Perspectives in Diabetes

Tumor Necrosis Factor \( \alpha \): A Key Component of the Obesity-Diabetes Link

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Recent data have suggested a key role for tumor necrosis factor (TNF)-\( \alpha \) in the insulin resistance of obesity and non-insulin-dependent diabetes mellitus (NIDDM). TNF-\( \alpha \) expression is elevated in the adipose tissue of multiple experimental models of obesity. Neutralization of TNF-\( \alpha \) in one of these models improves insulin sensitivity by increasing the activity of the insulin receptor tyrosine kinase, specifically in muscle and fat tissues. On a cellular level, TNF-\( \alpha \) is a potent inhibitor of the insulin-stimulated tyrosine phosphorylations on the \( \beta \)-chain of the insulin receptor and insulin receptor substrate-1, suggesting a defect at or near the tyrosine kinase activity of the insulin receptor. Given the clear link between obesity, insulin resistance, and diabetes, these results strongly suggest that TNF-\( \alpha \) may play a crucial role in the systemic insulin resistance of NIDDM. This may allow for new treatments of disorders involving resistance to insulin. *Diabetes* 43: 1271–1278, 1994

Non-insulin-dependent diabetes mellitus (NIDDM) is among the most common metabolic disorders in the industrial world (1). It is a major cause of morbidity and mortality, primarily through associated dyslipidemias, atherosclerosis, hypertension, cardiovascular disorders, and renal dysfunction (2). Obesity, found in \( \geq 80\% \) of NIDDM patients, constitutes the greatest risk factor for this disease. However, the connection between obesity and diabetes is poorly understood, and the molecular mechanisms that are involved in obesity-diabetes syndromes are still not known. Two demonstrable physiological defects that lead to the development of NIDDM are tissue resistance to the effects of insulin and altered secretion of insulin (2,3). Recent data have strongly suggested a role for tumor necrosis factor (TNF)-\( \alpha \) in the insulin resistance of obesity and NIDDM (4–8). In this perspective, we will review the evidence from experimental animal models and cultured cells that suggests TNF-\( \alpha \) is a key component in these disorders. Because the role of insulin resistance in diabetes is generally well-known to the readers of *Diabetes* and has been recently reviewed (3), we will focus our discussion on the specifics of TNF-\( \alpha \) signaling pathways and how they may interfere with insulin action.

TNF-\( \alpha \) AND ITS RECEPTORS

TNF-\( \alpha \), along with its close relative lymphotoxin (LT-\( \alpha \) or TNF-\( \beta \)), was first isolated as the active principal causing tumor necrosis in bacterially infected animals (9,10). Subsequently, TNF-\( \alpha \) was also isolated as the mediator of hypertriglyceridemia and wasting (cachexia) in parasitically infected animals (11). It is now recognized that TNF-\( \alpha \) defines a family of effectors among cytokines that modulate many immune functions (12). Like other cytokines, TNF-\( \alpha \) has tremendously diverse functions in both immune and extra-immune systems. Examples include the induction of apoptotic cell death, lysis of tumor cells, the growth of thymocytes, stimulation of production of other cytokines, such as granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-1 (IL-1), and the suppression of lipoprotein lipase activity (12–14). Recently, mice with targeted mutations at the TNF-\( \alpha \) and LT-\( \alpha \) loci have been generated (15,16). The LT-\( \alpha \)-deficient mice, as well as mice deficient in both LT-\( \alpha \) and TNF-\( \alpha \), exhibited abnormal development of peripheral lymphoid organs and altered immune responses (15,16). These animal models (along with a TNF-\( \alpha \)-deficient mouse that is yet to be developed) will assist the elucidation of the full spectrum of TNF-\( \alpha \) and LT-\( \alpha \) activities.

In pathophysiological states, TNF-\( \alpha \) has been associated with septic shock, rheumatoid arthritis, graft-versus-host disease, inflammatory bowel diseases, and various other disorders (17). TNF-\( \alpha \) also has profound effects on whole body lipid metabolism (5,18,19). In vivo, TNF-\( \alpha \) administration causes an increase in serum triglycerides and very-low-density lipoproteins in rats and humans (18,19). This hyperlipidemia is thought to be the result of increased hepatic lipogenesis and lipolysis rather than decreased peripheral clearance (18). In addition to TNF-\( \alpha \), IL-1 and interferon (IFN)-\( \gamma \) also stimulate hepatic fatty acid synthesis in rodents (19). Previous studies have associated TNF-\( \alpha \) expression with catabolic states (such as cancer and infection), leading to a wasting syndrome, termed "cachexia" (13,20). This effect of TNF-\( \alpha \) has been recently reviewed (5) and will be briefly discussed below.

TNF-\( \alpha \) and a number of other cytokines (e.g., TNF-\( \alpha \), IL-1, and IFN-\( \gamma \)) also affect glucose homeostasis in various tissues.
(19). TNF-α stimulates insulin-independent glucose utilization in macrophage-rich tissues, while inhibiting insulin-stimulated glucose uptake in fat and muscle (21,22). Therefore, TNF-α administration leads to a net increase in basal glucose turnover and a decrease in insulin-stimulated peripheral glucose utilization. Although this effect has been postulated to be the result of divergent regulation of glucose transporters in different tissues (23), TNF-α has now been shown to affect more proximal events that regulate insulin action (4,6,7,24) and glucose homeostasis (see below). TNF-α administration also causes an increase in hormones (counterregulatory hormones), such as glucagon, cortisol, and epinephrine, that regulate glucose metabolism and stimulate gluconeogenesis in liver (21,22). Finally, TNF-α and other cytokines alter pituitary-adrenal and pituitary-thyroid axis functions (19).

With respect to adipocytes, where most of our own work has been done, previous work has shown that TNF-α causes a suppression of most lipogenic enzymes, including lipoprotein lipase, and induces “de-differentiation” of adipocytes when applied at fairly high doses (25). This effect potentially involves the activation of phospholipase A2 and generation of arachidonic acid metabolites (26,27). However, at lower doses (≤100 pmol/l), changes in gene expression are more specific (e.g., suppression of GLUT4 and adipin expression) and occur in the absence of any alterations in adipocyte phenotype (4). These changes in gene expression patterns have been postulated to involve regulation of transcription factor C/EBP (23). The interference with insulin action by TNF-α (discussed below) also occurs at doses that are insufficient to cause a generalized suppression of adipocyte gene expression (6).

TNF-α is believed to function, like most peptide effectors, through transmembrane receptors. The two identified receptors for TNF, called TNF-R1 (p55 in rodents, p60 in humans, [28,31]) and TNF-R2 (p75 in rodents, p80 in humans [30–32]), are coexpressed in virtually all cells, albeit at different ratios. Both of these receptors can bind to TNF-α and also to a related molecule, LT-α (also called TNF-β). As might be expected for ligands binding overlapping receptors, there is a great deal of overlap of function between TNF-α and -β. The two receptors for TNF, however, share almost no homology outside the ligand binding domain, suggesting that they signal for different biological functions (12,33,34).

The TNF receptors belong to a larger family of receptors that comprise a total of at least 12 members, including TNF-receptor-related protein, that has been shown to preferentially bind LT-β (or LT-α-β heterotrimers), nerve growth factor, and the Fas receptor (12). One interesting feature of this family is that their intracellular domains do not immediately suggest a biochemical function; they apparently do not act directly as ligand-stimulated protein kinases. On the other hand, TNF-α is known to initiate a cascade of signal transduction that includes the activation of multiple protein kinases and phosphoprotein phosphatases (14). For example, in human fibroblasts, TNF-α stimulation results in changes in phosphorylation patterns of more than 60 different proteins (25). Multiple protein kinases are activated by TNF-α, including protein kinase A and C (36,37), mitogen-activated protein (MAP) kinases p38 (38), p42, p44 (35,39,40), and p54 (41), and MAP kinase kinase (42). A number of novel kinases have also been defined in TNF-α-stimulated cells, including an hsp27 (heat shock protein) kinase (43), a β-casein kinase (43), and a ceramide-activated protein kinase (44). In addition to the activation of various kinases, TNF-α also regulates phosphoprotein phosphatase activities during the early phases of its signal transduction cascades (35,45). Current evidence suggests that the sphingomyelin pathway is a critical component of the initiation of these TNF-α-induced phosphorylation/dephosphorylation cascades, using ceramide as a second messenger (46,47).

Further downstream, responses include the activation of certain transcription factors, such as NF-κB, the AP-1 family of transcription factors (c-fos and c-jun), interferon regulatory factor (IRF)-1, and IRF-2 (14). Finally, a long list of genes (e.g., TNF-α itself and other cytokines, growth factors and hormones, cell adhesion molecules, and inflammatory mediators) are regulated by TNF-α through the activity of one or more of these transcription factors (14,46). Apparently, TNF-α’s ability to cause a bewildering array of biochemical changes in a wide variety of cells is attributable to its capacity for using multiple signaling pathways through its cell surface receptors.

MEDIATORS OF INSULIN RESISTANCE IN NIDDM

The biochemical basis of insulin resistance in NIDDM has been the subject of many studies. While the quantitative regulation of the insulin-sensitive glucose transporters (GLUT4) and insulin receptors themselves may contribute to this disorder, these two factors are probably inadequate to explain insulin resistance. Although GLUT4 mRNA and protein have been demonstrated to be reduced in the adipose tissue of animals and humans with obesity and NIDDM (48), gross quantitative regulation of glucose transporters in muscle, the major site for glucose disposal, has not been observed in NIDDM (49). Therefore, it is generally accepted that the major defect in glucose transporters in NIDDM involves not the number but the translocation of these transporters to the plasma membrane upon insulin stimulation (49). Likewise, the decrease in insulin response in NIDDM is far greater than can be explained by the reductions observed in the number of insulin receptors, indicating the presence of additional post-receptor defects (50). Hence, recent attention has focused on the intrinsic catalytic activity of the insulin receptor and downstream signaling events. A reduction in tyrosine phosphorylation of both the insulin receptor and insulin receptor substrate (IRS)-1 has been noted in both animal and human NIDDM (51,52). Importantly, this appears to occur in all of the major insulin-sensitive tissues: muscle, fat, and liver. In addition, it has been recently demonstrated that stimulation of PI-3'-kinase activity by insulin is also decreased in rodent models of obesity and insulin resistance, further confirming defective insulin receptor signaling in this disease (53,54). It is now clear that decreases in the intrinsic tyrosine kinase activity of the insulin receptor are important components of this disease.

Because 70–80% of all NIDDM patients are obese, a central question in understanding NIDDM is how obesity can bring about resistance to insulin in the key tissues: muscle, fat, and liver. Previously, much attention has been focused on various lipids that are commonly elevated in obesity, such as free fatty acids (FFAs). It has been hypothesized that elevated levels of FFA can have adverse effects on glucose metabolism because of increased uptake and intracellular oxidation.
of fatty acids (Randle effect, 55). In support of this hypothesis, administration of FFA to normal individuals has been shown to induce a mild state of insulin resistance, as determined by insulin clamps (56). In addition, fatty acid treatment of cultured cells suppresses insulin action (57). However, there is no conclusive evidence supporting a major pathophysiological role for FFAs in NIDDM.

Another category of potential mediators of insulin resistance in obesity include hormonal antagonists of insulin (counterregulatory hormones), such as glucocorticoids, glucagon, growth hormone, and catecholamines (50). It is clear that elevated levels of these hormones can induce insulin resistance and diabetes (58,59). This is best illustrated in clinical syndromes such as Cushing's disease (elevated glucocorticoids), acromegaly (elevated growth hormone), multiple endocrine neoplasia, and pancreatic α-cell tumors (elevated glucagon). However, it is generally accepted that these hormones are not causally involved in the most common forms of NIDDM.

Finally, a small number of studies have reported the existence of inhibitor molecules, such as pp63 and a currently unidentified inhibitor, that act on insulin receptor tyrosine kinase. The former has been isolated from rat liver as a natural inhibitor of the insulin receptor tyrosine kinase (60,61) and the latter from the fibroblasts of a patient with severe insulin resistance (62). However, whether these molecules are regulated in obesity and generally contribute to insulin resistance and diabetes is not yet clear.

**EXPRESSION OF TNF-α IN ADIPOSE TISSUE**

Recent data have indicated a role for TNF-α in linking obesity with the insulin resistance of NIDDM (4-8). Earlier studies on an endogenous pathway of complement activation from adipose tissues of obese animals had suggested the possible presence of a cytokine in this tissue (63). It was then shown that adipose tissue of the obese-diabetic (db/db) mouse produced significant levels of TNF-α mRNA (4). Much less expression was seen in fat from the lean control animals. Although TNF-α mRNA was also observed in spleen, it was not elevated in the obese animals relative to the control animals. Increased TNF-α protein production was also demonstrated both by explanting adipose tissues into culture and by the measurement of plasma TNF-α (4).

Elevated TNF-α mRNA expression in fat appears to be a common, if not a universal, correlate of obesity attended by significant insulin resistance (Table 1). In addition to the db/db mouse, overexpression is seen in ob/ob, tub/tub, and KKYα mice, and the Zucker fa/fa rat (4,8). Besides these natural genetic models, elevated TNF-α mRNA expression has also been seen in a transgenic model of obesity/insulin resistance created by ablation of brown adipose tissue (BAT) via a bacterial toxin gene driven by the uncoupling protein promoter (64 and B.B. Lowel, J.S. Flier, personal communication). In contrast, significant overexpression of TNF-α mRNA is not observed in the monosodium glutamate (MSG)-injection mouse model, which at early stages induces a milder obesity with little or no insulin resistance (4). Similarly, the streptozotocin (STZ)-injected rat, a model of type I diabetes with β-cell loss and hyperglycemia, but no obesity, does not show elevated TNF-α gene expression. Although STZ injection induces a mild state of insulin resistance, this insulin resistance appears to be linked to the severe hyperglycemia shown in these animals (66).

Interestingly, those animals that demonstrated TNF-α mRNA expression from the fat tissues did not show evidence of altered expression of other cytokines, such as TNF-β, IL-1, or IFN-γ. In other physiological contexts in which cytokines are expressed, such as infection, there are usually several that are expressed simultaneously (13,14). This observation suggested that the regulation of cytokine cascades in adipose tissue during rodent obesity may differ from that in other cell types, such as immune cells.

**ROLE OF TNF-α IN THE INSULIN RESISTANCE OF NIDDM AND OTHER DISEASES**

The role of TNF-α in the obese-diabetic rodents was investigated by neutralization of TNF-α in vivo with a soluble TNF receptor (TNFR)-IgG fusion protein. This molecule is quite stable in the circulation and has been engineered specifically to function as a TNF-neutralizing agent in vivo (67). To analyze the possible role of abnormal TNF-α expression in systemic glucose homeostasis, Zucker fa/fa rats were treated for 3 days with this reagent and then were subjected to a two-step hyperinsulinemic-euglycemic clamp. Obese rats treated with the TNFR-IgG were notably more sensitive (two- to threefold) to insulin at both doses used in these studies (4). Through the use of a radioactive tracer, it was determined that insulin-stimulated peripheral glucose uptake was increased two- to threefold, while hepatic glucose output was unaffected. Hence, these experiments suggest an important role for TNF-α in the development of insulin resistance in extrahepatic tissues. An effect on hepatic insulin responsiveness, as measured by insulin-induced suppression of hepatic glucose output, cannot be ruled out definitively, but it was not readily apparent from these experiments. The increase in insulin sensitivity after TNF-α neutralization represents a substantial improvement, but not a complete reversal, because the absolute levels of insulin-stimulated glucose disposal in the obese animals do not reach those seen in their lean littermates. Additional experimental systems will be necessary to determine the full extent of this cytokine's role in obesity-linked insulin resistance at relevant sites of insulin action.

Other studies have also suggested a role for TNF-α in other insulin resistance states in vivo. Insulin resistance frequently develops during the course of certain cancers (68,69), infections (18,19), and trauma, such as burn injuries (70,71). Several studies have demonstrated elevated production of TNF-α in naturally occurring and experimentally induced sepsis and burn injury (13,72). Similarly, in certain cancers, a

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<th>Model</th>
<th>Obesity</th>
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<th>TNF-α mRNA expression in fat</th>
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Relative physiological characteristics of various rodent models of obesity or diabetes. −, absence of a trait; + to ++++, increasing amount of a trait or TNF-α mRNA.
correlation has been observed between TNF-α production and insulin resistance (73). However, a causal relationship between abnormal cytokine production and insulin resistance of these pathological states has not been established. In addition to these correlation studies, it has been reported that administration of TNF-α into humans induces a state of hyperinsulinemia without hyperglycemia, indicating reduced insulin sensitivity (74). Similar, but more dramatic, results have been obtained in studies with lambs (21). Finally, chronic infusion of TNF-α into normal rats led to development of severe hepatic and peripheral insulin resistance, determined by hyperinsulinemic-euglycemic clamp studies (22).

MECHANISMS OF TNF-α-INDUCED INSULIN RESISTANCE

The effects observed for TNF-α on insulin action in vivo may result from a direct effect of this cytokine on insulin-sensitive cells or indirectly, via substances produced by other cells or tissues in response to TNF-α. Indeed, these possibilities are not mutually exclusive.

The evidence for an indirect effect of TNF via other hormones is circumstantial. In animals infused with TNF-α and rendered insulin resistant, elevated levels of the stress hormones glucocorticoids and epinephrine are observed (21,22). Both of these hormones have been shown to cause insulin resistance in model cultured cells and whole organisms (50). In addition, catecholamines are known to generate intracellular cyclic AMP, which leads to the activation of protein kinase A, an enzyme that has been shown to inhibit the protein kinase activity of the insulin receptor (75).

Direct actions of TNF-α on insulin-sensitive cells have been shown in two systems of potential relevance: GLUT4 and the insulin receptor itself. TNF-α has been shown to downregulate GLUT4 mRNA levels in adipocyte and myocyte cultures (4,23,76). In fat cells, this effect occurs in the context of downregulation of expression of several fat-specific genes, such as aP2 or adipins (4,23), so it is not entirely specific. On the other hand, GLUT4 is not necessarily the only gene with aberrant expression in obesity; adipins is also downregulated in many animal models of obesity, although this has not been observed in human studies conducted to date (77,78). Furthermore, TNF-α can dramatically inhibit insulin-stimulated glucose transport at doses that have no effect on the cellular content of GLUT4 protein (6). Therefore, the mechanism of TNF-α-induced insulin resistance in cultured cells is unlikely to be only at the glucose transporter level.

Treatment of insulin-sensitive cells with TNF-α can clearly alter the catalytic activity of the insulin receptor. In adipocytes, TNF-α treatment leads to moderate reduction (20–50%) of insulin-stimulated insulin receptor autophosphorylation and a more pronounced effect on IRS-1 phosphorylation (6). This requires at least 3 days treatment, unlike many acute effects of this cytokine (13,14,18). Although certain doses of TNF-α can reduce the absolute level of insulin receptors, doses up to 200 pmol/l have little apparent effect on the numbers of receptors or their insulin binding, but reduce intrinsic catalytic activity (6). The actual defect induced by TNF-α in these studies is likely to be at or near the insulin receptor itself. Partially purified receptors isolated from TNF-α–treated cells show reduced autophosphorylation and phosphorylation of exogenously added recombinant IRS-1 (6). This suggests that the insulin receptor itself is modified or that TNF-α promotes the production of an inhibitor of the receptor that is associated with these preparations. Finally, this effect of TNF-α on insulin receptor signaling may not be entirely specific for TNF; IL-1 and IL-6 also reduce insulin receptor autophosphorylation and tyrosine phosphorylation of IRS-1 when applied to intact cells (6).

Sensitivity to TNF-α is also seen in insulin signaling in liver-derived cells (24). Treatment with TNF-α inhibits insulin-stimulated phosphorylation of the insulin receptor, as well as phosphorylation of IRS-1 in hepatoma cells, without affecting cell surface insulin-binding activity (24). One striking difference between these experiments and those cited above is that only 1 h of TNF-α treatment was required in hepatoma cells, compared with the 3–4 days of treatment necessary in adipocytes (6). These results suggest that the operative mechanisms in fat and liver cells may not be entirely identical.

In an attempt to evaluate these several possible mechanisms by which TNF-α might regulate insulin action in vivo, we again neutralized TNF-α in Zucker fa/fa rats and analyzed the expression of GLUT4 mRNA and the number and catalytic activity of the insulin receptors. Neutralization of TNF-α had no obvious effect on GLUT4 mRNA levels (7). However, the tyrosine phosphorylation of the insulin receptor and IRS-1 was dramatically increased in response to acute insulin injection in animals that were pretreated with the TNF-α IgG fusion versus vehicle alone (7). This effect was observed in fat and muscle, while no effect was visible in the liver. No changes in absolute levels of the insulin receptor or IRS-1 protein were observed after TNF-α IgG injection, indicating that the effect of this agent was on specific quantities of tyrosine phosphorylation per protein molecule. Indeed, each animal receiving the neutralizing agent showed increased tyrosine phosphorylations, and in some cases, the levels approached the insulin-stimulated phosphorylation of insulin receptor or IRS-1 observed in lean control animals. This amelioration of phosphorylation cascades was observable only in the obese animals and was not seen upon TNF-α IgG injection into lean control animals (7). Because TNF-α overexpression is observed only in the obese, this strongly suggests that the agent improved insulin sensitivity via neutralization of TNF-α, rather than through some other process unrelated to TNF-α.

The effects of these improvements in insulin-stimulated tyrosine phosphorylations appear to be of sufficient magnitude to cause improvements in the pathophysiology of NIDDM. Three days of TNF-α neutralization in fa/fa rats not clamped for glucose or insulin levels resulted in an improvement in the hyperglycemia that these rats display (7). A dramatic improvement was simultaneously observed in the marked hyperinsulinemia, with this parameter being reduced to near the control lean levels. In addition, the elevated FFA levels of the obese animals were greatly reduced by these treatments (7). Presumably, the simultaneous improvements shown in these three key metabolic parameters all reflect improvements in the sensitivity to insulin.

A MODEL FOR THE ROLE OF TNF-α IN OBESITY-DIABETES

The data described above, derived from animal studies and cultured cell systems, allow the description of a new model
Although an endocrine effect via circulating TNF-α could not be detected in assays of serum or plasma because of the presence of interfering binding proteins (81), a direct endocrine role for TNF-α appears to be somewhat unlikely in NIDDM.

On the other hand, TNF-α could act directly on muscle cells via a paracrine mechanism, because muscle tissue and fibers are often associated with a significant amount of adipose tissue. This is obvious to all customers shopping for meat at their local market, but the association of fat and muscle cells can also be documented at the microscopic level (82). Besides, if obesity tends to increase the number of adipose cells around or within muscle tissue, as has been noted, it is likely that a paracrine effect of TNF-α on muscle may be increased. Thus, in addition to the effects of obesity on the relative increases in specific TNF-α mRNA expression and increases in the total body adipose content, obesity may also play a third role, i.e., bringing fat cells into closer proximity with muscle.

Finally, the possibility must be considered that TNF-α acts directly on adipose cells to cause the production of other molecules that act directly on muscle. TNF-α neutralization causes a decrease in the levels of circulating FFAs, presumably due to a reduction in adipose lypolysis as a consequence of improved insulin action (7). As discussed above, fatty acids have been implicated as a potential causal agent in insulin resistance, so this lipid alone or in combination with TNF-α could inhibit insulin action on muscle (57). Further studies will be necessary to determine the role of fatty acids, and possibly additional novel mediators, in TNF-α-induced insulin resistance in muscle tissue.

Liver is more problematic. Neutralization studies of TNF-α done in obese-diabetic animals to date have not shown an effect on hepatic glucose output or insulin-stimulated phosphorylation cascades in liver (4,7). On the other hand, TNF-α infusion in normal rats causes insulin resistance, including an effect on liver (22). Moreover, TNF-α can influence insulin signaling in cultured hepatoma cells (24). Thus, whether and the extent to which TNF-α contributes to insulin resistance in the liver in NIDDM is unclear.

Several questions arise from this scheme. First, the detailed molecular mechanisms inhibiting insulin signaling by TNF-α will be important to delineate. It is possible that the insulin receptor is covalently modified in cells treated with TNF-α, since partially purified insulin receptor from these cells exhibits decreased kinase activity. For example, TNF-α is known to induce multiple protein kinases in certain cellular systems (36,37), and covalent modification of insulin receptors through serine and threonine phosphorylation has been shown to correlate with reduced tyrosine kinase activity (83,84). Recently, it has also been demonstrated that serine/threonine phosphorylation of IRS-1 can interfere with insulin action (85). These possibilities are currently under investigation. It is also possible that the decreased kinase activity is due to a co-purifying inhibitory molecule and/or tyrosine phosphatase (60–62). If such a protein or activity is observed upon TNF-α treatment, it will be important to purify and characterize these molecules. Obviously, an understanding of the signal transduction pathways that interfere with insulin action may be of broad significance in NIDDM, whether or not TNF-α is the physiological effector in humans. In addition to the induction of defects at or near the insulin receptor, it will be important to understand the upstream signals deriving from TNF-α and its receptors.
TNF-α has two identified receptors. So far, studies have shown that the majority of the biological actions of TNF-α can be mediated through TNFR-1, and very few activities have been assigned to TNFR-2 (33,34). Interestingly, it has been shown that TNFR-2 mRNA levels are increased in obesity and regulated in fat and muscle by diet and drug treatments that are known to modulate insulin sensitivity (8 and G.S.H., B.M.S., unpublished observations). Recently, mice carrying targeted mutations of both TNFR-1 and TNFR-2 have been generated (86-88 and J. Peschon, R.G. Goodwin, C.A. Smith, personal communication). The TNFR-1-deficient mice were resistant to endotoxin-induced shock, but exhibited susceptibility to Listeria monocytogenes (86,87). The TNFR-2-deficient mice had a minimal phenotype in terms of altered immune responses to infection and endotoxin (88). None of these genetic models exhibit any developmental abnormalities. These genetic models provide an excellent tool to examine the relative roles of the two TNF receptors in obesity-diabetes. It is possible to carry these mutations individually or together into obese-diabetic mice and definitively address the receptor systems involved in TNF-α-mediated insulin resistance. Although the relative roles of the two TNF receptors in the inhibition of the insulin receptor signaling is not yet clear, they are likely to provide important insights into the crosstalk between these two receptor systems.

As stated above, insulin resistance is an early and central component in NIDDM, but persistent fasting hyperglycemia must also involve a defect in insulin secretion. While there is no a priori reason why the mechanisms of insulin resistance and insulin secretion should be linked, it may be worth considering whether TNF-α may contribute to β-cell dysfunction in NIDDM. In this regard, several cytokines, including TNF-α, have been shown to interfere with insulin secretion by β-cells in response to glucose (89). Moreover, TNF-α alters the HLA (human leukocyte antigen) class II antigen expression and has toxic effects on human β-cells (90). Severe inflammatory changes in islets have also been observed in transgenic animals expressing TNF-α and TNF-β in pancreatic islet cells (91). Whether TNF-α or other cytokines play such a role in NIDDM is entirely unknown at present but represents an interesting area for future study.

Another key question suggested by the data presented is the nature of the physiological factors that cause adipose tissue to elevate the expression of TNF-α in obesity. Because this occurs in many different models of obesity, it is highly unlikely that this represents a proximal step in the action of an obesity gene. More likely, some common metabolic event in development of the obese state itself leads to the production of an inducing signal. At a cellular level, it is possible that the enlargement of fat cells themselves to an “obese” size triggers this change in gene expression. Alternatively, a hormone or hormones dysregulated in obesity, such as insulin, insulin-like growth factor-1, or glucocorticoids, may play an important role. Another candidate regulator may be the advanced glycosylation products that are increased in diabetes and are capable of inducing TNF-α in macrophages (92). Finally, because obesity often correlates with elevated lipoproteins or altered lipid profiles, it is conceivable that such molecules may play an important role in TNF-α induction. However, whether the obesity-linked induction of TNF-α is via transcriptional or posttranscriptional mechanisms is not yet clear.

It is entirely possible that obesity-related TNF-α expression by fat cells also affects obesity itself. The degree of insulin resistance in humans has been shown to correlate negatively with further weight gain, so it is possible that TNF-α expression is a compensatory mechanism to limit fat development (93). On the other hand, TNF-α has been shown to be a growth factor for cultured pre-adipocytes, to increase vascular permeability, and to induce angiogenesis, so it could potentially accelerate the development of obesity (94,95). In addition, it is likely that the hormonal milieu of obesity (such as elevated insulin and glucocorticoids) can cause some alterations in the biological actions of TNF-α. Experimental manipulation of TNF-α in fat via transgenic studies should be illuminating in this regard.

**TNF-α, OBESITY, AND CACHEXIA**

TNF-α expression has previously been associated with cachexia, a wasting disorder involving both muscle and fat tissues (13,20,25). How can the association with obesity be reconciled with a role for TNF-α in cachexia? Recent data suggest that aberrant TNF-α production can play a role in cachexia, primarily when it occurs in the context of a mixture of cytokines or other substances secreted by tumors (5). Administration of TNF-α alone causes anorexia, with no preferential loss of muscle tissue (5,19). In addition, when TNF-α has been associated with cachexia, there have been significant circulating levels (200 ng/ml) of this cytokine. In contrast, we have observed little or no expression of other cytokines in addition to TNF-α in models of obesity-diabetes (4), and circulating levels of TNF-α in these models are very low, in the range of 10-200 pg/ml when they are detectable. Furthermore, obesity is often associated with other hormonal abnormalities, including elevated glucocorticoids and insulin, that can alter the response to TNF-α. Previous reports have also suggested the site of expression as an important determinant of the metabolic responses to TNF-α (95). In this context, TNF-α expressed from fat tissue may induce a divergent spectrum of biological responses through an autocrine/paracrine mode of action. Nevertheless, the precise factors that permit one molecule to play roles in these different pathophysiological states remain to be determined.

**HUMAN DISEASE AND THERAPEUTICS**

Since TNF-α expression in rodent obesity is a common feature of many model systems, there is considerable interest in the possibility that the fat tissues of obese human populations may overexpress TNF-α and induce insulin resistance. In a first study done by biopsy of human abdominal fat tissue, there is a strong positive correlation between the degree of obesity (body mass index), hyperinsulinemia, and relative TNF-α mRNA levels in adipose tissue (G.S.H., B.M.S., unpublished observations). Further studies will be necessary to evaluate the detailed metabolic parameters in subtypes of obesity and NIDDM and their relation to TNF-α expression in fat tissue.

If fat-derived TNF-α is playing an important role in inducing insulin resistance in NIDDM patients, it may be worth speculating on possible therapeutic implications. First, good evidence from a variety of studies indicates that agents that improve insulin sensitivity may be of great value in the treatment of NIDDM (2). The most obvious therapeutic...
approach in the context discussed here would be to neutralize TNF-α with an antibody or immunoadsorbin, such as the soluble TNFRI-gG fusion protein (4,7,67). While such reagents would presumably require injection, they are quite stable in the bloodstream and would require only infrequent administration. Of course, chronic long-term neutralization of TNF-α could ultimately compromise the beneficial trophic functions of this cytokine in the immune system, such as the ability to fight certain infections and the stimulation of thymocyte growth (12,33,86-88). This problem could be circumvented by appropriate dosing of a neutralizing agent if less TNF-α is necessary to produce TNF’s beneficial effects than its deleterious ones. Alternatively, it may ultimately be possible to sort out these effects at the level of the receptors, by the creation of receptor-specific antagonists. Finally, since insulin resistance appears to be linked to inappropriate adipose-specific expression of TNF-α, the development of drugs that specifically modulate this process could, in theory, have the desired degree of specificity. Whether any of these classes of agents can be used to treat human NIDDM remains to be determined. However, earlier studies have incidentally provided some indications that TNF-α neutralization may be a valid therapeutic approach in NIDDM.

Pentoxifylline has been used in both insulin-dependent diabetes mellitus and NIDDM to treat vascular complications. During the course of some of these studies, an increase in insulin sensitivity has been observed in patients with NIDDM (96,97). This was explained by improvements in microcirculation, but it is now known that pentoxifylline is an inhibitor of TNF-α production, though this has not been studied in fat (98,99). Although speculative, the ability to affect insulin resistance by pentoxifylline may support a role for TNF-α in NIDDM of humans.

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