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Leptin in Human Physiology and Therapeutics

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Abstract

Leptin regulates energy homeostasis and reproductive, neuroendocrine, immune, and metabolic functions. In this review, we describe the role of leptin in human physiology and review evidence from recent “proof of concept” clinical trials using recombinant human leptin in subjects with congenital leptin deficiency, hypoleptinemia associated with energy-deficient states, and hyperleptinemia associated with garden-variety obesity. Since most obese individuals are largely leptin-tolerant or -resistant, therapeutic uses of leptin are currently limited to patients with complete or partial leptin deficiency, including hypothalamic amenorrhea and lipoatrophy. Leptin administration in these energy-deficient states may help restore associated neuroendocrine, metabolic, and immune function and bone metabolism. Leptin treatment is currently available for individuals with congenital leptin deficiency and congenital lipoatrophy. The long-term efficacy and safety of leptin treatment in hypothalamic amenorrhea and acquired lipoatrophy are currently under investigation. Whether combination therapy with leptin and potential leptin sensitizers will prove effective in the treatment of garden-variety obesity and whether leptin may have a role in weight loss maintenance is being greatly anticipated.

Keywords

leptin; leptin deficiency; obesity; leptin resistance; energy homeostasis; insulin resistance; adipokines; amenorrhea; lipoatrophy

INTRODUCTION

During the second half of the twentieth century, scientists studied extensively two phenotypes of obese mice. It was later discovered that their phenotypes derive from homozygous mutations of the mouse obese (*ob*) and diabetic (*db*) genes, respectively [104;148;103]. Using the parabiosis paradigm (i.e. connecting the vascular systems of two animals to permit the exchange of circulating hormones), scientists noted that *ob/ob* mice continued to be obese when joined with wild-type mice and lost weight when joined with *db/db* mice [39]. In contrast, *db/db* mice did not exhibit any change in weight when joined with either wild-type or *ob/ob* mice [39]. Furthermore, wild-type and *ob/ob* mice joined to

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db/db mice died of starvation [39]. From these findings, it was postulated that a circulating factor present in the wild-type mice was absent in *ob/ob* mice, and that this factor was produced in excess in *db/db* mice, which were resistant or tolerant to its effects [39;229].

In 1994, Zhang et al. at Rockefeller University discovered through positional cloning that the *ob/ob* mouse model has an inactivating mutation of the *ob/ob* gene and that its phenotype results from complete deficiency of the *ob* gene product [257]. This product became known as leptin, which is derived from the Greek root leptos, meaning thin [86]. The discovery that the mouse *db* gene codes for the leptin receptor followed soon after [119]. It shortly became evident that exogenous leptin administration reduces weight and reverses the metabolic, endocrine, and immune disturbances in *ob/ob* mice; however, it has no obvious effect in *db/db* mice [257;90;101].

The discovery that most obese humans are resistant or tolerant to leptin quickly dispelled the idea of leptin as a wonder drug for obesity, and leptin proved to be extremely effective only in the exceptionally rare cases of humans with congenital leptin deficiency [59]. Despite leptin's inability to induce weight loss in the majority of obese individuals [93], ongoing exploratory clinical trials are investigating whether combination therapy with leptin and potential leptin sensitizers will prove effective in the treatment of garden-variety obesity [191]. Furthermore, recent studies suggest that leptin could potentially have a role in weight loss maintenance [195]. Emerging research also suggests that leptin plays a more important role in acute (e.g. fasting) and chronic energy-deficient states (e.g. diet- or exercise-induced hypothalamic amenorrhea and lipoatrophy) than in energy-replete states (e.g. obesity) [31]. These energy-deficient states are associated with relative leptin deficiency, which, in turn, is associated with infertility and other neuroendocrine abnormalities, metabolic dysfunction, depressed immune function, and bone loss. Human recombinant leptin may serve as a treatment option in these conditions.

In this review, we offer a description of leptin physiology; an explanation of its role in energy homeostasis, reward processing, brain development, neuroendocrine function, metabolism, immune function, and bone metabolism; and insights into emerging clinical applications and therapeutic uses of recombinant leptin in humans.

LEPTIN BIOLOGY

Leptin, known as the prototypical adipokine, is a 167-amino acid peptide with a four-helix bundle motif similar to that of a cytokine [256;24]. It is produced primarily in adipose tissue but is expressed in a variety of tissues including the placenta, ovaries, mammary epithelium, bone marrow [143], and lymphoid tissues [145].

Leptin levels are pulsatile and follow a circadian rhythm, with highest levels between midnight and early morning and lowest levels in the early- to mid- afternoon [214;129;18]. Specifically, the concentration of circulating leptin may be up to 75.6% higher during the night as compared to afternoon trough levels [214]. The pulsatile characteristics of leptin secretion are similar in obese and lean individuals, except the obese have higher pulse amplitudes [214;129;18].

Leptin concentration reflects the amount of energy stored in body fat. Circulating leptin levels are directly proportional to the amount of body fat [41] and fluctuate with acute changes in caloric intake [19;30]. This system is especially sensitive to energy deprivation. In our initial study of six healthy, lean men, we measured leptin levels both in the baseline fed state and in the fasting state [30]. We noted that after only 2 or 3 days of fasting, leptin levels dropped to ~ 40 or 10% of baseline, respectively [30]. Women tend to have higher leptin levels than men, although women experience a significant decline in the amount of

circulating leptin after menopause [112]. This sexual dimorphism is largely independent of body mass index (BMI), and is due in part to differences in sex hormones, fat mass, and body fat distribution [196;199;112]. Women tend to accumulate body fat peripherally whereas men are prone to an abdominal or android distribution of fat. Subcutaneous fat expresses more leptin mRNA than omental fat, and this may partially explain the higher leptin concentrations in women compared to men [154;199]. Hormones and cytokines other than sex steroids also affect leptin secretion but to a smaller degree (Table 1).

Leptin binds to leptin receptors (*ObRs*) located throughout the central nervous system and several peripheral tissues [66]. At least six variations or isoforms of the leptin receptor have been identified (*ObRa, ObRb, ObRc, ObRd, ObRe*, and *ObRf)* [119]. These isoforms have homologous extracellular domains but distinct intracellular domains, which vary by length and sequence due to alternative mRNA splicing [119;226]. The short isoforms *ObRa* and *ObRc* are thought to play important roles in transporting leptin across the blood–brain barrier (BBB) [17;94]. The long leptin receptor isoform *ObRb* is primarily responsible for leptin signaling [119;226;17]. This functional leptin receptor *ObRb*, expressed in several organs, is strongly expressed throughout the central nervous system but particularly in the hypothalamus, where it regulates energy homeostasis and neuroendocrine function described further below [66;55]. In the *db/db* mouse model, the *ObRb* is dysfunctional, resulting in obesity and the metabolic syndrome [245].

LEPTIN SIGNALING

Leptin and STAT3 Signaling

Activation of *ObRb* sets off a cascade of several signal transduction pathways (Table 2), of which the best studied pathway is the Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway [72]. STAT3 has been shown to mediate the transcription of several genes that affect a number of cellular processes [124].

The JAK2/STAT3 pathway plays crucial roles in energy homeostasis and possibly neuroendocrine function. Activation of STAT3 by leptin induces transcription of proopiomelanocortin (POMC), an anorexigenic neuropeptide, in the arcuate nucleus (ARC) of the hypothalamus [56]. *S/s* mice with a targeted mutation at the key intracellular tyrosine residue Tyr^{1138} of the *ObRb*, which disrupts STAT3 signaling, are hyperphagic and obese, similar to *db/db* mice [14]. However, unlike *db/db* mice, these mice are fertile and of normal length, suggesting that STAT3-independent signals may be responsible for neuroendocrine regulation by leptin [14]. Gao et al. have studied mice with neural-specific STAT3 deletion [74]. These mice had decreased POMC expression in the ARC and also developed hyperphagia, obesity, and diabetes [74]. However, Gao et al. also observed neuroendocrine defects including decreased linear growth and infertility [74]. The discrepancies between the *s/s* mice and the mice with neural-specific STAT3 deletion were felt to be due to the fact that the mutation in the Tyr^{1138} residue of *s/s* mice may allow for a low level of STAT3 activation by leptin [74].

In addition to activating POMC neurons, the JAK2/STAT3 pathway likely also suppresses AgRP/NPY neurons in the ARC, which produce the orexigenic neuropeptides agouti-related peptide (AgRP) and neuropeptide Y (NPY), but to a lesser degree [14;80]. Gong et al. specifically deleted STAT3 from AgRP/NPY neurons in mice, which resulted in hyperleptinemia, hyperphagia, modest weight gain, and high-fat diet-induced hyperinsulinemia [14;80]. These mice were found to have increased NPY mRNA, although AgRP mRNA levels were unaffected [14;80]. It is likely that other pathways contribute to leptin's action on AgRP/NPY neurons as Munzberg et al. found that AgRP/NPY neurons were appropriately suppressed by leptin in *s/s* mice [161]. However, as mentioned above, a

mutation in Tyr^{1138} residue may allow for a low level of STAT3-induced activation by leptin.

Finally, Xu et al. bred mice lacking functional STAT3 in only POMC-expressing neurons [249;163]. These mice were found to have diminished expression of POMC but were responsive to the anorexigenic effects of leptin and were not hypersensitive to a high-fat diet [249;163].

These elegant experiments in mice have indicated that leptin binding to its *ObRb* receptor leads to STAT3 phosphorylation and activation and thus in turn upregulates POMC and downregulates NPY and AgRP, which mediate leptin's effect on energy homeostasis and neuroendocrine function. Moreover, leptin administration has been shown to phosphorylate STAT3 in several cell lines *in vitro* and a variety of animals *ex vivo* and *in vivo* [116]. For example, leptin has been shown to activate STAT3 *in vivo* not only in rat hypothalamic tissue but also in rat adipose tissue and liver [116;127]. Leptin has also been shown to activate STAT3 phosphorylation in human tissues and cells *in vitro* and *ex vivo*, but similar effects of leptin in humans *in vivo* remain to be explored and elucidated. Further studies of leptin signaling in humans with the ultimate goal of developing novel therapies for the treatment of human obesity are expected with great anticipation.

Leptin and PI3K Signaling

In vitro and *in vivo* studies have suggested that leptin stimulates the phosphatidylinositol 3-kinase (PI3K) pathway in a variety of murine cells and tissues, including myocytes [16], hepatocytes [242], and hypothalamic tissue [157]. Similarly, leptin may stimulate this pathway in human cancer cells [91;71] and in human adipose, liver, and muscle tissues [116]. The activation of PI3K occurs downstream from the insulin receptor substrate (IRS), particularly IRS-2 [157].

Activation of PI3K in the hypothalamus contributes to leptin's anoretic and weight loss effects. Similar to the STAT3 pathway, the PI3K pathway activates POMC neurons [157] by activating ATP-sensitive potassium channels [185] and voltage-gated calcium channels [240]. Similar to insulin [168], the appetite-suppressing effects of leptin [169] are attenuated by intracerebroventricular administration of PI3K inhibitors. Interestingly, the insulin-PI3K pathway hyperpolarizes POMC neurons, making them less sensitive to leptin [185], and this may be one common mechanism for concomitant leptin and insulin resistance in obesity [174]. More recently, forkhead box O1 (FOXO1), a transcriptional factor that stimulates the expression of AgRP and NPY and inhibits the expression of POMC, has been found to be an important downstream mediator of the PI3K pathway [157]. Expression and activity of hypothalamic FOXO1 is inhibited by leptin through the PI3K pathway [114]. Finally, PI3K may also be involved in the regulation of sympathetic outflow [189] but the exact mechanisms remain unclear.

Thus, activation of the P13K signaling pathway in the hypothalamus may influence energy homeostasis and neuroendocrine function whereas activation of this pathway in the periphery may mediate leptin's effect on insulin resistance.

Leptin and MAPK Signaling

Mitogen-activated protein kinases (MAPK) are a group of kinases that control a large number of cellular processes, including differentiation, proliferation, and apoptosis, and comprise of a number of signaling molecules including extracellular signal-related kinases 1 and 2 (ERK1/2), c-Jun amino-terminal kinases (JNK), and p38 [208;211]. MAPK can be activated by leptin [71], and leptin receptor-deficient obese rats have decreased or no activation of MAPK [190].

ERK1/2 is the major MAPK involved in leptin's central effects [116] and plays a key role in the regulation of food intake, body weight, and energy expenditure. ERK1/2 is stimulated by leptin downstream of src homology-2 containing protein tyrosine phosphatase 2 (SHP2) and Tyr^{985} of the *ObRb* after JAK2 activation [164;71;157]. When leptin is administered *in vivo*, both centrally and systemically, a response in ERK1/2 is seen in the POMC neurons of the ARC [190]. Inhibition of ERK1/2 reversed the decrease in food intake and body weight seen after leptin administration [190]. Furthermore, inhibition of ERK1/2 also prevented leptin-induced increase in sympathetic activity to brown adipose tissue [190].

The other kinases in the MAPK family, including p38 and JNK, have mainly peripheral actions. Unlike ERK1/2, p38 does not respond to leptin stimulation in neuronal cells, including the hypothalamus [190;241]. In rat cardiomyocytes, administration of leptin activates p38, which corresponds to an increase in fatty acid oxidation, and inhibition of p38 activation prevents leptin-induced fatty acid oxidation [211]. Activation of p38 by leptin is also seen in skeletal muscle of rats after intraperitoneal administration [144].

Similar to p38, JNK has not been shown to be activated in response to leptin treatment in neuronal cells [241], but leptin has been postulated to promote cancer via activation of the JNK pathway. Leptin has been demonstrated to activate JNK in human breast cancer cells in a both time- and dose-dependent manner, with greater levels of phosphorylated JNK after long-term leptin exposure [170;149]. In breast cancer, activation of the JNK pathway by leptin results in up-regulation of matrix metalloproteinase-2 (MMP-2) activity, promoting cancer cell invasion [149]. In addition, leptin stimulates proliferation and inhibits apoptosis, both of which are mediated in part by JNK activation, in human colon cancer cells [170].

Leptin and AMPK Signaling

Leptin's actions in peripheral tissues are in part due to its effects on 5'- adenosine monophosphate-activated protein kinase (AMPK), which, in addition to p38, contributes to leptin's effects on fatty acid oxidation and glucose uptake [152;2;130]. In mouse skeletal muscle, leptin activates AMPK, which leads to phosphorylation and inhibition of acetyl co-enzyme A carboxylase (ACC) and subsequent stimulation of fatty acid oxidation [152;219]. There appears to be two mechanisms by which leptin activates AMPK in muscle. One pathway involves the hypothalamus and the α-adrenergic pathway and results in a prolonged effect [152]. After intrahypothalamic or intravenous injection of supra-physiological and near-physiological doses of leptin in mice, increased AMPK activity was observed for up to 6 hours [152]. The second pathway is a direct effect on skeletal muscle, as evidenced in *ex vivo* experiments [152]. The ability of leptin to stimulate fatty acid oxidation in skeletal muscles may prevent lipotoxicity and subsequent insulin resistance [152] and may underlie leptin's ability to improve insulin resistance and metabolic syndrome in hypoleptinemic humans (see below). Interestingly, it has been demonstrated that suppressor of cytokine signaling 3 (SOCS3) inhibits leptin activation of AMPK in skeletal muscle in obese humans but not in lean humans, and this may also contribute to the dysregulation of fatty acid metabolism observed in obesity [219].

In contrast to its effects in muscle, leptin has been found to have an inhibitory effect on AMPK in the hypothalamus, including the ARC and PVH. This results in stimulation of hypothalamic ACC and subsequent reduction of food intake and weight gain [151;157]. It appears that leptin's effects on AMPK in the hypothalamus are at least partially mediated by the melanocortin-4 receptor (MC4R) pathway, which promotes anorexia [151].

In summary, leptin controls energy homeostasis and body weight primarily by activating *ObRb* in the hypothalamus [72]. The *ObRb* activate numerous JAK2/STAT3-dependent and -independent signaling pathways that act in coordination as a network to fully mediate

leptin's action. The activation of individual pathways in the leptin signaling network appears to be differentially regulated in discrete subpopulations of *ObRb*-expressing neurons. These pathways are also likely to be regulated by various other hormonal, neuronal, and metabolic signals that cross-talk with leptin. Hence, it is important to fully determine whether and how positive and negative regulators of *ObRb* signaling, metabolic state, and/or neuronal activity regulate leptin signaling networks in a cell/tissue type-specific manner and how activation of these signaling pathways mediates leptin's effects in humans.

THE ROLE OF LEPTIN IN HUMAN PHYSIOLOGY

The Role of Leptin in Energy Homeostasis

Leptin maintains energy homeostasis, primarily in the energy-deficient state, by adjusting appetite/food intake and energy expenditure. Leptin interacts with several neuronal pathways in and outside of the hypothalamus to regulate energy intake via orexigenic and anorexigenic neuropeptides (Figure 1).

The ARC of the hypothalamus is a critical site of leptin action. It lies adjacent to the third ventricle and immediately above the median eminence, a region where the blood brain barrier is specially modified to allow peripheral peptides (e.g. leptin and insulin) access to receptors [5]. Leptin acts on two populations of neurons in the ARC. Via *ObRb*-receptor binding, leptin directly stimulates neurons to secrete POMC, a precursor protein that is cleaved into α-melanocyte-stimulating hormone (α-MSH) [158]. α-MSH is an anorexigenic neuropeptide that decreases food intake by activating melanocortin-4 (MC4R) and melanocortin-3 receptors (MC3R) [218;46;163]. Leptin also stimulates POMC neurons to secrete cocaine- and amphetamine- regulated transcript (CART), which also suppresses appetite [194]. Both CART and POMC expression are decreased in states of leptin deficiency [51]. Although ablation of central POMC receptors results in profound obesity, the selected deletion of *ObRb* from POMC neurons results in only mild hyperphagia and obesity in mice [9;163]. Similarly, although mutation of the STAT3 binding site on *ObRb* leads to profound obesity [14], the selected deletion of STAT3 specifically from POMC neurons in the ARC only modestly impacts body weight [249;163].

Thus, it has been found that in conjunction with the stimulation of POMC neurons in the ARC, leptin also inhibits AgRP/NPY neurons that coexpress the orexigenic neuropeptides AgRP and NPY [46]. AgRP antagonizes α-MSH/MC4R signaling and inhibits endogenous MC4R activity [171;163], and NPY effectively increases appetite and reduces energy expenditure [217;73]. Leptin inhibits the orexigenic effect of these neurons by hyperpolarizing ATP-sensitive potassium channels, thus decreasing the action potential firing rate [45].

The ARC is not the only area of the hypothalamus involved in the regulation of energy homeostasis by leptin. *ObRb* is well-dispersed throughout the CNS with the ARC accounting for only 15-20% of *ObRb*-expressing neurons [126;163]. The ventromedial hypothalamus (VMH) is another key site of leptin action. Using laser scanning photostimulation of brain slices from transgenic mice, Sternson et al. found that *ObRb*-expressing neurons in the VMH project excitatory impulses onto POMC neurons in the ARC [223]. Leptin has also been shown to stimulate the secretion of two anorexigenic neuropeptides expressed in the VMH, steroidogenic factor-1 (SF-1) and brain-derived neurotrophic factor (BDNF). SF-1 is a transcription factor necessary for the development of the VMH [50;73]. Dhillon et al. found that mice lacking *ObRb* on SF-1 neurons are markedly hyperphagic and obese, and mice lacking *ObRb* on both SF-1 neurons and POMC neurons are more obese than either mutant alone [50]. BDNF is a neurotrophin known to promote brain development and has also been shown to regulate food intake [1]. Deletion of

the BDNF gene results in mice that are obese and hyperleptinemic [192], and mice with partial deficiency of the BDNF receptor tyrosine kinase B (TrkB) exhibit hyperphagia and obesity when fed a high-fat diet [250].

The paraventricular nucleus (PVN) also contains neurons that express *ObRb* and have numerous projections from neurons in the ARC that contribute to the regulation of food intake and weight [5;216;231]. Compared to mice that do not express MC4R, mice with selective MC4R expression in the PVN are significantly less hyperphagic and obese [10]. Leptin has also been shown to upregulate expression of proTRH [121] and corticotropin-releasing hormone (CRH) [42] in PVN neurons. Furthermore, injection of a CRH agonist into the third ventricle of fasted rats attenuates the anorectic effect of leptin [234]. Leptin may also indirectly regulate energy homeostasis via its influences on the thyroidal and adrenal axes through these mechanisms, and the role of leptin in mediating the thyroidal and adrenal axes is discussed further below.

The lateral hypothalamus (LHA), which has been described as a hunger center, contains neurons that express the orexigenic neuropeptides melanin-concentrating hormone (MCH) and orexin, both of which are influenced by leptin [1]. Intracerebroventricular administration of MCH leads to increased food intake in rats [187], and mice with homozygous deletion of the MCH gene are excessively hypophagic and lean [212]. Leptin decreases MCH gene expression *in vitro* [15], and intracerebroventricular administration of leptin prior to injection of MCH prevents an increase in food consumption in rats [200]. Leptin also inhibits orexin expression by hyperpolarizing and decreasing the firing rate of orexin neurons [252]. In rats, central administration of orexin stimulates food intake and fasting increases expression of orexin mRNA [201]. Leptin administration to *ob/ob* mice blunts the fasting-induced increase in orexin expression [252]. Recently, another population of neurons in the LHA that express *ObRb* has been identified and these neurons have a γ-aminobutyric acid (GABA)-releasing/inhibitory effect on food intake [123]. Finally, neurons in the LHA densely innervate the ventral tegmental area (VTA), part of the mesolimbic dopamine system which is involved in reward processing [123]. The role of leptin in reward processing is discussed further below.

In addition to the hypothalamus, *ObRb* is widely expressed throughout the dorsal vagal complex and other structures of the caudal brainstem [73]. The dorsal vagal complex includes the area postrema, nucleus of the solitary tract (NTS), and dorsal motor nucleus of the vagus nerve (DMV) and has been implicated as a crucial site of meal size control [83;73]. In mice, peripheral leptin administration has been shown to induce STAT3 phosphorylation in neurons of the NTS and DMV [98]. Another mice study showed that leptin administered directly into the dorsal vagal complex significantly reduces food intake and body weight [83]. At the NTS, leptin may work synergistically with peripheral satiety signals including glucagon-like peptide 1 (GLP-1) and cholecystokinin (CCK) to create a sense of fullness in response to food intake [235]. Williams et al. demonstrated that intraperitoneal leptin treatment strongly enhances the anorexic effects of GLP-1 in rats [246]. Leptin also enhances the effect of CCK on the activation of neurons in the NTS and area postrema, and this effect requires leptin signaling in the ARC [159]. Finally, leptin has also been proposed to act synergistically with amylin, a hormone secreted by pancreatic β-cells in response to caloric intake and contributes to the satiety signal short-term [35]. When co-administered in rodents, amylin increased leptin binding within the VMH and ARC, and the mice experienced an additive decrease in food intake and greater than additive reduction in body weight and epididymal fat [232]. The role of amylin as a potential leptin sensitizer in weight loss is discussed further below.

In addition to controlling energy intake via appetite and satiety, leptin also regulates energy expenditure via activation of the adrenergic system [141] and the melanocortin system [183]. In rodents, leptin increases sympathetic outflow to brown adipose tissue, which may contribute to the increased metabolic rate and core temperature observed after chronic leptin administration in animal studies [40]. In fact, leptin has been shown to increase oxygen consumption and expression of uncoupling protein 1 (UCP1), which is necessary for thermogenesis in brown adipose tissue, in rats [204]. It has also been proposed that these effects are due to the suppression of MCH by leptin [206]. Segal-Liberman et al. generated *ob/ob* mice that also lacked the MCH-1 receptor [206]. These “double null” mice had dramatically less body fat than *ob/ob* mice but were still hyperphagic [206]. The weight control appeared to result from increased energy expenditure, including increased locomotor activity and increased resting energy expenditure, compared to *ob/ob* mice [206].

Although these effects have not been confirmed in humans [33], recent evidence suggests that adult humans have functionally active brown adipose tissue [48;239]. More importantly, the presence of brown adipose tissue inversely correlated with BMI and fasting plasma glucose activity [48], and, furthermore, its activity is reduced in men who are overweight or obese [237]. It remains to be seen if leptin affects brown adipose tissue in humans and if it plays a role in the inverse association between brown fat and obesity.

The Role of Leptin in Reward Processing

Leptin also influences the hedonic aspects of feeding and thus contributes to the maintenance of energy homeostasis. Leptin interacts with the mesolimbic dopaminergic system, which is known to regulate arousal, mood, and reward [73]. Mesolimbic areas include the VTA, substantia nigra, amygdala, prefrontal cortex, anteromedial ventral striatum (nucleus accumbens and caudate nucleus), and posterolateral ventral striatum (globus pallidus and putamen) [58;124]. *ObRb* has been found in both the VTA and substantia nigra [67]. In rats, direct administration of leptin into the VTA reduces the excitability of dopamine neurons there and results in decreased food intake [97]. As previously mentioned, *ObRb*-expressing neurons in the LHA innervate the VTA and leptin administration directly into the LHA promotes expression of tyrosine hydroxylase, a rate-limiting enzyme involved in the production of dopamine, in the VTA [123]. Collectively, this suggests that leptin influences the mesolimbic dopamine system to decrease the incentive for food intake.

Consistent with the notion that leptin plays an important role in the mesolimbic system, research using functional magnetic resonance imaging (fMRI) is beginning to elucidate leptin's role in both the homeostatic and hedonic regulation of food intake. fMRI serves as an indirect marker of blood oxygen supply in the brain by measuring the hemodynamic response to brain activity as blood oxygen level-dependent (BOLD) signals [132]. Only three studies have directly studied the effect of leptin administration on brain activity using fMRI, two of which studied leptin administration in patients with congenital leptin deficiency and one of which studied leptin administration in subjects with garden-variety obesity who had recently lost 10% of their body weight [58;197].

Farooqi et al. [58] conducted an fMRI study of congenitally leptin-deficient subjects (n=2) who underwent fMRI testing before and after seven days of recombinant human leptin treatment. The anteromedial and posterolateral ventral striatum were markedly active in response to food images in the baseline leptin-deficient state relative the leptin-replaced state, but after seven days of leptin treatment, no differential activation of mesolimbic areas was noted [58]. Leptin treatment was also associated with lower food intake, decreased sense of hunger in the fasting state, increased postprandial satiety, and decreased liking of food images in the fed state [58]. Furthermore, prior to leptin treatment, activation of the

accumbens and caudate nuclei correlated with how much the subjects liked food images in the both the fed and fasting state, whereas after leptin treatment, the 'liking' ratings only correlated with activation in the accumbens-caudate in the fasting state. The authors thus suggest that leptin, via effects on the mesolimbic system, enhances the ability to discriminate the rewarding properties of food and may diminish the perception of food-related reward post-food consumption [58].

Similarly, Baicy et al. [7] studied three adults with congenital leptin deficiency and performed fMRI scanning following 57 months of leptin replacement, 33 days after discontinuing leptin replacement, and again 14 days after leptin replacement therapy was reinstated. In response to viewing high-calorie food images, leptin-replaced subjects showed decreased activation of the insula and increased activation of the prefrontal cortex [7]. The authors proposed that in states of leptin deficiency, the insular cortex, which has interoceptive function [47], may mediate enhanced feelings of hunger and also suggested that greater activation of the prefrontal cortex in the leptin-replaced state may mediate behavioral inhibition (i.e., the inhibition of consuming high-calorie foods) as well as satiety [7]. Consistent with the patterns of brain activation observed, subjects' hunger declined after leptin replacement [7].

Rosenbaum et al. [197] examined patterns of brain activation in response to food and nonfood images in a randomized, placebo-controlled, cross-over study of obese individuals who lost 10% of their body weight. fMRI imaging was performed pre-weight loss, after stabilization of a 10% weight loss with the administration of leptin or placebo, after crossing over from leptin to placebo and vice versa. Weight loss was associated with changes in neural activity in brain areas associated with the regulatory, emotional, and cognitive control of food intake [197]. Importantly, administration of leptin in weight-reduced subjects produced a pattern of brain activation that was similar to the pattern observed at baseline (pre-weight loss). The behavioral implications of this finding are unknown but may lead to decreased food intake, as decreased feelings of hunger in association with leptin administration were reported [117]. The authors propose that the weight-reduced state is one of leptin insufficiency and that administration of leptin decreases one's responsiveness to the emotional and sensory aspects of food and increases the cognitive control of food intake [197].

While these studies certainly support the idea that leptin influences both the hedonic and homeostatic regulation of food intake, we are only beginning to understand how leptin is involved in the complex interaction between the hedonic and homeostatic systems. Generally, evidence from fMRI studies suggests that in addition to its homeostatic actions (which primarily involve the hypothalamus and ARC), leptin activates brain regions which could improve cognitive control and behavioral inhibition as well as diminish the perceived rewarding value of food, as evidenced by leptin's effects on activity levels in limbic and cortical structures. However, each of the three fMRI studies involving leptin administration has identified distinct regions differentially activated by leptin administration, making it difficult to draw concrete conclusions. This, however, can be explained by many factors, including methodological discrepancies. For example, while all three studies utilized whole brain analyses and functional region of interest analyses, they differ with respect to whether the reported results focus on the whole brain versus region of interest analyses and further differ regarding the specific regions of interest chosen [7;58;197]. These studies also differ with respect to the control (or lack thereof) of dietary intake, presentation and nature of the visual stimuli, duration of leptin administration, and the inclusion or exclusion of behavioural tasks (e.g., hunger ratings) performed by subjects while in the scanner.

Another possibility to consider is that weight-reduced subjects and congenitally leptin-deficient subjects may not react similarly to leptin (and therefore show different patterns of brain activation in response to leptin administration) because of metabolic, physiological, and developmental differences between such individuals. The severity of leptin deficiency (in both duration and degree) [181] may have important implications for the efficacy and/or nature of the brain's response to leptin administration. Additionally, the effects of leptin deficiency on brain development during prenatal, perinatal, and early development may influence brain physiology long-term. Researchers have found that children with congenital leptin deficiency exhibit cognitive improvements [181] and adults exhibit increases in gray matter concentration in the anterior cingulate gyrus, cerebellum, and the inferior parietal lobule in association with leptin administration [146], suggesting that leptin plays a role in brain development over the lifespan. Changes in brain volume over the course of these fMRI studies as a result of leptin treatment may influence results and explain inconsistencies in findings. Additional fMRI studies with variable degrees of leptin deficiency are needed to better understand the effects of leptin on the brain. Larger sample sizes, although difficult to achieve when studying rare diseases such as congenital leptin deficiency, are also needed to draw more substantial conclusions about leptin and brain activity. Nevertheless, the brain's response to leptin in states of relative leptin deficiency is currently under investigation and remains an exciting area of research. Hopefully, fMRI technology will be able to shed light on how the hedonic aspects of eating interact with, and can even override homeostatic satiety signals in states of energy excess, such as overweight and obesity [58].

The Role of Leptin in Brain Development and Synaptic Connection Plasticity

Ob/ob and *db/db* mice have decreased brain weight, decreased cortical volume, decreased total brain DNA content, impaired myelination, and an immature pattern of expression of synaptic and glial proteins as compared to wild-type mice [3;222;233]. Leptin replacement in embryonic and juvenile *ob/ob* mice has been shown to increase brain weight and total brain DNA content [3;222;233], normalize expression of synaptic and glial markers [3;233], and promote migration of neurons to the cortical plate [233]. Leptin treatment has also been found to promote neurite outgrowth from the ARC in neonatal *ob/ob* mice [21]. Collectively, these studies signify the importance of leptin in early development of neurons. The effects of leptin on the brain may not be limited to the development stages. Using volumetric magnetic resonance imaging, Matochik et al. found that leptin treatment in human adults with congenital leptin deficiency results in increased gray matter tissue in the anterior cingulated gyrus, the inferior parietal lobule, and the cerebellum [147].

Leptin may also rearrange synaptic connections, or stimulate synaptic plasticity, in the hypothalamus, and this may contribute to leptin's regulation of energy homeostasis long-term [1]. Using electron microscopic stereology, Pinto et al. analyzed the synaptic density on POMC and AgRP/NPY neurons from the ARC of *ob/ob* and wild-type mice [184]. Compared to their wild-type littermates, *ob/ob* mice had significantly more inhibitory synapses and less excitatory synapses onto POMC neurons and more excitatory synapses and less inhibitory synapses onto AgRP/NPY neurons [184]. After 12 days of leptin treatment, the number of excitatory and inhibitory synapses onto POMC and NPY neurons in *ob/ob* mice were restored to the levels of their wild-type littermates [184]. Further studies are needed to determine whether or not these findings also apply to humans.

In addition to providing insight into the role of leptin in reward processing, fMRI can be used to assess synaptic plasticity in humans. Utilizing diffusion tensor imaging (DTI), potential changes in the anatomical connectivity between brain areas can be detected. Current studies are using DTI by fMRI to look for differences in white matter tracts in humans with relative leptin deficiency and for changes after leptin treatment.

The Role of Leptin in Neuroendocrine Physiology and Pathophysiology

Fasting results in rapid decline of leptin levels which occurs before and out of proportion to loss of fat mass [19], and this decline triggers an adaptive mechanism to conserve energy [30]. In mice and humans, this response includes decreasing reproductive hormone levels (which decreases fertility and prevents pregnancy), decreasing thyroid hormone levels that slow metabolic rate, increasing growth hormone (GH) level that may mobilize energy stores, and decreasing insulin-like growth factor-1 (IGF-1) level that may slow growth-related processes [4;150]. The interaction between leptin and the GH axis is likely less important in humans than in animal models since patients with congenital leptin deficiency have normal linear growth, unlike mice [59;61]. Similarly, humans, but not mice, with congenital leptin deficiency have normal adrenal function [59;61].

We originally observed a fall in leptin concentration and associated neuroendocrine abnormalities in acutely energy-deprived lean men (i.e. fasted for 3 days), and most of these abnormalities were reversed with physiologic replacement doses of leptin [30]. We also found similar neuroendocrine abnormalities in normal-weight women after caloric deprivation; however, replacement doses of leptin only modestly restored the changes in LH pulsatility and did not affect any other neuroendocrine axes [32].

We felt that the discrepancies between these two studies were due to leptin's permissive role in regulating neuroendocrine function. Only when the circulating leptin level falls below a certain threshold does it exert a physiologically important effect. In the former study on lean men, leptin levels fell to a mean of 0.34 ng/ml in the placebo group [30]. In the latter study on normal-weight women, leptin concentrations fell by approximately 80% from mid-physiologic (14.7 ± 2.6 ng/ml) to low-physiologic (2.8 ± 0.3 ng/ml) levels with fasting [32]. From these studies, we believe that a leptin threshold of ~3 ng/mL is necessary for normal reproductive function, perhaps signaling the brain that energy stores in adipose tissue are adequate to bring pregnancy to term, as well as other neuroendocrine functions.

We then extrapolated the concept that falling leptin levels may mediate the neuroendocrine response to energy deprivation to women who are chronically energy-deprived (i.e. women with diet- and exercise-induced amenorrhea). We first hypothesized that these conditions are associated with hypoleptinemia, which was confirmed by observational studies [136;6;150;110]. We then hypothesized that long-standing hypoleptinemia leads to the neuroendocrine dysfunction associated with hypothalamic amenorrhea. After performing and publishing the first pharmacodynamic studies of leptin administration in the fed [120] and fasting states [30;32], we conducted a proof-of-concept trial of leptin treatment in replacement doses in women with hypothalamic amenorrhea [244]. We also studied neuroendocrine function [23] and insulin sensitivity/metabolism in hypoleptinemic participants with lipoatrophy [120;138;139]. (see below).

Leptin in relation to the hypothalamic pituitary gonadal axis—Although leptin has been shown to stimulate luteinizing hormone (LH) secretion in rodents in *in vitro* and *in vivo* studies [254], hypothalamic gonadotropin-releasing hormone (GnRH) neurons do not express *ObRb* [68;85;188]. Therefore, leptin must regulate the reproductive axis via an indirect mechanism. Recent studies have proposed that leptin mediates reproductive function by activating neurons which project afferent input to GnRH neurons in the preoptic area (PO) and other hypothalamic areas [95]. Several neurons involved in energy homeostasis are anatomically associated with GnRH neurons (e.g. AgRP/NPY and POMC neurons) and may be the link between changes in energy balance and subsequent alterations in reproductive function [95].

In addition, recent evidence suggests that leptin may mediate the reproductive axis through regulation of kisspeptins, products of the Kiss1 gene, and neurokinin B. Mutations causing dysfunction of the kisspeptin receptor gene GPR54 result in hypogonadotrophic hypogonadism in both mice and humans [49;207], and in animals various kisspeptins have been shown to stimulate the release of GnRH and increase plasma levels of LH, follicule stimulating hormone (FSH), and total testosterone *in vivo* [82;105;228;209]. Approximately 40% of Kiss1 mRNA-expressing cells in the ARC co-express *ObRb* mRNA [215]. Moreover, *ob/ob* mice have lower levels of Kiss1 mRNA than wild-type mice, and when *ob/ob* mice were treated with leptin, levels of Kiss1 mRNA increased, suggesting that the reproductive dysfunction associated with leptin deficiency may be partially due to diminished expression of kisspeptins [215]. Similarly, human mutations in the gene encoding neurokinin B or its receptor leads to defective GnRH release and subsequent hypogonadism [122]. Furthermore, a subpopulation of neurons in the ARC co-express kisspeptin, neurokinin B, and dynorphin, another peptide that is implicated in the feedback regulation of GnRH neurons [122]. These neurons also contain leptin receptors [215], and, thus, may mediate the effects of nutritional status and stress on the hypothalamic-pituitary-gonadal axis [122]. Although the exact brain areas and molecular mechanisms involved remain to be elucidated [95], compelling evidence from basic and clinical studies indicate that leptin is an important mediator of the hypothalamic-pituitary-gonadal axis.

Ob/ob mice are infertile, and their fertility can be restored with exogenous leptin administration, independent of weight loss alone [36]. In humans, congenital leptin deficiency is associated with inadequate secretion of GnRH, manifesting in hypogonadotrophic hypogonadism and, in most of the subjects, resultant failure to reach puberty, including lack of growth spurt, secondary sex characteristics, and menarche [224]. Leptin replacement can permit the appropriate onset of puberty in individuals with congenital leptin deficiency [59;61;128]. An uncontrolled study observed the effects of leptin replacement in three children with congenital leptin deficiency [61]. One child, who was of appropriate pubertal age, exhibited a gradual increase in gonadotropins and estradiol during leptin therapy and exhibited normal pulsatile secretion of LH and FSH after 24 months of treatment [61]. However, the other two children, both of whom were prepubertal, did not respond to leptin treatment [61]. Another uncontrolled study of adults with congenital leptin deficiency demonstrated that leptin replacement in males increased testosterone levels; increased facial, pubic, and axillary hair; promoted of penal and testicular growth; and permitted normal ejaculatory patterns [128]. These findings are consistent with our longitudinal analysis that rising leptin levels herald the onset of puberty in normal boys [137] and indicate a crucial role for leptin in mediating reproductive function.

Fasting results in decreased gonadotropin pulsatility and secretion and blunts reproductive function [4]. In otherwise healthy but acutely energy-deprived mice, leptin administration normalizes LH levels and the estrous cycle in females and restores testosterone levels in males [4]. We have shown that caloric deprivation blunts LH pulsatility and testosterone secretion in healthy, lean male participants, and that leptin replacement can restore these hormones to pre-fasting baseline levels [30]. In normal-weight women, caloric deprivation decreases LH peak frequency, which is also fully corrected with leptin [32].

Leptin replacement has also been promising as a treatment for infertility due to chronic relative leptin deficiency. In our proof-of-concept trial of leptin replacement in women with hypothalamic amenorrhea, we found that leptin can normalize LH concentration and pulse frequency within weeks of treatment and can restore ovulatory function after only months of treatment [244]. These results are remarkable as many participants were amenorrheic for years [244]. Furthermore, these improvements were independent of any weight gain or

decrease in exercise activity [244]. Similar results have been seen in patients with congenital lipoatrophy [162]. An open-label study of individuals with congenital lipoatrophy found that leptin replacement increased LH response to GnRH in women and increased testosterone levels in men [162]. In addition, all eight women who had been amenorrheic prior to treatment resumed normal menses after approximately twelve months of leptin replacement [162].

As mentioned above, leptin's role in regulating reproductive function seems to be permissive with a leptin threshold of ~3 ng/mL. There are no studies using a dose-dependent design to study the effects of leptin on reproduction function in states of relative leptin deficiency. However, we have recently completed a randomized, placebo-controlled, double-blinded trial of leptin replacement in women with hypothalamic amenorrhea, and we increased the administered dose of leptin in women who had not begun menstruating with good effects (Mantzoros, unpublished data).

Leptin in relation to the hypothalamic pituitary thyroid axis—Leptin mediates its influences on the thyroidal axis by regulating thyrotropin-releasing hormone (TRH) expression [202]. *In vitro* and *in vivo* rodent studies have shown that leptin directly stimulates PVN neurons that express TRH to upregulate proTRH gene expression [121]. Leptin also indirectly influences TRH neurons in the PVN through signals from the ARC, as melanocortins can stimulate the thyroid axis and AgRP can inhibit it [115;180]. Leptin upregulates prohormone convertases 1 and 2 (PC1 and PC2), which cleave TRH from proTRH, in the PVN [202]. In states of fasting, PC1 and PC2 levels are decreased, and leptin has been shown to restore PC1 and PC2 to pre-fasting levels [202]. Rodent studies have shown that caloric deprivation rapidly suppresses TRH expression in the PVN, leading to decreased levels of T4 and T3 [69], and leptin has been shown to reverse these changes [4].

Both complete and relative leptin deficiencies are associated with hypothalamic hypothyroidism. *Ob/ob* mice are hypothyroid from birth [236], and normal mice experience a drop in T4 levels with fasting [4]. In healthy individuals, TSH is secreted in a pulsatile fashion similar to that of leptin, reaching a peak in the early morning hours and a nadir in late morning [140]. Individuals with congenital leptin deficiency have highly disorganized secretion of TSH, suggesting that leptin may regulate TSH pulsatility and circadian rhythm [140]. Despite the altered circadian rhythm of TSH, the thyroid phenotype varies among humans with congenital leptin deficiency. A small, uncontrolled interventional study showed no change in TSH after leptin replacement in children with congenital leptin deficiency, though participants did exhibit increased levels of T3 and T4 [61]. Another uncontrolled study of three adults and one boy with congenital leptin deficiency observed normal thyroid function and no changes with leptin replacement [121;180].

Humans with acute, relative leptin deficiency also have abnormalities in their hypothalamic-pituitary-thyroid axis. Our group has shown that leptin in replacement doses prevents the fasting-induced changes in TSH pulsatility and increases free T4 levels but does not affect the fasting-induced fall in T3 levels in lean males with starvation-induced leptin deficiency [30]. However, in our trial in which we studied seven normal-weight women, replacement leptin had no effect on the fasting-induced changes in TSH pulsatility or thyroid hormone levels [32]. We propose that the fall in serum leptin levels in the women did not reach the aforementioned threshold [32].

In women with hypothalamic amenorrhea, for which the average baseline leptin level was 3.4 ng/ml, leptin treatment significantly increased T3 and free T4 levels but did not affect TSH levels [244]. Rosenbaum et al. studied both lean and obese participants with relative

hypoleptinemia from 10% weight loss over an average of eight weeks. Although all participants experienced a fall in leptin levels, the obese participants still had leptin levels ranging from 10 to 60 ng/ml [195]. They found that circulating concentrations of T3, T4, and TSH were reduced compared to baseline and that leptin replacement increased both T3 and T4 levels but did not affect TSH levels [195].

The lack of a significant change in TSH levels in most studies may be due to the pulsatile nature of TSH. It has also been proposed that leptin may directly stimulate T4 release from the thyroid gland and/or increase the bioactivity of TSH [195].

Leptin in relation to the hypothalamic pituitary growth hormone axis—Both *ob/ob* and *db/db* mice are known to have stunted growth curves and fragile growth plates [75;76]. Leptin has been shown to enhance growth hormone-releasing hormone (GHRH)-induced GH secretion in rat anterior pituitary cells *in vitro* [186]. Furthermore, leptin has been shown to blunt the fasting-induced decrease in GH and to increase longitudinal bone growth in normal rodents [76].

The GH axis in humans, however, is not affected by leptin in the same manner or to the same extent as in mice. Children with congenital leptin deficiency have normal linear growth [59;61], but children with the leptin receptor mutation can experience an early growth delay with inadequate concentrations of GH, IGF-I, and insulin growth factor-binding protein 3 (IGF-BP3) [38]. In healthy men, leptin replacement attenuated the starvation-induced fall in IGF-I but had no detectable short-term effect on circulating GH levels [30]. In normal-weight women, leptin replacement did not prevent the starvation-induced fall in IGF-1 and IGF-BP3 levels [32]. In women with hypothalamic amenorrhea and chronic hypoleptinemia, we have observed a significant increase in IGF-I and IGF-BP3 levels after leptin administration [244].

Leptin in relation to the hypothalamic pituitary adrenal axis—Corticotropin-releasing hormone (CRH) is synthesized in the PVN, and leptin has been found to cause a dose-dependent stimulation of CRH release *in vitro* [42]. *Ob/ob* mice have evidence of increased adrenal stimulation by adrenocorticotropic hormone (ACTH) [22], and leptin administration blunts the stress-mediated increase in ACTH and cortisol in normal mice [92]. From our analysis of leptin's pulse parameters, we have identified an inverse relationship between circulating leptin and ACTH in healthy men [129]. However, studies in humans with mutations in the leptin or leptin receptor genes reveal that these subjects still maintain normal adrenal function [38;64]. Our studies of relative leptin deficiency in men with acute energy deprivation (i.e. starvation) [30] and women with chronic energy deprivation (i.e. hypothalamic amenorrhea) [30;244] showed no major abnormalities in the adrenal axis, and we observed no significant change in ACTH or cortisol levels after leptin replacement. Others found similar results in women with lipoatrophy and hypoleptinemia [172]. However, all these studies were open-label. We have recently conducted a larger, randomized, placebo-controlled trial in women with hypothalamic amenorrhea and found a small but statistically significant decrease in cortisol levels with leptin treatment (Mantzoros, unpublished observations).

The Role of Leptin in Metabolism

Several leptin-deficient states, including complete leptin deficiency and lipoatrophy, are associated with insulin resistance and diabetes, and studies in both rodents and humans have shown that administration of leptin may improve glucose metabolism either directly or indirectly via reduction of fat deposited ectopically, i.e. in tissues other than adipose tissue such as the liver [24]. After being injected with leptin, *ob/ob* mice exhibited significantly

greater reductions in serum glucose levels as compared to pair-fed *ob/ob* controls, even before any significant reduction in body weight [205]. Schwartz et al. calculated that 42% of leptin's hypoglycemic action was independent of weight reduction [205]. Like *ob/ob* mice, humans with congenital leptin deficiency are profoundly obese and can exhibit hyperinsulinemia and dyslipidemia. Leptin replacement can improve insulin, cholesterol, triglyceride, and LDL cholesterol levels in these individuals [61;79].

Mouse models of lipoatrophy also present with a similar array of metabolic disturbances. Serving as a model of lipoatrophy, AZIP/F-1 mice completely lack white adipose tissue and are, thus, unable to produce adequate amounts of leptin, resulting in a 20-fold reduction in leptin concentration [153;24]. Transplantation of white adipose tissue in physiologic quantities [78;113] as well as exogenous leptin administration [213] improves insulin sensitivity in these mice. Other studies have shown that insulin sensitivity is fully restored with administration of the combination of leptin and adiponectin to lipoatrophic mice, and only partially restored with either agent alone [253]. Studies on humans with congenital and acquired lipoatrophy have noted similar improvements after leptin administration and are described further below [173;182;54;108;109;120;160].

Leptin improves insulin resistance by not only decreasing body weight and fat mass, especially ectopic fat or intraabdominal fat [155], but also by activating insulin-sensitive tissues, including adipose tissue and liver tissue [116]. Furthermore, leptin activates signaling pathways that overlap with those of insulin, including STAT3, MAPK, and PI3K [116]. This overlap suggests a common pathogenesis of leptin and insulin resistance in obesity and needs to be explored in human studies as it may prove significant in emerging therapies [174].

The Role of Leptin in Immune Function

Hypoleptinemic states are associated with increased risk of infection [24]. It is likely that leptin directly affects immune function as a variety of immune cells have been found to express *ObRb* [133;24;178]. In *ex vivo* studies, leptin has been shown to enhance phagocytic activity in macrophages [135]; to promote production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-12 (IL-12) [131]; and to stimulate chemotaxis in polymorphonuclear cells [26;24]. In addition, we have shown that leptin promotes lymphocyte survival *in vitro* by suppressing Fas-mediated apoptosis [178]. Overall, leptin promotes Th1 cell differentiation and cytokine production [145]. An *in vivo* study showed that leptin administration improved the immune dysfunction of *ob/ob* mice and protected against immune dysfunction in acutely starved wild-type mice [100].

Individuals with congenital leptin deficiency have a higher incidence of infection than the general population due to decreased proliferation and function of CD4+ T cells, which normalize with exogenous leptin administration [61]. Our group has studied the effect of leptin replacement on immune function in chronically energy-deprived individuals. Leptin replacement in women with hypothalamic amenorrhea and chronic relative leptin deficiency leads to greater activation of the TNF-α system [34].

Leptin's role in immune function also seems to be a permissive one [18]. In normal-weight women with a starvation-induced decrease in leptin to 2.8 ng/ml, leptin replacement had no major effect on fasting-induced changes on most immunophenotypes and did not affect *in vitro* proliferative and cytokine-producing capacity of T cells, suggesting that this degree of acute leptin deficiency only minimally disrupts immune function [32]. Our group also showed that raising circulating leptin levels to between high physiologic (as seen in obesity) and low pharmacologic (as seen in extreme obesity) in lean men resulted in no significant increase in cytokines and inflammatory markers, including those known to be important in

insulin resistance and cardiovascular disease such as interferon-γ (IFN-γ), IL-10, and TNF-α [29]. We found similar results in obese diabetic subjects after 4 to 16 weeks of leptin administration [29]. As such, our data does not suggest a role for leptin in the pathogenesis of the obesity-related pro-inflammatory state.

The Role of Leptin in Bone Metabolism

Early research on the effect of leptin on bone metabolism in mice suggested that leptin may decrease bone mass. Initial radiographic and histological analyses suggested that *ob/ob* mice have a high bone mass phenotype, and intracerebroventricular injection of leptin appeared to reduce bone mass independent of weight loss [52]. However, it now appears that leptin's action in murine bone is not consistent throughout the skeleton, but varies among skeletal regions [37]. As compared to normal mice, *ob/ob* mice have significantly increased bone mineral density and trabecular bone volume in the lumbar area but lower bone mineral density, trabecular bone volume, and cortical thickness in the femora [37]. Since the appendicular skeleton constitutes 80% of total bone volume and is comprised primarily of cortical bone, *ob/ob* mice have lower bone mass than lean mice with regard to overall bone volume [37]. In the *ob/ob* and *db/db* mice models, there is a 20-25% decrease in total bone mass, primarily at the site of cortical bone [134;221;88]. Leptin treatment has been shown to increase bone mineral content and bone density in *ob/ob* mice [221;88].

Leptin affects bone metabolism via central and peripheral means. In mice, leptin appears to regulate cortical bone formation via sympathetic activation [87]. *Ob/ob* mice have diminished sympathetic tone [225], and leptin action in the VMH can increase sympathetic activity [87]. Furthermore, compared to normal mice, β1- and β2- adrenergic receptor knockout mice exhibit decreased femoral mass and cortical thickness [87] and low leptin levels [141]. Data from mice studies also indicate that leptin may mediate cortical bone formation by regulating the expression of several neuropeptides in the hypothalamus. One study found that the femora of mice lacking the NPY receptor Y2 have increased cortical bone mass and density [8], indicating that NPY inhibits cortical bone formation [87]. However, leptin injection into the PVN has been shown to increase expression of neuromedin U [248], which may decrease both cortical and trabecular bone [203]. Moreover, Yadav et al. have shown that brainstem-derived serotonin binds to Htr2c receptors on VMH neurons, favoring bone mass accrual, and that leptin inhibits this effect by reducing serotonin synthesis and firing of serotonergic neurons [251]. Thus, it appears that leptin affects the expression of many neuropeptides that affect bone metabolism either positively or negatively and more research is needed to fully elucidate the role of leptin in central regulation of bone metabolism.

Peripherally, leptin interacts with bone marrow stromal cells, osteoblasts, and osteoclasts to increase bone mass. Leptin increases expression of osteogenic genes in stromal cells and directs them to the osteogenic instead of the adipogenic pathway [227;88;89]. In *in vitro* and *in vivo* mice studies, leptin has also been shown to increase osteoblast proliferation, de novo collagen synthesis, and mineralization [81]. Finally, leptin stimulates stromal cells to increase osteoprotegerin expression and decrease RANK ligand, leading to decreased osteoclastogenesis [96].

To date, it is unclear if the data derived from rodent studies will also apply to humans. Indeed, the contrasting effects of leptin in the axial and appendicular skeletons of *ob/ob* mice do not appear to occur in humans, as individuals with leptin deficiency have decreased bone mass and density throughout the skeleton [87]. Using dual-energy x-ray absorptiometry, we assessed bone mineral content, density, and apparent density in normal weight children and adolescents. After adjusting for age, fat mass, bone-free fat mass, and serum IGF-1 and estradiol levels, we found no relationship between leptin and bone mineral

density or content for either total body or body regions [193]. It has also been observed that leptin levels do not correlate with bone mineral density or bone mineral content in healthy postmenopausal women after adjustment for body composition [111]. We interpret these data as indicating that leptin's effect on bone metabolism reflects that of fat mass and is possibly mediated through changes in estradiol and IGF-1 levels.

Interventional studies suggest that leptin may play a more important role in patients with leptin deficiency. Despite the fact that patients with congenital leptin deficiency have age- and gender-appropriate bone mineral content and bone mineral density, leptin treatment increases their skeletal maturation [61]. In women with hypothalamic amenorrhea, we reported a significant increase in bone-formation markers after leptin administration [244]. However, we were unable to determine whether this increase in bone-formation markers is a direct effect of leptin and/or mediated by restoration of estradiol and/or IGF-1 levels. In addition, the duration of the study was too short to evaluate whether the increase in bone markers would translate into increased bone density or decreased risk of fractures; therefore, long-term studies are needed to fully elucidate leptin's role in bone metabolism in these subjects.

LEPTIN RESISTANCE

In contrast to the obese subjects with congenital leptin deficiency, most obese individuals have higher leptin levels than lean individuals and are resistant or tolerant to the effects of leptin [41]. In a small, proof-of-concept, phase II clinical trial, leptin was administered in escalating doses to obese participants for up to 24 weeks [93]. Although a dose-dependent response was noted, participants exhibited only modest weight loss despite supra-physiologic doses of leptin [93]. With the highest dose of leptin at 0.30 mg/kg/day, obese participants who completed the study lost a mean of 7.1 kg; however, when participants who withdrew from the study were included in the analysis, there was only a mean weight loss of 3.3 kg [93]. Furthermore, there was significant variability among the participants [93]. We have observed a similar lack of weight-reducing effects of leptin in the context of two randomized, placebo-controlled trials—one in obese, otherwise healthy participants and the other in obese, diabetic participants (Mantzoros, unpublished observations). In accordance with leptin resistance or tolerance in obesity, the effects of leptin appear to be most pronounced during states of relative leptin deficiency, e.g. fasting and hypothalamic amenorrhea or lipoatrophy.

Leptin resistance was first thought to be due to mutations of the leptin receptor and other rare monogenic obesity syndromes (Table 3). Mutations of other genes downstream of leptin, including POMC and MC4R, also result in an obese phenotype with associated neuroendocrine dysfunction [65]. However, only a few cases of human obesity are due to monogenic syndromes; instead, most instances appear to be multifactorial [64].

First of all, leptin transport across the blood-brain barrier is impaired in obesity. This is partially due to saturation of the transporter by hyperleptinemia, which is associated with obesity, and subsequent decrease in transport activity [238;25;11]. Moreover, different brain regions may saturate at different concentrations. Banks et al. used a brain perfusion method in mice to deliver leptin at certain concentrations [12]. At a concentration of 1 ng/ml, a value seen in lean humans, the hypothalamus contained a higher concentration of leptin than any other brain region [12]. At 30 ng/mL, which represents a level in the obese range, the hypothalamus contained less leptin than other regions [12]. By selectively activating brain regions at certain concentrations, there can be different thresholds for different actions of leptin [12]. In addition to hyperleptinemia, hypertriglyceridemia also impairs the transport of

leptin [13;230]. Finally, the soluble leptin receptor isoform *ObRe* has also been shown to antagonize leptin transport by inhibiting surface binding and endocytosis of leptin [230].

Leptin signaling is subject to negative feedback regulation, which may be more pronounced in the obese state and associated hyperleptinemia [174]. SOCS3 is a major inhibitor of leptin signaling. SOCS3 expression is induced by the JAK2/STAT3 pathway, and SOCS3 acts to inhibit the phosphorylation and activation of JAK2 and *ObRb* tyrosine residue Tyr^{985} [163]. SOCS3 knock-out mice have been shown to have increased STAT3 expression and greater weight reduction in response to leptin administration [99;156]. SHP2, which is also activated by leptin, is another negative regulator of STAT3-mediated gene induction [28]. Finally, protein-tyrosine phosphatase 1B (PTP1B) is another molecule that may inhibit leptin signaling by dephosphorylating JAK2, and PTP1B knock-out mice also have increased leptin sensitivity and leanness [174]. These feedback mechanisms may explain why excessive leptin can actually result in the same disturbances as leptin deficiency (e.g. insulin resistance and infertility) [24].

Endoplasmic reticulum (ER) stress has recently been shown to play a role in the development of leptin resistance. Obese, diabetic animals and humans have been shown to have higher levels of ER stress in the liver, adipose tissue, and pancreatic β-cells [177;142;20;210]. Increased ER stress in obese mice has been shown to inhibit leptin receptor signaling in the hypothalamus, and chemical chaperones that decrease ER stress have been shown to increase leptin sensitivity [176]. Furthermore, intracerebrovetricular administration of thapsigargin, which induces ER stress, results in increased food intake and body weight [247]. Thus, the negative effect of ER stress on leptin sensitivity may also contribute to insulin resistance.

Targeting these mechanisms of leptin resistance will be important in the treatment for obesity and has led to development of insulin sensitizers, such as the chemical chaperones mentioned above. Roth et al. has also proposed that amylin may act synergistically with leptin to induce fat-specific weight loss [198], and the amylin analog pramlintide has been tried in conjunction with leptin in clinical trials for weight loss with modest effects [191]. However, given the complexity of leptin signaling and regulation, it is unlikely that any one target will be the key.

EMERGING CLINICAL APPLICATIONS

States of Leptin Deficiency

Congenital leptin deficiency—Complete congenital leptin deficiency from a homozygous mutation of the leptin gene is extremely rare and is often associated with consanguineous marriage [63]. Children with complete congenital leptin deficiency are born with normal weight but rapidly gain weight during the first year of life mainly due to hyperphagia [59;61]. Both children and adults exhibit profound obesity, hyperphagia, hyperinsulinemia, and hyperlipidemia as well as reproductive and immune dysfunction [61], and anecdotal evidence indicates that a number of children with congenital leptin deficiency may die early in life due to infections [175]. Leptin in replacement doses decreases appetite, food intake, weight, and serum insulin and cholesterol levels [61]. It also facilitates appropriate pubertal development [61;63]. Finally, leptin therapy also restores immune function by increasing the number of CD4+ T cells and memory T cells and the production of the proinflammatory cytokines IFN-γ, IL-4, and IL-10 [61]. Leptin is available for individuals with congenital leptin deficiency through an Amylin-sponsored compassionate use program.

Hypothalamic amenorrhea—Hypothalamic amenorrhea is characterized by the cessation of menstrual cycles due to dysfunction of the hypothalamic-pituitary-gonadal axis in the absence of organic disease. It occurs with excessive exercise, psychological stress, or decreased food intake and includes patients with anorexia nervosa, female athletes with the well-recognized triad (low energy availability with or without disordered eating, amenorrhea/neuroendocrine dysfunction, and osteoporosis), and normal-weight patients with ovulatory dysfunction.

Our proof-of-concept study demonstrated that replacement with human recombinant leptin in women with hypothalamic amenorrhea and chronic hypoleptinemia restores ovulatory menses [244]. After two weeks of leptin administration, mean LH concentration and pulse frequency were restored, and after three months of therapy, increases in endometrial thickness, ovarian volume, maximal follicular diameter, number of dominant follicles, and estradiol concentrations were observed [244]. Furthermore, leptin replacement normalized thyroid hormones and IGF-1 levels, but did not affect cortisol or ACTH levels [244]. Leptin replacement also increased bone-formation markers (e.g. bone alkaline phosphatase and osteocalcin) [244], and current trials are studying the effect of leptin replacement on bone mineral density. We have recently confirmed these findings in the context of a randomized, placebo-controlled trial and have found a small but statistically significant decrease in cortisol levels in response to leptin treatment (Mantzoros, unpublished observations). Thus, leptin may play a crucial role in neuroendocrine function and bone metabolism in these women and may prove to be a physiological replacement treatment for this and related conditions sharing the phenotype of “female triad.”

Lipoatrophy—Humans with congenital or HIV-associated lipoatrophy present with profound lack of subcutaneous fat, insulin resistance, fatty infiltration of the liver, and increased cardiovascular risk [27;84] and are also found to have relative leptin deficiency [179;165].

Congenital lipoatrophy is a rare autosomal recessive condition often associated with consanguineous marriage [179;167]. Approximately 100 subjects have been studied in the context of small, uncontrolled interventional studies, which have shown that leptin replacement dramatically improves dyslipidemia and insulin sensitivity in these individuals and reduces glycosylated hemoglobin, hepatic gluconeogenesis, and hepatic fat content [173;182;108;109]. The reduction of HbA1c by approximately 3% in these subjects who are resistant to other antihyperglycemic medications and/or high doses of insulin is impressive. Furthermore, leptin also normalizes testosterone levels and restores menstrual function in women with lipoatrophy who have polycystic ovarian syndrome [162]. Leptin is currently available through Amylin Pharmaceuticals, Inc., through an expanded access program for individuals with congenital lipoatrophy [139].

HIV-associated lipoatrophy is a much more prevalent condition, affecting an estimated 15-36% of HIV-infected patients [107]. In our initial randomized, placebo-controlled, interventional study, we found that leptin in physiologic doses improves insulin resistance, hyperlipidemia, and central fat mass in these patients [120]. A longer independent study of 6 months duration confirmed our results [160]. Studies performed to date indicate that leptin's ability to improve lipid levels and truncal fat mass is comparable to or better than that of insulin-sensitizers [138].

Current therapeutic options for patients with HIV-associated lipoatrophy and the metabolic syndrome include metformin, thiazolidinediones, GH, and synthetic GHRH [138]. We recently demonstrated that leptin acts independently of changes in the GH-IGF-1 pathway, inviting the possibility that combination therapy may produce an additive effect [23;138].

Pioglitazone, a thiazolidinedione that increases adiponectin levels, may have beneficial effects in HIV-associated lipoatrophy and the metabolic syndrome [77] and may be another option for combination therapy. Currently, there are no published studies using combination therapy with leptin for HIV-associated lipoatrophy. Larger, randomized, placebo-controlled clinical trials of leptin alone and in combination with other therapeutics are needed to elucidate the indications, optimal dosing, and duration of leptin therapy [139], which seems to represent a viable therapeutic option for these conditions.

States of Leptin Excess

Garden-variety obesity—Initial attempts to utilize leptin as a monotherapy for obesity included using supra-physiologic doses of leptin [93;70;125]; however, data from these trials revealed modest, if any, weight loss and with significant variability among the participants. Hukshorn et al. have also tried pegylated leptin, which has a much longer half-life [102]. In their randomized, double-blinded trial of weekly injections of pegylated leptin in obese men for 12 weeks, there were no significant differences in weight loss or percent body fat [102].

Most recently, human recombinant leptin has been combined with potential leptin sensitizers in an attempt to overcome the effects of leptin resistance. As mentioned above, a recent phase II clinical trial confirmed that the combination of pramlintide, an amylin analog, and human recombinant leptin in obese and overweight participants produces significantly more weight loss than either treatment alone [191]. The results appear additive, however, which suggests that amylin may not improve sensitivity to leptin. These clinical data are consistent with our *ex vivo* and *in vitro* signaling data (Mantzoros, unpublished data).

Emerging evidence suggests that leptin may play a more important role in weight loss maintenance as it regulates the body's adaptation to weight loss. Reduction in fat mass results in decreased leptin concentrations, and the weight-reduced state can be interpreted by the hypothalamus as one of relative leptin deficiency. As leptin levels fall, energy expenditure, sympathetic nervous system tone, and thyroid hormones decrease to collectively drive patients to regain weight [195]. In addition, as discussed above, fMRI data also suggests that leptin treatment can prevent the changes in brain activity involved in the regulatory, emotional, and cognitive control of food intake following weight loss [197]. Leptin replacement in patients actively losing weight may allow for weight loss maintenance and is currently under investigation.

CONCLUSION

Observational and interventional studies have shown that leptin contributes to the regulation of energy homeostasis, reward processing, brain development, neuroendocrine function, metabolism, immune function, and bone metabolism. Both complete (e.g. congenital leptin deficiency) and partial leptin deficiency states (e.g. female triad / exercise induced hypothalamic amenorrhea and lipoatrophy) present with dysfunction in these systems that can be reversed with leptin treatment. Thus, we propose that leptin deficiency should be considered (and treated as) as a clinical syndrome similar to other hormone deficiency states. Although the vast majority of obese individuals are resistant or tolerant to leptin, the use of leptin in a subset of garden-variety obesity, i.e. subjects who are heterozygotes for leptin gene mutations and are thus relatively hypoleptinemic, has been suggested as a promising treatment. Further research is needed to elucidate whether leptin sensitizers will be useful and if leptin has a role in weight loss maintenance. Hopefully, more randomized, placebo-controlled clinical trials will establish leptin's efficacy and safety in states of energy deficiency and obesity, so that leptin may become part of our therapeutic armamentarium for these conditions in the not so distant future.

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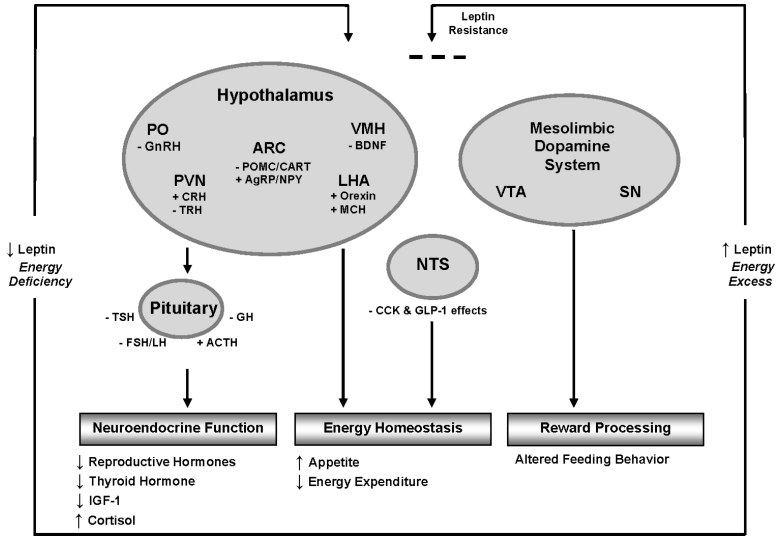

Figure 1.

Leptin's action in the brain during states of energy excess and energy deficiency. During states of leptin and energy excess, leptin's access to the hypothalamus and other brain areas is impaired and leptin's action is blunted. In states of leptin and energy deficiency, neuropeptides that are normally inhibited by leptin are elevated (+) and neuropeptides stimulated by leptin are suppressed (-). A change in the concentrations of these neuropeptides leads to alterations in neuroendocrine function and energy homeostasis. Alterations in leptin levels may also affect the hedonic aspects of feeding behavior. The role of leptin in the brain is discussed in greater detail in the text. Adapted from references [31;24;18].

Abbreviations: ACTH, adrenocorticotropic hormone; **AgRP**, agouti-related peptide; **ARC**, arcuate nucleus; **BDNF**, brain-derived neurotrophic factor; **CART**, cocaine- and amphetamine- regulated transcript; **CCK**, cholecystokinin; **CRH**, corticotropin-releasing hormone; **FSH**, follicle-stimulating hormone; **GH**, growth hormone; **GLP-1**, glucagon-like peptide 1; **GnRH**, gonadotropin-releasing hormone; **IGF-1**, insulin-like growth factor 1; **LH**, luteinizing hormone; **LHA**, lateral hypothalamic area; **MCH**, melanin-concentrating hormone; **NPY**, neuropeptide Y; **NST**, nucleus of the solitary tract; **PO**, preoptic area; **POMC**, proopiomelanocortin; **PVN**, paraventricular nucleus; **SN**, substantia nigra; **TRH**, thyrotropin-releasing hormone; **TSH**, thyrotropin-stimulating hormone; **VMH**, ventromedial hypothalamus; **VTA**, ventral tegmental area.

Table 1

Factors that regulate circulating leptin levels[1]

Factors promoting leptin secretion
*Excess energy stored as fat (obesity)
*Overfeeding
Glucose
Insulin
Glucocorticoids
Estrogens
Inflammatory cytokines, including Tumor Necrosis Factor-α and Interleukin-6 (acute effect)
Factors inhibiting leptin secretion
*Low energy states with decreased fat stores (leanness)
*Fasting
Catecholamines and adrenergic agonists
Thyroid hormones
Androgens
Peroxisome Proliferator-activated Receptor-γ (PPARγ) agonists†
Inflammatory cytokines, including Tumor Necrosis Factor-α (prolonged effect)

[1] Adapted from reference [24]

* Denotes major factor influencing leptin levels.

† Unlike animals, in humans PPARγ agonists decrease leptin gene expression but increase subcutaneous fat mass. Thus, the net effect is null.

Table 2

Leptin Signaling

Signaling Pathway	Primary Site of Action	Known Mechanisms of Action	Clinical Results
JAK-STAT3	Hypothalamus	Stimulates transcription of POMC [14;80] and suppresses transcription of NPY [14;80].	Regulates appetite and, thus, body weight [14]. May also contribute to neuroendocrine function as neural-specific STAT3 deletion results in decreased linear growth and infertility [74].
P13K	Hypothalamus	Stimulates POMC neurons [157] [185;240]. Inhibits FOXO1, an inhibitor of POMC transcription, to increase POMC expression [114].	Regulates appetite and body weight [169;114;157]. May contribute to leptin resistance in obesity, given the overlapping pathway with insulin [174]. May mediate the stimulation of sympathetic outflow [189].
MAPK	Hypothalamus, liver, pancreas, adipose tissue, and myocytes	Stimulates POMC neurons [190] and inhibits AgRP/NPY neurons [240].	Regulates appetite and body weight [190]. Increases sympathetic activity to brown adipose tissue [190]. Increases fatty acid oxidation in peripheral tissues [211]. Promotes cardiomyocyte hypertrophy [255].
AMPK	Hypothalamus, muscle	Stimulates ACC activity in the hypothalamus to regulate food intake and weight [151;157]. Inhibits ACC activity in muscle [152;220].	Regulates appetite and weight [151;157]. Stimulates fatty-acid oxidation in muscle [152;220] and may sensitize muscle to insulin [53].
mTOR	Hypothalamus	Induces phosphorylation of S6K1 to regulate protein synthesis [43].	Regulates appetite and weight [44].

Abbreviations: ACC, acetyl coenzyme A carboxylase; **AMPK**, 5'adenosine monophosphate-activated protein kinase; **ATP**, adenosine triphosphate; **FOXO1**, forkhead box O1; **JAK-STAT3**, janus kinase-signal transducers and activator of transcription 3; $\mathbf{K^+}$, potassium; **MAPK**, mitogen-activated protein kinase; **mTOR**, mammalian target of rapamycin; **NPY**, neuropeptide Y; **POMC**, pro-opiomelanocortin; **P13K**, phosphatidylinositol 3-kinase; **S6K1**, S6 Kinase 1.

Table 3

States of Leptin Deficiency and Leptin Resistance[1]

Syndrome	Estimated Prevalence	Associated Features
Obesity associated with leptin deficiency		
Complete congenital leptin deficiency	Rare	Early-onset morbid obesity, profound hyperphagia, hyperinsulinemia and type II diabetes, hypogonadotrophic hypogonadism, advanced bone age, immune dysfunction [62]
Heterozygous leptin deficiency	Unknown	Obesity with significantly lower than expected serum leptin levels with respect to % body fat, normal neuroendocrine function [62]
Fat loss associated with leptin deficiency		
Congenital generalized lipoatrophy	Rare	Generalized fat wasting, impaired glucose tolerance, insulin resistance/type II diabetes, hepatic steatosis, acanthosis nigricans [138]
HAART-induced lipoatrophy	15-36% of HIV-infected patients [107]	Fat wasting of the face, arms, legs, and buttocks; hypertriglyceridemia, impaired glucose tolerance, insulin resistance/type II diabetes, hepatic steatosis [138]
Hypothalamic amenorrhea (functional)	Up to 69% of trained female athletes [166]; 7.6% in women aged 15-24, 3.0% in women aged 25-34, and 3.7% in women aged 35-44 years [243]	Psychogenic stress, strenuous exercise, reduced fat mass, osteoporosis, amenorrhea with decreased GnRH pulsatility and estradiol levels, decreased thyroid hormone levels, increased growth hormone levels, and decreased IGF-1 [31]
Obesity associated with leptin resistance		
Leptin receptor deficiency	Rare	Phenotype similar to congenital leptin deficiency with early-onset obesity, hyperphagia but less remarkable hyperinsulinemia. In addition, exhibit hypogonadotrophic hypogonadism, mild growth retardation, hypothalamic hypothyroidism [38]
POMC deficiency	Rare	Early-onset obesity, hyperphagia, ACTH deficiency with adrenal insufficiency/crisis, pale skin and red hair from lack of MSH function at MC1Rs [118]
MC4R deficiency	0.5% of obese adults [57], 6% of early-onset, severe obesity [60]	Early-onset obesity, hyperphagia, increased lean mass, increased linear growth, and severe hyperinsulinemia [60]
Prohormone convertase 1 deficiency	Rare	Early-onset severe obesity, abnormal glucose homeostasis, hypoinsulinemia, hypogonadotropic hypogonadism, hypocortisolism [106]
Neurotrophin receptor tropomyosin-related kinase B mutations	Rare	BDNF deficiency resulting in severe obesity, hyperphagia, developmental delay, and cognitive dysfunction [57]

Abbreviations: BDNF, brain-derived neurotrophic factor; **HAART**, highly-active antiretroviral therapy; **IGF-1**, insulin-like growth factor 1; **MC4R**, melanocortin-4 receptor; **POMC**, pro-opiomelanocortin

[1] Adapted from references [24;18]