
Pasarica, M., Xie, H., Hymel, D., Bray, G., Greenway, F., Ravussin, E., Smith, S.R., 2009. Lower total adipocyte number but no evidence for small adipocyte depletion in patients with type 2 diabetes. Diabetes Care 32, 900–902.
Pedersen, S.B., 2005. Studies on receptors and actions of steroid hormones in adipose tissue. Dan. Med. Bull. 52, 258.
Pirola, L., Johnston, A.M., Van Obberghen, E., 2004. Modulation of insulin action. Diabetologia 47, 170–184.
Poirier, P., Giles, T.D., Bray, G.A., Hong, Y., Stern, J.S., Pi-Sunyer, F.X., Eckel, R.H., American Heart Association, Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism, 2006. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 14, 898–918.
Proud, C.G., Wang, X., Patel, J.V., Campbell, L.E., Kleijn, M., Li, W., Browne, G.J., 2001. Interplay between insulin and nutrients in the regulation of translation factors. Biochem. Soc. Trans. 29, 541–547.
Ravussin, E., Smith, S.R., 2002. Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. Ann. N. Y. Acad. Sci. 967, 363–378.
Reaven, G.M., 2005. The insulin resistance syndrome: definition and dietary approaches to treatment. Annu. Rev. Nutr. 25, 391–406.
Rebuffe-Scrive, M., Krotkiewski, M., Elfverson, J., Bjorntorp, P., 1988. Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. J. Clin. Endocrinol. Metab. W67, 1122–1128.
Rebuffe-Scrive, M., Andersson, B., Olbe, L., Bjorntorp, P., 1989. Metabolism of adipose tissue in intraabdominal depots of non-obese men and women. Metabolism 38, 453–461.
Rui, L., Aguirre, V., Kim, J.K., Shulman, G.I., Lee, A., Corbould, A., Dunaif, A., White, M.F., 2001. Insulin/IGF-1 and TNF-α stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. J. Clin. Invest. 107, 181–189.
Saltiel, A.R., Kahn, C.R., 2001. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414, 799–806.
Schenk, S., Saberi, M., Olefsky, J.M., 2008. Insulin sensitivity: modulation by nutrients and inflammation. J. Clin. Invest. 118, 2992–3002.
Shepherd, P.R., 2005. Mechanisms regulating phosphoinositide 3-kinase signalling in insulin-sensitive tissues. Acta Physiol. Scand. 183, 3–12.
Sierra-Honigmann, M.R., Nath, A.K., Murakami, C., García-Cardeña, G., Papapetropoulos, A., Sessa, W.C., Madge, L.A., Schechner, J.S., Schwabb, M.B., Polverini, P.J., Flores-Riveros, J.R., 1998. Biological action of leptin as an angiogenic factor. Science 281, 1683–1686.
Skolnik, E.Y., Marcusohn, J., 1996. Inhibition of insulin receptor signaling by TNF: potential role in obesity and non-insulin-dependent diabetes mellitus. Cytokine Growth Factor Rev. 7, 161–173.
Spalding, K.L., Arner, E., Westermark, P.O., Bernard, S., Buchholz, B.A., Bergmann, O., Blomqvist, L., Hoffstedt, J., Näslund, E., Britton, T., Concha, H., Hassan, M., Rydén, M., Frisén, J., Arner, P., 2008. Dynamics of fat cell turnover in humans. Nature 453, 783–787.
Standaert, M.L., Bandyopadhyay, G., Galloway, L., Soto, J., Ono, Y., Kikkawa, U., Farese, R.V., Leitges, M., 1999. Effects of knockout of the protein kinase C gene on glucose transport and glucose homeostasis. Endocrinology 140, 4470–4477.
Stephens, J.M., Lee, J., Pilch, P.F., 1997. Tumor necrosis factor-a-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. 272, 971–976.
Suganami, T., Ogawa, Y., 2010. Adipose tissue macrophages: their role in adipose tissue remodeling. J. Leukoc. Biol. 88, 33–39.
Summers, S.A., Kao, A.W., Kohn, A.D., Backus, G.S., Roth, R.A., Pessin, J.E., Birnbaum, M.J., 1999. The role of glycogen synthase kinase 3beta in insulin-stimulated glucose metabolism. J. Biol. Chem. 274, 17934–17940.
Sun, X.J., Goldberg, J.L., Qiao, L.Y., Mitchell, J.J., 1999. Insulin induced insulin receptor substrate-1 degradation is mediated by the proteasome degradation pathway. Diabetes 48, 1359–1364.
Tomlinson, J.W., Walker, E.A., Bujalska, I.J., Draper, N., Lavery, G.G., Cooper, M.S., Hewison, M., Stewart, P.M., 2004. 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. Endocr. Rev. 25, 831–866.
Trayhurn, P., Wood, I.S., 2004. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br. J. Nutr. 92, 347–355.
Ward, C.W., Lawrence, M.C., 2009. Ligand-induced activation of the insulin receptor: a multi-step process involving structural changes in both the ligand and the receptor. Bioessays 31, 422–434.
Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., Ferrante Jr., A.W., 2003. Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. 112, 1796–1808.
Wellen, K.E., Hotamisligil, G.S., 2003. Obesity-induced inflammatory changes in adipose tissue. J. Clin. Invest. 112, 1785–1788.
White, M.F., 2003. Insulin signaling in health and disease. Science 302, 1710–1711.
Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., Tartaglia, L.A., Chen, H., 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest. 112, 1821–1830.
Yudkin, J.S., 2003. Adipose tissue, insulin action and vascular disease: inflammatory signals. Int. J. Obes. 27 (Suppl. 3), S25–S28.
Yudkin, J.S., Stehouwer, C.D., Emeis, J.J., Coppack, S.W., 1999. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler. Thromb. Vasc. Biol. 19, 972–978.
Zuk, P.A., Zhu, M., Ashjian, P., De Ugarte, D.A., Huang, J.I., Mizuno, H., Alfonso, Z.C., Fraser, J.K., Benhaim, P., Hedrick, M.H., 2002. Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell 13, 4279–4295.