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REVIEW

The Role of TNF-α in Insulin Resistance

Stephen E. Borst

Department of Exercise & Sport Sciences, University of Florida, Gainesville, and Malcom Randall VA Medical Center, Gainesville, Florida

Insulin resistance is an important component of the metabolic syndrome associated with obesity. Early-stage insulin-resistance and related mild glucose intolerance may be compensated by increased insulin secretion. When combined with impaired insulin secretion, insulin resistance plays an important role in type 2 diabetes *(1)*. Insulin-resistance is also associated with a variety of pathological conditions, including trauma, infection, and cancer. Obesity and type 2 diabetes are the most common metabolic diseases in Western societies, together affecting as much as half of the adult population *(2)*. The prevalence of these conditions is not only high, but continues to increase. We have only recently come to appreciate the role of fat, especially visceral fat, as an endocrine organ. Visceral fat is the source of a number of substances which might play a role in the development of insulin resistance. Among the latter are tumor necrosis factor-α (TNF-α), adiponectin, IL-6, resistin and free fatty acids. This review will discuss the regulation of insulin responses by TNF-α and evidence supporting the hypothesis that over expression of TNF-α plays a role in the pathophysiology of insulin resistance.

Key Words: TNF-α; insulin resistance; type 2 diabetes; glucose transport.

Introduction

TNF-α is a proinflamatory cytokine that plays a complex role in the response to injury and infection, angiogenesis, apoptosis, and other physiological processes *(3)*. TNF-α has also been implicated in the pathology of endotoxin lethality *(4)*, rheumatoid arthritis *(5)*, Crohn's disease *(6)*, tumor-induced cachexia *(7),* and insulin resistance *(8,9)*. In the 1880s, the search for a "tumor necrosis factor" began with the observation that some cancer patients, who survived bacterial infections, would experience the surprising result of tumor regression *(3)*. It was later learned that the anti-tumor effect of infection was mediated by TNF-α released from macrophages in response to the lipopolysaccharide component of bacterial cell walls *(3)*. The search for "cachectin" began with the observation that cachexia, a catabolic condition characterized by loss of muscle and fat and by insulin resistance, is often associated with cancer and prolonged infection. Cachectin was identified by Beutler and Cerami as a factor that is produced by macrophages and has the property of inhibiting lipoprotein lipase, an enzyme that is required for storage and use of fats *(4)*. With the sequencing of TNF-α in 1984 and that of cachectin in 1985 *(4)*, it became apparent that these two hormones are one and the same. TNF-α is an inflammatory cytokine with a wide range of anti-tumor and immune functions. It is produced as a 26 kDa transmembrane protein (mTNF), and is cleaved by TNF-α converting enzyme (TACE) to form soluble, 17 Kda form of TNF-α (sTNF) *(10)*. It is thought that sTNF is the biologically active form of the hormone. Inhibition of TACE protects against a mouse model of endotoxin lethality *(11)*. Most biological effects are thought to occur through the binding of sTNF to either the 55 kDa TNF receptor (p55) or the 75 kDa receptors (p75) *(11)*.

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Author to whom all correspondence and reprint requests should be addressed: Stephen Borst, Ph.D., VA Medical Center, GRECC – 182, 1601 SW Archer Rd., Gainesville FL 32608-1197. E-mail: seborst@ufl.edu

Altered Insulin Signaling Contributes to Insulin Resistance

Insulin signaling may be impaired by at least four separate molecular modifications, some of which have been demonstrated following addition of TNF-α to cell cultures (see Fig. 1). The first modification identified was a serine phosphorylation of IRS-1. This alteration makes IRS-1 resistant to subsequent insulin-stimulated tyrosine phosphorylation. The result is a reduction in docking of PI-3 kinase and an impairment in insulin-stimulated glucose transport. TNF-α has been shown to induce this modification in the cell types that are of greatest interest in insulin resistance: murine adipocytes *(12)*, rat hepatoma cells *(13)*, and C_2C_{12} muscle cells *(14)*. Engelman et al. have shown that the MAP kinase pathway is required for TNF-mediated serine phosphorylation of IRS-1 in 3T3-L1 adipocytes *(15)*. Second, addition of TNF-α to rat hepatoma cultures has a second effect of phosphorylating and activating the protein tyrosine phosphatase SH-PTPase, which rapidly removes tyrosine phosphate groups from IRS-1 and FAK, thus terminating insulin action *(16)*. Third, TNF-α phosphorylates the protein phos-

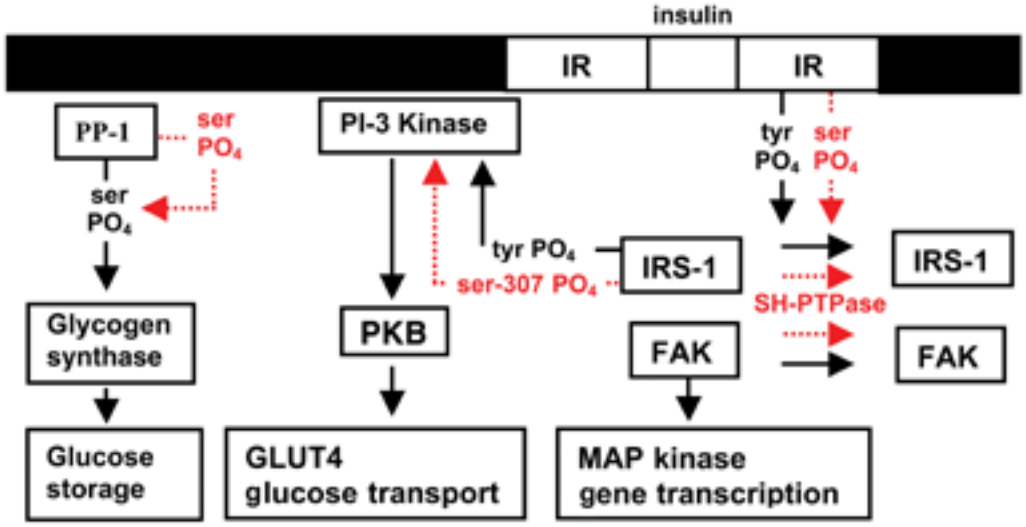

Fig. 1. Molecular mechanisms of insulin resistance. Sites of altered signaling are shown in light gray, with dashed lines representing inhibition. Abbreviations: IR = insulin receptor; IRS-1 = insulin receptor substrate 1; PTPase = protein tyrosine phosphates; FAK = focal adhesion kinase; PI3 kinase = phosphotidylinositide-3 kinase; PKB = protein kinase B; PP-1 = protein phosphatase 1; MAP kinase = mitogen-activated protein kinase.

phate PP-1 at site 2, resulting in its inactivation *(17)*. Insulin promotes glucose storage by phosphorylating PP-1 at site 1 and activating it. Activated PP-1 activates glycogen synthase and inhibits phosphorylase a. This mechanism of TNF-α action has been demonstrated in L6 muscle cells *(18)* and in rabbit skeletal muscle *(19)*. Finally, insulin receptors (IRs) isolated from muscle of insulin-resistant subjects are serine/threonine phosphorylated and have lower intrinsic tyrosine kinase activity, compared to IRs isolated from insulin-responsive muscle. Serine/threonine phosphorylation may inhibit the ability of IRs both to phosphorylate IRS-1 and to autophosphorylate *(20)*. Dohm et al. have shown that in vitro treatment with alkaline phosphatase can restore tyrosine kinase activity in IRs isolated from insulin-resistant muscle and that phorbyl esters can reduce tyrosine kinase in IRs isolated from healthy tissue *(21)*. Current evidence suggests that serine/threonine phosphorylation of IRs is mediated by protein kinase C theta *(21–23)*. TNF-α has not been implicated in the latter mechanism. Kanety et al. reported that treatment of Fao hepatoma cells with TNF-α caused a increase in serine phosphorylation of IRS-1 and a decrease in docking of PI-3 kinase, but did not affect tyrosine kinase activity of isolated IRs *(24)*.

Evidence that TNF-α Plays a Role in Insulin Resistance

Most of the available evidence supports the role of TNF in insulin resistance (see Table 1). As discussed above, TNF has been shown to impair insulin signaling by serine phosphorylation of IRS-1 and of PP-1 and by activation of SH-PTPase. Serine phosphorylation of IRs could also be mediated by TNF, but this possibility has not been tested. The role of TNF in inhibiting insulin signaling may be closely related to its role as a stimulator of cell division and inhibitor differentiation *(25)*. Halse et al. have observed that TNF inhibits differentiation of cultured human myoblasts into myotubes and inhibits insulin signaling if present during the differentiation, but does not inhibit signaling in mature myotubes *(26)*.

Binding of TNF to its receptors triggers a broad pattern of signaling cascades that ultimately results in the activation of two major transcription factors: nuclear factor kappa B (NF-κB) and c-Jun amino-terminal kinase (JNK) *(27,28)*. JNK activation causes phosphorylation of serine 307 in IRS-1, resulting in an impairment of IR-mediated tyrosine phosphorylation of IRS-1 *(27)*. Hotamisligil and colleagues found that JNK-1 activity is increase in muscle, liver, and fat of both genetically obese (ob/ob) mice and those made obese by a high-fat diet *(29)*.

Both TNF-α and insulin cause phosphorylation of IRS-1 at serine 307, an action that impairs insulin responsiveness. When induced by insulin, this phosphorylation may be a self-limiting feature of insulin signaling or a response to prolonged hyperinsulinemia. Phosphorylation of IRS-1 at serine 307 is increased in skeletal muscle of human subjects following insulin-clamp studies *(30)*. Interestingly, insulin and TNF-α phosphorylate IRS-I at serine 307 by different mechanisms. Rui et al. have shown that in 3T3 adipocytes, that the effect of insulin is blocked by wortmannin and thus is mediated by PI-3 kinase, whereas the effect of TNF-α is blocked by PD98059 and thus is mediated by MAP kinase kinase 1(MEK1) *(30)*. TNF-α is well known

Table 1
Evidence For and Against the Role of TNF-α in Insulin Resistance

	References for TNF-α effect	References for no TNF-α effect
TNF-α impairs insulin signaling.	Hotamisligil *(12),* Paz *(13),* Del Aguila *(14),* Engelman *(15),* Ahmed *(16),* Ragolia *(17),* Liu *(18),* Cohen *(19),* Youngren *(20),* Halse *(26),* Aguirre *(27),* Hirosumi *(29),* Rui *(30)*	
Tissue TNF-α is over expressed in insulin resistance.	Hotamisligil *(31,33),* Hofmann *(32),* Xu *(34),* Hamman *(35),* Begum *(36)*	Liu *(18),* Borst *(37)*
Circulating TNF-α is elevated in insulin resistance.	Tsigos *(43)*	Kern *(44),* Koistinen *(45)*
TNF administration causes insulin resistance.	Lang *(9),* Fan *(46)*	
TNF-α KO mice have improved glucose tolerance.	Ventre *(50),* Hotamisligil *(51)*	
TNF-α neutralization reverses insulin resistance.	Cheung *(8),* Borst *(47)* Hotamisligil *(31)*	Paquot *(54),* Ofei *(55)*

to activate the NF-κB pathway. Whether this arm of TNF-α signaling is involved is insulin resistance has not been explored.

Expression of TNF-α in Insulin Resistance

TNF-α overexpression has been observed in adipose tissue from several strains of insulin-resistant rodents. Hotamisligil et al. found that TNF-α mRNA is elevated in visceral fat from ob/ob mice and Zucker fatty rats, compared to lean counterparts *(31)*. Similarly, elevated message for TNF-α has been observed in visceral fat of KKY insulin-resistant mice *(32)*, KKA mice, tub/tub mice, ob/ob mice, and obese humans *(33,34)*. Making mice obese and insulin-resistant by introduction of a transgene that causes ablation of brown adipose tissue also causes an increase in TNF-α mRNA in white adipose tissue *(35).* Several groups have reported increased levels of TNF-α mRNA in adipose tissue of obese or insulin resistant subjects *(33,35,36)*.

The laboratories reporting overexpression of TNF-α have relied mainly on mRNA measurements, but changes in TNF-α message do not always reflect changes in TNF-α protein. Liu et al. reported that TNF protein in adipose tissue of Zucker fatty rats is decreased during the onset of diabetes *(18).* This is in distinct contrast to the findings of Hotamisligil et al. who observed an increase TNF message in the same animals *(31)*. We found that TNF protein is decreased in adipose tissue of Sprague–Dawley rats during the onset of insulin resistance *(37).* Thus, TNF protein content of adipose tissue is reduced in at least two models of insulin resistance. Morin et al. found that administering a high-fat diet to Wistar rats increased adipose expression of TNF mRNA, but decreased in vitro secretion of TNF protein by explants of adipose tissue *(38).* To our knowledge, TNF protein has not been measured in other insulin resistant strains.

Relationship of Circulating TNF-α to Insulin Responsiveness

In cachexia and other pathological states in which circulating TNF-α is elevated, insulin responsiveness is often impaired *(39,40).* The elevation of serum TNF-α in cachexia is sometimes dramatic. Bossola et al. reported serum TNF-α concentrations of 15–20 pg/mL for gastrointestinal cancer patients with weight loss, compared to undetectable levels in controls *(41).* Sharma and Anker reported that circulating TNF-α is approx 15 pg/mL in cachectic heart failure, compared to approx 6 pg/mL in heart failure patients who are not cachectic *(42).* In comparison to cachectic patients, circulating TNF is lower in insulin resistance associated with obesity and the metabolic syndrome. Some researchers have reported that TNF-α is elevated in this most common form of insulin resistance and some have reported that it is not. Tsigos et al. observed a circulating TNF-α concentration of approx 3 pg/mL in insulin resistant humans, compared to approx 1.6 pg/mL in controls *(43).* Two studies have shown no correlation between TNF-α mRNA levels and BMI in men and women *(44,45)*.

A popular hypothesis holds that overexpression of TNF-α in adipose tissue is a cause of insulin resistance. Because skeletal muscle is the main site of peripheral insulin resistance, it follows that TNF-α of adipose origin would cause its effects in muscle via the circulation. However, although

circulating TNF-α may elevated in insulin resistant humans, it is still quite low and circulating TNF-α is virtually undetectable in many rodent models of insulin resistance. Circulating levels of TNF-α in insulin-resistant rats are also quite low compared to the concentrations observed when TNF-α administration is used to create experimental insulin resistance. Fan et al. *(46)* and Lang et al. *(9)* have reported that TNF causes hepatic and peripheral insulin resistance, when infused in sufficient quantity to elevate circulating TNF-α into the range of 150–500 pg/mL *(9,46).* In contrast to the low circulating concentration of TNF-α, we found that concentrations of TNF-α protein in rat muscle and liver are several orders of magnitude higher, in the ng/g range *(47,48).* Taken together, these findings suggest that circulating TNF-α is low in insulin resistance and that most of the TNF-α acting in liver and muscle is probably produced locally. In support of this concept is the finding of Saghizadeh et al. that TNF-α mRNA is elevated sixfold in muscle biopsies obtained from type 2 diabetic subjects *(49).* The possible role of locally produced TNF-α in muscle insulin resistance is also supported by our findings that treatment of insulin-resistant Sprague–Dawley rats with anti-TNF reverses muscle insulin resistance, while reducing TNF-α protein content in muscle, but not in fat *(47).* Thus, most of the evidence available supports the hypothesis that locally produced TNF-α acts in a paracrine manner to cause insulin resistance.

Anti-TNF Strategies

A variety of anti-TNF strategies have successfully prevented or reversed insulin-resistance in rodents (see Table 1). Ventre et al. developed a strain of TNF-null (–/–) mice which are virtually devoid of TNF-α protein *(50).* These mice have improved glucose tolerance and lowered fed insulin concentrations. Significantly, TNF-null mice were protected against insulin resistance resulting from gold-thioglucose–induced hyperphagia. Hotamisligil et al. found that a knockout of the p75 TNF receptor restored insulin responsiveness to ob/ob mice, whereas knocking out the p55 receptor had little effect *(51).* We found that anti-TNF treatment of aging Sprague–Dawley rats reversed insulin resistance in skeletal muscle, while reducing TNF-α protein content in muscle, but not fat *(47).* Cheung et al. have similarly reported that TNF-neutralization reverses hepatic insulin resistance *(8).*

In humans, anti-TNF strategies have proven effective in treating a variety inflammatory conditions. Enbrel® (etanercept, a TNF-receptor p75-IgG Fc fusion product) and Remicade® (infliximab, an anti-TNF antibody) have been used effectively in Crohn's disease and rheumatoid arthritis *(52).* TNF-α plays a key role in granuloma formation by stimulating clonal expansion of T cells and elevated levels of TNF-α have been found in GI mucosa of Crohn's patients *(6,53).* Stack et al. observed a significant reduction in Crohn's symptoms after administering a single dose of 10 μg/kg of the anti-TNF antibody CDP571 *(6).* However, similar attempts to treat human insulin resistance with TNF-neutralization have not been successful to date. Paquot et al. treated obese humans with a single dose of a TNF receptor–IgG chimeric protein construct (Ro 45-2081) and found no improvement in insulin responsiveness *(54).* When Hotamisligil et al. treated Zucker fatty rats with the same construct, they found that the rate of insulin-stimulated glucose disposal was markedly improved *(31).* Although these findings are consistent with different etiologies for insulin resistance in humans vs Zucker rats, they may also be explained by the fact that Hotamisligil et al. used a repeated dosing regimen in treating Zucker rats. Paquot et al. did not assess whether their treatment was sufficient to cause depletion of TNF-α in either tissues or serum. Ofei et al. administered a single dose of 5 μg/kg anti-TNF antibody CDP571 to type II diabetics and found that it did not improve insulin responsiveness *(55)*. The dose of antibody was half of that used successfully by Stack et al. in Crohn's disease *(6)*. The failure of TNF-neutralization strategies in the studies by Ofei and Paquot does not mean that TNF-α is not a mediator of human insulin resistance, nor does it mean that TNF-neutralization cannot be successfully employed to treat human insulin resistance. Instead, a successful strategy may depend on the choice of the right neutralizing agent for a given species and on the dosing regimen. Because of metformin and rosiglitazone are effective in type 2 diabetes, testing of other anti-TNF strategies is not a high priority.

Other Possible Mediators of Insulin Resistance

In addition to TNF-α, visceral fat is a source of several other substances that are hypothesized to play a role in insulin resistance, including resistin, adiponectin, and free fatty acids (FFAs). Resistin is an adipocyte differentiation factor that was discovered by Lazar and coworkers in 2001 *(56).* At present, it is not clear whether resistin plays a role in insulin resistance. Arguing in favor of such a role are the observations that administration of resistin causes increased glucose production *(57)* and that administration of anti-resistin antibody reduces the extent of diet-induced insulin resistance *(56).* Arguing against the role of resistin in insulin resistance are the observations that the impairment of glucose tolerance by resistin is not robust *(56)* and that adipose expression of resistin is strongly suppressed in insulin resistance *(58).* Adiponectin is a fat-derived peptide that is decreased in the blood of obese mice *(59)* and patients with type 2 diabetes *(60).* Administration of adiponectin improves glucose tolerance and reduces tissue and serum triglycerides as well as reducing serum FFAs. Elevation of serum FFAs is often associated with insulin resistance and infusion of FFAs is known to decrease insulin-stimulated glucose disposal in humans *(61,62).* FFAs have been proposed to decrease insulin responsiveness by two mechanisms. The first is through an increase in fatty acid oxidation with a

resulting decrease in glucose oxidation and ultimately a lower driving force for glucose transport *(61,62)*. The second proposed mechanism is that FFAs might increase the intracellular concentration of metabolites, which share in common with diacylglycerol the property of stimulating protein kinase C, including PKC theta. The proposed result would be an impairment of insulin signaling via serine phosphorylation of IRs *(21)*.

Future Directions of Research

A role has been clearly established for TNF-α in the development of insulin resistance. However, it is not clear whether TNF-α acts locally or systemically. It is also unclear whether it is the expression and secretion of TNF-α protein or the responsiveness to TNF-α that is altered in insulin resistance. To date, no studies have examined tissue expression of TNF-α mRNA and TNF-α protein side by side to determine whether over expression of TNF-α is a common theme in age-induced, diet-induced, and genetic models of insulin resistance. Similarly, the potential roles of TNF-α receptors and signaling have not been explored. A great deal has been learned recently about underlying mechanisms of insulin resistance. Separate lines of evidence have demonstrated roles for TNF-α, adiponectin, FFAs, visceral adiposity, and possibly PKC theta and resistin. More research will be needed to learn how these are separate pathways work in concert to produce insulin resistance and whether, in doing so, they converge on some final common pathway.

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