Revisión

Contribution of TNF-α to Obesity-Associated Insulin Resistance

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ABSTRACT

Insulin resistance is an important contributor to the pathogenesis of type 2 diabetes, and obesity is a risk factor for its development, due in part to the fact that adipose tissue secretes proteins called adipokines, that may influence insulin sensitivity. Among these molecules, TNF-α has been proposed as a link between obesity and insulin resistance because TNF-α is overexpressed in adipose tissues of obese animals and humans, and obese mice lacking either TNF-α or its receptor show protection for developing insulin resistance. The direct exposure to TNF-α induced a state of insulin resistance on glucose uptake in myocytes and brown adipocytes, due to the activation of pro-inflammatory pathways that impair insulin-signaling at the level of the IRS proteins. In this regard the residue Ser307 in IRS-1 has been identified as a site for TNF-α-inhibitory effects in myotubes, with

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p38MAPK and IKK being involved in the phosphorylation of this residue. Conversely, serine phosphorylation of IRS-2 mediated by TNF-α activation of MAPKs was the mechanism found in brown adipocytes. The phosphatase PTP1B acts as a physiological negative regulator of insulin signaling by dephosphorylating the phosphotyrosine residues of the insulin receptor and IRS-1, and PTP1B expression is increased in muscle and white adipose tissue of obese and diabetic humans and rodents. Moreover, up-regulation of PTP1B expression was recently found in cells treated with TNF-α. Accordingly, myocytes and primary brown adipocytes deficient on PTP1B are protected against insulin resistance by this cytokine. Furthermore, down-regulation of PTP1B activity is also possible by the use of pharmacological agonists of nuclear receptors that restore insulin sensitivity in the presence of TNF-α. In conclusion, the lack of PTP1B in muscle and brown adipocytes increases insulin sensitivity and glucose uptake and could confer protection against insulin resistance induced by adipokines.

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

RESUMEN

Contribución del TNF-α a la insulinorresistencia asociada a la obesidad

Entre las complicaciones asociadas a la Obesidad**,** tiene una especial relevancia el desarrollo de resistencia a la insulina, siendo el primer eslabón de una amplia patología conocida como diabetes tipo 2. La Obesidad se considera como un estado crónico de inflamación de baja intensidad, como indican los niveles circulantes elevados de moléculas proinflamatorias. Se ha propuesto al TNF-α como el nexo de unión entre adiposidad y desarrollo de resistencia a insulina ya que la mayoría de los pacientes con diabetes tipo 2 son obesos y tienen aumentada la expresión de TNF-α en sus adipocitos, y los animales obesos delecionados para la función del TNF-α o su receptor no desarrollan resistencia a insulina. Las citocinas proinflamatorias producidas por los adipocitos y/o macrófagos activan quinasas de estrés, proinflamatorias y factores de transcripción que actúan sobre los tejidos periféricos (entre ellos el músculo, así como el propio tejido adiposo) produciendo resistencia a la acción de la insulina, que es un defecto en la señalización a varios niveles. En concreto, el TNF-α activa la quinasa p38MAPK que fosforila en residuos de serina a los IRSs, bloqueando su fosforilación en tirosina en respuesta a la insulina, tanto en adipocitos marrones como en miocitos. Muy recientemente hemos observado que la fosfatasa PTP1B también está implicada en la resistencia a insulina por TNF-α en ambos modelos. En la Clínica se está utilizando actualmente el tratamiento con tiazolidindionas en pacientes con diabetes tipo 2. Otros agonistas de receptores nucleares empiezan a aparecer en la bibliografía como potenciales sensibilizadores a acción de la insulina, entre ellos el LXR, que puede antagonizar la señalización proinflamatoria tanto en los propios adipocitos como en el músculo.

Palabras clave: transporte de glucosa, LXR, PTP1B, p38MAPK, rosiglitazona.

INTRODUCTION

Insulin exerts a dominant role in regulating glucose homeostasis though orchestrated effects on promotion of glucose uptake in peripheral tissues such as muscle and fat, and suppressing hepatic glucose uptake. Insulin initiates the biological effects in target cells by binding to, and activating endogenous tyrosine kinase receptors. Insulin receptors (IR) are believed to transduce signals by phosphorylation on tyrosine residues of several cellular substrates including insulin receptor substrate (IRS) proteins 1,2,3 and 4 (1). A number of signalling pathways can be activated downstream of IRS proteins. Molecules containing Src homology 2 domain, including the regulatory subunits of phosphatidylinositol 3-kinase (PI3K), Grb-2 and others, are recruited to the tyrosine phosphorylated IRSs and transmit a cascade of signals, which consists in two major elements, i.e., Ras/Raf/ extracelullar-signal regulated kinase (ERK) and PI3K/ AKT/p70S6 kinase pathways. A parallel mitogen-activated protein kinase (MAPK), p38MAPK, has been shown to be stimulated by insulin in several cell systems including skeletal muscle (2). On the other had, the insulin signaling cascade is negatively regulated by protein phosphatases, including tyrosine-, serine- and lipid-phosphatases. Most notably, protein-tyrosine phosphatase (PTP)1B acts dephosphorylating the phosphotyrosine residues of the IR and IRS-1 (Figure 1).

Both muscle and fat are target tissues for insulin action. In skeletal muscle insulin and insulin-like growth factors (IGF)s control differentiation and regeneration of the tissue. The signaling pathways that accompany the formation of myotubes in C2C12 cells involved sequential activation of PI3K, AKT, P70S6 kinase and p38MAPK cascade in parallel to the induction of muscle-specific proteins, with a concomitant inhibition of ERK (3). Adipose tissues, including the most abundant white adipose tissue (WAT) and the thermogenic one (BAT) are also under insulin control. In fetal brown adipocytes insulin and IGF-I, acting independently and thought the activation of the IRS-PI3K signaling pathway, up-regulated the expression of adipogenic-related genes at the transcriptional level, as reviewed (4). In addition to adipogenesis, insulin/IGF-I are thermogenic factors through the ability to increase the uncoupling-protein (UCP)-1 gene

transcription rate by a mechanism dependent on activation of PI3K and p21ras signalling cascades (4).
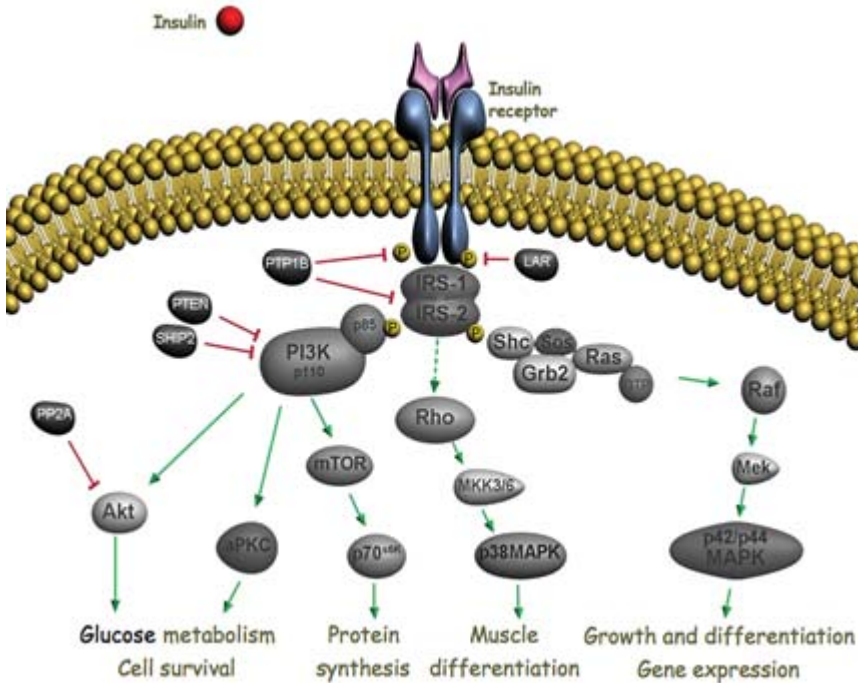

FIGURE 1. *A complex insulin intracellular signaling cascade controls the pleoitropic effects of this hormone.*

Glucose transport is the main metabolic aspect regulated by insulin in peripheral tissues and is maintained mainly by the activity of the insulin-regulated glucose transporter (GLUT)4, although the ubiquitous GLUT1 glucose transporter is often expressed at appreciable levels. The mechanisms by which insulin could modulate GLUT4 function contributing to increase glucose uptake included translocation of pre-existing intracellular retained populations of GLUT4 proteins to the cell surface and up-regulation of the amount of GLUT4 protein. It is well established the participation of PI3K and its downstream of targets, AKT and the atypical PKC isoforms z and l? in insulin-induced GLUT4 redistribution to the plasma membrane in adipocytes and myocytes (5, 6). The expression of GLUT4 is subjected to tissue-specific, hormonal and metabolic

regulation and, in states of relative insulin deficiency such as streptozotocin-induced diabetes and chronic fasting GLUT4 gene expression is down-regulated, a situation that is reversed by insulin treatment and refeeding (7). However, studies in cells failed to establish a stimulatory role of insulin on GLUT4 expression, suggesting that additional factors could be involved in this tissue. In this regard, dexamethasone, a synthetic glucocorticoid, has been shown to increase GLUT4 mRNA expression in myocytes, white adipocytes and brown adipocytes, in these cells acting as a co-regulator with insulin (8). This review is focused on examining alterations on insulin action in states of insulin resistance associated with obesity.

OBESITY, INFLAMATION AND INSULIN RESISTANCE

Type 2 diabetes mellitus is a complex metabolic disease with an environmental and genetic component affecting over 5% of the population in Western societies. This disease represents the final stage of long existing metabolic disturbances with deleterious effects on the vascular system, tissues and organs. Insulin resistance, 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 (1). At the molecular level, insulin resistance correlates with impaired insulin signalling in peripheral tissues (Figure 2). Insulin resistance in adipose tissues 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 increased hepatic glucose production (1). Both genetic and environmental factors can contribute to develop insulin resistance and in the second group, obesity has been proposed as an important contributor.
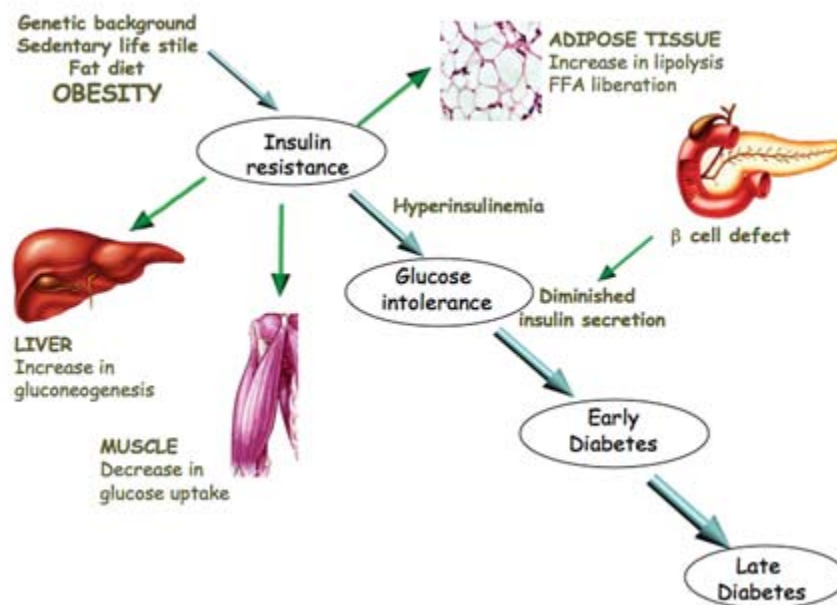
Figure 2. *The different steps in the development of type 2 diabetes.*

Obesity is a risk factor to develop type 2 diabetes, due in part 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 signaling altering insulin-mediated processes, including glucose homeostasis and lipid metabolism (9). Accordingly, obesity is being considered a chronic state of low intensity inflammation. On the other hand, recent studies reveal that obesity is also associated with an increase in adipose tissue infiltration of macrophages, which contribute to the inflammatory process through the additional secretion of cytokines (10). The mechanisms by which WAT recruits and maintains macrophages could involve expression of monocyte chemoattractan proteins and intercellular adhesion molecule-1, as has been recently described (11). Tumor necrosis factor (TNF)-α. has been proposed as a link between adiposity and

the development of insulin resistance because the majority of type 2 diabetic subjects are obese; TNF-α is highly expressed in adipose tissues from obese subjects and obese mice lacking either TNF-α or its receptors showed protection for developing insulin resistance (12). Rather than systemically, TNF-a seems to acts locally at the site of WAT through autocrine/paracrine mechanisms having effects on insulin resistance and inducing interleukin-6 (9).

On the other hand, TNF-α has lypolitic and anti-adipogenic effects on WAT (9). 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 had been reported to induce insulin resistance in peripheral tissues and the production of these molecules could be consequence of activation of sphingomyelinase or lipolysis by TNF-α (9). Several other mediators that are activated in response to TNF-α such as stress kinases and inflammatory pathways could also contribute to insulin resi. In this regard, an increased phosphorylation of p38MAPK in human adipocytes and muscle from type 2 diabetic subjects has been described (13).

Direct exposure of isolated cells to TNF-α inhibits insulin signalling and induces a state of insulin resistance in several systems including 3T3-L1 cells and human primary adipocytes by affecting IRS proteins (12). The mechanism of IRSs regulation involves proteasome-mediated degradation, phosphatase-mediated dephosphorylation and serine phosphorylation of IRS-1 that converts IRS-1 in an inhibitor of the IR tyrosine kinase activity, as reviewed (1, 14). Both ERK and c-Jun N-terminal kinase (JNK) have been proposed to mediate TNF-α serine/threonine phosphorylation of IRS-1 in white adipocytes, being identified the residue Ser307 as a site for TNF-α phosphorylation of IRS-1. In this regard, ablation of *jnk*1 decreases the development of insulin resistance associated with dietary obesity. Furthermore, ERK and p38MAPKs could also inhibit insulin signaling by TNF-α at the level of IRS-1 and IRS-2 in 3T3-L1 adipocytes, whereas JNK could mediate the feedback inhibitory effect of insulin (1, 14). Others works also implicate inhibitor kB kinases (IKK) activation by TNF-α on serine phosphorylation of IRS-1, meanwhile IKK inhibition with salicylate or targeted disruption of

*ikk*b?? reversed obesity and diet-induced insulin resistance (15). Our group has explored in deep the mechanism by which TNF-α produces insulin resistance on glucose uptake in two physiological models: murine neonatal myocytes and fetal brown adipocytes.

INSULIN RESISTANCE BY TNF-α IN MYOCYTES: AMELIORATION BY TREATMENT WITH SALICYLATE

Skeletal muscle is responsible for 80% of the glucose disposal of the body and is the organ where insulin resistance is first detectable. Acute insulin treatment stimulates glucose transport in myocytes largely by mediating translocation of GLUT4 to the plasma membrane, being accomplished by activation of PI3K, AKT and several PKC isoforms including ζ, λ, α and δ (16, 17). 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 (18). The AKT substrate of 160 kDa (AS160) has recently emerged as a point of convergence for both effectors of glucose transport and seems to modulate GLUT4 trafficking (18). While 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 signaling.

Both genetic and environmental factors have been identified to contribute to insulin resistance in skeletal muscle. The genetic approach shows that targeted disruption of the *igf-1* and *ir*, or of *glut4*, selectively, in murine skeletal muscle causes insulin resistance and insulin intolerance (19-21). Furthermore, IRS-1 is the key mediator of insulin action in muscle since IRS-1-deficient mice show insulin resistance in muscle. In this regard, IRS-1 silencing using small interfering RNA caused a marked reduction in insulin-inducing acting remodeling and GLUT4 translocation, but silencing IRS-2 was without effect (22). Among the environmental factors, adipokines secreted by adipocytes and/or macrophages in the obese state are

main candidates. In this regard, TNF-α blocks skeletal muscle differentiation causes sarcopenia and produces insulin resistance in skeletal muscle of healthy humans and in primary cultures of mouse skeletal muscle (23). Although ceramide and FFA have been reported to produce insulin resistance in skeletal muscle, a direct effect of TNF-α in this tissue has been the matter of controversia. Several reports did not detect TNF-α inhibitory action on insulin-induced glucose uptake, although TNF-α *per se* highly increased basal glucose uptake. However, others observed an inhibitory effect 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.

Consequently, in our laboratory we developed primary cultures of neonatal rat skeletal muscle that represented a suitable system for investigating the molecular basis of TNF-α-induced insulin resistance. When these cells were differentiated in low serum until the formation of myotubes and maintained in low glucose medium to mimick the physiological environment, responded to acute insulin stimulation by increasing glucose uptake and GLUT4 translocation to plasma membrane (24). Chronic exposure to TNF-α impaired both insulin-stimulated glucose uptake and GLUT4 translocation, without affecting the content of GLUT4 protein or the state of differentiation of the myotubes (24), in agreement with the effect produced in muscle *in vivo*. The molecular mechanism underlying TNF-α-mediated insulin resistance could involve activation of stress kinases and proinflamatory pathways, as was observed in neonatal myotubes (24). Acute insulin stimulation also produced a transient phosphorylation of p38MAPK (3), but insulin activated the isoform a meanwhile TNF-α activates the b. 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 signaling (24). In this regard, adenovirus-mediated transfections of constitutively active MKK6/3 mutants in L6 myotubes have been reported to diminish glucose transport induced by insulin via down-regulation of GLUT4 gene expression (25).

Furthermore, the residue Ser307 of IRS-1 seems to be one of the residues phosphorylated by TNF-α via p38MAPK, although other residues in either IRS-1 or IRS-2 or IR can not be discarding. The role of p38MAPK in inflamatory diseases, including obesity and cardiovascular dysfunction, is well recognized since this kinase regulated the biosynthesis of pro-inflamatory cytokines as well as is involved in the signaling transduction pathways activated by cytokines, as elegantly reviewed (2).

Several reports have also implicated IKK activation by TNF-α on serine phosphorylation of IRS-1, and aspirin rescues insulin-induced glucose uptake in 3T3-L1 adipocytes treated with TNF-α (15, 26). In this regard, activation of IKK dependent on the functionality of p38MAPK was observed during chronic treatment with TNF-α in neonatal myotubes. Moreover, inhibition of IKK activation with salicylate completely restores insulin signaling to normal levels, despite the presence of TNF-α (24) but salicylate does not affect p38MAPK activation by TNF-α. Then, IKK could act downstream of p38MAPK and could mediate TNF-α-induced insulin resistance on skeletal muscle, as summarized in Figure 3.
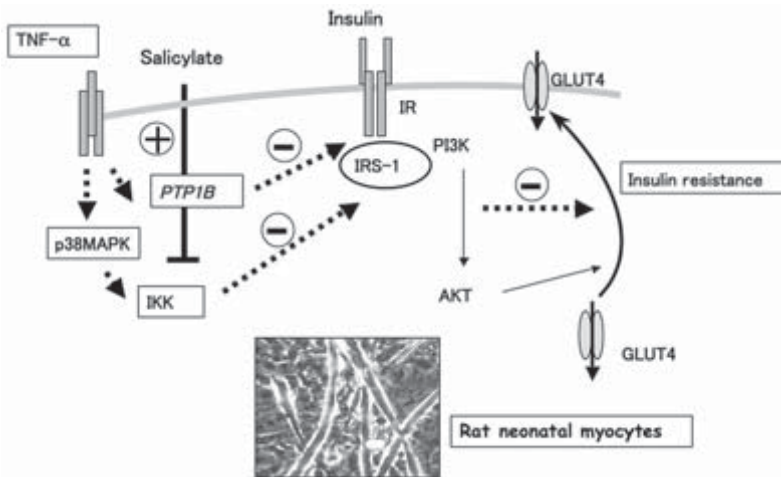

Figure 3.- *Treatment with TNF-α impairs insulin-stimulated glucose uptake in myocytes at the level of the IRS-1 by a double mechanism* that involves 1) serine phosphorylation by IKK and p38MAPK and 2) tyrosine dephosphorylation by the phosphatase PTP1B. Inhibition of IKK activation with salicylate, and ablation of PTP1B restores insulin sensitivity in the presence of the cytokine.

LACK OF PTP1B CONFERS PROTECTION AGAINST TNF-α-INDUCED INSULIN RESISTANCE IN SKELETAL MUSCLE

In pathological insulin-resistant states such as obesity, PTP1B expression and activity are increased in muscle and WAT of humans and rodents (27). Moreover, noncoding polymorphisms in the *PTP1B* gene have been found in different populations, displaying increased phosphatase muscle expression and being associated with insulin resistance (28). In this regard, transgenic overexpression of *ptp1b* in muscle causes insulin resistance, showing impaired insulin signaling and decreased glucose uptake in this tissue (29). By contrast, mice lacking PTP1B exhibit increased insulin sensitivity at 12 wk 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 (30, 31). 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 (30). Furthermore, PTP1B-deficiency also reduces the diabetic phenotype in mice with polygenic insulin resistance (32). Moreover, treatment with PTP1B antisense oligonucleotide improves insulin sensitivity in db/db mice and increases insulin signaling in WAT and liver in *ob*/*ob* mice (33).

The fact that primary neonatal myotubes developed in our laboratory have provided a unique tool for *in vitro* study of insulin sensitivity (24), prompted us to generate immortalized myocytes from wild-type and PTP1B-deficient neonates. Cell lines lacking PTP1B display enhanced insulin sensitivity in IR autophosphorylation and downstream signaling, including IRS-1 and IRS-2 tyrosine phosphorylation, PI3K associated activation and AKT serine/ threonine phosphorylation. The phosphorylation was detected at lower insulin doses and at shorter times in PTP1B-deficient cells than in wild-type cells (34). Because activation of PI3K and AKT controls glucose transport, we detect an increased insulin-stimulated glucose uptake and GLUT4 translocation to plasma membrane in PTP1B$^{-/-}$ cells versus wild-type cells (34). This result was not

unexpected since a decreased glucose uptake in skeletal muscle was observed when PTP1B was overexpressed selectively in muscle in transgenic mice (29). Moreover, recent data indicate that muscle-specific PTP1B-/- mice exhibed improved systemic insulin sensitivity and enhanced glucose tolerance under high-fat diet (35).

Given that TNF-α 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-α-induced insulin resistance. In this regard, chronic exposure to TNF-α does not induce insulin resistance either on glucose uptake or on insulin signaling in PTP1B-deficient myocytes (34). Moreover, PTP1B$^{-/-}$ mice showed complete protection against TNF-α-induced insulin resistance during the glucose and insulin tolerance tests (34). Accordingly, the lack of PTP1B expression confers protection against TNF-α-induced insulin resistance in skeletal muscle either *in vitro* or *in vivo*. However, PTP1B ablation in mice not only affects insulin sensitivity in muscle and liver, but also beta cell function, which might be contributing to this protective effect.

We have explored whether this protection against the deleterious effect of TNF-α was the molecular consequence of enhanced insulin signaling provoked by PTP1B deficiency or was produced by direct abolition of some effects of TNF-α. In this regard, treatment with TNF-α significantly enhanced PTP1B protein expression and activity either in neonatal myocytes or in adult mice, meanwhile the expression of other phosphatases such as PTEN, SH-PTP2 and protein-phosphatase (PP)2A were not affected (34). In consequence, the genetic ablation of PTP1B avoids this action of TNF-α and assures complete protection against insulin resistance by this cytokine. Therefore, 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.

Accordingly, TNF-α impairs insulin action in myocytes at the level of IRS-1 by a double mechanism that involves 1) serine phosphorylation by IKK and p38MAPK at the residue Ser307 and 2) tyrosine dephosphorylation by PTP1B. Therefore, 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 3. In this regard, new mono- and disalicylic acid derivates have been very recently used as PTP1B inhibitors and potential anti-obesity drugs (36).

INVOLVEMENT OF MAPK AND PHOSPHATASES IN THE DEVELOPMENT OF INSULIN RESISTANCE BY TNF-α IN BROWN ADIPOCYTES

BAT is present and active in mammal newborns and is responsible for their successful defense of body temperature without shivering. When BAT 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 BAT 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 (37). 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 (38). These findings point out that BAT is present and active in a substantial fraction of adult humans and that may thus be considered of metabolic significance in human physiology.

Glucose transport in brown adipocytes is maintained mainly by the activity of GLUT4, and insulin treatment stimulates glucose transport by mediating GLUT4 translocation in a PI3K-, AKT- and PKCζ-dependent manner (39, 40). Furthermore, inhibition of phospholipase C (PLC)γ activity precludes insulin stimulation of glucose uptake, GLUT4 translocation and actin reorganization, indicating that PLCγ, through the production of phosphatidic acid, is a link between IR and PKCζ (40). In addition, IRS-2 seems to be crucial in mediating glucose uptake in brown adipocytes (41).

TNF-α acts as a negative regulator of adipogenic and thermogenic differentiation and induces insulin resistance in BAT (4), in a similar fashion as reported in 3T3-L1 cells and in primary human adipocytes.

Moreover, TNF-α-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 (41). As a further step, we have identified ceramide production as one of the mediators of insulin-resistance by TNF-α and exogenously added C2-ceramide inhibited AKT activity throughout a ceramide-activated phosphatase (41). Furthermore, *de novo* ceramide generation produced by chronic treatment with TNF-α induces insulin resistance on GLUT4 gene expression in brown adipocytes by interfering C/EBPα acumulation (42). Moreover, stress kinases activated in response to TNF-α, meanly ERK and p38MAPKs also contribute to insulin resistance in brown adipocyte primary cultures (43).

Recently, a significant enhancement of PTP1B mRNA, protein and activity was observed in brown adipocytes treated with TNF-〈 (44). As expected, the lack of PTP1B these cells confered protection against TNF-α-induced insulin resistance on glucose uptake and insulin signalling (44). Therefore, modulation of genes such as PTP1B might contribute to the pathogenesis of TNF-〈-induced insulin resistance in murine brown adipocytes.

Accordingly, a complex mechanism impairs the normal response to insulin on GLUT4 translocation in brown adipocytes in the presence of TNF-α including 1) potential serine/treonine phosphory-lation of IRS-2 by MAPKs, weakening the tyrosine 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 4.

PPARγ AND LXR AGONISTS AMELIORATE TNF-α-INDUCED INSULIN RESISTANCE IN BROWN ADIPOCYTES

Nuclear receptors, such as retinoic acid receptor, peroxisome proliferator activated receptors (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 (TZD), such as pioglitazone and

rosiglitazone, 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 (45). Moreover, PPARγ plays a critical role in the adipogenic differentiation process since its deletion in WAT resulted in marked adipocyte hypocellularity and hypertrophy, elevated levels of FFA and decreased levels of plasma leptin (46). These mice showed insulin resistance and were more susceptible to high fat diet-induced liver steatosis. Thus, TZD may exert effects on adipogenesis and gene expression, such as formation of new, small, and insulin sensitive fat cells, reduced production of TNF- and/or increased expression of adiponectin in WAT (45). Moreover, TZD up-regulated lipoprotein lipase (LPL) and hormone sensitive lipase (HSL) gene expression and increased insulin responsiveness on lipolysis and lipogenesis in human subcutaneous adipocytes, consistent with the weight gain observed during the treatment (46). Nevertheless, the
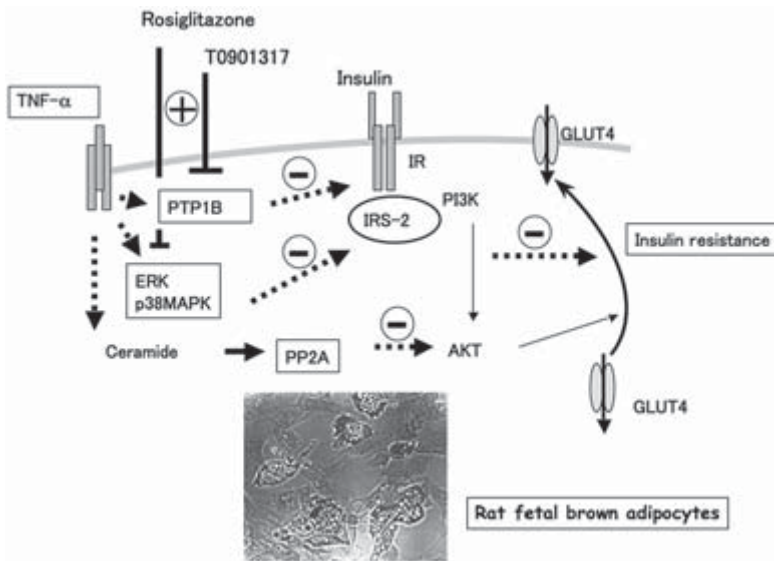

FIGURE 4. *Insulin resistance by TNF-α in brown adipocytes* involves 1) serine/ treonine phosphorylation of the IRS-2 by ERK and p38MAPK; 2) generation of ceramide and activation of the phosphatase PP2A, and 3) modulation of PTP1B activity. Inhibition of ERK and p38MAPK activation with rosiglitazone, and down-regulation of PTP1B with either rosiglitazone or T0901317? ameliorates TNF-α-induced insulin resistance.

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mechanisms employed for TZD to increase insulin sensitivity are still unclear. Rosiglitazone reduced FFA circulating levels and potentiated insulin-stimulated AKT phosphorylation in WAT and muscle from Zucker obese rats (46). Moreover, other evidence favors TZD effects on glucose uptake by modulating changes at the level of the expression of IRS-2, p85-PI3K, GLUT4 or GLUT1 either in white adipocyte or muscle cells (46). However, several limitations with this therapy, such as increased adiposity, secondary insulin resistance in WAT, and pro- and anti-atherogenic effects, are currently emerging.

LXRα is expressed predominantly in liver, WAT, and macrophages, and is activated by naturally produced oxysterols, as well as by synthetic compounds such as T0901317 and GW3965 (47). Although LXR function has been elucidated in detail with respect to cholesterol and lipid metabolism, new findings have emerged LXR as important regulators of glucose metabolism (47). Recent studies have reported low plasma glucose, 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. Several evidences suggest that LXR activity may be important in WAT because these nuclear receptors are abundant in subcutaneous fat preferentially (47), LXR expression is regulated by the key adipocyte transcription factor PPARγ and many LXR target genes are also highly expressed in adipocytes. 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.

BAT is also a target tissue for nuclear receptors agonists since highly expressed PPARγ, LXRα, and LXRβ (47). In this regard, the PPARγ agonist rosiglitazone up-regulates the expression of LPL, HSL and UCP-1 in brown adipocytes (48) as well as produces insulin sensitization by increasing the expression of IR and its tyrosine kinase activity (49). 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-α, and restored the insulin signaling cascade leading to normalization of insulin-induced glucose uptake (43). Furthermore,

rosiglitazone decreased the activity of PTP1B (49), and improved insulin sensitivity concomitant with an increase in thermogenic differentiation, contributing globally to an accelerated glucose disposal in BAT. 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 (50). On the other hand, synthetic LXR agonists ameliorate TNF-α–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 IR/IRS-2/PI3K/AKT, and could be due to the fact that T0901317 precludes the enlargement in PTP1B expression produced by TNF-α (44), supporting the hypothesis of nuclear receptors LXR are interesting targets for drug treatment of insulin-resistant conditions.

Therefore, inhibition of ERK and p38MAPK activation with rosiglitazone and down-regulation of PTP1B with either rosiglitazone or LXR agonists restores insulin sensitivity in brown adipocytes in the presence of TNF-, as summarized in Figure 4.

CONCLUSIONS

The mechanism by which TNF-α produces insulin resistance in murine skeletal muscle and in BAT shows tissue specificity. TNF-α impairs insulin-stimulated glucose uptake in myocytes at the level of IRS-1 by a double machinery that involves serine phosphorylation at the residue Ser307 (by IKK and p38MAPK) and tyrosine dephosphorylation (by PTP1B), weakening the tyrosine phosphorylation induced by insulin. Consequently, pharmacological inhibition of IKK with salicylate and ablation of PTP1B restores insulin sensitivity in the presence of the cytokine.

In brown adipocytes, TNF-α-induced insulin resistance involves serine/threonine phosphorylation of IRS-2 by MAPKs, and activation of the phosphatases PP2A and PTP1B that inactivate AKT and IR/IRS, respectively. Pharmacological inhibition of MAPKs and phosphatases with rosiglitazone and LXR agonists, respectively, recovers insulin sensitivity in the presence of TNF-α.

ACKNOWLEDGMENTS

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