

LEPTIN

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■ **Abstract** The discovery of the adipose-derived hormone leptin has generated enormous interest in the interaction between peripheral signals and brain targets involved in the regulation of feeding and energy balance. Plasma leptin levels correlate with fat stores and respond to changes in energy balance. It was initially proposed that leptin serves a primary role as an anti-obesity hormone, but this role is commonly thwarted by leptin resistance. Leptin also serves as a mediator of the adaptation to fasting, and this role may be the primary function for which the molecule evolved. There is increasing evidence that leptin has systemic effects apart from those related to energy homeostasis, including regulation of neuroendocrine and immune function and a role in development.

INTRODUCTION

Evidence for the existence of a physiological system for homeostatic regulation of body weight has accumulated over the past four decades. Kennedy (1) proposed that the amount of energy stored in adipose mass represented the balance between ingested calories and energy expenditure. Since adipose mass tends to be relatively stable over long periods in most mammals, he envisaged a homeostatic mechanism that monitors changes in energy stores and elicits compensatory changes in food intake and energy expenditure to maintain adipose mass at a set point. This adipostatic model of body weight regulation is consistent with the observation that a decrease in adiposity from fasting or surgical resection causes hyperphagia, decreases energy expenditure, and eventually restores body weight to the previous level (2-4). Conversely, weight gain from forced overfeeding inhibits voluntary food intake (3, 4). Hervey (5) demonstrated that cross-circulation (parabiosis) between rats rendered obese by lesions in the ventromedial hypothalamus (VMH) and control rats led to death from starvation in the latter. Based on these results he suggested that increased levels of a circulating satiety factor from the obese rats inhibited food intake in the non-lesioned lean rats. In contrast, the obese rats were incapable of responding to elevated endogenous levels of the presumed satiety factor because of the hypothalamic lesion. This latter view was

consistent with the known effects of VMH lesions on feeding and body weight regulation (6).

The concept of a circulating satiety factor was further strengthened by the discovery of recessive mutations, *obese* (*ob*) and *diabetes* (*db*), both of which led to hyperphagia, decreased energy expenditure, and early onset obesity in mice (7). Coleman (8, 9) and Hausberger (10) showed that parabiosis of wild-type mice and *ob/ob* mice suppressed weight gain in the latter, whereas parabiosis to *db/db* mice caused profound hypophagia in lean wild-type mice. Taken together, these results suggested that the *ob* locus was necessary for the production of a humoral satiety factor and that the *db* locus encoded a molecule required for response to this factor. Coleman's predictions more than two decades ago have been confirmed by the cloning of the *ob* and *db* genes (11). The product of the *ob* gene was named leptin (from the Greek root *leptos* meaning thin) because it caused marked reduction in food intake, body weight, and body fat when injected into leptin-deficient or normal mice (12). A rise in leptin levels was proposed to prevent obesity by decreasing appetite and increasing thermogenesis, through action in the brain (13, 14). However, as often happens when a new hormone or protein is discovered, the initial view of leptin as an anti-obesity hormone has been replaced by a more complex one. Here, we review major advances leading to our current understanding of the diverse roles of leptin in the regulation of metabolism, neuroendocrine, and immune function, and development.

THE *OB* GENE

The mouse *ob* gene was discovered through positional cloning and shown to encode a 4.5 kilobase mRNA transcript with a highly conserved 167-amino acid open reading frame that was unique in the GenBank database (11). Mouse and human *ob* genes have been localized to chromosomes 6 and 7q31.3, respectively (11, 15, 16). The *ob* gene encompasses 650 kb and consists of 3 exons separated by 2 introns. The coding region for Ob protein is located in exons 2 and 3. Several regulatory elements have been identified within the *ob* gene promoter, including cyclic AMP and glucocorticoid response elements, and CCATT/enhancer and SP-1 binding sites (17–19). The sites responsible for adipose-specific expression and for regulated expression in responses to changes in adipose size or energy balance have not been identified. Analysis of the *ob* gene product revealed characteristics consistent with a secreted protein and a high degree of homology among species. For example, human leptin is 84% identical to mouse leptin and 83% identical to rat leptin. Leptin is synthesized mainly, but not exclusively (see below), by white adipose tissue and circulates as a 16-kDa protein. There is a strong positive correlation between leptin mRNA and protein levels in adipose tissue and circulating leptin levels (20–22). Structural analysis indicates that leptin is similar to cytokines (23, 24). Moreover, leptin contains an intrachain disulfide bond that appears to be necessary for its biological activity (25). So far, it is not

known whether leptin is secreted by a constitutive or regulated mechanism; however, the former means is thought to be more likely because leptin does not appear to be stored in substantial amounts (26).

Mutations of the *ob* gene cause early onset obesity in mice (11–14). In C57Bl/6J *ob/ob* mice, a Cys-to-Thr substitution results in a stop codon at position 105 instead of arginine and in synthesis of a truncated protein that is incapable of being secreted (11, 27). mRNA expression of *ob* is increased in C57Bl/6J *ob/ob* mice, consistent with the view that the *ob* gene is under negative feedback regulation. In the *ob^{2J}/ob^{2J}* mouse mutant, a transposon inserted into the first intron of the *ob* gene prevents the synthesis of mature *ob* mRNA (11). Both *ob/ob* mouse mutants are leptin deficient, hyperphagic, hypothermic, and morbidly obese and have several metabolic and neuroendocrine abnormalities.

Human *ob* gene mutations are rare and were first reported in two children from a consanguineous Pakistani family in 1997 (28). In these patients, deletion of a single guanine nucleotide in codon 133 led to a frameshift mutation and synthesis of a truncated leptin protein that underwent proteosomal degradation (27). Strobel et al (29) identified three members of a Turkish family with a missense mutation (Cys-to-Thr) in codon 105. As with the *ob* mutation described above, the abnormal leptin protein encoded in these patients is incapable of being secreted (29). Human *ob* gene mutations cause hyperphagia, morbid obesity, and hypothalamic hypogonadism. Impairment of linear growth and decreased sympathetic tone (measured by cold pressor response) are less thoroughly documented (28, 29). However, unlike *ob/ob* mice (11–14) hyperinsulinemia, hyperglycemia, hypercorticism, and hypothermia have not as yet been reported in leptin-deficient humans (28, 29). The reasons for these species differences are not known, but they suggest substantial differences in the physiological actions of leptin between rodents and humans. As is discussed below, the relative importance of leptin as a signal for the adaptation to fasting may differ among these species.

REGULATION OF LEPTIN EXPRESSION

Leptin expression is influenced by the status of energy stores in fat, as evidenced by increased adipose *ob* mRNA and serum leptin levels in obese humans and other mammals (20–22, 30, 31) (Table 1). Moreover, adipocyte size is an important determinant of leptin synthesis, as larger adipocytes contain more leptin than smaller adipocytes in the same individual (30). Leptin levels in blood correlate with total body fat stores (20–22); however, it is not known whether increased triglyceride levels, lipid metabolites, or mechanical factors associated with increased adipocyte size influence leptin expression. Leptin levels increase within hours after a meal in rodents and after several days of overfeeding in humans (32–34). Leptin levels decrease within hours after initiation of fasting in both species (32, 35, 36). The changes in leptin expression in response to fasting and feeding are out of proportion to the corresponding changes in body weight or

TABLE 1 Regulation of leptin expression

| Site | Increase | Decrease |
|-----------------------|---|--|
| Adipose tissue | Overfeeding Obesity (except ob mutation) Insulin Glucocorticoids Acute infection Cytokines (TNF- α , IL-1, LPS) | Fasting Testosterone Beta-adrenergic agonists Thiazolidinediones (in vitro) ? Thyroid hormone Cold exposure |
| Placenta | Insulin Glucocorticoids Hypoxia | Smoking Low birth weight |
| Skeletal muscle (rat) | Glucosamine Glucose Lipids | |
| Stomach fundus (rat) | | Feeding Cholecystokinin |

body fat (34, 36), suggesting that leptin serves as an indicator of energy stores, as well as a mediator of energy balance. On the other hand, because leptin levels do not rise in response to individual meals (37), leptin is not likely to serve as a meal-related satiety signal.

Regulation of leptin expression by nutrition is probably mediated in part by insulin. Leptin expression increases after peak insulin secretion during the feeding cycle (32, 38). Insulin stimulates leptin expression directly in isolated adipocytes (39) and increases leptin levels when injected into rodents (32). In contrast, leptin is decreased in low insulin states, such as streptozotocin-induced diabetes, and increases after insulin treatment (40). In humans leptin expression is correlated with insulin levels, increases several days after insulin infusion, and may be predictive of insulin resistance (41–43). Conversely, the fall in insulin levels may mediate the decline in leptin levels during fasting (36).

Leptin levels are regulated by other factors (Table 1). Glucocorticoids directly stimulate leptin synthesis in cultured adipocytes (44–46), and leptin expression increases in response to chronic elevation of cortisol in humans (47). In contrast to this positive relationship in free-living animals, plasma leptin and glucocorticoid levels are inversely related. Peak glucocorticoid levels coincide with the nadir of leptin at the beginning of the light cycle in humans (dark cycle in rodents), and the nadir of glucocorticoids is related to peak leptin levels at night (light cycle in rodents) (48–51). An inverse correlation between pulsatile leptin secretion and cortisol and adrenocorticotropin (ACTH) has been reported in humans (50). Leptin levels are higher in prepubertal rodents and boys and does not appear to be dependent on adipose mass or triglyceride level (49, 52, 53). The prepubertal increase in leptin expression precedes the rise in testosterone and estradiol and is postulated to be involved in the maturation of the gonadal axis (49, 53). Females have higher leptin levels than males when matched by age, weight, or body fat

(54, 55). This may be attributable to sex differences in body fat distribution and testosterone level (54, 56). Subcutaneous adipose tissue is more abundant and contains higher levels of leptin in females. Leptin synthesis is inhibited by testosterone but is not affected by ovarian sex steroids (56, 57).

Administration of thyroid hormone decreases leptin levels in rodents (58). Although levels of plasma leptin, thyroid-stimulating hormone (TSH), and adiposity correlate in euthyroid patients (59), there are conflicting reports on the effect of hypothyroidism and hyperthyroidism on leptin levels and on the interaction between leptin and the pituitary-thyroid axis in humans. Some studies have described an elevation of plasma leptin in hypothyroid patients and a decrease in hyperthyroid patients, but others have reported no significant alteration in leptin levels in these conditions or in response to replacement doses of thyroxine (59–62).

Leptin synthesis is stimulated by infection, endotoxin, and cytokines, e.g. tumor necrosis factor (TNF), leukemia inhibitory factor (LIF), and interleukin 1 (IL-1) (63–66). In addition to regulating feeding behavior and energy balance directly, the rise in leptin as a result of elevation of cytokine levels may also contribute to anorexia and weight loss in these inflammatory conditions (65, 67). Troglitazone decreases leptin synthesis in vitro (68), and cold exposure and catecholamines decrease leptin expression, most likely through activation of β -adrenergic receptors (69–72). The effects of thiazolidinediones and catecholamines on leptin levels are likely to be mediated by direct action on the *ob* gene, since binding sites for the respective nuclear transcription factors are present in the *ob* gene promoter (17, 18).

Initial studies indicated that leptin expression was synthesized only in adipose tissue. However, leptin is also synthesized in extra-adipose tissues including placenta, gastric fundic mucosa, skeletal muscle, and mammary epithelium (73–76) (Table 1). Placental leptin expression is stimulated by hypoxia, insulin, and glucocorticoids (77, 78). Leptin is synthesized by mammary epithelium, secreted in colostrum, and absorbed by the neonate (74). Feeding and administration of cholecystokinin or gastrin decrease leptin synthesis in the gastric fundus and increase plasma leptin (75). It is speculated that meal-related alterations in gastric and plasma leptin levels may be involved in the short-term regulation of appetite (75). Glucosamine infusion increases leptin expression in adipose tissue and induces de novo leptin synthesis in rat skeletal muscle (76). Glucose and lipid infusion have similar effects on leptin expression in these tissues, raising the possibility that leptin acts as a sensor of nutrient flux in adipose tissue and skeletal muscle (76).

THE *DB* GENE

Tartaglia et al (79) were the first to isolate the leptin receptor (Ob-R) from mouse choroid plexus using an expression cloning strategy. Because the sequence and expression of the initially cloned receptor are normal in *db/db* mice, they predicted

that the *db* mutation affected a different receptor or an alternatively spliced isoform. The latter explanation rapidly proved to be correct. Multiple splice variants of Ob-R mRNA encode at least six leptin receptor isoforms (80, 81) (Figure 1). The leptin receptor belongs to the family of class 1 cytokine receptors, which include receptors for interleukin 6 (IL-6), leukemia inhibitory factor (LIF), granulocyte-colony stimulating factor (GCSF), and glycoprotein 130 (for review see 82). Leptin receptor isoforms share an identical extracellular ligand-binding domain at the amino terminus but differ at the carboxy terminus. Five isoforms, Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, and Ob-Rf have transmembrane domains; however, only Ob-Rb (the long receptor isoform) contains intracellular motifs required for activation of the JAK (janus kinase)-STAT (signal transducers and activators of transcription) signal transduction pathway (83–86) (Figure 1). Activation of ObRb, and to a lesser extent ObRa, promotes JAK-dependent signaling to pathways other than STAT, such as MAP kinase (86), and the relative importance of these divergent signaling events in leptin action is unknown. Leptin binds with high affinity (nanomolar range) to an Ob-R homodimer, leading to activation of

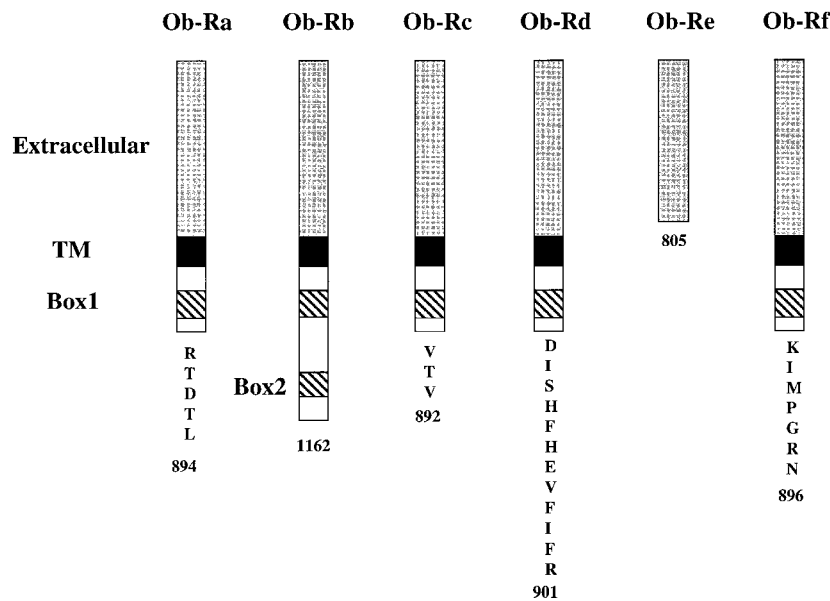


Figure 1 Domain structure of alternatively spliced leptin receptor (Ob-R) isoforms. Ob-R isoforms (ObRa-ObRf) share a common extracellular leptin-binding domain, but differ at the carboxy terminus (intracellular domain). Only Ob-Rb, the long isoform, has all intracellular protein motifs necessary for signaling via the Jak-STAT signal transduction pathway. Terminal amino acid residues for various Ob-R isoforms are denoted by the alphabet code. Ob-Re lacks a transmembrane domain (TM) as well as intracellular motifs. Intracellular Box1 and Box2 domains are indicated by hatched boxes.

JAK2. It is not known whether Ob-Rb is capable of forming heterodimers with other receptor isoforms. Ob-Re lacks transmembrane and intracellular domains and circulates as a soluble receptor (80, 87).

Leptin receptor mutations cause early onset obesity in rodents (83, 88, 89). In C57Bl/Ks *db/db* mice, a premature stop codon inserted in the 3'-end of the Ob-Rb mRNA transcript leads to the synthesis of a truncated receptor that replaces the Ob-Rb isoform with the Ob-Ra isoform, which is incapable of mediating JAK-STAT signaling (84, 85). Ob-Ra and other splice variants are expressed normally in these *db/db* mice. Mice with *db^{Pas}/db^{Pas}* and *db^{3J}/db^{3J}* mutations lack transmembrane and intracytoplasmic domains and thus lack all receptor species (87, 90). *db* mutations cause leptin insensitivity, hyperphagia, metabolic derangement, morbid obesity, and neuroendocrine abnormalities, including hypercorticism and hypothalamic hypogonadism (12, 14, 91). A Gln-to-Pro substitution at amino acid position 269 in the extracellular domain decreases cell surface Ob-R expression in *fa/fa* rats and additionally reduces the signaling capacity of the receptor (83, 89). There are conflicting reports on the effect of intracerebroventricular (icv) leptin administration on feeding in *fa/fa* rats. Some studies have described a decrease in food intake in response to icv leptin injection; however, others have observed no such effect in these rats (93, 94). Obese Koletsky rats have a point mutation at amino acid position 763, which results in a stop codon in the extracellular domain and failure of expression of all isoforms of Ob-R (88, 95).

Ob-R mutations are extremely rare in humans. Clement et al (96) described the first cases of human Ob-R mutations in three sisters from a consanguineous Kabilian family. In these patients, a G→A substitution in the splice donor site of exon 16 resulted in a truncated leptin receptor lacking both transmembrane and intracellular domains. The mutant leptin receptor circulates at high concentrations and is capable of binding leptin (96). As with human *ob* gene mutations, human *db* mutation causes hyperphagia, early onset obesity, and hypothalamic hypogonadism. In addition, the secretion of thyrotropin and growth hormone is impaired in these patients. Unlike *db/db* mice, the human *db* mutation is not associated with hyperglycemia, hypercorticism, and hypothermia (91, 96).

LEPTIN TRANSPORT AND SITES OF ACTION

Leptin circulates as a 16-kDa protein and is partially bound to plasma proteins (97, 98). In one study, the proportion of bound leptin was reported to be higher in lean (~45%) compared with obese (~20%) individuals (98). The bound fraction of leptin can be as high as 80% in humans with a leptin receptor mutation due to binding of leptin to circulating leptin receptors (96). An additional pool of leptin is bound to tissue-binding sites and is likely to contribute to the maintenance of steady-state plasma leptin levels (99). Based on anatomic and functional data, it appears that leptin exerts its effects on energy balance mainly by

acting in the brain. Intravenous leptin injection activates neurons in the arcuate, ventromedial, and dorsomedial hypothalamic nuclei and in brainstem neuronal circuits implicated in the regulation of feeding behavior and energy balance (100, 101). The long form leptin receptor (Ob-Rb) is present in these hypothalamic regions and colocalized with STAT3 and neuropeptide mediators of leptin action, such as neuropeptide Y (NPY) and proopiomelanocortin (POMC) (102–106). In contrast, short leptin receptor isoforms are expressed in choroid plexus, vascular endothelium, and peripheral tissues, such as kidney, liver, lung, and gonads, where they may serve a transport and/or clearance role (104, 107). It is of interest that intracerebroventricular leptin injection inhibits food intake and decreases adiposity more potently than peripheral leptin administration (12–14).

Leptin enters the rat brain by a saturable transport mechanism (108), possibly by receptor-mediated transcytosis across the blood-brain barrier, as is the case for some other large proteins (109, 110). In support of this view, brain microvessels express high levels of the short form leptin receptor Ob-Ra and are also capable of binding and internalizing leptin (107, 111). Leptin target neurons in the arcuate, dorsomedial, ventromedial, and ventral premammillary nuclei lie within close proximity to the median eminence. Because capillaries in the median eminence lack tight junctions, as in other circumventricular organs, leptin may reach neurons in the adjacent ventrobasal hypothalamus through diffusion. Leptin may also be transported into the brain via cerebrospinal fluid (CSF) (112). It has also been suggested that Ob-Ra, which is highly expressed in the choroid plexus (the site of CSF production), could mediate blood-to-CSF leptin transport (107). However, leptin is present (albeit at a much lower fraction of the elevated circulating levels) in CSF of obese Koletsky rats, which totally lack leptin receptors, indicating that leptin receptors are not essential for leptin transport into CSF (95). More importantly, CSF leptin concentration is ~ 100 -fold lower than plasma leptin and less than the K_d of the leptin receptor (79, 112). Therefore, it is unlikely that CSF leptin is a major source of leptin for targets in the brain.

Leptin is distributed widely in various tissues and is cleared mainly by the kidney (99, 113, 114). A role for the kidney in leptin clearance is consistent with elevation of leptin levels in patients with renal impairment and end-stage renal disease (115, 116). Leptin is filtered by the glomeruli and is thought to be degraded by renal epithelial cells (114). High levels of short form leptin receptors are present in the kidney, and leptin binds to the corticomedullary junction and renal papilla (117). These receptors may internalize and degrade leptin, as occurs in Chinese hamster ovary (CHO) cells expressing Ob-Ra (118). Leptin levels are higher in patients with liver cirrhosis (119, 120), suggesting that the liver may be involved in leptin synthesis or clearance. The liver is unlikely to be involved in leptin clearance because there is no net uptake of leptin by normal human liver (113). A study in rats has demonstrated that transdifferentiated stellate cells obtained from rat liver following injury are capable of synthesizing leptin (121). This finding in humans raises the possibility that the rise in leptin levels in cirrhosis is derived in part from hepatic synthesis.

LEPTIN ACTION IN THE BRAIN

The discovery of leptin has provided insight into the interaction between energy stores and hypothalamic centers that regulate feeding behavior and energy balance. Abnormalities of energy balance resulting from ventrobasal hypothalamic lesions resemble those in *ob/ob* and *db/db* mice (reviewed in 122, 123). All these conditions are characterized by hyperphagia, decreased energy expenditure, and increased adiposity, and these features underscore the critical roles of leptin as an indicator of energy stores and the hypothalamus as a major effector organ for energy homeostasis. Leptin-sensitive neurons in the arcuate, ventromedial, and dorsomedial hypothalamic nuclei express neuropeptides/neurotransmitters implicated in the central regulation of energy balance. A detailed description of the neuroanatomy of leptin targets in the brain is beyond the scope of this article (122–124). Briefly, the long form leptin receptor (Ob-Rb) is coexpressed with neuropeptide Y (NPY), agouti-related peptide (AgRP), proopiomelanocortin (POMC), a precursor of α -melanocyte stimulating hormone (α -MSH), and cocaine- and amphetamine-regulated transcript (CART) in the arcuate hypothalamic nucleus (Figure 2). NPY and AgRP are expressed in the same neurons in the medial arcuate nucleus, whereas POMC and CART are coexpressed in the lateral arcuate nucleus. Intracerebral injection of NPY stimulates feeding (i.e. orexigenic). In contrast, α -MSH (a product of POMC) and CART inhibit feeding (i.e. anorexigenic). α -MSH is thought to regulate feeding through melanocortin 4 (MC4) receptors in the hypothalamus (125). AgRP antagonizes the actions of α -MSH at MC4 receptors and is therefore orexigenic (126).

Leptin-sensitive neurons in the arcuate hypothalamic neurons may influence feeding by regulating the expression of other orexigenic peptides, e.g. melanin-concentrating hormone (MCH) and possibly orexins/hypocretin in the lateral hypothalamic nucleus (Figure 2) (122, 123). Other neurotransmitters/neuropeptides suggested as potential mediators of leptin action in the brain include corticotropin-releasing hormone (CRH), cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), urocortin, bombesin, and serotonin (87, 127). The stimulatory effect of NPY and MCH on food intake is consistent with the elevation of the levels of these peptides in the hypothalamus in states of leptin deficiency, e.g. *ob/ob* mice and fasting, and the decrease in their expression in response to leptin administration (123, 124). In contrast, expression of the anorexigenic peptides POMC and CART is decreased in *ob/ob* mice and during fasting and increased in response to leptin administration (67, 123, 128).

Genetic ablation of putative neuropeptide mediators of leptin action in the brain does not always produce phenotypes predicted on the basis of pharmacological studies. For example, NPY deficiency or lack of Y1 and Y5 NPY receptors does not reduce feeding behavior or prevent weight gain, impair thermogenesis and neuroendocrine function, or impair responsiveness to leptin (129–131). Mice with mutation of the serotonin 5-HT_{2C} receptor develop hyperphagia, late onset obesity, and hyperleptinemia but are still capable of responding normally to exog-

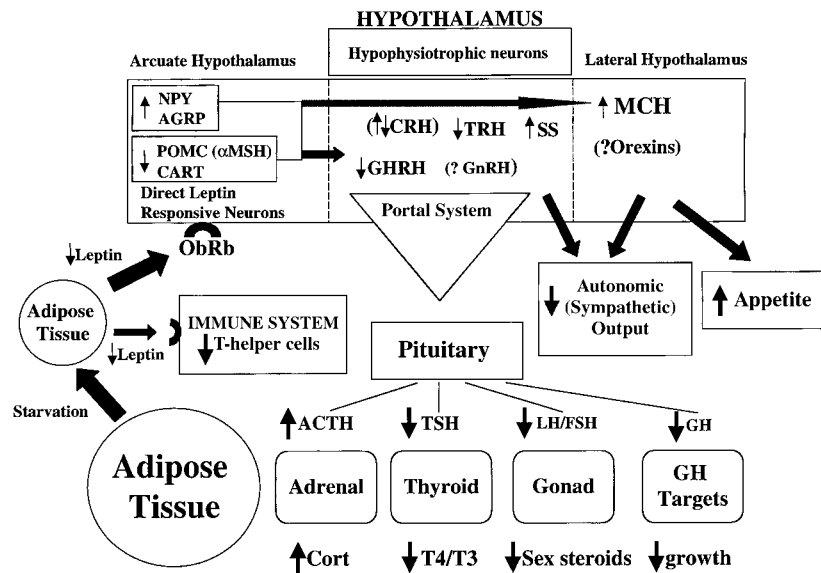


Figure 2 Role of leptin in the adaptation to starvation. The fall in leptin with starvation results in an increase in neuropeptide Y (NPY) and agouti-related peptide (AGRP) levels, and a decrease in proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) levels in the arcuate hypothalamic nucleus. NPY, POMC, AGRP, and CART neurons are directly responsive to leptin. NPY and AGRP stimulate feeding (orexigenic), whereas α -melanocyte stimulating hormone (a product of POMC) and CART inhibit feeding (anorexigenic). These neurons also project to the lateral hypothalamus and regulate the expression of melanin-concentrating hormone (MCH), a major stimulator of feeding. In addition, leptin targets in the arcuate hypothalamic nucleus respond to low leptin levels by regulating the neuroendocrine axis and decreasing sympathetic nervous output. The fall in leptin also leads to suppression of immune function. The metabolic and neuroendocrine adaptations to fasting mediated by leptin are likely to be of greater survival value in rodents since short-term starvation has more severe consequences in this species. CRH (corticotropin-releasing hormone), TRH (thyrotropin-releasing hormone), GHRH (growth hormone-releasing hormone), SS (somatostatin), GnRH (gonadotropin-releasing hormone), GH (growth hormone).

enous leptin (132). NPY appears to be partially responsible for hyperphagia, obesity, and neuroendocrine abnormalities in leptin-deficient *ob/ob* mice, as evidenced by partial amelioration of these abnormalities in NPY-deficient *ob/ob* mice (133). In contrast to NPY deficiency, MCH deficiency causes hypophagia and failure of weight gain, in agreement with its suggested role as an orexigenic peptide (134). These findings suggest that the central effects of leptin and other feeding signals are likely mediated by neuronal networks expressing a complex array of hypothalamic neuropeptides/neurotransmitters.

In addition to regulating food intake, leptin-sensitive NPY/AgRP and POMC/CART neurons project to the paraventricular hypothalamic nucleus and probably mediate the effects of leptin on the neuroendocrine axis and autonomic function (122, 123). The fall in leptin levels suppresses thyroid function, in part by decreasing thyrotropin-releasing hormone (TRH) formation in the paraventricular hypothalamic nucleus (135). Ablation of the arcuate hypothalamic nucleus with monosodium glutamate prevents the fall in TRH mRNA expression with fasting, indicating that the effect of leptin on the thyrotropic axis is mediated, at least in part, through leptin-sensitive NPY and POMC neurons (136). Leptin stimulates secretion of gonadotropin-releasing hormone (GnRH) in hypothalamic explants (137). However, this effect may not be mediated directly by leptin, since GnRH neurons do not express detectable levels of leptin receptors (138). Reports on regulation of CRH by leptin are variable. Leptin has been reported to increase basal CRH release from hypothalamic explants and to inhibit hypoglycemia-induced CRH release from hypothalamic explants (139, 140). Leptin is reported to stimulate CRH mRNA expression in the paraventricular hypothalamic nucleus in some studies (67), whereas others have not demonstrated a significant effect.

Although leptin activates STAT1, 3, and 5 in in vitro systems, intravenous leptin activates only STAT3 in the mouse hypothalamus (84, 85). STAT3 protein is expressed in NPY and POMC neurons in the arcuate hypothalamic nucleus, consistent with the concept that leptin regulates the transcription of these genes at least in part through the JAK-STAT signal transduction pathway (106). Leptin also regulates the expression of other STAT3 target genes in the hypothalamus, including *c-fos*, *c-jun*, *tis-11*, and SOCS-3 (a member of the suppressors of cytokine signaling family) (141). Leptin inhibits glucose-responsive neurons in the hypothalamus and insulin secretion by pancreatic β -cells, through effects on ATP-sensitive potassium channels (142, 143). In addition, leptin depolarizes paraventricular hypothalamic neurons (144), inhibits hypothalamic NPY release (145), and regulates vagal afferents in the stomach (146). The mechanisms underlying these rapid electrophysiological actions of leptin are not known but are not likely to involve STAT-mediated transcription.

Although studies in *ob/ob* mice and other rodents, often involving the administration of supraphysiological doses of leptin, have provided some insights into leptin action, it is not known how physiological alterations in leptin influence neuronal function. Ioffe et al (147) showed that there is a dose-effect of leptin such that neuroendocrine abnormalities, i.e. hypercorticism and central hypogonadism, are corrected by lower doses of leptin. In contrast, leptin concentrations above the normal fed level are required to inhibit feeding and prevent weight gain. Thermoregulation requires even higher leptin levels than are required for neuroendocrine regulation and maintenance of normal body weight. These results suggest that there are different thresholds for leptin for various functions and that responses to physiological alterations in leptin may be mediated by different subsets of neurons.

THE PHYSIOLOGICAL ROLE OF LEPTIN IN ENERGY HOMEOSTASIS

Leptin As Anti-Obesity Hormone

Administration of leptin to rodents decreases food intake and increases energy expenditure (12–14, 148). In contrast to starvation, weight loss after peripheral or central leptin administration is restricted to adipose tissue, with no loss of lean mass (148). Leptin activates lipid oxidation, at least in part, by inducing the expression of enzymes of lipid oxidation (149). In rats leptin also stimulates apoptosis of adipocytes (150). The ability of leptin to decrease body weight and body fat content led to the prevailing view that leptin is an anti-obesity hormone (reviewed in 87, 151, 152). However, this view must be reconciled with the failure of high endogenous leptin levels to prevent obesity in humans and other obese mammals (20–22). Hyperleptinemia is thought to be indicative of leptin resistance, which may play a role in the development of obesity (87, 151, 152). Mechanisms thought to underlie leptin resistance include dysregulation of leptin synthesis and/or secretion, abnormalities of brain leptin transport, and abnormalities of leptin receptors and/or post-receptor signaling.

Studies in obese rodents have provided some insights into the potential mechanisms of leptin resistance. However, there is as yet no direct explanation of the apparent lack of sensitivity of individuals to elevated leptin levels during the course of diet-induced obesity. CSF leptin transport may be limited in obesity, as evidenced by decreased plasma:CSF leptin ratio in obese individuals (112, 153). Because plasma:CSF ratios are markedly decreased in *fa/fa* and Koletsky rats with abnormalities of membrane leptin receptor expression, leptin resistance may arise from defects of receptor-mediated leptin transport into the brain (95). A polygenic mutation that leads to late onset obesity in New Zealand obese (NZO) mice may also offer some insight into the role of brain leptin transport in obesity. These mice are resistant to peripheral leptin administration, but do respond to intracerebroventricular leptin injection, consistent with defective brain leptin transport (148). Similarly, diet-induced obesity in rodents is characterized by insensitivity to peripheral leptin injection (154) but respond to intracerebroventricular leptin (154). In contrast, agouti (*Ay/a*) mice have impaired melanocortin (MC4) receptor signaling in the brain and are resistant to both peripheral and central leptin injection (148). Studies of leptin transport into brain in these models have not been reported.

A member of the suppressors of cytokine signaling family, SOCS-3, is a potential molecular mediator of leptin resistance (141). SOCS-3 mRNA levels are conserved by leptin-mediated STAT3 activation, and SOCS-3 protein potently inhibits leptin signaling. In support of its role as an inhibitor of leptin action in the brain, SOCS-3 expression is induced in the arcuate and dorsomedial hypothalamic nuclei of mice after leptin treatment (141). Leptin resistance may also be mediated by the SH2-containing tyrosine phosphatase, SHP-2, since leptin

signaling is reported to be enhanced when the binding site on Ob-Rb for SHP-2 is mutated (155). Leptin resistance could also reside at steps downstream from the initial step of receptor interaction.

The role of leptin in body weight regulation may involve interactions with other metabolic signals, notably insulin and glucocorticoids (67). These hormones regulate the expression of similar neuropeptides in brain regions involved in feeding behavior and body weight regulation. Glucocorticoids have a permissive effect on obesity, as evidenced by the ability of adrenalectomy to ameliorate obesity (156–158). Conversely, hypercortisolism leads to abnormalities of adipose distribution (159). Further studies are needed to understand the interactions among these metabolic hormones.

Leptin As a Signal for Adaptation to Fasting

The widespread occurrence of leptin-resistant obesity may reflect the fact that inability to store energy efficiently at times of abundance is evolutionarily disadvantageous (151). According to this view, the dominant role of leptin in energy homeostasis is likely to be as a mediator of the adaptation to fasting (48, 151). Starvation triggers complex neural, metabolic, hormonal, and behavioral adaptations with the goal of maintaining the supply of energy substrates for use by the brain, protecting lean mass, and promoting survival. A major aspect of this adaptation is the capacity to switch from carbohydrate- to fat-based metabolism during fasting. This change is mediated predominantly by a fall in insulin and rise in counteregulatory hormones, i.e. glucagon, epinephrine, and glucocorticoids (67, 151, 160). Other adaptations to starvation include a decrease in thyroid and gonadal hormones, increased adrenal glucocorticoids, decreased body temperature, and increased appetite. The net effect of these adaptations is to stimulate gluconeogenesis to provide glucose for vital cellular function and supply fatty acids for use by skeletal muscle. Energy utilization is minimized during fasting, in part through suppression of thyroid thermogenesis and curtailment of procreation and growth. In addition, starvation is characterized by immune suppression, including decreased lymphocyte proliferation and helper T-cell cytokine production (161). The changes in thyroid hormones, glucocorticoids, and in body temperature are prominent in rodents but limited in humans (48, 162–164). Similarly, perturbations of the reproductive axis as a result of starvation develop more rapidly in rodents than humans (48, 165).

Starvation decreases leptin levels (21, 48). Because leptin-deficient *ob/ob* mice have similar metabolic, neuroendocrine, and immune abnormalities as those resulting from starvation (91), we reasoned that leptin deficiency is perceived as a state of continuous starvation in *ob/ob* mice and that the fall in leptin mediates the adaptation to fasting (48). To test this hypothesis, leptin was administered twice daily by intraperitoneal injection to mice during a 48-h fast. Prevention of the characteristic fall in leptin during fasting blunted the expected rise in corticosterone and ACTH and prevented the decrease in the levels of thyroid

hormone, testosterone, and luteinizing hormone (48). Leptin administration during fasting also prevented a prolongation of estrus cycles (48). Studies in rats and non-human primates have also demonstrated that the fall in leptin with fasting is an important mediator of the somatotrophic, thyroid, and reproductive alterations (135–138, 166, 167). Leptin stimulates inflammatory response, T-lymphocyte proliferation, and Th1 cytokine production during fasting in normal mice and in fed *ob/ob* mice, indicating that leptin is an important link between nutrition and the immune system (161).

Low leptin levels may contribute to the development of obesity. Leptin is inappropriately low (as a function of body fat) in some obese individuals (168); however, it is not known whether these individuals have defective leptin synthesis and/or release, and this finding has not yet been observed in all populations (169). Although the functional implications of this observation are yet to be determined, it is plausible that relatively low leptin is perceived as a starvation signal, leading to increased appetite and efficient energy utilization. In contrast, elevation of leptin levels may predispose to cachexia in patients with renal failure and infections by inhibiting appetite and increasing energy expenditure (65, 116). The plasma:CSF leptin ratio is normal in patients with anorexia nervosa during refeeding (prior to weight restoration) and may create a premature sense of satiety during refeeding (112, 170).

OTHER ACTIONS OF LEPTIN

Leptin has diverse effects on the neuroendocrine axis in addition to appetite and body weight regulation. Leptin appears to be necessary for maturation of the reproductive axis, as evidenced by its ability to restore puberty and fertility in *ob/ob* mice, accelerate puberty in wild-type mice, and facilitate reproductive behavior in rodents (171–174). Mutations of *ob* and *db* genes result in hypothalamic hypogonadism in humans (29), and low leptin and absence of a diurnal leptin rhythm occur with exercise-induced amenorrhea (51). Leptin stimulates gonadotropin release and inhibits insulin-like growth factor-mediated release of estradiol in ovarian follicular cells (137, 175). These results indicate that leptin signals the adequacy of energy stores for reproduction, by interacting with different target organs in the hypothalamic-pituitary-gonadal axis.

In rodents leptin is also capable of regulating the hypothalamic-pituitary-adrenal axis independent of its role in the adaptation to starvation. For example, leptin injection blunts ACTH and corticosterone secretion during restraint stress in rats and directly inhibits glucocorticoid synthesis and ACTH-stimulated glucocorticoid secretion in adrenal cortical cells (140, 176, 177). Taken together with its ability to regulate CRH synthesis and secretion (139, 140), these results show that leptin is capable of interacting with various components of the hypothalamic-pituitary-adrenal (HPA) axis. The absence of HPA activation in leptin-deficient humans suggests species differences that require additional study.

Leptin exerts acute effects on metabolism, independent of its role in long-term body weight regulation. For example, leptin decreases glucose and insulin levels acutely in *ob/ob* mice before detectable weight loss (49) and also stimulates gluconeogenesis and glucose metabolism in wild-type rodents (178–180). Leptin stimulates lipolysis, alters lipid partitioning in skeletal muscle, and is capable of increasing fatty acid synthesis in the liver (13, 180, 181). The extent to which these effects are mediated directly on peripheral targets or through the central nervous system is as yet unsettled. Local leptin expression in the stomach has been postulated to regulate satiety (75). The long form leptin receptor (Ob-Rb) is expressed in jejunal epithelium and responds to intravenous leptin administration by inducing STAT3, STAT5, and immediate early genes (182). Direct leptin signaling in the intestine may be involved in the regulation of nutrient absorption and intestinal motility. Leptin also increases sympathetic nerve activity in brown adipose tissue, adrenal gland, kidney, and hindlimb skeletal muscle (183). Thus in addition to increasing energy expenditure (at least in rodents) (13, 184), leptin may be involved in the regulation of cardiovascular and renal function via the central nervous system (183). Such a role may have important implications for the development of cardiovascular and renal complications in obesity and related diseases.

Leptin may play an important role in development, as evidenced by formation of leptin in placenta, widespread expression of leptin and its receptors in fetal tissues, and stimulation of hematopoiesis and angiogenesis by leptin (73, 185–187). Leptin is also likely to be involved in brain development. *ob/ob* and *db/db* mice have decreased brain weight, structural neuronal defects, impaired myelination, and deficiency of several neuronal and glial proteins (188–191). Chronic leptin administration increases brain weight, and restores whole brain protein content and the levels of some neuronal proteins, e.g. growth-associated protein (GAP-43), syntaxin 1, and SNAP-25 in *ob/ob* mice (190, 191).

CONCLUSION

The discovery of leptin marks an important milestone in our understanding of metabolic physiology and will stimulate further research into effector mechanisms in the brain and other organs involved in energy homeostasis. Our current understanding of the roles of leptin in metabolism and neuroendocrine regulation derives mainly from studies in rodents. Although there have been many reports of associations between leptin levels and various physiological and disease states in humans, there are no studies designed to evaluate the role of leptin directly in humans. Human recombinant leptin has been used in phase 1 trials for obesity. It is hoped that future studies will examine the effects of leptin administration on metabolism and on neuroendocrine and immune systems in humans. It is likely that the most important physiological role of leptin is as a signal for the switch between the starved and fed states. Although some leptin is thought to prevent

obesity, most obesity occurs in the presence of increased leptin levels. Major tasks ahead include the determination of the molecular mechanisms for leptin resistance and the role, if any, of leptin in the pathophysiology of common obesity.

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