Review

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

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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 $\beta_3$-adrenoceptors and, partly, to reduced activity of $\alpha_2$-adrenoceptors. As a consequence, more free fatty acids (FFA) are released into the portal system. Hypertrophic adipocytes begin to secrete low levels of TNF-$\alpha$, 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-$\alpha$ 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-$\Theta$, 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-$\alpha$, 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.

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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) (Vaghar 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; Ferramini et al., 1983; Kellet al., 1993; Berg and Scherer, 2005; Bays and Ballantyne, 2006; Schenck 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 (Fujiki 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, perimysial, 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 aird 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 (Reubuck-Schive et al., 1988).

3.2. β3 and α2-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 α2-adrenoceptors. In men and women, the lipolytic response to noradrenaline, which acts through α2- and β-adrenoceptors, is more marked in the visceral than in the gluteal or femoral fat (Krotkiewski et al., 1983). Lonngqvist 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 β3-adrenoceptor sensitivity and coupling efficiency in visceral adipocytes. The authors suggested that this enhanced β3-adrenoceptor 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 β3-adrenoceptors and, partly, to a reduced activity of α2-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-Scribe et al., 1989). Other factors, however, intervene in the metabolic dysfunction of VAT IR, e.g., the chronic elevation of FFA and inflammatory adipokines, particularly TNF-α.

An increase of FFA level in VAT, primarily due to excess of lipolysis promoted by the enhanced β3-adrenoceptors 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 chemotactic protein-1 (MCP-1); and a diverse range of other protein factors, which have been called adipokines or adipocytokines. The adipokineome 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
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 serine307–312 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).

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 to -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 biphosphate [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 isofrom theta (θ) of PKC (PKC-θ), which is known to be negatively associated with insulin sensitivity in the cells (Standarta et al., 1999; Lehtes 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-1-protein serine307 in rodents and serine312 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 serine307 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 IRS-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 threonine323 residue. Phosphorylated PKCθ starts a downstream activation of other two serine-kinase, the c-jun NH2-terminal kinase (JNK) and the inhibitor Kβ kinase (IKK). JNK and IKK associate with IRS-1, promoting its serine-phosphorylation on serine312 in humans (Greene et al., 2003) and serine293 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 serine307 residue in rodents and serine312 in humans; and (b) tyrosine dephosphorylation by protein-tyrosine phosphatase 1B (PTP1B). Inhibition of IKK activity 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
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.

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References


