Leptin at the Intersection of Neuroendocrinology and Metabolism: Current Evidence and Therapeutic Perspectives

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Since its discovery as an adipocyte-secreted hormone, leptin has been found to impact food intake, energy homeostasis, and metabolism through its effects on the central nervous system and peripheral organs. Recent research indicates that leptin may also be involved in cognition, immune function, and bone metabolism. These findings place leptin at the intersection of neuroendocrinology and metabolism, and possibly immune function, and render it an appealing therapeutic target for several niche areas of unmet clinical need. Current evidence regarding classic and emerging roles of leptin as well as the pros and cons of its potential clinical use are summarized herein.

Introduction
As a weight loss drug, leptin has proven to be efficacious in patients with congenital and acquired leptin deficiency (Chan et al., 2006; Farooqi et al., 2002; Licinio et al., 2004) but not in most obese humans, who are found to be resistant to the effects of leptin (Heymsfield et al., 1999). Leptin plays an important role in maintaining energy homeostasis in energy-deficient states, which include acute fasting, exercise- or diet-induced hypothermia, and also improves metabolism in lipodystrophy, all of which are associated with relative hypoleptinemia (Kelesidis et al., 2010). In these leptin-sensitive states, leptin regulates food intake, energy expenditure, neuroendocrine function, metabolism, bone metabolism, and immune function. Given the evolution in our understanding of leptin physiology, the leptin field has shifted its focus from leptin’s role as an antiobesity hormone to its role in weight loss maintenance. Importantly, the mechanisms underlying leptin resistance in obesity still remain to be elucidated. Here, we offer a description of leptin physiology; an overview of its multiple roles, focusing on its role in energy homeostasis and neuroendocrine regulation; and insights into its emerging role in the neurobiology of eating behavior.

Leptin Biology
Leptin is a 167-amino-acid peptide that is primarily produced in adipose tissue. Circulating leptin levels are directly proportional to the amount of body fat and fluctuate with acute changes in caloric intake (Chan et al., 2003), thereby signaling the amount of stored energy. This is evidenced by our initial study of six healthy, lean men, in whom 3 days of fasting resulted in leptin levels falling to 10% of baseline (Chan et al., 2003). Women tend to have higher leptin levels than men even after correction for differences in body fat composition, as subcutaneous fat has been associated with higher levels of leptin messenger RNA (mRNA) expression compared to visceral fat. However, leptin levels decline significantly after menopause, and it has been proposed that estrogen increases leptin levels, while androgens suppress them (Rosenbaum and Leibel, 1999).

Leptin binds to its receptors located throughout the central nervous system as well as in several peripheral tissues. Numerous splice variants have been identified, but the LepRb isoform is the main receptor isoform responsible for leptin signaling. It is strongly expressed throughout the central nervous system, particularly in the hypothalamus, where it regulates energy homeostasis and neuroendocrine function (Kelesidis et al., 2010). Binding to LepRb activates a number of signaling pathways (Figure 1) that facilitate leptin’s central and peripheral effects. These include the janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3), src homology-2-containing protein tyrosine phosphatase 2 (SHP2)/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3 kinase (PI3K)/Akt, and mammalian target of rapamycin (mTOR) pathways (Kelesidis et al., 2010) (Table 1).

Disrupted leptin signaling may result in leptin resistance, which is generally defined as the failure of endogenous or exogenous leptin to promote anticipated salutary metabolic outcomes, such as suppression of appetite and weight gain and stimulation of energy expenditure. Leptin resistance typically accompanies states of energy excess, such as garden-variety obesity, and may result from interference with JAK2 phosphorylation by several intracellular signaling molecules, including suppressor of cytokine signaling 3 (SOCS3), SHP2, and protein tyrosine phosphatase 1B (PTP1B) (Myers et al., 2010). Other mechanisms that may contribute to leptin resistance include...
impaired blood brain barrier transport, impaired LepRb trafficking from the trans-Golgi network to the cellular membrane, and obesity-associated endoplasmic reticulum (ER) stress and chronic low-level inflammation, both of which can impair LepRb signaling (Moon et al., 2011). It is important to distinguish these mechanisms from several models of genetic obesity, in which mutations in or downstream of the LepRb gene block proper leptin response (Myers et al., 2010). The role in and partial contribution of each mechanism to leptin resistance remain to be fully elucidated. Table 2 shows disease states associated with leptin deficiency and leptin resistance.

**The Role of Leptin in Regulating Food Intake and Energy Expenditure**

In response to energy deprivation, leptin levels fall before and out of proportion to loss of fat mass (Boden et al., 1996), initiating several mechanisms to increase energy intake and conserve energy expenditure. These adaptations have been noted in healthy subjects in response to a short-term fast, women with hypothalamic amenorrhea who are chronically energy deprived, patients with lipodystrophy, and those with recent weight loss.

**The Role of Leptin in Regulating Food Intake**

Numerous studies of leptin-deficient individuals have shown that leptin treatment affects food intake and satiety. In patients with congenital leptin deficiency, which is well characterized by hyperphagia and severe, early-onset obesity, treatment with leptin results in decreased food intake, increased physical activity, and decreased weight (Farooqi et al., 2002). In subjects with relative hypoleptinemia due to 10% weight loss, exogenous leptin replacement blunted the decrease in satiation observed after weight loss, although it did not change absolute food intake (Kissileff et al., 2012).

These effects are mainly central. Leptin interacts with several neuronal pathways in the brain, particularly the hypothalamus, to regulate food intake via orexigenic and anorexigenic neuropeptides (Figure 2). In the arcuate nucleus (ARC) of the hypothalamus, leptin activates neurons that express pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), both of which suppress appetite, and inhibits neurons that express agouti-related peptide (AgRP) and neuropeptide Y (NPY), both of which stimulate appetite (Cowley et al., 2001). The effect of leptin on POMC neurons is partly mediated via GABAergic presynaptic neurons found in the ARC, dorsomedial hypothalamos, and lateral hypothalamus (LHA); via GABAergic neurons, leptin reduces inhibitory tone to POMC neurons (Vong et al., 2011), thereby enhancing their antiorexigenic action. In the LHA, leptin also decreases the expression of the orexigenic neuropeptides melanin-concentrating hormone (MCH) (Bayer et al., 1999) and orexin (Yamanaka et al., 2003). In the ventromedial hypothalamos (VMH), leptin acts on neurons that express steroidogenic factor-1 (SF-1) (Kim et al., 2011), through which it possibly induces thermogenesis after acute exposure to high-fat diet, and brain-derived neurotrophic factor (BDNF) (Liao et al., 2012), through which it inhibits food intake.

LepRb-expressing neurons have also been found in the nucleus of the solitary tract (NTS) of the hindbrain and include subpopulations that express POMC, progluacagon/glucagon-like peptide 1 (GLP-1), and cholecystokinin (CCK) (Garfield et al., 2012). Leptin may also act synergistically with gastrointestinal hormones in this region to promote satiety, and leptin administration to mice has been found to enhance the anorexic and weight loss responses to intraperitoneally administered CCK, GLP-1, and amylgin (Figure 2).

Leptin also influences the hedonic aspects of feeding via interactions with the mesolimbic dopaminergic system, which is known to regulate reward, mood, and arousal (Dardeno et al., 2010) (Figure 2). In rats, direct administration of leptin into the ventral tegmental area (VTA) reduces the excitability of dopamine neurons in the area and decreases food intake, while knockdown of LepRb in the VTA increases food intake and preference for highly palatable food (Hommel et al., 2006). In addition, leptin administration directly into the LHA promotes expression of tyrosine hydroxylase, a rate-limiting enzyme involved in the production of dopamine, in the VTA (Leinninger et al., 2013).

**Figure 1. Intracellular Leptin Signaling Pathways**

Binding of leptin to the LepRb receptor results in its dimerization and prompts JAK2 phosphorylation and activation of the JAK2/STAT3 pathway. STAT3 phosphorylation causes it to dissociate from the LepRb receptor and form active dimers, which regulate gene expression after translocation to the nucleus. STAT3 signaling may activate STAT5, which may also regulate STAT3-dependent gene expression. JAK2 phosphorylation also activates SHP2, which then recruits the adaptor protein Grb2 to prompt activation of ERK1/2. SOCS3 expression is induced by STAT3 upon LepRb dimerization and acts as a negative-feedback signaling compound by inhibiting leptin-stimulated phosphorylation of JAK2. Leptin-mediated PI3K activation via IRS1/2 causes Akt/mTOR phosphorylation and subsequent regulation of S6 and FOXO1 activity. PTP1B may interfere with PI3K activation by inhibiting leptin-stimulated IRS1/2 phosphorylation. Akt, v-Akt murine thymoma viral oncogene homolog;
Table 1. Leptin-Activated Signaling Pathways

<table>
<thead>
<tr>
<th>Signaling Pathway</th>
<th>Effects of Pathway Activation</th>
<th>Evidence</th>
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<tbody>
<tr>
<td>JAK2/STAT3: tyrosine kinase signaling pathway responsible for leptin signal transduction</td>
<td>CNS regulation of adipose tissue maintenance</td>
<td>Neural disruption of the STAT3 pathway results in severe obesity in mice</td>
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<td></td>
<td>Stimulation of anorectic pathways in ARC of hypothalamus</td>
<td>POMC transcription; AgRP/NPY transcription</td>
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<tr>
<td>SHP2/MAPK: upstream activator of the MAPK pathway</td>
<td>CNS regulation of energy homeostasis</td>
<td>ERK1/2 activation by JAK2; ERK1/2 is the primary MAPK implicated in leptin’s central regulation of energy homeostasis</td>
</tr>
<tr>
<td>PI3K/Akt/mTOR: kinase pathway that mediates leptin’s effects on insulin metabolism and cellular proliferation</td>
<td>Activation of growth pathways/cellular proliferation pathways</td>
<td>Akt activation corresponds with cellular proliferation in several murine peripheral tissue cell lines</td>
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<td></td>
<td>Anorectic central effects</td>
<td>Initiation of the PI3K pathway activates POMC-expressing neurons</td>
</tr>
<tr>
<td></td>
<td>Mediation of leptin’s impacts on insulin metabolism</td>
<td>The PI3K/Akt/mTOR pathway may play a role in pancreatic islet function</td>
</tr>
<tr>
<td>FoxO1: orexigenic downstream mediator of the PI3K pathway</td>
<td>Orexigenic central effects</td>
<td>FoxO1 activation the expression of POMC and promotes the expression of AgRP and NPY in the CNS; leptin administration inhibits FoxO1 activity and expression in a PI3K-dependent manner</td>
</tr>
<tr>
<td></td>
<td>Point of intersection for leptin and insulin signaling</td>
<td>Deletion of IRS2 in LepRb CNS neurons deregulates PI3K signaling and results in increased FoxO1 signaling</td>
</tr>
<tr>
<td>AMPK: serine/threonine protein kinase that modulates energy regulation</td>
<td>Peripheral adipose tissue maintenance</td>
<td>Peripherally promotes increased fatty-acid oxidation and various insulin-sensitizing effects</td>
</tr>
<tr>
<td></td>
<td>Orexigenic central effects</td>
<td>In the CNS promotes increased food intake and weight gain; leptin inhibits AMPK centrally and activates it peripherally</td>
</tr>
<tr>
<td>SOCS3: key negative feedback molecule in leptin signaling</td>
<td>Possibly mediates leptin resistance in obesity</td>
<td>Interferes with JAK2/STAT3 activation SOCS3 mRNA is increased in leptin-resistant mouse model of obesity</td>
</tr>
<tr>
<td>SHP2: Src homology-2-containing tyrosine phosphatase implicated in growth-factor and cytokine signaling</td>
<td>May be a therapeutic target for treatment of leptin resistance; possibly mediates leptin resistance in obesity</td>
<td>SHP2’s interference in JAK2/STAT3 activation is counteracted by the SHP2-mediated upregulation of the leptin-ERK1/2 pathway</td>
</tr>
<tr>
<td>PTP1B: negative regulator of leptin-stimulated pathways</td>
<td>Possibly mediates leptin resistance in obesity</td>
<td>PTP1B binds to and dephosphorylates JAK2</td>
</tr>
</tbody>
</table>

AgRP, agouti-related peptide; Akt, v-Akt murine thymoma viral oncogene homolog; AMPK, 5’, adenosine monophosphate-activated protein kinase; ARC, arcuate nucleus; CNS, central nervous system; ERK, extracellular-signal-regulated kinase; FoxO1, forkhead box protein O1; IRS, insulin receptor substrate; JAK, Janus kinase; LepR, leptin receptor; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NPY, neuropeptide Y; PI3K, phosphatidylinositol 3 kinase; POMC, pro-opiomelanocortin; PTP1B, protein tyrosine phosphatase 1B; SHP, src-homology-2-containing protein tyrosine phosphatase; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription.

Collectively, these data suggest that leptin decreases both the incentive to feed and pleasure from eating through influencing the mesolimbic dopamine system. 

**The Role of Leptin in Regulating Energy Expenditure**

In addition to controlling energy intake via central regulation of appetite and satiety, leptin also regulates energy expenditure via the central nervous system, possibly through the suppression of MCH and FoxO1. Compared to ob/ob mice, which are leptin deficient, double-null mice to ob/ob and MCH-1 receptor have increased locomotor activity, increased resting energy expenditure, and dramatically less body fat, despite similar food intake (Segal-Lieberman et al., 2003). Mice with FoxO1 deletion in SF-1 neurons of the VMH had higher energy expenditure during fasting, likely through activation of the sympathetic nervous system (Kim et al., 2012). Finally, administration of leptin has been shown to increase sympathetic outflow to brown adipose tissue, which may mediate increased expression of uncoupling protein in rodents (Scarpace et al., 1997).

Similar findings have been more difficult to confirm in humans. In two children with congenital leptin deficiency, leptin administration did not have a major effect on basal metabolic rate or total energy expenditure (Farooqi et al., 2002). Interestingly, in adults with congenital leptin deficiency, leptin replacement prevented the fall in energy expenditure observed after weight loss (Galgani et al., 2010). In our experience with healthy, normal-weight women, leptin replacement did not prevent the decrease in catecholamine levels, the increase in heart rate, or the decrease in cardiac vagal modulation observed after a short-term fast (Chan et al., 2007). On the other hand, in subjects on a longer-term weight loss program, leptin replacement has been shown to reverse in part the decrease in energy expenditure in addition to the changes in skeletal muscle work efficiency and sympathetic nervous system tone associated with weight loss (Kissileff et al., 2012; Rosenbaum et al., 2005), but these data need to be confirmed by studies by other, independent groups.
Lessons from Neuroimaging Studies

Functional magnetic resonance imaging (fMRI) examines local neuronal activity as an indirect marker of blood oxygen supply in the brain from blood-oxygen-level dependent (BOLD) changes in the paramagnetic properties of hemoglobin (Carnell et al., 2012). fMRI studies are commonly used to evaluate the brain’s response to food imagery or to different categories of food cues. Structural MRI and subsequent voxel-based morphometry (VBM) analysis may be used to obtain anatomical details, based upon the different paramagnetic properties of brain tissues comprising white and gray matter.

Table 2. Disease States Associated with Leptin Deficiency and Leptin Resistance

<table>
<thead>
<tr>
<th>Disease States</th>
<th>Estimated Prevalence</th>
<th>Clinical Features</th>
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<tbody>
<tr>
<td>Fat Loss Associated with Leptin Deficiency</td>
<td></td>
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<tr>
<td>Congenital generalized lipoatrophy</td>
<td>Rare</td>
<td>Generalized fat wasting, insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus, dyslipidemia, hepatic steatosis, acanthosis nigricans</td>
</tr>
<tr>
<td>HAART-induced lipoatrophy</td>
<td>15%–36% of HIV-infected patients</td>
<td>Fat wasting of the face, arms, legs and buttocks, insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus, hypertriglyceridemia, hepatic steatosis</td>
</tr>
<tr>
<td>Hypothalamic amenorrhea (functional)</td>
<td>3%–7.8% in women aged 13–44 years; up to 69% of trained female athletes</td>
<td>Strenuous exercise, psychogenic stress, energy deficit, low bone mass and increased bone loss, neuroendocrine dysfunction with decreased GnRH pulsatility and estradiol levels, decreased thyroid and IGF-1 levels, increased growth hormone levels</td>
</tr>
<tr>
<td>Anorexia nervosa</td>
<td>1.2%–2.2% lifetime prevalence for women</td>
<td>Weight loss to body weight &lt;85% of expected, refusal to maintain normal body weight, fear of gaining weight, disturbed body image, severe restriction of food intake, amenorrhea and other neuroendocrine dysfunction</td>
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<tr>
<td>Obesity as a Manifestation of Leptin Deficiency</td>
<td></td>
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<tr>
<td>Complete congenital leptin deficiency</td>
<td>Rare</td>
<td>Hyperphagia, early-onset morbid obesity, hypogonadotrophic hypogonadism, hyperinsulinemia and type 2 diabetes mellitus, dyslipidemia, immune dysfunction</td>
</tr>
<tr>
<td>Heterozygous leptin deficiency</td>
<td>≤6% in obese subjects</td>
<td>Garden-variety obesity with hyperleptinemia relative to fat mass, normal neuroendocrine function</td>
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<tr>
<td>Obesity Associated with Leptin Resistance (Involving Leptin and Molecular Signaling Pathways Downstream of the Leptin Receptor)</td>
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<tr>
<td>Leptin receptor gene mutations</td>
<td>Rare</td>
<td>Phenotype similar to congenital leptin deficiency, hyperphagia, less-remarkable hyperinsulinemia, hypogonadotrophic hypogonadism, abnormal growth hormone secretion with mild growth delay, hypothalamic hypothyroidism, immune dysfunction</td>
</tr>
<tr>
<td>POMC mutations</td>
<td>Rare</td>
<td>Hyperphagia, early-onset obesity, ACTH deficiency with adrenal insufficiency/crisis, lack of MSH function at MC1Rs resulting in pale skin and red hair</td>
</tr>
<tr>
<td>Prohormone convertase 1 deficiency</td>
<td>Rare</td>
<td>Hyperphagia, early-onset obesity, hypogonadotrophic hypogonadism, abnormal glucose homeostasis, hypoinsulinemia, hypocortisolemia</td>
</tr>
<tr>
<td>Melanocortin 4 receptor mutations</td>
<td>5%–8% of childhood obesity</td>
<td>Hyperphagia, early-onset obesity, increased fat and lean body mass, increased linear growth and bone density, severe hyperinsulinemia</td>
</tr>
<tr>
<td>Melanin-concentrating hormone receptor-1 mutations (loss of function)</td>
<td>Rare</td>
<td>Markedly underweight individuals</td>
</tr>
<tr>
<td>Neurtropeptide-related kinase B mutations</td>
<td>Rare</td>
<td>BDNF deficiency resulting in hyperphagia, severe obesity, developmental delay, cognitive dysfunction</td>
</tr>
<tr>
<td>Mutations of other molecules downstream of leptin receptor</td>
<td>Rare</td>
<td>Obesity with onset in childhood</td>
</tr>
<tr>
<td>Mechanism to be discovered</td>
<td>&gt;90% of obese individuals</td>
<td>Garden-variety obesity</td>
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The table was adapted from the following references: Bührer and Mantzoros (2009), Brennan and Mantzoros (2006), Dardeno et al. (2010), and Smink et al. (2012). ACTH, adrenocorticotropic hormone; BDNF, brain-derived neurotrophic factor; GnRH, gonadotropin-releasing hormone; HAART, highly active antiretroviral therapy; IGF, insulin-like growth factor; MC1R, melanocortin 1 receptor; MSH, melanocyte-stimulating hormone; POMC, pro-opiomelanocortin.
fMRI studies have introduced what is referred to as the “obesogenic phenotype” of brain activity, which is generally characterized by (1) hyperactivity in a food-related network of brain areas involved in reward (e.g., striatum, insula, orbitofrontal cortex [OFC]), motor/sensor processing (e.g., insula, precentral gyrus) and emotion/memory (e.g., amygdala, hippocampus) and (2) hypoactivity in brain regions associated with homeostatic control (e.g., hypothalamus) and attention/cognitive control (e.g., prefrontal cortex [PFC], cingulate gyrus) (Carnell et al., 2012). The obesogenic phenotype is influenced by emotion, cognition, and behavior, as well as by genetic and environmental parameters.

Given that leptin may regulate hedonic response to food via the mesolimbic system, neuroimaging studies have attempted to illuminate leptin’s role in homeostatic regulation of feeding in the context of (1) congenital or acquired leptin deficiency and (2) leptin resistance states.

**Neuroimaging Studies in Patients with Congenital Leptin Deficiency.** Three neuroimaging studies have investigated the functional effects of leptin treatment on the brain in congenitally leptin-deficient patients using fMRI (Baicy et al., 2007; Farooqi et al., 2007; Frank et al., 2011), and three other studies conducted in individuals with congenital leptin deficiency have investigated the structural effects associated with leptin deficiency and replacement using MRI/VBM to assess increased gray matter tissue concentration (London et al., 2011; Matochik et al., 2005; Paz-Filho et al., 2008b); these data are summarized in Table 3. In brief, based on these fMRI studies, leptin seems to increase satiety, direct homeostatic- and reward-related brain areas toward lower intake and selection of lower-caloric foods through interactions with cortical regions (Table 3). Based on MRI/VBM studies, leptin may regulate brain plasticity—and thereby play a role in normal brain development in children—and may increase gray matter in adults in regions regulating hunger and satiation (Table 3). The major limitation of all these studies is their very small sample size; however, due to disease rarity, it is rather difficult to perform clinical trials with large sample sizes in patients with congenital leptin deficiency. A better understanding of leptin’s effects on neurogenesis, axon growth, synaptogenesis, dendritic morphology, neuron excitability, and regulation of β-amyloid levels in both subjects with leptin deficiency and those with adequate leptin levels may lead to leptin-based therapies for not only congenital leptin deficiency, but also mood and neurodegenerative disorders.

**Neuroimaging Studies in Leptin-Resistance States.** Obesity, which is probably the most prevalent leptin-resistant state, is notably associated with numerous alterations to brain activity. Late-life obesity is associated with smaller global and regional gray matter volumes (Brooks et al., 2013) and long-term obesity has been associated with cerebral atrophy (Matochik et al., 2005). Obese, in comparison to lean, individuals have reduced gray matter in several brain areas, including regions involved in the regulation of taste (i.e., inferior frontal operculum and postcentral gyrus), reward (i.e., putamen) and behavioral processing (i.e., middle frontal gyrus) (Pannacciulli et al., 2006). Other changes seen in obese individuals include enlarged volumes in the orbitofrontal white matter (Pannacciulli et al., 2006) and neuronal and myelin abnormalities in the frontal lobes (Gazdzinski et al., 2008). These abnormalities may be the consequence of several underlying parameters/comorbidities, such as subclinical inflammation, reduced physical activity, impaired respiratory function, hypercortisolemia, hypertension, hyperlipidemia, cardiovascular disease, type 2 diabetes mellitus, and impaired leptin signaling and function manifested in garden-variety obesity.

Notably, a VBM-based study showed an independently negative association between fasting plasma leptin concentrations, which are increased in garden-variety obesity, and the volume of gray matter in brain areas in which obese people typically exhibit reduced gray matter compared to their lean counterparts (Pannacciulli et al., 2007); although this cannot directly prove a
Table 3. Summary of Neuroimaging Studies in Patients with Congenital Leptin Deficiency

<table>
<thead>
<tr>
<th>Reference*</th>
<th>Main Findings</th>
<th>Main Highlights</th>
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<tr>
<td><strong>fMRI Studies</strong></td>
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<tr>
<td>Farooqi et al. (2007): two patients, 7 days</td>
<td>(1) The anteromedial and posterolateral ventral striatum were markedly activated in response to food pictures in the baseline leptin-deficient state compared to the leptin-replaced state; (2) leptin reduced food intake, decreased hunger in the fasting state, increased postprandial satiety, and decreased liking of food pictures in the fed state</td>
<td>Leptin via the mesolimbic system (striatal regions) reduces the perception of food reward while enhancing the response to satiety signals produced during food consumption</td>
</tr>
<tr>
<td>Baicy et al. (2007): three patients, 57 months followed by 1 month of discontinuation and 14 days of reinstitution</td>
<td>(1) Leptin reduced activation in brain regions linked to hunger, including the insular cortex and parietal and temporal cortex; (2) leptin increased activation in brain regions linked to satiety, including the PFC (middle, superior, and medial frontal gyr) in response to high-caloric pictures</td>
<td>The greater activation of the PFC in the leptin-replaced state may mediate behavioral inhibition and satiety control (i.e., inhibition of consuming high-caloric foods) in accordance with the generally accepted belief that high-caloric foods are unhealthy and should be avoided</td>
</tr>
<tr>
<td>Frank et al. (2011): one patient, 6 months</td>
<td>(1) Leptin decreased activation in amygdala (area responsible for evaluation of emotional salience) and VTA/substantia nigra, whereas increased activation in the OFC (area involved in reward and food-related responses); (2) low- versus high-caloric cues provided differential effect in VTA and OFC and hypothalamus (for example, in hypothalamus, activation after low-caloric cues was gradually increased, whereas activation after high-caloric cues was gradually decreased)</td>
<td>Leptin treatment resulted in activity changes in both homeostatic- and reward-related brain areas, which generally favored reward system toward a low caloric diet together with weight loss</td>
</tr>
<tr>
<td><strong>MRI/VBM Studies</strong></td>
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<tr>
<td>Matochik et al. (2005): three patients (with a recessive mutation in the ob gene), 18 months</td>
<td>Increased gray matter concentration, reflecting an increase in number or size of neurons, within the anterior cingulate gyrus, the inferior parietal lobule, and the cerebellum</td>
<td>Leptin treatment increases number or size of neurons of brain regions implicated in leptin’s overhypothalamic neural pathways, regulating hunger and satiation</td>
</tr>
<tr>
<td>Paz-Filho et al. (2008b): one patient; 2 years</td>
<td>Substantial increase in the rates of development in most neurocognitive domains, together with some skills at or exceeding expectations based on chronological age</td>
<td>Leptin treatment improved neurocognition</td>
</tr>
<tr>
<td>London et al. (2011): three patients, 3 years (cycle of treatment withdrawal and reinstitution)</td>
<td>(1) Withholding of leptin treatment increased BMI and reversed the initial effects of leptin in the cerebellum and the anterior cingulate gyrus; (2) leptin restoration did not lead to a short-term recovery of gray matter, but to an unexpected gray matter increase in the posterior half of the left thalamus, particularly the pulvinar nucleus</td>
<td>Leptin treatment may play a therapeutically valuable role in regulating plasticity-dependent brain functions</td>
</tr>
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*BMI, body mass index; fMRI, functional magnetic resonance imaging; MRI, magnetic resonance imaging; OFC, orbitofrontal cortex; PFC, prefrontal cortex; VBM, voxel-based morphometry; VTA, ventral tegmental area.

cause-effect relationship, it may suggest a role for leptin in the reorganization of brain composition in regions recently found to be associated with excess body fatness. Leptin supplementation may blunt brain activity in response to food stimuli in obese adults after weight loss. In an fMRI-based study, six obese individuals receiving leptin treatment or placebo (in a crossover fashion), after being stabilized at a weight of approximately 10% lower of their initial weight, were evaluated. Leptin administration to these weight-reduced individuals reversed the increased hunger and decreased satiety that are characteristic of the weight-reduced state. Moreover, in response to visual food cues, there were observable leptin-reversible increases in neural activity of the brainstem, culmen, parahippocampal gyrus, inferior, and middle frontal gyri, middle temporal gyrus, and lingual gyrus and leptin-reversible decreases in activity of the hypothalamus, cingulate gyrus, and middle frontal gyrus (Rosenbaum et al., 2008). Weight loss is linked to neural activity changes in these brain regions, which are associated with the emotional, regulatory, and cognitive control of food intake. Despite the small sample size, the authors proposed that, after weight loss, which is accompanied by decreases in leptin due to decreases in stored endogenous energy, leptin administration decreases the responsiveness to the emotional and sensory aspects of food and increases the cognitive control of food intake (Rosenbaum et al., 2008).

Limitations and Future Directions for Neuroimaging Studies. Overall, current studies provide evidence that leptin treatment in severe leptin-deficient or leptin-resistant states may alter the
activity patterns in brain areas related to homeostatic, emotional, and reward-related processes. It seems that leptin enhances satiety and cognitive responses to food stimuli, while it diminishes responses in hedonic, reward-related, and sensory brain regions. These studies also suggest that leptin may engender extrahypothalamic effects on human brain tissue composition and may improve neuroregeneration. It seems that neuroplasticity within the human brain partly depends on leptin.

A significant drawback of the above-mentioned neuroimaging studies is their very small sample size, which prohibits generalization of the reported results. Moreover, it is difficult to draw concrete conclusions based on current fMRI studies, as they have each identified distinct and separate brain regions that are differentially activated or inhibited by leptin treatment. Methodological discrepancies could explain these observed differences. For example, the above-discussed fMRI studies differed with respect to whether they reported results on whole brain or regions of interest analyses (Baicy et al., 2007; Farooqi et al., 2007; Frank et al., 2011; Rosenbaum et al., 2008). Other differences include the control (or lack of control) of dietary intake, duration of leptin administration, presentation and nature of the visual stimuli, and the inclusion or exclusion of behavioral tasks (e.g., hunger ratings) performed by subjects while in the scanner. Furthermore, congenitally leptin-deficient patients and weight-reduced subjects may not react similarly to leptin administration and may exhibit different patterns of brain activation, because of metabolic, physiological, and developmental differences; such differences obfuscate our ability to draw a cohesive conclusion about leptin’s effects on brain activity in states of severe and partial leptin insufficiency. Moreover, the severity of leptin deficiency (degree and duration) may also have important implications on the efficacy and/or nature of the brain’s response to leptin treatment (Paz-Filho et al., 2008b).

Additional neuroimaging studies that examine variable degrees of leptin deficiency are needed to better understand leptin’s effects on the brain. More studies with a larger sample size, although difficult to achieve when performing studies for rare diseases such as congenital leptin deficiency, are also needed in order to draw more substantial conclusions about the interplay between leptin and brain activity. Hopefully, future neuroimaging studies will reveal the pathways that mediate the interaction between hedonic/reward and homeostatic/satiety signaling in states of energy excess, such as obesity (Carnell et al., 2012; Dardeno et al., 2010; Farooqi et al., 2007).

The Role of Leptin in Regulating Neuroendocrine Function
Leptin also regulates energy homeostasis by moderating neuroendocrine adaptations seen in leptin-deficient states, such as starvation, hypothalamic amenorrhea, and human immunodeficiency virus (HIV)-associated lipodystrophy, to prevent overconsumption, slow metabolism, and inhibit growth-related processes via decreased hypothalamic-gonadal signaling, decreased thyroid hormone levels, and decreased insulin-like growth factor-1 (IGF-1) levels (Chan et al., 2003). Patients with congenital leptin deficiency have several neuroendocrine disorders, including hypogonadotropic hypogonadism with failure to reach puberty (Licinio et al., 2004) and disorganized secretion of thyroid-stimulating hormone (TSH), but not necessarily thyroid dysfunction (Mantzoros et al., 2001). Leptin replacement may permit the appropriate onset of puberty in individuals with congenital leptin deficiency (Farooqi et al., 2002; Licinio et al., 2004) and may increase thyroid hormone levels but does not affect TSH levels (Farooqi et al., 2002). Unlike mice, children with congenital leptin deficiency have normal linear growth and adrenal function (Farooqi et al., 2002).

An acute fall in leptin levels in response to starvation results in similar neuroendocrine abnormalities. In healthy men, leptin replacement prevented the fasting-induced fall in testosterone levels, the changes in LH and TSH pulsatility, and the fall in total IGF-1 levels, but did not prevent the changes in GH pulsatility, the decline in free IGF-1 levels, or the increase in 24 hr cortisol concentration (Chan et al., 2003). In healthy women, leptin replacement also prevented fasting-induced decreases in LH pulsatility (Chan et al., 2006). However, administration of leptin in overweight and obese subjects during weight loss has not been shown to reverse these changes in thyroid, corticotropic, and somatotropic axes associated with weight loss, perhaps because even after weight loss such individuals remain relatively leptin resistant (Shetty et al., 2011). In contrast, leptin administration during weight loss maintenance, a time of relative leptin deficiency, was found to reverse the decreases in both triiodothyronine (T3) and thyroxine (T4) levels but not the decrease in TSH levels (Rosenbaum et al., 2005), but this study was not randomized and only of sequential design. This suggests a potential pleiotropic role for leptin in weight loss maintenance, especially since leptin levels are found to be significantly decreased for over a year after weight loss (Sumithran et al., 2011), but this remains to be shown by well-designed randomized clinical trials.

We have shown that leptin replacement has similarly positive effects on neuroendocrine regulation among women with hypothalamic amenorrhea, who are chronically energy deprived and have relative hypo leptinemia (Miller et al., 1998). In our proof-of-concept trial, we showed that leptin treatment in replacement doses for 12 weeks resulted in increased mean LH levels, LH pulse frequency, estradiol levels, number of dominant follicles, free T3 and free T4 levels, and IGF-1 and IGF-binding protein 3 (IGFBP-3) levels; no changes in cortisol or ACTH levels were found in that initial study, but this might have been due to the specific study design (Welt et al., 2004). This study was followed by a randomized, placebo-controlled 36-week trial that confirmed increases in the levels of estradiol, progesterone, and free T3, and in the ratio of IGF1:IGFBP-3 and found a decrease in cortisol levels with leptin treatment (Chou et al., 2011). These improvements were independent of any weight gain or decrease in exercise activity (Chou et al., 2011; Welt et al., 2004). Finally, 70% of subjects on leptin treatment menstruated during the course of the study compared to 22% of those on placebo (Chou et al., 2011).

Lipoatrophy is another condition associated with hypo leptinemia caused by deficiency or destruction of adipose cells and the resultant inability to produce leptin (Pardini et al., 1998). In open-label studies, leptin treatment in female patients with lipodystrophy and hypoleptinemia has been shown to restore menses, increase estradiol levels, decrease testosterone levels, increase sex hormone binding globulin (SHBG) levels, and normalize LH response to LH releasing hormone (Musso et al., 2005); fewer
changes were seen in male patients with lipodystrophy (Musso et al., 2005). In response to leptin treatment, IGF-1 levels, but not GH levels, have been found to increase in patients with generalized or partial lipodystrophy (Musso et al., 2005) but did not change in patients with highly active antiretroviral-therapy-induced lipodrophy with less-profound leptin deficiency (Brennan et al., 2009). No changes were observed in the hypothalamic-pituitary-adrenal axis (Musso et al., 2005).

These findings indicate that in states of severe hypoleptinemia leptin may act as a “master switch” for the hypothalamic neuro-endocrine axes that control metabolism, growth, and reproduction. Only through increased study of leptin may we come to harness this physiological feature for therapeutic advantage.

The Role of Leptin in Cognition

Leptin has been associated with beneficial effects on cognition and memory. Administration of leptin in rabbits reduced accumulation of β-amyloid and phosphorylated tau protein, two major peptides implicated in Alzheimer’s disease, and improved cognitive performance compared to saline-treated littermates (Marwarha et al., 2010). Epidemiological studies have reported that higher leptin levels are prospectively associated with lower odds of dementia in nonobese persons only (Lieb et al., 2009). These findings corroborate those from fMRI studies, which have indicated that plasma leptin levels are positively associated with total cerebral brain volume (Lieb et al., 2009). This signifies a therapeutic potential for leptin in the treatment of cognitive decline and in the optimization of mental function. As the use of sophisticated neuroimaging techniques further refines our understanding of leptin’s role in the neurobiology of eating behavior, this may prove useful in the fields of psychology and dementia. Additional interventional trials will be necessary to assess the effects of leptin on mental health and cognition in the general population or in obese individuals at risk for late-in-life cognitive decline.

The Role of Leptin in Metabolism

Carbohydrate Metabolism

Leptin administration may improve glucose metabolism in several leptin-deficient states, including complete leptin deficiency and lipodrophy, which are associated with insulin resistance and impaired glucose metabolism. In addition to improving insulin resistance by decreasing body weight and fat mass, especially ectopic or intra-abdominal fat (Moran et al., 2004), leptin can also activate insulin-sensitive tissues. Leptin signaling in mouse muscle cells has been found to parallel that of insulin via PI3K activation (Maroni et al., 2005). Similarly, SOCS3, whose expression is enhanced through the JAK2/STAT3 pathway, acts as a negative feedback mechanism by attenuating leptin signaling, which induces leptin resistance; SOCS3 may possibly also attenuate insulin signaling by inhibiting PI3K, thereby simultaneously inducing insulin resistance (Polyzos et al., 2011). Furthermore, in vitro studies have shown that leptin inhibits insulin gene expression and glucose-stimulated insulin secretion from pancreatic β cells, and in vivo rodent studies have shown that leptin protects β cells from lipotoxicity (Lee et al., 2011). These findings suggest that particular mechanisms exist through which leptin may enhance or modify insulin’s metabolic effects.

Patients with congenital leptin deficiency are profoundly obese and can exhibit insulin resistance with hyperinsulinemia and diabetes mellitus; leptin replacement has been shown to improve insulin sensitivity in these individuals (Farooqi et al., 2002; Licinio et al., 2004). Using mathematical modeling, Andreev et al. demonstrated that hepatic extraction of insulin increased 2-fold in a man with congenital leptin deficiency after 1 week of leptin treatment (Andreev et al., 2009). After 24 months of treatment, insulin sensitivity increased by 10-fold and insulin secretion decreased by 2-fold, while insulin hepatic extraction returned to normal (Andreev et al., 2009).

In patients with congenital and acquired lipodystrophies, including HIV-associated lipodystrophy, leptin treatment results in significant improvements in glycemia, insulin resistance, and central obesity, the effects of which appear to be time and dose dependent (Brennan et al., 2009; Lee et al., 2006; Mulligan et al., 2009; Musso et al., 2005). In patients with severe, generalized lipodystrophy, improvement in glycemic control has been attributed to a 2-fold increase in insulin suppression of hepatic glucose production, as well as insulin-stimulated peripheral glucose disposal (Petersen et al., 2002). These improvements in hepatic and skeletal muscle sensitivity were associated with 85% reduction in hepatic triglyceride content and 30% reduction in intramyocellular triglyceride and fatty acyl CoA content (Petersen et al., 2002).

These improvements in glycemia and insulin resistance have prompted further research into leptin’s role in diabetes mellitus. Studies in lipotoxic mice showed that leptin monotherapy at pharmacological doses normalizes glucose levels and improves glucose variability, a finding attributable to leptin’s ability to suppress glucagon (German et al., 2010; Wang et al., 2010). In response to physiological doses of leptin, blood glucose levels are only slightly lowered, despite similar decreases in glucagon levels and corticosterone levels (German et al., 2010). They did find that leptin replacement prevented insulin resistance at the level of the liver, independently of its effects on food intake and body weight, via reduction of hepatic triglyceride content, decreases in hepatic expression of gluconeogenic genes (i.e., glucose-6-phosphatase and phosphoenolpyruvate kinase), and improvement of insulin signaling at the level of PI3K; these effects were not shown in the skeletal muscle or adipose tissue. Intracerebroventricular leptin treatment has been shown to normalize glucose levels by suppressing hepatic glucose production, but also by increasing insulin-independent glucose uptake in peripheral tissues, including the skeletal muscle, heart, and brown adipose tissue, via the sympathetic nervous system activation (German et al., 2011).

In “experiments of nature” studies, two patients with concomitant type 1 autoimmune diabetes and acquired generalized lipodystrophy demonstrated severe insulopenia and extreme insulin resistance, requiring higher insulin dosing than expected based on weight (Park et al., 2008). After the initiation of leptin treatment, insulin requirements decreased dramatically. In contrast, although leptin treatment has resulted in dramatic improvement in insulin resistance in patients with congenital leptin deficiency and lipodystrophy, leptin treatment has been ineffective in improving glycemic control and body weight in patients with type 2 diabetes mellitus, likely due to leptin resistance (Moon et al., 2011). No studies have been performed in patients suffering from lipodystrophy due to HIV and insulin resistance.
with type 1 diabetes mellitus alone, and it would be interesting to see whether leptin can serve as an adjunct to insulin therapy. **Lipid Metabolism**

Leptin regulates lipid metabolism in conjunction with insulin, but they do so in opposing ways. While insulin acutely stimulates lipogenesis, leptin stimulates fatty-acid oxidation via AMP-activated protein kinase pathway to prevent the accumulation of lipids in nonadipose tissue and the resultant lipotoxicity (Mino-koshi et al., 2002). Leptin replacement in patients with congenital leptin deficiency has been shown to decrease insulin-mediated stimulation of lipogenesis (Paz-Filho et al., 2008a). These patients have mild dyslipidemia and particularly low HDL cholesterol levels, and leptin replacement has been shown to improve their triglyceride and HDL and LDL cholesterol levels (Farooqi et al., 2002; Licinio et al., 2004). Additionally, uncontrolled, open-label studies in humans with congenital and acquired lipodystrophies and complete leptin deficiency have shown that leptin treatment results in significant improvement in hepatic steatosis and intramyocellular lipid content (Musso et al., 2005; Petersen et al., 2002).

Similar but less-robust effects are seen in patients with HIV-associated lipodystrophy, as these patients do not have an absolute leptin deficiency relative to those with congenital lipodystrophy. In a 6-month open-label study of eight men with HIV-associated lipodystrophy, leptin treatment decreased lipolysis and LDL and non-HDL cholesterol and increased HDL cholesterol (Mulligan et al., 2009). In our randomized, placebo-controlled, crossover study in seven patients with HIV-associated lipodystrophy, 2-month metreleptin treatment improved truncal obesity and insulin sensitivity, and its positive effects on HDL, LDL, and triglycerides were mediated through changes in intrabdominal fat (Lee et al., 2006). Results similar to ours were reported in another recent 4-month, randomized, placebo-controlled trial, which found a modest decrease in non-HDL levels, although no changes in triglyceride or HDL levels were observed (Sekhar et al., 2012). The varying efficacies of leptin in these metabolic abnormalities need to be studied further to determine which subset of patients with HIV-associated lipodystrophy would benefit the most, which may be primitively related to the severity of leptin deficiency.

**The Role of Leptin in Bone Metabolism**

Leptin affects bone metabolism differently via central and peripheral means. Centrally, mouse studies suggest that leptin induces cortical bone formation via β1, sympathetic activation and/or the GH-IGF-1 axis, but also induces trabecular bone remodeling with loss in volume via β2 sympathetic activation (Hamrick and Ferrari, 2008). Data from mouse studies also indicate that leptin may mediate bone metabolism either positively or negatively by regulating the expression of several neuropeptides in the hypothalamus, including NPY (Ballock et al., 2006) and neurenomedin U (Sato et al., 2007).

Peripherally, leptin interacts with bone marrow stromal cells and osteoblasts to increase overall bone mass. Leptin increases expression of osteogenic genes in stromal cells, directing them down the osteogenic instead of adipogenic pathway (Bartell et al., 2011). Leptin also stimulates stromal cells to increase osteoprotegerin expression and decrease RANK ligand, resulting in decreased osteoclastogenesis (Holloway et al., 2002). In vitro and in vivo mouse studies, leptin has also been shown to increase osteoblast proliferation, de novo collagen synthesis, and mineralization (Gordeladze et al., 2002).

Although no correlation between bone mineral density and leptin has been found in normal weight children or adolescents or healthy postmenopausal women (Roemmich et al., 2003), interventional studies suggest that leptin may be more important for bone health 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, unlike ob/ob mice, which have 25% lower bone mass (Hamrick et al., 2005), leptin treatment increases their skeletal maturation (Farooqi et al., 2002). In women with hypothalamic amenorrhea, we reported significant changes in markers of bone turnover, suggestive of bone formation, compared to placebo-treated subjects (Chou et al., 2011; Welt et al., 2004) and, over the course of 2 years of treatment, increases in lumbar spinal bone mineral density and bone mineral content compared to baseline (Sienkiewicz et al., 2011). However, it remains to be determined whether this increase in bone-formation markers and bone mineral density and content is a direct effect of leptin and/or mediated by restoration of estradiol and/or IGF-1 and cortisol levels.

An important issue that remains to be elucidated is whether hypoleptinemia increases fracture risk, which is the hard endpoint of metabolic bone disease. Only recently was impaired fracture healing (delay in callus maturation and increase in total callus volume) shown radiographically and histologically in ob/ob compared with control mice subjected to open stabilized middiaphyseal femur fractures; local leptin application reversed the delay in healing in ob/ob mice (Khan et al., 2013). Given that currently only cross-sectional studies have evaluated leptin levels in patients with low-energy fractures and have provided controversial results, it remains to be elucidated whether leptin administration in patients with lipodystrophy and hypoleptinemia might have an effect on fracture prevention or therapy.

**The Role of Leptin in Immune Function**

Leptin has also been proposed to link energy metabolism with the inflammatory and immune responses, particularly the CD4+ T-helper-1 response (Carbone et al., 2012). In ex vivo studies, leptin has been shown to promote proliferation and interleukin-2 (IL-2) secretion by naive T cells, enhance phagocytic activity in macrophages, enhance proliferation and cytotoxicity of natural killer cells, stimulate chemotaxis in polymorphonuclear cells, and promote production of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), IL-6, and IL-12 (Carbone et al., 2012). In vitro, we have shown that leptin promotes lymphocyte survival by suppressing Fas-mediated apoptosis (Papathanassoglou et al., 2006). Additionally, 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 (Howard et al., 1999).

Compared to the general population, individuals with congenital leptin deficiency have a higher incidence of infection. One leptin-deficient patient was reported to have decreased proliferation and function of CD4+ T cells at baseline, which normalized after exogenous leptin replacement (Farooqi et al., 2002).
Although patients with severe lipodystrophy have no clinically evident immune dysfunction and exhibit normal absolute number and percentage of T cells, leptin treatment has been shown to increase the number of T cells, both CD4+ and CD8+, and increase TNF-α secretion from peripheral blood mononuclear cells in vitro to normal levels in response to lipopolysaccharide and lipopolysaccharide plus IFN-γ treatment, suggesting that the response is mediated through the Toll-like receptor pathway (Oral et al., 2006). Overall, these immunomodulatory effects in lipodystrophic patients were modest (Oral et al., 2006). Our group has studied the effect of leptin administration on the immune system in women with hypothalamic amenorrhea and relative leptin deficiency and found that it leads to greater activation of the TNF-α system (Chan et al., 2005) and to the restoration of CD4+ T cell counts and their in vitro proliferative responses via specific transcriptional changes (Matarese et al., 2013).

However, leptin’s role in immune function seems to be permissive, as leptin replacement had no major effect on fasting-induced changes on most immunophenotypes in normal-weight women with fasting-induced hypoleptinemia (Chan et al., 2006). Our group also showed that leptin administration to raise circulating levels in lean and obese men resulted in no significant increase in cytokines and inflammatory markers (Chan et al., 2005). As such, our data to date do not suggest a role for leptin in the pathogenesis of the obesity-related proinflammatory state.

**Emerging Clinical Applications**

Since its discovery in 1994 as an adipocyte-secreted hormone, leptin has been implicated in various aspects of energy homeostasis and metabolism through numerous regulatory pathways involving several organs. Recent research further implicates leptin as a key player in yet other diverse functions, such as the regulation of the immune system or bone metabolism; however, this may be an early speculation, even these leptin actions may be a function of energy status/availability. Nevertheless, despite significant progress in elucidating the pathophysiological role of leptin, many issues still remain to be explored.

Its role at the intersection of neuroendocrinology and metabolism renders leptin an appealing therapeutic target for the management of disorders related to energy homeostasis. Leptin treatment has been shown to correct the neuroendocrine and metabolic abnormalities seen in patients with leptin deficiency and lipodystrophy. Currently, leptin is available to the rare patient with congenital leptin deficiency through a compassionate-use program at Amylin Pharmaceuticals (San Diego), a company recently acquired by Bristol-Myers Squibb. These hypoleptinemic patients—who have severe neuroendocrine abnormalities and develop extreme obesity very early in life—exhibit decreased appetite, diminished food intake, marked decreases in fat mass, and resolution of their neuroendocrine irregularities, including the inability to progress through puberty, with leptin treatment (Ozata et al., 1999). Leptin’s utility in this setting is highly apparent.

Amylin Pharmaceuticals also has a FDA-approved expanded access program for the use of leptin in patients with congenital lipodystrophy. Though congenital lipodystrophy is rare, acquired lipodystrophy as a result of highly active antiretroviral therapy (HAART) for the treatment of HIV/AIDS has become more common. Individuals with HIV-associated lipodystrophy, who comprise a much larger and growing population, are leptin deficient and, upon treatment with recombinant human leptin, exhibit improvements in insulin resistance and hyperlipidemia comparable to those seen with other treatments, including metformin and thiazolidinediones (Lee et al., 2006; Mulligan et al., 2009). Hopefully, therapeutic use of leptin will expand to patients with HIV-associated lipodystrophy once further clinical trials establish treatment protocols (Brennan et al., 2009).

In addition to treatment for patients with congenital and other acquired leptin deficiency, recombinant human leptin is emerging as a potential treatment for conditions associated with relative hypoleptinemia, such as lipodystrophy and hypothalamic amenorrhea. We have found that in women with hypothalamic amenorrhea and in individuals with acquired lipodystrophy leptin administration in replacement doses may correct the metabolic abnormalities observed in these leptin-deficient states (Chou et al., 2011; Oral et al., 2006; Sienkiewicz et al., 2011; Welt et al., 2004). Leptin replacement therapy may also have numerous other observed physiological benefits, including improved immune status in patients with generalized and severe lipodystrophy, normalized menstrual patterns in young women with lipodystrophy and polycystic ovary syndrome (Mittendorfer et al., 2011), and improved hypothalamic neuroendocrine axes in women with HA (Kusakabe et al., 2009). Large phase III clinical trials are needed to confirm these findings and to determine the clinical efficacy of leptin replacement therapy in these populations.

Leptin sensitivity remains an issue when considering the potential for leptin treatment in subjects with hyperleptinemia and energy excess, such as obesity. Clinical trials exploring leptin monotherapy for weight loss have subsided due to underwhelming results (Heymsfield et al., 1999; Moon et al., 2011). The amount of weight loss was not markedly more than that observed as a result of behavioral interventions or pharmacological treatments using orlistat, sibutramine, or a combination of phentermine and topiramate (up to 5–10 kg) (Leblanc et al., 2011). Leptin resistance is still obviously a potent and recalcitrant factor, the underlying molecular mechanisms of which are now being actively explored (Moon et al., 2011).

Given that leptin monotherapy does not seem to be efficacious in promoting weight management among leptin-resistant obese individuals, leptin combination therapy for obesity may be a promising treatment for garden-variety obesity, which, aside from costly and risky bariatric surgery, is a chronic disease with few successful long-term therapeutic options. Newer trials have focused on the weight loss effects of leptin coadministration with possible leptin sensitizers like amylin, a hormone secreted by the pancreas that also is involved in the regulation of energy homeostasis (Ravussin et al., 2009; Roth et al., 2008). In a study conducted by Amylin Pharmaceuticals, overweight and obese subjects lost significantly more weight on a combination of leptin and pramlintide, an amylin analog, compared to either agent alone, though in an additive, and not synergistic, fashion, suggesting that amylin does not improve the sensitivity to leptin (Ravussin et al., 2009). Roth et al. found similarly additive results in humans, but did find a synergistic weight loss response in diet-induced obese rats, which was
accompanied by enhanced leptin-induced pSTAT3 signaling in the ventromedial hypothalamus and hindbrain area postrema (Roth et al., 2008).

Other combinations of leptin and leptin-sensitizers have been studied in rodents, and although some are promising, their effects also may not be well translated in humans. In addition to augmenting leptin’s anorectic and weight-loss-promoting effects, metformin seems to restore leptin sensitivity in diet-induced obese rats (Kim et al., 2006). In these rats, leptin monotherapy did not increase POMC expression, while leptin and metformin combination therapy did. Additionally, when used in combination with metformin a lower dose of leptin was required to decrease the hypothalamic pAMPK level. These data collectively indicate that leptin plus metformin combination therapy may be particularly useful in promoting anorexigenic and suppressing orexigenic pathways within the CNS (Anderson et al., 2008; Kim et al., 2006). As further evidence, additional studies performed in rats have shown that combined leptin therapy with previously approved medications for weight loss, including sibutramine or rimonabant, a cannabinoid CB1 receptor antagonist, results in greater decreases in food intake and weight loss compared to monotherapy (Boustany-Kari et al., 2011). Combined leptin and rimonabant treatment was also found to synergistically increase and decrease neuronal firing in the ventromedial hypothalamus and ARC, respectively, signifying a highly potent antiobesity effect (Boustany-Kari et al., 2011). Additionally, in diet-induced obese mice, combination therapy of leptin analog with either exendin-4, a long-acting glucagon-like peptide 1 receptor agonist, or FGF21, another important modulator of glucose metabolism and energy expenditure, additively enhanced weight loss (Müller et al., 2012). However, this benefit did not emerge until after an initial weight loss of 30%, which seemed to be the threshold for the pharmacologic restoration of leptin sensitivity in these mice. Larger trials in this capacity are needed to determine the utility of leptin in the context of weight loss management and to determine whether a threshold amount of initial weight loss is necessary to reinstate leptin sensitivity in obese or overweight subjects. Finally, large randomized leptin administration studies are needed to obtain accurate information on potential adverse effects and long-term outcomes of combination therapy.

The notion of a threshold in terms of BMI levels or leptin levels needed for the restoration of leptin sensitivity has prompted focus on leptin’s role in weight loss maintenance. Increased hunger and the danger of increased food intake are consistently problematic throughout weight loss maintenance (Kissileff et al., 2012; Rosenbaum et al., 2005). However, these may arise as a direct result of the decreases in circulating leptin levels that occur due to weight loss and energy deprivation. This is a very acute area of research which is expected to result in major therapeutic advances in the not so distant future.

Although leptin administration has proven helpful in the management of lipodystrophy and other acquired hypoleptinemic states, the overall efficacy of leptin therapy in the treatment of these states remains unclear, since large, well-controlled, randomized studies have yet to show whether all patients with lipodystrophy or only those who are extremely leptin deficient would benefit from leptin therapy. Moreover, a growing body of evidence from preclinical, in vitro, and animal studies indicates that leptin administration in such states may be associated with possible sideeffects, and this needs to also be studied in the context of large future trials.

Notably, any potential adverse effects associated with leptin administration may differ among hypoleptinemic versus hyperleptinemic states. Leptin administration in hyperleptinemic states has also been hypothesized to contribute to a host of side effects including hypertension, thrombosis/angiogenesis, and increased inflammation (Ravussin et al., 2009), although we have failed to observe these effects in our clinical studies (Aronis et al., 2011; Chan and Mantzoros, 2005; Chan et al., 2007; Moon et al., 2011).

Investigators have also examined concerns for the carcinogenic potential of leptin administration in patients with garden-variety obesity. In vitro studies suggest that leptin may prompt neoplasia through two mechanisms: (1) activation of receptor-mediated growth and survival signaling pathways, such as JAK2/STAT3, PI3k/Akt, and MAPK/extracellular-signal-regulated kinase (ERK) (Gibson et al., 2004) and (2) increasing inflammatory and cytokine response (Hwang et al., 2008). Notably, most in vitro studies that have observed carcinogenic effects have used very high leptin levels, whereas in vivo clinical studies using physiologic or pharmacologic doses of leptin have not revealed any consistent associations between leptin levels and cancer risk. Larger prospective, mechanistic, and longitudinal studies are urgently needed to thoroughly evaluate this risk before leptin can be implemented as a treatment approach in patients with diabetes or obesity.

In conclusion, future large-scale clinical and epidemiological trials are required to assess the full spectrum of potential adverse effects associated with leptin replacement therapy and to determine its efficacy and safety in addition to the best safety monitoring practices. Given the lack of efficacy of leptin in garden-variety obesity and/or diabetes, trials are also needed to determine whether the discovery and concomitant use of other leptin sensitizers would be useful in the treatment of obesity or in the promotion of weight maintenance. Similarly, large-scale clinical trials are needed to determine not only (1) the appropriate upper and lower doses of leptin needed to optimize metabolic response and safely overcome leptin resistance, but also (2) the diagnostic criteria for hypo- and hyperleptinemia in order to provide the basis for inclusion criteria in future investigative trials, given that the definition of relative deficiency or excess of leptin levels (i.e., specific leptin cutoff points) seem to be critical to maximizing the benefits and minimizing the drawbacks of leptin use. Hence, although great progress has been made in terms of understanding leptin’s role in (patho-)physiology, several fundamental questions still remain unanswered.

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