Leptin: a pivotal regulator of human energy homeostasis

I Sadaf Farooqi and Stephen O’Rahilly

ABSTRACT
The identification of the hormone leptin by Friedman et al (1) in 1994 has proved to be a seminal observation in biomedical science. The discovery that a circulating protein secreted almost exclusively by adipocytes could regulate body weight through its effects on food intake and energy expenditure represented a remarkable breakthrough in our understanding of the molecular components of the systems involved in energy homeostasis. In this article, we describe how the identification of humans with mutations in the gene encoding leptin and the characterization of the associated clinical phenotype of congenital leptin deficiency, which includes hyperphagia, severe obesity, hypogonadism, and impaired immunity, has provided insights into the role of leptin-responsive pathways in the regulation of eating behavior, intermediary metabolism, and the onset of puberty. We and others have also been able to demonstrate that leptin signaling plays a critical role in the regulation of reproductive and immune function in humans, which places leptin at the center of the complex networks that coordinate changes in nutritional state with many diverse aspects of mammalian biology. Am J Clin Nutr 2009;89(suppl):980S–4S.

INTRODUCTION
In 1994 Friedman et al (1) showed that an inbred strain of severely obese mice, ob/ob, harbored mutations in the obese (ob) gene, which resulted in a complete lack of its protein product leptin. Subsequently, 3 independent research groups (led by Friedman, Mary Pelleymounter, and Art Campfield, respectively) went on to show that peripheral administration of recombinant leptin reduced the food intake and body weight of leptin-deficient ob/ob mice and corrected all of their neuroendocrine and metabolic abnormalities (2–4). These effects were replicated by small doses of centrally administered leptin, which suggests that the main effect of leptin was to act as a signal from adipose tissue to the brain regarding the quantity of fat tissue stored (4). Flier and Ahima (5) elegantly showed that reduced leptin also acts as a signal of nutritional deprivation, with low leptin concentrations initiating an adaptive response to conserve energy, which is manifested by increased food intake, decreased energy expenditure, and suppression of the reproductive and certain other endocrine axes. These phenotypes were similar to those seen in the obese leptin-deficient ob/ob mouse, thereby establishing that starvation/fasting could be considered a state of relative leptin deficiency. With the identification of leptin, the molecular and physiologic circuits controlling energy homeostasis could be dissected. Leptin was shown to signal through the long isoform of the leptin receptor, a member of the interleukin-6 receptor family of class 1 cytokine receptors cloned in 1995 by Tartaglia (6). The signaling form of the leptin receptor is deleted in db/db mice, which are consequently unresponsive to endogenous or exogenous leptin (7, 8). Studies by many groups demonstrated that leptin stimulates the expression of pro-opiomelanocortin (POMC) in primary neurons located in the arcuate nucleus of the hypothalamus (9–12). POMC is extensively posttranslationally modified to generate a range of smaller biologically active peptides, such as the melanocortins, which are agonists at melanocortin receptors and mediate an anorectic response (13, 14). Leptin inhibits orexigenic pathways mediated by neurons expressing the melanocortin antagonist Agouti-related peptide and neuropeptide Y (15). Both sets of primary leptin-responsive neurons project to second-order neurons expressing the melanocortin 4 receptor (MC4R) (16). These pathways interact with other brain centers to coordinate appetite and modulate effluent signals to the periphery regulating intermediary metabolism and energy expenditure (these pathways are discussed in more detail in the review by Williams et al in this supplement).

HUMAN CONGENITAL LEPTIN DEFICIENCY
Early studies in obese humans showed that, in general, leptin mRNA concentrations in adipose tissue and serum leptin concentrations correlated positively and closely with fat mass (17, 18). In 1997 we were referred to 2 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. Aware of Friedman’s work (1), we went on to measure serum leptin in these children and found that they had undetectable concentrations despite their severe obesity (19). Both children were homozygous for a frameshift mutation in the LEP gene, which resulted in a truncated protein that was not secreted (20). We have since identified 5 other affected individuals from 4 other families (reference 21 and IS Farooqi and S O’Rahilly, 1 From the University of Cambridge Metabolic Research Laboratories, Institute of metabolic Science, Addenbrooke’s Hospital, Cambridge, United Kingdom.
3 Reprints not available. Address correspondence to IS Farooqi, University of Cambridge Metabolic Research Laboratories, Institute of metabolic Science, Box 289, Addenbrooke’s Hospital, Cambridge CB2 0QQ, United Kingdom. E-mail: isf20@cam.ac.uk
First published online February 11, 2009; doi: 10.3945/ajcn.2008.26788C.
unpublished observations, 2007) who are also homozygous for the same mutation in the gene encoding leptin. All the families are of Pakistani origin but not known to be related over 5 generations. A large Turkish family in which 3 adults carry a homozygous missense mutation (Arg105Trp) in the LEP gene has been described by Ozata et al (22). These patients allowed us to show that leptin is essential for the regulation of body weight in humans as complete deficiency leads to severe obesity. We were privileged to be able to administer daily injections of recombinant human leptin to these patients as part of a clinical trial with the support of Amgen Inc (Thousand Oaks, CA) and subsequently Amylin Pharmaceuticals Inc (San Diego, CA). Recombinant leptin therapy led to remarkable beneficial effects for the patients involved (Figure 1 and provided proof of principle for the pivotal role of leptin action in humans (21, 23). The study of patients with congenital leptin deficiency before and after leptin treatment allowed us to report the major physiologic actions of leptin in humans, which have been supported and extended by the findings from elegant studies by many other investigators in normal weight and obese volunteers in the context of fasting or a weight-reduced state (24–26) and in patients with lipodystrophic syndromes characterized by partial leptin deficiency due to a loss of adipose tissue mass (27) (as reviewed by Blüher and Mantzoros in this supplement).

**PHYSIOLOGIC ACTIONS OF LEPTIN**

**Regulation of energy intake**

Leptin-deficient subjects are born with a normal birth weight but exhibit rapid weight gain in the first few months of life, which results in severe obesity. All leptin-deficient subjects exhibit intense hyperphagia with food-seeking behavior and aggressive behavior when food is denied (21). Increased food-seeking behavior continues into later life in the adult subjects that have been reported (28). We have shown that energy intake at an ad libitum meal is markedly elevated in leptin-deficient subjects with both an increase in hunger and in impaired satiety seen after meals of fixed quantity and composition. The major effect of leptin administration is on food intake with normalization of hyperphagia. Leptin therapy reduced energy intake during an 18 MJ ad libitum test meal by up to 84% (5 MJ ingested pretreatment compared with 0.8 MJ after treatment in the child with the greatest response) (21). Leptin treatment was associated with reduced hunger scores with no change in satiety in adults with leptin deficiency (29).

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 unappetizing, although they do not exhibit pica behavior. We observed that within 7 d of leptin administration this behavior changed and patients were able to discriminate more readily between foods they did and did not like. We wondered whether leptin might be involved in mediating the rewarding properties of food and, working with colleagues Paul Fletcher and Ed Bulmore in Cambridge, United Kingdom, we designed a study to examine the pattern of brain activation in patients with leptin deficiency before and 7 d after leptin treatment. We used functional magnetic resonance imaging scans to measure changes in blood flow, which reflect changes in neural activation, in response to the visual presentation of pictures of food compared with pictures of nonfood items while in the scanner. We used 10-cm visual analog scores to rate hunger, satiety, and the preference for food images; and to examine the interaction with eating, we studied 2 subjects in the fasted state and after eating (30). In the leptin-deficient state, images of food (compared with nonfood 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 normalized by 7 d of leptin treatment. When asked to rate how much they liked each of the food images, leptin-deficient subjects after eating gave high ratings to all food images ($\bar{x} = 8.9 \pm 0.5$). After leptin, the preference ratings were reduced ($\bar{x} = 5.9 \pm 0.4$), which is consistent with our previous behavioral observations. These behavioral responses were accompanied by a region-specific change in the pattern of brain activation (30). In the leptin-deficient state, nucleus accumbens activation correlated positively with preference ratings in the fasted and fed states. In the leptin-treated state, nucleus accumbens activation correlated positively with preference 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 preference but rather the motivational salience or desire for food (30). Recent studies by Rosenbaum and Leibel (31) 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.
Regulation of energy expenditure and fat oxidation

Although in rodents leptin plays a key role in thermogenesis, 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 labeled water method in leptin-deficient humans (21). However, because weight loss by other means is associated with a decrease in basal metabolic rate (32), the fact that energy expenditure did not fall in our leptin-deficient subjects is notable. Furthermore, Ozata et al (28, 29) reported abnormalities of sympathetic nerve function in leptin-deficient adults, which is consistent with defects in the different sympathetic limb of thermogenesis. Also relevant to the regulation of human energy expenditure are the changes in thyroid function that are seen in patients with leptin deficiency. In leptin-deficient children, plasma free-thyroxine concentrations were within the normal range, but 4 children had significantly elevated thyroid-stimulating hormone (TSH) concentrations (21), and the pulsatility of TSH secretion, studied in a single adult with congenital leptin deficiency, was characterized by a markedly disorganized secretory pattern (33). In the 3 previously reported children, there were small, but sustained, increases in free thyroxine and tri-iodothyronine and TSH that occurred ≤1 mo of leptin therapy (21). A fourth patient had substantial elevation of TSH before treatment, such that thyroxine therapy was commenced but was discontinued when thyroid function tests normalized after leptin treatment (34). Evidence from rodents suggests that leptin is necessary for the normal biosynthesis and secretion of thyrotropin-releasing hormone (35, 36) and that complete leptin deficiency is associated with a moderate degree of hypothalamic hypothyroidism characterized by low free-thyroxine and high serum TSH concentrations, which is relatively bio-inactive (37).

Body composition measurements in patients show that leptin deficiency is characterized by the preferential deposition of fat mass. The mean percentage of body fat among homozygous carriers of LEPMutations was high at 58% [compared with 45% for equally obese children of the same age (38)], and weight loss led to a preferential loss of fat mass: 98% compared with 70–75% in common obesity (21). These findings are consistent with a role for leptin in fatty acid oxidation (39). In rodents, leptin stimulates fatty acid oxidation in skeletal muscle via the stimulation of AMP-kinase activity (40, 41).

Leptin as a metabolic gate for the onset of puberty in humans

Leptin deficiency is associated with hypogonadotropic hypogonadism and a failure of normal pubertal development (22). The administration of leptin permits progression of appropriately timed pubertal development in male and female patients of appropriate age (23) and does not cause the early onset of puberty in younger children, which suggests that leptin is a permissive factor for the development of puberty in humans (21). In adults with leptin deficiency, leptin induced the development of secondary sexual characteristics and pulsatile gonadotrophin secretion (29).

However, there is some evidence for the delayed but spontaneous onset of menses in 1 and 3 leptin-receptor-deficient adults who had estradiol, luteinizing hormone, and follicle stimulating hormone concentrations that were consistent with their age (38). It is plausible that the excess adipose tissue mass leads to the production of sufficient estrogen (due to the action of aromatase) to result in uterine development and irregular menses in the absence of fully developed secondary sexual characteristics.

Leptin as a mediator of the nutritional regulation of immune function

Leptin stimulates inflammatory responses, T lymphocyte proliferation, and T helper (Th)-1 cytokine production during fasting in normal mice and in ob/ob mice, which indicates that leptin is an important link between nutrition and the immune system (42, 43). We demonstrated that children with leptin deficiency had an increased frequency of infections and marked abnormalities of T cell number and function in vitro, which were normalized with leptin treatment (21). Congenital leptin deficiency is associated with a predominant Th2 cytokine response, which switches to a Th1 response with leptin administration (21). Several studies of ob/ob mice have shown that leptin deficiency protects against experimental autoimmune encephalomyelitis and other models of autoimmune disease (44). These multiple effects of leptin on innate and adaptive immunity suggest that immunomodulation by leptin may have therapeutic potential in a range of human diseases (45).

LEPTIN ACTION IN COMMON HUMAN OBESITY

The major question with respect to the potential therapeutic use of leptin in more common forms of obesity relates to the shape of the leptin dose-response curve. We have shown that raising leptin concentrations from undetectable to detectable has profound effects on appetite and weight. We studied the heterozygous relatives of our leptin-deficient patients and showed that they had partial leptin deficiency, a higher prevalence of obesity than that seen in a control population of similar age and ethnicity, and an increased percentage of body fat compared with control subjects of the same ethnicity and body mass index (46). These findings are consistent with the findings of Chung et al (47), who demonstrated an increase in fat mass in both ob/+ and db/+ mice. These data provide further support for the possibility that leptin can produce a graded response in terms of changes in fat mass across a broad range of plasma concentrations.

All heterozygous subjects had normal thyroid function and appropriate gonadotropins, normal development of secondary sexual characteristics, normal menstrual cycles, and fertility, which suggests that their low leptin concentrations were sufficient to preserve these functions (46). This is consistent with the data of Ioffe et al (48), who demonstrated that several of the neuroendocrine features associated with leptin deficiency were abolished in low-concentration-leptin transgenic mice, which were fertile with normal corticosterone concentrations. However, these low-concentration-leptin transgenic mice still exhibited abnormal thermoregulation in response to cold exposure and had mildly elevated plasma insulin concentrations, which suggests that there are different thresholds for the various biological responses elicited by changes in serum leptin concentration and that these could be reversed by leptin administration.

Our findings in the heterozygous individuals have some potential implications for the treatment of common forms of obesity. Although serum leptin concentrations correlate positively with fat
mass, there is considerable interindividual variation at any particular fat mass (18). Leptin is inappropriately low in some obese individuals (49), and the relative hypo leptinemia in these subjects, which may actively contribute to their obesity, may be responsive to leptin therapy.

Heymsfield et al (50) administered supraphysiologic doses (0.1–0.3 mg/kg body weight) of leptin to obese subjects for 28 wk. On average, some subjects lost weight, but the extent of weight loss and the variability between subjects has led many to conclude that the leptin resistance of common obesity cannot be usefully overcome by leptin supplementation, at least when administered peripherally. However, it is of interest that there was a significant effect on weight in some subjects with low serum leptin concentrations (50), which suggests that leptin can continue to have a dose-response effect on energy homeostasis across a wide serum concentration range. Recent studies of leptin in combination with other agents suggest that leptin may yet find a role in the treatment of more common forms of obesity (51).

SUMMARY

Although it had been recognized for a long time that the hypothalamus plays a key role in the regulation of energy homeostasis (52) and that body fat status plays an important role in regulating immune and reproductive function in many species (53), the precise molecular mechanisms by which energy availability is translated into a physiologic signal became apparent only with the discovery of leptin (1). Subsequent work showing the mechanisms involved in leptin signaling and the physiologic responses mediated by leptin has provided a robust framework on which our current understanding of the mechanisms involved in energy homeostasis has been built (54). Although congenital leptin deficiency is rare, the characterization of these disorders has provided insights into the role of leptin in human physiology and shown that, for the most part, leptin-mediated responses are highly conserved in mammalian species. 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 continued to show that human obesity can result from a multiplicity of defects in the pathways downstream of leptin signaling within the brain. These include mutations in the leptin receptor (38, 55); pro-opiomelanocortin (56–58); prohormone convertase-1/3 (59–61); the melanocortin 4 receptor (62, 63), which is activated by products of cleavage of the POMC gene; and, more rarely, defects in signaling systems that are thought to be downstream of the melanocortin 4 receptor, which include the brain-derived neurotrophic factor (64) and its receptor (65). Interestingly, all of these disorders are characterized by hyperphagia and have allowed us to demonstrate unequivocally that human appetite and eating behavior is in part biologically determined. Our growing understanding that human appetite varies markedly between individuals as does interindividual susceptibility to obesity should lead to a more enlightened and sympathetic approach toward obese patients and the problems that they endure. Finally, the discovery of leptin and the central pathways involved in energy homeostasis will contribute to the development of more rationale pharmacologic approaches toward the prevention and treatment of obesity and its complications. (Other articles in this supplement to the Journal include references 66–69.)

Both authors contributed to the writing of this manuscript. Neither author had a conflict of interest.

REFERENCES


