

Leptin and the regulation of body weight in mammals

Jeffrey M. Friedman & Jeffrey L. Halaas

The assimilation, storage and use of energy from nutrients constitute a homeostatic system that is essential for life. In vertebrates, the ability to store sufficient quantities of energy-dense triglyceride in adipose tissue allows survival during the frequent periods of food deprivation encountered during evolution. However, the presence of excess adipose tissue can be maladaptive. A complex physiological system has evolved to regulate fuel stores and energy balance at an optimum level. Leptin, a hormone secreted by adipose tissue, and its receptor are integral components of this system. Leptin also signals nutritional status to several other physiological systems and modulates their function. Here we review the role of leptin in the control of body weight and its relevance to the pathogenesis of obesity.

There is intense pressure to be thin in late-twentieth-century 'western' societies. Nevertheless, obesity is a prevalent condition which is often stigmatized. In addition, actuarial data indicate that life expectancy is reduced when body-mass index (BMI, body mass in kg/square of the height in metres) is 20% or more above the ideal¹. The ideal BMI is the level at which life expectancy is maximal and a BMI of >28 is now considered to represent obesity. The health risk of obesity is largely a consequence of the morbidities of diabetes, hypertension and heart disease, whose incidence increases with BMI.

The belief that obesity is largely the result of a lack of willpower, though widely held, is unsatisfactory. Studies of twins, analyses of familial aggregation, adoption studies and animal models of obesity all indicate that obesity is the result of both genetic and environmental factors^{2,3}. Moreover, weight is stable in lean and obese individuals even though much of the population actively practises weight control. Dieting is not usually successful in long-term maintenance of reduced body weight and most reduced obese individuals eventually regain the lost weight⁴.

The relative stability of weight in individuals indicated that energy balance may be controlled by a feedback loop, which maintains constancy of total body energy stores. It has been proposed that signals reflecting nutritional state are sensed by the hypothalamus, which, in turn, modulates food intake and energy expenditure^{5,6}. The identification of these signals proved difficult and for many decades their existence was widely questioned.

The *ob* gene and leptin

Recessive mutations in the mouse *obese* (*ob*) and *diabetes* (*db*) genes result in obesity and diabetes in a syndrome resembling morbid human obesity^{3,7}. *ob/ob* and *db/db* mice have identical phenotypes, each weighing three times more than normal mice (even when fed the same diet) and exhibiting a fivefold increase in body fat content. Data from cross-circulation (parabiosis) experiments suggested that the *ob* gene encoded, or was responsible for the generation of, a circulating factor that regulated energy balance and that the *db* gene encoded the receptor for this factor³. However, these conclusions were viewed with scepticism by many and their confirmation awaited the identification of the *ob* and *db* genes.

The cloning and characterization of the *ob* gene showed that it encodes a hormone, leptin (leptos (Greek): thin), that is expressed in adipose tissue and, at lower levels, in gastric epithelium and placenta⁸⁻¹⁰. The *ob* gene was isolated by positional cloning and found to encode a new, 167-amino-acid secreted protein⁸. The *ob* transcript is mutant in both of the available strains of *ob/ob* mice. In *ob^{2j}* mice, a ~5-kilobase (kb) ETn transposon is inserted into the first intron of *ob*¹¹. This mutation results in the synthesis of hybrid RNAs in which the splice donor of the non-coding *ob* first exon is joined to splice acceptors in the transposon. Mature *ob* RNA is not

synthesized in this mutant. In the C57BL/6J *ob/ob*^{1j} mutant, a nonsense mutation results in the synthesis of a truncated protein that is apparently degraded in the adipocyte⁸. In this mutant, the levels of *ob* RNA were increased, indicating that the gene might be under feedback control.

Initial data indicated that leptin might be an afferent signal in a negative-feedback loop regulating the size of adipose tissue mass (Fig. 1). If true, leptin RNA should be expressed in the principal site of fat storage, adipocytes; leptin should circulate in plasma; plasma leptin levels should correlate with adipose tissue mass; and recombinant leptin should reduce body fat content when injected into *ob/ob* and wild-type, but not into *db/db*, mice.

Leptin RNA is expressed in adipocytes, as shown using *in situ* hybridization, cell fractionation and immunohistochemistry¹². Leptin circulates as a protein of relative molecular mass 16,000 (M_r 16K) in mouse and human plasma¹³. A 19K form has also been identified in extracts of rat stomach, although the molecular nature and functional significance of this form are not known¹⁹. The 16K moiety is not modified post-translationally, as the relative molecular mass of the native protein is identical to that predicted by the primary structure (without the signal sequence)¹⁴. The plasma levels of leptin are highly correlated with adipose tissue mass and fall in both humans and mice after weight loss¹⁵. Levels of leptin are increased in obese humans and in several genetic and environmentally induced forms of rodent obesity^{2,15}.

Administration of leptin by injection or, with greater potency, as a constant subcutaneous infusion results in a dose-dependent decrease in body weight at incremental increases of plasma leptin levels within the physiological range^{13,16-19}. In both *ob/ob* and wild-type mice, leptin-induced weight loss is restricted to adipose tissue with sparing of lean body mass¹³.

There appears to be both a short-term and a long-term system for controlling feeding behaviour and energy balance. Plasma glucose concentration, body temperature, plasma amino acids, cholecystokinin (CCK) and other hormones can all modulate meal patterns (hunger and satiety) and are components of the short-term system^{20,21}. Leptin does not increase significantly after a meal and does not, by itself, lead to the termination of a meal^{15,22}. Leptin appears to function largely within the long-term system and influences the quantity of food consumed relative to the amount of energy expended. However, leptin is depleted from the stomach of rats after a meal or administration of CCK, indicating that it could also function in the short-term system or locally in the gastrointestinal tract⁹.

A broader role for leptin

ob/ob mice show many of the abnormalities seen in starved animals, including decreased body temperature, hyperphagia, decreased

energy expenditure (including activity), decreased immune function, and infertility³. Leptin replacement corrects all of these abnormalities, implying that *ob* mice exist in a state of 'perceived starvation' and that the resulting biological response in the presence of food leads to obesity^{13,17,23,24}. The idea that decreased plasma leptin levels signal nutrient deprivation is supported by the observation that exogenous leptin attenuates the neuroendocrine responses to food restriction²⁵. Fasted wild-type mice receiving leptin continue to ovulate, whereas fasted controls given saline experience an ovulatory delay of several days. Leptin treatment blunts the changes in circulating thyroid hormone and corticosterone levels that are normally associated with food deprivation²⁵. Starvation is also associated with decreased immune function and leptin corrects these abnormalities²⁴. Leptin stimulates proliferation of CD4⁺ T cells and increases production of cytokines by T-helper-1 cells²⁴. These results indicate that leptin may also be a key link between nutritional state and the immune system.

Leptin is also important in regulating the onset of puberty. Extremely thin women often stop ovulating and abnormally thin adolescent women enter puberty later than their heavier counterparts, indicating that fat tissue may produce a signal that regulates reproduction. This factor may be leptin. Treatment of mice with leptin accelerates the maturation of the female reproductive tract and leads to an earlier onset of the oestrous cycle and reproductive capacity^{26,27}. In humans, a surge in plasma leptin concentration is seen in prepubertal males²⁸. Leptin RNA and the leptin receptor have been identified in mouse and human placenta, but the functional significance of this is unknown^{10,29}.

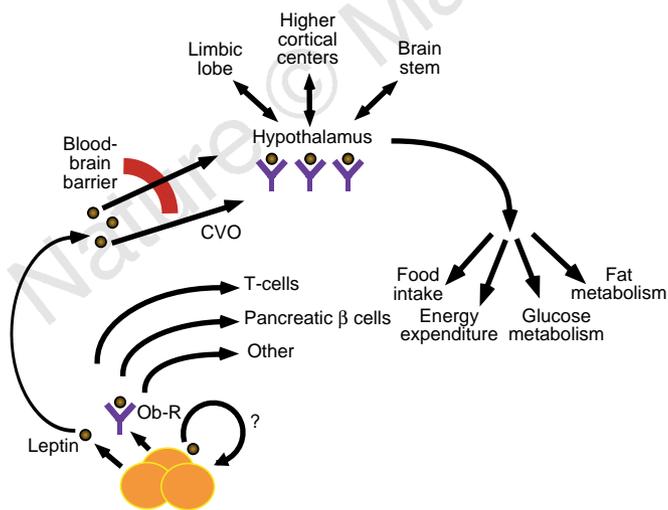


Figure 1 Adipocyte leptin and the regulation of adipose tissue mass. The cloning of the *ob* gene and the characterization of its gene product, leptin, indicated that body fat content may be under homeostatic control. Available data indicate that leptin is the afferent signal in a negative feedback loop that maintains constancy of adipose tissue mass. Leptin is secreted from adipocytes (bottom left) either as a 16K protein or bound to a soluble form of its receptor (Ob-R). The level of leptin is positively correlated with differences in body fat. Increased leptin results in negative energy balance (energy expenditure > food intake), whereas decreased levels lead to positive energy balance (food intake > energy expenditure). Leptin acts mainly on the hypothalamus (top). Extensive connections exist between the hypothalamus and other brain regions. Leptin acts centrally to decrease food intake and modulate glucose and fat metabolism (right). Peripheral effects on T cells, pancreatic islets and other tissues have also been demonstrated. CVO, circumventricular organ.

These observations led to speculation that leptin's main physiological role is to signal nutritional status during periods of food deprivation²¹. However, leptin's role in preventing excess weight gain has been shown to be physiologically significant. Lean mice given chronic infusions of leptin lose adipose mass in a dose-dependent manner at leptin levels within the physiological range¹⁶. Therefore, dynamic changes in plasma leptin concentration act to resist weight change in either direction.

Leptin receptor and leptin's sites of action

The leptin receptor (Ob-R) was first isolated from mouse choroid plexus by expression cloning³⁰. It was identified as a member of the cytokine family of receptors and binds leptin with nanomolar affinity³⁰. The stoichiometry of binding is unknown, but the Ob-R sequence probably includes two ligand-binding domains. Positional cloning of *db*, the Ob-R gene, showed that this gene encodes five alternatively spliced forms, Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd and Ob-Re (refs 30, 31). Ob-Rb (also known as Ob-RL) has a long cytoplasmic region containing several motifs required for signal transduction. The other forms lack some or all of these motifs (Fig. 2).

Mutations in Ob-R result in an obese phenotype identical to that of *ob* mice³². C57Bl/Ks *db/db* mice are phenotypically the same as other strains of *db* mice. In this co-isogenic strain, the Ob-Rb transcript contains an insert with a premature stop codon, as a result of abnormal splicing^{31,33}. Expression of Ob-Ra and the other splice forms is unaffected in this mutant. Thus Ob-Rb is essential for leptin's weight-reducing effects.

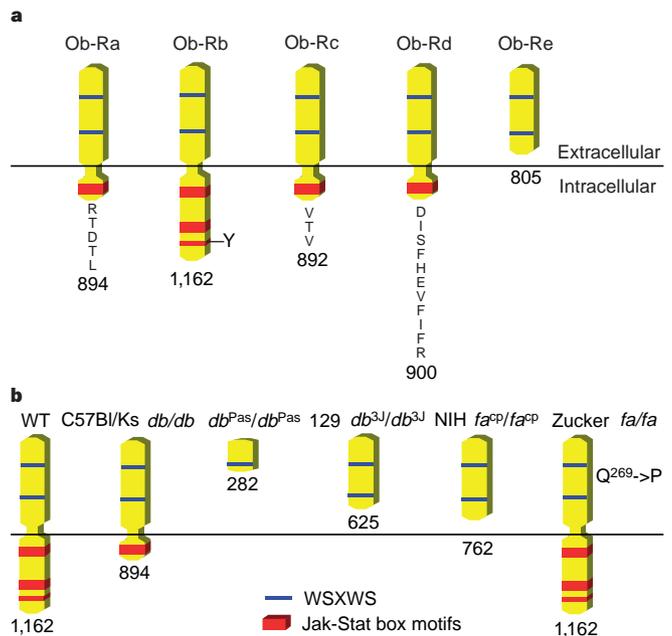


Figure 2 Leptin-receptor isoforms and receptor mutations in rodent models of obesity. **a**, There are at least five different isoforms of the leptin receptor in mouse. All share identical extracellular, ligand-binding domains but they differ at the C terminus. Four of the five have transmembrane domains, but only Ob-Rb encodes all protein motifs capable of activating the Jak-Stat signal transduction pathway. The remaining isoform, Ob-Re, is truncated before the membrane-spanning domain and is secreted. **b**, Mutations in Ob-R lead to massive obesity in *db* mice and *fa* rats. Most of the mutations affect all of the splice forms. However, in obese C57Bl/Ks *db/db* mice, the mutation creates a new splice donor that inserts a premature stop codon into the Ob-Rb 3'-end, resulting in the replacement of the Ob-Rb isoform by the Ob-Ra isoform. This mutation establishes the Ob-Rb isoform as being critical for leptin function. WT, wild type.

Although the Ob-Ra isoform, which has a short cytoplasmic domain, is expressed in the choroid plexus and many other tissues, its significance is unknown. Ob-Ra can activate gene expression and signal transduction in cultured cells, albeit weakly³⁴. It is unknown whether this occurs *in vivo*. The function of the other forms is likewise unclear³². They may function in the transport of leptin across the blood–brain barrier or form heterodimers with other cell-surface proteins.

Ob-Rb is normally expressed at high levels in hypothalamic neurons and in other cell types, including T cells and vascular endothelial cells^{24,31,35,36}. *In situ* hybridization was used to identify the hypothalamic arcuate nucleus, dorsomedial hypothalamic nucleus (DMH), paraventricular nucleus (PVN), ventromedial hypothalamic nucleus (VMH) and lateral hypothalamic nucleus (LH) as principal sites of Ob-Rb expression in the central nervous system (CNS). Each of these nuclei is important in regulating body weight^{5,37,38}. Leptin induces a dose-dependent activation of the transcription factor Stat3 in the hypothalamus of mice within 15 minutes of a single intraperitoneal injection³⁹. Stat3 was not activated in any other tissues. Leptin also increases the expression of *fos*, a Stat3 target, and several other genes in the hypothalamus specifically⁴⁰.

Synaptic transmission from neurons in the arcuate nucleus is altered by leptin⁴¹, which also leads to the hyperpolarization of some hypothalamic neurons and replicates the effect of glucose on these cells⁴². This effect depends on an ATP-dependent potassium channel, as tolbutamide, which blocks these channels, inhibits the effect⁴². These electrophysiological effects are rapid and unlikely to require new transcription through activation of STAT proteins. Although the components of leptin-mediated signal-transduction pathways that alter electrical activity in neurons are not yet known, their identification could provide new therapeutic targets.

Taken together with the fact that *db* mice and mice with hypothalamic lesions are leptin-resistant, these findings support the conclusion that the hypothalamus is an important site of leptin action^{12,13,43}. This conclusion is further supported by the observation that a single intracerebroventricular (i.c.v.) injection of leptin reduces food intake at doses that have no effect when delivered peripherally^{16,18,19}. Chronic i.c.v. infusion of leptin at a dose of 3 ng per hour results in total depletion of body adipose stores, whereas peripheral administration requires doses >500 ng h⁻¹ to achieve the same effect¹⁶. The effects of leptin on energy metabolism are the same regardless of the route of administration (refs 16 and 44, and our unpublished observations).

Low i.c.v. doses of leptin lead to a transient reduction in food intake that persists only until the adipose tissue mass has been depleted, after which food intake returns to normal¹⁶. The attenuation of the leptin response may be explained by the presence of other, undiscovered, signals, perhaps from skeletal muscle. These results also indicate that the effects of recombinant leptin are qualitatively different from those seen after parabiosis in which lean mice receiving *db/db* (hyperleptinaemic) plasma become anorectic and die of apparent starvation. A factor(s) other than leptin may be required for lethality after parabiosis of wild-type mice to *db* mice.

The mechanisms of leptin transport into the CNS is unknown. As leptin uptake occurs in the capillary endothelium of mouse and human brain, active transport by Ob-Ra or other proteins has been suggested as a possible mechanism⁴⁵. Some Ob-Rb-positive neurons project to the median eminence, the site of hypothalamic circumventricular organs, and may be in front of the blood–brain barrier⁴⁶. Circumventricular organs have fenestrations in their capillaries that allow direct communication between the blood and specific CNS neurons.

Leptin also acts on peripheral cell types and has direct mitogenic effects on CD4⁺ human T cells²⁴. Leptin also affects endothelial cells directly and increases angiogenesis, although high doses are

required³⁶. Leptin modulates pancreatic β -cell function *in vivo*⁴⁷. Leptin also has direct effects on other cell types *in vitro*. The leptin receptor is widely expressed, although the Ob-Ra (short) form of the receptor predominates in many of these tissues^{31,35,38}. Although the potency of i.c.v. leptin indicates that direct peripheral effects are not required for weight loss, the full spectrum of its actions is not known. It is thus possible that leptin acts on many tissues and functions in other physiological systems. The use of the *Lox-cre* system to introduce tissue-specific mutations into Ob-R may be required to establish the relevance of non-CNS sites of leptin action *in vivo*.

The introduction of mutations into Ob-Rb *in vitro* has been used to identify several sequences in the carboxy-terminal domain that are required to activate signal transduction^{34,48}. Activation of the leptin receptor depends on phosphorylation of the kinase Jak2 after ligand binding to an Ob-Rb homodimer. Receptor homodimerization may or may not be ligand-dependent: a baculovirus-expressed receptor spontaneously forms a homodimer⁴⁹. *In vitro*, binding of leptin to its receptor leads to the activation of Stat1, 3 and 5, whereas leptin activates only Stat3 *in vivo*^{35,39,48}. Leptin also leads to tyrosine phosphorylation of SHP-2, a phosphotyrosine phosphatase, which decreases both the state of Jak2 phosphorylation and transcription of a leptin-inducible reporter gene (ref. 50, and our unpublished observations). SOC-3 may also be involved in leptin-mediated signal transduction and it, as well as SHP-2, may downregulate the response to leptin⁵¹.

Leptin and the neuronal circuit regulating weight

Leptin receptors have been found in several hypothalamic nuclei, including the arcuate nucleus, VMH, LH, DMH and PVN^{5,37,38}. The LH and VMH project both within and outside the hypothalamus and modulate activity of the parasympathetic and sympathetic nervous systems, respectively. The DMH also has inputs to the parasympathetic nervous system and may be involved in integrating information among the VMH, LH and PVN. The PVN controls secretion of peptides from both the posterior and anterior pituitary and projects to nuclei with sympathetic or parasympathetic efferents.

These hypothalamic nuclei express one or more neuropeptides and neurotransmitters that regulate food intake and/or body weight (Table 1). Genetic data indicate that neuropeptide Y (NPY) and one or more of its receptors act in the response to absent (and possibly low) leptin, whereas melanocyte-stimulating hormone (MSH), its receptor, the melanocortin-4 receptor, and possibly the agouti-related transcript (ART, also known as AGRP; another melanocortin-4 receptor) are required for the response to an increased plasma leptin concentration (Fig. 3)⁵². NPY is the most potent orexigenic agent known when administered intrathecally. NPY RNA is increased in *ob/ob* mice and decreases after leptin treatment¹⁹. An NPY-knockout attenuates the obesity and other features of *ob/ob* mice⁵³.

Table 1 Hypothalamic modulators of food intake

Increase food intake	Decrease food intake
NPY	CART
MCH	CCK
Galanin	CRH
Orexin a and b	α -MSH
Peptide YY	Insulin
Noradrenaline	GLP-1
($\alpha 2$ receptor)	Bombesin
	Urocortin
	Serotonin

Many factors regulate feeding behaviour. The neurotransmitters and neuropeptides known to regulate food intake are shown. Leptin probably modulates the activity some or all of these factors (and vice versa). A detailed understanding of the functional relationships among leptin and these (and other) neuropeptides and neurotransmitters will be necessary to determine the mechanisms regulating food intake and body weight. GLP-1, glucagon-like peptide-1.

Abnormal melanocortin signalling in yellow agouti (A^Y) or melanocortin-4-knockout mice leads to obesity and leptin resistance^{16,54,55}. A subset of neurons expresses both Ob-R and proopiomelanocortin (POMC), and leptin modulates POMC gene expression (POMC is the precursor of MSH)⁴⁶. Agonists of α -MSH and MSH decrease food intake and pretreatment of animals with an α -MSH antagonist blunts the anorectic effect of injected leptin⁵⁶. ART, an endogenous antagonist of melanocortin signalling, is also implicated in the regulation of weight, as transgenic mice overexpressing ART are markedly obese⁵⁷. Levels of messenger RNA encoding this hypothalamic peptide are increased eightfold in *ob/ob* mice⁵⁸.

Other neurotransmitters and neuropeptides also function in this homeostatic system (Table 1)²¹. Leptin may stimulate the action of the anorexigenic agents and antagonize the orexigenic effects of others. Cholecystokinin (CCK) potentiates the anorectic effect of leptin⁵⁹. In addition, CCK-8 decreases stores of leptin in the stomach⁹. CART (cocaine- and amphetamine-regulated transcript), a hypothalamic peptide, decreases food intake; anti-CART antibodies increase food intake and levels of its mRNA are increased in *ob/ob* mice⁶⁰. Bombesin reduces food intake and induced mutations of the bombesin-3 receptor result in mild obesity⁶¹. Insulin acts on the hypothalamus to decrease food intake⁶². Levels of melanin-concentrating hormone (MCH), a neuropeptide expressed in the lateral hypothalamus, are increased in *ob/ob* mice and injections of it increase food intake in mice⁶³. Orexin-a and orexin-b are also expressed in the lateral hypothalamus and increase food intake⁶⁴. Leptin increases levels of corticotropin-releasing hormone (CRH) mRNA in the PVN and stimulates release of CRH from perfusion

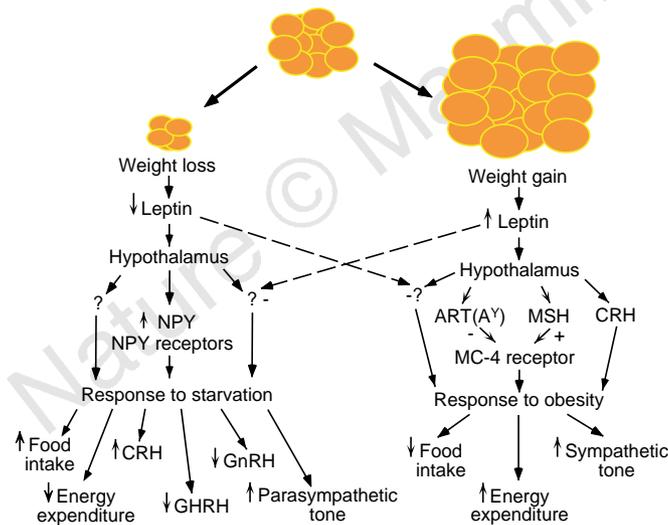


Figure 3 Biological response to high versus low leptin levels. Leptin acts as part of a feedback loop to maintain constant stores of fat. A loss of body fat (starvation) leads to a decrease in leptin, which in turn leads to a state of positive energy balance wherein food intake exceeds energy expenditure. A range of other metabolic and endocrine responses is also seen. Conversely, an increase in adiposity leads to an increase in the levels of leptin and a state of negative energy balance, with energy expenditure exceeding food intake. Genetic evidence indicates that different hypothalamic neuropeptides may mediate these responses. The melanocortin-4 (MC-4) receptor and its ligands, MSH and ART, are probably necessary for the biological response to increasing leptin levels. CRH also mediates some of leptin's effects, as pretreatment with an anti-CRH antibody blunts the anorectic effects of an i.c.v. dose of leptin. Other studies indicate that NPY is an important component of the biological response to low levels of leptin and possibly starvation. Other molecules are likely to play a role in the leptin response. These other factors are largely unknown but probably include some of the molecules shown in Table 1. GnRH, gonadotropin-releasing hormone; GHRH, growth-hormone-releasing hormone.

slices of both amygdala and the PVN^{65,66}. Delivery of CRH to the PVN results in reduced food intake and increased energy expenditure in lean and obese rats⁶⁷. Pretreatment with anti-CRH antibodies decreases the anorectic effect of a single i.c.v. dose of leptin⁶⁸.

High levels of glucocorticoids are seen in most strains of genetically obese mice and adrenalectomy and glucocorticoid antagonists blunt the obesity evident in *ob/ob*, *db/db* and other obese mice⁶⁹. Low-dose glucocorticoid replacement restores the obese phenotype, indicating that glucocorticoids have a permissive role in the development of the obese phenotype⁶⁹. It is unknown whether the requirement for glucocorticoids in the development of the full *ob/ob* phenotype depends on the suppression of CRH or on other known effects of glucocorticoids, or whether another mechanism is involved.

The functional relationship among these molecules will have therapeutic implications. Antagonists of NPY and its receptors are being developed at present⁷⁰. Serotonin, which reduces food intake, and noradrenaline, which is orexigenic, are targets for known weight-reducing agents such as fen-fen (dexfenfluramine and phentermine). A knockout of the HT-2c form of the serotonin receptor leads to a form of mild obesity⁷¹.

In summary, leptin concentrations are sensed by groups of neurons in the hypothalamus. During starvation, leptin levels fall, thus activating a behavioural, hormonal and metabolic response that is adaptive when food is unavailable. Weight gain increases plasma leptin concentration and elicits a different response, leading to a state of negative energy balance. It is not yet known whether the same (or different) neurons respond to increasing and decreasing

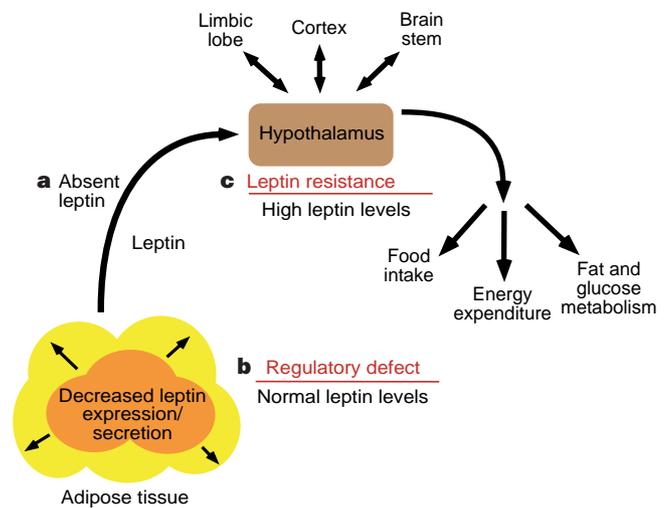


Figure 4 Pathogenesis of obesity. There are three general ways in which alterations of the leptin regulatory loop could lead to obesity. **a**, Failure to produce leptin, as occurs in *ob/ob* mice, would result in obesity, as would **b**, inappropriately low leptin secretion for a given fat mass. In the latter case, the fat mass would expand until 'normal' leptin levels are reached, resulting in obesity (also see Fig. 5). **c**, Finally, obesity could result from relative or absolute insensitivity to leptin at its site of action. Such resistance would be associated with increased circulating leptin, analogous to the increased insulin levels seen with insulin-resistant diabetes. In general, high plasma leptin levels are evident in obese rodents and humans. In a subset of cases, obesity is associated with normal levels of leptin. Differences in leptin production and leptin sensitivity could be the result of genetic, environmental and psychological factors.

leptin levels. The range of leptin's effects is likely to be complex, as different thresholds exist for several of leptin's actions⁷².

CNS efferent pathways regulating metabolism

The evidence that leptin acts centrally to reduce weight raises the question of how the CNS regulates peripheral metabolism in response to differences in leptin concentration. Increasing leptin leads to fatty acid oxidation and a reduction in adipose tissue mass, whereas leptin deficiency, seen in *ob/ob* mice or in mice receiving a leptin antagonist, is associated with an increase in fat deposition^{3,73}. It is unclear whether the metabolic derangements of *ob* mice are solely the result of increased food intake: other factors are likely to be important as food-restricted *ob* mice still deposit excess adipose tissue³.

Also, the mechanism by which centrally administered leptin leads to lipolysis and the loss of adipose tissue mass is unknown. The metabolic response to leptin is markedly different from the response to reduced food intake. Whereas food restriction (dieting) leads to the loss of both lean body mass and adipose tissue mass, leptin-induced weight loss is specific for the adipose tissue mass^{13,16}. Leptin also prevents the reduced energy expenditure normally associated with decreased food intake¹⁶. Finally, in contrast to food-restricted (pair-fed) animals, which show a marked increase in serum-free fatty acids, hyperleptinaemic animals undergoing a rapid period of weight loss fail to show a rise in serum-free fatty acids or ketones⁷⁴.

Leptin also has new effects on glucose metabolism. *ob/ob* mice are diabetic and the severity of the diabetes depends on the background strain carrying the mutation³. Leptin normalizes the hyperglycaemia and hyperinsulinaemia seen in C57BL/6J *ob/ob* mice at doses that do not decrease weight¹⁷. Treatment of lean animals with leptin leads to a reduction in serum glucose levels, without changing insulin levels, and increases glucose usage during euglycaemic clamp experiments⁷⁵. These effects are also seen after acute i.c.v. infusion of leptin, which leads to a significant increase in glucose turnover without altering the plasma insulin concentration⁴⁴. Previous data have indicated that the CNS has important effects on insulin secretion and glucose metabolism⁷⁶. If leptin increases glucose uptake in peripheral tissues in humans independently of weight loss, it could be of benefit to some patients suffering from non-insulin-dependent diabetes mellitus.

The mechanisms by which the CNS regulates fat and glucose metabolism in response to leptin are also unknown. Direct measurement of nerve activity has indicated that infusion of leptin increases sympathetic activity to brown adipose tissue, kidney, hind limb and adrenal gland⁷⁷. It is not yet known whether blockade of β -adrenergic receptors attenuates (or blocks) leptin-induced weight loss. Thus the role of the sympathetic nervous system in mediating the weight-reducing effect of leptin is not yet established.

An increase in sympathetic activity in brown-fat adipose tissue has also been observed in leptin-treated *ob/ob* mice⁷⁸. This is associated with an increase in the levels of uncoupling protein (UCP)-1 mRNA. Uncoupling proteins disrupt the mitochondrial proton gradient in brown fat (and possibly other tissues), resulting in the generation of heat rather than ATP. There is no evidence that leptin treatment of wild-type animals increases the activity of UCP-1. Levels of UCP-2 RNA may increase in leptin-treated mice, but the implications of this change are unknown⁷⁹. Leptin treatment does not cause a net increase in 24-hour energy expenditure but instead blunts the decreased energy expenditure that generally accompanies food restriction¹⁶. It is thus uncertain whether leptin increases energy expenditure or activates uncoupling protein.

Leptin and the pathogenesis of obesity

The role of leptin in the pathogenesis of obesity can be inferred by measurement of plasma leptin (Fig. 4). An increase in plasma leptin

suggests that obesity is the result of resistance to leptin. A low or normal plasma concentration of leptin in the context of obesity suggests decreased production of leptin. This interpretation is similar to that used in studies of insulin and the pathogenesis of type I and type II diabetes.

Plasma leptin has been measured in rodents and humans^{15,22}. Leptin circulates in a complex with a soluble form of Ob-R (Ob-Re) and it is not known whether assays of plasma leptin levels distinguish between bound and free leptin³². In general obese animals have higher leptin levels than controls (with the exception of *ob/ob* mice), indicating that these forms of animal obesity are associated with leptin resistance^{15,80}. Administration of leptin to obese hyperleptinaemic *A^y*, New Zealand obese (NZO) and diet-induced obese (DIO) mice confirmed that they are leptin-resistant^{16,81}. DIO mice are only partially leptin-resistant and lose moderate amounts of weight at high intraperitoneal doses of leptin. *A^y* mice (in which the response of the melanocortin-4 receptor to MSH is inhibited) are completely resistant to high i.c.v. doses of leptin, indicating that the normal function of the melanocortin-4 receptor is required for the response to exogenous leptin. NZO mice are resistant to peripherally administered leptin but respond normally to centrally administered leptin, suggesting that impaired leptin transport into the CNS causes this obesity¹⁶.

The cellular basis for leptin resistance in other obese animals is not known. Mutant *fat* and *tubby* mice are hyperleptinaemic¹⁵. Carboxypeptidase E (CPE), the gene product of the *fat* locus, alters post-translational processing of many peptides, including insulin, neurotensin, POMC and MCH^{82,83}. Mutations in PC-1, another peptide-processing enzyme, are also associated with obesity and increased leptin concentrations⁸⁴. The substrates of CPE and PC-1 that regulate weight and lead to obesity when these enzymes are defective are unknown. The *tub* gene product is highly expressed in the PVN^{85,86}. Further studies may reveal a link between this gene product and the response to leptin.

The leptin resistance seen in diet-induced obese AKR/J mice emphasizes the fact that environmental factors can modulate sensitivity to leptin^{16,81}. AKR/J mice remain lean when fed a standard chow diet but become obese when fed a high-fat diet. Other mouse strains do not become obese when fed an identical diet. This indicates that the pathogenesis of diet-induced obesity is the result of an interaction between genetic and environmental factors. An understanding of the mechanisms by which fat content in the diet modulates weight is likely to be relevant to human obesity as the incidence of obesity increases in many populations in proportion to the high-fat content of a 'western' diet. How might genes interact with environmental factors to cause obesity? A highly palatable diet often leads to transient weight gain. In most cases, the gained weight is rapidly lost. However, it is possible that in some cases, the induced increase in endogenous leptin levels (which accompanies weight gain) leads to a downregulation of the leptin response and a failure to return to the starting weight. If the downregulation of the leptin response is variable and influenced by genetic factors, one might predict that a subset of individuals would be particularly susceptible to diet-induced obesity. Alternative explanations are possible and further investigation is necessary. Identification of the *AKR* alleles that predispose AKR/J mice to diet-induced obesity may illuminate the underlying mechanisms⁸⁷.

The basis for leptin resistance in obese, hyperleptinaemic human subjects is unknown. Data from studies of animals indicate that this condition is likely to be heterogenous and that many factors may influence the activity of the neural circuit that regulates body weight. The entry of leptin into cerebrospinal fluid may be limiting in some obese subjects and morbid obesity could result when the plasma leptin levels exceed the capacity of the transport system^{88,89}. Factors that directly modulate energy expenditure or activate adipogenesis and lipogenesis could also result in apparent leptin

resistance. Finally, leptin's actions are likely to be influenced by psychological factors through connections between the higher cortical centres, which modulate an animal's motivational state, and the hypothalamus.

Regulation of leptin production

The physiological importance of quantitative changes in leptin concentration indicates that regulation of the *ob* gene is a critical control point^{16,25,72}. Decreased leptin expression per adipocyte could lead to obesity with normal (but inappropriately low) plasma leptin concentrations (Fig. 5). This prediction is supported by the observation that *ob/ob* mice carrying a poorly expressed leptin transgene are obese, despite having relatively normal leptin levels⁷².

The amount of *ob* gene expression per cell correlates with the lipid content and the corresponding size of individual adipocytes¹². The signal-transduction system by which fat cells sense their lipid content and adjust the level of *ob* expression is unknown. Studies of the regulation of *ob* expression have been hindered by the fact that cultured adipocyte cell lines deposit very little lipid relative to adipocytes *in vivo* and hence express only small amounts of leptin¹². The observation that cultured adipocytes express physiological levels of *ob* RNA when introduced directly into animals suggests an approach for studying quantitative changes in *ob* gene expression⁹⁰.

Extrinsic factors also modulate the expression of leptin. Leptin levels increase by ~30% at night²². Although feeding does not

appear to increase leptin expression, fasting acutely decreases plasma leptin concentrations^{15,22}. High intracellular glucosamine concentrations increase leptin production in adipose tissue and skeletal muscle⁹¹. Tumour-necrosis factor, insulin, glucocorticoids, interleukin-1 and other proteins also modulate *ob* gene expression^{92,93}. The *ob* promoter is responsive to several transcription factors, including C/EBP and peroxisome proliferator-activated receptor- γ ⁹²⁻⁹⁵. It is not known whether leptin is regulated and released from storage vesicles or secreted through a constitutive pathway.

Leptin in human physiology

Plasma leptin concentration correlates with body fat content and is usually increased in obese subjects^{15,22}. This suggests that human obesity is generally associated with an insensitivity to leptin. However, 5–10% of obese human subjects have relatively low levels of leptin, indicative of a reduced rate of leptin production in this subgroup^{15,22}. Low leptin levels also predispose pre-obese Pima Indians to weight gain⁹⁶.

In humans, diet-induced weight loss results in a decrease in plasma leptin concentration^{15,22}. This may explain the high failure rate of dieting, as low leptin is likely to be a potent stimulus to weight gain. Anorexia nervosa patients also have extremely low leptin levels^{97,98}. Refeeding of these patients results in a rapid increase in plasma leptin concentration to roughly normal levels before normal weight is achieved. Thus, excessive leptin production could play a permissive role in the pathogenesis of this condition.

In almost all cases, obese subjects express at least some leptin, indicating that human *ob* mutations are likely to be rare. Indeed there were no *ob* mutations in one study in which ~500 obese subjects were tested⁹⁹. Nevertheless, a few *ob* mutations have been described. Two cousins homozygous for a frameshift mutation in the leptin gene are markedly obese and do not have any circulating leptin¹⁰⁰. Three members of a Turkish kindred with a missense mutation in the leptin gene are extremely obese and amenorrhoeic, indicating that leptin is important in modulating human reproductive function¹⁰¹. Three massively obese members of a French family carry mutations in the leptin receptor and have reproductive abnormalities¹⁰². It is not yet known whether heterozygous members of these families show a more subtle phenotype. Hypercortisolaemia, cold intolerance and severe diabetes, abnormalities of *ob/ob* mice, are not seen in leptin-deficient humans.

The *ob* gene has been linked to severe obesity in some, but not all, family studies, but mutations in the leptin-coding sequence were not identified^{103,104}. The molecular basis for this linkage is unknown but could relate to differences in the amount of expression of leptin mRNA. Loci on human chromosome 2 may be linked to leptin levels and, to a lesser extent, to BMI¹⁰⁵. These loci are near to the human POMC gene, and two obese, red-haired subjects with POMC mutations have been identified¹⁰⁶.

The association of mutations in leptin and its receptor with massive obesity confirms its importance in regulating human body weight. However, these syndromes are rare. The pathogenesis of most human obesity is unknown and likely to be the result of differences in leptin secretion and/or leptin sensitivity. Both genetic and physiological studies are required to confirm this prediction.

Prospects for leptin treatment of human obesity

The possible therapeutic benefit of leptin treatment in humans is now being studied in clinical trials. Early data show that 4 weeks of daily leptin injections are safe and cause small but significant weight loss in lean and obese subjects, compared with placebo effects ($P < 0.02$) (A. S. Greenberg *et al.*, unpublished observations). A subset of eight obese subjects treated for a total of six months (0.3 mg kg^{-1} leptin subcutaneously) lost 7.1 kg compared with 1.7 kg in a group receiving placebo. Some of the subjects in this group lost substantial amounts of weight, but others did not. This

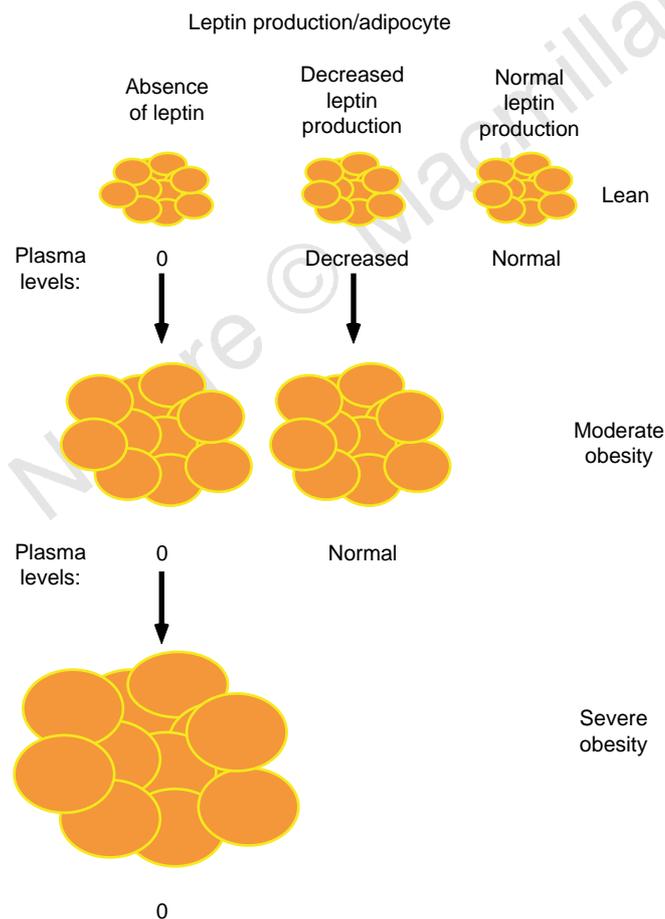


Figure 5 Regulation of leptin expression and the development of obesity. A relative decrease in leptin production by adipose tissue would be expected to lead to obesity. In such a case, the fat cell mass would be predicted to expand until the set point for plasma leptin levels was reached. Thus obesity with normal leptin levels is inferred to result from a relative decrease in leptin production by adipose tissue.

limited study indicates that leptin might be an effective therapy for some obese subjects, although more patients need to be investigated.

The therapeutic response has not yet been related to the initial plasma leptin level. Perhaps only the subset of obese individuals with low plasma leptin (5–10%) will be completely sensitive to exogenous leptin. This possibility is supported by the robust response to leptin treatment of obese transgenic mice that hypo-secrete leptin⁷². Leptin administration could also produce a biological response in those obese individuals who have high circulating levels of leptin, as is the case with insulin treatment of many type II diabetes patients. This may require the administration of relatively high doses.

The amount of protein that can be delivered is limited by its solubility and by local reactions in the skin that occur in response to high doses. Improvements in the leptin formulation and refinements in the dosing may mitigate these difficulties. Animal studies indicate that the ability to optimize the pharmacokinetics of recombinant leptin may greatly influence its usefulness. For example, leptin is more potent when administered as a subcutaneous infusion, whereas once-daily intraperitoneal injections are largely ineffective^{13,16–19}. Direct administration of leptin into cerebrospinal fluid is very effective but its use would require that the drug be delivered safely¹⁶. Gene therapy may provide an alternative means of delivering leptin. Adenovirus and adeno-associated virus vectors expressing leptin both exert potent weight-reducing effects in rodents^{74,107}.

Prospective epidemiological studies are required to define the indications for antiobesity treatments. The health risk of obesity is greatly diminished when even moderate amounts of weight (5% of total weight) are lost¹⁰⁸. This is the result of a marked improvement in the diabetic, hypertensive and cardiovascular status of obese subjects affected by these conditions¹⁰⁸. In rodents, leptin can increase glucose metabolism independently of effects on weight, an observation that indicates a possible use for it in the treatment of type II diabetes^{17,44,75}. The presence of co-morbidities is an obvious indication because dieting, by itself, is only rarely effective for the long-term maintenance of weight loss⁴. It is less clear whether leptin (or any other antiobesity treatment) is indicated in the absence of co-morbid conditions. This issue is complicated by the observation in one study that women weighing 15% less than average had reduced mortality rates, irrespective of co-morbidities¹⁰⁹.

Whether leptin finds its way into general usage as an antiobesity drug, the use of modern methods to identify and target the components of the leptin signalling pathway will form the basis for new pharmacological approaches to the treatment of obesity and other nutritional disorders. Further studies of leptin are also likely to reveal additional links between nutritional state and animal physiology.

J. M. Friedman is at the Laboratory of Molecular Genetics and Howard Hughes Medical Institute, and J. L. Halaas is at the Laboratory of Molecular Genetics, The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA.

- Harris, T. *et al.* Body mass index and mortality among nonsmoking older persons: the Framingham Heart Study. *J. Am. Med. Assoc.* **259**, 1520–1524 (1988).
- Stunkard, A. J., Harris, J. R., Pedersen, N. L. & McClearn, G. E. The body-mass index of twins who have been reared apart. *N. Engl. J. Med.* **322**, 1483–1487 (1990).
- Coleman, D. L. Obese and Diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* **14**, 141–148 (1978).
- Wadden, T. A. Treatment of obesity by moderate and severe caloric restriction. Results of clinical research trials. *Ann. Intern. Med.* **119**, 688–693 (1993).
- Hetherington, A. W. & Ranson, S. W. The spontaneous activity and food intake of rats with hypothalamic lesions. *Am. J. Physiol.* **136**, 609–617 (1942).
- Kennedy, G. C. The role of depot fat in the hypothalamic control of food intake in the rat. *Proc. R. Soc. Lond. B* **140**, 578–592 (1953).
- Friedman, J. M. & Leibel, R. L. Tackling a weighty problem. *Cell* **69**, 217–220 (1992).
- Zhang, Y. *et al.* Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425–432 (1994).
- Bado, A. *et al.* The stomach is a source of leptin. *Nature* **394**, 790–793 (1998).
- Masuzaki, H. *et al.* Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nature Med.* **3**, 1029–1033 (1997).
- Moon, B. C. & Friedman, J. M. The molecular basis of the obese mutation in *ob²* mice. *Genomics* **42**, 152–156 (1997).
- Maffei, M. *et al.* Increased expression in adipocytes of *ob* RNA in mice with lesions of the hypothalamus and with mutations at the *db* locus. *Proc. Natl Acad. Sci. USA* **92**, 6957–6960 (1995).
- Halaas, J. L. *et al.* Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **269**, 543–546 (1995).
- Cohen, S. L. *et al.* Characterization of endogenous human leptin. *Nature* **382**, 589 (1996).
- Maffei, M. *et al.* Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nature Med.* **1**, 1155–1161 (1995).
- Halaas, J. L. *et al.* Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proc. Natl Acad. Sci. USA* **94**, 8878–8883 (1997).
- Pelleymounter, M. A. *et al.* Effects of the obese gene product on body weight regulation in *ob/ob* mice. *Science* **269**, 540–543 (1995).
- Campfield, L. A. *et al.* Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* **269**, 546–549 (1995).
- Stephens, T. W. *et al.* The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* **377**, 530–534 (1995).
- Schneider, B. S., Friedman, J. M. & Hirsch, J. in *Brain Peptides* (eds Krieger, D., Martin, J. & Brownstein, M.) 251–280 (Wiley, USA, 1983).
- Spiegelman, B. M. & Flier, J. S. Adipogenesis and obesity: rounding out the big picture. *Cell* **87**, 377–389 (1996).
- Considine, R. V. *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N. Engl. J. Med.* **334**, 324–325 (1996).
- Chehab, F. F., Lim, M. E. & Lu, R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nature Genet.* **12**, 318–320 (1996).
- Lord, G. M. *et al.* Leptin modulates the T-cell immune response and reverses starvation induced immunosuppression. *Nature* **394**, 897–891 (1998).
- Ahima, R. S. *et al.* Role of leptin in the neuroendocrine response to fasting. *Nature* **382**, 250–252 (1996).
- Chehab, F. F., Mounzih, K., Lu, R. & Lim, M. E. Early onset of reproductive function in normal female mice treated with leptin. *Science* **275**, 88–90 (1997).
- Ahima, R. S. *et al.* Leptin accelerates the onset of puberty in normal female mice. *J. Clin. Invest.* **99**, 391–395 (1997).
- Mantzoros, C. S., Flier, J. S. & Rogol, A. D. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. Rising leptin levels may signal the onset of puberty. *J. Clin. Endocrinol. Metab.* **82**, 1066–1070 (1997).
- Gavrilou, O., Barr, V., Marcus-Samuels, B. & Reitman, M. Hyperleptinemia of pregnancy associated with the appearance of a circulating form of the leptin receptor. *J. Biol. Chem.* **272**, 30546–30551 (1997).
- Tartaglia, L. A. *et al.* Identification and expression cloning of a leptin receptor, OB-R. *Cell* **83**, 1263–1271 (1995).
- Lee, G. H. *et al.* Abnormal splicing of the leptin receptor in *diabetic* mice. *Nature* **379**, 632–635 (1996).
- Li, C. *et al.* Absence of soluble leptin receptor in plasma from *db^{fa}/db^{fa}* and other *db/db* mice. *J. Biol. Chem.* **273**, 10078–10082 (1998).
- Chen, H. *et al.* Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in *db/db* mice. *Cell* **84**, 491–495 (1996).
- Bjorbaek, C., Uotani, S., da Silva, B. & Flier, J. S. Divergent signaling capacities of the long and short isoforms of the leptin receptor. *J. Biol. Chem.* **272**, 32686–32695 (1997).
- Ghilardi, N. *et al.* Defective STAT signaling by the leptin receptor in *diabetic* mice. *Proc. Natl Acad. Sci. USA* **93**, 6231–6235 (1996).
- Sierra-Honigsmann, M. R. *et al.* Biologic action of leptin as an angiogenic factor. *Science* **281**, 1683–1685 (1998).
- Mercer, J. G. *et al.* Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by *in situ* hybridization. *FEBS Lett.* **387**, 113–116 (1996).
- Fei, H. *et al.* Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proc. Natl Acad. Sci. USA* **94**, 7001–7005 (1997).
- Vaisse, C. *et al.* Leptin activation of Stat3 in the hypothalamus of wild-type and *ob/ob* mice but not *db/db* mice. *Nature Genet.* **14**, 95–97 (1996).
- Woods, A. J. & Stock, M. J. Leptin activation in hypothalamus. *Nature* **381**, 745 (1996).
- Glaum, S. R. *et al.* Leptin, the obese gene product, rapidly modulates synaptic transmission in the hypothalamus. *Mol. Pharmacol.* **50**, 230–235 (1996).
- Spanswick, D. *et al.* Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature* **390**, 521–525 (1997).
- Satoh, N. *et al.* Pathophysiological significance of the obese gene product, leptin, in ventromedial hypothalamus (VMH)-lesioned rats: evidence for loss of its satiety effect in VMH-lesioned rats. *Endocrinology* **138**, 947–954 (1997).
- Kamohara, S., Burcelin, R., Halaas, J. L., Friedman, J. R. & Charron, M. J. Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature* **389**, 374–377 (1997).
- Banks, W. A. *et al.* Leptin enters the brain by a saturable system independent of insulin. *Peptides* **17**, 305–311 (1996).
- Hakansson, M.-L. *et al.* Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. *J. Neurosci.* **18**, 559–572 (1998).
- Wang, M. Y. *et al.* Ob-Rb gene transfer to leptin-resistant islets reverses diabetogenic phenotype. *Proc. Natl Acad. Sci. USA* **95**, 714–718 (1998).
- White, D. W. *et al.* Leptin receptor (OB-R) signaling. Cytoplasmic domain mutational analysis and evidence for receptor homo-oligomerization. *J. Biol. Chem.* **272**, 4065–4071 (1997).
- DeVos, R. *et al.* Ligand-independent dimerization of the extracellular domain of the leptin receptor and determination of the stoichiometry of leptin binding. *J. Biol. Chem.* **272**, 18304–18310 (1997).
- Carpenter, L. R. *et al.* Enhancing leptin response by preventing SH2-containing phosphatase 2 interaction with ob receptor. *Proc. Natl Acad. Sci. USA* **95**, 6061–6066 (1998).
- Bjorbaek, C. *et al.* Identification of SOC-3 as a potential mediator of central leptin resistance. *Mol. Cell* **1**, 619–625 (1998).
- Friedman, J. M. The alphabet of weight control. *Nature* **385**, 119–120 (1997).
- Erickson, J. C., Holoopeter, G. & Palmiter, R. D. Attenuation of the obesity syndrome of *ob/ob* mice by the loss of neuropeptide Y. *Science* **274**, 1704–1707 (1996).
- Fan, W. *et al.* Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* **385**, 165–168 (1997).
- Huszar, D. *et al.* Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* **88**, 131–141 (1997).
- Satoh, N. *et al.* Satiety effect and sympathetic activation of leptin are mediated by hypothalamic melanocortin system. *Neurosci. Lett.* **249**, 107–110 (1998).
- Ollmann, M. M. *et al.* Antagonism of central melanocortin receptors *in vitro* and *in vivo* by agouti-related protein. *Science* **278**, 135–138 (1997).

58. Shutter, J. R. *et al.* Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes Dev.* **11**, 593–602 (1997).
59. Matson, C. A., Wiater, M. F., Kuijper, J. L. & Weigle, D. S. Synergy between leptin and cholecystokinin (CCK) to control daily caloric uptake. *Peptides* **18**, 1275–1278 (1997).
60. Kristensen, P. *et al.* Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* **393**, 72–76 (1998).
61. Ohi-Hamazaki, H. *et al.* Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. *Nature* **390**, 165–169 (1997).
62. Woods, S. C., Chavez, M. & Park, C. R. The evaluation of insulin as a metabolic signal influencing behavior via the brain. *Neurosci. Biobehav. Rev.* **20**, 139–144 (1996).
63. Qu, D. *et al.* A role for melanin-concentrating hormone in the central regulation of feeding behavior. *Nature* **380**, 243–247 (1996).
64. Sakurai, T. *et al.* Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* **92**, 573–585 (1998).
65. Schwartz, M. W. *et al.* Identification of targets of leptin action in rat hypothalamus. *J. Clin. Invest.* **98**, 1101–1106 (1996).
66. Elmquist, J. K. *et al.* Leptin activates neurons in ventrobasal hypothalamus and brainstem. *Endocrinology* **138**, 839–842 (1997).
67. Rohner-Jeanrenaud, F., Walker, C.-L., Greco-Perotto, R. & Jeanrenaud, B. Central corticotropin-releasing factor administration prevents the excessive body weight gain of genetically obese (*fa/fa*) rats. *Endocrinology* **124**, 733–739 (1989).
68. Gardner, J. D., Rothwell, N. J. & Luheshi, G. N. Leptin affects food intake via CRF-receptor-mediated pathways. *Nature Neurosci.* **1**, 103 (1998).
69. Freedman, M. R., Horwitz, B. A. & Stern, J. S. Effect of adrenalectomy and glucocorticoid replacement on development of obesity. *Am. Physiol. Soc.* R595–R607 (1986).
70. Gerald, C. *et al.* A receptor subtype involved in neuropeptide-Y induced food intake. *Nature* **382**, 168–171 (1996).
71. Tecott, L. H. *et al.* Eating disorder and epilepsy in mice lacking 5-HT_{2c} serotonin receptors. *Nature* **374**, 542–546 (1995).
72. Ioffe, E., Moon, B., Connolly, E. & Friedman, J. M. Abnormal regulation of the leptin gene in the pathogenesis of obesity. *Proc. Natl Acad. Sci. USA* **95**, 11852–11857 (1998).
73. Verploegen, S. *et al.* A human leptin mutant induces weight gain in normal mice. *FEBS Lett.* **405**, 237–240 (1997).
74. Chen, G. *et al.* Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. *Proc. Natl Acad. Sci. USA* **93**, 14795–14799 (1996).
75. Sivitz, W. I. *et al.* Effects of leptin on insulin sensitivity in normal rats. *Endocrinology* **138**, 3395–3401 (1997).
76. Miles, P. D. G., Yamatani, K., Lickley, H. L. A. & Vranic, M. Mechanism of glucoregulatory responses to stress and their deficiency in diabetes. *Proc. Natl Acad. Sci. USA* **88**, 1296–1300 (1991).
77. Haynes, W. G. *et al.* Receptor-mediated regional sympathetic nerve activation by leptin. *J. Clin. Invest.* **100**, 270–278 (1997).
78. Collins, S. *et al.* Role of leptin in fat regulation. *Nature* **380**, 677 (1996).
79. Zhou, Y.-T. *et al.* Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. *Proc. Natl Acad. Sci. USA* **94**, 6386–6390 (1997).
80. Frederich, R. C. *et al.* Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nature Med.* **1**, 1311–1314 (1995).
81. Van Heek, M. *et al.* Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *J. Clin. Invest.* **99**, 385–390 (1997).
82. Naggert, J. K. *et al.* Hyperproinsulinaemia in obese *fat/fat* mice associated with a carboxypeptidase E mutation which reduces enzyme activity. *Nature Genet.* **10**, 135–142 (1995).
83. Rovere, C., Viale, A., Nahon, J. L. & Kitabgi, P. Impaired processing of brain proneurotensin and melanin-concentrating hormone in obese *fat/fat* mice. *Endocrinology* **137**, 2954–2958 (1996).
84. Jackson, R. S. *et al.* Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nature Genet.* **16**, 303–306 (1997).
85. Kleyn, P. W. *et al.* Identification and characterization of the mouse obesity gene *tubby*: a member of a novel gene family. *Cell* **85**, 281–290 (1996).
86. Noben-Trauth, K., Naggert, J., North, M. & Nishina, P. A candidate for the mouse mutation *tubby*. *Nature* **380**, 534–538 (1996).
87. West, D. B., Boozer, C. N., Moody, D. L. & Atkinson, R. L. Dietary obesity in nine inbred mouse strains. *Am. J. Physiol.* **262**, R1025–R1032 (1992).
88. Schwartz, M. W. *et al.* Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nature Med.* **2**, 589–593 (1996).
89. Caro, J. F. *et al.* Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* **348**, 159–161 (1996).
90. Mandrup, S. *et al.* Obese gene expression at *in vivo* levels by fat pads derived from Sc implanted 3t3-F442a preadipocytes. *Proc. Natl Acad. Sci. USA* **94**, 4300–4305 (1997).
91. Wang, J. *et al.* A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature* **393**, 384–388 (1998).
92. Saladin, R. *et al.* Transient increase in *obese* gene expression after food intake or insulin administration. *Nature* **377**, 527–529 (1995).
93. Kiess, W. *et al.* High leptin concentrations in serum of very obese children are further stimulated by dexamethasone. *Horm. Metab. Res.* **28**, 708–710 (1996).
94. Miller, S. G. *et al.* The adipocyte specific transcription factor C/EBP α modulates human *ob* gene expression. *Proc. Natl Acad. Sci. USA* **93**, 5507–5511 (1996).
95. Devos, P. *et al.* Thiazolidinediones repress *ob* gene expression in rodents via activation of peroxisome proliferator-activated receptor gamma. *J. Clin. Invest.* **98**, 1004–1009 (1996).
96. Ravussin, E. *et al.* Relatively low plasma leptin concentrations precede weight gain in Pima Indians. *Nature Med.* **3**, 238–240 (1997).
97. Casanueva, F. F. *et al.* Serum immunoreactive leptin concentrations in patients with anorexia nervosa before and after partial weight recovery. *Biochem. Mol. Med.* **60**, 116–120 (1997).
98. Mantzoros, C. *et al.* Cerebrospinal fluid leptin in anorexia nervosa: correlation with nutritional status and potential role in resistance to weight gain. *J. Clin. Endocrinol. Metab.* **82**, 1845–1851 (1997).
99. Maffei, M. *et al.* Absence of mutations in the human *ob* gene in obese/diabetic subjects. *Diabetes* **45**, 679–682 (1996).
100. Montague, C. T. *et al.* Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* **387**, 903–908 (1997).
101. Strobel, A., Camoin, T. I. L., Ozata, M. & Strosberg, A. D. A leptin missense mutation associated with hypogonadism and morbid obesity. *Nature Genet.* **18**, 213–215 (1998).
102. Clement, K. *et al.* A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* **392**, 398–401 (1998).
103. Clement, K. *et al.* Indication for linkage of the human *OB* gene region with extreme obesity. *Diabetes* **45**, 687–690 (1996).
104. Reed, D. R. *et al.* Extreme obesity may be linked to markers flanking the human *OB* gene. *Diabetes* **45**, 691–694 (1996).
105. Comuzzie, A. G. *et al.* A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. *Nature Genet.* **5**, 273–276 (1997).
106. Krude, H. *et al.* Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nature Genet.* **19**, 155 (1998).
107. Murphy, J. E. *et al.* Long-term correction of obesity and diabetes in genetically obese mice by a single intramuscular injection of recombinant adeno-associated virus encoding mouse leptin. *Proc. Natl Acad. Sci. USA* **94**, 13921–13926 (1997).
108. Blackburn, G. Effect of degree of weight loss on health benefits. *Obes. Res.* **3** (suppl. 2), 211–216 (1995).
109. Manson, J. E. *et al.* Body weight and mortality among women. *New Engl. J. Med.* **333**, 677–685 (1995).

Acknowledgements. We thank S. Korres for assistance in preparing this manuscript and J. Froude for editing it, and D. Luck, J. Breslow, B. Schneider and M. Stoffel for critically reading it and for thoughtful comments. This work was supported by a grant from NIH/NIDDK (J.M.E.) and in part by an NIH MSTP grant (to J.L.H.).

Correspondence should be addressed to J.M.E. (e-mail: friedj@rockvax.rockefeller.edu).