International Journal of Obesity (2003) **27**, 875–888
© 2003 Nature Publishing Group All rights reserved 0307-0565/03 $25.00
www.nature.com/ijo

**REVIEW**

# Integrative physiology of human adipose tissue

KN Frayn[1]*, F Karpe[1], BA Fielding[1], IA Macdonald[2] and SW Coppack[3]

[1]*Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, UK;* [2]*University of Nottingham Medical School, Nottingham, UK; and* [3]*St Bartholomew's and the Royal London School of Medicine, London, UK*

Adipose tissue is now recognised as a highly active metabolic and endocrine organ. Great strides have been made in uncovering the multiple functions of the adipocyte in cellular and molecular detail, but it is essential to remember that adipose tissue normally operates as a structured whole. Its functions are regulated by multiple external influences such as autonomic nervous system activity, the rate of blood flow and the delivery of a complex mix of substrates and hormones in the plasma. Attempting to understand how all these factors converge and regulate adipose tissue function is a prime example of integrative physiology. Adipose tissue metabolism is extremely dynamic, and the supply of and removal of substrates in the blood is acutely regulated according to the nutritional state. Adipose tissue possesses the ability to a very large extent to modulate its own metabolic activities, including differentiation of new adipocytes and production of blood vessels as necessary to accommodate increasing fat stores. At the same time, adipocytes signal to other tissues to regulate their energy metabolism in accordance with the body's nutritional state. Ultimately adipocyte fat stores have to match the body's overall surplus or deficit of energy. This implies the existence of one (or more) signal(s) to the adipose tissue that reflects the body's energy status, and points once again to the need for an integrative view of adipose tissue function.

*International Journal of Obesity* (2003) **27,** 875–888. doi:10.1038/sj.ijo.0802326

**Keywords:** adipose tissue; integrative physiology; fat balance; blood flow; autonomic innervation; secretory activity

## Introduction

Over the past decade, remarkable strides have been made in recognising the complex nature of the adipocyte as a secretory cell as well as a site of the regulation of energy storage. Many informative studies have been performed with cultured adipocytes. However, there are some aspects of adipose tissue function that require a more integrative approach to their study. The regulation of adipose tissue metabolism *in vivo*, for instance, must involve the activity of the autonomic nervous system, the delivery of a complex mixture of substrates and hormones to adipose tissue in the plasma, feedback from autocrine and paracrine effectors secreted by adipocytes and also blood flow through adipose tissue. All of these influences are likely to change with time and nutritional state. In addition, factors such as leptin secreted by adipocytes may feed back to other bodily systems to regulate energy balance. It seems unlikely that cellular or molecular studies of adipocytes will ever be able to elucidate the multitude of factors that integrate to regulate adipocyte metabolism *in vivo*. Trying to understand the regulation of adipose tissue function is therefore a prime example of integrative physiology.

In this review we have attempted to illustrate the complexity of adipose tissue function, with an emphasis on the dynamic nature of this tissue. Our aim is not to duplicate the many excellent reviews that have been written recently about adipocytes, but rather to bring together those aspects of adipose tissue function that must interact *in vivo* for it to fulfil its biological functions. Thus, we will cover some aspects of the regulation of fat storage and mobilisation; the regulation of adipose tissue blood flow and the role of adipose tissue innervation; some aspects of the secretory functions of adipose tissue as they relate to regulation of fat balance; and finally, to demonstrate the integrative nature of adipose tissue function, possible oscillatory behaviour of the adipose tissue mass. Our review builds upon our joint experiences of studying human adipose tissue function *in vivo*.

## Regulation of fat stores

The regulation of whole-body fat stores illustrates the complex nature of adipose tissue integrative physiology.

---

***Correspondence: Dr KN Frayn, Oxford Lipid Metabolism Group, Oxford Centre for Diabetes Endocrinology and Metabolism, Churchill Hospital, Old Road, Oxford OX3 7LJ, UK.**
**E-mail: keith.frayn@oxlip.ox.ac.uk**

**Received 1 November 2002; revised 5 March 2003;**
**accepted 9 March 2003**

Glycogen stores are small in relation to energy throughput, and adipose tissue triacylglycerol (TG) is therefore the long-term repository for excess energy. The amount of TG stored within adipocytes must be an accurate reflection of the imbalance between energy intake and energy expenditure, integrated over a long period. The TG store of adipocytes in turn reflects the net balance between fat deposition and fat mobilisation. The pathways of fat storage and mobilisation in adipocytes are therefore regulated in accordance with whole-body energy balance. Recent studies demonstrate that this is indeed the case, and give insights into the mechanisms of this regulation.

The individual pathways of TG deposition and TG mobilisation and their regulation have been reviewed in detail elsewhere.[1–3] In human adipose tissue, the capacity for *de novo* lipogenesis (DNL) appears to be low,[4] and the contribution of DNL generally to metabolism appears not to be significant except perhaps under conditions of overfeeding with a high-carbohydrate diet.[5] There is one report that considerable whole-body DNL during carbohydrate overfeeding did not occur in the liver, suggesting adipose tissue as a likely site.[6] However, during normal, eucaloric conditions it seems that most of the TG deposited in adipose tissue arises from the pathway mediated by lipoprotein lipase (LPL)—hydrolysis of circulating lipoprotein-TG, followed by uptake of the fatty acids, and esterification to glycerol 3-phosphate. Both the activities of adipose tissue LPL and the pathway of esterification are stimulated by insulin,[7–10] leading to a very energy-efficient pathway for storage of dietary TG in the postprandial period. Increased adipose tissue blood flow (ATBF) in the postprandial period may also be involved, and is discussed below. Net fat storage must also be regulated on a site-specific basis by steroid hormones (glucocorticoids and sex steroids), reviewed below. Recently, a novel stimulator of the pathway of fat deposition has been identified, the 76-amino-acid peptide, acylation-stimulating protein (ASP).[11] ASP was initially recognised as an activity in plasma that stimulated fatty acid esterification in adipose tissue.[12] Later it was found to be identical to the peptide C3a-desarg,[11] a known product of the interaction of components B, $C_3$ and D of the alternative complement pathway. Component D had already been identified as a major protein expressed in adipocytes, initially called adipsin, and it had been recognised that all these three components were produced by adipocytes.[13] The production of ASP *in vitro* is stimulated by the presence of chylomicrons,[14] and its production by adipose tissue *in vivo* increases in the postprandial period,[15] giving ASP a potential role in coordination of postprandial fat storage. However, physiological importance in humans remains unclear at present.

The pathway of fat mobilisation is exquisitely sensitive to suppression by insulin. The half-maximal insulin concentration for suppression of fat mobilisation in humans is around 90 pmol/l,[16] whereas that for glucose production is around 200 pmol/l.[17] When insulin is infused to moderately high physiological concentrations, there is therefore complete suppression of nonesterified fatty acid (NEFA) release from subcutaneous adipose tissue.[18] This is brought about by a dual action, inhibition of hormone-sensitive lipase (HSL) by dephosphorylation, and stimulation of the re-esterification of fatty acids by the pathway described earlier. Stimulation of fat mobilisation requires activation of HSL by phosphorylation,[3] and involves increased gene transcription in the longer term.[19] Phosphorylation of HSL by protein kinase A is accompanied by translocation of HSL from the adipocyte cytosol to the surface of the lipid droplet[20] and also by phosphorylation of perilipin, a protein that appears to coat the lipid droplet and to move away upon stimulation, to allow HSL access.[20] Acute activation of lipolysis, via perilipin and HSL phosphorylation, may be brought about by catecholamines acting through $\beta$-adrenergic receptors, although in the situation of overnight fasting, when lipolysis increases steadily, $\beta$-adrenergic stimulation appears not to be involved;[21] progressive removal of insulin inhibition may be more important. Overnight secretion of growth hormone[22] and the morning rise in cortisol[23] play additional modulatory roles. Atrial natriuretic peptide has been suggested[24] as an activator of HSL, but its physiological importance remains uncertain.

These pathways have been further elucidated recently using data from human genetics and from genetic manipulations in animals. Clearly, fat deposition can still occur in the absence of LPL, because it has long been known that patients with complete LPL deficiency have relatively normal adipocytes.[25,26] Mice lacking LPL specifically in adipose tissue have normal fat mass, but this is achieved by upregulation of *de novo* fatty acid synthesis.[27] Diacylglcyerol acyltransferase (DGAT) is the terminal enzyme in TG deposition whatever the source of the fatty acids. There are two isoforms of DGAT, DGAT1 and DGAT2, both expressed in white adipose tissue. Adipose tissue fat storage is reduced but not absent in mice deficient in DGAT1.[28] Energy balance in these mice is attained, despite an increase in energy intake, by an increase in energy expenditure.[28,29] Murine adipocytes lacking HSL are enlarged. They display normal basal lipolytic activity, suggesting that other lipases may play a role, but catecholamine stimulation of lipolysis is severely reduced.[30,31] Mice lacking perilipin are lean, but their adipocytes display elevated basal lipolysis and, again, impaired catecholamine stimulation.[32] These findings accord with the view that phosphorylation of both HSL and perilipin is important for catecholamine stimulated lipolysis. Neutral lipases present in the cell may take over the role of HSL in basal lipolysis, and may even to some extent be stimulated by catecholamines because 'access' to the fat droplet will be allowed when phosphorylated perilipin moves away.

The aim of this brief description of the pathways of fat deposition and mobilisation, summarised in Figure 1, is not to be comprehensive, but rather to show that a multiplicity of hormonal and neural influences may be involved. The adipocyte regulates how much fat it will store, partly

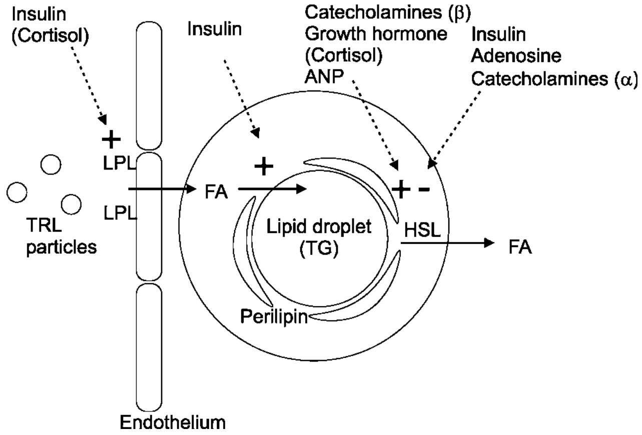

**Figure 1** Multiple influences on net fat storage in adipocytes. In general insulin stimulates net fat deposition. Other influences may be site-specific, for example, cortisol probably has net anabolic effects in some adipose depots and net catabolic in others, or dependent upon other factors such as receptor expression or concentration, for example, catecholamines may stimulate fat mobilisation via $\beta$-adrenoceptors or inhibit via $\alpha$-adrenoceptors. Lipolysis is activated via phosphorylation of both HSL and perilipin, which coats the fat droplet. ANP, atrial natriuretic peptide, FA, fatty acids; LPL, lipoprotein lipase; TG, triacylglycerol; TRL, TG-rich lipoproteins.

through its own gene products. LPL in the adipose tissue capillaries, itself a product of adipocyte gene expression, generates a surplus of fatty acids, and the adipocyte takes up and esterifies a proportion of these: the remainder mix with NEFAs released from adipocyte lipolysis.[33] Production of ASP (an adipocyte-derived stimulator of fat storage) represents one way in which the adipocyte may regulate how much fat it takes up. The opposing pathway, lipolysis of adipocyte TG, is again a function of the adipocyte. In order to regulate how much fat to store and mobilise, the adipocyte must respond to signalling mechanisms. If fat storage is impaired, for instance in the situation of DGAT deficiency, then other pathways come into play to maintain energy balance (in that case, increased energy expenditure including an increase in physical activity[28]). The integrative nature of adipose tissue physiology is apparent.

While individual adipocytes may expand or contract by the pathways described in this section, at some point in the process of fat storage there is a need for more adipocytes. The differentiation of new adipocytes is regulated by many factors in common with the pathway of fat deposition.

## Adipocyte differentiation

Preadipocytes, fibroblast-like cells present in the stromal-vascular fraction of adipose tissue, can differentiate to form mature adipocytes and this capacity is present throughout life.[34] The topic of fat cell differentiation has been covered in detail elsewhere,[35–37] but one important point will be emphasised here. The signal for differentiation of new adipocytes is related to nutritional state. Important stimuli for differentiation include insulin and fatty acids. Fatty acids act through members of the peroxisome proliferator-activated receptor (PPAR) family, PPAR$\delta$ (also known as fatty acid-activated receptor, FAAR[38]) and particularly PPAR$\gamma$. The natural ligand for PPAR$\gamma$ is probably a fatty acid derivative.[39] In addition, differentiation is regulated by a pathway involving the sterol regulatory element binding protein-1c (SREBP-1c, also known as adipocyte determination and differentiation factor-1, ADD-1), a pathway that in adipocytes is regulated by insulin.[40,41] Given that individual adipocytes can also expand over a very large range as they store more TG, the net effect is that the capacity to store fat can increase seemingly without limit.

Expansion of fat stores, especially if differentiation of new adipocytes is involved, requires new blood vessels. Angiogenesis in adipose tissue appears also to be regulated in part by factors produced within the tissue. Leptin has angiogenic properties,[42,43] and adipocytes secrete matrix metalloproteinases that may be involved in vascular remodelling.[44] Expression of genes involved in angiogenesis is upregulated during weight gain in animals,[45,46] and inhibition of angiogenesis reduces fat deposition in various obesity models in mice.[47]

Situations of negative energy balance involve loss of adipocyte TG stores. While this involves shrinkage of adipocytes, there is now considerable evidence that apoptosis is also involved in the turnover of adipocytes.[48,49] The subject of adipocyte apoptosis has been reviewed previously in this journal and we will not cover it in more detail here.[50]

## Adipose tissue blood flow

Adipose tissue blood flow after an overnight fast is typically around 3 ml blood per 100 g tissue per minute, whereas in resting skeletal muscle the value is more like 1.5 ml blood per 100 g tissue per minute.[51] Skeletal muscle blood flow can increase many-fold (perhaps 20-fold) during exercise. Adipose tissue blood flow is also very labile. In some lean, healthy subjects subcutaneous abdominal blood flow increases several-fold (up to four-fold) in response to a meal[52–57] (Figure 2). There are similar increases in thigh and forearm adipose tissue blood flow in response to feeding.[52,58,59] Adipose tissue blood flow also increases during exercise,[60,61] although the increase is not particularly marked except during very prolonged exercise. Adipose tissue blood flow increases steadily during the night, presumably reflecting increasing duration of fasting.[62] Extending an overnight fast (14 h) fast to 22 h causes no significant change in flow,[63] but more prolonged (72 h) fasting increases blood flow further (approximately 1.5-fold).[64] Thus, it appears that there is some minimum value for adipose tissue blood flow at rest in a late postprandial state: blood flow increases as fasting continues, or when a meal is eaten, or during exercise. In all these states, the increase in adipose tissue blood flow may be related to the

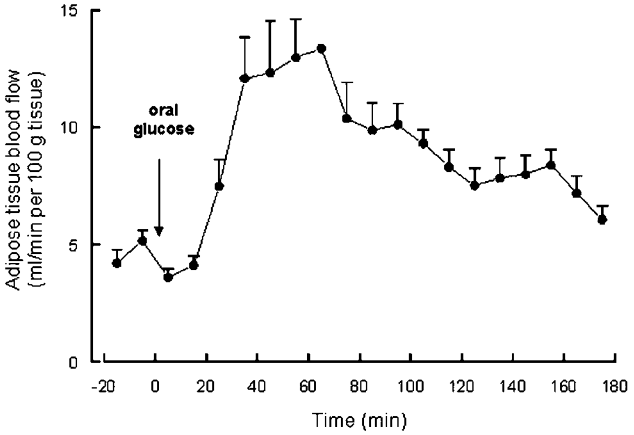

**Figure 2** Increase in adipose tissue blood flow after 75 g oral glucose in five healthy subjects. These data represent the five greatest responders out of 15 subjects studied[57] (Reproduced from Karpe *et al*[57] with permission).

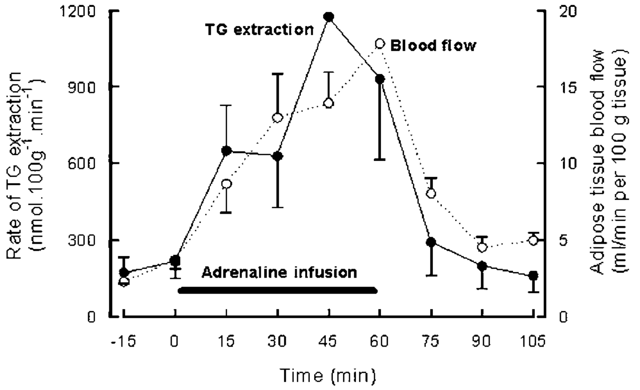

**Figure 3** Parallel increases in adipose tissue blood flow (open circles, dotted line) and in TG extraction across adipose tissue (solid points, solid line) during infusion of adrenaline at 25 ng kg$^{-1}$ min$^{-1}$. Data are from six healthy subjects.[65]

metabolic activity of the tissue. During fasting or during exercise, adipose tissue releases NEFA, and requires a supply of plasma albumin for transport of these NEFA into the circulation. After feeding, there is a need to increase substrate delivery for TG clearance. When adipose tissue blood flow was increased by infusion of adrenaline, TG extraction increased exactly in parallel with increased blood flow[65] (Figure 3), implying that TG extraction is normally limited by substrate delivery. It must be pointed out that the peak in adipose tissue blood flow after eating is early, and does not correspond closely to the later peak in plasma TG concentration, so there may be some role for the increased postprandial blood flow that we do not yet understand. For instance, it might serve to deliver a signal, such as insulin, to initiate the postprandial increase in LPL activity.

Regulation of adipose tissue blood flow has been studied extensively (reviewed by Frayn and Macdonald[66]). In humans, adrenergic influences are predominant, with $\beta$-mediated vasodilatation[67] and $\alpha$2-mediated vasoconstriction.[68] These influences may explain the increased blood flow during fasting or exercise. Studies in patients with spinal cord lesions suggest that circulating catecholamines are more important in the exercise-induced increase in adipose tissue blood flow than sympathetic nerve activity.[69] The increase in blood flow following feeding has not been fully explained. Its time course parallels that of plasma insulin concentrations,[54] although insulin itself is not the local signal responsible.[56] The blood flow response to feeding is blocked by propranolol infusion[58] (completely in some depots, partially in others) suggesting that it is dependent upon sympathetic activation induced by postprandial hyperinsulinaemia. Again, therefore, the need for an integrated approach to studying adipose tissue function is evident. The global regulation of blood flow is probably modulated by locally produced factors including unbound NEFA,[70] adenosine, nitric oxide and prostaglandins. Adipocytes also express and secrete angiotensinogen and angiotensin-II, which may relate to vasomotor control.[71]

## Innervation of white adipose tissue

One obvious signalling pathway relevant to regulation of net fat deposition would be via the autonomic nervous system. Both sympathetic (adrenergic)[21] (Figure 4) and parasympathetic (cholinergic)[72] activation affect adipose tissue lipolysis.

There has been little histological work on the innervation of human adipose tissue, so the following information is based mainly on work in rats and hamsters. The similar effects of catecholamines and adrenoceptor agonists and antagonists on human and animal adipose tissue *in vitro* and *in vivo* provide a reasonable indication that the patterns of innervation seen in experimental animals also occur in human white adipose tissue.

It has been known for many years that white adipose tissue is innervated by the sympathetic nervous system (SNS).

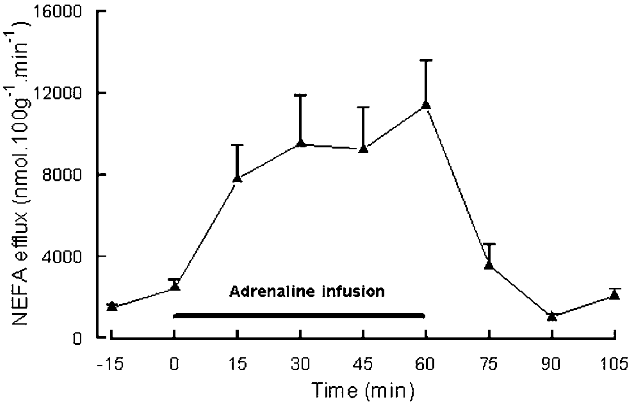

**Figure 4** Stimulation of NEFA release from adipose tissue during adrenaline infusion at 25 ng kg$^{-1}$ min$^{-1}$. Data are from six healthy subjects.[65]

However, there has been some controversy as to whether these nerves only terminate on the blood vessels[73] or whether they also synapse with the adipocytes.[74] It has been suggested that there might be two pools of adipocytes, only one of which is innervated,[74] and electron microscopy suggests that only approximately 3% of adipocytes receive direct innervation.[75] If this were the case, it would not be surprising that some authors failed to observe innervation of adipocytes. However, some preliminary data show more extensive cellular innervation, with marked regional differences.[76] Histofluorescence and confocal microscopy were used to demonstrate catecholamine-containing nerves in direct contact with adipocytes, taken from epididymal, perirenal, mesenteric and inguinal regions of the rat. The highest level of innervation was observed in the mesenteric depot, and the lowest in the inguinal. Further evidence of direct innervation of adipocytes was provided by injection of fluorescent tracers into the sympathetic chain of hamsters, with the observation of rings of fluorescence around fat cells in both epididymal and inguinal regions.[77] The same authors showed that some of the innervation of these two regions arose from different parts of the sympathetic chain, providing the structural basis for selective activation of adipose tissue.[77] This possibility was confirmed by the demonstration of higher rates of noradrenaline turnover in epididymal compared to inguinal fat pads of hamsters acutely exposed to a reduced day length.

The SNS innervation of white adipose tissue leads to the release of the neurotransmitter noradrenaline, and drugs that stimulate or antagonise adrenoceptors have the potential for directly affecting adipose tissue function. In addition, such drugs and the catecholamines, adrenaline and noradrenaline, have the potential to influence the blood flow through adipose tissue and other parts of the cardiovascular system and could thus indirectly affect adipose tissue function. Adrenergic receptors expressed within adipose tissue have been extensively studied.[78,79] Obesity is associated with changes in adrenoceptor numbers and subtypes.[80] This suggests that the autonomic signals to adipose tissue are modulated in response to energy stores and adipocyte size.

While receptor numbers are probably regulated over periods of days and weeks, changing local sympathetic activity is a more acute regulator of adipose tissue activity. Local noradrenaline spillover from a subcutaneous adipose tissue bed does not change significantly between the overnight fasted state (14 h fast) and 22 h fast.[81] In contrast, there is increased noradrenaline spillover from adipose tissue associated with (and possibly causing) increased local blood flow 60–120 min after eating and after 72 h fasting.[64,82]

In addition to acute effects on adipose tissue function, the SNS innervation appears to affect cell numbers. Denervation of retroperitoneal adipose tissue in rats increases fat pad weight.[83] Within 1 week of denervation there was proliferation of preadipocytes and after 1 month there was an increase in the numbers of mature adipocytes. Denervation did not affect glucose metabolism, nor LPL and HSL expression and activity. Denervation also increased fat pad weight in the hamster, even in conditions of reduced food intake produced by exposing the animals to a short day length.[84] Specific removal of the parasympathetic innervation has the opposite effects (see below).

As well as its effects on adipose tissue function, the sympathetic innervation of white adipose tissue appears to have an afferent component, which presumably sends signals to either the spinal cord or the brain. Injection of small amounts of leptin into perirenal adipose tissue in rats was associated with increased renal sympathetic efferent activity without altering plasma leptin concentration.[85] Studies on hamsters and rats have demonstrated direct neural connections between white adipose tissue and several different brain regions involved in the SNS regulation of the cardiovascular and other systems.[86] There was little difference between the species, and the areas of the brain involved were similar to those previously shown to be neurally connected to the adrenal medulla.[87]

Considerably less attention has been paid to parasympathetic innervation of adipose tissue, which historically was considered to be less important than sympathetic innervation (reviewed in Frayn and Macdonald[66]). More recently this view has changed. Cholinergic stimuli affect lipolysis *in vivo* as determined by microdialysis: nicotinic receptor stimulation increases and muscarinic receptor stimulation decreases lipolysis in humans.[72] Newer studies in the rat show clearly parasympathetic innervation, which can be traced to origins in the brain stem.[88] When this vagal innervation was severed, fat pad anabolic processes (glucose and fatty acid uptake) were impaired and HSL activity increased.[88] The implication is of a dual innervation, the sympathetic controlling catabolic processes and the parasympathetic anabolic.[89]

It is clear, therefore, that the autonomic innervation of white adipose tissue is of major importance in the regulation of tissue mass and function. It is less clear how this relates to the autonomic control of other physiological processes, or the extent to which acute activation or suppression of adipose tissue by the autonomic nervous system affects metabolic processes such as insulin sensitivity, peptide secretion or even lipolysis.

## Site-specific properties of adipose tissue depots

Adipose tissue, like skeletal muscle, is distributed through the body in discrete depots. Although the different depots may assume different importance in various species,[90,91] nevertheless there are homologous depots in all mammals. Some of these are small (eg the popliteal) and seem to have some primary function other than as a reserve of fat, others are larger and contribute more to fat storage and mobilisation. We have chosen in this review to concentrate on the similarities among adipose tissue depots rather than their differences. For instance, NEFA release from one particular, relatively large depot, the subcutaneous anterior abdominal depot, correlates strongly with systemic plasma NEFA concentrations,[92] arguing for common control of lipolysis in all large depots. Similarly, when blood flow has been studied in various depots, there are qualitative similarities in the responses to nutrient ingestion.[58]

Nevertheless, since the early clinical observations of Vague[93,94] that upper-body obesity was associated with a much greater incidence of adverse consequences (largely related to insulin resistance and dyslipidaemia) than was lower-body obesity, much attention has been directed towards trying to understand the physiological differences between these sites. Much of this work has been carried out by preparing isolated adipocytes from biopsies from the various sites removed at surgery.[95–98] In view of the complex integration of adipose tissue metabolism *in vivo*, stressed in this review, results from such *in vitro* observations must be interpreted with some caution. The general picture that emerges is, however, consistent: intra-abdominal 'visceral' adipocytes have the highest metabolic activity followed by upper-body subcutaneous adipocytes, and lowest response in lower-body adipocytes.[99] Metabolic activity here refers mainly to lipolysis: intra-abdominal adipocytes have the highest rates of lipolysis when stimulated with a $\beta$-adrenoceptor agonist, and lipolysis is least susceptible to suppression by insulin. Thus, it could be simplistically argued that upper-body adipocytes discharge fatty acids at a high rate, and interfere with insulin-sensitive glucose metabolism. Lower-body fat depots may be efficient at removing fat from the circulation and they are relatively resistant to loss during weight loss.[100] Their predominance in women may imply that these are long-term fat reserves to cover eventualities such as child-bearing and nurturing if food supply runs short.

However, this view is necessarily simplistic.[101] To make one obvious point, continued high rates of lipolysis would lead to disappearance of the fat depot unless they were matched at some other time by high rates of fat deposition. Fat deposition is much less easily studied *in vitro*. Measurements of LPL activity in intra-abdominal adipose tissue show no consistent pattern relative to subcutaneous tissue,[102–105] and measurements of fatty acid incorporation into TG in isolated adipocytes show a lower rate of TG synthesis in omental than subcutaneous tissue.[106] Such studies as have been performed *in vivo* lend general support to the picture. When subjects were given isotopically labelled fatty acids, and biopsies of different depots were taken at abdominal surgery 24 h later,[102] accumulation of label was most marked (per g TG) in the omental and retroperitoneal depots. This is in agreement with a high rate of lipid turnover. Microdialysis measurements show considerably greater lipolysis during exercise in subcutaneous abdominal fat than in gluteal (lower-body).[21]

Whole-body physiological studies of fat mobilisation provide a clearer picture of the relative roles of lower and upper-body fat depots. Jensen and co-workers have studied lean people, and people with upper and lower-body obesity using depot-specific catheterisations and labelled fatty acid tracers. The contribution of the visceral fat depot was assessed by hepatic venous catheterisation. It was concluded that the upper-body subcutaneous adipose tissue provides the major proportion of the systemic NEFA, whereas the lower-body fat only provides a small proportion.[107,108] The visceral adipose tissue depot provides little NEFA to the systemic circulation and even in women with upper-body obesity, the specific contribution to NEFA delivery to the liver from the visceral depot was small relative to upper-body subcutaneous fat.[108]

Another view is that the relative importance of the different depots with regard to adverse effects may reflect differential expression of secreted peptides. There has been a large literature on this topic and we will refer to it in the appropriate section below. It should be noted, however, that for the one adipose tissue-derived peptide clearly shown to have physiological relevance in man, leptin, secretion is considerably greater from subcutaneous than from visceral fat depots.[109,110]

## Adipose tissue and peptide secretion

Adipose tissue was first suggested to have endocrine functions by Siiteri,[111] who pointed to the tissue's ability to interconvert steroid hormones. More recently interest has moved to adipose tissue production and secretion of a wide range of proteins. Many of these are classical cytokines and others, including leptin, are structurally related to cytokines. This has led to the introduction of the term adipocytokines to describe this wide range of proteins produced by adipose tissue. The use of the term adipocytokine also highlights that several proteins produced by adipocytes may act locally as autocrine and paracrine factors rather than remote-acting endocrine factors.

Before discussing adipose tissue production of adipocytokines and other endocrine factors, it is worth reiterating that adipose tissue characteristics often differ between species, between depots within an individual and even between cells in different parts of a depot. In addition, adipocytokine production may differ between adipocytes at different stages of their development, for example, preadipocytes producing less tumour necrosis factor (TNF) than mature cells.

### Adipocytokines

Adipose tissue's endocrine function is now well established for some protein hormones, most notably leptin. The identification of leptin as an adipostatic hormone[112] transformed views of the tissue and of obesity. To summarise, leptin is a hormone that arises predominantly ($>95\%$) from adipose tissue (especially subcutaneous depots), and its secretion is under regulation by the size of the whole-body adipose tissue mass. There are also short-term regulators of leptin concentration: these are mostly influences of food and energy balance. Food, food composition, ambient temperature, exercise, sleep pattern and recent net energy balance have all been shown to regulate plasma leptin concentration.[63,113–115] These influences appear to be mediated mostly by insulin, by glucose entry rates into adipocytes and by sympathetic regulators.[63,113] The clear regulation of leptin secretion by these factors that reflect whole-body energy status suggests that adipose tissue is capable of determining short- and long-term energy status and changing its endocrine activity accordingly. Leptin's actions are mostly beyond the scope of the article and the reader is referred to other reviews of this topic.[116,117] In summary, leptin clearly has major effects reducing food intake in both rodents and man. In rodents (but possibly not in man) it stimulates the SNS to increase energy expenditure[118] and it may increase fatty acid oxidation in muscle.[119] Leptin is required for fertility; leptin deficiency (as seen in undernutrition or anorexia nervosa, or in people with leptin or leptin-receptor mutations) causes delayed sexual maturity and anovulation.[120–124]

The recognition of leptin's important roles stimulated the search for other proteins secreted by adipose tissue and/or adipocytes. It appears that a large proportion of the genetic material expressed within adipose tissue encodes secretory proteins, but at present there is some uncertainty as to whether all the proteins secreted by adipose tissue or adipocytes *in vitro* are released into the systemic circulation.[125]

Several proteins have been demonstrated to be secreted into the circulation by human subcutaneous abdominal tissue *in vivo*. This group includes interleukin-6 (IL-6) and soluble receptors for TNF$\alpha$.[126,127] IL-6, which is structurally related to leptin, appears to act as a hormone.[128] For each of these secreted adipocytokines, it appears from whole-body extrapolations that obese subjects release more protein from their expanded adipose tissue mass into the general circulation. However, in regard to acute regulation, there is little evidence that IL-6 and soluble receptors for TNF$\alpha$ are regulated by food intake.[126,127]

Several other proteins are secreted by adipose tissue *in vitro*. This group includes TNF$\alpha$, plasminogen activator inhibitor-1 and components of the renin–angiotensin system.[71,129] It remains to be established which of these are secreted into the systemic circulation by adipose tissue. For example, although adipocytes secrete TNF$\alpha$, and this cytokine can induce insulin resistance,[130] it may act more as a paracrine than as an endocrine factor.[126,127] TNF$\alpha$ production is increased in obesity and does respond to insulin and other energy-balance signals[130] and has several effects on adipocytes including inducing apoptosis,[48] suggesting that it is one means by which adipocytokines respond to limit further adiposity.

Recently, several additional proteins have been described that are secreted by adipose tissue *in vitro*, adiponectin, fasting-induced adipose factor (FIAF) and resistin: each appears to reach the systemic circulation from adipose tissue.

Adiponectin, secreted solely by adipose tissue, is the gene product of the most abundant gene transcript-1 (apM1), which is abundantly expressed in white adipose tissue. The protein has structural homology with collagen VIII, collagen X, complement C1q as well as with TNF$\alpha$.[131] mRNA expression of adiponectin increases as preadipocytes differentiate and is reduced by TNF.[132] Adiponectin protein is abundant in human plasma, typically accounting for 0.01% of total plasma protein and circulating adiponectin concentrations are reduced in obesity.[133] These concentrations are further reduced in diabetes mellitus and/or in insulin resistance,[133] and in coronary artery disease including myocardial infarction.[134] Furthermore, administration of recombinant adiponectin reverses insulin resistance of glucose metabolism[135,136] in part by increasing fatty acid oxidation and energy dissipation in skeletal muscle and in part by inhibiting hepatic glucose output. The diurnal profiles of adiponectin are relatively flat,[134] which suggest that adiponectin acts as an adipostat sensing either adipose tissue mass or the resulting insulin resistance. Adiponectin signalling from adipose tissue appears to preserve fat utilisation in muscle and promotes glucose utilisation. These two events may provide unified dysregulation of the metabolic abnormalities in the insulin resistance syndrome.

Adiponectin may have a protective role in the vascular wall.[137,138] It can accumulate within injured vascular walls[139] and can suppress TNF$\alpha$-induced expression of adhesion molecules in vascular endothelial cells.[137] This action is on the postreceptor part of TNF signalling, although the 'adiponectin receptor' has not yet been identified. Other actions of adiponectin include suppression of macrophage to foam-cell transformation *in vitro*,[131,137] and inhibition of endothelial signalling through cAMP. In addition to its effects on the endothelium and vascular wall, adiponectin can downregulate haematopoeisis and the immune system.[140]

FIAF is another factor that has recently been identified as a secreted product of white (and brown) adipose tissue. Other tissues, such as liver and lungs, appear to express the mRNA for this protein to a much lower extent than does adipose tissue. As its name implies, FIAF is strongly induced by fasting. The protein is a member of the fibrinogen/angiopoietin-like proteins. It is suggested that it is a sort of 'antileptin' being stimulated by undernutrition and responsible for orchestrating the metabolic adaptation to fasting.[141,142]

Resistin mRNA is found in white but not in brown adipose tissues,[143] and is found in high concentrations in the adipocyte cytoplasm. Resistin mRNA content varies from depot to depot, but, as the gene is PPAR$\gamma$-regulated, it is reduced by thiazolidinediones.[143] Circulating resistin concentrations increase with fat feeding and in obese animals. Antiresistin antibodies improve insulin resistance both *in vitro* and *in vivo*.[143] However, it should be noted that there is no universal agreement with this story. Others have not confirmed this in rodents[144] and it seems that resistin expression in mature human adipocytes or adipose tissue is very low or absent,[145–147] although one report suggests it is higher in abdominal than in lower-body adipose tissue.[148]

It was initially suggested that resistin is secreted specifically by rodent adipocytes. However, the structurally related protein RELM$\alpha$ is secreted by the heart, liver and tongue, as well as adipose tissue and breast. RELM$\alpha$ is not found in preadipocytes. mRNA for RELM$\alpha$ is found only in the intestine. The receptor/s for resistin and RELMs is/are not yet understood. There is no consensus yet as to whether resistin secretion by adipose tissue is acutely regulated by food.

As reviewed earlier, some important proteins involved in local lipid metabolism are secreted *in vivo*, notably adipsin and ASP (reviewed above[15]), and LPL.[149,150] These proteins, which are not usually considered adipocytokines, probably function mostly within the adipose tissue to regulate lipid metabolism. Again, it appears from whole-body extrapolations that obese subjects release more of these proteins from their expanded adipose tissue mass into the general circulation. In regard to acute regulation, release of both LPL[149] and ASP[15] increases postprandially. In addition, both leptin and matrix metalloproteinases (also secreted from adipocytes[44]) may be involved with angiogenesis, as reviewed earlier.

Finally when discussing adipocytokines, it is important to recall that 'adipose tissue' is not purely adipocytes, and some of the nonadipocyte cells within the tissue may be critical players in both the secretion of adipocytokines and in the tissue response to adipocytokines and other signals. Of especial interest in this respect is the observation by Pond and co-workers[151,152] that the behaviour of adipocytes depends upon their proximity to lymph nodes. Larger and many smaller lymph nodes are embedded in adipose tissue.[151,152] It appears both that adipocytes affect the activity of the lymph node they surround, and that the lymph node affects the surrounding adipocytes.[151] Activation of lymph nodes increases local adipocyte lipolysis, presumably by means of local release of cytokines including TNF$\alpha$ and IL-6,[153,154] which may increase the sensitivity of lipolysis to noradrenaline.[152] Adipose tissue surrounding lymph nodes may therefore provide fatty acids locally as a nutritive fuel for immune cells,[152] and may by this means also suppress lymphocyte proliferation.[151]

## Other endocrine functions

Recent studies have confirmed that human adipose tissue *in vivo* does produce sex-steroids and glucocorticoids from precursors (eg androgens to oestrogens, cortisone to cortisol).[155,156] However, whole-body extrapolations from individual depots suggest that while the sex-steroid conversion by adipose tissue is quantitatively important (10–20% of whole-body production), net glucocorticoid conversion is minor.[156] The conversion of sex steroids is increased by obesity and acutely regulated by insulin.[157]

Table 1 summarises some of the endocrine activities of adipose tissue although this is a rapidly expanding area and the reader is referred to reviews of this topic.[125,158,159] For several of these secreted products, the factors regulating the secretion have not been fully established, and their full role in the integrated physiology of adipose tissue remains to be established.

Summarising what is known about adipose tissue's endocrine functions, it appears that the secretion of adipocytokines and other proteins by adipose tissue depends upon several factors: (1) the size of the TG stores, (2) recent whole-body energy balance and insulin/glucose signals, and (3) other 'descending' influences from the SNS and other endocrine systems such as hypothalamo-pituitary axis and growth hormone axis. Thus, the endocrine functions are another clear example of the integrative physiology of adipose tissue.

## Pulsatility

There is growing evidence for pulsatility in several aspects of adipose tissue function. Leptin secretion may have an

**Table 1** Adipose tissue secreted products regulated by energy balance

| | *Endocrine/paracrine* | *Effect of obesity* | *Acute affect of food* | *Main regulators* |
|---|---|---|---|---|
| Leptin | Endocrine and paracrine | Increased | Increased | Multiple (please see text) |
| Adiponectin | Endocrine | Decreased | None | |
| Adipsin | Endocrine and paracrine | Increased | Increased | |
| TNF$\alpha$ | Paracrine | Increased | Increased | Multiple |
| IL-6 | Endocrine and paracrine | Increased | None | Multiple |
| TNF-soluble receptors | Endocrine | Increased | None | |
| LPL | Mostly paracrine | Probably increased | Increased | Insulin |
| Resistin | Not known | Increased | Not known | |
| FIAF | Endocrine? | | Reduced | |
| PAI-1 | Paracrine | Increased | | |

This is not an exhaustive list of factors secreted by adipose tissue, but reflects those whose expression/secretion is regulated by energy balance. IL, interleukin; LPL, lipoprotein lipase; PAI-1, plasminogen activator inhibitor-1; TNF, tumour necrosis factor.

ultradian pattern[160,161] and show pulsatility with a periodicity of less than 1 h.[162] For such leptin pulsation, it has been suggested that growth hormone pulsation is the pacemaker,[163] but this remains to be proven. Systemic plasma NEFA and glycerol concentrations show pulsatility,[164] as does lipolysis in omental adipose tissue in dogs, arguing for coordinated action of adipose tissue throughout the body.[165] Some of these apparent pulsations have a periodicity of only a few minutes,[165] which would suggest a neural pacemaker mechanism. This has recently been confirmed in dogs; $\beta$3-adrenoceptor blockade removes the oscillatory component with little effect on 'basal' plasma NEFA concentrations.[164] The existence of pulsatile behaviour emphasises the complexity of the integrative physiology within adipose tissue. Further studies in this area are needed.

## Integration of adipose tissue fat stores in relation to energy balance

We can now summarise our understanding of how the pathways of fat deposition and fat mobilisation can be regulated in accordance with the integrated imbalance between whole-body energy intake and energy expenditure. The multiplicity of factors affecting adipose tissue metabolism, some generated in adipose tissue, seem to offer many opportunities for modulation of energy utilisation in other organs (for instance, adiponectin and resistin signalling to the liver and skeletal muscle). Effects on overall fat stores are less convincing in humans: possibly leptin becomes ineffective once a certain level of obesity is reached, confirming that leptin is essentially a 'starvation hormone' rather than one of abundance. Ultimately, it would seem that there must be some controlling signal originating outside adipose tissue that regulates net adipose tissue TG deposition. A role for the SNS in regulation of net fat stores would seem to be likely, especially with current understanding that the sympathetic system is not a coherent whole but different efferent branches can be activated independently.[166,167] Such a system would have to involve the brain in sensing energy balance independently of the leptin system, which basically responds to TG storage although with 'fine tuning' by insulin. It is not immediately clear how this would operate. Alternatively insulin itself might be the signal. An excess of energy intake over energy expenditure will generally lead to an increase in glycogen stores, small elevation of (fasting) plasma glucose concentration and a rise in insulin. Insulin, as noted several times above, has a strong effect in stimulating net fat storage in adipocytes. This hypothesis does not, however, explain how fat accumulation would occur if someone were to over eat solely fat. This is clearly an unphysiological situation, but if it is mimicked acutely by intravenous infusion of a TG emulsion, producing no detectable increase in plasma insulin, there is a marked decrease in NEFA release from adipose tissue, reflecting a suppression of HSL activity.[168,169] This experiment seems to imply that there is some signal that responds to increased nutrient availability to regulate net fat storage independently of insulin, and in this case presumably also independently of the SNS.

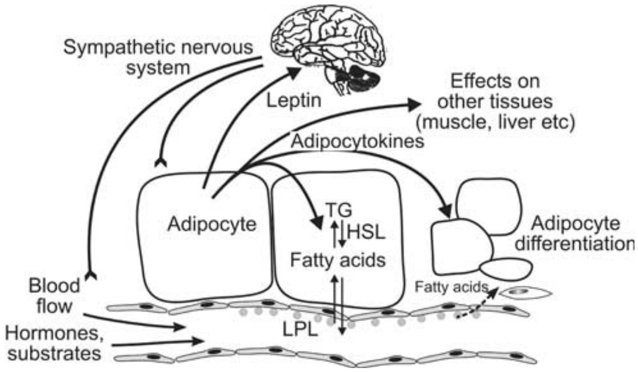

**Figure 5** Overview of the integrative physiology of adipose tissue.

In conclusion, adipose tissue function can only be truly understood when studied in an integrated way (Figure 5). There is much to be learned still about the regulation of TG storage in adipose tissue. Key questions for the future are (1) whether there is a single or multiple, signals from outside the tissue that regulate adipocyte fat storage pathways appropriately and, if so, what the nature of such a signal might be; and (2) whether indeed adipose tissue, through secretion of peptides and metabolites, regulates metabolic processes in other tissues to help to achieve appropriate fat balance. As our understanding of the integrative physiology of adipose tissue increases, we hope that these, and many other aspects of adipose tissue function, will be clarified.

### Acknowledgements

We thank the Wellcome Trust for support of our studies on the integrative physiology of human adipose tissue.

### References

1 Coleman RA, Lewin TM, Muoio D. Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. *Ann Rev Nutr* 2000; **20**: 77–103.
2 Langin D, Holm C, Lafontan M. Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. *Proc Nutr Soc* 1996; **55**: 93–109.
3 Holm C, Osterlund T, Laurell H, Contreras JA. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Ann Rev Nutr* 2000; **20**: 365–393.
4 Shrago E, Spennetta T, Gordon E. Fatty acid synthesis in human adipose tissue. *J Biol Chem* 1969; **244**: 2761–2766.
5 Hellerstein MK, Schwarz J-M, Neese RA. Regulation of hepatic *de novo* lipogenesis in humans. *Annu Rev Nutr* 1996; **16**: 523–557.
6 Aarsland A, Chinkes D, Wolfe RR. Hepatic and whole-body fat synthesis in humans during carbohydrate overfeeding. *Am J Clin Nutr* 1997; **65**: 1774–1782.

7 Leboeuf B. Regulation of fatty acid esterification in adipose tissue incubated *in vitro*. In: Renold AE, Cahill GF (eds). *Handbook of physiology Section 5: adipose tissue*. American Physiological Society: Washington DC, 1965. pp 385–391.

8 Harper RD, Saggerson ED. Factors affecting fatty acid oxidation in fat cells isolated from rat white adipose tissue. *J Lipid Res* 1976; **17**: 516–526.

9 Sadur CN, Eckel RH. Insulin stimulation of adipose tissue lipoprotein lipase. Use of the euglycemic clamp technique. *J Clin Invest* 1982; **69**: 1119–1125.

10 Frayn KN, Shadid S, Hamlani R, Humphreys SM, Clark ML, Fielding BA, Boland O, Coppack SW. Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am J Physiol* 1994; **266**: E308–E317.

11 Baldo A, Sniderman AD, St-Luce S, Avramoglu RK, Maslowska M, Hoang B, Monge JC, Bell A, Mulay S, Cianflone K. The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. *J Clin Invest* 1993; **92**: 1543–1547.

12 Cianflone K, Vu H, Walsh M, Baldo A, Sniderman A. Metabolic response of acylation stimulating protein to an oral fat load. *J Lipid Res* 1989; **30**: 1727–1733.

13 Choy LN, Rosen BS, Spiegelman BM. Adipsin and an endogenous pathway of complement from adipose cells. *J Biol Chem* 1992; **267**: 12736–12741.

14 Maslowska M, Scantlebury T, Germinario R, Cianflone K. Acute *in vitro* production of acylation stimulating protein in differentiated human adipocytes. *J Lipid Res* 1997; **38**: 1–11.

15 Saleh J, Summers L, Cianflone K, Fielding B, Sniderman A, Frayn K. Coordinated release of acylation stimulating protein (ASP) and triacylglycerol clearance by human adipose tissue *in vivo* in the postprandial period. *J Lipid Res* 1998; **39**: 884–891.

16 Campbell PJ, Carlson MG, Hill JO, Nurjhan N. Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. *Am J Physiol* 1992; **263**: E1063–E1069.

17 Rizza RA, Mandarino LJ, Gerich JE. Dose–response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol* 1981; **240**: E630–E639.

18 Coppack SW, Frayn KN, Humphreys SM, Dhar H, Hockaday TDR. Effects of insulin on human adipose tissue metabolism *in vivo*. *Clin Sci* 1989; **77**: 663–670.

19 Sztalryd C, Kraemer FB. Regulation of hormone-sensitive lipase during fasting. *Am J Physiol* 1994; **266**: E179–E185.

20 Clifford GM, Londos C, Kraemer FB, Vernon RG, Yeaman SJ. Translocation of hormone-sensitive lipase and perilipin upon lipolytic stimulation of rat adipocytes. *J Biol Chem* 2000; **275**: 5011–5015.

21 Arner P, Kriegholm E, Engfeldt P, Bolinder J. Adrenergic regulation of lipolysis *in situ* at rest and during exercise. *J Clin Invest* 1990; **85**: 893–898.

22 Samra JS, Clark ML, Humphreys SM, Macdonald IA, Bannister PA, Matthews DR, Frayn KN. Suppression of the nocturnal rise in growth hormone reduces subsequent lipolysis in subcutaneous adipose tissue. *Eur J Clin Invest* 1999; **29**: 1045–1052.

23 Samra JS, Clark ML, Humphreys SM, Macdonald IA, Matthews DR, Frayn KN. Effects of morning rise in cortisol concentration on regulation of lipolysis in subcutaneous adipose tissue. *Am J Physiol* 1996; **271**: E996–E1002.

24 Sengenes C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. *FASEB J* 2000; **14**: 1345–1351.

25 Julien P, Despres J-P, Angel A. Scanning electron microscopy of very small fat cells and mature fat cells in human obesity. *J Lipid Res* 1989; **30**: 293–299.

26 Peeva E, Brun DL, Ven Murthy MR, Després J-P, Normand T, Gagné C, Lupien P-J, Julien P. Adipose cell size and distribution in familial lipoprotein lipase deficiency. *Int J Obes Relat Metab Disord* 1992; **16**: 737–744.

27 Weinstock PH, Levak Frank S, Hudgins LC, Radner H, Friedman JM, Zechner R, Breslow JL. Lipoprotein lipase controls fatty acid entry into adipose tissue, but fat mass is preserved by endogenous synthesis in mice deficient in adipose tissue lipoprotein lipase. *Proc Natl Acad Sci USA* 1997; **94**: 10261–10266.

28 Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, Sanan DA, Raber J, Eckel RH, Farese Jr RV. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nat Genet* 2000; **25**: 87–90.

29 Chen HC, Ladha Z, Smith SJ, Farese Jr RV. Analysis of energy expenditure at different ambient temperatures in mice lacking DGAT1. *Am J Physiol Endocrinol Metab* 2003; **284**: E213–E218.

30 Osuga J, Ishibashi S, Oka T, Yagyu H, Tozawa R, Fujimoto A, Shionoiri F, Yahagi N, Kraemer FB, Tsutsumi O, Yamada N. Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. *Proc Natl Acad Sci USA* 2000; **97**: 787–792.

31 Wang SP, Laurin N, Himms-Hagen J, Rudnicki MA, Levy E, Robert M-F, Pan L, Oligny L, Mitchell GA. The adipose tissue phenotype of hormone-sensitive lipase deficiency in mice. *Obes Res* 2001; **9**: 119–128.

32 Tansey JT, Sztalryd C, Gruia-Gray J, Roush DL, Zee JV, Gavrilova O, Reitman ML, Deng CX, Li C, Kimmel AR, Londos C. Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc Natl Acad Sci USA* 2001; **98**: 6494–6499.

33 Frayn KN, Coppack SW, Fielding BA, Humphreys SM. Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue *in vivo*: implications for the control of fat storage and fat mobilization. *Adv Enzyme Regul* 1995; **35**: 163–178.

34 Hauner H, Entenmann G, Wabitsch M, Gaillard D, Ailhaud G, Negrel R, Pfeiffer EF. Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. *J Clin Invest* 1989; **84**: 1663–1670.

35 Ailhaud G. Molecular mechanisms of adipocyte differentiation. *J Endocrinol* 1997; **155**: 201–202.

36 Darlington GJ, Ross SE, MacDougald OA. The role of C/EBP genes in adipocyte differentiation. *J Biol Chem* 1998; **273**: 30057–30060.

37 Rosen ED, Spiegelman BM. Molecular regulation of adipogenesis. *Annu Rev Cell Dev Biol* 2000; **16**: 145–171.

38 Amri EZ, Bonino F, Ailhaud G, Abumrad NA, Grimaldi PA. Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors. *J Biol Chem* 1995; **270**: 2367–2371.

39 Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 1995; **83**: 803–812.

40 Kim JB, Sarraf P, Wright M, Yao KM, Mueller E, Solanes G, Lowell BB, Spiegelman BM. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J Clin Invest* 1998; **101**: 1–9.

41 Gondret F, Ferré P, Dugail I. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. *J Lipid Res* 2001; **42**: 106–113.

42 Bouloumié A, Drexler HC, Lafontan M, Busse R. Leptin, the product of Ob gene, promotes angiogenesis. *Circ Res* 1998; **83**: 1059–1066.

43 Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR. Biological action of leptin as an angiogenic factor. *Science* 1998; **281**: 1683–1686.

44 Bouloumié A, Sengenès C, Portolan G, Galitzky J, Lafontan M. Adipocyte produces matrix metalloproteinases 2 and 9: involvement in adipose differentiation. *Diabetes* 2001; **50**: 2080–2086.

45 Morimura M, Ishiko O, Sumi T, Yoshida H, Ogita S. Angiogenesis in adipose tissues and skeletal muscles with rebound weight-gain after diet-restriction in rabbits. *Int J Mol Med* 2001; **8**: 499–503.

46 Li J, Yu X, Pan W, Unger RH. Gene expression profile of rat adipose tissue at the onset of high-fat-diet obesity. *Am J Physiol Endocrinol Metab* 2002; **282**: E1334–E1341.

47 Rupnick MA, Panigrahy D, Zhang C-Y, Dallabrida SM, Lowell BB, Langer R, Folkman MJ. Adipose tissue mass can be regulated through the vasculature. *Proc Natl Acad Sci USA* 2002; **99**: 10730–10735.

48 Prins JB, Niesler CU, Winterford CM, Bright NA, Siddle K, O'Rahilly S, Walker NI, Cameron DP. Tumor necrosis factor-$\alpha$ induces apoptosis of human adipose cells. *Diabetes* 1997; **46**: 1939–1944.

49 Wabitsch M. The acquisition of obesity: insights from cellular and genetic research. *Proc Nutr Soc* 2000; **59**: 325–330.

50 Sorisky A, Magun R, Gagnon AM. Adipose cell apoptosis: death in the energy depot. *Int J Obes Relat Metab Disord* 2000; **24**(Suppl 4): S3–S7.

51 Elia M, Kurpad A. What is the blood flow to resting human muscle? *Clin Sci* 1993; **84**: 559–563.

52 Bülow J, Astrup A, Christensen NJ, Kastrup J. Blood flow in skin, subcutaneous adipose tissue and skeletal muscle in the forearm of normal man during an oral glucose load. *Acta Physiol Scand* 1987; **130**: 657–661.

53 Samra JS, Frayn KN, Giddings JA, Clark ML, Macdonald IA. Modification and validation of a commercially available portable detector for measurement of adipose tissue blood flow. *Clin Physiol* 1995; **15**: 241–248.

54 Summers LKM, Samra JS, Humphreys SM, Morris RJ, Frayn KN. Subcutaneous abdominal adipose tissue blood flow: variation within and between subjects and relationship to obesity. *Clin Sci* 1996; **91**: 679–683.

55 Summers LKM, Callow J, Samra JS, Macdonald IA, Matthews DR, Frayn KN. The effect on adipose tissue blood flow of isoenergetic meals containing different amounts and types of fat. *Int J Obes Relat Metab Disord* 2001; **25**: 1294–1299.

56 Karpe F, Fielding BA, Ardilouze J-L, Ilic V, Macdonald IA, Frayn KN. Effects of insulin on adipose tissue blood flow in man. *J Physiol* 2002; **540**: 1087–1093.

57 Karpe F, Fielding BA, Ilic V, Macdonald IA, Summers LKM, Frayn KN. Impaired postprandial adipose tissue blood flow response is related to aspects of insulin sensitivity. *Diabetes* 2002; **51**: 2467–2473.

58 Simonsen L, Bülow J, Astrup A, Madsen J, Christensen NJ. Diet-induced changes in subcutaneous adipose tissue blood flow in man: effect of $\beta$-adrenoceptor inhibition. *Acta Physiol Scand* 1990; **139**: 341–346.

59 Romanski SA, Nelson RM, Jensen MD. Meal fatty acid uptake in adipose tissue: gender effects in nonobese humans. *Am J Physiol Endocrinol Metab* 2000; **279**: E455–E462.

60 Bülow J, Madsen J. Adipose tissue blood flow during prolonged, heavy exercise. *Pflügers Arch* 1976; **363**: 231–234.

61 Al Mulla N, Simonsen L, Bülow J. Post-exercise adipose tissue and skeletal muscle lipid metabolism in humans: the effects of exercise intensity. *J Physiol* 2000; **524**: 919–928.

62 Hagström-Toft E, Bolinder J, Ungerstedt U, Arner P. A circadian rhythm in lipid mobilization which is altered in IDDM. *Diabetologia* 1997; **40**: 1070–1078.

63 Klein S, Horowitz JF, Landt M, Goodrick SJ, Mohamed Ali V, Coppack SW. Leptin production during early starvation in lean and obese women. *Am J Physiol Endocrinol Metab* 2000; **278**: E280–E284.

64 Patel JN, Coppack SW, Goldstein DS, Miles JM, Eisenhofer G. Norepinephrine spillover from human adipose tissue before and after a 72-hour fast. *J Clin Endocrinol Metab* 2002; **87**: 3373–3377.

65 Samra JS, Simpson EJ, Clark ML, Forster CD, Humphreys SM, Macdonald IA, Frayn KN. Effects of epinephrine infusion on adipose tissue: interactions between blood flow and lipid metabolism. *Am J Physiol* 1996; **271**: E834–E839.

66 Frayn KN, Macdonald IA. Adipose tissue circulation. In: Bennett T, Gardiner SM (eds). *Nervous control of blood vessels*. Harwood Academic: Amsterdam, 1996. pp 505–539.

67 Hjemdahl P, Linde B, Daleskog M, Belfrage E. Sympatho-adrenal regulation of adipose tissue blood flow in dog and man. *Gen Pharmacol* 1983; **14**: 175–177.

68 Galitzky J, Lafontan M, Nordenström J, Arner P. Role of vascular alpha-2 adrenoceptors in regulating lipid mobilization from human adipose tissue. *J Clin Invest* 1993; **91**: 1997–2003.

69 Stallknecht B, Lorentsen J, Enevoldsen LH, Bülow J, Biering-Sørensen F, Galbo H, Kjaer M. Role of the sympathoadrenergic system in adipose tissue metabolism during exercise in humans. *J Physiol* 2001; **536**: 283–294.

70 Bülow J, Madsen J, Astrup A, Christensen NJ. Vasoconstrictor effect of high FFA/albumin ratios in adipose tissue *in vivo*. *Acta Physiol Scand* 1985; **125**: 661–667.

71 Boschmann M, Ringel J, Klaus S, Sharma AM. Metabolic and hemodynamic response of adipose tissue to angiotensin II. *Obes Res* 2001; **9**: 486–491.

72 Andersson K, Arner P. Cholinoceptor-mediated effects on glycerol output from human adipose tissue using *in situ* microdialysis. *Br J Pharmacol* 1995; **115**: 1155–1162.

73 Wirsén C. Adrenergic innervation of adipose tissue examined by fluorescence microscopy. *Nature* 1964; **202**: 913.

74 Ballard K, Malmfors T, Rosell S. Adrenergic innervation and vascular patterns in canine adipose tissue. *Microvasc Research* 1974; **8**: 164–171.

75 Slavin BG, Ballard KW. Morphological studies on the adrenergic innervation of white adipose tissue. *Anat Rec* 1978; **191**: 377–389.

76 Rebuffé-Scrive M. Neuroregulation of adipose tissue: molecular and hormonal mechanisms. *Int J Obes Relat Metab Disord* 1991; **15**(Suppl 2): 83–86.

77 Youngstrom TG, Bartness TJ. Catecholaminergic innervation of white adipose tissue in Siberian hamsters. *Am J Physiol* 1995; **268**: R744–R751.

78 Barbe P, Millet L, Galitzky J, Lafontan M, Berlan M. *In situ* assessment of the role of the $\beta$1-, $\beta$2- and $\beta$3-adrenoceptors in the control of lipolysis and nutritive blood flow in human subcutaneous adipose tissue. *Br J Pharmacol* 1996; **117**: 907–913.

79 Langin D, Lucas S, Lafontan M. Millennium fat-cell lipolysis reveals unsuspected novel tracks. *Horm Metab Res* 2000; **32**: 443–452.

80 Lafontan M, Bousquet-Melou A, Galitzky J, Barbe P, Carpene C, Langin D, Berlan M, Valet P, Castan I, Bouloumie A. Adrenergic receptors and fat cells: differential recruitment by physiological amines and homologous regulation. *Obes Res* 1995; **3**(Suppl 4): 507S–514S.

81 Horowitz JF, Coppack SW, Paramore D, Cryer PE, Zhao G, Klein S. Effect of short-term fasting on lipid kinetics in lean and obese women. *Am J Physiol* 1999; **276**: E278–E284.

82 Patel JN, Eisenhofer G, Coppack SW, Miles JM. Norepinephrine spillover in forearm and subcutaneous adipose tissue before and after eating. *J Clin Endocrinol Metab* 1999; **84**: 2815–2819.

83 Cousin B, Casteilla L, Lafontan M, Ambid L, Langin D, Berthault MF, Penicaud L. Local sympathetic denervation of white adipose tissue in rats induces preadipocyte proliferation without noticeable changes in metabolism. *Endocrinology* 1993; **133**: 2255–2262.

84 Youngstrom TG, Bartness TJ. White adipose tissue sympathetic nervous system denervation increases fat pad mass and fat cell number. *Am J Physiol Reg Integr Comp Physiol* 1998; **275**: R1488–R1493.

85 Tanida M, Iwashita S, Ootsuka Y, Terui N, Suzuki M. Leptin injection into white adipose tissue elevates renal sympathetic nerve activity dose-dependently through the afferent nerves pathway in rats. *Neurosci Lett* 2000; **293**: 107–110.

86 Bamshad M, Aoki VT, Adkison MG, Warren WS, Bartness TJ. Central nervous system origins of the sympathetic nervous

system outflow to white adipose tissue. *Am J Physiol* 1998; **275**: R291–R299.

87 Strack AM, Sawyer WB, Hughes JH, Platt KB, Loewy AD. A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. *Brain Res* 1989; **491**: 156–162.

88 Kreier F, Fliers E, Voshol PJ, Van Eden CG, Havekas LM, Kalsbeek A, Van Heijningen CL, Sluiter AA, Mettenleiter TC, Romijn JA, Sauerwein HP, Buijs RM. Selective parasympathetic innervation of subcutaneous and intra-abdominal fat—functional implications. *J Clin Invest* 2002; **110**: 1243–1250.

89 Bartness TJ. Dual intervention of white adipose tissue: some evidence for parasympathetic nervous system involvement. *J Clin Invest* 2002; **110**: 1235–1237.

90 Pond CM. The natural history of adipocytes. *Sci Prog Oxford* 1986; **70**: 45–71.

91 Pond CM. An evolutionary and functional view of mammalian adipose tissue. *Proc Nutr Soc* 1992; **51**: 367–377.

92 Frayn KN, Coppack SW, Humphreys SM, Clark ML, Evans RD. Periprandial regulation of lipid metabolism in insulin-treated diabetes mellitus. *Metabolism* 1993; **42**: 504–510.

93 Vague J. La différenciation sexuelle, facteur déterminant des formes de l'obésité. *Presse Méd* 1947; **30**: 339–340.

94 Vague J. The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout and uric calculous disease. *Am J Clin Nutr* 1956; **4**: 20–34.

95 Östman J, Arner P, Engfeldt P, Kager L. Regional differences in the control of lipolysis in human adipose tissue. *Metabolism* 1979; **28**: 1198–1205.

96 Mauriège P, Galitzky J, Berlan M, Lafontan M. Heterogeneous distribution of beta and alpha-2 adrenoceptor binding sites in human fat cells from various fat deposits: functional consequences. *Eur J Clin Invest* 1987; **17**: 156–165.

97 Engfeldt P, Arner P. Lipolysis in human adipocytes, effects of cell size, age and of regional differences. *Horm Metab Res Suppl* 1988; **19**: 26–29.

98 Van Harmelen V, Lönnqvist F, Thörne A, Wennlund A, Large V, Reynisdottir S, Arner P. Noradrenaline-induced lipolysis in isolated mesenteric, omental and subcutaneous adipocytes from obese subjects. *Int J Obes Relat Metab Disord* 1997; **21**: 972–979.

99 Arner P. Catecholamine-induced lipolysis in obesity. *Int J Obes Relat Metab Disord* 1999; **23**(Suppl 1): 10–13.

100 Jones PR, Edwards DA. Areas of fat loss in overweight young females following an 8-week period of energy intake reduction. *Ann Hum Biol* 1999; **26**: 151–162.

101 Frayn KN. Visceral fat and insulin resistance—causative or correlative? *Br J Nutr* 2000; **83**(Suppl 1): S71–S77.

102 Mårin P, Andersson B, Ottosson M, Olbe L, Chowdhury B, Kvist H, Holm G, Sjöström L, Björntorp P. The morphology and metabolism of intraabdominal adipose tissue in men. *Metabolism* 1992; **41**: 1242–1248.

103 Pedersen SB, Jønler M, Richelsen B. Characterization of regional and gender differences in glucocorticoid receptors and lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab* 1994; **78**: 1354–1359.

104 Mauriège P, Marette A, Atgie C, Bouchard C, Theriault G, Bukowiecki LK, Marceau P, Biron S, Nadeau A, Després JP. Regional variation in adipose tissue metabolism of severely obese premenopausal women. *J Lipid Res* 1995; **36**: 672–684.

105 Lefebvre A-M, Laville M, Vega N, Riou JP, van Gaal L, Auwerx J, Vidal H. Depot-specific differences in adipose tissue gene expression in lean and obese subjects. *Diabetes* 1998; **47**: 98–103.

106 Maslowska MH, Sniderman AD, MacLean LD, Cianflone K. Regional differences in triacylglycerol synthesis in adipose tissue and in cultured preadipocytes. *J Lipid Res* 1993; **34**: 219–228.

107 Martin ML, Jensen MD. Effects of body fat distribution on regional lipolysis in obesity. *J Clin Invest* 1991; **88**: 609–613.

108 Guo Z, Hensrud DD, Johnson CM, Jensen MD. Regional postprandial fatty acid metabolism in different obesity phenotypes. *Diabetes* 1999; **48**: 1586–1592.

109 Montague CT, Prins JB, Sanders L, Zhang J, Sewter CP, Digby J, Byrne CD, O'Rahilly S. Depot-related gene expression in human subcutaneous and omental adipocytes. *Diabetes* 1998; **47**: 1384–1391.

110 Van Harmelen V, Reynisdottir S, Eriksson P, Thörne A, Hoffstedt J, Lönnqvist F, Arner P. Leptin secretion from subcutaneous and visceral adipose tissue in women. *Diabetes* 1998; **47**: 913–917.

111 Siiteri PK. Adipose tissue as a source of hormones. *Am J Clin Nutr* 1987; **45**: 277–282.

112 Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse *obese* gene and its human homologue. *Nature* 1994; **372**: 425–432.

113 Coleman RA, Herrmann TS. Nutritional regulation of leptin in humans. *Diabetologia* 1999; **42**: 639–646.

114 Hilton LK, Loucks AB. Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. *Am J Physiol Endocrinol Metab* 2000; **278**: E43–E49.

115 Coppack SW, Stanner S, Rawesh A, Goodrick S, Mohamed-Ali V. *In vivo* adipose tissue leptin production before and after a high carbohydrate meal. *Int J Obesity Relat Metab Disord* 1997; **21**: S33.

116 Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998; **395**: 763–770.

117 Auwerx J, Staels B. Leptin. *Lancet* 1998; **351**: 737–742.

118 Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995; **269**: 543–546.

119 Minokoshi Y, Kim Y-B, Peroni OD, Fryer LGD, Müller C, Carling D, Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 2002; **415**: 339–343.

120 Bray GA. Obesity and reproduction. *Hum Reprod* 1997; **12**(Suppl 1): 26–32.

121 Balligand JL, Brichard SM, Brichard V, Desager JP, Lambert M. Hypoleptinemia in patients with anorexia nervosa: loss of circadian rhythm and unresponsiveness to short-term refeeding. *Eur J Endocrinol* 1998; **138**: 415–420.

122 Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guy-Grand B. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 1998; **392**: 398–401.

123 Ozata M, Ozdemir IC, Licinio J. Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. *J Clin Endocrinol Metab* 1999; **84**: 3686–3695.

124 Hileman SM, Pierroz DD, Flier JS. Leptin, nutrition, and reproduction: timing is everything. *J Clin Endocrinol Metab* 2000; **85**: 804–807.

125 Mohamed-Ali V, Pinkney JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. *Int J Obes Relat Metab Disord* 1998; **22**: 1145–1158.

126 Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppack SW. Subcutaneous adipose tissue secretes interleukin-6 but not tumor necrosis-factor-$\alpha$ *in vivo*. *J Clin Endocr Metab* 1997; **82**: 4196–4200.

127 Mohamed-Ali V, Goodrick S, Bulmer K, Holly JM, Yudkin JS, Coppack SW. Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue *in vivo*. *Am J Physiol* 1999; **277**: E971–E975.

128 Yudkin JS, Kumari M, Humphries SE, Mohamed Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 2000; **148**: 209–214.

129 Yudkin JS, Coppack SW, Bulmer K, Rawesh A, Mohamed Ali V. Lack of evidence for secretion of plasminogen activator inhibitor-1 by human subcutaneous adipose tissue *in vivo*. *Thromb Res* 1999; **96**: 1–9.

130 Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-$\alpha$ in human obesity and insulin resistance. *J Clin Invest* 1995; **95**: 2409–2415.

131 Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation* 2001; **103**: 1057–1063.

132 Kappes A, Loffler G. Influences of ionomycin, dibutyryl-cycloAMP and tumour necrosis factor-$\alpha$ on intracellular amount and secretion of apM1 in differentiating primary human preadipocytes. *Horm Metab Res* 2000; **32**: 548–554.

133 Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001; **86**: 1930–1935.

134 Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1595–1599.

135 Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001; **7**: 947–953.

136 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7**: 941–946.

137 Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999; **100**: 2473–2476.

138 Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 2001; **50**: 1126–1133.

139 Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, Igura T, Inui Y, Kihara S, Nakamura T, Yamashita S, Miyagawa J, Funahashi T, Matsuzawa Y. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. *Horm Metab Res* 2000; **32**: 47–50.

140 Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 2000; **96**: 1723–1732.

141 Kersten S, Mandard S, Tan NS, Escher P, Metzger D, Chambon P, Gonzalez FJ, Desvergne B, Wahli W. Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. *J Biol Chem* 2000; **275**: 28488–28493.

142 Yoon JC, Chickering TW, Rosen ED, Dussault B, Qin Y, Soukas A, Friedman JM, Holmes WE, Spiegelman BM. Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. *Mol Cell Biol* 2000; **20**: 5343–5349.

143 Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Rexford SA, Lazar MA. The hormone resistin links obesity to diabetes. *Nature* 2001; **409**: 307–312.

144 Way JM, Görgün CZ, Tong Q, Uysal KT, Brown KK, Harrington WW, Oliver Jr WR, Willson TM, Kliewer SA, Hotamisligil GS. Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor $\gamma$ agonists. *J Biol Chem* 2001; **276**: 25651–25653.

145 Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. *Biochem Biophys Res Commun* 2001; **285**: 561–564.

146 Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, O'Rahilly S. Resistin Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes* 2001; **50**: 2199–2202.

147 Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Resistin gene expression in human adipocytes is not related to insulin resistance. *Obes Res* 2002; **10**: 1–5.

148 McTernan CL, McTernan PG, Harte AL, Levick PL, Barnett AH, Kumar S. Resistin, central obesity, and type 2 diabetes. *Lancet* 2002; **359**: 46–47.

149 Coppack SW, Yost TJ, Fisher RM, Eckel RH, Miles JM. Periprandial systemic and regional lipase activity in normal humans. *Am J Physiol* 1996; **270**: E718–E722.

150 Karpe F, Olivecrona T, Olivecrona G, Samra JS, Summers LK, Humphreys SM, Frayn KN. Lipoprotein lipase transport in plasma: role of muscle and adipose tissues in regulation of plasma lipoprotein lipase concentrations. *J Lipid Res* 1998; **39**: 2387–2393.

151 Pond CM, Mattacks CA. Interactions between adipose tissue around lymph nodes and lymphoid cells *in vitro*. *J Lipid Res* 1995; **36**: 2219–2231.

152 Pond CM. Physiological specialisation of adipose tissue. *Prog Lipid Res* 1999; **38**: 225–248.

153 Pond CM, Mattacks CA. *In vivo* evidence for the involvement of the adipose tissue surrounding lymph nodes in immune responses. *Immunol Lett* 1998; **63**: 159–167.

154 Mattacks CA, Pond CM. Interactions of noradrenalin and tumour necrosis factor alpha, interleukin 4 and interleukin 6 in the control of lipolysis from adipocytes around lymph nodes. *Cytokine* 1999; **11**: 334–346.

155 Boulton KL, Hudson DU, Coppack SW, Frayn KN. Steroid hormone interconversions in human adipose tissue *in vivo*. *Metabolism* 1992; **41**: 556–559.

156 Katz JR, Mohamed-Ali V, Wood PJ, Yudkin JS, Coppack SW. An *in vivo* study of the cortisol–cortisone shuttle in subcutaneous abdominal adipose tissue. *Clin Endocrinol* 1999; **50**: 63–68.

157 Katz JR. *Production and interconversion of steroid hormones in obesity and polycystic ovary syndrome* [MD]. University of London, London; 1999.

158 Frühbeck G, Gomez-Ambrosi J, Muruzabal FJ, Burrell MA. The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J Physiol Endocrinol Metab* 2001; **280**: E827–E847.

159 Trayhurn P, Beattie JH. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. *Proc Nutr Soc* 2001; **60**: 329–339.

160 Sinha MK, Sturis J, Ohannesian J, Magosin S, Stephens T, Heiman ML, Polonsky KS, Caro JF. Ultradian oscillations of leptin secretion in humans. *Biochem Biophys Res Commun* 1996; **228**: 733–738.

161 Licinio J, Negrao AB, Mantzoros C, Kaklamani V, Wong ML, Bongiorno PB, Mulla A, Cearnal L, Veldhuis JD, Flier JS, McCann SM, Gold PW. Synchronicity of frequently sampled, 24-h concentrations of circulating leptin, luteinizing hormone, and estradiol in healthy women. *Proc Natl Acad Sci USA* 1998; **95**: 2541–2546.

162 Licinio J, Mantzoros C, Negrao AB, Cizza G, Wong ML, Bongiorno PB, Chrousos GP, Karp B, Allen C, Flier JS, Gold PW. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nat Med* 1997; **3**: 575–579.

163 Ahmad AM, Guzder R, Wallace AM, Thomas J, Fraser WD, Vora JP. Circadian and ultradian rhythm and leptin pulsatility in adult GH deficiency: effects of GH replacement. *J Clin Endocrinol Metab* 2001; **86**: 3499–3506.

164 Hücking K, Hamilton-Wessler M, Ellmerer M, Bergman RN. Burst-like control of lipolysis by the sympathetic nervous system *in vivo*. *J Clin Invest* 2003; **111**: 257–264.

165 Getty L, Panteleon AE, Mittelman SD, Dea MK, Bergman RN. Rapid oscillations in omental lipolysis are independent of changing insulin levels *in vivo*. *J Clin Invest* 2000; **106**: 421–430.

166 Muntzel MS, Morgan DA, Mark AL, Johnson AK. Intracerebroventricular insulin produces nonuniform regional increases in sympathetic nerve activity. *Am J Physiol* 1994; **267**: R1350–R1355.

167 Cox HS, Kaye DM, Thompson JM, Turner AG, Jennings GL, Itsiopoulos C, Esler MD. Regional sympathetic nervous activition after a large meal in humans. *Clin Sci* 1995; **89**: 145–154.

168 Samra J, Giles S, Summers L, Evans R, Arner P, Humphreys S, Clark M, Frayn K. Peripheral fat metabolism during infusion of an exogenous triacylglycerol emulsion. *Int J Obes Relat Metab Disord* 1998; **22**: 806–812.

169 Evans K, Clark ML, Frayn KN. Effects of an oral and intravenous fat load on adipose tissue and forearm lipid metabolism. *Am J Physiol* 1999; **276**: E241–E248.