Pro-inflammatory cytokines and adipose tissue

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Cytokines appear to be major regulators of adipose tissue metabolism. Therapeutic modulation of cytokine systems offers the possibility of major changes in adipose tissue behaviour. Cytokines within adipose tissue originate from adipocyte, preadipocyte and other cell types. mRNA expression studies show that adipocytes can synthesise both tumour necrosis factor α (TNF-α) and several interleukins (IL), notably IL-1β and IL-6. Other adipocyte products with 'immunological' actions include complement system products and macrophage colony-stimulating factor. Cytokine secretion within adipocytes appears similar to that of other cells. There is general agreement that circulating TNF-α and IL-6 concentrations are mildly elevated in obesity. Most studies suggest increased TNF-α mRNA expression or secretion *in vitro* in adipose tissue from obese subjects. The factors regulating cytokine release within adipose tissue appear to include usual 'inflammatory' stimuli such as lipopolysaccharide, but also the size of the fat cells *per se* and catecholamines. There is conflicting data about whether insulin and cortisol regulate TNF-α. The effects of cytokines within adipose tissue include some actions that might be characterised as metabolic. TNF-α and IL-6 inhibit lipoprotein lipase, and TNF-α additionally stimulates hormone-sensitive lipase and induces uncoupling protein expression. TNF-α also down regulates insulin-stimulated glucose uptake via effects on glucose transporter 4, insulin receptor autophosphorylation and insulin receptor substrate-1. All these effects will tend to reduce lipid accumulation within adipose tissue. Other effects appear more 'trophic', and include the induction of apoptosis, regulation of cell size and induction of de-differentiation (the latter involving reduced peroxisome proliferator-activated receptor γ). Cytokines are important stimulators and repressors of other cytokines. In addition, cytokines appear to modulate other regulatory systems. Examples of the latter include effects on leptin secretion (probably stimulation followed by inhibition) and reduction of β_3-adrenoceptor expression. There seems to be no clear agreement as to which cytokines derived from adipose tissue act as remote regulators, i.e. hormones. Leptin, which is structurally a cytokine, is also a hormone. IL-6 appears to be released systemically by adipose tissue, but TNF-α is probably not. Both leptin and IL-6 appear to act on the hypothalamus, IL-6 acts on the liver, while leptin may have actions on the pancreas. The importance of the immune system in whole-body energy balance provides a rationale for the links between cytokines and adipose tissue. It seems clear that TNF-α is a powerful autocrine and paracrine regulator of adipose tissue. Other cytokines, notably leptin, and possibly IL-6, have lesser actions on adipose tissue. These cytokines act as hormones, reporting the state of adipose tissue stores throughout the body.

Cytokines: Adipose tissue: Tumour necrosis factor α: Interleukin 6: Leptin

Cytokines have been described (McDermott, 2001) as 'A vast array of relatively low molecular weight, pharmacologically active proteins that are secreted by one cell for the purpose of altering either its own functions (autocrine effect) or those of adjacent cells (paracrine effect). In many instances, individual cytokines have multiple biological activities. Different cytokines can have the same activity, which provides for biological redundancy within the inflammatory and immune systems. As a result, it is infrequent that loss or neutralization of one cytokine will markedly interfere with either of these systems. This fact has great significance in the development of therapeutic strategies.' Over 200 cytokine ligands have been recognised. With regard to adipose tissue, only a handful of

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these cytokines have been studied, notably tumour necrosis factor (TNF) α and various interleukins (IL) including IL-1β, IL-6 and IL-8. Fortunately, there appears to be a hierarchy of cytokines, so that some of the 'higher order' cytokines, such as TNF-α, orchestrate the synthesis, secretion and activity of others.

The present review will address: (1) the regulation of cytokine production in adipose tissue; (2) the effects of cytokines within adipose tissue; (3) the possible role of cytokines as remote signals from adipose tissue.

The different 'families' of cytokines have characteristic protein structures for the ligands and receptors. Leptin and its receptor have been shown to have structures resembling pro-inflammatory cytokines and receptors respectively. As leptin has a structural relationship with pro-inflammatory cytokines, the present review will allude to leptin as a possible indicator of the way such cytokines behave.

Cytokine production in adipose tissue and its regulation

White adipocytes *per se*, especially those in subcutaneous tissue, are the main source of leptin from adipose tissue (Montague *et al*. 1998). However, approximately 50 % of the nuclei and protein synthetic components in adipose tissue are found in non-adipocyte cells such as immunocytes, vascular, stromal and other cells which are also able to synthesise cytokines. For TNF-α, the most-studied cytokine in adipose tissue, it has been shown that most of the mRNA in adipose tissue is found within adipocytes *per se* (Hotamisligil *et al*. 1993; Kern *et al*. 1995; Montague *et al*. 1998). However, mRNA abundance is very low compared with that of other proteins in human white adipocytes (Montague *et al*. 1998) and unstimulated adipose tissue releases relatively small amounts of TNF-α *in vitro* (Crawford *et al*. 1997).

IL-6 is another pro-inflammatory cytokine secreted by adipose tissue in culture (Purohit *et al*. 1995; Fried *et al*. 1998), and its mRNA has also been demonstrated within adipocytes (Stephens *et al*. 1992; Purohit *et al*. 1995; Fried *et al*. 1998; Bastard *et al*. 2000). However, for IL-6 and other cytokines there is still uncertainty as to the proportion of the total tissue cytokine production that comes from adipocytes *per se*. For example, Fried *et al*. (1998) reported that isolated adipocytes release only 10 % as much IL-6 as adipose tissue in culture.

This sort of quantification is made more complex by the mechanisms by which cytokines can be secreted. For example, TNF-α is secreted as a membrane-bound precursor (26 kDa, 233 amino acids) (Gearing *et al*. 1994; McDermott, 2001). Acting on the membrane-bound substrate, a matrix metalloproteinase known as TNF-α-converting enzyme cleaves off soluble TNF-α which has 157 amino acids (17 kDa) (Gearing *et al*. 1994; Fried *et al*. 1998).

Once generated, membrane-bound TNF-α and soluble TNF-α in man can interact with receptors such as TNF receptors (TNF-R) 1 and 2. In man these are known as gp60 or TNFR60 and gp80 or TNFR80 respectively). In mice, the p55 receptor corresponds to TNF-R1 and p75 receptor to TNF-R2. Adipose tissue expresses mRNA for both these two receptors (Hotamisligil *et al*. 1997).

TNF-α and its main receptors also form part of a super-family of related cytokines and receptors (McDermott, 2001). It is known that in other (non-adipose) tissues there are nineteen different ligands that can interact with TNF receptors and that TNF-α can interact with a total of over twenty receptors (McDermott, 2001). It is unclear to what extent other members of these superfamilies are active in adipose tissue. However, of the major TNF receptors that are known to be active in adipose tissue, TNF-R1 mediates apoptosis, whilst TNF-R2 induces one or more mitogen-associated protein kinases (Hube & Hauner, 1999). There is no clear consensus as to how the different receptors relate to different actions of TNF-α. Pharmacological studies concluded that the TNF-R2 was involved with induction of insulin resistance (Hube & Hauner, 1999), although studies with knockout animals suggested that the TNF-R1 is crucial for stimulating lipolysis (Sethi *et al*. 2000). Whilst it is clear that membrane-bound TNF-α can interact with both TNF-R1 and TNF-R2, it has been suggested that soluble TNF-α preferentially binds to TNF-R1 rather than TNF-R2 (Hube & Hauner, 1999). More recently, it has been suggested that there are 60 kDa proteins that bind TNF-α in the mito-chondria, the significance of which is undetermined as yet (Ledgerwood *et al*. 1998).

TNF-α synthesis within adipose tissue has been more extensively studied than that of other cytokines. Its production can be shown to be responsive to both 'nutritional' and 'immunological' regulators. Additionally, there are regional effects such that subcutaneous adipose tissue expresses mRNA for TNF-α and TNF-R1 and TNF-R2 to a greater extent than omental tissue (Hube *et al*. 1999a). The reverse is true for IL-6 mRNA (Fried *et al*. 1998).

Regulation by nutritional factors

The synthesis of TNF-α can be shown to be related both to adiposity and to fatty acid metabolism. In many situations, adiposity and fatty acid metabolism are themselves linked. Several studies demonstrate that obese animals have increased TNF-α mRNA and protein synthesis within adipose tissue, as reviewed by Hotamisligil (1999). While mRNA expression is higher in obese human subjects (Hotamisligil *et al*. 1995; Hotamisligil, 1999; Hube *et al*. 1999a), it is not clear whether the protein release *in vitro* shows the same trend (Sewter *et al*. 1999). Circulating concentrations of TNF-α are usually reported to be slightly greater in more obese subjects (Hotamisligil *et al*. 1995; Mohamed-Ali *et al*. 1997), but the correlation between adiposity and plasma TNF-α is relatively weak, typically r_s <0·40; Mohamed-Ali *et al*. 1997; Hauner *et al*. 1998) and some researchers do not report this effect in human subjects (Hauner *et al*. 1998). Weight loss reduces plasma TNF-α and IL-6 concentrations (Kern *et al*. 1995; Bastard *et al*. 2000), whilst feeding animals high-fat diets will increase the TNF-α content of their adipose tissue (Morin *et al*. 1997b). Both adiposity and adipose tissue TNF-α increase as rats age (Morin *et al*. 1997a). Transgenic animals with the adipocyte fatty acid-binding protein knocked out of their adipose tissue have no TNF-α in their adipose tissue (Hotamisligil *et al*. 1996a). *n*-3 Fatty acids reduce production, as does infusion of Intralipid (Nisoli *et al*.

2000*b*). Insulin stimulates adipose tissue to produce more TNF-α, although the effect is less obvious in isolated adipocytes (Sewter *et al.* 1999). Circulating TNF-α concentration tends to correlate with circulating insulinaemia (Hotamisligil *et al.* 1995), although this relationship is difficult to interpret in view of the close inter-correlation of obesity and insulinaemia. Thiazolidinediones have been reported to stimulate adipose tissue TNF-α production in lean human subjects (Sewter *et al.* 1999), but to depress it in obese rodents (Peraldi *et al.* 1997*a*).

Regulation by immunological factors

In macrophages and monocytes many immunological regulators are known to affect cytokine production. Endotoxin induces TNF-α mRNA expression (Berkowitz *et al.* 1998) and protein secretion from both adipose tissue and isolated adipocytes within 2 h (Berkowitz *et al.* 1998; Sewter *et al.* 1999). Stimulation of TNF-α protein synthesis *in vitro* by lipopolysaccharide can be blocked by inhibitors of metalloproteinases (Sewter *et al.* 1999). Phorbol esters and phosphatase inhibitors induce TNF-α production in adipose tissue. Cortisol has no significant effect on adipose tissue TNF-α production, although it depresses production by macrophages (Beutler *et al.* 1986; Sewter *et al.* 1999). By contrast, glucocorticoid reduces adipose tissue IL-6 release *in vitro* (Fried *et al.* 1998).

TNF-α is able to induce not only its own synthesis, but is also a powerful regulator of other cytokines, e.g. stimulating the production of IL-6 (Stephens & Pekala, 1991). TNF-α may also regulate the synthesis of its own receptors. The expression of the TNF-R1 receptor may (Hube *et al.* 1999a) or may not (Hotamisligil *et al.* 1997) correlate with adiposity. Expression of the TNF-R2 receptor may correlate more closely with adipocyte diameter (Hube *et al.* 1999a) or obesity (Hotamisligil *et al.* 1997). These associations notwithstanding, in general the synthesis of TNF receptor mRNA within adipose tissue appears to be linked with that of TNF-α (Hube *et al.* 1999a), although this area has not been extensively studied. Little is known about deactivation of TNF and IL-6 receptors, but metalloproteinases cleave them from the cell surface (Mullberg *et al.* 1995).

Effects of cytokines within adipose tissue

For purposes of discussion, cytokines may be considered as having 'metabolic' effects, 'trophic' effects and effects on other regulatory systems within adipose tissue, although patently such divisions are somewhat artificial and arbitrary, and do not reflect physiology.

As discussed earlier, there is still uncertainty about which TNF-α receptor mediates individual effects of the cytokine within adipose tissue. It is known that a variety of cytoplasmic and nuclear 'second messengers' are involved in the TNF-α signal transductions. Within adipose tissue these messengers include CCAAT-enhancer-binding proteins α and β, the sphingomyelinase leading to the generation of ceramide and the proliferative and cell growth regulating p44/42 mitogen-associated protein kinase cascade (Jain *et al.* 1998, 1999). This topic has been authoritatively reviewed by Qi & Pekala (2000).

Metabolic effects of cytokines

There are more data relating to the metabolic actions of TNF-α than those of other cytokines (Table 1). Most of these effects have been demonstrated by *in vitro* studies of rodent adipose tissue. In these studies TNF-α can reduce expression of mRNA for glucose transporter 4 (Stephens & Pekala, 1991) as well as stimulate hormone-sensitive lipase expression to increase lipolysis (Sumida *et al.* 1997). TNF-α has long been known to decrease lipoprotein lipase activity (Kawakami *et al.* 1982; Fried & Zechner, 1989; Feingold & Grunfeld, 1992). The net effect is to induce insulin resistance and mobilise lipids (Ogawa *et al.* 1989; Hauner *et al.* 1995) from within the adipocytes. TNF-α appears to orchestrate these effects via inhibition of CCAAT-enhancer-binding protein (Stephens & Pekala, 1991) and peroxisome proliferator-activated receptor γ (Zhang *et al.* 1996). This suppression may down regulate many adipocyte-specific proteins such as fatty acid-binding protein, fatty acid synthase, acetyl-CoA carboxylase and glucose-6-phosphate dehydrogenase as well as lipoprotein lipase and glucose transporter 4 (Hube & Hauner, 1999). TNF-α also reduces tyrosine phosphorylation of the insulin receptor and increases phosphorylation of insulin receptor substrate-1 at serine residues, actions which contribute to the insulin-resistant state.

TNF-α can also inhibit insulin action and insulin receptor signalling in cultured human adipocytes (Liu *et al.* 1998). This inhibition of insulin action is thought to be mainly mediated by TNF-R1 (Kanety *et al.* 1996; Peraldi *et al.* 1997*b*). Administration of a soluble TNF-R–immunoglobulin G fusion protein improved insulin sensitivity in obese rodents *in vivo* (Hotamisligil *et al.* 1993). However, attempts to demonstrate the importance of TNF-α in human subjects *in vivo* by infusion of anti-TNF-α antibodies showed no effect (Ofei *et al.* 1996). It is not clear whether this finding should be interpreted as showing that TNF-α has no metabolic effects in man. Alternative explanations might be that the antibody concentrations and binding characteristics were not adequate, or that they were not able to penetrate the tissues to adequately block both membrane-bound TNF-α and soluble TNF-α.

IL-6 and IL-1β also reduce adipose tissue lipoprotein lipase activity (Feingold *et al.* 1992; Greenberg *et al.* 1992; Hardardottir *et al.* 1994).

Trophic effects of cytokines

The trophic effects of TNF-α have been studied more extensively than those of the other cytokines. In mature white adipocytes TNF-α is reported to regulate cell size (Hotamisligil *et al.* 1995), the suggestion being that as the cells get bigger they produce more TNF-α, which in turn initiates changes to limit adipocyte size or to induce apoptosis.

The regulation of adipose cell numbers is complex, with regulation occurring through multiple mechanisms (Prins & O'Rahilly, 1997). However, TNF-α may be a major factor, with actions on both adipocyte precursors and mature adipocytes. There are three main ways in which cell numbers can be regulated within adipose tissue:

(1) blocking differentiation of new adipocytes; (2) promoting de-differentiation of existing adipocytes or preadipocytes; (3) inducing apoptosis of adipocytes or preadipocytes. TNF-α appears to do all three, at least *in vitro*. TNF-α has been shown to induce apoptosis in pre-adipocytes (Prins *et al.* 1997), with omental preadipocytes more sensitive to this effect than subcutaneous pre-adipocytes (Niesler *et al.* 1998). Additionally, it can both block differentiation and induce de-differentiation in pre-adipocytes (Pekala *et al.* 1983; Torti *et al.* 1989; Petruschke & Hauner, 1993). TNF-α can induce both de-differentiation (Petruschke & Hauner, 1993), and also apoptosis of mature adipocytes (Prins *et al.* 1997). Prins *et al.* (1997) demonstrated a dose–response effect, with a maximal effect at 25 nM-TNF-α and an effect within 2 h.

In brown adipocytes TNF-α appears to have the slightly 'contradictory' actions of both mediating apoptosis and stimulating uncoupling protein-1 expression (Nisoli *et al.* 2000*b*).

Insulin-like growth factor-1 potentiates the pro-apoptotic effect of TNF-α (Niesler *et al.* 2000), but thiazolidinediones can block the effects of TNF-α on regulation of adipocyte number (Szalkowski *et al.* 1995).

Cytokine effects on other regulators

In addition to the previously described metabolic and trophic effects, cytokines can also have important actions via their impact on other regulators. These actions include the abilities of cytokines to affect signals coming into adipose tissue. An example of this effect is the ability of TNF-α to repress the expression of β_3-adrenoceptors in white and brown adipocytes (Berkowitz *et al.* 1998; Nisoli *et al.* 2000*a*). These receptors are important for sympathetically-mediated lipolysis in white adipose tissue and thermogenesis in brown adipose tissue (Strosberg & Pietri-Rouxel, 1996). TNF-α can also regulate signals efferent from adipose tissue by inducing leptin mRNA expression in, and protein secretion from, adipocytes (Grunfeld *et al.* 1996; Kirschgessner *et al.* 1997; Berkowitz *et al.* 1998). A complication of this effect is that it is not seen during longer incubations of fat cells, but the reason for this situation has not been established (Fawcett *et al.* 2000). Exogenous IL-1 is also able to stimulate leptin secretion (Sarraf *et al.* 1997).

Knockout animals

Transgenic animals have been bred with TNF-α protein (Uysal *et al.* 1997; Ventre *et al.* 1997) or its receptors (Uysal *et al.* 1998) knocked out. The TNF-α protein knockout animals are the same weight as their wild-type littermates when placed on equivalent diets (Uysal *et al.* 1997). They have low plasma concentrations of insulin and of non-esterified fatty acids (Uysal *et al.* 1997; Ventre *et al.* 1997). These animals have increased insulin sensitivity, this change being mediated through rodent TNF-R1 (Uysal *et al.* 1997; Hotamisligil, 1999). The rodents with TNF receptors knocked out similarly have normal adiposity, but also show better insulin sensitivity even when obese (Uysal *et al.* 1998).

Questions about the importance of cytokines in adipose tissue

If we are to consider that cytokines are important regulators within adipose tissue, we need to be sure that cytokines have the proposed regulatory effects at concentrations (or 'doses') actually found in adipose tissue. Many actions of cytokines listed earlier have been demonstrated at concentrations which may be much greater than those known to exist in adipose tissue. The fact that TNF-α knockout animals do have abnormal insulin resistance and lipid metabolism suggests that cytokines have constitutive effects under physiological conditions (Uysal *et al.* 1997; Ventre *et al.* 1997). However, these animals apparently had normal adiposity. This factor would suggest either that TNF-α has only a minor role on adipose tissue mass, or that the redundancy of the system for adipose tissue regulation is such that removing TNF-α as a single factor has little impact.

There is evidence that at least some cytokines do not have the same effects in different adipose tissue depots or adipocytes. There is the possibility that the effects of cytokine on adipose tissue differ between species (Hube & Hauner, 1999), but this factor has not been extensively studied. Even within one mammal, different adipose depots show different responses to cytokines and levels of cytokine expression (Fried *et al.* 1998; Hube *et al.* 1999*b*). Furthermore, there is data that preadipocytes and mature adipocytes can differ in their cytokine expression characteristics (Hube *et al.* 1999*b*). Finally, except for the knockout animals, all the other studies have examined short-term effects of cytokines, and we know little of the longer-term actions of cytokines on adipose tissue. Indeed, there is some evidence that TNF-α can have a biphasic action on leptin secretion, with intial stimulation being followed by suppression (Fawcett *et al.* 2000).

Tumour necrosis factor α as an anti-adipogenic regulator

Notwithstanding these limitations in the current state of knowledge, it has been postulated that TNF-α acts as a regulator of adipose tissue mass through its anti-adipogenic effects (Hube & Hauner, 1999). TNF-α has a wide range of actions which all combine to reduce adiposity. Some of these effects are listed in Table 1.

Many of these actions have been mentioned earlier and extend from actions within adipose tissue to remote effects on appetite. Induction of uncoupling protein-1 would tend to promote 'wasting' of energy by uncoupled respiration. Naturally, such energy wastage will tend to reduce the size of adipose tissue stores. The propensity of TNF-α to prevent both differentiation of preadipocytes and promotion of apoptosis within mature adipocytes will tend to reduce adipocyte number. Finally, TNF-α production in adipose tissue can potentially have effects on distant tissues either directly by TNF-α acting as a hormone, or indirectly by TNF-α affecting other hormones such as leptin.

Do cytokines act as hormones from adipose tissue?

The discovery of leptin, which is undoubtedly a hormone released by adipose tissue (Zhang *et al.* 1994; Friedman,

Table 1. Some actions of tumour necrosis factor α as an anti-adipogenic agent

	Examples	References
Local metabolic actions: induction of insulin resistance	Reduces expression of GLUT4	Stephens & Pekala (1991)
	Stimulates HSL expression to increase lipolysis	Sumida et al. (1997)
	Decreases LPL activity	Kawakami et al. (1982), Fried & Zechner (1989), Feingold & Grunfeld (1992)
	Reduced insulin signalling:	
	Reduced tyrosine phosphorylation of insulin receptor	Hotamisligil et al. (1994)
	Increased phosphorylation of IRS-1	Hotamisligil et al. (1996b)
	Decreased C/EBP	Stephens & Pekala (1991)
	Decreased PPARγ	Zhang et al. (1996)
Local trophic actions	Apoptosis of mature white adipocytes	
	De-differentiation of preadipocytes	
Local effects on other signalling systems	Reduces expression of β₃-adrenoceptors in white and brown adipocytes	Berkowitz et al. (1998), Nisoli et al. (2000a)
Energy 'wasting'	Induction of uncoupling protein-1	Nisoli et al. (2000a)
Remote actions	Anorectic actions	Van der Poll et al. (1991), Plata-Salaman (2000)
Indirect actions	Anti-adipogenic actions via stimulation of leptin production	Grunfeld et al. (1996), Sarraf et al. (1997)

GLUT4, glucose transporter 4; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; IRS-1, insulin receptor substrate-1; C/EBP, CCAAT-enhancer-binding protein; PPARγ, peroxisome proliferator-activated receptor γ.

2000), demonstrated unequivocally that adipose tissue can act as an endocrine organ (Mohamed-Ali et al. 1998; Lawrence & Coppack, 2000). More importantly for the present article, leptin appears to be strongly related in a structural way to pro-inflammatory cytokines. This factor clearly raises the question as to whether other cytokines can behave as hormones. Interest has focused on TNF-α and IL-6. For TNF-α it is clear that this cytokine has actions in several organs and tissues in vitro, and that it is able to induce insulin resistance by multiple effects on insulin signalling (Hotamisligil, 1999). Injections of TNF-α to mimic the order of circulating TNF-α concentrations seen in sepsis have an anorectic effect (as mentioned earlier; Van der Poll et al. 1991; Plata-Salaman, 2000) and induce elevations in plasma non-esterified fatty acid concentrations and whole-body insulin resistance (Hotamisligil, 1999). Likewise injections of IL-6 can increase circulating concentrations of fatty acids, C-reactive protein and fibrinogen (Strassmann et al. 1993). IL-1 and IL-6 are two of the several cytokines shown to have anorectic effects on the brain (Plata-Salaman, 2000) and to induce gastric stasis.

However, there is little evidence of net release of TNF-α from human subcutaneous adipose tissue (Mohamed-Ali et al. 1997, 1999), and injection of TNF-α-neutralising antibody (Ofei et al. 1996) has no measurable effect on systemic insulin resistance and lipids. The fact that adipose tissue is actually a net exporter of soluble TNF-R1 (Mohamed-Ali et al. 1999) suggests that TNF-α produced locally within adipose tissue is sequestered, possibly by the large excess of TNF receptors within the tissue. As a result of the local production of soluble receptors, TNF-α ligand:soluble receptors is lower in venous blood draining subcutaneous adipose tissue than in arterial blood (Mohamed-Ali et al. 1999). Likewise, obese subjects have lower circulating TNF-α:soluble TNF receptors (Hauner

et al. 1998; Mohamed-Ali et al. 1999). Any release into the circulation of TNF-α produced in adipose tissue is probably balanced by comparable clearance of circulating TNF-α by the same tissue.

For IL-6, a stronger case can be made for it having a role as a circulating hormone (Jones, 1994; Yudkin et al. 2000). As for TNF-α, IL-6 injected systemically has significant effects on a variety of tissues, suppressing appetite, increasing lipolysis and suppressing lipoprotein lipase. Additionally, it is a powerful regulator of the hypothalamus–pituitary–adrenal axis (Jones & Kennedy, 1993; Rothwell, 1994; Papanicolaou et al. 1998). As the protein is thought to be little expressed within the central nervous system, it can be inferred that IL-6 acts remotely in this way. IL-6 concentrations correlate well with concentrations of C-reactive protein, even in apparently-healthy subjects without an obvious infection (Mohamed-Ali et al. 1997, 1999). This finding suggests that IL-6 may have actions on the liver to stimulate the secretion of acute-phase proteins (Mohamed-Ali et al. 1998). IL-6 can also affect steroid hormone conversion, changing the balance of sex hormones, which is well known to regulate adipose tissue distribution (Purohit et al. 1995). The correlation of circulating IL-6 concentrations with adiposity appears stronger than those of TNF-α (Mohamed-Ali et al. 1997). This finding implies that a higher proportion of circulating IL-6 is synthesised by adipose tissue.

Interactions between adipose tissue stores and immunology

It is apparent that extremely-malnourished individuals have impaired immune function. Even in better-nourished individuals there are several indications of interaction between the immune system and adipose tissue. Adipose

tissue secretes into the general circulation several factors that might appear to be predominantly involved with immune functions. One example of this action is the secretion of complement components, some of which make adipsin, a component of adipocyte-stimulating protein (Saleh *et al*. 1998). Another example is the secretion of IL-6 discussed earlier. IL-6 appears to act as both a pro-inflammatory cytokine and as an important hypothalamic regulator (Jones & Kennedy, 1993; Rothwell, 1994). This fact may give a clue to the ontological development of leptin, a hormone with a cytokine structure having major effects on the hypothalamus but whose relation to inflammation appears to be somewhat peripheral. Pond, whose work is described elsewhere in the present symposium (Pond, 2001), has shown that there is a tight coupling of adipocyte metabolic activity with their distance to the nearest lymph node (Pond, 1999), and that TNF-α, and IL-4 and IL-6 may be part of this interaction (Mattacks & Pond, 1999).

These facts demonstrate the linkage between adipose tissue and the immune system. As yet, it is not clear why such links exist. However, the immune system is a major user of energy (it has been estimated that it consumes about 15 % of resting metabolic rate; about the same percentage as that of defence spending as a percentage of gross domestic product in the USA). One can therefore speculate that this level of energy usage needs to be linked to the availability of energy, especially the size of the adipose tissue energy stores. This requirement could explain the evolutionary development of 'cross-talk' between adipose tissue and the immune system. Such communication would need to be two way. When the immune system is stressed, it needs to call on energy from the stores, hence cytokines such as TNF-α have anti-adipogenic actions. Conversely, when the adipose tissue stores are undesirably small, signals that would reduce immunological preparedness and reduce energy expenditure would be appropriate. This type of communication is the likely origin of leptin and IL-6 as adipose tissues signals. Originally in evolution these proteins may have had a primary immunological message, but they may have evolved to become recognised by other tissues including the hypothalamus.

Although speculative, these reasons can explain why it is now becoming apparent, perhaps unexpectedly, that cytokines have important roles in adipose tissue. For adipose tissue biologists, cytokines are both a challenge and an opportunity. The challenge is to understand such a complicated and redundant system of regulation. The opportunity may be that they could offer a significant therapeutic target to combat the current expansion in the adipose tissue that we all are carrying around.

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