

Horizons in Nutritional Science

Adipokines: inflammation and the pleiotropic role of white adipose tissue

Paul Trayhurn* and I. Stuart Wood

Neuroendocrine and Obesity Biology Unit, Liverpool Centre for Nutritional Genomics, School of Clinical Sciences, University of Liverpool, Daulby Street, Liverpool L69 3GA, UK

(Accepted 4 May 2004)

White adipose tissue is now recognised to be a multifunctional organ; in addition to the central role of lipid storage, it has a major endocrine function secreting several hormones, notably leptin and adiponectin, and a diverse range of other protein factors. These various protein signals have been given the collective name 'adipocytokines' or 'adipokines'. However, since most are neither 'cytokines' nor 'cytokine-like', it is recommended that the term 'adipokine' be universally adopted to describe a protein that is secreted from (and synthesised by) adipocytes. It is suggested that the term is restricted to proteins secreted from adipocytes, excluding signals released only by the other cell types (such as macrophages) in adipose tissue. The *adipokinome* (which together with lipid moieties released, such as fatty acids and prostaglandins, constitute the *secretome* of fat cells) includes proteins involved in lipid metabolism, insulin sensitivity, the alternative complement system, vascular haemostasis, blood pressure regulation and angiogenesis, as well as the regulation of energy balance. In addition, there is a growing list of adipokines involved in inflammation (TNF α , IL-1 β , IL-6, IL-8, IL-10, transforming growth factor- β , nerve growth factor) and the acute-phase response (plasminogen activator inhibitor-1, haptoglobin, serum amyloid A). Production of these proteins by adipose tissue is increased in obesity, and raised circulating levels of several acute-phase proteins and inflammatory cytokines has led to the view that the obese are characterised by a state of chronic low-grade inflammation, and that this links causally to insulin resistance and the metabolic syndrome. It is, however, unclear as to the extent to which adipose tissue contributes quantitatively to the elevated circulating levels of these factors in obesity and whether there is a generalised or local state of inflammation. The parsimonious view is that the increased production of inflammatory cytokines and acute-phase proteins by adipose tissue in obesity relates primarily to localised events within the expanding fat depots. It is suggested that these events reflect hypoxia in parts of the growing adipose tissue mass in advance of angiogenesis, and involve the key controller of the cellular response to hypoxia, the transcription factor hypoxia inducible factor-1.

White adipose tissue: Adipokines: Inflammation: Obesity: Hypoxia: Cytokines: Acute phase proteins

White adipose tissue (WAT) is the main site of energy storage in mammals and birds, substrate being deposited as triacylglycerols at a high energy density. Until the last decade energy storage was seen as essentially the only role of white fat, apart from providing thermal and mechanical insulation. A revolution has occurred recently, however, in our understanding of the biological function of WAT; the tissue is now seen as a highly dynamic organ, being involved in a wide range of physiological and metabolic processes far beyond the paradigm of fuel storage. This changed perspective has occurred through the recognition that WAT is an endocrine organ; white adipocytes secrete several major hormones, most notably leptin and adiponectin, together with a diverse range of other protein signals and factors. This is in addition to the adipocytes' central role in the deposition and release of fatty acids

(Mohamed-Ali *et al.* 1998; Frühbeck *et al.* 2001; Trayhurn & Beattie, 2001).

In the present article we consider one of the key recent developments in the function of white fat, i.e. inflammation and the role of the tissue as a source of proteins characteristic of the inflammatory response. Specific aspects of this area have also been addressed in other recent reviews (Coppack, 2001; Frühbeck *et al.* 2001; Rajala & Scherer, 2003; Klaus, 2004). We consider first some important issues of definition and nomenclature which we believe require clarification and agreement.

Definitions: 'adipokines' not 'adipocytokines'?

As the number of protein signals recognised to be secreted from adipose tissue rapidly increased it became helpful to

Abbreviations: CRP, C-reactive protein; HIF-1, hypoxia-inducible factor-1; NGF, nerve growth factor; PAI-1, plasminogen activator inhibitor-1; SAA, serum amyloid A; VEGF, vascular endothelial growth factor; WAT, white adipose tissue.

* **Corresponding author:** Professor Paul Trayhurn, fax +44 151 706 5802, email p.trayhurn@liverpool.ac.uk

accord them a collective name. The term initially introduced was 'adipocytokines' (Funahashi *et al.* 1999), and this has been used extensively. Although the name has merit, it is potentially misleading since there is an inference that the adipocyte-secreted proteins are cytokines, or cytokine-like. While this is the case for some, such as TNF α and IL-6, it is clearly not so with the majority. The alternative name coined is 'adipokines', and this is rather more satisfactory since it does not imply that the proteins belong to a particular functional group. *We therefore recommend that the term 'adipokine' be universally adopted to describe a protein that is secreted from (and synthesised by) adipocytes.*

Secretion is the critical characteristic of an adipokine, and we emphasise this since the term has also been used in connection with other adipocyte proteins such as adiponutrin (Wiesner *et al.* 2004), which is a transmembrane protein rather than a secretory product. 'Adipokine' has also been employed to describe a protein that is secreted by adipose tissue, rather than by adipocytes. However, it is preferable to restrict the term to those proteins that are released by adipocytes themselves. The principal reason for this is that cells such as macrophages which also secrete protein signals are found in a number of organs, as well as being present in adipose tissue (Weisberg *et al.* 2003; Xu *et al.* 2003). There is, therefore, a lack of specificity in giving the secreted proteins a special designation when such cells happen to be within fat depots, even though their presence may well be of considerable functional importance.

There is also a question of whether the term adipokine should be restricted to proteins released from white adipocytes, or include those secreted from *brown* adipocytes. White and brown adipocytes are functionally different, of course, with the latter being specialised for the production of heat through the presence of the mitochondrial uncoupling protein, UCP-1. In practice, no proteins appear to be secreted from brown adipocytes which are not also released by white fat cells, so in effect this may not be an issue.

Given the current focus on 'omic' approaches in biology (genome, transcriptome, proteome, metabolome/metabonome), the totality of secreted proteins may be described as constituting the *adipokinome*. Proteins are, however, clearly not the only class of molecule secreted from adipocytes. In addition to fatty acids, which quantitatively are much the largest secretory product, there are other lipid substances, including cholesterol, steroid hormones, prostaglandins and prostanoids, and retinol (neither retinol nor cholesterol are actually synthesised within adipocytes, but are stored and released). The lipid substances and adipokinome together can be said to constitute the '*secretome*' of the adipocyte.

Identification of an adipokine

The identification of a protein as an adipokine requires that secretion from adipocytes be demonstrated. In practice, this will generally reflect selective release from adipocytes *in vitro*. This may be either from freshly isolated adipocytes, or adipocytes derived by differentiation from fibroblastic

pre-adipocytes – whether primary culture or from clonal cell lines. Alternative approaches, which are more challenging, include detection of the protein in the venous drainage from WAT at a concentration higher than in arterial blood, or following microdialysis.

The initial stage in the identification of a candidate adipokine is frequently detection of the expression of a gene in adipose tissue, or in adipocytes differentiated in culture. Such a gene may encode either: (i) a product recognised to be secreted from other tissues (for example, IL-6), or (ii) reflect a protein found in the circulation, or (iii) if a novel gene, the derived protein should contain a signal sequence. When expression is first identified in adipose tissue itself, it is essential to determine whether that expression occurs within mature adipocytes or in the other cells that constitute the tissue, either histologically (*in situ* hybridisation) or by separation of the adipocytes from the stromal vascular fraction by collagenase digestion. Equally, expression in an adipocyte clonal line needs to be verified for the native tissue. Gene expression must, of course, be followed by detection of the encoded protein in adipocytes.

When a protein which is present in the circulation, or recognised to be secreted from other tissues, is synthesised in adipocytes there is an *a priori* case for it being considered an adipokine. Nevertheless, secretion from the adipocyte needs to be directly demonstrated before such a protein should be accepted as a genuine adipokine.

Adipokines: a diverse group of proteins

The total number of adipokines, both documented and putative, is now well over fifty; the main functional categories are summarised in Fig. 1. The earliest to be identified was in practice the enzyme lipoprotein lipase, responsible for the hydrolysis of circulating triacylglycerols to NEFA; this was followed in the mid 1980s by adipsin, a serine protease and part of the alternative complement pathway (Cook *et al.* 1985, 1987). The key development which led to the current focus on adipose tissue as a major site of the secretion of protein signals was, however, the discovery in 1994 of leptin (Zhang *et al.* 1994). Leptin, which is a 16 000 molecular-weight cytokine-like hormone with a wide range of biological functions, established adipocytes as endocrine cells.

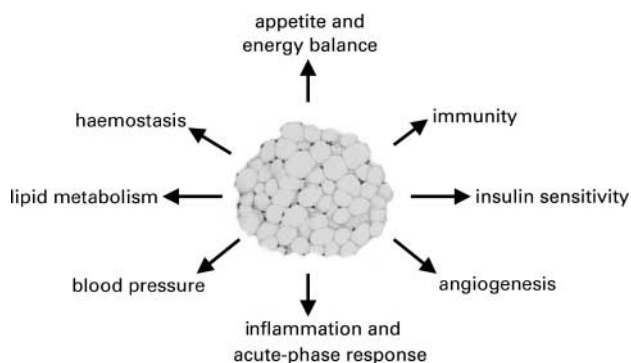


Fig. 1. Adipokines classified by functional role.

The diversity of the adipokines (Fig. 2), both in terms of protein structure and of putative function, is considerable (Frühbeck *et al.* 2001; Trayhurn & Beattie, 2001). The group includes: classical cytokines (e.g., TNF α , IL-6, IL-8), growth factors (e.g., transforming growth factor- β ; TGF- β) and proteins of the alternative complement system (e.g., adipsin, acylation-stimulating protein). The group also includes: proteins involved in vascular haemostasis (e.g., plasminogen activator inhibitor-1 (PAI-1), tissue factor), the regulation of blood pressure (angiotensinogen), lipid metabolism (e.g., retinol-binding protein, cholesteryl ester transfer protein), glucose homeostasis (e.g., adiponectin, possibly resistin) and angiogenesis (e.g., vascular endothelial growth factor; VEGF), as well as acute-phase and stress responses (e.g., haptoglobin, metallothionein).

From the wide range of protein signals and factors already identified, it is 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. It is not easy to put forward a coherent framework for why such a diversity of factors is secreted by white adipocytes. However, one hypothesis would be that the various factors may relate ultimately to the central lipid storage and release function of the tissue (Trayhurn & Beattie, 2001). A corollary to the secretion of such a wide range of adipokines is that WAT has an extensive system for communication with other tissues and organs. Co-culture studies have indicated, for example, that adipocytes directly signal to other tissues such as skeletal muscle and the adrenal cortex (Dietze *et al.* 2002; Ehrhart-Bornstein *et al.* 2003). There is also, in particular, a distinct cross-talk between white adipocytes and the brain through leptin and the sympathetic nervous system (Rayner & Trayhurn, 2001).

Inflammation and obesity

An important recent development in our understanding of obesity is the emergence of the concept that it (and diabetes) is characterised by a state of chronic low-grade inflammation (Yudkin *et al.* 1999; Das, 2001; Festa *et al.* 2001; Engström *et al.* 2003). The basis for this view is that increased circulating levels of several markers of inflammation, both pro-inflammatory cytokines and acute-phase proteins, are elevated in the obese; these markers include IL-6, the TNF α system, C-reactive protein (CRP) and haptoglobin (Das, 2001; Bulló *et al.* 2003). The implications in terms of the site of inflammation itself, whether systemic or local, are unclear. Nevertheless, it is increasingly evident that the inflammatory state may be causal in the development of insulin resistance and the other disorders associated with obesity, such as hyperlipidaemia and the metabolic syndrome (Hotamisligil, 2003; Yudkin, 2003). While the general assumption is that inflammation is consequent to obesity, it has been suggested that obesity is in fact a result of inflammatory disease (Das, 2001).

A central question is the origin of the inflammatory markers in obesity, and there are three possibilities. The first is that it reflects production and release from organs other

than adipose tissue, primarily the liver (and immune cells). The second explanation is that WAT is secreting factors that stimulate the production of inflammatory markers from the liver and other organs; this may well be the case with CRP, where it is argued that hepatic production is stimulated by increased IL-6 from the expanded fat mass of the obese (Yudkin *et al.* 2000; Yudkin, 2003). The third possibility is that adipocytes themselves are the immediate source of some, or most, of these inflammatory markers, raised circulating levels in obesity reflecting production from the increased WAT mass. There is also, of course, the possibility of there being a combination of production in adipose tissue and other organs.

From the perspective of adipose tissue biology, a key question is whether adipocytes (or adipose tissue) directly contribute to the raised circulating levels of specific inflammatory markers and, if so, to what extent? Although obtaining quantitative information on the contribution from particular cells within adipose tissue is difficult, the issue that can be readily addressed is whether adipocytes express certain inflammatory genes and their encoded proteins secreted. Recent reports demonstrating that WAT is infiltrated by macrophages in obesity clearly suggest that the non-adipocyte fraction may be a significant component of the inflammatory state within adipose tissue (Weisberg *et al.* 2003; Xu *et al.* 2003).

Cytokines

Tumour necrosis factor- α and interleukin-6

Several inflammatory cytokines are now recognised to be expressed in, and secreted by, white adipocytes, the first to be identified being TNF α (Hotamisligil *et al.* 1993). TNF α expression in WAT was initially demonstrated in rodents, and found to be markedly increased in obese models (Hotamisligil *et al.* 1993). From this it was proposed that TNF α is linked to the development of insulin resistance. The cytokine has been extensively examined in relation to insulin action, and multiple effects have been described, including the inhibition of the insulin receptor signalling pathway (Coppack, 2001; Hotamisligil, 2003). In man, the secretion of TNF α is reported to be mainly due to the cells of the stromal vascular and matrix fractions, including the macrophages, despite the fact that previously most of the mRNA for TNF α was thought to be found within the adipocytes themselves (Weisberg *et al.* 2003; Fain *et al.* 2004a). An apparent disparity between mRNA and protein secretion in human WAT is evident for several adipokines in recent reports by Fain *et al.* (2004a,b) and requires further examination.

TNF α is a powerful local regulator within adipose tissue, acting in both an autocrine and a paracrine manner to influence a range of processes, including apoptosis (Prins *et al.* 1997; Coppack, 2001). There appears to be a hierarchy of cytokines within WAT, with TNF α playing a pivotal role in relation to the production of several cytokines and other adipokines (Coppack, 2001). Thus, for example, TNF α is a key regulator of the synthesis of IL-6, of the acute-phase protein, haptoglobin (Chiellini *et al.* 2002; Oller do Nascimento *et al.* 2004), and of the

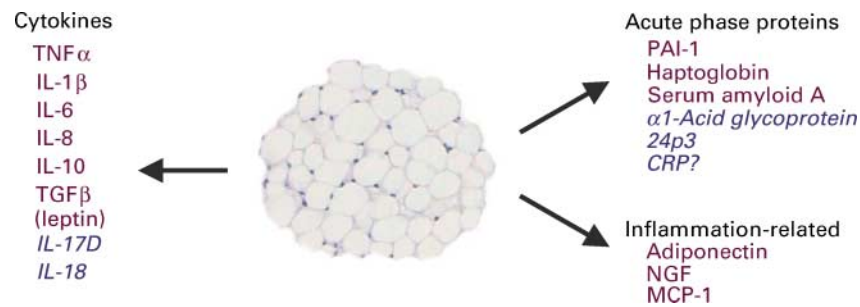


Fig. 2. Inflammatory and acute-phase response proteins secreted from adipocytes. The proteins in red have been clearly identified as adipokines (i.e. shown to be released by adipocytes) while those in blue are putative adipokines. CRP, C-reactive protein; IL, interleukin; NGF, nerve growth factor; PAI-1, plasminogen activator inhibitor-1; TGF- β , transforming growth factor- β ; TNF- α , tumour necrosis factor- α ; MCP-1, monocyte chemoattractant protein 1.

neurotrophin, nerve growth factor (NGF; Peeraully *et al.* 2004). The extent to which TNF α produced in WAT is released into the circulation has been a matter of debate, but a relationship between the plasma TNF α system (including the soluble receptors) and indices of obesity has been reported (Bulló *et al.* 2003).

The other cytokine that has been the subject of major interest in WAT is IL-6. It is expressed in, and secreted by, adipocytes and although it has local actions within the tissue, it is released into the circulation (Mohamed-Ali *et al.* 1997). Both plasma levels of IL-6 and expression in WAT are elevated in obesity and insulin resistance (Mohamed-Ali *et al.* 1997; Bastard *et al.* 2000; Vozarova *et al.* 2001). It has been proposed that IL-6 has direct central actions, as IL-6 receptors are found in the hypothalamus in mice (Mohamed-Ali *et al.* 1997; Wallenius *et al.* 2002). As such, it is a candidate molecule for conveying information from adipocytes to the hypothalamus in the regulation of energy balance, additional to leptin.

Other cytokines

Although there has been considerable focus on TNF α and IL-6, several other cytokines and related factors are synthesised within adipose tissue, including IL-1 β , TGF- β and, of course, leptin. Recent reports have included IL-8, IL-10 and IL-17D. The IL-8 gene is expressed in human adipocytes and the protein released from both fat cells and adipose tissue fragments (Bruun *et al.* 2000, 2001). IL-1 β and TNF α stimulate IL-8 release, while dexamethasone is inhibitory (Bruun *et al.* 2001). As with IL-6, the plasma level of IL-8 is increased in obesity (Strackowski *et al.* 2002).

Circulating levels of IL-18 are also increased in obesity and fall with weight reduction (Esposito *et al.* 2002). As a consequence, it is speculated that WAT is a probable site of production of IL-18. Although there are no published reports on this, we have recently observed IL-18 gene expression in human WAT (IS Wood and P Trayhurn, unpublished results). Similarly, the levels of IL-10, an anti-inflammatory cytokine, are also raised in the obese (Esposito *et al.* 2003); the secretion of IL-10 from human adipocytes, as well as from the stromal vascular fraction and tissue matrix of human fat depots, has been reported (Fain *et al.* 2004b).

The expression of IL-17D, believed to be the last member of the IL-17 family to be identified, has recently been described in (human?) adipocytes (Starnes *et al.* 2002).

This cytokine stimulates the production of IL-6 and IL-8 from endothelial cells (Starnes *et al.* 2002). However, its release from adipocytes has not been documented, so at present it can only be considered as a putative adipokine.

Acute-phase proteins

There are a number of acute-phase proteins whose plasma concentration increases substantially during the early stages of the inflammatory response, and a small number where the level falls (Gabay & Kushner, 1999). Several of these proteins are now recognised as adipokines, with adipose tissue being a potential contributor (either major or minor) to the raised circulating levels in obesity; recently described examples are summarised here. Expression of the genes encoding some acute-phase reactants, such as α 1-acid glycoprotein and 24p3, has been observed in either adipocyte cell culture or in murine tissues (Soukas *et al.* 2000; Lin *et al.* 2001), but secretion as such has not yet been reported.

Plasminogen activator inhibitor-1

PAI-1 is an important factor in the maintenance of vascular haemostasis, inhibiting the activation of plasminogen, the precursor of plasmin, which is involved in the breakdown of fibrin (Mutch *et al.* 2001). The expression and secretion of PAI-1 by adipocytes, both rodent and human, is well documented (Lundgren *et al.* 1996; Eriksson *et al.* 1998; Cigolini *et al.* 1999; Mutch *et al.* 2001). The circulating level of PAI-1 is increased in obesity and synthesis in WAT is also raised (Alessi *et al.* 2000). This has led to the view that adipose tissue is the major source of the elevated PAI-1 levels in the obese (Lundgren *et al.* 1996; Samad *et al.* 1996; Alessi *et al.* 2000). As the risk of atherothrombotic disease is increased in obesity, this is a potent example of how the co-morbidities, such as diabetes and cardiovascular risk, associated with a high body fat can be directly linked to alterations in the production of specific adipokines. In addition to its role in haemostasis, PAI-1 is also an acute-phase response protein, the levels rising in inflammation (Gabay & Kushner, 1999).

Haptoglobin

Several studies have now reported that the haptoglobin gene is expressed in murine adipose tissue (Friedrichs

et al. 1995; Chiellini *et al.* 2002; Oller do Nascimento *et al.* 2004). Similarly, gene expression has also been shown in human WAT (Oller do Nascimento *et al.* 2004). A very recent study has demonstrated direct release of haptoglobin from human adipose tissue explants (Fain *et al.* 2004a) and release of haptoglobin into the medium has been observed in 3T3-L1 adipocytes by a proteomic approach (Kratchmarova *et al.* 2002). Both transgenic studies and studies on 3T3-L1 adipocytes indicate that TNF α is a key factor in the stimulation of haptoglobin expression (Chiellini *et al.* 2002; Chinetti *et al.* 2003; Oller do Nascimento *et al.* 2004), with IL-6 also being stimulatory.

Stimulation of the PPAR γ nuclear receptor through the administration of the thiazolidinedione, rosiglitazone, strongly inhibits haptoglobin gene expression (Oller do Nascimento *et al.* 2004). This is consistent with the emerging view that PPARs have substantial anti-inflammatory actions (Moller & Berger, 2003). Indeed, several other inflammation-related adipokines are down regulated by PPAR γ ligands, including TNF α , leptin and NGF, while there is evidence that adiponectin is up regulated (Moller & Berger, 2003).

Serum amyloid A

Serum amyloid A (SAA), the precursor to amyloid A protein found in secondary amyloid plaques, consists of a family of apolipoproteins which bind to, and substitute for, apo A-I in HDL. These apolipoproteins are expressed as either major acute-phase reactants SAA, or constitutive SAA, the functions of which are largely unknown. However, a few clinically important functions have been suggested that include pro-inflammatory and anti-inflammatory roles. A number of genes have now been identified in man and mice which share very similar sequence identities and genomic organisation (Uhlir & Whitehead, 1999). These genes are up regulated by pro-inflammatory cytokines such as TNF α and IL-6, as well as by glucocorticoids. The expression of SAA, as with other acute-phase reactants, is predominantly in the liver. However, extrahepatic expression of SAA, including adipocytes, has been reported; expression and release of SAA3 occurs in murine adipocytes, and this is up regulated under hyperglycaemic conditions (Lin *et al.* 2001).

C-reactive protein

The circulating level of CRP rises with BMI (Visser *et al.* 1999; Pannacciulli *et al.* 2001; Bulló *et al.* 2003), and elevated levels of this inflammatory marker have been associated with both obesity and diabetes, falling with weight loss (Tchernof *et al.* 2002). There is evidence from a study using real-time PCR that the gene encoding CRP is expressed in adipose tissue, an inverse correlation between the levels of the mRNA for CRP and adiponectin being apparent (Ouchi *et al.* 2003). This raises the possibility that adipose tissue contributes directly to the circulating pool of CRP. Unfortunately, it is not clear whether CRP expression in adipocytes is in practice significant; our own studies using conventional RT-PCR have found that there is little expression in human WAT, or adipocytes

(IS Wood and P Trayhurn, unpublished results). It should be noted that murine CRP is not regarded as an acute-phase protein due to its very low expression (Volanakis, 2001).

Very low levels of expression would suggest that adipocytes are unlikely to be a significant direct contributor to circulating CRP levels. However, IL-6 is secreted by adipose tissue in increased amounts in obesity, as noted earlier, and this is the major cytokine regulating the hepatic production of CRP (Heinrich *et al.* 1990; Yudkin *et al.* 2000). Thus WAT may be a major player in the raised circulating levels of CRP in obesity, but through the indirect route of adipocyte-derived IL-6.

Additional inflammation-related proteins

There are several other adipokines involved in the inflammatory response that are neither cytokines nor acute-phase proteins. Interestingly, adiponectin (also known as Acrp30, AdipoQ, ApM1 or GBP28), which is synthesised only in adipose tissue, appears to have an anti-inflammatory effect, inhibiting phagocytic activity and TNF α production in macrophages (Ouchi *et al.* 1999; Yokota *et al.* 2000). This adipokine is now very much centre stage; this is partly because, in contrast to many other adipokines, its expression and release fall in obesity (Arita *et al.* 1999; Hotta *et al.* 2000). However, the principal reason for the recent focus on adiponectin is the evidence that it is involved in modulating insulin sensitivity (Berg *et al.* 2001; Yamauchi *et al.* 2001), as well as having anti-atherogenic properties (Engeli *et al.* 2003).

We have recently observed that the target-derived neurotrophin, NGF, is synthesised by the main adipose tissue depots in both rodents and man, and is secreted from white adipocytes (Peeraully *et al.* 2004). This protein, which was the first of the family of neurotrophins to be discovered, was originally linked to the growth and survival of sympathetic neurones; however, although not a cytokine, it is now recognised as also being involved in immune and inflammatory responses (Levi-Montalcini *et al.* 1996; Vega *et al.* 2003). Indeed, NGF is expressed in adipocytes specifically associated with wound healing and with atherosclerotic lesions (Hasan *et al.* 2000; Chaldakov *et al.* 2001). Importantly, TNF α has a strong stimulatory effect on NGF gene expression and NGF release from 3T3-L1 adipocytes, suggesting that the neurotrophin is an inflammatory response protein in adipose tissue (Peeraully *et al.* 2004).

Why inflammation in obesity: a response to hypoxia?

Much attention has been directed towards unravelling the pathological and clinical implications of inflammation in obesity, and establishing the links with insulin resistance and other metabolic disorders – the metabolic syndrome. However, a central issue is why does WAT release pro-inflammatory cytokines and acute-phase proteins, and why do these rise sharply as fat mass increases? Linked to this is the question of why obesity should be associated with chronic low-grade inflammation. Adipose tissue may, of course, be contributing inflammation-related factors

to a specific site of inflammation in an organ (or organs) elsewhere, or as part of a systemic state of inflammation. However, an alternative view is that the inflammatory state is mainly within WAT itself. If the inflammatory response is primarily local to adipose tissue, at least in terms of its initiation, then the elevated circulating levels of inflammation-related products may in effect reflect spillover from the tissue, and the link with insulin resistance would be an incidental consequence.

In the absence of any specific indication to the contrary, the parsimonious view is that the secretion of inflammatory cytokines and acute-phase proteins by adipocytes in obesity relates to events within WAT itself. If this is correct, what could be the rationale for a local effect? A possible explanation is that it is a response to hypoxia in areas of the fat depots as the tissue mass increases during the progressive development of obesity. The sequence of events might be that as the tissue expands, the vasculature (which is less extensive in WAT than in brown fat) is insufficient to maintain normoxia throughout the organ. Clusters of adipocytes then become relatively hypoxic, and an inflammatory response ensues which serves to increase blood flow and to stimulate angiogenesis. This has some parallels with tumour growth in cancer. That vascular development is an important issue in WAT function is indicated by the fact that adipose tissue mass is sensitive to angiogenesis inhibitors and can be regulated by its vasculature (Rupnick *et al.* 2002), and that several angiogenic factors are secreted by adipocytes (Claffey *et al.* 1992; Rupnick *et al.* 2002; Lolmede *et al.* 2003). These factors include recognised angiogenic signals (VEGF, PAI-1 and leptin), as well as putative signals such as metallothionein and haptoglobin.

A pivotal signal in the cellular response to hypoxia is hypoxia-inducible factor-1 (HIF-1); this transcription factor is a heterodimeric protein consisting of α and β subunits. The β subunit is constitutively expressed, but the α subunit is highly induced by hypoxia leading to the formation of functional HIF-1 (Semenza, 2001; Wenger, 2002; Höpfl *et al.* 2004). Hypoxia is characteristic of tumours, where HIF-1 expression is increased, as well as in other disorders such as ischaemic heart disease (Binley *et al.* 2003; Höpfl *et al.* 2004). A number of genes are regulated by HIF-1, which acts as a central controller of oxygen-regulated gene expression. The transcription factor is stabilised and its expression stimulated by cytokines such as TNF α and IL-1 β (Hellwig-Bürgel *et al.* 1999). The target genes for HIF-1 include VEGF and PAI-1 (Höpfl *et al.* 2004). In addition, there is now evidence for the transcriptional activation of leptin through HIF-1 α in response to hypoxia (Ambrosini *et al.* 2002).

Immunoreactive HIF-1 α has been reported in 3T3-F442A adipocytes and hypoxia results in an increase in the amount of the protein in the cultured cells (Lolmede *et al.* 2003). Furthermore, hypoxia leads to an induction of leptin and VEGF expression in these adipocytes, raising the likelihood that a low oxygen tension leads to the stimulation of angiogenesis in adipose tissue through the HIF-1 pathway (Lolmede *et al.* 2003).

HIF-1 α expression is not just a feature of cultured adipocytes, since we have recently observed that the HIF-1 α gene is expressed in mouse WAT depots. Expression

occurs in both the adipocytes and in the stromal vascular cells, and in the WAT of obese (*ob/ob*) mice the level of the mRNA is markedly increased compared with lean siblings (L Hunter, IS Wood and P Trayhurn, unpublished results). Thus a link between the increased WAT mass in obesity, adipocyte hypoxia, inflammation and the stimulation of angiogenesis is plausible.

Other key developments

We have focused in the present paper on the role of WAT in inflammatory responses. There have, however, been a number of other important recent developments in the adipokine field. For example, a recent report has indicated that human white adipocytes secrete mineralocorticoid-releasing factors, aldosterone secretion by adrenocortical cells being stimulated (Ehrhart-Bornstein *et al.* 2003). These results indicate a direct link between obesity and hypertension through adipose tissue regulating mineralocorticoid production.

There is continuing interest in whether adipocytes secrete centrally acting signals in the regulation of appetite and energy balance additional to leptin, as noted earlier. An initial candidate for such a role was fasting-induced adipose factor (also known as PPAR γ angiopoietin-related gene) (Kersten *et al.* 2000; Yoon *et al.* 2000). Fasting-induced adipose factor is an angiopoietin-related protein, the expression of which is strongly stimulated by fasting in a manner which may be reciprocal to leptin. Fasting-induced adipose factor is potentially of considerable interest, not only as a putative signal in energy balance, but as part of the adaptive response to nutritional deprivation. In this regard, since it is induced by fasting, it is a positive signal of the fasted state, in contrast to leptin which signals food deprivation through a reduction in its expression (Ahima *et al.* 1996).

An intriguing report using DNA microarrays for gene expression profiling of human visceral adipose tissue has suggested that a constellation of neuroendocrine factors may be produced by white adipocytes (Yang *et al.* 2003). These factors included cholecystokinin, neurotensin and neuropeptide Y. If correct, it suggests that the adipocyte is even more remarkable as a secretory cell than currently envisaged; indeed, it would be a veritable powerhouse in the secretion of neuroendocrine signals. However, our attempts to identify transcripts of some of these genes using conventional RT-PCR have proved unsuccessful (L Hunter, IS Wood and P Trayhurn, unpublished results), and the reported expression may in practice reflect the problems inherent in setting a suitable (arbitrary) threshold in microarray studies when comparisons between groups are not part of the experimental paradigm.

Conclusions

The biology of WAT seems ever more rich and complex. The family of adipokines is increasing rapidly, these proteins being highly diverse in structure and in function. A critical issue is the physiological role that many of them play. The role may be local (through an autocrine or paracrine function) or endocrine, or involve multiple

levels (as with leptin and IL-6). An important development is the recognition of the extensive and direct involvement of white adipocytes in inflammation and the acute-phase response. In some cases it seems probable that adipocytes contribute substantially to the raised circulating levels of particular pro-inflammatory cytokines and acute-phase reactants in obesity, such as IL-6, PAI-1 and haptoglobin. In others, adipose tissue appears to have an indirect role; for example, hepatic production of CRP may be stimulated by the increased release of IL-6 from adipocytes.

The inflammatory state that is characteristic of obesity may primarily relate to local events within adipose tissue, raised circulating levels of inflammatory cytokines and acute-phase proteins that are adipokines reflecting spillover from the tissue. Hypoxia consequent upon the expansion of white fat mass in advance of angiogenesis could be a key trigger for the inflammation-related events in WAT in obesity. Adipose-derived inflammatory cytokines may lead to a direct stimulation of angiogenic factors, such as VEGF and leptin, as well as through the activation of HIF-1, the central controller of the cellular response to hypoxia.

Acknowledgements

We thank Dr Mònica Bulló Bonet for her helpful comments on the manuscript. Our work is funded by grants from the BBSRC, European Union and the University of Liverpool.

References

- Ahima RS, Prabakaran D, Mantzoros C, Qu DQ, Lowell B, Maratos-Flier E & Flier JS (1996) Role of leptin in the neuroendocrine response to fasting. *Nature* **382**, 250–252.
- Alessi MC, Bastelica D, Morange P, Berthet B, Leduc I, Verdier M, Geel O & Juhan-Vague I (2000) Plasminogen activator inhibitor 1, TGF β 1, and BMI are closely associated in human adipose tissue during morbid obesity. *Diabetes* **49**, 1374–1380.
- Ambrosini G, Nath AK, Sierra-Honigmann MR & Flores-Riveros J (2002) Transcriptional activation of the human leptin gene in response to hypoxia. Involvement of hypoxia-inducible factor 1. *J Biol Chem* **277**, 34601–34609.
- Arita Y, Kihara S, Ouchi N, *et al.* (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* **257**, 79–83.
- Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, Vidal H & Hainque B (2000) Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab* **85**, 3338–3342.
- Berg AH, Combs TP, Du X, Brownlee M & Scherer PE (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nature Med* **7**, 947–953.
- Binley K, Kan O, White J & Naylor S (2003) Exploiting the hypoxia response. *Curr Opin Mol Ther* **5**, 650–656.
- Bruun JM, Pedersen SB & Richelsen B (2000) Interleukin-8 production in human adipose tissue. Inhibitory effects of anti-diabetic compounds, the thiazolidinedione ciglitazone and the biguanide metformin. *Horm Metab Res* **32**, 537–541.
- Bruun JM, Pedersen SB & Richelsen B (2001) Regulation of interleukin 8 production and gene expression in human adipose tissue in vitro. *J Clin Endocrinol Metab* **86**, 1267–1273.
- Bulló M, Garcia-Lorda P, Megias I & Salas-Salvado J (2003) Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. *Obesity Res* **11**, 525–531.
- Chaldakov GN, Stankulov IS, Fiore M, Ghenev PI & Aloe L (2001) Nerve growth factor levels and mast cell distribution in human coronary atherosclerosis. *Atherosclerosis* **159**, 57–66.
- Chiellini C, Bertacca A, Novelli SE, *et al.* (2002) Obesity modulates the expression of haptoglobin in the white adipose tissue via TNF α . *J Cell Physiol* **190**, 251–258.
- Chinetti G, Fruchart JC & Staels B (2003) Peroxisome proliferator-activated receptors and inflammation: from basic science to clinical applications. *Int J Obesity* **27**, Suppl. 3, S41–S45.
- Cigolini M, Tonoli M, Borgato L, *et al.* (1999) Expression of plasminogen activator inhibitor-1 in human adipose tissue: a role for TNF- α ? *Atherosclerosis* **143**, 81–90.
- Claffey KP, Wilkison WO & Spiegelman BM (1992) Vascular endothelial growth factor. Regulation by cell differentiation and activated second messenger pathways. *J Biol Chem* **267**, 16317–16322.
- Cook KS, Groves DL, Min HY & Spiegelman BM (1985) A developmentally regulated mRNA from 3T3 adipocytes encodes a novel serine protease homologue. *Proc Natl Acad Sci USA* **82**, 6480–6484.
- Cook KS, Min HY, Johnson D, Chaplinsky RJ, Flier JS, Hunt CR & Spiegelman BM (1987) Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. *Science* **237**, 402–405.
- Coppack SW (2001) Pro-inflammatory cytokines and adipose tissue. *Proc Nutr Soc* **60**, 349–356.
- Das UN (2001) Is obesity an inflammatory condition? *Nutrition* **17**, 953–966.
- Dietze D, Koenen M, Rohrig K, Horikoshi H, Hauner H & Eckel J (2002) Impairment of insulin signaling in human skeletal muscle cells by co-culture with human adipocytes. *Diabetes* **51**, 2369–2376.
- Ehrhart-Bornstein M, Lamounier-Zepter V, Schraven A, Langenbach J, Willenberg HS, Barthel A, Hauner H, McCann SM, Scherbaum WA & Bornstein SR (2003) Human adipocytes secrete mineralocorticoid-releasing factors. *Proc Natl Acad Sci USA* **100**, 14211–14216.
- Engeli S, Feldpausch M, Gorzelnik K, Hartwig F, Heintze U, Janke J, Mohlig M, Pfeiffer AFH, Luft FC & Sharma AM (2003) Association between adiponectin and mediators of inflammation in obese women. *Diabetes* **52**, 942–947.
- Engström G, Hedblad B, Stavenow L, Lind P, Janzon L & Lindgärde F (2003) Inflammation-sensitive plasma proteins are associated with future weight gain. *Diabetes* **52**, 2097–2101.
- Eriksson P, Reynisdottir S, Lonnqvist F, Stemme V, Hamsten A & Arner P (1998) Adipose tissue secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. *Diabetologia* **41**, 65–71.
- Esposito K, Pontillo A, Ciotola M, Di Palo C, Grella E, Nicoletti G & Giugliano D (2002) Weight loss reduces interleukin-18 levels in obese women. *J Clin Endocrinol Metab* **87**, 3864–3866.
- Esposito K, Pontillo A, Giugliano F, Giugliano G, Marfella R, Nicoletti G & Giugliano D (2003) Association of low interleukin-10 levels with the metabolic syndrome in obese women. *J Clin Endocrinol Metab* **88**, 1055–1058.
- Fain JN, Bahouth SW & Madan AK (2004a) Haptoglobin release by human adipose tissue in primary culture. *J Lipid Res* **45**, 536–542.
- Fain JN, Madan AK, Hiler ML, Cheema P & Bahouth SW (2004b) Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral

- and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* **145**, 2273–2282.
- Festa A, D'Agostino R Jr, Williams K, Karter AJ, Mayer-Davis EJ, Tracy RP & Haffner SM (2001) The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obesity* **25**, 1407–1415.
- Friedrichs WE, Navarajo-Ashbaugh AL, Bowman BH & Yang F (1995) Expression and inflammatory regulation of haptoglobin gene in adipocytes. *Biochem Biophys Res Commun* **209**, 250–256.
- Frühbeck G, Gómez-Ambrosi J, Muruzabal FJ & Burrell MA (2001) The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J Physiol* **280**, E827–E847.
- Funahashi T, Nakamura T, Shimomura I, Maeda K, Kuriyama H, Takahashi M, Arita Y, Kihara S & Matsuzawa Y (1999) Role of adipocytokines on the pathogenesis of atherosclerosis in visceral obesity. *Intern Med* **38**, 202–206.
- Gabay C & Kushner I (1999) Acute-phase proteins and other systemic responses to inflammation. *New Engl J Med* **340**, 448–454.
- Hasan W, Zhang R, Liu M, Warn JD & Smith PG (2000) Coordinate expression of NGF and alpha-smooth muscle actin mRNA and protein in cutaneous wound tissue of developing and adult rats. *Cell Tissue Res* **300**, 97–109.
- Heinrich PC, Castell JV & Andus T (1990) Interleukin-6 and the acute phase response. *Biochem J* **265**, 621–636.
- Hellwig-Bürgel T, Rutkowski K, Metzger E, Fandrey J & Jelkmann W (1999) Interleukin-1 β and tumor necrosis factor- α stimulate DNA binding of hypoxia-inducible factor-1. *Blood* **94**, 1561–1567.
- Höpfel G, Ogunshola O & Gassmann M (2004) HIFs and tumors - causes and consequences. *Am J Physiol* **286**, R608–R623.
- Hotamisligil GS (2003) Inflammatory pathways and insulin action. *Int J Obesity* **27**, Suppl. 3, S53–S55.
- Hotamisligil GS, Shargill NS & Spiegelman BM (1993) Adipose expression of tumor necrosis factor- α - direct role in obesity-linked insulin resistance. *Science* **259**, 87–91.
- Hotta K, Funahashi T, Arita Y, *et al.* (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscl Thromb Vasc Biol* **20**, 1595–1599.
- Kersten S, Mandart S, Tan NS, Escher P, Metzger D, Chambon P, Gonzalez FJ, Desvergne B & Wahli W (2000) Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. *J Biol Chem* **275**, 28488–28493.
- Klaus S (2004) Adipose tissue as a regulator of energy balance. *Curr Drug Targets* **4**, 1–10.
- Kratchmarova I, Kalume DE, Blagoev B, *et al.* (2002) A proteomic approach for identification of secreted proteins during the differentiation of 3T3-L1 preadipocytes to adipocytes. *Mol Cell Proteomics* **1**, 213–222.
- Levi-Montalcini R, Skaper SD, Dal Toso R, Petrelli L & Leon A (1996) Nerve growth factor: from neurotrophin to neurokine. *Trends Neurosci* **19**, 514–520.
- Lin Y, Rajala MW, Berger JP, Moller DE, Barzilai N & Scherer PE (2001) Hyperglycemia-induced production of acute phase reactants in adipose tissue. *J Biol Chem* **276**, 42077–42083.
- Lolmede K, Durand de Saint Front V, Galitzky J, Lafontan M & Bouloumie A (2003) Effects of hypoxia on the expression of proangiogenic factors in differentiated 3T3-F442A adipocytes. *Int J Obesity* **27**, 1187–1195.
- Lundgren CH, Brown SL, Nordt TK, Sobel BE & Fujii S (1996) Elaboration of type-1 plasminogen activator inhibitor from adipocytes - a potential pathogenetic link between obesity and cardiovascular disease. *Circulation* **93**, 106–110.
- Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S & Coppack SW (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- α , *in vivo*. *J Clin Endocrinol Metab* **82**, 4196–4200.
- Mohamed-Ali V, Pinkney JH & Coppack SW (1998) Adipose tissue as an endocrine and paracrine organ. *Int J Obesity* **22**, 1145–1158.
- Moller DE & Berger JP (2003) Role of PPARs in the regulation of obesity-related insulin sensitivity and inflammation. *Int J Obesity* **27**, Suppl. 3, S17–S21.
- Mutch NJ, Wilson HM & Booth NA (2001) Plasminogen activator inhibitor-1 and haemostasis in obesity. *Proc Nutr Soc* **60**, 341–346.
- Oller do Nascimento C, Hunter L & Trayhurn P (2004) Regulation of haptoglobin gene expression in 3T3-L1 adipocytes by cytokines, catecholamines, and PPAR γ . *Biochem Biophys Res Commun* **313**, 702–708.
- Ouchi N, Kihara S, Arita Y, *et al.* (1999) Novel modulator for endothelial adhesion molecules - adipocyte-derived plasma protein adiponectin. *Circulation* **100**, 2473–2476.
- Ouchi N, Kihara S, Funahashi T, *et al.* (2003) Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* **107**, 671–674.
- Pannacciulli N, Cantatore FP, Minenna A, Bellacicco M, Giorgino R & De Pergola G (2001) C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. *Int J Obesity* **25**, 1416–1420.
- Peeraully MR, Jenkins JR & Trayhurn P (2004) NGF gene expression and secretion in white adipose tissue: regulation in 3T3-L1 adipocytes by hormones and inflammatory cytokines. *Am J Physiol* **287** (In the Press).
- Prins JB, Niesler CU, Winterford CM, Bright NA, Siddle K, Orhilly S, Walker NI & Cameron DP (1997) Tumor necrosis factor- α induces apoptosis of human adipose cells. *Diabetes* **46**, 1939–1944.
- Rajala MW & Scherer PE (2003) Minireview: the adipocyte - at the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology* **144**, 3765–3773.
- Rayner DV & Trayhurn P (2001) Regulation of leptin production: sympathetic nervous system interactions. *J Mol Med* **79**, 8–20.
- Rupnick MA, Panigrahy D, Zhang C-Y, Dallabrida SM, Lowell BB, Langer R & Folkman MJ (2002) Adipose tissue mass can be regulated through the vasculature. *Proc Natl Acad Sci USA* **99**, 10730–10735.
- Samad F, Yamamoto K & Loskutoff DJ (1996) Distribution and regulation of plasminogen activator inhibitor-1 in murine adipose tissue *in vivo* - induction by tumor necrosis factor- α and lipopolysaccharide. *J Clin Invest* **97**, 37–46.
- Semenza GL (2001) HIF-1 and mechanisms of hypoxia sensing. *Curr Opin Cell Biol* **13**, 167–171.
- Soukas A, Cohen P, Socci ND & Friedman JM (2000) Leptin-specific patterns of gene expression in white adipose tissue. *Genes Dev* **14**, 963–980.
- Starnes T, Broxmeyer HE, Robertson MJ & Hromas R (2002) Cutting edge: IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. *J Immunol* **169**, 642–646.
- Straczkowska M, Dzienis-Straczkowska S, Stepień A, Kowalska I, Szlachowska M & Kinalska I (2002) Plasma interleukin-8 concentrations are increased in obese subjects and related to fat mass and tumor necrosis factor- α system. *J Clin Endocrinol Metab* **87**, 4602–4606.
- Tchernof A, Nolan A, Sites CK, Ades PA & Poehlman ET (2002) Weight loss reduces C-reactive protein levels in obese postmenopausal women. *Circulation* **105**, 564–569.
- Trayhurn P & Beattie JH (2001) Physiological role of adipose

- tissue: white adipose tissue as an endocrine and secretory organ. *Proc Nutr Soc* **60**, 329–339.
- Uhlir CM & Whitehead AS (1999) Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem* **265**, 501–523.
- Vega JA, Garcia-Suarez O, Hannestad J, Perez-Perez M & Germana A (2003) Neurotrophins and the immune system. *J Anat* **203**, 1–19.
- Visser M, Bouter LM, McQuillan GM, Wener MH & Harris TB (1999) Elevated C-reactive protein levels in overweight and obese adults. *J Am Med Assoc* **282**, 2131–2135.
- Volanakis JE (2001) Human C-reactive protein: expression, structure, and function. *Mol Immunol* **38**, 189–197.
- Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C & Pratley RE (2001) Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obesity Res* **9**, 414–417.
- Wallenius K, Wallenius V, Sunter D, Dickson SL & Jansson JO (2002) Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochem Biophys Res Commun* **293**, 560–565.
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL & Ferrante AW Jr (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* **112**, 1796–1808.
- Wenger RH (2002) Cellular adaptation to hypoxia: O₂-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O₂-regulated gene expression. *FASEB J* **16**, 1151–1162.
- Wiesner G, Morash BA, Ur E & Wilkinson M (2004) Food restriction regulates adipose-specific cytokines in pituitary gland but not in hypothalamus. *J Endocrinol* **180**, R1–R6.
- Xu H, Barnes GT, Yang Q, *et al.* (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* **112**, 1821–1830.
- Yamauchi T, Kamon J, Waki H, *et al.* (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipodystrophy and obesity. *Nature Med* **7**, 941–946.
- Yang YS, Song HD, Li RY, Zhou LB, Zhu ZD, Hu RM, Han ZG & Chen JL (2003) The gene expression profiling of human visceral adipose tissue and its secretory functions. *Biochem Biophys Res Commun* **300**, 839–846.
- Yokota T, Oritani K, Takahashi I, *et al.* (2000) Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* **96**, 1723–1732.
- Yoon JC, Chickering TW, Rosen ED, Dussault B, Qin Y, Soukas A, Friedman JM, Holmes WE & Spiegelman BM (2000) Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. *Mol Cell Biol* **20**, 5343–5349.
- Yudkin JS (2003) Adipose tissue, insulin action and vascular disease: inflammatory signals. *Int J Obesity* **27**, Suppl. 3, S25–S28.
- Yudkin JS, Kumari M, Humphries SE & Mohamed-Ali V (2000) Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* **148**, 209–214.
- Yudkin JS, Stehouwer CD, Emeis JJ & Coppack SW (1999) C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* **19**, 972–978.
- Zhang YY, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM (1994) Positional cloning of the mouse obese gene and its human homolog. *Nature* **372**, 425–432.