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Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ

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The traditional role attributed to white adipose tissue is energy storage, fatty acids being released when fuel is required. The metabolic role of white fat is, however, complex. For example, the tissue is needed for normal glucose homeostasis and a role in inflammatory processes has been proposed. A radical change in perspective followed the discovery of leptin; this critical hormone in energy balance is produced principally by white fat, giving the tissue an endocrine function. Leptin is one of a number of proteins secreted from white adipocytes, which include angiotensinogen, adipsin, acylation-stimulating protein, adiponectin, retinol-binding protein, tumour neorosis factor α, interleukin 6, plasminogen activator inhibitor-1 and tissue factor. Some of these proteins are inflammatory cytokines, some play a role in lipid metabolism, while others are involved in vascular haemostasis or the complement system. The effects of specific proteins may be autocrine or paracrine, or the site of action may be distant from adipose tissue. The most recently described adipocyte secretory proteins are fasting-induced adipose factor, a fibrinogen–angiopoietin-related protein, metallothionein and resistin. Resistin is an adipose tissue-specific factor which is reported to induce insulin resistance, linking diabetes to obesity. Metallothionein is a metal-binding and stress-response protein which may have an antioxidant role. The key challenges in establishing the secretory functions of white fat are to identify the complement of secreted proteins, to establish the role of each secreted protein, and to assess the pathophysiological consequences of changes in adipocyte protein production with alterations in adiposity (obesity, fasting, cachexia). There is already considerable evidence of links between increased production of some adipocyte factors and the metabolic and cardiovascular complications of obesity. In essence, white adipose tissue is a major secretory and endocrine organ involved in a range of functions beyond simple fat storage.

Cytokines: Fasting-induced adipose factor: Leptin: Metallothionein: Resistin

The growing concern with obesity has led to an emphasis on the 'undesirability' of white adipose tissue (WAT). Nevertheless, the tissue plays several key roles in mammalian physiology. The classical view of the function of WAT is that it provides a long-term fuel reserve which can be mobilised during food deprivation with the release of fatty acids for oxidation in other organs. Thus, the size of the adipose tissue stores increases in periods of positive energy balance and declines when energy expenditure is in excess of intake. WAT can also provide thermal insulation, and this is particularly evident in the case of the blubber of marine mammals such as seals and whales. Additional functions recently attributed to WAT include a mechanical role (blubber in whales), a role in inflammatory processes through preadipocytes acting as macrophage-like cells (Cousin *et al*. 1999) and a role in glucose homeostasis (Fig. 1). The last of these functions is elegantly demonstrated by transgenic mice containing little WAT; such lipodystrophic animals are diabetic, exhibiting both hyperglycaemia and substantial hyperinsulinaemia (Moitra *et al*. 1998; Shimomura *et al*. 1998).

The critical change in our perspectives on WAT came with the discovery of the cytokine-like factor, leptin (Zhang *et al*. 1994). An endocrine role for WAT in the regulation of energy balance and other physiological processes (see p. 332) has been established through the identification of leptin, the hormone being secreted principally from adipocytes, with actions both centrally (particularly in the

Abbreviations: FIAF, fasting-induced adipose factor; MT, metallothionein; TGFβ, transforming growth factor β; TNF-α, tumour necrosis factor α; WAT, white adipose tissue.

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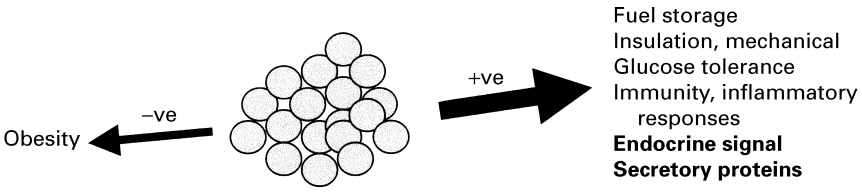

Fig. 1. Functions of white adipose tissue. A negative view of the tissue has been prevalent with the concern about obesity.

hypothalamus) and in peripheral organs (Fig. 1). Leptin is not, however, the only protein factor secreted by WAT. Indeed, there is a growing list of protein signals and factors that are released from white adipocytes (see Mohamed-Ali *et al*. 1998; Ahima & Flier, 2000; Ailhaud, 2000; Trayhurn *et al*. 2001). The implication of these findings is clear, that white fat plays a wide-ranging role in metabolic regulation and physiological homeostasis, far beyond the simple paradigm of fat storage.

The present article considers the principal protein factors secreted from WAT, including the recently-documented secretory proteins, fasting-induced adipose factor (FIAF), metallothionein (MT) and resistin.

Fatty acids and steroid secretions

Before discussing protein factors, it is emphasised that quantitatively fatty acids are the major secretory product of WAT, reflecting the role of the tissue as a fuel reserve. White fat also stores cholesterol and is involved in the metabolism of steroid hormones. The tissue does not synthesise steroid hormones *de novo* but it does express enzymes which are involved in the conversion of both glucocorticoids and sex hormones, which are subsequently released (see Mohamed-Ali *et al*. 1998). Oestrone is converted to oestradiol and androstenedione to testosterone, while androgens can be aromatised to oestrogens.

The factors responsible for fatty acid release from WAT through the stimulation of lipolysis have been the subject of considerable scrutiny. It does, however, appear that physiologically the sympathetic nervous system is the key regulator of the breakdown of triacylglycerols (Hales *et al*. 1978). Recent studies involving direct measurements of tissue noradrenaline turnover have demonstrated that there is a marked sympathetic activation in white fat in specific situations in which there is net lipolysis, i.e. fasting and cold exposure (Garofalo *et al*. 1996; Migliorini *et al*. 1997). In fasted animals this sympathetic activation is highly selective to WAT, since noradrenaline turnover falls in other tissues such as brown fat and the heart (Landsberg & Young, 1984).

Methodological considerations

There are several methodological considerations in exploring the endocrine and secretory role of adipose tissue. WAT is a heterogeneous organ, and this is the case both in terms of differences between individual depots and in the range of cells which are present within the tissue. Given the metabolic heterogeneity of the various WAT depots, it is important to establish the pattern of secretory proteins from each site and the relative importance of different sites in the synthesis of specific factors. The significance of heterogeneity is particularly evident in relation to an abdominal fat distribution, with the pathophysiological implications of such a distribution.

WAT does not consist only of adipocytes. Indeed, the tissue is composed of several cell types in addition to mature white adipocytes, with the stromal-vascular fraction which includes fibroblasts and macrophages accounting for at least half the total cell number (Hausman, 1985). The implication is that when adipose tissue is found to express a particular gene, it is important to determine whether the expression occurs within the mature adipocytes or in the other cell types that are present. The customary approach is to separate adipocytes from the stromal-vascular component by collagenase digestion and then to probe for the mRNA of interest in the two fractions. An alternative approach is to employ *in situ* hybridisation for the direct localisation of the mRNA within a cell type. Similarly, when a protein is identified in WAT, Western blotting on the proteins from the separate fractions or immunohistochemistry of the intact tissue can be used to establish whether it is localised to adipocytes.

Both *in vivo* and *in vitro* studies may be employed to investigate secretory proteins from WAT, although each clearly has its own specific objectives and limitations. *In vivo* approaches such as the collection of the venous drainage from WAT depots, or microdialysis, provide means by which the direct production of secreted proteins from the tissue can be determined. Collection of the venous drainage with the measurement of the arterio–venous difference in concentration of a protein (or other molecular species) has been fruitful in identifying net release in human studies (Frayn, 1999; see also Coppack, 2001). For *in vitro* studies, mature adipocytes may be harvested and incubated for short periods, with 'ceiling culture' providing a viable longer-term preparation (Zhang *et al*. 2000), and tissue explants have also been used. Alternatively, fibroblastic preadipocytes can be induced to differentiate into adipocytes in primary culture, or murine adipocyte clonal cell lines (e.g. 3T3-L1 or F442A) may be employed. Primary cell culture is the only option for long-term *in vitro* studies on human adipose tissue and the adipocytes of other frequently-used species such as pigs.

Leptin: the adipocyte hormone

Leptin (also termed OB protein) was discovered in 1994 by Friedman and colleagues (Zhang *et al*. 1994), with the identification of the mutant gene which underlies the development of the obesity of the *ob/ob* mouse. The

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hormone, which is widely viewed as the most important protein factor secreted by WAT, has been extensively reviewed (see Friedman & Halaas, 1998; Trayhurn *et al*. 1999; Ahima *et al*. 2000; Harris, 2000), and it is therefore our intention to summarise here only the central elements of its biology, primarily from the perspective of adipose tissue.

The leptin gene (*Lep(ob)*) encodes a protein of molecular weight 18 000 containing a signal sequence which is cleaved to produce the mature hormone of molecular weight 16 000 (Zhang *et al*. 1994). Initial studies suggested that leptin was only synthesised in WAT, but it is now recognised that the hormone is produced in several additional sites. Synthesis occurs in brown adipose tissue, the stomach, placenta, mammary gland, ovarian follicles and certain fetal organs such as the heart and bone or cartilage, and perhaps even the brain (Trayhurn *et al*. 1999, 2001). Nevertheless, WAT is the principal site of production and the major determinant of the level of the circulating hormone. This fact is evident from the correlation between plasma leptin and indices of body fatness in both human subjects and experimental animals (Considine *et al*. 1996; Ostlund *et al*. 1996). It is also implicit in the observation that transgenic mice with little or no adipose tissue have very low circulating leptin levels (Moitra *et al*. 1998; Shimomura *et al*. 1998).

Leptin production in adipose tissue

The *ob* gene is expressed in all WAT depots, but there are substantial differences in the levels of *ob* mRNA between sites, as well as differences according to developmental stage and between species. In mature rodents the levels of *ob* mRNA are highest in the gonadal and perirenal adipose tissue, and lowest in the subcutaneous depots (Trayhurn *et al*. 1995*b*). In human subjects, however, the subcutaneous tissue exhibits higher levels of *ob* mRNA than omental fat (Hube *et al*. 1996; Montague *et al*. 1997). Rodent studies indicate that there are major developmental changes; in suckling rats, in contrast to adult animals, the level of *ob* mRNA is much higher in the subcutaneous adipose tissue than in the internal fat (Rayner *et al*. 1997). Assuming that levels of mRNA reflect rates of leptin production, it would seem that in the suckling rodent subcutaneous adipose tissue is the main site of production of the hormone, but that after weaning the internal depots are more important.

There are a number of factors which acutely influence leptin synthesis in WAT, and these are superimposed on the endogenous level of production associated with the amount of body fat (see Trayhurn *et al*. 1999). Fasting leads to a rapid inhibition of *ob* gene expression in WAT, and there is a concomitant fall in the level of circulating leptin; these effects are reversed on refeeding (Becker *et al*. 1995; Trayhurn *et al*. 1995*b*; Hardie *et al*. 1996). Acute exposure to cold also leads to a suppression of *ob* gene expression and a decline in circulating leptin level (Trayhurn *et al*. 1995*a*; Hardie *et al*. 1996; Bing *et al*. 1998). Several hormones have been shown to affect leptin production by WAT. Insulin, glucocorticoids and cytokines such as tumour necrosis factor α (TNF-α) stimulate leptin production, as do oestrogens (see Trayhurn *et al*. 1999). In contrast, a major suppressive effect on leptin production occurs with catecholamines, both noradrenaline and adrenaline (Trayhurn *et al*. 1995*a*, 1999). The effect of catecholamines on leptin synthesis is mediated primarily through the β3-adrenoceptor, the dominant β-receptor subtype in rodent adipose tissue (Giacobino, 1996), since selective β3-agonists strongly suppress *ob* gene expression and reduce circulating leptin levels (Gettys *et al*. 1996; Mantzoros *et al*. 1996; Trayhurn *et al*. 1996).

It is proposed that the sympathetic nervous system is the main physiological regulator of leptin production and that it provides a negative feedback loop to adipose tissue in the production of the hormone (Trayhurn *et al*. 1998). Evidence for this proposition comes in part from the effects of blocking noradrenaline production with α-methyl-*p*-tyrosine, which leads to the rapid induction of hyperleptinaemia with increased levels of *ob* mRNA in WAT (Rayner *et al*. 1998). Similarly, the administration of β-adrenoceptor antagonists, both the general antagonist propranolol and the selective β3-antagonist SR 59230A, inhibits the fall in circulating leptin level occurring with cold exposure and on fasting (Evans *et al*. 1999; DV Rayner and P Trayhurn, unpublished results). This finding suggests that the reduction in leptin production with these stimuli is mediated primarily through sympathetic activation. The regulatory role of the sympathetic system is, however, complex (Sivitz *et al*. 1999).

The view that the sympathetic nervous system is an important regulator of leptin production in WAT reflects the current recognition that there is a significant sympathetic innervation of the tissue (Bartness & Bamshad, 1998), there being direct nerve endings adjacent to adipocytes. Mapping studies with retrovirus techniques have indicated that part of the sympathetic innervation of WAT arises in hypothalamic areas associated with the regulation of energy balance (Bartness & Bamshad, 1998). The sympathetic system is considered to be the main physiological mediator of lipolysis in WAT, as indicated earlier, and it also plays a trophic role in the tissue (Bartness & Bamshad, 1998; Youngström & Bartness, 1998).

The interaction between the sympathetic system and leptin is two-way, the hormone stimulating sympathetic activity in WAT and other tissues through its hypothalamic receptors (Haynes *et al*. 1997), while afferent signals from leptin sensors in white fat exert a reflex effect (Niijima, 1998). There is, therefore, communication from adipose tissue to the nervous system as well as in the opposite direction.

Functions of leptin

One or more isoforms of the leptin receptor are found in most tissues (Hoggard *et al*. 1997), including WAT, suggesting that the hormone may have an autocrine or paracrine function in adipose tissue. The leptin receptor long form (OB-Rb) splice variant, which is the key signalling form of the receptor (Lee *et al*. 1996), is found particularly in regions of the hypothalamus such as the arcuate nucleus and paraventricular nucleus (Mercer *et al*. 1996; Guan *et al*. 1997; Håkansson *et al*. 1998). Thus, parts of the brain associated with the central control of energy balance are a major target for leptin.

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Leptin interacts with several central neuroendocrine systems, including neuropeptide Y (Stephens *et al*. 1995; Ahima *et al*. 2000), leading to the inhibition of food intake (Campfield *et al*. 1995; Halaas *et al*. 1995; Pelleymounter *et al*. 1995). The functions attributed to the hormone, however, are extensive. Leptin affects energy expenditure, and acts as a major signal to the reproductive system (particularly in relation to sexual maturation in females) and as a factor in angiogenesis and in the immune system (Stehling *et al*. 1997; Bouloumie *et al*. 1998; Lord *et al*. 1998; Sierra-Honigmann *et al*. 1998). In addition to influencing these general physiological systems, leptin has been reported to affect a diverse spectrum of metabolic processes, ranging from the inhibition of insulin secretion by pancreatic β-cells to the stimulation of sugar transport and platelet aggregation (Emilsson *et al*. 1997; Lostao *et al*. 1998; Nakata *et al*. 1999).

In view of the many effects attributed to leptin the question has arisen as to whether there is a unifying function of the hormone, and two such ideas have been advanced. The first suggests that leptin is primarily a starvation signal (Ahima *et al*. 1996), the evidence for which includes the observation that several of the neuroendocrine and metabolic changes associated with fasting, as well as the immunosuppressive effects of starvation (Lord *et al*. 1998), can be reversed in the fasted animal by administering leptin. Another proposal suggests that a core role of leptin is to channel fatty acids into adipose tissue and limit triacylglycerol deposition in other tissues (Unger *et al*. 1999). This concept is based on the view that the function and viability of non-adipocytes can be compromised when the triacylglycerol content extends beyond the 'physiological' range.

Other proteins secreted from adipose tissue

A wide range of protein factors are secreted from WAT, in addition to leptin, and these are summarised in Fig. 2. They include other cytokines as well as proteins directly involved in lipid metabolism, in the complement system and in vascular haemostasis. The role of most of the diverse proteins secreted from WAT is not established, in terms of the physiological significance of production by adipose tissue. In principle, adipocyte factors could play either an autocrine or paracrine role within WAT, or have a broader endocrine function (or a combination thereof).

To date, because obesity has been a key focus of studies on adipose tissue, emphasis has been placed on the pathological significance of changes in the production of different secreted factors in the face of a greatly expanded adipose mass. However, it is also important to consider the implications on protein secretion of major reductions in the amount of WAT, for example as in chronic fasting, malnutrition and cancer cachexia. A key challenge is to provide a rationale in functional terms as to why particular factors are secreted from WAT, especially when they are but one element in a process involving a number of different proteins (as in vascular haemostasis).

Proteins of lipid and lipoprotein metabolism

Several proteins which play an important role in lipid and lipoprotein metabolism are released from white adipocytes. The enzyme lipoprotein lipase was in effect the earliest recognised protein secretory product of adipocytes. Lipoprotein lipase is responsible for the breakdown of circulating triacylglycerols, in the form of chylomicrons and VLDL, to fatty acids. A number of factors which regulate the expression of the lipoprotein lipase gene and the level and activity of the protein have been identified, with insulin playing an important role (Enerback & Gimble, 1993). Other secreted proteins from WAT directly involved in lipid and lipoprotein metabolism include cholesteryl ester transfer protein and apolipoprotein E. Cholesteryl ester transfer protein plays an important role in the accumulation of cholesteryl ester by adipose tissue (see Mohamed-Ali *et al*. 1998; Radeau *et al*. 1998*a,b*). Indeed, WAT is a substantial site of the synthesis of cholesteryl ester transfer protein (Radeau *et al*. 1998*a*).

Retinol is stored in WAT, and the gene encoding the plasma retinol-binding protein is strongly expressed in adipocytes (Makover *et al*. 1989; Tsutsumi *et al*. 1992; Zovich *et al*. 1992). Retinol-binding protein mRNA is reported in practice to be one of the most abundant transcripts in rodent and human adipose tissue (Makover *et al*. 1989; Montague *et al*. 1998). Cell culture studies indicate that plasma retinol-binding protein is secreted from white

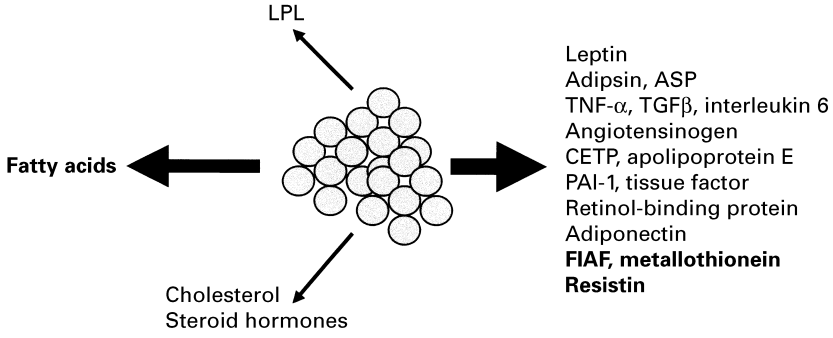

Fig. 2. Proteins factors secreted from white adipose tissue. ASP, acylation-stimulating protein; CETP, cholesteryl ester transfer protein; FIAF, fasting-induced adipose factor; LPL, lipoprotein lipase; PAI-1, plasminogen activator inhibitor-1; TGFβ, transforming growth factor β; TNF-α, tumour necrosis factor α.

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adipocytes, and WAT presumably contributes to the total circulating pool (Tsutsumi *et al*. 1992; Zovich *et al*. 1992). Quantitatively, the liver and kidney have been regarded as the main sites of plasma retinol-binding protein production (Blomhoff *et al*. 1990), and the physiological significance of synthesis in adipose tissue is unclear.

Angiotensinogen

White adipocytes appear to be an important source of angiotensinogen, the substrate for renin in the renin–angiotensin system which plays a central role in blood pressure regulation. Indeed, WAT may be second only to the liver with respect to the production of angiotensinogen (Safonova *et al*. 1997). The activation product of angiotensinogen, angiotensin II, stimulates the production and release of prostacyclin which acts as a signal in the differentiation of preadipocytes to adipocytes (Zorad *et al*. 1995; Ailhaud *et al*. 2000). The circulating level of angiotensinogen is raised in obesity and this is thought to reflect the rise in adipose tissue mass. Thus, hypertension in the obese may result from the increased secretion of angiotensinogen (Engeli *et al*. 2000).

Adipose tissue expresses the genes encoding angiotensin converting enzyme and type 1 angiotensin receptor, in addition to angiotensinogen itself (Harp & DiGirolamo, 1995; Karlsson *et al*. 1998; Engeli *et al*. 1999). This finding suggests that a local renin–angiotensin system is present in WAT (Ailhaud *et al*. 2000).

Plasminogen activator inhibitor-1 and tissue factor

At least two proteins involved in the fibrinolytic system and vascular haemostasis are secreted by WAT, i.e. tissue factor and plasminogen activator inhibitor-1. Tissue factor is the key cellular initiator of the coagulation cascade and acts as a cell-surface receptor for the activation of factor VII. Expression of the gene encoding tissue factor is higher in WAT of *ob/ob* mice than in normal animals (Samad *et al*. 1998).

Plasminogen activator inhibitor-1, as its name implies, inhibits the activation of plasminogen, the precursor of plasmin which breaks down fibrin (see Booth, 2001). The plasminogen activator inhibitor-1 gene has been shown to be expressed and the encoded protein released in both human and rodent WAT (Lundgren *et al*. 1996; Eriksson *et al*. 1998; Cigolini *et al*. 1999). A number of factors stimulate plasminogen activator inhibitor-1 gene expression and the production of the protein by adipose tissue, including transforming growth factor β (TGFβ) and TNF-α which are themselves produced by adipose tissue (Samad *et al*. 1997; Sakamoto *et al*. 1999; Birgel *et al*. 2000).

The incidence of cardiovascular disease is increased in obesity, and this has been linked to the rise in the circulating level of plasminogen activator inhibitor-1 (Juhan-Vague & Alessi, 1997); indeed, there is a correlation between plasminogen activator inhibitor-1 levels in plasma and BMI (Alessi *et al*. 1997). WAT appears to be a quantitatively significant site of plasminogen activator inhibitor-1 production, and the tissue may well be the source of the elevated levels in obesity (Lundgren *et al*. 1996; Samad *et al*. 1996; Samad & Loskutoff, 1997; Alessi *et al*. 2000), although definitive evidence for this proposition is lacking.

Cytokines and growth factors

Several important 'classical' cytokines and growth factors are synthesised in WAT, particularly TNF-α, interleukin 6 and TGFβ (see Mohamed-Ali *et al*. 1998; Coppack, 2001). TNF-α production is increased in obesity, and the cytokine has been implicated in the development of insulin resistance in the adipocyte of the obese by altering insulin signalling through an autocrine or paracrine action (Hotamisligil *et al*. 1993; Hotamisligil, 2000). The cytokine may also efflux from adipose tissue to contribute to the circulating level. The TGFβ gene is expressed in white fat and the level of both the mRNA and the protein are increased in genetically-obese rodents (*ob/ob* and *db/db*) compared with their lean counterparts (Samad *et al*. 1997). TGFβ is released from adipocytes and TNF-α stimulates expression of the gene and production of the protein (Samad *et al*. 1997). Interleukin 6 has recently been shown to be synthesised in WAT, both the mRNA and the protein being identified in the tissue, with increased levels of production in obesity (Mohamed-Ali *et al*. 1998, 1999; Bastard *et al*. 2000).

Given the multiplicity of effects now attributed to TNF-α and TGFβ, including a role in the regulation of the synthesis of other adipose tissue-derived factors, these cytokines may play an important integrative function in WAT (Samad *et al*. 1999; Hotamisligil, 2000; Sethi & Hotamisligil, 1999). Interaction is evident, with TNF-α stimulating the synthesis of TGFβ, which in turn leads to an increase in the production of plasminogen-activator inhibitor-1, as noted earlier.

Adipsin and acylation-stimulating protein

Adipsin was the first major protein secreted from white fat to be identified (Cook *et al*. 1985), after lipoprotein lipase. It was discovered as a factor expressed in a differentiation-dependent manner in adipocyte cell lines (Cook *et al*. 1985). An early observation was that expression of the adipsin gene is greatly decreased in animal models of obesity, with reduced levels of the circulating protein, leading to the initial view that it might be a lipostatic signal (Cook *et al*. 1987; Flier *et al*. 1987). However, adipsin, which is a serine protease and part of the alternative complement pathway (complement factor D), is not reduced in human obesity and is no longer regarded as a signalling molecule in energy balance.

Another protein of the alternative complement system is synthesised by WAT, i.e. acylation-stimulating protein, or C3ades-Arg, which is derived from the C3 complex through the action of adipsin, factor B and a carboxypeptidase. Several roles in lipid metabolism have been proposed for acylation-stimulating protein (Cianflone *et al*. 1999). Studies on transgenic mice lacking the protein through a deficiency of C3 support the hypothesis that acylation-stimulating protein is important in the postprandial clearance of triacylglycerols (Murray *et al*. 1999*a,b*). The protein stimulates the uptake of fatty acids into white adipocytes and their esterification (Cianflone *et al*. 1999).

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Adiponectin

Adiponectin was originally identified from a cDNA, adipose most-abundant gene transcript 1, reflecting a gene which is abundantly and specifically expressed in adipose tissue. The protein has homology with collagen VIII and collagen X, as well as with complement factor C1q (Maeda *et al*. 1997). It is suggested that adiponectin may modulate endothelial adhesion molecules and inhibit inflammatory responses, and there are also proposals that the protein is involved in the link between atherosclerosis and obesity (Ouchi *et al*. 1999; Yokota *et al*. 2000). In contrast to many proteins secreted by adipose tissue, expression of the adiponectin gene and the circulating level of the protein fall in obesity (Arita *et al*. 1999) and in diabetes (Hotta *et al*. 2000).

Novel adipocyte-secreted proteins

It is widely thought that there are likely to be a number of other proteins secreted from WAT additional to those identified to date. The discovery of new secreted proteins will come from the application of several experimental approaches, including microarrays to identify the range of genes expressed, specific differential expression studies, and proteomics to determine the protein complement of adipose tissue.

The three most recently described (late 2000 to early 2001) secretory proteins from WAT are FIAF, MT and resistin. The discovery of FIAF came from a subtractive hybridisation study (Kersten *et al*. 2000), while resistin was found in a screen for genes whose expression is induced during adipocyte differentiation but which are down regulated in mature fat cells in response to thiazolidinediones (Steppan *et al*. 2001*a*). In contrast, MT was identified following investigation of whether the MT gene is expressed in white fat, as it is in brown adipose tissue (Trayhurn *et al*. 2000*a*).

Fasting-induced adipose factor

The synthesis of FIAF, as implied by its name, is increased in response to fasting, and the factor itself belongs to the family of fibrinogen–angiopoietin-like proteins (Kersten *et al*. 2000). The gene encoding FIAF is predominantly expressed in WAT, although strong expression also occurs in brown fat. It is a target gene of the peroxisome proliferator-activated receptor α transcription factor; indeed, FIAF was discovered in a study aimed at identifying unknown target genes for peroxisome proliferator-activated receptor α. The protein is present in plasma, where the concentration is increased on fasting but decreased on feeding a high-fat diet (Kersten *et al*. 2000). It has been speculated that FIAF has an endocrine role and may be a signalling molecule which operates reciprocally to leptin.

Resistin

Resistin is a small, and potentially highly important, protein (molecular weight <10 000) which is secreted from adipocytes, with the factor being present in serum (Steppan *et al*. 2001*a*). The initial report suggests that resistin is produced only by adipocytes, based on the tissue pattern of gene expression, with minimal expression in brown fat as compared with WAT. Expression of the resistin gene is reduced on fasting, with a parallel fall in the circulating level of the protein. In contrast, both gene expression and the plasma resistin level are increased in obese animals. Resistin induces insulin resistance; administration of the recombinant protein impairing glucose tolerance and insulin action (Steppan *et al*. 2001*a*). Correspondingly, treatment with antibodies to resistin improves glycaemia and reverses insulin resistance.

It is proposed that resistin is a key adipocyte signal in the induction of insulin resistance, and as such provides a molecular link between diabetes and obesity (Steppan *et al*. 2001*a*). The significance of this new factor could well prove to mirror that of leptin.

Metallothionein

MT, a low-molecular-weight (6000) stress-response and metal-binding protein, has long been recognised to be synthesised in several tissues, particularly the liver and kidney (Bremner & Beattie, 1990). Among the tissues in which the MT gene is expressed is brown fat, where it is suggested that the protein may play an important antioxidant role (Beattie *et al*. 1996, 2000). In very recent studies we have demonstrated that both the MT-1 and MT-2 genes are also expressed in WAT, the mRNA being present in each of the main depots, with no substantial site differences (Trayhurn *et al*. 2000*a*). The MT genes are expressed in the adipocytes themselves (Fig. 3) rather than in the cells of the stromal-vascular fraction (Trayhurn *et al*. 2000*a*).

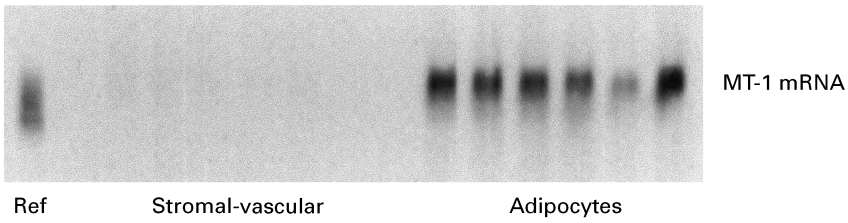

Fig. 3. Metallothionein (MT)-1 gene expression in white adipocytes. Mouse white adipose tissue was digested with collagenase and mature adipocytes separated from cells of the stromal-vascular fraction by centrifugation. Total RNA was extracted and Northern blots probed for MT-1 mRNA using a 28-mer antisense oligonucleotide (end labelled with digoxigenin) with chemiluminescence-based detection. Ref, reference white adipose tissue.

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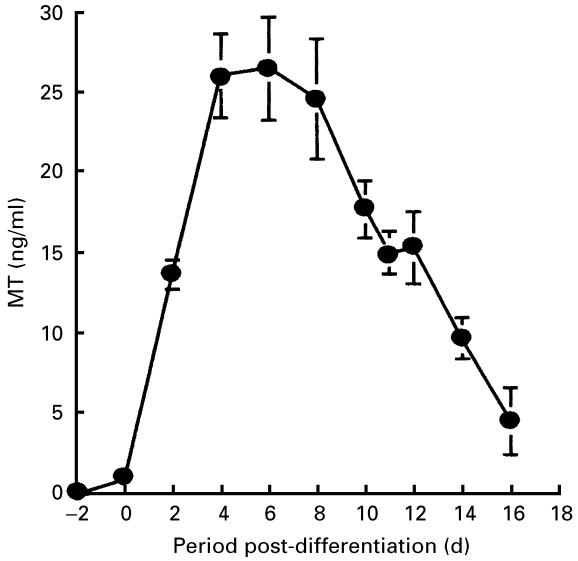

Fig. 4. Release of metallothionein (MT) into the medium on the differentiation of rat preadipocytes to adipocytes in primary culture. Fibroblastic preadipocytes from 2-week-old rats were induced to differentiate, the culture medium changed every 2d and MT measured by radioimmunoassay. Values are means with their standard errors represented by vertical bars for six samples per group. (Adapted from Trayhurn *et al*. 2000*a*.)

In vivo studies have indicated that the level of MT-1 mRNA in WAT is unaltered by fasting, by the injection of noradrenaline, or even on administration of Zn, which is a powerful inducer of MT production in the liver and kidney. However, injection of a β3-agonist induced a modest increase in MT-1 mRNA level, suggesting that expression of the MT gene can be subject to adrenergic activation, given a sufficiently potent stimulus (Trayhurn *et al*. 2000*a*). In *in vitro* studies the differentiation of fibroblastic preadipocytes to adipocytes in primary culture resulted in a high level of MT mRNA and the release of MT protein into the medium (Fig. 4). This release occurred before the secretion of leptin and was not a reflection of the general leakage of cell contents (Trayhurn *et al*. 2000*b*). Both gene expression and the release of MT protein into the medium were stimulated by the glucocorticoid dexamethasone, and by forskolin and bromo-cAMP, agents which increase or mimic cAMP (Trayhurn *et al*. 2000*b*).

These observations indicate that MT is a previously unrecognised secretion product of white adipocytes, notwithstanding the lack of a signal sequence in the protein. A central question is the physiological role of MT both within adipocytes and as a secretory protein. We have suggested that the main function of MT in WAT may be as an antioxidant protecting fatty acids from oxidative damage (Trayhurn *et al*. 2000*a*), a proposition reflecting one of the general roles postulated for the protein (Miles *et al*. 2000). Certainly, there is a rapid induction of MT gene expression in brown adipocytes on acute exposure of rodents to the cold, consistent with a role for the protein in countering free radical damage during the high rates of O_2 utilisation required for non-shivering thermogenesis (Beattie *et al*. 1996). In the case of white adipocytes, a housekeeping function is envisaged both within adipocytes and in adipose tissue during the transport of fatty acids. An alternative hypothesis for secreted MT is a signalling function, and there is some initial evidence for such a possibility (El Refaey *et al*. 1997).

Coda

There are several key challenges in the continuing investigation of the secretory functions of WAT: (1) to identify the full constellation of proteins secreted from adipocytes; (2) to determine the physiological role of each secreted protein; (3) to assess the pathophysiological consequences of changes in adipocyte protein production in response to major changes in adipose tissue mass; (4) to determine how the production of diverse proteins in different depots is coordinated. The pathophysiological implications of substantial changes in adipose tissue mass on the production and secretion of adipose tissue-derived factors are clearly important, and there is much evidence of links with the cardiovascular and metabolic complications of obesity. Although obesity has been the main focus to date, there is the possibility that changes in protein secretion associated with reductions in adipose tissue mass are also of significance. Thus, in conditions such as cancer cachexia, malnutrition and anorexia and during prolonged fasting there may be substantial alterations in the production of most of the proteins secreted from adipocytes (and this has been documented in some cases, such as leptin) with important metabolic and regulatory implications.

It is not easy to put forward a rational framework for why such a range of factors are secreted by white adipocytes, given the evident diversity of the proteins concerned. However, one hypothesis would be that the various factors may relate in some manner to the central lipid storage (and release) function of the tissue. It is now evident that WAT is a secretory and endocrine organ of considerable complexity which is highly integrated into the overall physiological and metabolic control systems of mammals, and much more so than the simple storage of fuel would imply. We now recognise there to be a distinct two-way communication between white adipocytes and the brain, through leptin and the sympathetic nervous system, with leptin stimulating sympathetic activity and the sympathetic system regulating the production of leptin (and other secretions). In essence, the white adipocyte can be seen to be a very ‘smart’ cell.

Note added in proof

A new report has indicated that there is a family of resistin-like molecules (REML), one of which, REMLα, is also expressed in WAT (Steppan *et al*. 2001*b*). This family is the same as the recently described FIZZ gene family of cysteine-rich secreted proteins (Holcomb *et al*. 2000), with FIZZ1 equating to REMLα and FIZZ3 to resistin.

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