NEW PERSPECTIVES ON ENDOCRINE SIGNALLING

Tumour necrosis factor α : a key regulator of adipose tissue mass

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Abstract

In addition to its established role in the immune system, tumour necrosis factor α (TNF α) exerts complex regulatory actions on adipose tissue. TNF α is produced in and secreted by the adipocyte and thus is in a position to exert a paracrine and/or autocrine role within adipose tissue. TNF α affects many aspects of adipocyte function, from adipocyte development to lipid metabolism. Bringing

together all of these diverse actions, TNF α appears to play a general role in reducing adipose tissue mass. Dysregulation of TNF α production and/or action could be one facet in the development of cachexia and obesity, as well as associated metabolic disorders such as insulin resistance.

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Introduction

Over the past decade, there has been a shift in the understanding of the role of adipose tissue in metabolic homeostasis. The concept of adipose tissue as merely an energy store has now been surpassed by the realisation that the tissue has an important endocrine role. However, the exact endocrine nature of adipose tissue remains to be fully elucidated.

Adipose tissue secretes a variety of products that can exert both local and systemic effects. Amongst this ever-increasing list of secretory products are a number of pro-inflammatory cytokines, such as tumour necrosis factor α (TNF α). TNF α has received particular attention over the past decade due to its role as a key regulator of adipose tissue mass. TNF α is synthesised and secreted from adipocytes (Kern *et al.* 1995) and, hence, is in a key position to play a paracrine and/or autocrine role in the control of adipocyte function.

Changes in adipose tissue mass may be associated with a change in adipocyte number and/or a change in adipocyte volume. Changes in adipocyte number are governed by changes in preadipocyte maturation by adipogenesis, preadipocyte replication and cell deletion by apoptosis. The processes of fatty acid uptake, lipogenesis and lipolysis may alter adipocyte volume. These processes can be modulated by a range of stimuli, including insulin, glucocorticoids, catecholamines and cytokines (Sethi & Hotamisligil 1999). TNF α has been found to play a key modulatory role in many of these processes.

Adipogenesis

Adipogenesis refers to the process whereby preadipocytes, which are stem cells that have become committed to the adipocyte lineage, further develop into functioning adipocytes. Adipogenesis is initiated by the production of two key transcription factors, CCAAT/enhancer-binding protein α and peroxisome proliferator-activated receptor γ (PPAR γ), which are responsible for inducing the expression of adipocyte-specific genes. Adipogenesis is regulated by a myriad of signals, which exert positive or negative effects on adipogenesis. These factors can be either of adipocyte or preadipocyte origin, or from other sources. TNF α acts to inhibit the process of adipogenesis (MacDougald & Mandrup 2002). Either secreted or membrane-bound TNF α can act through the TNF α receptor (TNFR)1 to inhibit adipogenesis via a mechanism involving the activation of the extracellular regulated kinase (ERK)1/2 pathway (Xu *et al.* 1999). This sustained activation of the ERK1/2 pathway has been suggested to inhibit adipogenesis, at least in part, through the phosphorylation and thus functional inhibition of PPAR γ (Hu *et al.* 1996). However, other studies have suggested that activation of the ERK1/2 pathway promotes adipogenesis (Bost *et al.* 2002). In order to consolidate these findings, the timing of activation of the ERK1/2 pathway has been suggested to be critical to the outcome. Early activation of the ERK1/2 pathway during the differentiation process might promote whereas late activation might inhibit adipogenesis (Prusty *et al.* 2002), an idea that remains to be

fully investigated. Alternatively, it is possible that the distinction between enhancement and inhibition of adipogenesis exists in differential activation of TNFR1 and its sister receptor, TNFR2. The two receptor subtypes have been shown to have different effects on adipocyte function (Fruhbeck *et al.* 2001).

Apoptosis

Increasing concentrations of TNF α have been shown to augment apoptosis of both human preadipocytes and adipocytes from subcutaneous and omental adipose tissue (Prins *et al.* 1997). The mechanism through which this is achieved is unclear. Studies in the rat have suggested that adipocytes, but not preadipocytes, can be induced to undergo apoptosis by TNF α via a mechanism involving caspase 3, a protease involved in the proteolytic cascade leading to apoptosis (Qian *et al.* 2001). However, studies utilising subcutaneous adipose tissue from human subjects have shown that TNF α can up-regulate the expression of pro-apoptotic genes, such as *bcl-2* and caspase 1, in both preadipocytes and adipocytes (Zhang *et al.* 2001). The ability of human, but not rat, preadipocytes to respond could be associated with the doses employed in the different studies or, alternatively, there may be fundamental differences in the mechanisms by which TNF α initiates apoptosis in rat and human preadipocytes.

Lipid metabolism

Lipid metabolism is a complex sequence of events that determine whether the triglyceride pool within the adipocyte increases, due to the processes of free fatty acid (FFA) uptake and lipogenesis, or if the pool decreases, due to the process of lipolysis. Circulating lipoprotein and triglyceride are first converted into FFA by the action of lipoprotein lipase (LPL), which is secreted by the adipocyte. FFA can then enter the adipocyte via a fatty acid transporter. Once inside the adipocyte, the FFA is converted into the triglyceride by a multi-step-regulated enzymatic reaction, one of the enzymes involved being acyl-CoA synthetase. In addition, triglyceride can be formed from the uptake of glucose, via glucose transporters (GLUT)1 and 4, into the adipocyte. The glucose can then be converted into triglyceride by the actions of a series of enzymes, which include acetyl-CoA carboxylase and fatty acid synthase (Ramsay 1996, Sethi & Hotamisligil 1999).

A large body of evidence now supports a role for TNF α in modulating these processes (Sethi & Hotamisligil 1999). Studies utilising mammary adipose tissue from human subjects have now shown that TNF α inhibits LPL activity by down-regulating its protein expression (Hauner *et al.* 1995). Indeed, increasing TNF α mRNA levels are correlated with decreasing LPL activity in human subcutaneous

adipose tissue (Kern *et al.* 1995). In addition, TNF α has also been shown to reduce the expression of FFA transporters in adipose tissue of the Syrian hamster (Memon *et al.* 1998a). TNF α could thus hinder the synthesis and entry of FFA into the adipocyte, curtailing an increase in the intracellular triglyceride pool size. Studies have also suggested that TNF α may decrease the expression of enzymes involved in lipogenesis. Specifically, it has been suggested that acetyl-CoA carboxylase and fatty acid synthase are down-regulated. However, it is unclear if this occurs in mature adipocytes (Sethi & Hotamisligil 1999). Acyl-CoA synthetase expression and activity have also been suggested to be down-regulated by TNF α (Memon *et al.* 1998b).

The triglyceride pool can be reduced by the process of lipolysis. The triglyceride is converted into FFA and glycerol, by the actions of hormone-sensitive lipase (HSL) and 2-monoacylglycerol lipase, which are released from the adipocyte (Ramsay 1996). TNF α has been found to promote lipolysis. However, the mechanisms by which this is achieved are unclear. Studies utilising human subcutaneous adipocytes have shown that, concomitant with an increase in lipolysis induced by TNF α , there is activation of mitogen-activated protein kinase and ERK1/2, leading to a decrease in the expression of phosphodiesterase 3B, together with an elevation in intracellular cAMP, hence activation of protein kinase A (Zhang *et al.* 2002). Since these two pathways are classically not coupled, the significance of changes in both is unclear in relation to an increase in lipolysis. Conversely, TNF α has been found to down-regulate key lipolytic proteins. The close association between the expression of enzymes involved in lipolysis and lipogenesis with adipogenesis have made it difficult to tease out process-specific actions of TNF α (Sethi & Hotamisligil 1999). TNF α can thus act on the adipocyte to shift lipid metabolism away from lipid accumulation and towards a reduction in the triglyceride pool, which could represent one mechanism by which TNF α can reduce total adipose tissue weight.

Obesity

The role of TNF α in human obesity is unclear. Studies in women have shown that subcutaneous adipose tissue TNF α mRNA levels in obese subjects are higher than those found in lean subjects, but are normalised after weight loss (Hotamisligil *et al.* 1995). These findings are paralleled in the plasma, where TNF α levels are increased in obese women compared with lean controls (Bastard *et al.* 2000). In addition, studies in women have shown an increase in TNFR2 expression in adipose tissue and a sixfold increase in circulating soluble TNFR2 levels, but no change in TNFR1, with obesity. This rise in TNFR2 may modulate the actions of TNF α (Hotamisligil *et al.* 1997). However, other studies have reported no

Figure 1 Proposed mechanisms by which TNF α may act to decrease adipocyte mass. TNF α has been found to promote (+) apoptosis of both preadipocytes and mature adipocytes, inhibit (–) the processes of adipogenesis and lipogenesis and promote (+) the process of lipolysis. TNF α is thus in a position to maintain adipose tissue mass by curtailing excessive mature adipocyte production and lipid accumulation. TNF α may be of adipocyte origin or from other sources, accessing the tissue via the circulation.

correlation between TNF α mRNA levels and body mass index (BMI) in studies using mixed sex groups (Koistinen *et al.* 2000). Interpreting these apparently contradictory data is difficult. There could be a sexual dimorphism in the alteration of TNF α gene expression and protein secretion with obesity. This sexual dimorphism could be responsible for the lack of any strong association between BMI and TNF α mRNA in studies that have utilised mixed sex groups. Also, BMI may not be sufficiently indicative of total body fat. Indeed, one study has shown that although there was a lack of association between TNF α mRNA and BMI, there was a positive correlation between total body fat and TNF α mRNA (Kern *et al.* 1995).

Studies utilising animal models of obesity have also produced conflicting results. The *db/db*, *ob/ob*, *tub/tub* and *fa/fa* genetic mouse models of obesity all exhibit a similar obesity-related increase in expression of TNF α mRNA in adipose tissue, compared with lean controls. However, in the more modest forms of obesity induced by administration of monosodium glutamate and streptozotocin, no changes in TNF α mRNA have been observed (Hotamisligil *et al.* 1993). TNF α mRNA expression has been shown to be only increased in the morbidly obese,

but not in mildly obese or lean human subjects (Koistinen *et al.* 2000). Thus, in both the human and the mouse, it would appear that TNF α gene expression is increased only in the more extreme forms of obesity. Another possibility could be associated with the root cause of the obesity – genetic or environmental. The importance of genetics is exemplified in studies of patients with familial combined hyperlipidaemia, in which there is an increase in the gene expression of TNF α in subcutaneous adipose tissue (Eurlings *et al.* 2002). Changes in TNF α appear to be more consistent when there is a predominant genetic basis to the obesity.

Insulin resistance

TNF α has been implicated as a factor associated with the development of insulin resistance in obesity. Studies in women have found a positive association between plasma insulin levels and TNF α mRNA from subcutaneous adipose tissue (Hotamisligil *et al.* 1995), which is supported by a study showing increased adipose TNF α secretion in obese patients with insulin resistance (Kern *et al.* 2001).

Further supporting evidence comes from studies in TNF α -deficient mice that are protected from the obesity-induced insulin resistance (Hotamisligil *et al.* 1993). However, studies in men utilising subcutaneous adipose tissue have found no association between TNF α and insulin sensitivity (Koistinen *et al.* 2000). This further underscores the importance of gender when considering the actions of TNF α . Extensive research has highlighted several potential mechanisms by which TNF α induces insulin resistance. These include: accelerated lipolysis and a concomitant increase in circulating FFA concentrations, down-regulation of GLUT4 synthesis, down-regulation of insulin receptor and insulin receptor substrate 1 (IRS-1) synthesis and increased Ser/Thr phosphorylation of IRS-1 (Moller 2000).

Cachexia

Severe weight loss, or cachexia, is a detrimental end-point of several diseases, including cancer, infection and congestive heart failure. Unlike starvation, cachexia results from a severe loss of lean body mass (mostly skeletal muscle) and the depletion of the fat depots (Tisdale 2002). The pathogenesis of this weight loss is multifactorial, but evidence suggests that cytokines are key players. The predominant reason for the loss of adipose tissue is the fall in the activity of LPL and an increase in the activity of HSL. Since TNF α has been shown to promote lipolysis and inhibit lipogenesis, it is an ideal player in the depletion of adipose tissue mass seen with cachexia. It has been proposed that an elevation in plasma levels of TNF α , as opposed to adipose tissue-derived TNF α , is responsible for the metabolic alterations in adipose tissue seen with cachexia (Argiles *et al.* 1997).

Summary

The actions of TNF α on adipocyte function are diverse and together they all may promote weight loss, as illustrated in Fig. 1. TNF α could curtail any increase in adipose tissue mass by reducing the triglyceride pool of adipocyte. Alternatively or in addition, TNF α may prevent an increase in adipocyte number by inhibiting adipogenesis. In conditions of extreme weight loss (cachexia) or gain (obesity), the role of TNF α is not clear. In cachexia, non-adipocyte-derived TNF α may contribute to the wasting observed. However, the role in obesity is more elusive. There does not appear to be any consensus on the relationship between TNF α and BMI. Important factors in the role of TNF α in obesity could be gender, the underlying cause of the obesity (genetic *vs* environmental) and the adipose tissue depot under investigation. It is clear, however, that TNF α is an important member of

an ever-growing list of factors that modulate adipocyte function. Thus a better understanding of the interplay between these factors, as well as their alterations in pathological states, is critical to our understanding of metabolic disorders and may provide an opportunity to develop novel therapies for these wide-ranging conditions.

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