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REVIEW ARTICLE

Insulin resistance associated to obesity: the link TNF-alpha

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Abstract
Adipose tissue secretes proteins which may influence insulin sensitivity. Among them, tumour necrosis factor (TNF)-alpha has been proposed as a link between obesity and insulin resistance because TNF-alpha is overexpressed in adipose tissue from obese animals and humans, and obese mice lacking either TNF-alpha or its receptor show protection against developing insulin resistance. The activation of proinflammatory pathways after exposure to TNF-alpha induces a state of insulin resistance in terms of glucose uptake in myocytes and adipocytes that impair insulin signalling at the level of the insulin receptor substrate (IRS) proteins. The mechanism found in brown adipocytes involves Ser phosphorylation of IRS-2 mediated by TNF-alpha activation of MAPKs. The Ser307 residue in IRS-1 has been identified as a site for the inhibitory effects of TNF-alpha in myotubes, with p38 mitogen-activated protein kinase (MAPK) and inhibitor kB kinase being involved in the phosphorylation of this residue. Moreover, up-regulation of protein-tyrosine phosphatase (PTP)1B expression was recently found in cells and animals treated with TNF-alpha. PTP1B acts as a physiological negative regulator of insulin signalling by dephosphorylating the phosphotyrosine residues of the insulin receptor and IRS-1, and PTP1B expression is increased in peripheral tissues from obese and diabetic humans and rodents. Accordingly, down-regulation of PTP1B activity by treatment with pharmacological agonists of nuclear receptors restores insulin sensitivity in the presence of TNF-alpha. Furthermore, mice and cells deficient in PTP1B are protected against insulin resistance induced by this cytokine. In conclusion, the absence or inhibition of PTP1B in insulin-target tissues could confer protection against insulin resistance induced by cytokines.

Key words: Glucose uptake, LXR, PTP1B, p38MAPK, rosiglitazone.

Abbreviations: AMPK, AMP-activated protein kinase; AS160, AKT substrate of 160 kDa; ERK, extracellular-signal regulated kinase; FFA, free fatty acids; FATP, fatty acid transporter protein; FAT, fatty acid translocase; GLUT4, insulin-regulated glucose transporter; HSL, hormone sensitive lipase; IKK, inhibitor kB kinase; IR, insulin receptor; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; LPL, lipoprotein lipase; LXR, liver X receptor; MAPK, mitogen-activated protein kinase; PIP3K, phosphatidylinositol 3-kinase; PLC, phospholipase C; PP, protein-phosphatase; PPAR, peroxisome proliferator activated receptor; PTP, protein-tyrosine phosphatase; TNF, tumour necrosis factor; TZD, thiazolidinediones; UCP, uncoupling-protein.

Introduction
Tumour necrosis factor (TNF)-alpha was first described as an endotoxin-induced serum factor that causes necrosis of tumours (Carswell et al., 1975). Later, TNF-alpha was shown to be identical to the protein called cachectin, which is found in cultured macrophages exposed to endotoxin. Cachectin, or TNF-alpha, was initially found to induce cachexia in animals (Tracey et al., 1988).

The human gene of TNF-alpha, TNFA, was cloned in 1984 (Old, 1985). TNF-alpha is expressed as a 26 kDa plasma membrane-bound monomer. Proteolytic cleavage by the TNF-alpha converting enzyme results in the formation of a 17 kDa soluble trimer of the membrane-bound precursor protein of TNF-alpha. Two TNF-alpha receptors, denominated type I (TNFR1) and type II (TNFR2), mediate the TNF-alpha signal by forming protein complexes with cytoplasmic adaptor proteins. The
fact that TNF-alpha signalling involves two receptors has been complicating the dissection of its effects (reviewed in Aggarwal, 2003). Depending on which receptor is activated opposing intracellular pathways with partially dramatically differential effects can be triggered. Studies, so far, have addressed this possibility only partially and, to date, most known functions of TNF-alpha have been ascribed to signals transduced by TNFR1 with TNFR2 playing primarily a modulatory role in ligand passing. Interestingly, utilizing the two different receptor types TNF-alpha can affect cells pro-apoptotic or anti-apoptotic, depending on conditions. This has recently been shown to be the case in human skeletal muscle sarcopenia, liver, and human adipocytes (for review see Dirks and Leeuwenburgh, 2006).

Recent studies revealed that the pro-inflammatory cytokine TNF-alpha secreted by the adipose tissue does not only play a key role in the mediation of immune response as a multi-functional regulator of inflammation, cell apoptosis and survival, cytotoxicity, and production of other cytokines, such as interleukin (IL)-1 and IL-6, but also in the induction of obesity-induced insulin resistance.

**Obesity, TNF-alpha, and insulin resistance**

Insulin resistance, which can be defined as a diminished ability of the cell to respond to the action of insulin, is the most important pathophysiological feature in many prediabetic states and is the first detectable defect in type 2 diabetes. The pathogenesis of type 2 diabetes involves abnormalities in both insulin action and secretion. Insulin resistance is usually compensated by hyperinsulinemia. Although moderate hyperinsulinemia might be tolerated in the short term, chronic hyperinsulinemia exacerbates insulin resistance and contributes directly to beta-cell failure and diabetes (White, 2003). At the molecular level, insulin resistance correlates with impaired insulin signalling in peripheral tissues (Figure 1). Insulin resistance in the adipose tissue leads to an increase of lipolysis, with subsequent release of glycerol and free fatty acids (FFA) into the circulation. It is widely accepted that increased availability and utilization of FFA contribute to the development of skeletal muscle insulin resistance, as well as to augmented hepatic glucose production (White, 2003). In fact, the progression of insulin resistance in high-fat diet rats is closely related to plasma FFA rather than TNF-alpha and IL-6 levels (Jiao et al., 2008). Both genetic and environmental factors can contribute to develop insulin resistance, and in the second group, obesity has been proposed to be an important contributor.

Obesity is a risk factor to develop type 2 diabetes, in part due to the fact that adipose tissue secretes proteins named adipokines that may influence insulin sensitivity. Adipose tissue, in particular the visceral compartment, is now recognized as the primary contributor to the insulin resistance syndrome. Several factors secreted from adipose tissue, including cytokines, chemokines and FFA, can impair insulin signalling altering insulin-mediated processes, including glucose homeostasis and lipid metabolism (Arner, 2003). Accordingly, obesity is now being considered a chronic state of low-intensity inflammation. In this regard, recent studies reveal that obesity is also associated with an increase in adipose tissue infiltration of macrophages, which contributes to the inflammatory process through the additional secretion of cytokines (Lumeng et al., 2007). The mechanisms by which adipose tissue recruits and maintains macrophages could involve expression of monocyte chemoattractant proteins and intercellular adhesion molecule-1, as was described recently (Weisberg et al., 2006).

TNF-alpha has been proposed as a link between adiposity and the development of insulin resistance.

Figure 1. The different steps in the development of type 2 diabetes.
because the majority of type 2 diabetic subjects are obese, is highly expressed in adipose tissues from obese subjects, and obese mice lacking either TNF-alpha or its receptors showed protection for developing insulin resistance (Hotamisligil, 2003). Rather than acting systemically, TNF-alpha seems to act locally at the site of adipose tissue through autocrine or paracrine mechanisms, having effects on insulin resistance and inducing IL-6 (Arner, 2003). Circulating levels of soluble TNF-alpha receptors now seems well correlated with BMI, and impairment in TNF-alpha processing can improve systemic insulin sensitivity (Zahorska-Markiewicz et al., 2000; Serino et al., 2007). On the other hand, TNF-alpha has lypolitic and antiadipogenic effects on adipose tissue (Arner, 2003). This paradox could be due to proliferative and anti-apoptotic effects of this cytokine in the obese adipocyte, and could be mediated by the differential expression of its soluble and membrane-anchored receptors. Both ceramides and FFA were reported to induce insulin resistance in peripheral tissues, and the production of these molecules could be the consequence of activation of sphingomyelinase or lipolysis by TNF-alpha (Arner, 2003). Several other mediators that are activated in response to TNF-alpha such as stress kinases and inflammatory pathways, could also contribute to insulin resistance. In this regard, increased phosphorylation of p38MAPK in adipocytes and muscle from type 2 diabetic subjects has been reported (Carlson et al., 2003).

Hence, TNF-alpha initiated pathways are thought to contribute at least partially, to the metabolic consequences of obesity in causing decreased adipocyte and skeletal muscle insulin sensitivity. However, the picture is far from being straight forward, and rodent versus human data does not always match up. Hence many questions remain, some of which we will attempt to address here.

TNF-alpha deficient mice exhibit lower circulating FFA and triglycerides than wild-type animals (Uysal et al., 1997). Furthermore, infusion of TNF-alpha in rodents leads to impairment of insulin stimulated skeletal muscle glucose uptake. Accordingly, neutralisation of TNF-alpha with specific antibodies has the opposite effect and improves an insulin resistant state in rats (Hotamisligil et al., 1994). However, in contrast to the findings in rodent models, TNF-alpha neutralisation has been of no beneficial effect in terms of insulin sensitivity in humans (Rosenvinge et al., 2007). The effects of neutralisation of TNF-alpha on the expression levels of other adipokines were differential being suggestive for why TNF-alpha neutralisation gave no positive result. The fact that TNF-alpha is a regulator of expression of other adipokines, which also have effects on insulin sensitivity, lipid metabolism and energy homeostasis control, complicates studies on the effects of TNF-alpha, especially in genetically diverse human subjects. While neutralisation of TNF-alpha increased total adiponectin, the high molecular weight form of adiponectin, which is thought to have positive effects on insulin sensitivity was unaltered (Lo et al., 2007). Also IL-6 expression was decreased but did not result in any change in insulin sensitivity either (Rosenvinge et al., 2007). Furthermore, muscle adiposity was increased, leptin content was unaltered and resistin levels were decreased. Additionally, in order to induce insulin resistance using TNF-alpha infusion requires concentrations that exceed the physiological range of TNF-alpha usually observed in insulin resistant rodent models (Altomonte et al., 2003).

However, TNF-alpha expression is increased in adipose tissue from insulin-resistant obese individuals (Kern et al., 2001). Interestingly, these studies have mostly investigated male subjects while studies performed with females have suggested that there might be a lack of such a correlation of TNF-alpha with insulin resistance in women. It has been suggested that some seemingly conflicting findings in humans may be based on a differential expression of the TNFR in males compared to females (Kern et al., 2001). Further investigation will be required to address this possibility. In order to understand the possible mechanisms by which TNF-alpha acts upon metabolism and insulin sensitivity, we will review the direct and indirect effects of TNF-alpha in adipose tissue and skeletal muscle.

The role of TNF-alpha in insulin resistance in adipose tissue

While many secreted factors including RBP4, leptin and IL-6 are elevated in adipose tissue from obese humans and rodents, TNF-alpha has been most strongly implicated in the pathogenesis of insulin resistance, because its expression is strongly correlated to reduced insulin-stimulated glucose disposal (Kern et al., 2001). TNF-alpha expression is increased in adipose tissue of humans with insulin resistance (Hotamisligil et al., 1995). In adipose tissue TNF-alpha is the main factor that triggers enhanced lipolysis leading to increased secretion of FFA from adipose tissue into the circulation (Table I) (Ruan et al., 2003). TNF-alpha has been proposed to stimulate lipolysis by inhibiting insulin receptor signalling, by inhibiting signalling through the G-protein-coupled adenosine receptor and via direct stimulation of basal (nonhormonal) lipolysis through interactions with the lipid-binding protein perilipin. Lipids released from adipocytes as FFA are transported as triglycerides by VLDL and shifted to non-adipose tissues like skeletal muscle (Sharma and Chetty, 2005).

Concomitantly, lipoprotein lipase (LPL), the enzyme which hydrolyses lipids in lipoproteins into fatty acids and glycerol and facilitates lipid FA uptake is inhibited by TNF-alpha in 3T3-L1 adipocytes and adipose tissue (Ramsay, 1996). Furthermore, increased circulating TNF-alpha contributes also to an
inhibition of adipogenesis, possibly via inhibition of peroxisome proliferator activated receptor (PPAR) (Zhang et al., 1996) and liponeogenesis from glucose is reduced (Hotamisligil, 1999). Taken together, this leads to diminished ability of adipose tissue to accumulate lipids and thereby render clearance of FFA from circulation impossible leading to increased FFA in the blood stream.

Many of these described effects are a sign of an impairment of the insulin signalling network. Insulin initiates the biological effects in target cells by binding to, and activating, its endogenous Tyr kinase receptors. Insulin receptors (IR) are believed to transduce signals by phosphorylation on Tyr residues of several cellular substrates including insulin receptor substrate (IRS) proteins 1, 2, 3 and 4 (White, 2003). A number of signalling pathways can be activated downstream of IRS proteins organized in two major elements, Ras/Raf/extracellular-signal regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3K)/AKT/p70S6 kinase pathways. On the other hand, the insulin-signalling cascade is negatively regulated by protein phosphatases, including Tyr, Ser and lipid phosphatases. Most notably, protein-tyrosine phosphatase (PTP)1B acts by dephosphorylating the phosphotyrosine residues of the IR and IRS-1 (Figure 2).

It is apparent that TNF-alpha is important for regulating insulin action in human adipose tissue because there is a strong negative relationship between the release of the cytokine and insulin-induced glucose metabolism in this tissue (Lof gren et al., 2000). The interaction between TNF-alpha and insulin signalling is above all important for local insulin resistance in obesity. When cells are exposed directly to TNF-alpha, this adipokine inhibits insulin signalling by affecting IRS proteins (Hotamisligil, 2003). The mechanisms affecting IRSs involve proteasome-mediated degradation, phosphatase-mediated dephosphorylation, and Ser phosphorylation of IRS-1, which converts IRS-1 in an inhibitor of the IR Tyr kinase activity, as reviewed previously (White, 2003; Pirola et al., 2004). Both ERK and c-Jun N-terminal kinase (JNK) have been proposed as mediating TNF-alpha Ser/Thr phosphorylation of IRS-1 in white adipocytes, with the Ser307 residue

Table I. Effect of TNF-alpha in adipose tissue and skeletal muscle.

<table>
<thead>
<tr>
<th>Adipose tissue</th>
<th>Skeletal muscle</th>
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<tbody>
<tr>
<td>Insulin sensitivity</td>
<td>Insulin sensitivity</td>
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<tr>
<td>TNF-alpha expression</td>
<td>TNF-alpha expression</td>
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<tr>
<td>Lipolysis</td>
<td>Long term FA oxidation</td>
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<tr>
<td>LPL activity</td>
<td>AMPK activity</td>
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<td>Liponeogenesis</td>
<td>Glycogen synthesis</td>
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<td>GLUT4 expression</td>
<td>Glucose uptake</td>
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<tr>
<td>FAT/FATP expression</td>
<td>ADC activity</td>
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<tr>
<td>IRS-2 Ser phosphorylation</td>
<td>IRS-1 Ser phosphorylation</td>
</tr>
<tr>
<td>PTP1B expression</td>
<td>PTP1B expression</td>
</tr>
<tr>
<td>Ceramide levels</td>
<td>Diacylglycerol levels (DAG)</td>
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</tbody>
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Figure 2. A complex insulin intracellular signalling cascade controls the pleiotropic effects of this hormone.
being identified as the site for TNF-alpha phosphorylation of IRS-1. In this regard, ablation of jnk1 decreases the development of insulin resistance associated with dietary obesity. Furthermore, ERK and p38MAPKs could inhibit insulin signalling by TNF-alpha at the level of IRS-1 and IRS-2 in 3T3-L1 adipocytes, whereas JNK could mediate the feedback inhibitory effect of insulin (White, 2003; Pirola et al., 2004). Others work has also implicated activation of inhibitor kB kinases (IKK) by TNF-alpha on Ser phosphorylation of IRS-1. Meanwhile, IKK inhibition with salicylate or targeted disruption of ikkb reversed obesity and diet-induced insulin resistance (Gao et al., 2003).

In addition to these signalling impairments long-term effects take place. Long term effects of TNF-alpha are mediated via repression or stimulation of expression of many genes responsible for glucose and FFA uptake and storage in adipose tissue. For 3T3-L1 adipocytes it has been shown that TNF-alpha dependent gene up- and downregulation occurs by regulation of the NF-kB (Ruan et al., 2002). For example, TNF-alpha has been shown to down-regulate the genes for adiponectin, GLUT4, IRS-1, C/EBPα, PPARγ, and perilipin in adipocytes. Interestingly, in TNF-alpha injected syrian hamster fatty acid transporter protein (FATP), and fatty acid translocase (FAT) mRNA expression was reduced approximately 50% in adipose tissue (Memon et al., 1998).

TNF-alpha action on adipose tissue can alter the production of many adipokines, and this is relevant to the systemic effects of this cytokine on insulin sensitivity and whole body energy homeostasis as review in Cawthorn and Sethi (2008). On the other hand TNF-alpha upregulates the expression of genes like vascular cell adhesion molecule-1, plasminogen activator inhibitor-1, IL-6, IL-1β, angiotensinogen, resistin and leptin (Ruan et al., 2002). Interestingly, the drug metformin, which exerts its antihyperglycemic effect by improving insulin sensitivity, which is associated with decreased level of circulating FFA, attenuated TNF-alpha-mediated lipolysis by suppressing phosphorylation of ERK and reversing the downregulation of perilipin protein in TNF-alpha-stimulated adipocytes (Ren et al., 2006). Activators of PPARγ have been shown to inhibit the effects of a number of the above mentioned effects and ultimately the TNF-alpha induced inhibition of insulin sensitivity (Souza et al., 1998). This data indicates that TNF-alpha action via TNFR1 might have an inhibitory effect on PPAR-gamma mediated expression control.

Finally, the role of variations in TNF-alpha genotypes in the mediation of the TNF-alpha action has recently become the focus of attention. In a recent study subjects were genotyped for the TNF-alpha-238G > A, -308G > A, and -863C > A polymorphisms. Compared with subjects who were homozygous for the -238G allele, carriers of the -238A allele had an altered ability to suppress postprandial FFA. This effect was observed in obese but not in non-obese individuals with type 2 diabetes (men, n = 56 and women, n = 67) (Fontaine-Bisson et al., 2007). Patients with coronary artery disease showed a higher frequency of the TNF-308A allele than healthy controls, while coronary artery disease patients with diabetes had a higher frequency of the TNF-308AA genotype compared with healthy controls (Sbarsi et al., 2007).

**Molecular mechanisms involved in insulin-resistance by TNF-alpha in brown adipocytes: amelioration by agonists of nuclear receptors**

Brown adipose tissue is present and active in mammal newborns and is responsible for their successful defence of body temperature without shivering. When this tissue is not adrenergically stimulated, brown adipocytes suffer apoptosis or transform into white adipocyte-like cells that gradually loose many brown characteristics. This phenomenon is particularly noticeable in adult humans, in which brown adipose tissue is thought to be rapidly lost postnatally, so that humans later in life do not possess more than vestigial amounts of this tissue, located within the white fat depots (Cannon and Nedergaard, 2004). However, the use of fluorodeoxyglucose positron emission tomography has revealed the presence of symmetrical areas of increased tracer uptake in the upper parts of the human body, which correspond to brown adipose tissue. The human depots are differently located from those in rodents, mainly in the supraclavicular and the neck regions, but no interscapular (Nedergaard et al., 2007). These findings point out that brown adipose tissue is present and active in a substantial fraction of adult humans and that may thus be considered of metabolic significance in human physiology.

Insulin exerts a dominant role in regulating glucose homeostasis though orchestrated effects on the promotion of glucose uptake in peripheral tissues, such as muscle and fat, and suppressing hepatic glucose production. Glucose transport in brown adipocytes is maintained mainly by the activity of insulin-regulated glucose transporter (GLUT)4. Insulin treatment stimulates glucose transport by mediating GLUT4 translocation in an AKT- and PKCζ-dependent manner (Hernandez et al., 2001; Lorenzo et al., 2002). Furthermore, inhibition of phospholipase C (PLC)ζ; activity precludes insulin stimulation of glucose uptake, GLUT4 translocation and actin reorganization, indicating that PLCζ through the production of phosphatidic acid, is a link between IR and PKCζ (Lorenzo et al., 2002). In addition, IRS-2 seems to be crucial in mediating glucose uptake in brown adipocytes (Teruel et al., 2001).

TNF-alpha acts as a negative regulator of adipogenic and thermogenic differentiation and induces insulin resistance in brown adipose tissue.
(Valverde et al., 2005), in a similar fashion as reported in 3T3-L1 cells and in primary human adipocytes. Moreover, TNF-alpha-induced insulin resistance on glucose uptake in brown adipocytes seems to be due to the hypophosphorylation of the IR and IRS-2 in response to insulin, resulting in an impairment of IRS-2-associated PI3K activity (Teruel et al., 2001). As a further step, we have identified ceramide production as one of the mediators of insulin-resistance by TNF-alpha and exogenously added C2-ceramide inhibited AKT activity throughout a ceramide-activated protein-phosphatase (PP)2A (Teruel et al., 2001). Furthermore, de novo ceramide generation produced by chronic treatment with TNF-alpha induces insulin resistance on GLUT4 gene expression in brown adipocytes by interfering C/EBPz accumulation (Fernandez-Veledo et al., 2006a). Moreover, stress kinases activated in response to TNF-alpha, meanly ERK and p38MAPKs also contribute to insulin resistance in brown adipocyte primary cultures (Hernandez et al., 2004).

Modulation of genes such as PTP1B might contribute to the pathogenesis of TNF-alpha-induced insulin resistance. In this regard, brown adipocytes treated with TNF-alpha showed a significant enhancement of PTP1B mRNA, protein and activity (Fernandez-Veledo et al., 2006b). As expected, the lack of PTP1B these cells conferred protection against TNF-alpha-induced insulin resistance on glucose uptake and insulin signalling (Fernandez-Veledo et al., 2006b). Accordingly, a complex mechanism impairs the normal response to insulin on GLUT4 translocation in brown adipocytes in the presence of TNF-alpha including: (1) potential Ser/Thr phosphorylation of IRS-2 by MAPKs, weakening the Tyr phosphorylation induced by insulin, (2) generation of ceramide and activation of PP2A maintaining AKT in an inactive dephosphorylated state and (3) modulation of PTP1B protein expression and activity, as summarized in Figure 3.

Nuclear receptors, such as PPAR, and liver X receptor (LXR), comprise a superfamily of related proteins, which act as transcription factors to activate expression of target genes in response to binding of ligands. Thiazolidinediones are agonists for PPARγ that display insulin-sensitizing actions across a wide spectrum of insulin-resistant states, and have recently been introduced as therapeutic agents for the treatment of type 2 diabetes (Olefsky, 2000). However, several limitations with this therapy, such as increased adiposity, secondary insulin resistance in adipose tissue, and pro- and anti-atherogenic effects, are currently emerging. LXRz has been proposed recently as important regulator of glucose metabolism (Steffensen and Gustafsson, 2004). In this regard, improved glucose tolerance and increased glucose-induced insulin secretion by islets in genetic and dietary models of type 2 diabetes treated with synthetic LXR agonists was reported. Moreover, ligand activation of LXR regulates the expression of GLUT4 either in vivo as well as in murine and human adipocytes, through direct interaction with a conserved LXR response element in the GLUT4 promoter. In addition, the ability of LXR ligands to regulate GLUT4 expression was abolished in mice lacking LXRs.

Brown adipose tissue is a target tissue for nuclear receptors agonists since highly expressed PPARγ, LXRz, and LXRβ (Steffensen and Gustafsson, 2004). In this regard, the PPARγ agonist rosiglitazone up-regulates the expression of LPL, HSL and uncoupling protein-1 in brown adipocytes (Teruel et al., 2005) as well as produces insulin sensitization by increasing the expression of IR and its tyrosine

Figure 3. Insulin resistance by TNF-α in brown adipocytes involves: (1) Ser/Thr phosphorylation of the IRS-2 by ERK and p38MAPK; (2) generation of ceramide and activation of the phosphatase PP2A, and (3) modulation of PTP1B activity. Inhibition of ERK and p38MAPK activation with rosiglitazone, and down-regulation of PTP1B with either rosiglitazone or T0901317 ameliorates TNF-α-induced insulin resistance.
kinase activity (Hernandez et al., 2003). The effectiveness of rosiglitazone to treat TNF-α-induced insulin resistance in these cells was due to the fact that this TZD impaired the activation of p38MAPK and ERK produced by TNF-alpha, and restored the insulin signalling cascade leading to normalization of insulin-induced glucose uptake (Hernandez et al., 2004). Furthermore, rosiglitazone decreased the activity of PTP1B (Hernandez et al., 2003), and improved insulin sensitivity concomitant with an increase in thermogenic differentiation, contributing globally to an accelerated glucose disposal in brown adipose tissue. Moreover, recent studies have demonstrated increased levels and activities of PTP1B in skeletal muscle and liver of diabetic rats whereas rosiglitazone treatment decreases this enlargement in muscle but not in liver (Wu et al., 2005). On the other hand, synthetic LXR agonists ameliorate TNF-alpha-induced insulin resistance in fetal brown adipocytes restoring completely insulin-stimulated GLUT4 translocation to plasma membrane. This effect was parallel to the recovering of insulin signalling cascade and could be due to the fact that T0901317 precludes the enlargement in PTP1B expression produced by TNF-alpha (Fernandez-Veledo et al., 2006b), supporting the hypothesis of nuclear receptors LXR are interesting targets for drug treatment of insulin-resistant conditions. Therefore, inhibition of ERK and p38MAPK activation by rosiglitazone and down-regulation of PTP1B with either rosiglitazone or LXR agonists restores insulin sensitivity in brown adipocytes in the presence of TNF-alpha as summarized in Figure 3.

**Contribution of p38MAPK and PTP1B to TNF-α-induced insulin resistance in skeletal muscle**

The organ in which insulin resistance is first detectable is skeletal muscle because this tissue is responsible for the highest glucose disposal of the body. Acute insulin treatment stimulates glucose transport in myocytes largely by mediating translocation of GLUT4 to the plasma membrane, which is accomplished by activation of PI3K, AKT and several PKC isoforms including ζ, λ, α and δ (Khan and Pessin, 2002; Vollenweider et al., 2002). Moreover, skeletal muscle has an insulin-independent mechanism to increase glucose transport that involves the activation of AMP-activated protein kinase (AMPK) by stimuli such as exercise, hypoxia or ischemia (Fujii et al., 2006). The AKT substrate of 160 kDa (AS160) has emerged recently as a point of convergence for both effectors of glucose transport and seems to modulate GLUT4 trafficking (Fujii et al., 2006). Whereas the GLUT4 protein content is normal in muscle from subjects with type 2 diabetes, the capacity of insulin to stimulate translocation of GLUT4 to plasma membrane is impaired. In contrast to the effect of insulin, contraction-stimulated glucose uptake and GLUT4 translocation in diabetic patients is normal, providing evidence that exercise might be able to bypass defects in insulin signalling.

TNF-alpha expression in skeletal muscle of insulin resistant subjects and diabetic patients was fourfold higher than in the insulin sensitive subjects (Kern et al., 2001). TNF-alpha blocks skeletal muscle differentiation, causes sarcopenia and produces insulin resistance in skeletal muscle of healthy humans and in primary cultures of mouse skeletal muscle (Table I) (Dirks and Leeuwenburgh, 2006; Plomgaard et al., 2005). Although ceramide and FFA have been reported to produce insulin resistance in skeletal muscle, a direct effect of TNF-alpha in this tissue has been a matter of controversy. Several reports did not detect an inhibitory action of TNF-alpha on insulin-induced glucose uptake, although TNF-alpha per se highly increased basal glucose uptake. However, others observed an inhibitory effect of TNF-alpha on insulin action without modifying basal glucose uptake in muscle cells. Moreover, in most of these studies insulin stimulation of glucose uptake was very poor because virtually all cultured skeletal muscle cell lines, including L6 and C2C12 myotubes, have been found to be deficient in GLUT4 expression.

Accordingly, in our laboratory we developed primary cultures of neonatal rat skeletal muscle that represented a suitable system for investigating the molecular basis of TNF-alpha-induced insulin resistance. When these cells were differentiated until the formation of myotubes in low-serum medium and then maintained in low-glucose medium to mimic the physiological environment, they responded to acute insulin stimulation by increasing glucose uptake and GLUT4 translocation to plasma membrane (de Alvaro et al., 2004). Chronic exposure to TNF-alpha impaired both insulin-stimulated glucose uptake and GLUT4 translocation, without affecting the content of GLUT4 protein or the state of differentiation of the myotubes (de Alvaro et al., 2004), in agreement with the effect produced in muscle in vivo. The molecular mechanism underlying TNF-alpha-mediated insulin resistance could involve activation of stress kinases and proinflammatory pathways, as was observed in neonatal myotubes (de Alvaro et al., 2004). Acute insulin stimulation also produced a transient phosphorylation of p38MAPK (Conejo et al., 2002), but insulin activated the isoform α meanwhile TNF-alpha activates the β. When chemical inhibitors were used to evaluate the contribution of sustained activation of stress kinases by TNF-α to insulin resistance, only the inhibition of p38MAPK completely restores insulin-stimulated glucose uptake and insulin signalling (de Alvaro et al., 2004). In this regard, adenovirus-mediated transfections of constitutively active MKK6/3 mutants in L6 myotubes were reported to diminish glucose transport induced by insulin via down-regulation of GLUT4 gene expression (Fujishiro et al., 2003). Furthermore, the Ser307...
residue of IRS-1 seems to be one of the residues phosphorylated by TNF-alpha via p38MAPK, although other residues in either IRS-1 or IRS-2 or IR cannot be excluded. The role of p38MAPK in inflammatory diseases, including obesity and cardiovascular dysfunction, is well recognized because this kinase regulates the biosynthesis of proinflammatory cytokines, as well as is involved in the signalling transduction pathways activated by cytokines, as elegantly reviewed (Cuenda and Rousseau, 2007).

Several reports have also implicated IKK activation by TNF-alpha on Ser phosphorylation of IRS-1, and aspirin rescues insulin-induced glucose uptake in 3T3-L1 adipocytes treated with TNF-alpha (Yuan et al., 2001; Gao et al., 2003). In this regard, activation of IKK dependent on the functionality of p38MAPK was observed during chronic treatment with TNF-alpha in neonatal myotubes. Moreover, inhibition of IKK activation with salicylate completely restored insulin signalling to normal levels, despite the presence of TNF-alpha (de Alvaro et al., 2004), but salicylate does not affect p38MAPK activation by the cytokine. Then, IKK could act downstream of p38MAPK and could mediate TNF-alpha-induced insulin resistance on skeletal muscle, as summarized in Figure 4. Similarly to this finding TNF-alpha also affects insulin signalling negatively in endothelial cells via p38 MAPK (Li et al., 2007).

Interestingly, knock-down of MAP4K4 in primary human skeletal muscle cells, the upstream kinase of ERK1/2 and JNK inhibits the effects of TNF-alpha, further acknowledging the involvement of these kinases in the transmission of TNF-alpha induced signalling (Bouzakri and Zierath, 2007).

Additionally, the phosphatase PTP1B, a negative regulator of insulin signalling acting, has been shown to be crucial for TNF-alpha signalling. The expression and activity of PTP1B has been found increased in pathological insulin-resistant states such as obesity (Ahmad et al., 1997). Moreover, noncoding polymorphisms in the PTP1B gene have been found in different populations, displaying increased phosphatase muscle expression and being associated with insulin resistance (Bento et al., 2004). In this regard, transgenic overexpression of ptp1b in muscle causes insulin resistance, showing impaired insulin signalling and decreased glucose uptake in this tissue (Zabolotny et al., 2004). By contrast, mice lacking PTP1B exhibit increased insulin sensitivity at 12 weeks of age (attributable to enhanced phosphorylation of IR in liver and skeletal muscle), resistance to weight gain on a high-fat diet, and an increased basal metabolic rate (Elchebly et al., 1999; Klaman et al., 2000). The PTP1B-deficient mice had circulating insulin concentrations that were about half those of control animals. Thus, these mice appeared to be more insulin sensitive, because they maintained lower glucose concentration with significantly reduced amounts of insulin. In the fasted state, there were no significant differences in concentrations of glucose or insulin (Elchebly et al., 1999). Furthermore, PTP1B-deficiency also reduces the diabetic phenotype in mice with polygenic insulin resistance (Xue et al., 2007). Moreover, treatment with PTP1B antisense oligonucleotide improves insulin sensitivity in db/db mice and increases insulin signalling in WAT and liver in ob/ob mice (Gum et al., 2003).

In our laboratory we generated myocytes lacking PTP1B that displayed enhanced insulin sensitivity in IR autophosphorylation and downstream signalling, including IRS-1 and IRS-2 Tyr phosphorylation, PI3K associated activation and AKT Ser/Thr phosphorylation. The phosphorylation was detected at lower insulin doses and at shorter times in PTP1B–/– cells than in wild-type cells (Nieto-Vazquez et al., 2007). Because activation of PI3K and AKT controls glucose transport, we detected an increased insulin-stimulated glucose uptake and GLUT4 translocation to plasma membrane in PTP1B–/– cells.

Figure 4. Treatment with TNF-α impairs insulin-stimulated glucose uptake in myocytes at the level of the IRS-1 by a double mechanism that involves: (1) Ser phosphorylation by IKK and p38MAPK and (2) Tyr dephosphorylation by the phosphatase PTP1B. Inhibition of IKK activation with salicylate, and ablation of PTP1B restores insulin sensitivity in the presence of the cytokine.
versus wild-type cells (Nieto-Vazquez et al., 2007). This result was expected because decreased glucose uptake in skeletal muscle was observed when PTP1B was overexpressed selectively in muscle of transgenic mice (Zabolotny et al., 2004). Moreover, recent data indicate that muscle-specific PTP1B−/− mice exhibited improved systemic insulin sensitivity and enhanced glucose tolerance under high-fat diet (Delibegovic et al., 2007).

As long as TNF-alpha is a strong candidate to produce insulin resistance in skeletal muscle for the reasons mentioned above, the lack of PTP1B might confer protection against TNF-alpha-induced insulin resistance. In this regard, chronic exposure to TNF-alpha does not induce insulin resistance either on glucose uptake or on insulin signalling in PTP1B-deficient myocytes (Nieto-Vazquez et al., 2007). Moreover, PTP1B−/− mice showed complete protection against TNF-alpha-induced insulin resistance during the glucose and insulin tolerance tests (Nieto-Vazquez et al., 2007). Accordingly, the lack of PTP1B expression confers protection against TNF-alpha-induced insulin resistance in skeletal muscle either in vitro or in vivo (Nieto-Vazquez et al., 2007). Consequently, at least part of the effects elicited by TNF-α on pathways involving reversible tyrosine phosphorylation may be exerted through the dynamic modulation of PTP1B expression.

Therefore, TNF-alpha impairs insulin action in myocytes at the level of IRS-1 by a double mechanism that involves: (1) Ser phosphorylation by IKK and p38MAPK at the Ser307 residue and (2) Tyr dephosphorylation by PTP1B. Accordingly, inhibition of IKK activation with salicylate and ablation of PTP1B restores insulin sensitivity in myocytes in the presence of the cytokine, as summarized in Figure 4. In this regard, new mono- and disalicylic acid derivatives have been used very recently as PTP1B inhibitors and potential antiobesity drugs (Shrestha et al., 2007).

**Effect of TNF-alpha on muscle lipid metabolism**

In contrast to the numerous investigations examining the effect of TNF-alpha glucose uptake into skeletal muscle, only few studies have investigated the effect of TNF-alpha on fatty acid (FA) metabolism in muscle. However, TNF-alpha does increase FA esterification into diacylglycerol (DAG) ultimately leading to increased DAG content while it does not affect FA oxidation, uptake or esterification into triacylglycerol (TAG) in skeletal muscle and other cell types (Bruce and Dyck, 2004). Since DAG is a potent activator of the PKC isoforms beta and theta, which Ser phosphorylate IRS-1, this offers further mechanistic insight into the pathway by which TNF-alpha potentially controls insulin sensitivity negatively. PKC has been shown to be able to phosphorylate PTP1B. Hence, these results would suggest a mechanism along the axis PKC, PTP1B, MAPK to an insulin signal inhibiting IRS-1 Ser phosphorylation. In addition to these insulin signal impairing actions leading to reduced glucose uptake, glycogen synthesis in muscle is inhibited by TNF-alpha which contributes greatly to hyperglycaemia.

As mentioned above, lipids released from adipocytes following TNF-alpha exposure are transported to non-adipose tissues like skeletal muscle as FFA (Sharma and Chetty, 2005). Excess FFA are stored as triglyceride droplets in muscles, in turn contributing to an enhanced insulin resistance.

In addition, direct inhibition of AMPK activity via transcriptional upregulation of PP2C by TNF-alpha in mice has been proposed (Steinberg et al., 2006). PP2C has only recently been indicated as a regulator of AMPK activity in cardiomyocytes and skeletal muscle cells (Steinberg et al., 2006). TNF-alpha treatment of L6 myocytes for 8 h impaired AMPK activity; thus, in turn leading to an increased activity of acetyl CoA carboxylase leading to a reduction of FA transport and subsequently to a diminished utilization of lipids. This contributes even further to the negative spiral of FA accumulation and insulin resistance. Mice lacking the TNF-alpha receptors were protected from this effect as one would expect when the observations are mediated by TNF-alpha (Uysal et al., 1997). Not surprisingly therefore, in humans intramyocellular lipid, and TNF-alpha concentrations were amongst the strongest predictors of insulin resistance.

As mentioned, it has been suggested that even though TNF-alpha has no direct effect on beta-oxidation in skeletal muscle (Bruce and Dyck, 2004) the high levels of circulating FFA would enhance beta-oxidation, which in turn would diminishes glucose uptake and oxidation. In fact, for example during the “first days” on a high-fat diet, when fat oxidation does not equal fat intake, subjects are in negative carbohydrate balance, leading to a decrease in the body’s glycogen stores, a decrease in glucose oxidation and to increased fat oxidation. However, the consumption of a high-fat diet for “one week” is accompanied by molecular changes that would favour fat storage in muscle rather than oxidation (Schrauwen-Hinderling et al., 2005). This circumstance is obviously similar to continuous exposure of skeletal muscle to FFA due to impaired adipocyte action as a result of high TNF-alpha levels, as described above.

Time dependent differences, i.e., early and late effects of TNF-alpha and lipid accumulation appear to be of crucial importance in this context. Concluding, the above findings rather suggest that in a long-term perspective beta-oxidation is impaired due to TNF-alpha exposure of skeletal muscle, ultimately enhancing the problem of lipid accumulation in skeletal muscle which causes insulin resistance to progress.

The clue to understanding impairment of insulin action in skeletal muscle due to TNF-alpha
possibility lies in the combination of two factors: downregulation of mitochondrial FA oxidative due to diminished AMPK activity and lipid accumulation due to increased FFA in circulation. AMPK is involved in the expression control of the PPARγ coactivator (PGC)-1alpha and even directly controls its function in skeletal muscle (Jager et al., 2007). PGC-1alpha in turn has been recognised to be the crucial determining factor of mitochondrial function. Hence, PGC-1alpha impairment ultimately leads to impaired mitochondrial function. The combination of lipid accumulation in skeletal muscle concomitantly with impaired mitochondrial function and reduced beta-oxidation has been described as the crucial trigger of impaired insulin sensitivity (reviewed in (Schrauwen-Hinderling et al., 2005)).

As described for adipocytes, activators of PPARs, especially PPARγ, seem to hold a potential in the treatment of TNF-alpha induced insulin resistance. In skeletal muscle, however, PPARγ appears to be the more predominant isoform and we have previously described the potential of PPARγ selective ligands in enhancing insulin sensitivity in human skeletal muscle cells (Kramer et al., 2005).

In cardiomyocytes a PPARγ selective ligand inhibited lipopolysaccharide (LPS)-induced TNF-alpha production from cardiomyocytes (Ding et al., 2006). Even more interesting was that adenoviral-mediated overexpression of PPARγ strongly inhibited TNF-alpha expression; whereas overexpression of a PPARγ mutant with ablated ligand binding domain did not show a similar effect. Hence it is tempting to speculate that PPAR-delta may exhibit similar effects on TNF-alpha in skeletal muscle. Unfortunately, no data is available as yet.

However, we have demonstrated that PPARγ activation using a specific ligand (which is currently under scrutiny in clinical trial) in human skeletal muscle cells enhances FA oxidation via expression changes, hence addressing the problem of diminished mitochondrial action as caused by TNF-alpha (Kramer et al., 2007). Further investigations will be necessary to address the question if PPARγ also is a regulator of TNF-alpha expression in skeletal muscle.

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Insulin resistance associated to obesity: the link TNF-alpha


