



## Review

# Adipose tissue as an endocrine and paracrine organ

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The discovery of leptin has imparted great impetus to adipose tissue research by demonstrating a more active role for the adipocyte in energy regulation. Besides leptin, however, the adipose tissue also secretes a large number of other signals. Cytokine signals, TNF $\alpha$  and IL-6, and components of the alternative pathway of complement influence peripheral fuel storage, mobilization and combustion, as well as energy homeostasis. In addition to the acute regulation of fuel metabolism, adipose tissue also influences steroid conversion and sexual maturation. In this way, adipose tissue is an active endocrine organ, influencing many aspects of fuel metabolism through a network of local and systemic signals, which interact with the established neuroendocrine regulators of adipose tissue. Thus, insulin, catecholamines and anterior pituitary endocrine axes interact at multiple levels with both cytokines and leptin. It may be proposed that the existence of this network of adipose tissue signalling pathways, arranged in an hierarchical fashion, constitutes a metabolic repertoire which enables the organism to adapt to a range of different metabolic challenges, including starvation, reproduction, times of physical activity, stress and infection, as well as short periods of gross energy excess. However, the occurrence of more prolonged periods of energy surplus, leading to obesity, is an unusual state in evolutionary terms, and the adipose tissue signalling repertoire, although sophisticated, adapts poorly to these conditions. Rather, the responses of the adipose tissue endocrine network to obesity are maladaptive, and lay the foundations of metabolic disease.

**Keywords:** adipose tissue; insulin; catecholamines; leptin; TNF $\alpha$ ; IL-6

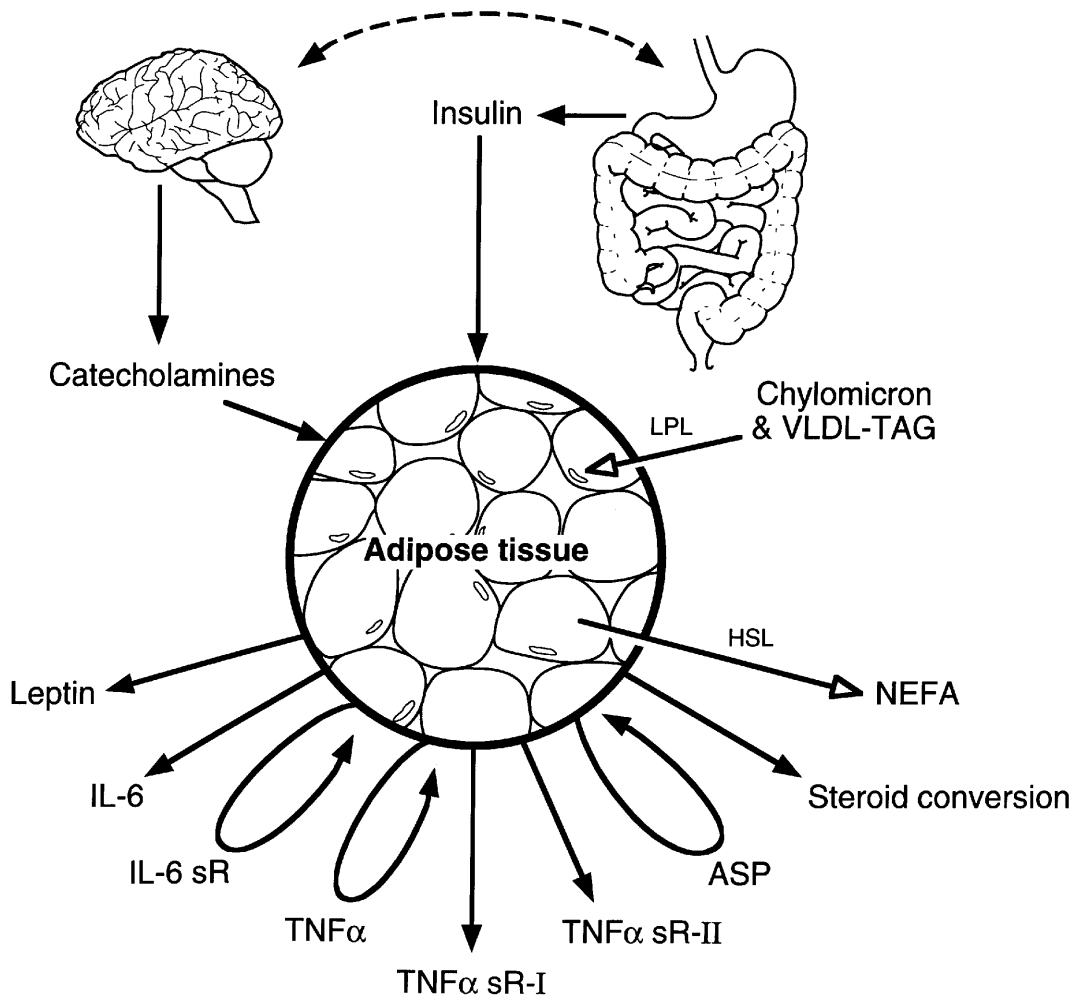
## Introduction

Adipose tissue participates actively in energy regulation, through a network of endocrine, paracrine and autocrine signals. This network, the complexity of which was not suspected until relatively recently, enables the adipose tissue to influence metabolic activity at many other sites, including skeletal muscle, liver and the brain. In 1987 Siiteri identified the endocrine role of adipose tissue in relation to sex steroids.<sup>1</sup> A more modern view would include a diverse range of signals emanating from adipose tissue, such as leptin,<sup>2</sup> tumour necrosis factor- $\alpha$  (TNF $\alpha$ ),<sup>3</sup> interleukin-6 (IL-6),<sup>4</sup> and their respective soluble receptors, non-esterified fatty acids (NEFA)<sup>5</sup> and acylation stimulating protein (ASP).<sup>6</sup> The relationships between sex steroids and glucocorticoids, and adipose tissue distribution and heterogeneity are better understood. Adipose tissue also secretes important regulators of lipoprotein metabolism including lipoprotein lipase (LPL), cholesteryl ester transfer protein (CETP) and apolipoprotein E.<sup>7–9</sup> The increasing number of adipose tissue products also includes

plasminogen activator inhibitor-1 (PAI-1),<sup>10</sup> transforming growth factor- $\beta$  (TGF $\beta$ ),<sup>9</sup> angiotensinogen<sup>11</sup> and possibly insulin-like growth factor-1 (IGF-1),<sup>12</sup> the roles of which remain to be fully defined. Afferent signals modulating adipocyte function include catecholamines, insulin, and anterior pituitary endocrine axes. Recent investigations strongly suggest that several of these afferent signals also influence efferent signalling by adipose tissue. Thus the adipose tissue lies at the heart of a network of autocrine, paracrine and endocrine signals (Figure 1).

There are two types of adipose tissue, white and brown, with different physiological roles. In this article, we shall firstly focus on the evidence for the synthesis and secretion by white adipose tissue of endocrine and paracrine signals involved in the regulation of energy balance, with particular reference to cytokines and leptin. Secondly, we shall examine the evidence for interactions between these adipose tissue-derived mediators and other neuroendocrine pathways. An exhaustive review of adipose tissue products such as the enzymes of lipoprotein metabolism, angiotensinogen, and growth factors TGF $\beta$  and IGF-1, however, is beyond the scope of this article, and the reader is referred elsewhere.<sup>7–12</sup> We propose an hypothesis of adipose tissue endocrine function in which an hierarchy of signals enables the adipose

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**Figure 1** Adipose tissue: principal efferent and afferent signals. **Afferent signals:** The principal afferent regulators of adipocyte metabolism are extrinsic to adipose tissue, and include insulin, catecholamines, and the anterior pituitary endocrine axes. Additional paracrine and autocrine regulatory influences include local adipose tissue products such as  $TNF\alpha$  and ASP. **Efferent signals:** Adipose tissue synthesises a wide range of signalling molecules, from both the adipocyte and stromovascular compartments. See text for other signals secreted by adipose tissue. LPL = lipoprotein lipase; HSL = hormone-sensitive lipase; NEFA = non-esterified fatty acids; IL-6 = interleukin-6; TNF = tumour necrosis factor; sR = soluble receptor; ASP = acylation stimulating protein; VLDL = very low density lipoprotein; TAG = triacylglycerol.

tissue both to regulate and co-ordinate peripheral fuel metabolism at different sites (Figure 2), appropriately to the prevailing metabolic circumstances. It is suggested that a prominent role has evolved for leptin and other cytokines in the regulation of energy expenditure in the lean state. Obesity, however, gives rise to quantitative and qualitative alterations in adipose tissue signalling, not only failing to restore energy equilibrium, but giving rise to maladaptive effects which predispose to metabolic disease.

## Signals emanating from adipose tissue

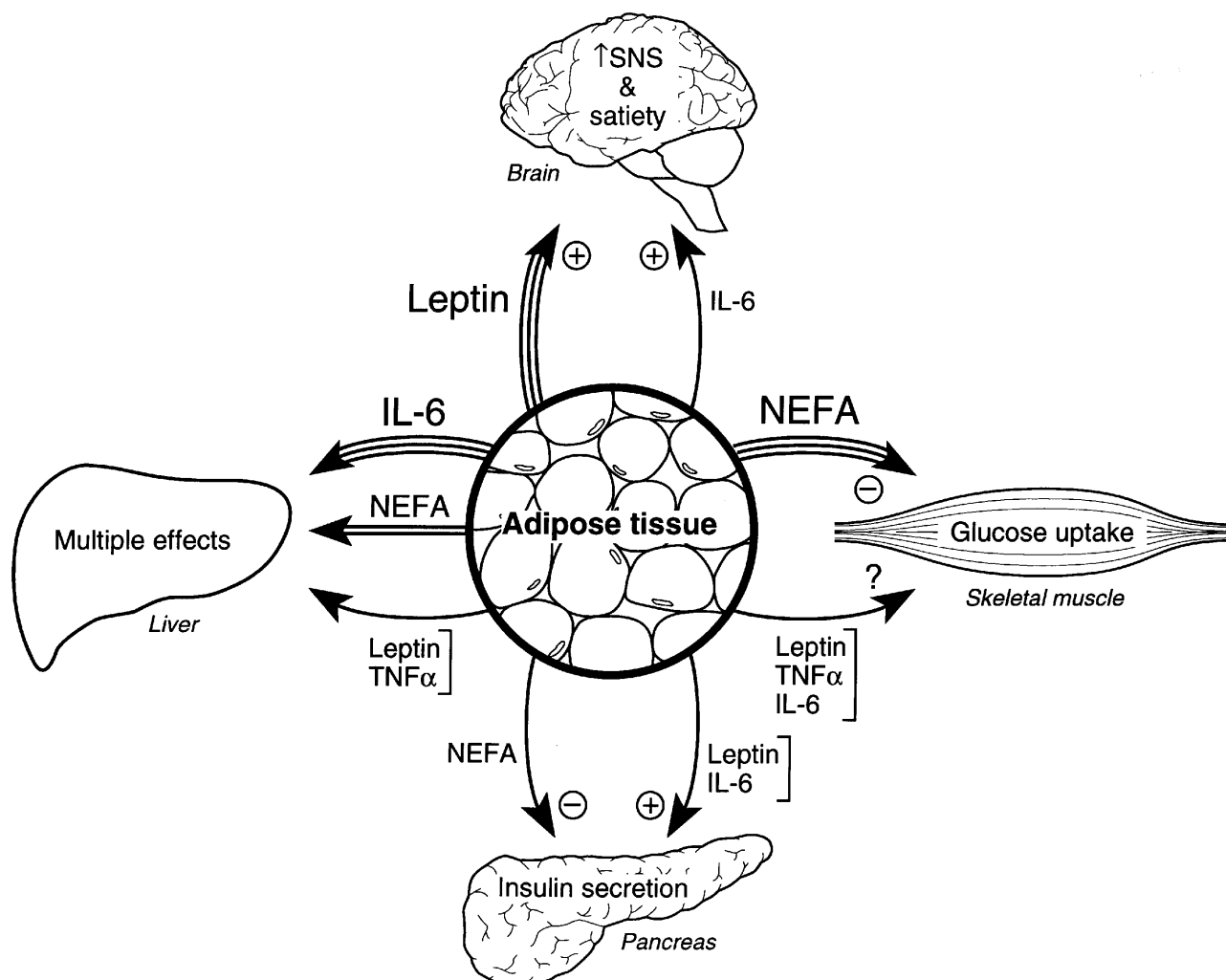
### (a) Leptin

Leptin, the circulating product of the obesity (*ob*) gene, is a 16 kDa glycoprotein expressed and secreted primarily by the adipocyte. The two most-discussed

actions of leptin have been its feedback effect on hypothalamic energy regulation and its role in the maturation of reproductive function. For excellent accounts of both the fascinating story of the discovery of leptin, and the elucidation of its central actions on energy regulation, the reader is referred to recent reviews.<sup>2,13</sup> The permissive role of leptin in reproductive function has been observed in animal studies. Thus, the female *ob/ob* mouse is infertile, and this infertility is reversed by the administration of leptin.<sup>14</sup> Furthermore, in normal mice, injections of leptin have been shown to advance the onset of puberty.<sup>15</sup> Therefore, leptin appears to signal to the hypothalamus when sufficient energy has been stored to enable the organism to embark on the energy-intensive reproductive cycle.

### Regulation of leptin production and bioactivity

Leptin biosynthesis and release is governed by a complex array of neuroendocrine, endocrine and paracrine signals which impinge on the adipocyte.



**Figure 2** Endocrine and metabolic signals emanating from adipose tissue. The figure represents the major effects of signals from adipose tissue, and the hierarchy of the stronger and weaker signals. Brain, liver, skeletal muscle and pancreas are believed to be the main remote targets of adipose tissue-derived signals. The width of each arrow represents the relative strength of each signal. Please see text for discussion of the multiple effects of these signals on the liver. SNS = sympathetic nervous system. Other abbreviations as in Figure 1.

Insulin has been shown, both *in vitro* and *in vivo*, to stimulate the production of leptin.<sup>16–18</sup> In contrast, catecholamines, acting through  $\beta_2$  and  $\beta_3$  adrenoceptors, rapidly suppress leptin production<sup>13</sup> (see *Interactions between Catecholamines and Leptin*). However, additional regulators stimulating leptin production include  $\text{TNF}\alpha$ ,<sup>19</sup> and glucocorticoids,<sup>17</sup> whereas thyroid hormones probably suppress leptin production.<sup>21–23</sup> Furthermore, it appears likely that the bioactivity of secreted leptin may be further potentiated or retarded by binding to soluble forms of its receptor and specific leptin binding proteins.<sup>24–26</sup> Thus, leptin production is influenced by nutritional status, stress, and immune activation.

#### Peripheral metabolic actions of leptin

The presence of receptors for leptin, not only in the hypothalamus, but also in peripheral tissues, including adipose tissue, liver, skeletal muscle and islet cells, suggests that leptin has peripheral, as well as central, actions.<sup>27–30</sup> Such actions have been confirmed by experimental studies which have suggested that leptin can impair insulin signalling, both in skeletal muscle

and adipocytes. Leptin has been found to impair insulin-mediated glucose uptake in mouse skeletal muscle myotubules.<sup>29</sup> Furthermore, in both Hep-G2 cells<sup>30</sup> and skeletal muscle myotubules<sup>29</sup> leptin was found to inhibit phosphorylation of insulin receptor substrate-1 (IRS-1). In rat adipocytes leptin has also been found to inhibit insulin-mediated glucose uptake, as well as lipogenesis, and to stimulate lipolysis and protein kinase A (PKA) activation.<sup>28</sup> Leptin stimulates lipogenic enzymes in adipocyte cell lines.<sup>31</sup> These studies suggest a role for leptin in peripheral metabolic regulation, and furthermore, have raised the question whether the impaired insulin signalling in obese subjects might result, in part, from increased circulating leptin levels. At the present time, however, the *in-vivo* relevance of such mechanisms remains to be determined. Perhaps consistent with an *in vivo* relationship between impaired insulin signalling and increased plasma leptin levels are the results of retrospective studies in Pima Indians, in which insulin resistance was found to be associated with reduced subsequent weight gain,<sup>32</sup> and lower plasma leptin levels to precede weight gain.<sup>33</sup> Thus, insulin

resistance may be, in part, a maladaptive consequence of high leptin levels, in response to overfeeding.

## (b) Adipose tissue cytokines

### Adipose tissue as a source of cytokines

TNF $\alpha$  and IL-6 are pro-inflammatory cytokines with potent actions in host defence.<sup>34</sup> Both these cytokines may have important effects on lipid and glucose metabolism.<sup>35</sup> IL-6 and TNF $\alpha$  stimulate basal glucose uptake into cultured adipocytes.<sup>36,37</sup> Both cytokines have been shown to inhibit LPL activity and TNF $\alpha$  has been shown to stimulate lipolysis.<sup>38</sup> In humans IL-6 was also found to stimulate glucose and fatty acid oxidation, as well as to induce the release of glucagon and cortisol.<sup>39–41</sup> Additionally, IL-6 has been shown to stimulate insulin release from a hamster islet cell line.<sup>42</sup> Adipose tissue is a significant source of endogenous TNF $\alpha$  production, and its expression is elevated in most rodent models of obesity and implicated in human obesity.<sup>3,43</sup> IL-6 is also expressed in and released by human adipose tissue and its circulating concentrations increase with obesity.<sup>44,45</sup> IL-6 mRNA and protein have both been demonstrated in human adipose tissue.<sup>4,44</sup> Remaining uncertainties include whether these cytokines emanate from the adipocytes themselves or from associated lymphoid tissue, and whether they are able to act in an endocrine fashion to influence metabolism in remote tissues. The metabolic effects of the modest elevations of systemic cytokines seen in obesity need to be more fully explored.

### Regulation of cytokine production and bioactivity

Cytokine action is tightly controlled by regulation at the levels of both transcription and release, and by counteracting mechanisms which limit their bioactivity.<sup>46</sup> However, relatively little is yet known of the regulation of cytokine release specifically by adipose tissue. There are reports that claim both induction and suppression of cytokine release by catecholamines. Data from human studies suggests that isoprenaline, the  $\beta$ -adrenergic agonist, increases IL-6 release, with little or no effect on TNF $\alpha$  release.<sup>47</sup> Although glucocorticoids down-regulate cytokine production in immune tissue, we are not aware of any data on this effect in adipose tissue.

Soluble receptors for TNF $\alpha$ , IL-6 and leptin may be important regulators of bioactivity. Most of the information about cytokine soluble receptors has derived from the study of immune cells, whereas very little is yet known about the regulation of these soluble receptors in adipose tissue. By binding to their ligands, cytokine soluble receptors can act either as antagonists or as carrier proteins. Cytokine-binding proteins may produce agonist-like activities, instead of the expected antagonist-like effects, when they extend the half-life of an otherwise short-lived cyto-

kine.<sup>48</sup> The soluble receptor systems for TNF $\alpha$  and IL-6 will be discussed separately.

(i) *TNF $\alpha$  soluble receptors* TNF $\alpha$  interacts with two cell–surface receptors, p55 (type 1) and p 75 (type 2).<sup>49</sup> Expression of these two receptors seems to be regulated by separate mechanisms, as they differ in their cellular and tissue distribution. Arteriovenous difference studies show *in-vivo* secretion of the soluble receptors from human subcutaneous adipose tissue. Release was observed from adipose tissue of both isoforms of TNF $\alpha$  receptor,<sup>50</sup> but not of TNF $\alpha$  itself.<sup>4</sup> The circulating levels of both soluble receptors correlate with measures of adiposity.

The physiological role of the soluble receptors of cytokines is controversial. It is known that both types of soluble receptors can bind to TNF $\alpha$  *in vitro* and inhibit its biological activity by competing with cell–surface receptors for TNF $\alpha$ . Consequently, the release of soluble receptors could serve as a mechanism for binding and inhibiting the TNF $\alpha$  not immediately bound to surface receptors, thus protecting other cells and tissues and localising its action.<sup>51,52</sup> It has been suggested also, that release of soluble receptors may be a mechanism for desensitizing the cell from the effects of TNF $\alpha$ .<sup>52</sup> On the other hand, it has been reported that at low concentrations of TNF $\alpha$ , binding to soluble receptors can stabilize TNF $\alpha$  and augment some of its activities.<sup>48</sup>

(ii) *Interleukin-6 soluble receptors* The biological activities of IL-6 are initiated by binding to a high-affinity receptor complex, consisting of two membrane glycoproteins.<sup>53</sup> The 80 kDa ligand binding component (IL-6R) binds IL-6 with low-affinity, while a second 130 kDa signal-transducing component (gp130), although not binding free IL-6, is required for high-affinity binding of gp80-bound IL-6. The cDNAs for both IL-6R and gp130, have been cloned and sequenced.<sup>54,55</sup> A soluble form of the IL-6R with a molecular weight of approximately 50 kDa has been found,<sup>56</sup> apparently arising from proteolytic cleavage of the membrane-bound IL-6R. Recombinant soluble IL-6R (IL-6Rs) has been shown to bind IL-6 in solution and to augment the activity of the IL-6 as a result of the binding of the IL-6/IL-6Rs complex to the membrane-bound gp130.<sup>57,58</sup> It has been suggested that elevated levels of IL-6 are associated with increased production of IL-6Rs.<sup>59</sup> Recently, evidence has been found also for a soluble form of the gp130 receptor, which may have antagonist properties.<sup>60</sup>

However, neither the regulation, *in vivo*, of soluble receptor release, nor their functional significance, are clearly understood. Few data have been published regarding the regulation of these molecules in adipose tissue. *In-vivo* studies in humans showed release from sub-cutaneous adipose tissue of IL-6, but not its soluble receptors,<sup>4,50</sup> in contrast to the results obtained

with TNF $\alpha$  and its soluble receptors. These data suggest a novel regulatory role for adipose tissue in the bioavailability of these cytokines.

### Roles of cytokines in the regulation of energy metabolism

(i) *Cytokine actions on insulin signaling* Studies *in-vitro* have suggested that TNF $\alpha$  alters insulin signaling, but the mechanism of this effect is still under review. *In-vitro* studies on skeletal muscle suggest that TNF $\alpha$  impairs insulin signalling by decreasing phosphorylation of the insulin receptor and IRS-1.<sup>61,62</sup> Other evidence suggests that TNF $\alpha$  may increase tyrosine phosphorylation of IRS-1 and activate phosphatidylinositol 3-kinase.<sup>63</sup> Alternatively there is evidence that TNF $\alpha$  may produce insulin resistance by decreasing IRS-1 and GLUT4 expression.<sup>64</sup> Recently TNF $\alpha$  and ceramides were shown to increase basal glucose uptake by adipose tissue. Their insulinomimetic effect may be via stimulation of phosphatidylinositol 3-kinase and thereby increasing the synthesis of GLUT 1, thus accounting for the increased basal glucose uptake.<sup>37</sup> High concentrations of both TNF $\alpha$  and IL-6 have been shown also to increase basal intracellular calcium, which negatively modulates insulin-mediated stimulation of GLUT 4-dependent glucose transport.<sup>65</sup> Increased intracellular calcium in skeletal myocytes can alter phosphorylation of GLUT 4, effectively blocking insulin stimulated glucose uptake, and thereby contributing further to the impairment of insulin signalling. However, the *in-vivo* significance of these data are as yet unclear. Indeed, whether such effects could result from the release of TNF $\alpha$  from local or more distant adipose tissue depots is still under investigation.

(ii) *Cytokine actions on adipose tissue and lipid metabolism* Both TNF $\alpha$  and IL-6 in high concentrations inhibit LPL activity and decrease its production in murine adipocyte cell-lines, as well as increasing lipolysis.<sup>66,67</sup> This may down-regulate triglyceride deposition, promote futile cycling, and increase fuel mobilization from the adipose tissue.<sup>66,67</sup> Consistent with these actions, both TNF $\alpha$  and IL-6 cause weight loss in mice,<sup>68</sup> and this is inhibited by pre-treatment with either anti-TNF $\alpha$  or anti-IL-6 monoclonal antibodies. Administration of lipopolysaccharide (LPS) to mice induced a transient weight loss, hypoglycaemia, hypertriglyceridaemia and an increase in the hepatic acute phase protein, fibrinogen. Pre-treatment with an anti-IL-6 antibody resulted in a reduction in the LPS-induced hypoglycaemia and weight loss, as well as decreasing plasma fibrinogen, but had no effect on the hypertriglyceridaemia. In similar studies, an anti-TNF $\alpha$  antibody completely inhibited the elevation of triglycerides, with only modest effects on weight loss, and no effect on hypoglycaemia and fibrinogen

production.<sup>69</sup> These results suggest distinct, but related, roles for these two cytokines in metabolic regulation.

(iii) *Neuroendocrine actions of cytokines* IL-6 receptors are present in the hypothalamus, which supports a direct central role for this cytokine.<sup>70</sup> IL-6 stimulates both thermogenesis and satiety, through a range of central effects, including prostaglandin synthesis, corticotrophin releasing hormone (CRH) release and activation of the hypothalamic–pituitary–adrenal (HPA) axis.<sup>71</sup> Cytokine modulation of neuroendocrine mechanisms is further discussed in the ‘Interactions of cytokines and leptin with endocrine pathways’.

Therefore, adipose tissue synthesises significant quantities of both IL-6 and TNF $\alpha$ . Both molecules may have local paracrine/autocrine effects within adipose tissue, and it may be suggested, furthermore, that adipose tissue contributes significantly to their circulating levels. Cytokines of non-adipose tissue origin may also play a significant role in the systemic metabolic adaptation to infection, including fuel mobilization, insulin resistance in insulin-sensitive tissues, and thermogenesis. Both cytokines are strongly implicated in the regulation of energy balance at multiple sites (Figure 2). Although obesity may increase circulating levels of these cytokines, and this may contribute to some of the maladaptive consequences of obesity, such as dyslipidaemia and insulin resistance, the net biological effects of increased circulating cytokine concentrations remain to be clarified. Not only the absolute rate of cytokine secretion by adipose tissue, but also the relative rates of production of cytokines and their soluble receptors will determine local, and contribute to systemic, bioactivity of the cytokines.

### (c) Acylation stimulating protein

Adipose tissue expresses a range of components of the Alternative Pathway of Complement. Of these, much attention has focused on C3adesArg, also known as acylation stimulating protein (ASP). C3adesArg is derived from the cleavage of the C3 complex, which requires factors B and D (adipsin) to form C3a, which is cleaved by a carboxypeptidase to yield C3adesArg. Preadipocytes produce both C3 and adipsin. *In-vitro* studies have shown that small amounts of ASP are expressed by both fibroblasts and preadipocytes, but ASP formation is, predominantly, a feature of the mature and fully differentiated adipocyte.<sup>72</sup>

Several roles have been proposed for ASP in adipocyte metabolism. ASP may play a role in the uptake and esterification of fatty acids to make triacylglycerol to facilitate fatty acid storage in the post-prandial state.<sup>73</sup> ASP has been shown to stimulate triglyceride synthesis via diacylglycerol

acyl transferase (DGAT).<sup>74</sup> ASP also stimulates translocation of glucose transporters to the cell surface.<sup>75</sup> These effects may be mediated by activation of the diacylglycerol/protein kinase C (DAG/PKC) pathway.<sup>76</sup> *In-vitro* experiments have shown that several lipoproteins, including very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL), increase ASP release, but the greatest effect is seen with chylomicrons. Arteriovenous studies<sup>6</sup> have demonstrated *in-vivo* release of ASP both in the basal state and after a mixed meal, concurrent with uptake chylomicron triacylglycerol. The increase in ASP production in this situation corresponds inversely with the uptake of triacylglycerol, perhaps suggesting a cause–effect relationship. There is evidence that ASP can act on several different cell types of non-adipose tissues. This suggests it can function as an endocrine signal.<sup>77</sup>

In support of the putative role for ASP in triglyceride storage, studies in ASP functional knockout mice have shown delayed triglyceride clearance compared to wild-type mice, and this difference is further exaggerated in female mice. When ASP was injected into the mice, an increased clearance of triglycerides was observed. The delay in triglyceride clearance in this model may be due to the effect on LPL of increased concentrations of NEFA. Lastly, although a receptor for ASP has not yet been identified, differences between adipose tissue depots have been observed, with greater degrees of ASP binding in subcutaneous compared to omental fat, in females compared with males, and in morbidly obese compared to non-obese individuals.

#### (d) Non-esterified fatty acids (NEFA)

The regulation of lipolysis, which determines production of non-esterified fatty acids (NEFA), has been reviewed in detail.<sup>78</sup> Although not regarded traditionally as an ‘endocrine’ signal, elevated concentrations of NEFA in the systemic circulation are associated with impaired insulin sensitivity, and therefore represent a major determinant of carbohydrate storage and oxidation.<sup>79</sup> NEFA impairs insulin-stimulated glucose uptake and glycogen synthase activity in skeletal muscle, whilst in the liver gluconeogenesis is enhanced, together with an increase in hepatic glucose output. Furthermore,  $\beta$ -cell insulin secretion is also stimulated by circulating NEFA. Therefore, increased circulating NEFA likely contributes to the development of insulin resistance in both skeletal muscle and liver, and to hyperinsulinaemia, which are such prominent abnormalities in obese individuals. This topic also has been well reviewed more recently.<sup>5,80,81</sup> It has also been recognised that the local NEFA in adipose tissue may stimulate the activity of LPL,<sup>82</sup> and the activity of uncoupling proteins (UCPs).<sup>83</sup> In this sense, NEFA is an important systemic and ‘autocrine’ signal derived from adipose tissue.

## Interactions of cytokines and leptin with endocrine pathways

Considerable evidence may be adduced in support of important interactions between the classical endocrine pathways regulating adipose tissue (the anterior pituitary endocrine pathways, catecholamines and insulin) and both leptin and the cytokines. Thus both leptin and cytokines interact with pituitary–adrenal, pituitary–gonadal, and pituitary–thyroid axes, whilst cytokines additionally influence the complement pathway in adipose tissue.

#### Leptin and the HPA axis

The results of studies looking at the relationship between plasma leptin and cortisol levels are conflicting.<sup>84,85</sup> The diurnal variation of leptin and cortisol is reciprocal, leptin levels peaking during the nadir of cortisol secretion. In cultured adrenocortical cells, physiological doses of leptin were found to bring about a dose-dependent inhibition of adrenocorticotropin (ACTH)-stimulated cortisol production and P450 17 $\alpha$ -hydroxylase mRNA expression.<sup>86</sup> In contrast, leptin increases CRH expression in the para-ventricular nucleus.<sup>87</sup> However, peripheral CRH administration in humans appears not to influence plasma leptin levels acutely,<sup>88</sup> despite the observation that dexamethasone can stimulate leptin production in cultured adipocytes. Thus, leptin and HPA axis may be reciprocally related, but with interactions at several levels.

An intact HPA axis may be necessary for the normal actions of leptin on energy balance. Zakrzewska *et al* injected leptin, intracerebroventricularly, into adrenalectomised and sham-operated rats and found that the hypophagic and weight-reducing effect of leptin was significantly amplified in the adrenalectomised group, and that this effect was partly abolished by treatment with dexamethasone.<sup>89</sup> These observations led the investigators to postulate that glucocorticoids exert a counter-regulatory influence on leptin action, and that activity of the HPA axis may set the level of target-organ sensitivity to leptin. Thus, a picture is emerging of reciprocal interactions between energy balance and the HPA axis, in which leptin may play an important role.

#### Cytokines and anterior pituitary function

Interactions between cytokines and the anterior pituitary endocrine axes occur at multiple levels and in both directions. TNF $\alpha$ , and particularly, IL-6 are known to affect the release of anterior pituitary hormones by an action on the hypothalamus and/or the pituitary gland. They stimulate the HPA axis and suppress the hypothalamic–pituitary–thyroid and gonadal axes, and possibly growth hormone release. The relative importance of systemically and locally produced cytokines in achieving these responses, and their precise sites of action, are as yet unclear.

ACTH secretion from the pituitary gland is controlled, in part, by the stimulatory effect of CRH from the hypothalamus, and by the negative feedback of glucocorticoids. The release of CRH, stimulated by IL-6 is mediated by an eicosanoid cyclo-oxygenase pathway.<sup>90</sup> IL-6 and TNF $\alpha$  also stimulate ACTH release in rats, when given intravenously or intracerebroventricularly.<sup>91,92</sup> Furthermore, co-administration of an anti-CRH antibody with the IL-6 or TNF $\alpha$  blocks the effect of these cytokines on ACTH secretion, suggesting that their actions are mediated by CRH.<sup>89,91</sup> Further support for this comes from *in-vitro* studies, where IL-6 has been shown to stimulate CRH release from rat medial basal hypothalamic fragments.<sup>93</sup> Also, recombinant human IL-6 increased ACTH secretion by human fetal pituitary cultures.<sup>94</sup> Lastly, glucocorticoids inhibit cytokine synthesis and gene expression from immune cells.<sup>95</sup>

Evidence for the effects of TNF $\alpha$  and IL-6 on the hypothalamic–pituitary–gonadal axis are conflicting. Their effects may be indirect via CRH which has been shown to inhibit gonadotrophin releasing hormone (GnRH) and luteinising hormone (LH) release.<sup>96,97</sup> But, IL-6 infusions in human volunteers have been shown to acutely stimulate growth hormone (GH) and prolactin secretion.<sup>98</sup> Also, in one study IL-6 and TNF $\alpha$  injected intracerebroventricularly into ovariectomised rats lowered serum LH, whereas in another, IL-6 administered in a similar manner had no effect on either LH or follicle stimulating hormone (FSH) secretion.<sup>99,100</sup> Gonadal function is often suppressed during conditions where inflammatory cytokines are raised, such as infections, and it is suggested that the inhibitory effects of cytokines on the hypothalamic–pituitary–gonadal axis may mediate this effect.

Both TNF $\alpha$  and IL-6 appear to influence elements of the hypothalamic–pituitary–thyroid axis. IL-6 inhibited thyroid stimulating hormone (TSH) release, whereas it stimulated thyrotropin releasing hormone (TRH) release both *in vivo* and *in vitro*.<sup>93,98,101</sup> IL-6 also stimulates TSH secretion from anterior pituitary cells *in vitro*.<sup>102</sup> TNF $\alpha$  has been found to have a direct inhibitory effect on thyroid hormone secretion, and TNF $\alpha$  inhibits deiodinase activity in thyroid gland.<sup>103,104</sup> Thus, while TNF $\alpha$  may have inhibitory effects on the hypothalamic–pituitary–thyroid axis, the net effects of IL-6 are unclear.

In summary, cytokines synthesised in adipose tissue may influence anterior pituitary endocrine pathways, by contributing to circulating concentrations, which influence endocrine function at hypothalamic, pituitary, and target organ level. At present, however, the *in-vivo* significance, for energy metabolism, of much of the above work remains to be determined.

### Catecholamines and leptin

In studies on mouse adipocytes, catecholamines and synthetic  $\beta$ 3-adrenergic agonists have been shown to rapidly suppress levels of leptin mRNA.<sup>105</sup> These

effects of catecholamines are partially inhibited by the  $\beta$ -adrenergic antagonist propranolol.<sup>106</sup> These studies implicate both  $\beta$ 3 and  $\beta$ 2 in the regulation of leptin. Studies, in humans, show acute suppression of plasma leptin by isoprenaline, concurrent with increased lipolysis.<sup>107,108</sup> These studies suggest that leptin production may be regulated very acutely by sympathetic stimulation. The physiological significance of this interaction is uncertain, but the possibility is raised that adrenergic stimulation may, by suppressing plasma leptin levels, feed back to the brain to reduce sympathetic outflow and thermogenesis. The SNS has not been regarded as a feedback-regulated system, but these data suggest that the activity of its adipose tissue division may be regulated in this fashion.

It is recognised also that the actions of catecholamines on adipose tissue are modulated by thyroid hormone. At the present time, however, the interaction of leptin with the pituitary–thyroid axis is controversial. *In-vivo* studies in humans,<sup>23</sup> *in-vitro* studies on cultured adipocytes,<sup>21</sup> and experimental studies on rodents<sup>22</sup> have suggested that thyroid hormones may suppress the production of leptin in adipose tissue. However, not all investigators have observed this relationship.<sup>109–111</sup> We have interpreted our observations as suggesting that thyroid hormones may suppress leptin production through enhanced  $\beta$ -adrenergic sensitivity in the adipocyte.<sup>23,108</sup>

### Catecholamines and cytokines

While there are data suggesting that catecholamines may regulate the production of IL-6 and TNF $\alpha$ , there is no consensus as to whether this effect is stimulatory or inhibitory. In primary cultures of murine adipocytes, noradrenaline, isoprenaline and a  $\beta$ 3-selective agonist, CGP-12117 stimulated IL-6 gene expression and protein secretion, while stimulation of the  $\alpha$ -adrenergic receptors had no effect.<sup>112</sup> In human volunteers who underwent strenuous exercise, after pre-treatment with placebo, hydrocortisone or dexamethasone, plasma noradrenaline and adrenaline peaked after 15 min, but IL-6 peaked at 15 min and 45 min. There was no effect of treatment on catecholamine levels, but both hydrocortisone and dexamethasone pre-treatment inhibited IL-6. In all three groups, IL-6 levels correlated positively with catecholamine levels at 15 min.<sup>113</sup> In contrast, in a study of LPS-induced release of TNF $\alpha$  and IL-6 from human whole blood, both noradrenaline and isoprenaline inhibited cytokine production.<sup>114</sup> Studies with isoprenaline infusions in human volunteers have shown a stimulatory effect on circulating IL-6 levels, but little or no change in TNF $\alpha$  levels.<sup>47</sup> Because these cytokines are produced by several different cell types it is possible that the regulation is different in adipocytes compared to macrophages or endothelial cells, perhaps accounting for contrasting results of different studies.

### Insulin, leptin and cytokines

Abundant experimental data support a stimulatory effect of insulin on leptin secretion.<sup>2,16,17</sup> In human studies using the euglycaemic clamp technique, increases in plasma leptin have been observed after 48 h and 72 h.<sup>16</sup> Increases in plasma leptin in the fed state and reductions in the fasting state, may be explained by insulin. This subject has been reviewed in detail elsewhere<sup>2</sup> and will not be discussed further here. Although several studies have observed relationships between hyperglycaemia, hyperinsulinaemia and elevated circulating levels of cytokines,<sup>115,116</sup> the mechanisms responsible for these relationships are poorly understood.

### Relationship between leptin, interleukin-6 and TNF $\alpha$

Several lines of evidence suggest that leptin is a helical cytokine-like molecule, and that it shares both structural and functional similarities with other cytokines. These include receptor homology, signalling via the JAK/STAT system, growth factor properties, and their circulation in plasma, either in the free form or bound to specific serum proteins.<sup>117,118</sup> Leptin, IL-6 and TNF $\alpha$  all play important signalling roles in the regulation of fat mass (Table 1). All three are expressed and released by the adipose tissue (Table 2), and have been implicated in impairment of insulin action in liver and skeletal muscle. TNF $\alpha$  induces the release of both leptin and IL-6 from adipose tissue<sup>19,119,120</sup> (Figure 3). A recent study on the regulation of leptin release by TNF $\alpha$  suggested that the induction occurs acutely at the post-

translational level. Mice with TNF $\alpha$  gene knockout had reduced circulating levels of leptin compared with obese wild-type mice.<sup>121</sup> Furthermore, as already elaborated, all three molecules may influence hypothalamic neuroendocrine mechanisms (Table 1).

## Adipose tissue distribution and steroid conversion

Although adipose tissue does not synthesise steroid hormones, *de novo*, it expresses enzymes which metabolise both sex steroids and glucocorticoids,<sup>122</sup> as well as receptors for oestrogens,<sup>123</sup> androgens and glucocorticoids.<sup>124</sup> Björntorp has argued that changes in glucocorticoid or sex steroids are a major determinant of adipose tissue distribution, and has recently reviewed this area.<sup>125</sup> This hypothesis suggests that both glucocorticoids and sex steroids, whose metabolism is itself influenced by adipose tissue, exert a powerful influence on regional adipose tissue development. HPA axis activation might play a primary role, interacting with other factors, in the expansion of adipose tissue at visceral sites.<sup>125</sup>

### Sex steroids

Adipose tissue possesses two enzymes of importance to sex steroid metabolism, 17 $\beta$ -hydroxysteroid oxidoreductase and cytochrome-p450-dependent aromatase.<sup>122,124</sup> 17 $\beta$ -hydroxysteroid oxidoreductase converts androstenedione, synthesised in the adrenal

**Table 1** Leptin, TNF $\alpha$  and IL-6 as adipostatic agents. Summary of (i) some of the effects of cytokine-like molecules relevant to energy balance, and (ii) some of the factors regulating their release

	Leptin	TNF $\alpha$	IL-6
Action of hormone/cytokine:			
Appetite	↓↓	↓	↓
Energy expenditure	↑↑	↑	↑
Lipolysis (NEFA concentrations)	↑	↑	↑
Lipogenesis	↓	?	?
Reproduction	↑	?	?
Factors regulating hormone/cytokine:			
Effect of food ( <i>cf</i> fasting)	↑	none	none
Effect of prolonged fast	↓	?	none
Effect of isoprenaline	↓	±	↓ from monocytes and ↑ from adipocytes

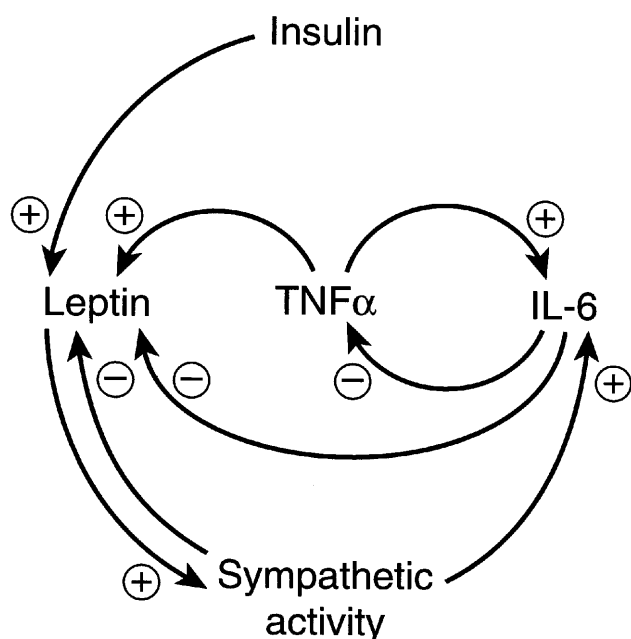
Arrows indicate whether effect is to stimulate or suppress, double arrows indicate stronger effects. ? indicates no clear data. ± indicates no clear effect. Please see text for references.

**Table 2** Leptin, TNF $\alpha$  and IL-6 as hormones released by adipose tissue. Summary of evidence that these cytokine-like molecules are endocrine signals from adipose tissue

	Leptin	TNF $\alpha$	IL-6
Release by adipose tissue	yes	±	yes
Proportion of circulating levels attributable to adipose tissue	> 95%	±	10–30% (rest monocytes, endothelial cells and fibroblasts)
Release of its soluble receptors by adipose tissue	±	yes	±
Correlation of circulating concentrations with adiposity (Pearsons 'r' value)	0.60–0.95	0.12–0.45	0.25–0.65

± indicates no clear effect. Please see text for references.





**Figure 3** Interactions of cytokine-like molecules, insulin and sympathetic activity.

cortex, to testosterone. This conversion may be a significant source of testosterone in normal women. The same enzyme converts the relatively inactive oestrogen, oestrone, to the more active oestradiol. There is also aromatisation of androgens to oestrogens, by aromatase, in adipose tissue. Confirmation of a net release of testosterone, oestradiol and oestrone from abdominal subcutaneous adipose tissue, in women, but not in men, comes from arteriovenous studies.<sup>126</sup> However, continuing uncertainty exists as to regional variation of sex steroid conversion and to its contribution to whole body sex steroid production.

Excess body fat is associated with reduced fertility, hyperandrogenism and hormone-sensitive cancers.<sup>125,126</sup> The effect of obesity on sex steroid profile is associated with ‘feminization’ in men and ‘masculinization’ in women.<sup>127–129</sup> The percentage contribution of adipose tissue as a source of sex steroids may be greatest in post-menopausal women. However, the extent to which testosterone production in women, or oestrogen production in men, significantly effects reproductive function is controversial. Relative hyperandrogenism has been associated with central obesity, with its attendant metabolic disturbances,<sup>125,130</sup> although what is cause and what is effect remains undetermined.

### Glucocorticoids

Although adipose tissue possesses 11-hydroxysteroid dehydrogenase enzymes capable of inter-converting cortisol and cortisone, it is uncertain whether cortisone/cortisol inter-conversion in adipose tissue significantly influences glucocorticoid bioavailability and bioactivity. However, local 11- $\beta$  HSD activity may influence local cortisol-induced stimulation of aromatase activity.<sup>131</sup> It has also been suggested that increased

11- $\beta$ HSD expression in visceral adipose tissue could contribute to the development of central obesity.<sup>132</sup> Although obesity is associated with increased activity of the hypothalamo–pituitary axis,<sup>133</sup> plasma concentrations of cortisol are found to be normal in obese individuals. Obesity is also associated with increased levels of cortisol binding globulin. However, cortisol binding globulin levels fall with weight reduction, with no net change in free cortisol concentrations.<sup>134</sup>

The physiological significance, if any, of the involvement of adipose tissue in steroid hormone metabolism in lean subjects is uncertain. It may be suggested, however, that the changes in sex hormones, and perhaps glucocorticoid metabolism observed in obese subjects may influence local steroid bioactivity and general adipose tissue distribution. Whether such changes are beneficial or not is often unclear.

## The effects of obesity on the endocrine function of adipose tissue

In obesity, not only is adipose tissue function quantitatively increased, but changes in both the relative sizes of different adipose tissue depots, together with changes at the cellular level, give rise to qualitative alterations in its metabolism.

### Endocrine consequences of an increased adipose tissue mass

Increased production from adipose tissue of NEFA,<sup>5</sup> leptin<sup>2</sup> and cytokines<sup>4</sup> contributes to changes in systemic metabolism of obese subjects causing insulin resistance. Insulin resistance prevents further weight gain<sup>135</sup> and mobilizes energy stores. Impaired insulin signalling, increased lipolysis, and perhaps central neuroendocrine effects, such as HPA activation, may be adaptive under certain circumstances, including the early stages of fuel storage and in acute inflammation, whereas such changes may become maladaptive during sustained weight gain. Insulin resistance is one particularly maladaptive consequence of obesity, in terms of its predisposition to cardiovascular disease.<sup>136</sup> Other endocrine products of adipose tissue may have similar initially beneficial, but later maladaptive effects. Thus, IL-6 may reduce LPL action, reducing fuel storage and thereby limiting weight gain,<sup>67</sup> but IL-6 may also increase hepatic synthesis of pro-coagulant molecules and contribute to dyslipidaemia.

Adipose tissue in obese subjects may behave in a qualitatively different manner to that of lean subjects; for example there is little change in adipose tissue post-prandial blood flow,<sup>137</sup> and markedly altered post-prandial NEFA release.<sup>5,81</sup> While there are certainly quantitative changes in the endocrine functions of adipose tissue in obesity, it is less clear whether there are also qualitative endocrine changes.

### Endocrine consequences of regional adiposity

The relative sizes of the various adipose tissue depots may exert a powerful influence on the signalling properties of the tissue. The contrasting metabolic consequences of android and gynoid obesity have long been recognised.<sup>138</sup> Although visceral obesity has been suggested to be more pathological than generalised obesity, there are generally close relationships between adipose tissue mass and distribution, such that within each sex, there is a strong tendency for more obese subjects to have proportionately more upper-body, abdominal and visceral fat.<sup>139–142</sup> These relationships confound distinctions between the consequences of generalised, as opposed to local, obesity. While some authors have emphasised especially deleterious consequences of visceral obesity,<sup>125,132</sup> others have suggested there may be few, or negligible, effects once total obesity, sex, and level of physical fitness and social class have been taken into account.<sup>141,142</sup>

Differential adipose tissue distribution appears to have significant effects upon the endocrine function of adipose tissue. Regional variations in adipose tissue signalling functions include increased expression of leptin<sup>143</sup> and binding of ASP<sup>77</sup> in subcutaneous adipose tissue, and increased expression of 11- $\beta$ HSD<sup>132</sup> and glucocorticoid receptors<sup>144</sup> in visceral adipose tissue. Differences in glucocorticoid sensitivity may underlie differences in growth characteristics of visceral and subcutaneous adipose tissue.<sup>145</sup> A large visceral adipose tissue depot is thought to increase hepatic exposure to NEFA,<sup>146</sup> with secondary impairment of hepatic insulin clearance,<sup>147</sup> increased hepatic synthesis of VLDL triglyceride,<sup>148</sup> and impaired peripheral glucose disposal.<sup>79</sup> Furthermore, the increased  $\beta_3$  adrenoceptor sensitivity of visceral adipose tissue may account for its increased lipolytic activity and release of NEFA.<sup>149</sup> The release of other mediators, including cytokines,<sup>143</sup> by visceral adipose tissue may also have important metabolic effects on the liver. Obesity is associated also with reduced adipocyte  $\beta_2$ -adrenergic receptor sensitivity<sup>150</sup> and an impaired lipolytic response to adrenergic stimulation.<sup>151</sup> These defects may be caused by adipocyte adrenoceptor down-regulation in the face of the increased sympathetic activation of obesity.<sup>141</sup> Adipose tissue at different sites also differs with respect to the sensitivity of LPL release to modulation by sex steroids.<sup>152</sup> Lastly, it is possible to speculate that visceral adipose tissue is less effective than subcutaneous adipose tissue in regulating energy balance through its production of leptin.<sup>143</sup>

### Thrifty, surplus and maladaptive signalling

Many of these endocrine and metabolic changes, as they increase in degree, will further impair energy homeostasis. Obesity increases adipose tissue production of leptin,<sup>2</sup> NEFA<sup>5</sup> and IL-6,<sup>4</sup> and may further modulate cytokine bioactivity through altered release of soluble receptors. Thus, the normal profiles of hormones, fatty acids and cytokines released by

adipose tissue, instead of being determined by the prevailing metabolic needs of the individual (state of feeding, levels of stress, physical activity and inflammatory response, and reproductive activity) will be shaped primarily by the degree and distribution of obesity, together with qualitative changes in adipose tissue behaviour at the cellular level. In this way, it may be proposed that adipose tissue metabolism is intrinsically *thrifty*, having evolved in an energy deficient environment. In the face of sustained *surplus* energy intake, however, many of the changes in adipose tissue signalling observed in obese subjects are *maladaptive*, and predispose to metabolic disease. One can only speculate whether the coexistence of hyperleptinaemia with elevated NEFA and cytokines may cause synergistic abnormalities and metabolic problems.

## Conclusions

Adipose tissue releases a wide range of signals, some clearly endocrine, some probably auto- or paracrine. It seems clear that this network of signals has profound and widespread effects on energy balance. This network, in which individual signals may operate in an hierarchical fashion, appears to represent a metabolic repertoire which may enable the organism to make adaptive changes to fuel metabolism, one regulator modulating the effects of another. Many of the maladaptive metabolic consequences of obesity may arise from dysfunction of the adipose tissue endocrine network. We are presently in a very exciting period where the place of novel signals is being determined. Some of these signals are recently recognised and the physiological regulation has not been determined, much less the effect of obesity on such regulation. As yet, we know little about the interactions between the signals, but advances are occurring rapidly. Adipose tissue is increasingly being recognised as a sophisticated endocrine organ, capable of orchestrating multiple effects, initially adipostatic, but likely to become maladaptive in circumstances of continued positive energy balance.

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### 9th International Congress on Obesity

Further information about the role of adipose tissue as an endocrine organ emerged at the 9th ICO and its satellite meeting 'Endocrinology of Obesity' in September 1998. It is not possible to mention all these new data, however of particular note there was further evidence that leptin production is primarily from

subcutaneous rather than visceral adipose tissue (Van Harmelen *et al*, *Int J Obes* **22**(suppl 3);1998: S41, Jensen *et al*, *op cit* S81). Differential production of secretory products by adipocytes from various depots was also a theme of Hauner (*op cit* S45). Negrel considered the possible paracrine functions of angiotensinogen, PGI<sub>2</sub>, PGF<sub>2α</sub>, and PAI-1 (*op cit* S20) as well as discussing other secretory products whose physiological roles remain uncertain. Matsuzawa and colleagues (*op cit* S5, S42, S56 and others) reported an exciting new adipose tissue secretory product that they have termed adiponectin. This factor is present in the systemic circulation and has remote effects, especially on vascular tissue.

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