

The role of TNF α in adipocyte metabolism

Jaswinder K. Sethi and Gökhan S. Hotamisligil*

Tumor necrosis factor-alpha (TNF α) is a multifunctional cytokine which exerts a myriad of biological actions in different tissues and species. Many of these actions can perturb the normal regulation of energy metabolism. In adipose tissue, in particular, TNF α has been demonstrated to regulate or interfere with adipocyte metabolism at numerous sites including transcriptional regulation, glucose and fatty acid metabolism and hormone receptor signaling. The implications of these perturbations in disease states and the current understanding of the molecular mechanisms utilised by TNF α are discussed herein.

Key words: adipose tissue / glucose metabolism / lipid metabolism / insulin resistance / tumor necrosis factor- α (TNF α)

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Introduction

SINCE ADIPOSE TISSUE primarily functions to manage energy homeostasis, it is not surprising that this specialised organ has received much attention in recent years. It is now apparent that adipose tissue serves not only as a storage depot but also as an endocrine organ.^{1,2} It is actively involved in sensing the nutritional and metabolic status of the organism through several different signaling pathways and regulates energy metabolism by secreting molecules in response to these cues. Thus, it is able not only to respond locally to metabolic stimuli by altering the expression of genes important in the mobilization or storage of energy, but can also activate signals to other tissues thereby regulating energy balance, systemically. One signaling molecule produced by adipose tissue is TNF α which has been demonstrated to modulate

almost every aspect of adipose biology. For example, it can directly alter glucose homeostasis and lipid metabolism and plays an important role in the development of insulin resistance. As TNF α is also emerging to be a key component in a number of metabolic diseases, such as obesity, diabetes, dislipidemia and atherosclerosis, it is crucial to understand the molecular mechanisms by which its actions are mediated. Only then would it be possible to identify specific targets for therapeutic intervention. For these reasons we have sought to review the current evidence and understanding of the molecular mechanisms utilised by TNF α in adipose tissue.

The TNF ligands and receptors

TNF α is synthesized as a 26-kDa transmembrane pro-hormone, which undergoes proteolytic cleavage to yield a 17-kDa soluble TNF α molecule. Despite the differences in size and location, both forms of TNF α are capable of mediating biological responses^{3–5} and together may be responsible for both local and systemic actions of this cytokine.⁶ To date most (if not all) of the cellular actions of TNF α have been attributed to the activities of two distinct receptors: type 1 (TNFR1, a 55- or 60-kDa peptide in rodents and humans, respectively); and a type 2 (TNFR2, a 75- or 80-kDa in rodents and humans, respectively). Both of these receptors are expressed ubiquitously (albeit at different ratios) and oligomerise upon ligand binding.^{7,8} The extracellular domains of these two receptors exhibit some sequence homology, while the intracellular domains appear to be quite dissimilar. The latter has been interpreted as an indication that they might signal for different biological functions.⁸ Studies, so far, have addressed this possibility only partially and, to date, most known functions of TNF α have been ascribed to signals transduced by TNFR1 with TNFR2 playing primarily a modulatory role in ligand passing.⁹ However, both receptors do seem to activate multiple kinases and phosphoprotein phosphatases and can utilize all major transduction pathways.¹⁰

*From the *Division of Biological Sciences and Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA*

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Both TNF receptors can also be released from the cell surface through proteolytic cleavage and exist in soluble form. The circulating levels of both soluble TNF receptors are elevated in many pathological states, including sepsis,¹¹ cancers,¹² autoimmune diseases,¹² fever,¹³ chronic lymphocytic leukemia¹² and obesity^{14,15}, and may serve to modulate TNF α bioactivity both temporally and spatially.

Role of TNF α in lipid metabolism

A key function of adipocytes is the regulation of lipid metabolism according to the physiological energy requirements. The three biochemical sites of regulation are fatty acid uptake, lipogenesis and lipolysis

(Figure 1). Each of these can be altered in response to extracellular stimuli such as insulin, cortisol, catecholamines, growth hormone, testosterone, free fatty acids (FFA) and cytokines.¹⁶ There is also an increasing body of evidence to support a role for TNF α in modulating lipid metabolism. First, treatment of tumor-bearing (cachectic) rodents with anti-TNF α antibodies protects against abnormalities in lipid metabolism.¹⁷ In obesity, the elevated levels of TNF α may also contribute to the elevated basal lipolysis that is a characteristic of adipocytes from obese subjects.¹⁶ This is further supported by studies in which the administration of the sTNFR-IgG chimera,^{18,71} but not anti-TNF α antibody,¹⁹ decreases serum FFA levels of obese rodents. Moreover, TNF α deficient mice exhibit lower circulating FFA and triglycerides than

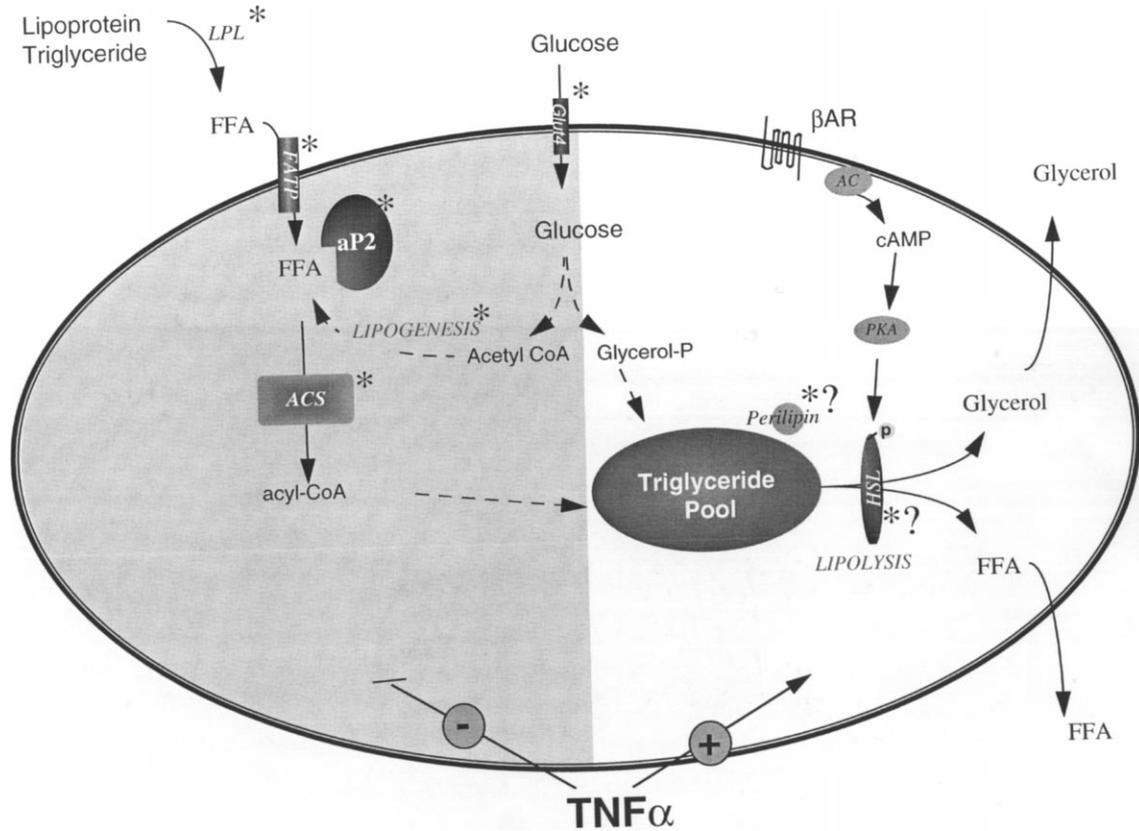


Figure 1. Actions of TNF α on lipid metabolism in adipocytes. As indicated by the shaded area, the net action of TNF α in adipocytes is to decrease FFA uptake and triglyceride synthesis (lipogenesis) whilst increasing lipolysis. LPL, lipoprotein lipase; FFA, free fatty acids; FATP, fatty acid transporter protein; aP2, adipocyte fatty acid binding protein; ACS, acyl-CoA synthetase; HSL, hormone sensitive lipase; β AR, β_3 adrenergic receptor; AC, adenylate cyclase; PKA, protein kinase A; Glut 4, insulin sensitive glucose transporter. An asterisk indicates proteins whose activity and/or expression is down-regulated by TNF α but upregulated by PPAR γ and its ligands. This also includes lipogenic enzymes such as acetyl-CoA carboxylase and fatty acid synthetase.

their wild-type littermates.^{20,21} Finally, the exogenous application of TNF α can stimulate lipolysis and increase circulating FFA levels *in vivo* and *in vitro*.^{22–26} Since FFA can also mediate insulin resistance,²⁶ the actions of TNF α on insulin sensitivity (see ‘Role of TNF α in mediating insulin resistance’ below) may be potentiated by increased lipolysis.^{27,28}

In adipocytes, fatty acids are derived predominantly via uptake from the circulation or from intracellular lipolysis and to a lesser extent by *de novo* synthesis from glucose (Figure 1). Fatty acid uptake is facilitated by the extracellular activity of lipoprotein lipase (LPL) which varies with nutritional and endocrine status.¹⁶ TNF α has been shown to inhibit LPL activity and to down-regulate its protein expression *in vitro*, and *in vivo*.^{25,29,30} However, this action in human adipocytes remains controversial.³¹ More recently, TNF α has also been shown to decrease the expression of FFA transporters (such as FATP and FAT) in adipose tissue.³² Together, these actions of TNF α could decrease FFA uptake from the circulation (Figure 1) and contribute to the hyperlipidemia that is observed during infections and also in obesity.

In addition to inhibiting FFA uptake, TNF α also acts to decrease the expression of key enzymes involved in lipogenesis (Figure 1), namely acetyl-CoA carboxylase³³ and fatty acid synthase.³⁴ However, this may not occur in mature adipocytes.³³ Nonetheless, a recent report suggests that TNF α can also decrease acyl-CoA synthetase (ACS) mRNA and activity in hamster adipose tissue.³⁵ This would result in reduced re-esterification of FFA and together with the decreased substrate availability (through inhibition of insulin-sensitive glucose uptake) may be the cause of suppressed triglyceride accumulation.

The third target of TNF α action is the lipolytic machinery of adipocytes. While the lipolytic process is predominantly regulated by adrenergic stimulation and mediated by a cAMP-dependent pathway (Figure 1), the mechanism of TNF α -induced lipolysis appears to be distinct and less well understood. Despite this, biochemical and genetic studies have clearly shown that the lipolytic actions are mediated by TNFR1.^{36,37} This is consistent with earlier studies which used human TNF α (selective for TNFR1 in murine systems) to stimulate lipolysis in murine cultures.²² The actions downstream of TNFR1 may involve transcriptional regulation of key proteins involved in lipolysis, since a chronic exposure is required to induce lipolysis in fat cell cultures.^{23–25} This is supported by the demonstration that TNF α -induced lipolysis is blocked by activators of the transcription factor, PPAR γ , such

as indomethacin, thiazolidinediones and 15-dPGJ2.^{23,26,38}

The rate limiting enzyme, hormone sensitive lipase (HSL) is a candidate protein whose levels may be regulated by TNF α . However, this action remains controversial since studies have either shown no regulation by TNF α (in human cultured adipocytes²⁴), or a significant decrease (in 3T3-L1 adipocytes²⁶). It remains to be seen if TNF α can modulate the activity of HSL directly or acts by up-regulating other, as yet unidentified, modulatory proteins. Perilipin is another protein implicated in lipolysis, although its exact function remains unknown. It is localized at the surface of lipid droplets³⁹ and downregulated by TNF α ²⁶ but not by catecholamines. Intriguingly, over-expression of perilipin in 3T3-L1 adipocytes selectively blocks TNF α - but not catecholamine-stimulated lipolysis.¹³²

Whilst the downregulation of key lipolytic proteins is not consistent with the stimulation of lipolysis by TNF α , these studies do highlight one problem facing mechanistic investigations on differentiated adipocytes: since the expression of lipogenic and lipolytic proteins is intrinsically linked to adipocyte differentiation, and TNF α can suppress the expression of adipogenic genes, it is difficult to identify the direct targets of TNF α signaling. Indeed, the observations discussed above (particularly on cultured 3T3-L1 adipocytes) may be the causal effects of modifying the differentiation program and this may not be representative of actions *in vivo*. Nonetheless, the possibility remains that the lipolytic actions of TNF α are mediated not by upregulation of key lipolytic enzymes but by the downregulation of proteins involved in the trapping of FFA in adipocytes (e.g. ACS and perilipin). Interestingly, β_2 adrenergic receptor (β_2 -AR) expression has been reported to be up-regulated by TNF α in adipocytes. However, this is accompanied by a significant decrease in β_1 and β_3 adrenoceptor expression.⁴⁰ Whether this differential regulation contributes to metabolic disorders mediated by TNF α *in vivo* remains to be determined.

Role of TNF α in mediating insulin resistance

Elevated levels of TNF α have been postulated to induce insulin resistance in a variety of catabolic disease states, including cancer,⁴¹ sepsis^{42,43} and trauma.^{43,44} This is supported by the observation that direct exposure of healthy individuals, animals or isolated cells to TNF α induces a state of insulin

resistance.^{18,25,45-49} Recent studies have also firmly established TNF α as an important mediator of obesity-related insulin resistance.^{18,20,48,50-53} There now exists a substantial body of evidence to support this latter hypothesis. First, in obese individuals and rodent models of obesity, TNF α is over-expressed in adipose and muscle tissue.^{50,52-58} This has been demonstrated at both mRNA and cellular protein levels and is consistent with recent reports which suggest that circulating levels of TNF α are increased in individuals with NIDDM.⁵⁹⁻⁶⁴ Furthermore, the levels of TNF α expression strongly correlate with hyperinsulinemia and decreased insulin sensitivity.

Dietary and chemical treatment of obesity improves insulin sensitivity and correlates with a decreased production of TNF α .^{53,57,58,61,65} The inhibitory actions of TNF α can also be reversed by insulin sensitizing drugs such as thiazolidinediones.^{27,28,52,66-69} *In vivo*, these actions coincide with a decrease TNF α expression.^{28,52,69} Interestingly, in obese mice deficient in the adipocyte-specific fatty acid binding protein, aP2, there is substantial protection from obesity-related insulin resistance and in this model, adipose TNF α level does not increase with weight gain.⁷⁰

The neutralisation of endogenous TNF α with the sTNFR-IgG chimera also appears to improve insulin sensitivity in obese and insulin resistant rats.^{18,50,71} In contrast, one report suggests that the administration of a neutralizing anti-TNF α antibody fails to improve insulin sensitivity in obese NIDDM subjects.⁷² Although it is possible that the actions of TNF α may differ among species, these divergent results also highlight the disadvantage of studies using neutralizing antibodies or binding proteins, since it is difficult to ascertain whether the injected substance has completely antagonised the antigen at all sites within the body before being degraded. However, a recent study has successfully illustrated that these problems may be overcome by using an effective delivery system to neutralize TNF α action in obesity.⁷¹

Perhaps the most definitive evidence of a role for TNF α in obesity-linked insulin resistance has come from recent studies in ligand or receptor deficient mice.^{20,21,73} These independent reports demonstrate that the absence of either TNF α or its functional receptors affords considerable protection from the insulin resistance that occurs in at least three models of rodent obesity (namely, genetic, dietary and chemically-induced obesity). These studies also demonstrate that TNF α influences insulin sensitivity through its actions on multiple targets involved in

insulin action including glucose transport, leptin production, insulin receptor signaling and improved lipid metabolism. However, in the absence of TNF α function, the extent of protection from obesity-related insulin resistance is not complete and may depend on other, as yet unidentified, factors or on the severity of the obesity model. For example, a recent report could not demonstrate the effects of the lack of both TNFRs in a dietary model of rodent obesity where the leptin system is intact.⁷⁴ Further investigations are required to definitively resolve this issue.

In normal insulin sensitive tissues, insulin binding to its receptor triggers signaling cascades which result in a number of cellular responses. One of these end responses is the increased translocation of Glut 4 (the insulin sensitive glucose transporter) to the plasma membrane which facilitates insulin-stimulated glucose uptake (Figure 2). A number of *in vitro* studies, in adipocytes, suggest that TNF α may act directly to down-regulate Glut 4 gene expression (Figure 2), thereby decreasing insulin-stimulated glucose transport.^{25,75,76} Intriguingly, the Glut 4 content is reduced in adipocytes from obese NIDDM subjects.⁷⁷ Whether this is the main mode of TNF α action in human adipose tissue in obesity remains unclear. However, in obese rodents lacking TNF α , adipose levels of Glut 4 remain similar to wild-type animals.²⁰ This suggests that in adipose tissue (*in vivo*) TNF α acts via a mechanism independent of Glut 4 expression.

TNF α has also been shown to act on the proximal steps of insulin signaling. In cultured adipocytes TNF α increases the phosphorylation of insulin receptor substrate-1 (IRS-1) at serine residues (Figure 2). This converts this multi-functional docking protein into an inhibitor of the insulin receptor (IR) tyrosine kinase.^{48,78,79} Such a modification of IRS-1 has been observed in obesity and burn-induced insulin resistance^{44,49} and is sufficient to block the downstream events of IR signaling, including the association of IRS-1 with phosphatidylinositol (PI) 3-kinase.⁴⁷⁻⁴⁹ That these actions of TNF α play a role in insulin resistance *in vivo* is supported by investigations in which both pharmacological and genetic blockade of TNF α function results in increased signaling capacity of insulin receptors.^{18,20,71} Conversely, in 3T3-L1 adipocytes, acute treatment with TNF α (30 min) can enhance IRS-1 tyrosine phosphorylation and its association to the p85 PI3-kinase subunit.⁸⁰ Currently, very little is known about the mediators of the signal transduction pathway that forms this crosstalk between TNFR and insulin receptors. Since neither

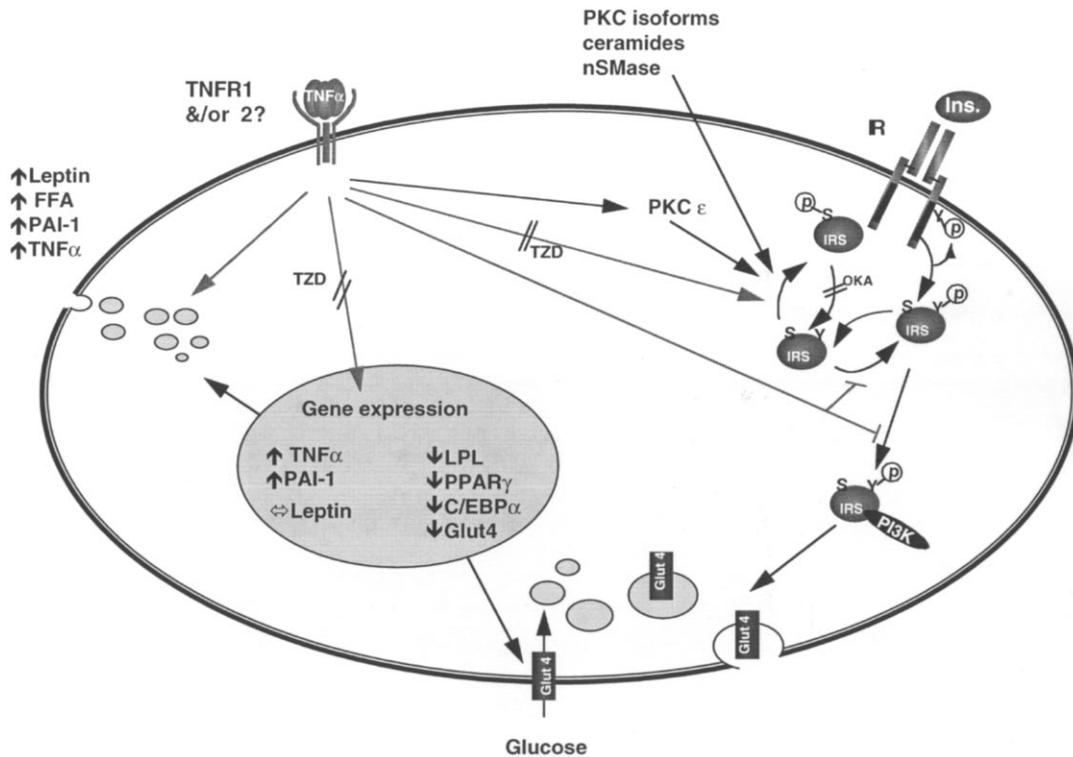


Figure 2. Potential mechanisms of TNF α -induced insulin resistance in adipocytes. TNF α has been demonstrated to disrupt insulin signaling at a number of peripheral sites which are dependent on IRS phosphorylation. As indicated, some of these actions can be mimicked by protein kinase C (PKC) isoforms, cell permeable ceramides, neutral sphingomyelinase (nSMase) and the protein phosphatase inhibitor, okadaic acid (OKA). In addition, chronic exposure to TNF α can suppress the expression of many adipocyte-specific genes including the insulin-sensitive glucose transporter (Glut 4). Pre-treatment with the insulin sensitizing thiazolidinediones (TZD) has been shown to be reverse at least two actions of TNF α . TNF α can also stimulate the production of additional mediators such as free fatty acids (FFA) and leptin which may act to potentiate its actions on insulin resistance in adipocytes. IR, insulin receptor; IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3-kinase.

TNFR1 nor TNFR2 exhibits intrinsic kinase activity, the search is on for receptor associated proteins that couple TNFR activation to IRS-1 phosphorylation. To this end, a few candidate kinases have been implicated. Of particular interest is PKC ϵ which can facilitate TNF α -induced inhibition of IR signaling in human embryonic kidney (HEK293) cells and whose translocation is stimulated by TNF α .⁸¹ Other PKC isoenzymes, namely α , δ , β_2 and θ , have recently been shown to inhibit IR kinase activity in an IRS-1 dependent manner.⁸² Alternatively, TNF α may be inhibiting IR signaling by inhibiting serine/threonine phosphatases⁸³ and/or recruiting the activity of protein tyrosine phosphatases (PTPases).^{84,85} However, it remains to be seen whether any of these enzymes are involved in TNF α -induced inhibition or simply medi-

ate the endogenous downregulation (negative feedback) of adipose IR activity in pathological states such as obesity and NIDDM.⁸⁶

Sphingolipid metabolism may also play a role in TNF α -induced insulin resistance (Figure 2). TNF α increases intracellular ceramide levels and the treatment of cells with neutral sphingomyelinase and synthetic analogs of ceramides can mimic the actions of TNF α on IR and IRS-1 phosphorylation.^{87,88} Whether ceramides then act directly on IR signaling or downregulate Glut 4 gene expression remains controversial.⁸⁹ However, Ceramide generation is known to regulate the activities of numerous other serine/threonine protein kinases, such as PKC ζ ⁹⁰ and Raf1 kinase,⁹¹ as well as phosphatases, such as protein phosphatase 2A.^{92,93} It is likely that one or more of

these enzymes could be involved in modulating IRS-1 in response to TNF α .

In addition to adipocytes, TNF α -induced insulin resistance has also been demonstrated in a number of other cell types including; hepatoma cells (FAO and KRC-7),^{47,78,85,88} fibroblasts,⁹⁴ myeloid cells (32D)^{49,87} and rat muscle cells (L6).⁹⁵ The factors recruited to mediate its actions appear to vary depending not only on the duration and concentration of TNF α but may also be specific to the tissue under investigation. For example, in fetal brown adipocytes, TNF α inhibition of IR phosphorylation is mediated via IRS-2⁹⁶ whilst in cultured white adipocytes and 32D cells, IRS-1 is the major substrate that is modulated.^{45,49} It has also been suggested that chronic treatment with TNF α can quantitatively regulate IR and IRS-1 in cultured adipocytes.⁹⁷ Furthermore, TNF α may employ additional extracellular mediators by altering free fatty acid and leptin secretion from adipose tissue. Whether these act directly with TNF α signaling pathways or indirectly as a means of potentiating its effects on adipose (or other tissues) remains to be determined.

Role of TNF α in regulating leptin production

Like TNF α , leptin also has significant effects on energy metabolism and appetite. Adipose tissue is considered to be the major source of circulating leptin.⁹⁸ An increasing number of reports (from rodents and humans) suggest that leptin expression and secretion can be positively regulated by insulin, glucocorticoids and glucosamine.⁹⁹⁻¹⁰¹ Cytokines including TNF α ^{60,102,103} also regulate leptin production. Recently, we and others have demonstrated that TNF α treatment increases leptin production *in vivo*.¹⁰²⁻¹⁰⁴ In contrast, treatment of cultured adipocytes produces a transient response, with a chronic treatment inhibiting leptin gene expression and decreasing secreted protein.^{104,105} Nevertheless, obese mice lacking TNF α or TNF receptors exhibit significantly lower circulating leptin levels.^{21,74,104} This strongly suggests that TNF α does have a physiological role in positively regulating leptin production in rodent models of obesity. That TNF α plays a similar role in human obesity has also recently been suggested.^{60,106} Indeed a significant correlation exists between circulating leptin, TNF α and body mass index (BMI) in humans.^{60,106} Interestingly, both TNF α and circulating leptin exhibit numerous parallels in their biology; they both show a strong correlation with percent

body fat, can modulate insulin sensitivity, can decrease food intake and regulate other aspects of energy metabolism (as cited in ref 104). It is therefore tempting to speculate that TNF α may recruit leptin to potentiate its insulin resistance effects through an autocrine or paracrine action. However, since obese TNF α -deficient mice still exhibit higher leptin levels than their lean counterparts,¹⁰⁴ TNF α may not be the master regulator of adipose derived leptin.

TNF α -mediated release of leptin from adipose tissue may be part of the adipostat mechanism that relates circulating leptin concentrations to triglyceride stores. It was postulated that cytokine-induced anorexia which occurs during infection and inflammation, may be mediated by enhanced leptin secretion.^{102,103} However, recent studies using mice deficient in either leptin (*ob/ob*) or its functional receptor (*db/db*), show that leptin is unlikely to be the sole mediator of LPS-induced appetite suppression.¹⁰⁷

We have used TNF receptor deficient mice to identify the receptor responsible for TNF α -induced leptin production *in vivo*. This function of TNF α appears to be predominantly attributed to the activity of TNFR1.¹⁰⁸ However, unlike most other actions of TNF α , transcriptional regulation does not seem to be the main mode of action. Indeed, the increased levels of leptin secreted in response to TNF α are not reflected by increased leptin mRNA expression levels in adipose tissue.¹⁰⁴ This discordance has been demonstrated *in vitro* in 3T3-L1 adipocytes, and *in vivo* in obese rodents¹⁰⁴ and humans.¹⁰⁶ Further pharmacological characterization with 3T3-L1 adipocytes has lent some clues as to how TNF α may influence leptin production.¹⁰⁴ These have demonstrated that the TNF α -stimulated leptin production is insensitive to the protein synthesis inhibitor, cyclohexamide, but can be blocked by the secretion inhibitor, brefeldin A. This indicates that TNF α is acting post-translationally perhaps by mobilizing preformed pools of leptin.

Role of TNF α in regulating PAI-1 biosynthesis

Adipose tissue expression and circulating levels of plasminogen activator inhibitor (PAI-1) is elevated in obese individuals¹⁰⁹ and may represent the main source of the elevated circulating plasma PAI-1 observed in obesity.¹¹⁰ This hypothesis is supported by previous studies which demonstrate that PAI-1 levels can be reduced significantly in parallel to weight loss.¹¹⁰ The expression of TNF α also shows a strong

correlation with PAI-1 expression, particularly in the context of obesity.¹¹⁰ Indeed, treatment with TNF α stimulates PAI-1 biosynthesis in numerous cell types, including 3T3-L1 adipocytes,¹¹¹ adventitial cells and vascular smooth muscle cells in adipose tissue.¹¹⁰ Furthermore, inhibiting TNF α synthesis with pentoxifylline treatment also reduces PAI-1 expression. These observations are in line with the hypothesis that locally elevated TNF α in adipose tissue acts in an autocrine fashion to stimulate PAI-1 production. Thus, in obesity, the increased levels of TNF α may be contributing to the elevated plasma PAI-1 levels and hence increased risk of coronary heart disease. Additional factors may also potentiate the actions of TNF α on PAI-1 synthesis. These include TGF β , insulin, triglycerides and FFA.^{110,112} At least the latter three are known to be elevated in obesity and modulated by TNF α . Studies are currently underway using ligand and receptor knockout obese mice to further address the role of TNF α in PAI-1 production in obesity.

Role of TNF α in adipocyte differentiation

TNF α has been shown not only to be a potent inhibitor of adipocyte differentiation but capable of suppressing the expression of some adipocyte-specific genes in fully differentiated adipocytes.¹¹³⁻¹¹⁵ Interestingly, the anti-adipogenic action of TNF α also appears to be reversed by subsequent treatment with adipogenic agents such as dexamethasone and indomethacin.¹¹⁶ This raises the intriguing possibility that adipocyte differentiation may be a reversible process *in vivo* and under the control of both positive and negative regulators.^{113,116} This long-standing debate has yet to be resolved.

As is the case for the action of other growth factors on development and differentiation, the exact mechanism(s) by which TNF α alters the adipocyte differentiation program is not yet clear. However, much attention has been focused on its effects on transcriptional regulation. TNF α has been shown to downregulate the expression of transcription factors, such as PPAR γ (peroxisome proliferator activated-receptor γ) and C/EBP α (CCAAT/enhancer-binding protein α), which are involved in the early stages of adipocyte conversion.^{115,117,118} This may explain the parallel downregulation of those adipose-specific genes (such as Glut 4 and aP2) that contain binding sites for PPAR γ or C/EBP α in their promoters^{76,117} and is consistent with the observation that PPAR γ

ligands, such as troglitazone and indomethacin, can prevent the actions of TNF α in cultured adipocytes as well as in whole animals.^{28,66} Interestingly, TNF α has recently been shown to directly modulate prostaglandin synthesis at the level of PGD₂ and/or PGE₂ synthesis in murine macrophages.¹¹⁹ Since, PGD₂ is metabolized to PGJ₂ and its derivatives, the putative endogenous ligands for PPAR γ , it is tempting to speculate that this may be the primary target for TNF α action in adipocytes. However, since TNF α -responsive kinases (MAPK and PKC) have been implicated in blocking adipogenesis¹²⁰ and their phosphorylation cascades can regulate transcription factor expression, the possibility remains that TNF α may also be acting through these alternative pathways. The TNF α receptor primarily involved in mediating the effects of TNF α on adipocyte differentiation is clearly TNFR1. Recent studies with cell lines deficient only in this TNF receptor have definitively demonstrated that this is the case with both soluble and transmembrane TNF α .^{5,121} These experimental systems should be instrumental in dissecting the signaling pathways involved in this action.

TNF α -induced apoptosis of adipose tissue

While the cytotoxic actions of TNF α toward certain cells and tumors are well documented,^{122,123} the potential apoptotic action on adipocytes has received little attention. However, reports are beginning to emerge which suggest that, under culture conditions, TNF α can also be cytotoxic to both adipocytes and pre-adipocytes.^{124,125} Most recently, TNF α -induced apoptosis has also been implicated in rodent brown adipocytes^{126,127} and increased brown fat apoptosis is correlated to obesity.¹²⁶ This, together with the actions of TNF α on adipocyte differentiation, could represent a possible mechanism by which TNF α may be acting to minimize adipose tissue mass.

Since both TNF receptors are capable of initiating apoptotic signals,¹²⁸⁻¹³⁰ they are both equally likely to be involved in adipocyte apoptosis. With the availability of receptor deficient mice, studies are currently underway to identify which receptor is involved in adipocyte apoptosis *in vivo* and whether this action is involved in obesity. Although very little is known about TNF α -induced cytotoxic signaling in adipocytes, a substantial amount of research has been conducted on other cells and tissues. Here the apoptotic signaling cascades emanating from each TNF receptor have been extensively studied. The reader is

referred to the excellent recent reviews by Hale *et al*¹³¹ and Wallach.¹²⁹

Conclusions

TNF α plays a key role in regulating energy metabolism under both physiological and pathological states. Recent developments of several transgenic models, with functionally-modified TNF α systems, have illustrated the definitive involvement of this cytokine in lipid and glucose metabolism, *in vivo*. These together with newly developed *in vitro* systems should aid future mechanistic studies of TNF α biology. The molecular dissection of the signaling events mediating these actions promises to offer novel targets for treatment of metabolic disorders in which TNF α plays a key role.

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