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Appetite and energy balance signals from adipocytes

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Interest in the biology of white adipose tissue has risen markedly with the recent surge in obesity and its associated disorders. The tissue is no longer viewed simply as a vehicle for lipid storage; instead, it is recognized as a major endocrine and secretory organ. White adipocytes release a multiplicity of protein hormones, signals and factors, termed adipokines, with an extensive range of physiological actions. Foremost among these various adipokines is the cytokine-like hormone, leptin, which is synthesized predominantly in white fat. Leptin plays a critical role in the control of appetite and energy balance, with mutations in the genes encoding the hormone or its receptor leading to profound obesity in both rodents and man. Leptin regulates appetite primarily through an interaction with hypothalamic neuroendocrine pathways, inhibiting orexigenic peptides such as neuropeptide Y and orexin A, and stimulating anorexigenic peptides such as proopiomelanocortin. White fat also secretes several putative appetite-related adipokines, which include interleukin-6 and adiponectin, but whether these are indeed significant signals in the regulation of food intake has not been established. Through leptin and the other adipokines it is evident that adipose tissue communicates extensively with other organs and plays a pervasive role in metabolic homeostasis.

Keywords: adipocytes; adipokines; appetite; energy balance; leptin; obesity

1. INTRODUCTION: ENERGY BALANCE

The control of appetite and energy balance are key biological processes in higher animals, and unravelling the critical mechanisms involved represents a continuing challenge in fundamental physiology. Recently, two practical issues have become major drivers in work on appetite and energy balance. The first relates to animal husbandry and food production, with the demand for the provision of meat animals of low carcass fat to meet nutritional recommendations for a reduction in dietary lipid intake, particularly of saturated fatty acids. The second, and now central, driver reflects the rapid rise in obesity in affluent societies, this being the major nutritional disorder in the developed world.

Obesity is fundamentally a problem of energy balance in that self-evidently it can develop only when energy intake is in excess of energy expenditure. This has led to a major focus on the mechanisms controlling intake and the components and regulatory mechanisms of energy expenditure. Much recent progress has been made in identifying the central neuroendocrine pathways involved both in the control of energy intake and of expenditure (see Trayhurn 2005*a*). Thus orexigenic pathways involving neuropeptide Y (NPY), melanin concentrating hormone (MCH), orexin A, agouti-related peptide (AgRP) and the endogenous cannabinoid system have each been identified (Ahima *et al*. 2000; Schwartz *et al*. 2000; Harrold & Williams 2003; Arch 2005). Similarly, anorexigenic pathways involving proopiomelanocortin (POMC) and the melanocortin system, cocaine and amphetamine regulated transcript (CART) and corticotrophin releasing hormone (CRH) are also recognized (Schwartz *et al*. 2000; Porte *et al*. 2005). Much effort is currently being directed towards defining the networks and the hierarchies involved in these neuroendocrine systems.

Several peripheral signals to the central neuroendocrine pathways of appetite and energy balance control have been identified. These signals, which include cholecystokinin, ghrelin, peptide YY and insulin, are predominantly associated with the gastrointestinal tract (Badman & Flier 2005; Otto *et al*. 2005; Wynne *et al*. 2005). A source of a further major peripheral signal is white adipose tissue; conceptually, as with the gut, this involves a direct link with an organ central to nutritional status (in the form of ingested nutrients in the case of the gastrointestinal tract, and the size of the lipid stores with adipose tissue). In this article, we specifically consider signals in appetite and energy balance, both established and putative, emanating from white fat.

2. ADIPOSE TISSUE

The adipose organ consists of two apparently distinct tissue types—brown and white adipose tissue (Cinti 2001). Brown adipose tissue is concerned functionally with thermogenesis, or adaptive heat production, and this is achieved through the regulated uncoupling of mitochondrial oxidative phosphorylation via the presence of the tissue-specific uncoupling protein, UCP-1 (Cannon & Nedergaard 2004). While brown

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One contribution of 16 to a Theme Issue 'Appetite'.

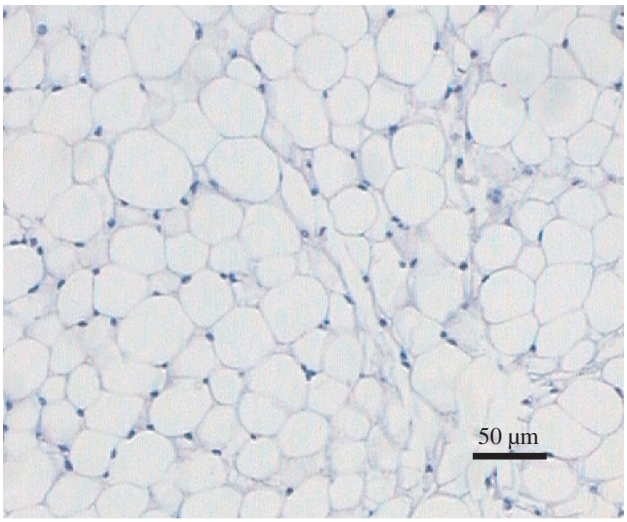

Figure 1. Histological section of white adipose tissue illustrating, at the level of light microscopy, the apparent simplicity of the tissue. A haematoxylin–eosin stained section of epididymal adipose tissue from a NMRI mouse is shown.

adipose tissue is a net consumer of fatty acids, white fat is the central organ for fuel storage in mammals. The major fuel storage role of white adipose tissue led to lipogenesis and lipolysis long being viewed as the key metabolic processes within the tissue.

Adipose tissue, and white fat in particular, is widely distributed throughout the body and is present in a number of distinct depots, both subcutaneously and around internal organs. In addition, adipocytes may be embedded within other tissues such as around skeletal muscle fibres. This locational diversity is increasingly considered to also reflect a degree of functional heterogeneity, with visceral fat being strongly associated with the metabolic syndrome and other obesity-linked disorders. Adipose tissue is the main variable in overall body composition in mammals, varying by an order of magnitude or more. Even in normal, lean individuals it is a substantial proportion of total tissue mass. For example, in an adult male with a body mass index (BMI) of 22 (close to ideal), some 20% of total tissue mass is white fat. In an obese individual, BMI = 30, up to one half of total body tissue mass may be due to white fat.

At the histological level, white adipose tissue seems remarkably simple in its structure (figure 1). There appears to be little other than mature adipocytes, filled with a large single (unilocular) lipid droplet, and this is reflected in the fact that the tissue may comprise 85% lipid by weight, enabling fuel to be stored at a high energy density. Despite superficial appearances, there is in practice considerable cellular heterogeneity, with mature adipocytes constituting no more than 50% of the total cell content of the tissue (Hausman 1985). The apparent simplicity of white fat at a histological level is partly why it is only recently that the complexity of adipocytes as secretory cells has become recognized.

3. ADIPOKINES: PROTEIN SIGNALS RELEASED FROM ADIPOCYTES

Fatty acids are quantitatively the most important secretory product from white adipocytes. In addition, certain other lipid moieties, including cholesterol, retinol, prostanoids and steroid hormones, are also released. Not all of these, however, are synthesized *de novo* within the fat cell; cholesterol and retinol, for example, are taken up from the circulation by adipocytes and stored.

The first protein recognized to be secreted from white adipocytes was lipoprotein lipase, which is responsible for the hydrolysis of circulating tricylglycerols (in the form of lipoproteins) with the subsequent uptake of the fatty acids released (Eckel 1989). A further secreted protein from adipocytes was identified in the late 1980s, namely the complement-related factor adipsin (Cook *et al.* 1987; Flier *et al.* 1987). Adipsin was initially thought to be the long sought adipocyte-derived signal in energy balance, but this was subsequently seen not to be the case. A major step forward in the recognition of the endocrine and secretory role of adipose tissue occurred in the early 1990s with the discovery that the pro-inflammatory cytokine tumour necrosis factor-α (TNFα) is synthesized and released by adipocytes (Hotamisligil *et al.* 1993, 1995). TNFα has subsequently been shown to have extensive metabolic effects in adipose tissue, these effects including the stimulation of both lipolysis and apoptosis (Prins *et al.* 1997; Gasic *et al.* 1999; Ryden *et al.* 2004). In addition, this cytokine plays an important role in the induction of insulin resistance in fat cells (Hotamisligil 2003; Hotamisligil *et al.* 1995).

Despite the initial expectations with adipsin, it was evident that the early protein factors identified as being secreted from adipocytes did not relate directly to the signalling of appetite or energy balance *per se*. However, the situation changed radically in 1994 with the discovery of the major adipocyte-derived hormone leptin. Leptin was identified during the characterization of the *Ob* gene, a mutation in which is responsible for the obesity of the *ob*/*ob* mouse (Zhang *et al.* 1994)—perhaps the most widely used animal model in obesity research. In the decade subsequent to the discovery of leptin, a number of other protein signals and factors secreted from adipocytes have been identified (Frühbeck *et al.* 2001; Trayhurn & Beattie 2001; Rajala & Scherer 2003; Trayhurn & Wood 2004; Hauner 2005; Trayhurn 2005*b*). These secretory proteins, which are generally known by the collective term 'adipokines', now number in excess of 50 different molecular entities (Trayhurn & Wood 2004; Hauner 2005); thus the adipocyte is a secretory cell of considerable richness and complexity.

4. LEPTIN

The discovery of leptin resulted in a radical shift in our perspectives on the physiological role of white fat, the tissue now being recognized as a major endocrine organ, which plays a direct—and critical—role in the regulation of energy balance. Indeed, white fat can be viewed as the largest endocrine organ in man, and very particularly in the obese.

Leptin (Greek *leptos*, meaning thin or small) is synthesized as an 18 000 mol. wt. pro-hormone which is cleaved to yield a 16 000 mol. wt. mature product which is 'cytokine-like'. White adipose tissue (hereafter referred to simply as adipose tissue) is the main site of

Ob gene expression and leptin secretion. Expression and secretion occur exclusively within the differentiated adipocytes. Leptin, however, is also produced in several cell types in other organs. For example, it is produced by gastric cells in the walls of the stomach (Bado *et al.* 1998; Cinti *et al.* 2000), in follicular papilla cells of hair follicles (Iguchi *et al.* 2001), in osteoblasts (Reseland *et al.* 2001) and in the placenta (Hassink *et al.* 1997; Hoggard *et al.* 1997; Masuzaki *et al.* 1997). In addition, it is produced in certain foetal organs in which synthesis does not occur in the adult (Hoggard *et al.* 1997). In each of these cases, it is probable that the effect of leptin is essentially local rather than systemic, i.e. a paracrine or autocrine action, rather than endocrine.

Despite the range of specific sites in which leptin is synthesized, white adipose tissue is quantitatively much the most important source of the hormone and the primary determinant of the circulating levels. Indeed, one of the earliest observations following the discovery of leptin was that of a direct relationship between BMI, or percent body fat, and circulating level of the hormone (Considine *et al.* 1996; Ostlund *et al.* 1996). In addition to the importance of body fat in determining circulating leptin levels, production of the hormone is subject to acute regulation by other factors. This includes nutritional regulation, as would be expected for a factor involved in the regulation of energy balance, the most potent example of which is the response to fasting; fasting leads to a marked reduction in *ob* mRNA level in adipose tissue and a rapid fall in circulating leptin levels, changes which are reversed on refeeding (Becker *et al.* 1995; Trayhurn *et al.* 1995; Hardie *et al.* 1996).

A range of hormones and drugs have been shown to directly influence *Ob* gene expression and leptin secretion from adipocytes, particularly from *in vitro* studies using adipocyte cell culture systems. The thiazolidinediones, which are PPARγ agonists, strongly inhibit leptin production indicating that this nuclear receptor is involved in the control of leptin gene transcription (Kallen & Lazar 1996; De Vos *et al.* 1996). Glucocorticoids on the other hand, including dexamethasone, stimulate leptin production (De Vos *et al.* 1995; Wabitsch *et al.* 1996). Much emphasis has also been placed on the role of insulin, which like glucocorticoids is stimulatory, and it is suggested that this hormone is particularly important physiologically in regulating circulating leptin levels (Saladin *et al.* 1995; Leroy *et al.* 1996; Havel 2000).

Considerable importance has also been given to the regulatory role of catecholamines and the sympathetic nervous system (Trayhurn *et al.* 1998; Rayner & Trayhurn 2001). Administration of noradrenaline (norepinephrine), or the β-adrenoceptor agonist isoprenaline, potently suppresses *Ob* gene expression and lowers circulating leptin levels (Trayhurn *et al.* 1998). In the case of rodents, this suppression operates principally through the β3-adrenoreceptor subtype, since β3-selective agonists are at least as effective as isoprenaline (Giacobino 1996; Mantzoros *et al.* 1996; Trayhurn *et al.* 1996). This led to the proposal that the sympathetic nervous system is a key component of the regulation of leptin production in adipocytes and, importantly, provides a negative feedback loop from the brain to fat cells in the control of *Ob* gene transcription (Trayhurn *et al.* 1998; Rayner & Trayhurn 2001; Mark *et al.* 2003). Further evidence for this proposition comes from several sources, including the observation that the blockade of noradrenaline synthesis through the administration of α-methyl-*p*-tyrosine, a selective tyrosine hydroxylase inhibitor, leads to hyperleptinaemia and an increase in *ob* mRNA level in white adipose tissue (Rayner *et al.* 1998; Sivitz *et al.* 1999).

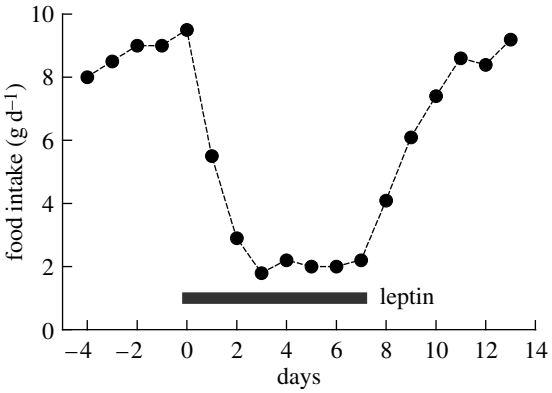

Figure 2. Appetite suppressing effects of leptin. The figure illustrates the powerful inhibitory effect of leptin on food intake in *ob*/*ob* mice, which lack the functional hormone. Mice were injected with vehicle from days −4 to 0 and then with recombinant leptin (1.25 μg g^{-1} body wt, twice daily) from days 0 to 7; they were then returned to vehicle injections. Adapted from Mercer *et al.* (1997).

(a) *Targets for leptin: appetite*

The initial proposition for the central physiological action of leptin was as a satiety factor, and as such the discovery of the hormone provided a molecular basis for the lipostatic theory of the regulation of energy balance. This theory, developed in the 1950s, proposed that there is a signal from adipose tissue to the hypothalamus in proportion to tissue mass, resulting in a feedback loop to the brain in the control of energy balance (Kennedy 1953). However, until the discovery of leptin no molecular entity had been identified which could meet the criteria for such a signal.

There is no doubt that leptin has a powerful inhibitory effect on food intake, and this is illustrated in figure 2 (adapted from Mercer *et al.* 1997), which shows the effects of administering recombinant leptin to obese *ob*/*ob* mice which lack the functional hormone. Leptin reduces the food intake of these mutant animals by approximately 75%, an effect which ceases once the injection is switched to vehicle. Administering leptin to normal animals also inhibits food intake, but the effect is considerably less dramatic, as would be expected since in this case the endogenously produced hormone is present.

In addition to the effects on food intake, there has been some focus on whether leptin also affects energy expenditure, i.e. the other side of the energy balance relationship. The view has developed that leptin does not stimulate expenditure *per se*, but may be involved in inhibiting fasting-induced adaptations in expenditure (Doring *et al.* 1998). One of the strongest pieces of evidence for a key role for leptin in expenditure is that

ob/*ob* mice develop obesity on a normal energy intake, as documented in pair-feeding studies in which young obese mutants were pair-fed to the *ad libitum* food intake of lean siblings (Thurlby & Trayhurn 1979). This difference in energetic efficiency can only reflect a lower energy expenditure in the leptin-deficient mutants. In addition, the early development of obesity in *ob*/*ob* mice takes place on a normal energy intake, hyperphagia only developing some days after weaning at three weeks of age (Trayhurn 1984). These studies were undertaken in the pre-leptin era (i.e. before leptin was discovered), but merit re-interpretation.

A further report from the same period illustrates the importance of leptin in diet-induced thermogenesis. When normal lean mice are fed a cafeteria diet—a mixed diet of highly palatable human-type food items, as pioneered by Rothwell and Stock (Rothwell & Stock 1979)—hyperphagia is induced. In a study on lean and obese *ob*/*ob* mice, the lean animals increased their energy intake by approximately 70% on the cafeteria diet. Nevertheless, there was no significant difference in energy deposition, because of the hyperphagia-induced stimulation of energy expenditure in the form of diet-induced thermogenesis (Trayhurn *et al.* 1982). In this particular experiment the lean animals receiving the cafeteria diet serendipitously had the same energy intake as *ob*/*ob* mice consuming the normal diet; in other words, they were 'pair-fed' on an energy basis (isocaloric intakes). However, the *ob*/*ob* mutants deposited considerably more energy than their lean wild type counterparts, indicating that diet-induced thermogenesis was substantially reduced in the absence of functional leptin (Trayhurn *et al.* 1982).

Pronounced effects of leptin on food intake have also been observed in studies on humans. Obese children who had been identified as having a mutation in the leptin gene, resulting in a non-functional hormone (Montague *et al.* 1997), had their food intake substantially reduced by the administration of recombinant leptin, and this was accompanied by a reduction in body weight and body fat (Farooqi *et al.* 1999, 2002). Thus, as predicted on the basis of rodent studies, leptin is a powerful anorexigenic signal in humans. Again, as with rodents, the effects of leptin administration are not restricted to appetite and energy balance, but include neuroendocrine actions and effects on T-cell responsiveness (Farooqi *et al.* 2002).

(b) *Leptin receptors*

The leptin receptor, a member of the cytokine receptor family, was first cloned a year after leptin itself (Tartaglia *et al.* 1995). It was quickly recognized that the receptor exists as several splice variants: Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re and Ob-Rf (Lee *et al.* 1996; Chua *et al.* 1997; Tartaglia 1997). Ob-Rb has received most attention since it is the long form of the receptor, with an intracellular signalling domain that contains all the motifs required for the action of leptin on appetite (Lee *et al.* 1996). The intracellular domain interacts with the Janus kinases (JAK; Lee *et al.* 1996) and signal transducer and activators of transcription-3 (STAT3) transcription factor (Vaisse *et al.* 1996), necessary for leptin signal transduction. The JAK/STAT pathway induces expression of the suppressor of cytokine signalling-3, a leptin signalling inhibitor that has been identified as a potential mediator of central leptin resistance (Bjorbaek *et al.* 1999). Leptin signalling has, however, also been reported with other isoforms of the receptor (Murakami *et al.* 1997).

Leptin receptors are expressed ubiquitously; indeed, it is difficult to identify a tissue in which they are not present. Following the cloning of the receptor, and the identification of splice variants, strong receptor expression was demonstrated in regions of the hypothalamus, including of the Ob-Rb isoform (Mercer *et al.* 1996*b*). Ob-Rb is highly abundant in the hypothalamus, particularly the arcuate nucleus (ARC), ventromedial nucleus, dorsomedial nucleus (DMH) and the lateral hypothalamic area (LHA; Fei *et al.* 1997; Elmquist *et al.* 1998). Studies on the co-localization between the leptin receptor and specific neuroendocrine systems have shown that Ob-Rb is expressed by many of the major appetite-regulating neuronal pathways. For example, Ob-Rb mRNA is expressed in the ARC by orexigenic NPY/AgRP neurons (Mercer *et al.* 1996*a*) and by anorexigenic POMC/CART neurons (Cheung *et al.* 1997). This is consistent with the powerful satiety effects of leptin operating through key hypothalamic nuclei.

(c) *Leptin entry into the CNS*

One of the issues in interpreting the central actions of leptin is the mechanism by which this peripheral signal crosses the blood-brain barrier. Circulating leptin is transported into the brain via a saturable process independent of insulin (Banks *et al.* 1996). The short form of the receptor, Ob-Ra, is considered to play a key role in the transport of leptin across the blood–brain barrier. Regulation of brain leptin transport appears to be important in mediating the effects of leptin on appetite. Brain transport is abolished during fasting in parallel with the fall in circulating leptin, but transport is increased on refeeding when circulating leptin levels rise (Kastin & Pan 2000). The dynamic changes in brain transport might be also influenced by other nutritional factors, since diet-induced obesity is associated with impaired leptin transport across the blood–brain barrier (Banks *et al.* 1999).

(d) *Leptin–neuroendocrine interactions*

Leptin interacts with several central neuroendocrine pathways, both orexigenic and anorexigenic (Ahima *et al.* 2000; Schwartz *et al.* 2000; Arch 2005). Orexigenic neurons are inhibited by leptin, and this was initially demonstrated with the NPY pathway. NPY, a powerful stimulator of food intake, is mainly synthesized in the ARC neurons that project to the paraventricular nucleus (PVN), DMH and other areas within the hypothalamus (Bai *et al.* 1985). Leptin, administered either intracerebroventricularly (ICV) or systemically, reduces the abnormally high NPY mRNA levels in the ARC, and the release of NPY from the PVN in *ob*/*ob* mice, but not in leptin resistant *db*/*db* mice (Stephens *et al.* 1995; Schwartz *et al.* 1996), while ICV injection of leptin decreased NPY protein levels in the ARC and PVN of lean and *fa*/*fa* Zucker rats (Cusin *et al.* 1996). Subsequently, the appetite lowering and thermogenic actions of leptin, via inhibition of the

NPY neurons in the ARC, have been demonstrated in normal rats; ICV administration of leptin reduced NPY protein levels in the ARC, PVN and DMH, and this was accompanied by a rapid decline in food intake and increased UCP-1 mRNA levels in brown fat (Wang *et al.* 1997). Moreover, starvation is normally associated with the fall in leptin and elevated NPY neuronal activity thereby stimulating the drive to eat, whereas leptin blocks the increase in NPY mRNA induced by fasting in normal mice (Ahima *et al.* 1996). It was, therefore, proposed that regulation of the neuroendocrine system during starvation could be the key physiological role of leptin (Ahima *et al.* 1996).

In addition to NPY, another orexigenic neuropeptide, AgRP, is a hypothalamic target of leptin. AgRP is co-expressed in 90% of the ARC–NPY neurons (Broberger *et al.* 1998) and appears to function as an endogenous antagonist to the melanocortin (MC) 4-R receptor that mediates the appetite-suppressing action of α-melanocyte-stimulating hormone (α-MSH). AgRP, like NPY, stimulates feeding and is elevated in both leptin-deficient *ob*/*ob* and leptin-resistant *db*/*db* mice, and its overexpression in transgenic mice leads to obesity (Ollmann *et al.* 1997). During fasting, AgRP gene expression and peptide release from the hypothalamus increases, but these effects can be reversed by leptin infusion (Mizuno & Mobbs 1999; Korner *et al.* 2001). Furthermore, an *in vitro* inhibitory effect of leptin perfusion on AgRP peptide release from hypothalamic slices has been observed in fed rats (Breen *et al.* 2005).

Other orexigenic peptides, such as MCH and orexin A, synthesized in the LHA, are probably inhibited by leptin. MCH is overexpressed in the hypothalamus of leptin-deficient *ob*/*ob* mice (Qu *et al.* 1996) and in rats where hypoleptinaemia has been induced by food-deprivation (Presse *et al.* 1996). Leptin replacement blunts increases in MCH mRNA levels in *ob*/*ob* mice and fasted mice (Tritos *et al.* 2001). Interestingly, ablation of the MCH gene in the *ob*/*ob* mouse results in a marked reduction in body fat, and the leanness of such combined leptin- and MCH-knockouts is a consequence of a substantial increase in energy expenditure, rather than through an attenuation of hyperphagia. These observations suggest that MCH might integrate energy homeostasis downstream of leptin (Segal-Lieberman *et al.* 2003). Orexin neurons co-express leptin receptors (Funahashi *et al.* 2000) and express STAT3, the transcription factor for leptin signal transduction. Orexin A induces feeding (Edwards *et al.* 1999) but hyperphagia is transient (Haynes *et al.* 1999), and leptin, when given peripherally, decreases orexin A levels in the LHA (Beck & Richy 1999).

The endocannabinoid system is also considered to be inhibited by leptin since acute treatment with the hormone decreases the hypothalamic endogenous cannabinoids, anandamide and 2-arachidonoyl glycerol, in normal rats as well as in *ob*/*ob* mice (Di Marzo *et al.* 2001).

Anorexigenic systems are, in contrast, stimulated by leptin. POMC is the precursor of α-MSH, an important appetite regulator that acts through binding to the melanocortin receptor family. POMC is synthesized in the ARC and in the nucleus of the solitary tract. In the ARC, approximately 30% of the POMC-expressing neurons carry the Ob-Rb receptor (Cheung *et al.* 1997). Intraperitoneal leptin administration upregulates hypothalamic POMC mRNA (Schwartz *et al.* 1997), while situations associated with a fall in leptin, such as fasting or the loss of the leptin signal (*ob*/*ob* mouse and *fa*/*fa* rat), show decreased POMC mRNA levels (Mizuno *et al.* 1998). Leptin therefore appears to activate POMC neurons, probably resulting in elevated α-MSH production, thereby inhibiting food intake via its interactions with MC4-R and/or MC3-R.

CART is found to be co-expressed with α-MSH in the lateral ARC (Elias *et al.* 1998; Kristensen *et al.* 1998), and neurons expressing CART are also present in the LHA and PVN (Couceyro *et al.* 1997). Food-deprivation induces a pronounced decrease in CART mRNA within the ARC, while peripheral administration of leptin stimulates CART gene expression in *ob*/*ob* mice (Kristensen *et al.* 1998). Second order neurons, such as those synthesizing CRH in the PVN, are controlled indirectly by leptin targets in the ARC, and mediate the inhibitory effects of leptin on food intake, and the stimulation of thermogenesis and neuroendocrine secretions (Ahima *et al.* 2000). Thus POMC, CART and CRH are each upregulated by leptin.

The net effect from these interactions of leptin with central neuroendocrine systems is that there is a powerful, integrated peripheral signal which serves to suppress food intake (figure 3).

(e) *Electrophysiological effects of leptin*

The initial studies on the central effects of leptin focused on the expression of neuropeptide genes and on the level of the encoded proteins. Recent studies using electrophysiological techniques have demonstrated effects of the hormone on hypothalamic neurotransmission. Fasting significantly increased the basal spike frequency of ARC NPY/AgRP neurons in mice, whereas treatment with leptin induced a dose-dependent decrease in firing frequency in these fasted-animals (Takahashi & Cone 2005), in agreement with the proposed role of leptin as a starvation signal. In leptin deficient and resistant mice (*ob*/*ob* and *db*/*db*, respectively), NPY/AgRP neuron spike frequency is increased analogous to fasting, which may underlie their hyperphagic phenotype (Takahashi & Cone 2005).

(f) *Leptin and the development of hypothalamic feeding circuits*

Recent observations have indicated a novel regulatory role of leptin in neuronal plasticity in hypothalamic neurons that regulate appetite. These studies, using electrophysiological approaches, have revealed leptin-mediated links between nutrition and neuro-development by demonstrating that the lack of leptin in *ob*/*ob* mice increases the number of excitatory synapses and decreases the inhibitory inputs on NPY/AgRP neurons while exerting opposite effects on POMC neurons (Pinto *et al.* 2004). It is likely that leptin can modulate both synapse number and the activity of cells, and this is supported by the observation that leptin repletion in

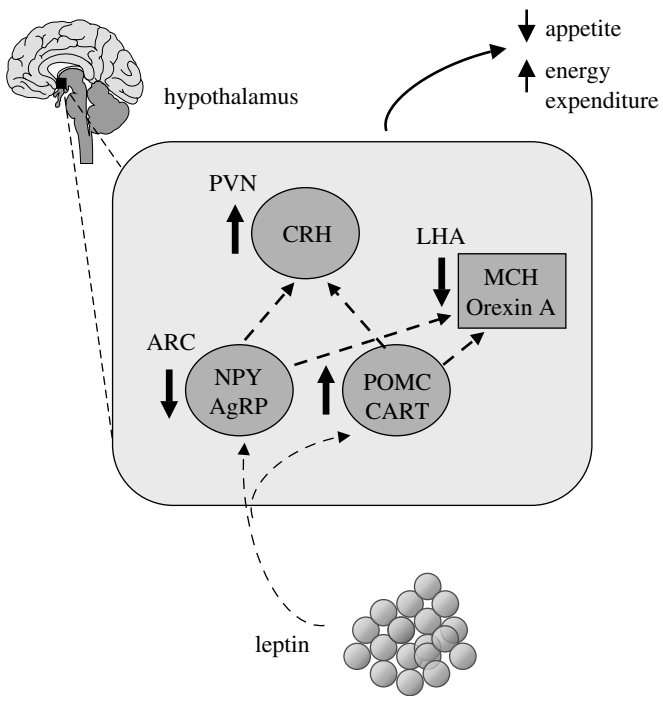

Figure 3. Schematic view of the integrated signalling effect of leptin on appetite through the central hypothalamic neuroendocrine pathways. AgRP, agouti-related peptide; CART, cocaine- and amphetamine-regulated transcript; CRH, corticotrophin releasing hormone; MCH, melanin concentrating hormone; NPY, neuropeptide Y; POMC, pro-opiomelanocortin.

ob/*ob* mice rapidly reverses both electrophysiological and axosomatic characteristics.

As mentioned earlier, ARC neurons have extensive connections with other hypothalamic nuclei, including the PVN, DMH and LHA, each of which is involved in appetite regulation. A recent study, using a lipophilic tracer that labels axonal projections, has shown that leptin deficiency causes profound and permanent disruption in the development of ARC projections to all three nuclei (Bouret *et al.* 2004). Interestingly, leptin replacement to neonatal *ob*/*ob* mice restored the density of projections, but failed to normalize fibre density in adult *ob*/*ob* mice, indicating a critical timing in leptin guidance in the development of hypothalamic neurons.

(g) *The leptin system and obesity*

It has been widely supposed that the administration of exogenous leptin, thereby increasing the circulating level of the hormone, should inhibit food intake and serve as an approach to obesity therapy. However, little success has been obtained through this approach and the concept of leptin resistance has been introduced. Whether there is resistance in any meaningful sense is open to debate (Arch *et al.* 1998). Certainly, there is not an unlimited response to leptin and there is no doubt that mutations in the leptin receptor result in a 'resistance' to the action of the hormone. This is evident in mutant rodents—the *db*/*db* mouse and the *fa*/*fa* rat—as indicated earlier, as well as in humans with a leptin receptor mutation (Chua *et al.* 1996; Lee *et al.* 1996; Clément *et al.* 1998). It seems increasingly likely, however, that in terms of energy balance only a small level of leptin is required with the appetite effects plateauing at, or below, normal physiological levels (Leibel 2002).

The increases in leptin that occur in obesity could primarily reflect the fact that there is an expansion of the major tissue in which the hormone is produced, rather than relate to an attempt to overcome a putative resistance. If the levels of circulating leptin physiologically are much higher than is required for the satiety effect, this may reflect the fact that there are many actions of leptin outwith appetite control. In practice, leptin is a pleiotropic hormone and a large number of effects have been described (Trayhurn *et al.* 1999; Harris 2000). These include as a signal in angiogenesis, immunity, reproduction and in insulin secretion (Barash *et al.* 1996; Chehab *et al.* 1997; Pallett *et al.* 1997; Bouloumie *et al.* 1998; Lord *et al.* 1998; Sierra-Honigmann *et al.* 1998; Morton *et al.* 1999). A broad range of leptin actions is consistent with the widespread distribution of receptors for the hormone in organs not related directly to appetite and energy balance.

(h) *Leptin and the sympathetic nervous system*

We have mentioned earlier the link between the sympathetic nervous system and white adipose tissue in terms of the control of leptin production, the sympathetic system providing a feedback loop from the brain to the tissue (Trayhurn *et al.* 1998; Rayner & Trayhurn 2001; Mark *et al.* 2003). The interaction between the hormone and the sympathetic system is, however, bi-directional with leptin having been shown to stimulate the sympathetic output to several tissues; these tissues include the kidneys, brown adipose tissue and white fat (Haynes *et al.* 1997*a*,*b*; Mark *et al.* 2003).

The general view has long been that white adipose tissue, in contrast to brown fat, is sparsely innervated by the sympathetic system (Youngström & Bartness 1998; Bartness *et al.* 2005). However, the extent and

importance of the sympathetic innervation is now recognized, with the sympathetic system playing a key role physiologically in the control of lipolysis (Youngström & Bartness 1998; Bartness *et al.* 2005). It has also been proposed that adipose tissue contains a parasympathetic innervation (Kreier *et al.* 2002), but the proposition is somewhat controversial and the evidence has not yet been substantiated (Bartness 2002). The sympathetic origins of white adipose tissue have been elegantly explored by Bartness and colleagues using pseudorabies virus as a retrograde tract tracer (Bartness & Bamshad 1998; Bamshad *et al.* 1998; Bartness *et al.* 2005). A number of areas of the brain have been identified from which the sympathetic innervation to adipose tissue (both brown and white) originates, and these include areas within the hypothalamus associated with energy balance regulation. This illustrates the extent to which adipose tissue is under direct central control.

5. PUTATIVE APPETITE SIGNALS FROM ADIPOSE TISSUE

We have commented above on the large number of protein signals and factors which are now recognized to be secreted from white adipocytes. These adipokines include proteins involved in insulin sensitivity (particularly adiponectin), angiogenesis (e.g. vascular endothelial growth factor), lipid metabolism (e.g. cholesteryl ester transfer protein, retinol binding protein), vascular haemostasis (e.g. plasminogen activator inhibitor 1, tissue factor), inflammation (e.g. TNFα, interleukin (IL)-1β, IL-6) and the acute phase response (e.g. haptoglobin, serum amyloid A) (Trayhurn & Beattie 2001; Frühbeck *et al.* 2001; Rajala & Scherer 2003; Trayhurn & Wood 2004; Hauner 2005; Trayhurn 2005*b*). The implication of such a diverse range of proteins being secreted from white fat is that the tissue is involved in extensive cross-talk with other tissues and organs and is intimately involved in general metabolic homeostasis—certainly beyond the basic paradigm of fat storage and release.

There continues to be much interest in whether there are signals from adipocytes in appetite and energy balance beyond leptin. However, the evidence for other factors is as yet limited. Fasting induced adipose factor (FIAF, or angiopoietin-like protein 4) appeared a candidate in that its expression in adipose tissue and the circulating levels were reported to dramatically increase in fasting, responses to food deprivation that are reciprocal to that of leptin itself (Kersten *et al.* 2000). However, it is now evident that FIAF is not always induced by fasting, the response apparently being limited to certain strains of mice. Furthermore, this protein, which like leptin is subject to regulation through the PPARγ nuclear receptor (Kersten *et al.* 2000; Yoon *et al.* 2000), is increasingly implicated in the regulation of lipid metabolism, particularly through the inhibition of lipoprotein lipase (Yoshida *et al.* 2002; Xu *et al.* 2005).

Adiponectin, a complement-like protein produced exclusively by adipocytes, was discovered by several groups who each proposed differing names, including Arp30 and AdipoQ (Scherer *et al.* 1995; Hu *et al.* 1996; Maeda *et al.* 1996). This hormone has been shown to have a wide range of roles, including in insulin sensitivity, inflammation and vascular function (Scherer *et al.* 1995; Ouchi *et al.* 1999; Yokota *et al.* 2000; Berg *et al.* 2001; Yamauchi *et al.* 2001). Adiponectin has strong sequence homology with C1q and types VIII and X collagen (Maeda *et al.* 1996). In contrast to leptin and a number of other adipokines, the circulating level of adiponectin and the adipose tissue expression of the adiponectin gene are inversely related to adiposity (Arita *et al.* 1999; Hotta *et al.* 2000, 2001). Thus adiponectin levels fall with obesity, while increases occur during weight loss induced by a calorie-restricted diet (Arita *et al.* 1999; Hotta *et al.* 2000, 2001).

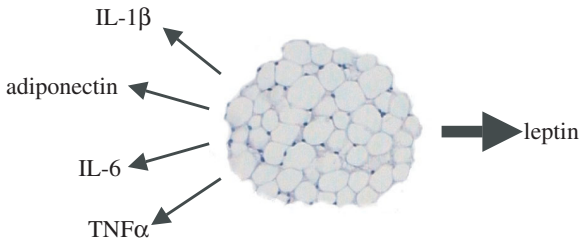

Figure 4. Leptin and putative appetite signals from white adipose tissue. IL, interleukin; TNFα, tumour necrosis factor-α.

Importantly, adiponectin may be a direct signal in appetite and the control of body weight (Shklyaev *et al.* 2003; Qi *et al.* 2004). In addition, peripheral adiponectin administration has been reported to reduce body weight through increased fatty acid combustion and energy dissipation (Berg *et al.* 2001; Yamauchi *et al.* 2001; Tomas *et al.* 2002)—without apparent effects on feeding. However, sustained peripheral expression of transgene adiponectin through a viral vector inhibits food intake and reduces body weight, concomitantly with improved insulin sensitivity and decreased lipid levels, in diet-induced obese rats (Shklyaev *et al.* 2003).

There is some recent evidence that adiponectin may cross the blood–brain barrier (Qi *et al.* 2004), and moreover the cloned adiponectin receptors 1 and 2 have been found to be expressed in the CNS (Yamauchi *et al.* 2003). It is, therefore, proposed that adiponectin could act centrally in the regulation of appetite and energy balance. In this regard, recent work has shown that adiponectin induces *c-fos* immunoreactivity in the PVN, and stimulates hypothalamic CRH synthesis (Qi *et al.* 2004). In addition, agouti ($A^{y/a}$) mice are not responsive to adiponectin, indicating that the melanocortin system might be involved in a central effect of the adipocyte hormone (Qi *et al.* 2004).

Other putative adipose tissue-derived signals in appetite and energy balance include several cytokines, such as IL-1β, IL-6 and TNFα (figure 4). Interleukin-6 is perhaps the most interesting of these candidates since it induces weight loss, as well as producing insulin resistance in adipocytes (Lagathu *et al.* 2003; Rotter *et al.* 2003). IL-6 is expressed together with its receptor in neurons of hypothalamic nuclei that regulate body composition (Shizuya *et al.* 1998), implying a role in the central modulation of energy homeostasis. This has been demonstrated in IL-6 knockout mice that develop

late-onset obesity with abnormal carbohydrate and lipid metabolism (Wallenius *et al.* 2002*b*). Chronic ICV administration of IL-6 reduces body fat through upregulation of energy expenditure without causing an acute phase reaction (Wallenius *et al.* 2002*a*).

Interleukin-1β and TNFα have been implicated in the anorexia of disease; these cytokines are also produced by adipose tissue. TNFα synthesis in white fat is increased in obesity in rodents and in humans (Hotamisligil *et al.* 1995; Kern *et al.* 1995). TNFα inhibits lipoprotein lipase activity, decreases insulin receptor tyrosine kinase activity and stimulates lipolysis in adipose tissue (Kern 1997). ICV infusion of IL-1β or TNFα inhibits feeding (Plata-Salaman *et al.* 1996; Sonti *et al.* 1996), while chronic peripheral administration is usually associated with tolerance to their anorectic effects (Weingarten *et al.* 1992). There is, however, a conceptual difficulty in invoking a specific role in appetite for classical inflammation-related factors such as IL-6 and TNFα; these proteins are released by a range of cells and tissues (including skeletal muscle in severe exercise), and as such it is difficult to attribute to them a specific role as adipocyte-derived signals in food intake and energy balance.

6. PERSPECTIVES

Leptin is undoubtedly a powerful peripheral signal from adipose tissue to the brain in the long-term control of appetite and energy balance. It has not, however, so far proved possible to convincingly titrate small or rapid fluctuations in circulating leptin levels with specific changes in appetite or food intake. Leptin is synthesized in brown, as well as white, adipocytes (Deng *et al.* 1997), but whether this is significant in terms of signalling to appetite is unclear. Given the limited importance of brown adipose tissue quantitatively relative to white fat, particularly in humans, it is unlikely that brown adipocytes are a significant contributor to the circulating levels of the hormone. As with other cells outwith white adipose tissue, it is possible that the role of leptin synthesized and released by brown adipocytes is local rather than endocrine.

The extent to which there are important signals from adipocytes to the brain in the control of intake, additional to leptin, is still uncertain, although indirect effects via peripheral actions on other hormones may occur. An example of a signal indirect to adipocytes is insulin. The circulating levels of insulin, secreted of course not from adipocytes but by pancreatic β-cells, are generally elevated in obesity as a consequence of insulin resistance, and this hormone has long been invoked as a signal to the brain in the control of energy balance and fat stores (Schwartz *et al.* 1992, 2000; Porte *et al.* 2005). Indeed, insulin enters the brain and acts so as to reduce food intake (Schwartz *et al.* 2000).

The discovery of leptin has resulted in a major change in perspectives on the biological role of white adipose tissue—as a key endocrine and secretory organ (Trayhurn & Beattie 2001; Frühbeck *et al.* 2001; Rajala & Scherer 2003; Trayhurn & Wood 2004). From the wide range of adipokines now recognized to be secreted from white adipocytes it is evident that adipose tissue is tightly integrated into overall metabolic control and communicates extensively with other organs and cell types. Signalling to appetite and energy balance is but one—albeit critically important—component of the physiological role of white adipose tissue. The significance of adipose tissue in homeostatic control is underscored by the sheer size of the organ and its major impact on overall body composition.

A question of growing importance in adipose tissue biology is the extent of cross-talk between mature adipocytes and the other cell types within the organ. The issue has been highlighted by the recent reports that white fat becomes infiltrated by macrophages during the development of obesity, with the macrophages amplifying the inflammatory response within the expanding tissue (Weisberg *et al.* 2003; Xu *et al.* 2003). This almost certainly involves the release of inflammatory cytokines and associated factors from macrophages which modulate adipocyte function, as well as the secretion of chemokines such as monocyte chemoattractant protein 1 and migration inhibitory factor from mature fat cells which in turn will act as attractant signals to the macrophages. Conversations between different cell types within adipose tissue will, in all probability, extend beyond mature adipocytes and macrophages, and will involve pre-adipocytes in particular. A key question is the extent to which cellular cross-talk, especially between macrophages and mature adipocytes, is likely to modify the release of appetite-related signals from adipose tissue—and this may be particularly relevant to IL-6 and TNFα in the anorexia of cachexia.

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