Abstract
The discovery of leptin has provided a robust framework upon which our current understanding of the mechanisms involved in energy homeostasis has been built. In this review, we describe how the identification of humans with mutations in the genes encoding leptin and the leptin receptor and the characterisation of the associated clinical phenotypes have provided insights into the role of leptin-responsive pathways in the regulation of eating behaviour, intermediary metabolism and the onset of puberty. Importantly, administration of recombinant human leptin in leptin deficiency represents the first mechanistically based targeted therapy for obesity and has provided immense clinical benefits for the patients concerned. In subsequent years, we and others have shown that human obesity can result from a multiplicity of defects in the pathways downstream of leptin signalling within the brain.

Key Words
- leptin
- receptors
- obesity
- signal transduction

Discovery of mutations in leptin and the leptin receptor in humans
Early studies carried out in obese humans showed that leptin mRNA concentrations in adipose tissue and serum leptin concentrations correlated positively and very closely with the amount of fat mass (Maffei et al. 1995, Considine et al. 1996). In 1997, we studied two severely obese cousins from a highly consanguineous UK family of Pakistani origin, in whom the known central and endocrine causes of obesity had been excluded (Montague et al. 1997). Being aware of Friedman’s work (Zhang et al. 1994), we measured the serum leptin concentration in these children and found that they had undetectable leptin concentrations despite their severe obesity and were homozygous for a frameshift mutation in the LEP gene, which resulted in a truncated protein that was not secreted (Montague et al. 1997). In 1998, three adults carrying a homozygous missense mutation in the LEP gene were reported in a family of Turkish origin (Strobel et al. 1998) and since that time, several other affected individuals from consanguineous families (Farooqi et al. 2002, Mazen et al. 2009, Frank et al. 2011, Saeed et al. 2012; and I S Farooqi, unpublished observations) have been studied. Clement et al. (1998) identified the first patients with homozygous mutations in the gene encoding the leptin receptor (LEPR) in 1998. Additional homozygous frameshift, nonsense and missense LEPR mutations have been identified in ~2–3% of severely obese patients from consanguineous families (Farooqi et al. 2007a, Mazen et al. 2011). LEPR mutations have been found in some non-consanguineous families, where both parents were unrelated but carried rare heterozygous alleles that result in a loss of function (Farooqi et al. 2007a).

Analysis of serum leptin levels is a useful test in patients with severe early-onset obesity because an undetectable serum leptin level is highly suggestive of a diagnosis of congenital leptin deficiency. It is plausible, that mutations in the LEP gene could result in a...
bio-inactive form of the hormone in the presence of apparently appropriate leptin levels; however, no such cases have been reported to date and, based on comparable hormone deficiency syndromes, if they do exist, such patients are likely to be very rare.

Serum leptin concentrations are appropriate for the degree of obesity in LEPR-deficient patients and as such an elevated serum leptin concentration is not necessarily a predictor of LEPR deficiency (Farooqi et al. 2007a). However, in some patients, particularly LEPR mutations that result in abnormal cleavage of the extracellular domain of LEPR (which then can act as a leptin-binding protein) are associated with markedly elevated leptin levels (Clement et al. 1998, Lahlou et al. 2000).

Characteristic clinical features seen in leptin and LEPR deficiency

The clinical phenotypes associated with leptin and LEPR deficiencies are broadly similar (Montague et al. 1997, Ozata et al. 1999, Farooqi et al. 2007a). Patients are born of normal birth weight but exhibit rapid weight gain in the first few months of life, resulting in severe obesity (mean BMI SDS: 5.8–7.8). The patients often have a distinctive clinical appearance with excessive amounts of subcutaneous fat over the trunk and limbs. Body composition measurements have shown that these disorders are characterised by the preferential deposition of fat mass; indeed the mean percentage body fat among homozygous carriers of LEP mutations is very high at 58% (compared with 45% for equally obese children of the same age (Farooqi et al. 2007a)).

In the clinical history, early development is usually normal. The most notable feature is intense hyperphagia with food-seeking behaviour and aggressive behaviour when food is denied. In the research setting, measurements of energy intake at ad libitum test meals reveal the extent of hyperphagia with food intake three to five times that of children of the same age with both an increase in hunger and impaired satiety seen after meals of fixed quantity and composition (Farooqi et al. 2002). Increased food-seeking behaviour continues into later life in the adult subjects who have been reported (Ozata et al. 1999). Children with leptin deficiency have marked abnormalities of T cell number and function (Farooqi et al. 2002), consistent with high rates of childhood infection and a high reported rate of childhood mortality from infection (Ozata et al. 1999). In those who survive, obesity continues in adult life, with hepatic steatosis (von Schurbein et al. 2013) and hyperinsulinaemia consistent with the severity of obesity. Some adults have developed type 2 diabetes in the third to fourth decade (Strobel et al. 1998).

Leptin and LEPR deficiency are associated with hypothalamic hypothyroidism characterised by a low free thyroxine (T₄) and a high serum thyroid-stimulating hormone (TSH) levels which are bio-inactive (Farooqi et al. 2002). The pulsatility of TSH secretion, studied in a single adult with congenital leptin deficiency, was characterised by a markedly disorganised secretory pattern (Mantzoros et al. 2001). Generally, normal pubertal development does not occur in adults with leptin or LEPR deficiency (Strobel et al. 1998), with biochemical evidence of hypogonadotropic hypogonadism (von Schnurbein et al. 2012). However, there is some evidence for the delayed but spontaneous onset of menses in a small number of leptin and LEPR-deficient adults (Farooqi et al. 2007a); the mechanisms underlying this are unclear. Linear growth is appropriate in childhood; however, due to the absence of a pubertal growth spurt, final height is reduced.

Response to leptin administration in leptin deficiency

Although leptin deficiency is rare, it is entirely treatable with daily s.c. injections of recombinant human leptin (Farooqi et al. 1999, Licinio et al. 2004). Such treatment is currently available to patients on a named-patient basis. In 1997, we started the first clinical trial administering daily injections of recombinant human leptin to leptin-deficient patients, (Farooqi et al. 1999) with the support of Amgen, Inc. (Thousand Oaks, CA, USA) and subsequently Amylin Pharmaceuticals, Inc. (San Diego, CA, USA) and Bristol Myers Squibb/AstraZeneca. Recombinant leptin therapy led to remarkable beneficial effects for the leptin-deficient patients (Fig. 1) and provided proof of principle for the pivotal role of leptin action in humans (Farooqi et al. 1999, 2002). Studies carried out in patients with congenital leptin deficiency before and after treatment with leptin paved the way for understanding the major actions of leptin in humans, which have been supported and extended by elegant studies carried out by many investigators in normal weight and obese volunteers in the context of fasting or a weight-reduced state (Rosenbaum et al. 2002, 2005) and in patients with lipodystrophic syndromes characterised by partial leptin deficiency due to a loss of adipose tissue mass (Oral et al. 2002).
Energy intake

The major effect of leptin administration in leptin deficiency is on food intake with normalisation of hyperphagia (Farooqi et al. 1999). Leptin therapy reduced energy intake during an 18 MJ ad libitum test meal by up to 84% (Farooqi et al. 2002) and was associated with reduced hunger scores with no change in satiety in adults with leptin deficiency (Licinio et al. 2004). We also observed that as well as severe hunger, patients with leptin deficiency like all types of food, even foods that other children of a similar age would usually find unappetising. Notably, this behaviour changed within 7 days of leptin administration, and patients were able to discriminate more readily between foods they liked and did not like. To investigate whether leptin might be involved in the mediation of the rewarding properties of food, working with Paul Fletcher in Cambridge, we designed a study to examine brain activation responses in patients with leptin deficiency before and 7 days after leptin treatment. We used functional magnetic resonance imaging to measure changes in blood flow, which reflect changes in neural activation, in response to the visual presentation of food pictures compared with pictures of non-food items, while in the scanner (Farooqi et al. 2007b). We used 10-cm visual analogue scores to rate hunger, satiety and liking ratings for food images; to examine the interaction with eating, we studied two subjects in the fasted state and after eating (Farooqi et al. 2007b). In the leptin-deficient state, images of food (compared with non-food items) were associated with a marked increase in neuronal activation in the anteromedial ventral striatum (nucleus accumbens and caudate nucleus) and the posterolateral ventral striatum (putamen and globus pallidus), which are areas associated with pleasure and reward. This response was normalised by 7 days of leptin treatment, before weight loss occurred. When asked to rate how much they liked each of the food images, leptin-deficient subjects gave high ratings to all food images in both the fasted and fed states (Farooqi et al. 2007b). After 7 days of leptin treatment, the liking ratings were reduced, in keeping with our previous behavioural observations. These behavioural responses were accompanied by a region-specific change in the pattern of brain activation (Farooqi et al. 2007b). In the leptin-deficient state, nucleus accumbens activation correlated positively with liking ratings in the fasted and fed states. In the leptin-treated state, nucleus accumbens activation correlated positively with liking ratings only in the fasted state, an effect that was also seen in normal weight controls who were studied using the same paradigm. Thus, as well as having profound effects on hunger and satiety, leptin administration results in an increased ability to discriminate between the rewarding properties of food and, at the neural level, in the modulation of activation in the ventral striatum. Our findings are consistent with the view that activation in the ventral striatal region does not directly encode the liking but rather the motivational salience or desire for food. Studies by Rosenbaum et al. (2008) in obese volunteers in an energy-restricted, partially leptin-deficient state are consistent with the view that these responses are part of the physiologic response to energy restriction.

Energy expenditure

Although leptin plays a key role in thermogenesis in rodents, we were unable to demonstrate a major acute effect of leptin administration on basal metabolic rate as measured by indirect calorimetry, total energy expenditure using chamber calorimetry, or free-living energy expenditure using the doubly-labelled water method in leptin-deficient humans after adjusting for body composition (Farooqi et al. 2002). However, as weight loss by other means is associated with a decrease in basal metabolic rate, the fact that energy expenditure did not fall in leptin-deficient subjects is an unusual finding.

Figure 1
A 3-year-old boy with congenital leptin deficiency, weighing 42 kg before (left) and 32 kg after (right) 4 years of treatment with recombinant leptin therapy.
In keeping with these findings, Ravussin et al. showed that before weight loss, leptin-deficient adults and matched controls had similar energy expenditures (Galgani et al. 2010). Whilst energy expenditure did not change after leptin-deficient patients were treated with leptin, controls who lost a comparable amount of weight on an energy-restricted diet had lower energy expenditures than expected for their new weight and body composition (Galgani et al. 2010). This response is often referred to as the metabolic adaptation to weight loss, i.e. a decrease in metabolic rate beyond that expected on the basis of the decrease in fat-free mass and fat mass. Furthermore, Ozata et al. (1999) reported abnormalities of sympathetic nerve function in leptin-deficient adults. Cumulatively, these findings are consistent with defects in the efferent sympathetic limb of thermogenesis. Body composition measurements show that leptin deficiency is characterised by the preferential deposition of fat mass (compared with lean mass), and weight loss leads to a preferential loss of fat mass (Faroopi et al. 1999). In rodents, leptin stimulates fatty acid oxidation in skeletal muscle via the stimulation of AMP kinase activity (Minokoshi et al. 2002). In leptin-deficient adults, impaired fat oxidation has been measured by chamber calorimetry (Galgani et al. 2010).

Regulation of neuroendocrine function

Also relevant to the regulation of energy expenditure are the changes in thyroid function that are seen in patients with leptin deficiency. In children, there were small, but sustained, increases in free T₄ and tri-iodothyronine and TSH levels, which occurred within 2 months of the commencement of leptin therapy (Faroopi et al. 2002). One patient had substantial elevation of TSH levels before treatment, such that T₄ therapy was commenced but was discontinued when thyroid function tests normalised after leptin treatment (Gibson et al. 2004). Evidence from rodents studies suggests that leptin is necessary for the normal biosynthesis and secretion of thyrotropin-releasing hormone (Nillni et al. 2000, Harris et al. 2001).

The administration of leptin permits progression of appropriately timed pubertal development, suggesting that leptin is a permissive factor for the development of puberty in humans (Faroopi et al. 1999, 2002). In adults with leptin deficiency (Licinio et al. 2004), leptin induced the development of secondary sexual characteristics and pulsatile gonadotrophin secretion. Leptin may exert these effects on the reproductive system through a number of molecules including kisspeptin, which signals through GPR54, to modify the release of gonadotrophin-releasing hormone, and through LEPR expressing neural pathways involving the ventral premamillary nucleus. In adults with leptin deficiency, leptin induced the development of secondary sexual characteristics and pulsatile gonadotrophin secretion (Licinio et al. 2004). However, there is some evidence for the delayed but spontaneous onset of menses in some LEPR-deficient adults who had oestradiol, luteinising hormone, and follicle-stimulating hormone concentrations that were consistent with their age (Faroopi et al. 2007a). It is plausible that the excess adipose tissue mass leads to the production of sufficient oestrogen (due to the action of aromatase) to result in uterine development and irregular menses in the absence of fully developed secondary sexual characteristics.

Regulation of immunity

Leptin stimulates inflammatory responses, T lymphocyte proliferation and Th1 cytokine production during fasting in normal mice and in Ob/Ob mice, indicating that it is an important link between nutrition and the immune system. Recent studies have shown that leptin is also involved in the mediation of the systemic response to sepsis (Tschop et al. 2010). Patients with leptin deficiency have an increased frequency of infections and marked abnormalities of T cell number and function in vitro, which are normalised with leptin treatment (Faroopi et al. 2002). The multiple effects of leptin on innate and adaptive immunity suggest that immunomodulation by leptin may have therapeutic potential in a range of diseases (Tschop et al. 2010).

Mutations disrupting leptin signalling

Although serum leptin concentrations correlate positively with fat mass, there is considerable inter-individual variation at any particular fat mass (Maffei et al. 1995). Leptin is relatively low in some obese individuals (Ravussin et al. 1997) who may be responsive to leptin therapy, although this has not formally been tested. One key question with respect to the potential therapeutic use of leptin in subgroups of people with more common forms of obesity relates to the nature of the dose–response curve for leptin. We studied the heterozygous relatives of leptin-deficient patients and showed that they had partial leptin deficiency (lower leptin levels for a given BMI), and an increased percentage body fat compared with controls of the same ethnicity and BMI (Faroopi et al. 2001). These findings are consistent with the findings in heterozygous Ob and Db mice (Coleman 1979). These data suggest the
possibility that leptin may produce a graded response in terms of changes in fat mass across a broad range of plasma leptin concentrations. All heterozygous subjects had normal thyroid function and appropriate gonadotropins, normal development of secondary sexual characteristics, normal menstrual cycles and fertility, which suggest that their low leptin concentrations were sufficient to preserve these functions (Farooqi et al. 2001). Ob/Ob mice modified by transgenesis to constitutively secrete low levels of leptin continued to be obese, but did not show the neuroendocrine features of leptin deficiency (Ioffe et al. 1998).

Mutations in molecules involved in leptin signalling

Leptin mediates its effects on body weight and neuroendocrine axes by binding to the long form of the LEPR (LEPRb) and activating receptor-associated JAK2. JAK2 phosphorylates multiple tyrosines in LEPRb, enabling the recruitment of downstream effectors. JAK2 also autophosphorylates on Tyr813, allowing the binding of the adapter protein Src homology 2 (SH2) B adapter protein 1 (SH2B1), which enhances JAK2 activation and helps to recruit insulin receptor substrate 1 (IRS1) and IRS2 to the LEPRb/JAK2 complex (Maures et al. 2007). This facilitates JAK2-mediated tyrosine phosphorylation of IRS1/2 and subsequent activation of the phosphoinositide 3-kinase pathway. SH2B1 is a key endogenous positive regulator of leptin sensitivity. Targeted deletion of Sh2b1 in mice results in impaired leptin signalling and severe obesity (Ren et al. 2007). Sh2b1-null mice are also insulin resistant and exhibit impaired insulin signalling (Morris et al. 2009). We have found that deletion of a 220-kb segment of 16p11.2, which includes SH2B1 (Bochukova et al. 2010) and mutations in the gene itself (Doche et al. 2012), is associated with highly penetrant familial severe early-onset obesity which may in part be due to altered leptin signalling. However, SH2B1 modulates signalling by a variety of ligands that bind to receptor tyrosine kinases or JAK-associated cytokine receptors, including insulin, growth hormone (GH) and nerve growth factor (Chua 2010); as such mutations in the SH2B1 gene have been associated with additional phenotypes including severe insulin resistance and behavioural abnormalities in some patients (Doche et al. 2012).

![Diagram of the leptin signalling pathway](image)

**Figure 2**

A schematic of the leptin signalling pathway. Leptin is secreted by adipose tissue, circulates in the bloodstream to act on neurons in the arcuate nucleus of the hypothalamus which express the signalling form of the leptin receptor. SH2B1 is involved in leptin signalling. Activation of the leptin receptor leads to phosphorylation and activation of the transcription factor STAT3, which dimerises and translocates to the nucleus where it activates POMC gene transcription. POMC is post-translationally processed to yield the melanocortin peptides, which act on MC4R, located on the surface of neurons in the paraventricular nucleus of the hypothalamus. Activation of signalling through MC4R leads to a reduction in food intake. *Genes in which mutations are associated with severe early onset obesity.
Disorders affecting melanocortin signalling

Leptin stimulates primary neurons in the arcuate nucleus of the hypothalamus which express pro-opiomelanocortin (POMC) which is post-translationally processed to yield the melanocortin (MC) peptides, which are agonists at MC3 receptor (MC3R) and MC4 receptor (MC4R) (Fig. 2; see chapter Myers). In addition, leptin inhibits neurons expressing the MC antagonist agouti-related protein and neuropeptide Y (NPY); NPY can suppress the expression of POMC. These primary leptin-responsive neurons project to second-order neurons expressing the MC4R (Barge-Schaapveld et al. 2011). Targeted genetic disruption of MC4R in mice leads to the lack of leptin and the LEPR (Huszar et al. 1997). These studies established the importance of MC signalling in the mediation of some, but not all, of the effects of leptin in the brain.

In humans, disruption of POMC results in hyperphagia and early-onset obesity due to loss of MC signalling at MC4R (Krude et al. 1998). As POMC is a precursor of adrenocorticotrophin (ACTH) in the pituitary, children who are homozygous or compound heterozygous for loss of function mutations in POMC present in neonatal life with hypoglycaemia, cholestatic jaundice or other features of adrenal crisis due to ACTH deficiency and require long-term corticosteroid replacement therapy (Krude et al. 2003). Such children have pale skin, and white Caucasians have red hair, due to the lack of MC function at MC1Rs in the skin. Although red hair may be an important diagnostic clue in patients of Caucasian origin, children from different ethnic backgrounds may have a less obvious phenotype such as dark hair with red roots (Farooqi et al. 2006). A failure of normal production of alpha and or β-MSH from its larger POMC precursor is a likely contributor to the obese, pale skinned phenotype of patients lacking the neuroendocrine-specific pro-protein convertase PCSK1 (Jackson et al. 1997). Elevated plasma levels of proinsulin and 32–33 split proinsulin in the context of low levels of mature insulin provide the basis for a diagnostic test for this disorder.

Heterozygous MC4R mutations have been reported in obese people from various ethnic groups. Prevalence estimates range from 0.5 to 2.5% of people with a BMI > 30 kg/m² in UK and European populations to 5% in patients with severe childhood obesity (Vaisse et al. 2000, Farooqi et al. 2003). As MC4R deficiency is the most common genetic form of obesity, assessment of the sequence of the MC4R is increasingly seen as a necessary part of the clinical evaluation of the severely obese child. Other genetic and environmental modifiers can affect the degree of obesity associated with MC4R mutations in some pedigrees (Stutzmann et al. 2008), as such co-dominance, with modulation of expressivity and penetrance of the phenotype, seems an appropriate descriptor for the mode of inheritance (Farooqi et al. 2003).

The clinical features of MC4R deficiency include hyperphagia, which often starts in the first few years of life. Alongside the increase in fat mass, MC4R-deficient subjects also have an increase in lean mass and a marked increase in bone mineral density, thus they often appear ‘big-boned’ (Farooqi et al. 2003). They exhibit accelerated linear growth in early childhood, which may be a consequence of disproportionate early hyperinsulinaemia, and effects on pulsatile GH secretion, which paradoxically is retained in MC4R-deficient adults in contrast to more common forms of obesity (Martinelli et al. 2011). Reduced activity of sympathetic nervous system in MC4R-deficient patients is likely to explain the lower prevalence of hypertension and lower systolic and diastolic blood pressures (Greenfield et al. 2009). Thus, central MC signalling appears to play an important role in the regulation of blood pressure and its coupling to changes in weight.

Summary

Finally, the discovery of leptin, and the central pathways involved in energy homeostasis that it regulates, has paved the way for the understanding of the regulation of human energy homeostasis. Studies in patients with mutations which disrupt leptin action (Fig. 2) have demonstrated the relevance of studies in rodents for understanding the complex aetiology of human body weight regulation and of clinically significant disorders such as obesity. Studies in these patients, all of whom are characterised by hyperphagia, have demonstrated the importance of leptin in the regulation of human appetite and demonstrated the biological underpinnings of eating behaviour. The practical implications of these findings for genetic counseling and in some cases therapy are beginning to emerge. However, further studies in humans and mice are needed to understand the detailed neural networks by which leptin exerts multiple effects on physiology and behaviour; mechanisms which may subsequently be targeted for therapeutic benefit for patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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Thematic Review

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