Leptin’s Role in Lipodystrophic and Nonlipodystrophic Insulin-Resistant and Diabetic Individuals

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Leptin is an adipocyte-secreted hormone that has been proposed to regulate energy homeostasis as well as metabolic, reproductive, neuroendocrine, and immune functions. In the context of open-label uncontrolled studies, leptin administration has demonstrated insulin-sensitizing effects in patients with congenital lipodystrophy associated with relative leptin deficiency. Leptin administration has also been shown to decrease central fat mass and improve insulin sensitivity and fasting insulin and glucose levels in HIV-infected patients with highly active antiretroviral therapy (HAART)-induced lipodystrophy, insulin resistance, and leptin deficiency. On the contrary, the effects of leptin treatment in leptin-replete or hyperleptinemic obese individuals with glucose intolerance and diabetes mellitus have been minimal or null, presumably due to leptin tolerance or resistance that impairs leptin action. Similarly, experimental evidence suggests a null or a possibly adverse role of leptin treatment in nonlipodystrophic patients with nonalcoholic fatty liver disease. In this review, we present a description of leptin biology and signaling; we summarize leptin’s contribution to glucose metabolism in animals and humans in vitro, ex vivo, and in vivo; and we provide insights into the emerging clinical applications and therapeutic uses of leptin in humans with lipodystrophy and/or diabetes. (Endocrine Reviews 34: 377–412, 2013)
A. States of leptin deficiency
B. States of leptin excess
C. Benefits, potential adverse effects, and challenges in relation to leptin administration

VII. Future Directions

I. Introduction

Leptin has a crucial role in the regulation of energy homeostasis, insulin action and lipid metabolism (1). As a hormone secreted by adipocytes in quantities mainly correlated with fat cell mass, leptin serves as an important signal of body energy stores. Its importance is well illustrated by the physiological consequences of leptin deficiency in mice homozygous for a mutation in the obese (ob) gene, which prevents leptin production or leads to secretion of a truncated inactive leptin molecule (1–3). Ob/ob mice exhibit hyperphagia, early-onset obesity, insulin resistance, diabetes (1, 2), and several neuroendocrine abnormalities (3, 4). All these abnormalities can be corrected by exogenous leptin administration (1, 2, 5, 6), suggesting that leptin plays a role in glucose homeostasis and possibly in the pathogenesis of other obesity-related metabolic complications. Interestingly, leptin-induced normalization of hyperglycemia and hyperinsulinemia in ob/ob mice is observed even before body weight reduction, suggesting that leptin’s effects on glucose homeostasis are, in part, independent of its weight-reducing effects (5, 6). Similar to ob/ob mice, other mouse models of obesity and leptin resistance or tolerance (7–9) present abnormalities of leptin deficiency due to subnormal leptin action (7, 10, 11).

The importance of leptin is also evident in human physiology (3, 4, 7–18). Leptin administration has been demonstrated to successfully treat obesity and its complications in individuals with congenital leptin deficiency, and thus, leptin is available on a compassionate basis for these patients (5–7). Moreover, leptin is effective in correcting neuroendocrine abnormalities and insulin resistance in patients with HIV-associated lipodystrophy (11–13) and congenital lipodystrophy (8–10). Therefore, an application has been submitted to the U.S. Food and Drug Administration (FDA) for approval of leptin in replacement doses for the treatment of congenital lipodystrophy. In contrast, in subjects with garden-variety obesity or diabetes (who exhibit hyperleptinemia presumably due to leptin tolerance), treatment with additional exogenous leptin has not been associated with significant weight loss or reduction in metabolic complications (19–21). This suggests that leptin tolerance or resistance exists in these subjects (22, 23). Hence, although great progress has been made in understanding the role of leptin in many physiological systems, much research is currently being directed toward elucidating the mechanisms and pathophysiology of leptin’s effects on glucose metabolism. In this review, we describe the effects of leptin biology and signaling on glucose metabolism in animals and humans in vitro, ex vivo, and in vivo and provide novel insights into emerging clinical applications and therapeutic uses of recombinant leptin in the area of lipodystrophy, insulin resistance, and diabetes in humans.

II. Leptin Biology

A. Leptin discovery

In 1994, the mouse ob gene was found to encode a 16-kDa 167–amino-acid secreted protein with a 4-helix bundle motif similar to that of a cytokine, which was named leptin (from the Greek word leptos, meaning thin) (10, 24). Leptin signals primarily the status of body energy reserves in fat to the brain and other tissues so that appropriate changes in food intake, energy expenditure, and nutrient partitioning can occur to maintain whole-body energy balance (24). This system is especially sensitive to energy deprivation. The discovery of leptin in 1995 also defined a novel endocrine role for adipose tissue and thus subsequently increased our understanding of how food intake and energy metabolism are regulated (10).

B. Leptin production and circulation

Leptin is primarily produced in adipose tissue but is also expressed in many other tissues, including the placenta, mammary gland, testes, ovary, endometrium, stomach, hypothalamus, pituitary, and others (25). Leptin concentrations in blood are pulsatile and follow a circadian rhythm, with lowest levels in the early to midafternoon and highest levels between midnight and early morning (26). The pulsatile characteristics of leptin secretion are similar in lean and obese individuals with the exception that the obese exhibit higher pulse amplitudes of leptin (26). Circulating leptin levels are directly proportional to the amount of body fat (23) and fluctuate with acute changes in caloric intake (27). Women tend to have higher leptin concentrations than men, although a significant reduction in the amount of circulating leptin occurs after menopause (28). This sexual dimorphism, which is independent of body mass index (BMI), is partly attributed to differences in fat mass, body fat distribution, and sex hormones (29). Subcutaneous fat expresses more leptin mRNA than omental (visceral) fat, and this may partially contribute to higher leptin levels in women compared with men (29). Thus, compared with visceral fat, accumulation of sc fat is associated with higher leptin levels (26) and steatotic and lipotoxic complications appear more fre-
C. Leptin receptors

Leptin exerts its effects via binding to specific leptin receptors (obese receptors [ObRs]) located throughout the central nervous system (CNS), including the hypothalamus, and several peripheral organs (4, 10, 28). The leptin receptor is a receptor in the class 1 cytokine receptor family, with a single membrane-spanning domain (10, 24, 28, 29). At present, 6 ObR isoforms, produced by alternative splicing of the leptin receptor gene, have been identified in rats (ObRa–ObRf) (30). In mice and humans, only 5 (ObRa–ObRe) and 4 (ObRa–ObRd) alternative spliced isoforms have been described, respectively (30, 31). These isoforms present homologous extracellular leptin-binding domains but distinct intracellular domains varying in length and sequence due to alternative mRNA splicing (32). The short isoforms ObRa and ObRc may play an important role in transporting leptin across the blood-brain barrier (BBB) (32).

The long leptin receptor isoform, ObRb, which contains a full-length intracellular domain required for cell signal transduction, is primarily responsible for leptin signaling (30, 31, 33) (Figure 1). ObRb is strongly expressed throughout the CNS and particularly in the hypothalamus, where it regulates energy homeostasis (food intake and energy expenditure) and neuroendocrine function (30, 32–35). In the db/db mouse model, the ObRb is dysfunctional, resulting in obesity and the metabolic syndrome (36). ObRb mRNA is highly expressed in the hypothalamus, particularly in the arcuate nucleus (ARC), the ventromedial hypothalamus (VMH), the lateral hypothalamic area, the dorsomedial hypothalamus (DMH), and the ventral premammillary nucleus (32). Via its receptor, leptin directly targets hypothalamic neurons, particularly ARC neurons (proopiomelanocortin [POMC] and agouti-related protein [AgRP] neurons), which are critical mediators of leptin action (37). POMC neurons are anorexigenic (appetite suppressor) neurons coexpressing POMC and cocaine- and amphetamine-regulated transcript. After proteolytic cleavage, POMC generates α-MSH, which stimulates melanocortin-3 and -4 receptors (MC3R and MC4R), which are important in energy homeostasis (37). Genetic defects in the melanocortin system (POMC or MC4R genes) are related to obesity in humans (38). Conversely, AgRP neurons are orexigenic (appetite stimulator) neurons that inhibit POMC activity by releasing inhibitory γ-aminobutyric acid and coexpress the orexigenic AgRP and neuropeptide Y (NPY), antagonists of both MC3R and MC4R (39). Leptin inhibits AgRP/NPY expression and AgRP neuronal excitability as well as γ-aminobutyric acid release from AgRP neurons (39). Moreover, in the VMH, leptin stimulates the expression of anorexigenic brain-derived neurotrophic factor (BDNF) through MC4R signaling (40). Finally, in the hypothalamus, leptin acts on neurons that directly or indirectly regulate levels of other circulating hormones (eg, thyroid hormone, sex steroids, and GH) (30, 32, 33, 41). Leptin action on these hypothalamic neurons also controls the activity of the autonomic nervous system; direct effects of leptin on ObRb-containing neurons in the brainstem and elsewhere may also play an important role (31, 33).

ObRb is also expressed in multiple peripheral tissues, including pancreatic islets, adipose tissue, skeletal muscle, liver, and immune cells (36, 37, 42). In the pancreatic islets, leptin directly inhibits insulin expression and secretion (42). In liver and white adipose tissue, leptin inhibits lipogenesis and stimulates lipolysis (36, 37) and adipocyte-specific overexpression of ObRb prevents diet-induced obesity (DIO) in mice (38). Leptin directly promotes fatty acid oxidation in isolated adipocytes and skeletal muscle (39, 40) and decreases lipid levels in isolated livers (43). Liver-specific overexpression of ObRb prevents hepatic steatosis in ObRb-deficient fa/fa rats (44, 45). However, deletion of the ObRb in these peripheral tissues has no effect on energy balance, body weight, or glucose homeostasis in mice, indicating that these processes are

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Table 1. Factors That Regulate Serum Leptin Levels

<table>
<thead>
<tr>
<th>Factors Promoting Leptin Secretion</th>
<th>Factors Inhibiting Leptin Secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excess energy stored as fat (obesity)</td>
<td>Low energy states with decreased fat stored (leaness)</td>
</tr>
<tr>
<td>Overfeeding*</td>
<td>Fasting*</td>
</tr>
<tr>
<td>Glucose</td>
<td>Thyroid hormones</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Free fatty acids and other lipid metabolites</td>
</tr>
<tr>
<td>Insulin</td>
<td>Catecholamines and adrenergic agonists</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>Androgens</td>
</tr>
<tr>
<td>Estrogens</td>
<td>PPARγ agonists</td>
</tr>
<tr>
<td>Inflammatory cytokines TNF-α and IL-6 (acute effect)</td>
<td>Inflammatory cytokine TNF-α (prolonged effect)</td>
</tr>
</tbody>
</table>

* Denotes major factor influencing leptin levels.

b Unlike animals, PPARγ agonists in humans decrease leptin gene expression but increase sc fat mass, thus presenting a null effect.
mainly regulated by the central effects of leptin in the brain (46).

D. Leptin’s antisteatotic and antilipotoxicity role

Whenever leptin action is not present either due to hypoleptinemia or leptin resistance, overfeeding could lead to fat deposition to nonadipose tissues characterized by generalized steatosis, lipotoxicity, and lipoapoptosis. Generalized steatosis leading to a dysfunction of nonadipose tissues, ie, lipotoxicity, may affect pancreatic β-cells (47, 48), myocardium (49), liver, kidneys, skeletal muscle (50), and other nonadipose tissues (27). When there is ectopic lipid accumulation during overfeeding, the surplus of fatty acids follows the nonoxidative metabolic pathway resulting in metabolic trauma characterized by lipotoxicity and lipoapoptosis (51). Also, apoptosis results from excessive ceramide synthesis coupled with underexpression of the antiapoptotic factor bcl-2 (52). Ceramidotoxicity, which is not clearly identified in metabolic syndrome, has been shown to occur spontaneously in the pancreatic islets of Zucker diabetic fatty (ZDF) rodents with type 2 diabetes and metabolic syndrome (53). In humans, the constellation of lipotoxic abnormalities that leads to insulin resistance, type 2 diabetes mellitus, and fatty liver is referred to as the metabolic syndrome, suggesting that this syndrome includes lipotoxicity of pancreatic β-cells, liver, myocardium, and skeletal muscle in a manner similar to lipotoxicity observed in rodents (27, 54, 55).
Leptin constitutes a liporegulatory hormone that controls lipid homeostasis in nonadipose tissues particularly during periods of overfeeding (27). It has been suggested that leptin regulates intracellular fatty acid homeostasis and prevents tissues from lipotoxicity, similar to insulin, which regulates intracellular glucose homeostasis and prevents glucotoxicity (56). Leptin activates AMP-activated protein kinase (AMPK), arresting lipogenesis and promoting fatty acid oxidation by inactivating acetyl coenzyme A (CoA) carboxylase (ACC) (99). In nonobese diabetic mice with uncontrolled diabetes type 1, leptin therapy normalizes the levels of a wide array of hepatic intermediary metabolites comprising acylcarnitines, tricarboxyl acid cycle intermediates, amino acids, and acyl CoA (58). Leptin reduces both lipogenic and cholesterologenic transcription factors and enzymes and lowers plasma and tissues lipids (58).

E. Leptin’s role in glucose homeostasis

Leptin may also directly regulate glucose homeostasis independently of its effects on adiposity; leptin regulates glycemia at least in part via the CNS, but it may also independently of its effects on adiposity; leptin regulates E. Leptin’s role in glucose homeostasis lipids (58).

Diabetes type 1 is characterized by loss of the endocrine and paracrine functions of insulin. α-Cells lack constant regulation of high insulin levels from juxtaposed β-cells and hypersecrete glucagon, which causes directly diabetic symptomatology (70). It has been proposed that insulin monotherapy corrects the endocrine insulin deficiency but may not fully correct the paracrine insulin deficiency that is responsible for the glycemic volatility and the catabolic syndrome of diabetes type 1 (66). If glucagon hypersecretion is the major cause of metabolic aberrations in human diabetes, glucagon inactivation or suppression could provide an attractive therapeutic vista for managing diabetic symptomatology compared with insulin monotherapy.

F. Leptin’s role in immune modulation

Leptin presents a pivotal role in the modulation of immune function. In normal subjects, leptin’s role is associated with a balance between the number of T helper (Th)1 cells and Th2/Treg (regulatory T cells), which are able to suppress immune and autoimmune responses (71). On one hand, leptin contributes to protection from infectious diseases whereas on the other hand to loss of tolerance and autoimmunity. Studies in mice have shown that effects of leptin on the immune system are implemented via central or peripheral pathways (72, 73). Leptin plays a role in the cells belonging to both the innate and the adaptive immune responses. In fact, as a cytokine, leptin affects thymic homeostasis and promotes Th1-cell immune responses by increasing interferon-γ, tumor necrosis factor (TNF)-α, and IgG2a production from B cells (74). Leptin is involved in all processes of NK cell development, differentiation, proliferation, activation and cytotoxicity (75). Leptin constitutes a negative signal for the expansion of naturally occurring human Foxp3+CD4+CD25+ regulatory T cells (Treg), a cellular subset suppressing autoreactive responses mediated by CD4+CD25— T cells (76). Hypo- and aleptinemia results in impaired Th1 response and induction of Treg cells; reducing thus the immunocompetence and autoimmunity in mice and humans and increasing susceptibility to infections such as tuberculosis (TB), pneumonia, candidiasis, and bacterial and viral diarrhea (77–79). Conversely, hyperleptinemia and leptin resistance are associated with a low proportion and proliferation of Treg cells, an expansion of Th1 cells and increased secretion of proinflammatory cytokines that sustain and enhance the development of immunoinflammatory responses and autoimmune diseases in subjects with predisposition to autoimmunity (72).
III. Leptin Signaling

A. Leptin and JAK2/STAT3 signaling

It has been shown that ObRb activates a cascade of several signal transduction pathways, including Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) (58) (Table 2 and Figure 1). The STAT family comprises Src homology-2 (SH2) domain-containing transcription factors situated in the cytoplasm in quiescent cells. STAT activation results in homo- or heterodimerization, nuclear translocation, and transcriptional activation. STAT3 is a transcription factor that is encoded by the STAT3 gene in humans (80). It mediates the expression of a variety of genes in response to cell stimuli and thus plays a key role in many cellular processes such as cell growth and apoptosis (80, 81). Constitutive STAT3 activation is associated with various human cancers and indicates poor prognosis, suggesting that STAT3 has antiapoptotic and proliferative effects (82–84). In contrast, loss-of-function mutations in the STAT3 gene result in hyper-IgE syndrome, associated with recurrent infections as well as disordered bone and tooth development (85, 86).

The JAK2/STAT3 pathway is the first identified signaling mechanism associated with the leptin receptor and plays a pivotal role in energy homeostasis and neuroendocrine function (80, 81, 84) (Table 2 and Figure 1). It has been well established that leptin stimulates ObRb dimerization, which results in JAK2 activation, autophosphorylation, and phosphorylation of Tyr985, Tyr1077, and Tyr1138, which act as docking sites for key downstream signaling molecules (59). Also, in response to leptin, STAT3 binds to phospho-Tyr1138, allowing JAK2 to phosphorylate and stimulate STAT3. Leptin binds to its ObRb receptor and activates the JAK2/STAT3 pathway in the ARC of the hypothalamus, inducing the transcription of the anorexigenic POMC and suppressing the orexigenic AgRP/NPY (65). Moreover, leptin administration induces phosphorylation of JAK2/STAT3 in several cell lines including keratinocytes, endometrial cancer cells, murine adipocytes, and mouse adipose tissue (87). We have recently demonstrated for the first time that leptin activates STAT3 signaling in mouse GT1-7 hypothalamic, C2C12 muscle, and AML12 liver cell lines (19).

Table 2. Main Signaling Pathways, Main Sites of Action, and Main Effects of Activation by Leptina

<table>
<thead>
<tr>
<th>Signaling Pathway</th>
<th>Primary Site of Action</th>
<th>Effects</th>
</tr>
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<tbody>
<tr>
<td>JAK2/STAT3</td>
<td>Hypothalamus, adipocytes, PBMCs, cancer cells, muscle cells, liver cells, etc</td>
<td>Regulates appetite, energy balance, and body weight. Regulates neuroendocrine function. Regulates cell proliferation. Regulates cell growth and apoptosis. Regulates bone and tooth development.</td>
</tr>
<tr>
<td>STAT5</td>
<td>Hypothalamus, adipocytes, PBMCs, cancer cells, muscle cells, liver cells, etc</td>
<td>Regulates energy balance and body weight. Regulates onco genesis.</td>
</tr>
<tr>
<td>MAPK</td>
<td>Hypothalamus, adipocytes, PBMCs, cancer cells, muscle cells, liver cells, etc</td>
<td>Regulates appetite and body weight. Regulates fatty acid oxidation. Regulates cell hypertrophy. Regulates cell proliferation and differentiation.</td>
</tr>
<tr>
<td>AMPK</td>
<td>Hypothalamus, adipocytes, PBMCs, cancer cells, muscle cells, liver cells, etc</td>
<td>Regulates appetite, energy balance, and body weight. Regulates gluconeogenesis and energy homeostasis. Regulates fatty acid oxidation. Regulates insulin resistance. Regulates cell proliferation and survival. Regulates onco genesis.</td>
</tr>
<tr>
<td>FoxO1</td>
<td>Hypothalamus, adipocytes, PBMCs, cancer cells, muscle cells, liver cells, etc</td>
<td>Regulates appetite and body weight. Regulates energy expenditure. Regulates cell proliferation and differentiation. Regulates insulin signaling.</td>
</tr>
<tr>
<td>SHP2/MAPK</td>
<td>Hypothalamus, adipocytes, PBMCs, cancer cells, muscle cells, liver cells, etc</td>
<td>Regulates appetite and body weight. Regulates STAT3 signaling with SOCS3. Regulates mitosis, proliferation, and differentiation. Regulates cell apoptosis and survival. Regulates neuroendocrine function.</td>
</tr>
</tbody>
</table>

a Adapted from Refs. 3 and 19–21.
demonstrated that leptin activates STAT3 signaling in human adipose tissue (hAT) in vivo and ex vivo and in human primary adipocytes in vitro (20, 21). Conversely, disrupting the ability of the leptin receptor to activate the STAT3 pathway in mice leads to severe obesity and several neuroendocrine abnormalities including decreased linear growth and infertility (88).

Leptin may also activate phosphorylation of ObRb on Tyr1077, which binds to STAT5 and stimulates STAT5 phosphorylation (32) (Figure 1). STAT5 deletion in the brain causes hyperphagia and obesity to a lesser extent than STAT3 deletion, suggesting that the ObRb/JAK2/STAT5 pathway may also play a role in the regulation of body weight and energy balance by leptin (70) (Table 2). It is important to study in depth the effects of leptin on human hypothalamic or other CNS cells; leptin increased STAT3/STAT5 signaling in several tissues and cells may have important clinical implications including oncogenesis.

B. Leptin and AMPK signaling

AMPK is an evolutionarily conserved serine/threonine protein kinase to the regulation of cell growth, proliferation, differentiation, motility, and survival (71) (Table 2 and Figure 1). AMPK is considered a cellular energy sensor that is stimulated by an increase in the intracellular AMP to ATP ratio (71). In its classical role as an intracellular metabolic stress-sensing kinase, AMPK switches on fatty acid oxidation and glucose uptake while switching off hepatic gluconeogenesis. It also exerts a broader role in metabolism by controlling appetite (89, 90). AMPK is also involved in cellular energy homeostasis through AMPK phosphorylation and inhibition of ACC, thus stimulating fatty acid oxidation (91–93).

Leptin’s actions in peripheral tissues are partly due to its effects on AMPK, which contribute to leptin’s effects on fatty acid oxidation and glucose uptake (94). In mouse skeletal muscle, leptin activates AMPK, which leads to phosphorylation and inhibition of ACC and subsequent stimulation of fatty acid oxidation. There appear to be 2 mechanisms through which leptin activates AMPK in muscle (95, 96). One pathway involves the hypothalamus including the α-adrenergic pathway, resulting in a prolonged effect, and as shown in our previous ex vivo and in vitro experiments (84), the second mechanism encompasses leptin’s direct effect on skeletal muscle. The ability of leptin to stimulate fatty acid oxidation in skeletal muscle, which plays a pivotal role in the pathophysiology of insulin resistance (97, 98), may prevent lipotoxicity and subsequent insulin resistance and may underlie leptin’s ability to improve insulin resistance and metabolic syndrome in hypoleptinemic humans (99, 100), thus supporting the potential use of leptin in treating lipodystrophy and, possibly, obesity and type 2 diabetes.

In contrast to its actions in muscle, leptin has been shown to exert an inhibitory effect on AMPK in the hypothalamus that results in stimulation of the hypothalamic ACC and subsequent reduction of food intake and weight gain (86). Leptin’s hypothalamic effects on AMPK are in part mediated by the MC4R pathway, which promotes anorexia (86). Moreover, the Ca²⁺/calmodulin-dependent protein kinase (CaMKK2) is an upstream activator of AMPK in the hypothalamus, whereas CAMKK2 inhibition reduces appetite and body weight (88). These observations indicate that the hypothalamic CaMKK2/AMPK/ACC pathway may also mediate leptin’s anorexigenic action. Further in vivo studies in humans are needed to elucidate whether leptin administration may alter in vivo signaling by altering a third intermediate factor that possibly could not be studied in vitro.

C. Leptin and PI3K/Akt/mTOR signaling

Phosphatidylinositol-3 kinase (PI3K) has been linked to an extraordinarily diverse group of cellular functions, including cell growth, proliferation, differentiation, motility, survival, and intracellular trafficking (101–103) (Table 2 and Figure 1). These functions are related to the ability of class I PI3Ks, which are responsible for the production of phosphatidylinositol 3-phosphate, phosphatidylinositol (3,4)-bisphosphate, and phosphatidylinositol (3,4,5)-trisphosphate, to modulate protein kinase B, suggesting that PI3K/Akt/mammalian target of rapamycin (mTOR) signaling pathway play an important role in diabetes mellitus (103, 104). Leptin activates ribosomal S6 kinase, an important physiological substrate of mTOR kinase in the hypothalamus (92). The PI3K/Akt/mTOR signaling pathway is required for an extremely diverse array of cellular activities including proliferation and survival (105–107).

Leptin increases cell proliferation in mouse GT1-7 hypothalamic, C2C12 muscle, and AML12 liver cell lines through mTOR and Akt signaling pathways (19). Moreover, in vitro and in vivo studies have suggested that leptin stimulates cell proliferation through PI3K signaling in myocytes (19, 108) and hepatocytes (109). Leptin may also stimulate this pathway in human cancer cells (110, 111) and in human adipose (21), liver (112), and muscle tissues (19, 113). Similar to the JAK2/STAT3 pathway, the PI3K pathway is also important in central leptin action. This pathway activates POMC-expressing neurons by activating ATP-sensitive potassium channels and voltage-gated calcium channels (114). Also, similar to insulin, the appetite-suppressing effects of leptin are attenuated by in-
tracerebroventricular (icv) administration of PI3K inhibitors (116, 286). Interestingly, the insulin-PI3K pathway hyperpolarizes POMC neurons, which makes them less sensitive to leptin, and may be one common mechanism for both leptin and insulin resistance in obesity (117, 118). Chronic activation of the PI3K pathway in POMC neurons may cause leptin resistance and hyperphagia in mice with POMC neuron-specific deletion of phosphatase and tensin homolog (PTEN) (105). Hence, activation of the PI3K signaling pathway in neuronal cells may influence energy homeostasis and neuroendocrine function, whereas activation of this pathway in the peripheral tissues may mediate leptin’s effect on insulin resistance.

D. Leptin and FoxO1 signaling

Forkhead box protein O1 (FoxO1) belongs to the forkhead family of transcription factors that are characterized by a distinct forkhead domain (119) (Table 2 and Figure 1). The specific function of this transcription factor has not yet been determined; however, it may contribute to myogenic growth and differentiation (119). FoxO1 is a downstream effector of insulin signaling with an important role in the regulation of metabolism in various organs including liver (120), pancreas (121), muscle (122), adipose tissue (122), and hypothalamus (123). Also, FoxO1, as a transcription factor, is one of the substrates for Akt phosphorylation resulting in cytoplasmic shuttling from the nucleus, thereby inactivating FoxO1 (124, 125). FoxO1, which stimulates the expression of AgRP and NPY and inhibits POMC expression, is an important downstream mediator of the PI3K pathway (286). It has been demonstrated that the activation of hypothalamic FoxO1 is inhibited by leptin via the PI3K pathway (123). In contrast, overexpression of constitutively active FoxO1 in the mediobasal hypothalamus of rats by adenoviral microinjection leads to a loss of the inhibitory effect of leptin on feeding and results in body weight gain (126). Moreover, hypothalamic-specific FoxO1 knock-in mice have increased food intake and decreased energy expenditure (127).

E. Leptin and SHP2/MAPK signaling

MAPKs are serine/threonine-specific protein kinases that respond to extracellular stimuli (mitogens, osmotic stress, heat-shock, and proinflammatory cytokines) and regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, and cell survival/apoptosis (19, 21, 128, 129). MAPKs encompass a number of signaling molecules that include ERK1/2, p38 and c-Jun N-terminal kinase (JNK) (21). ERK1/2 represents the major MAPK involved in leptin’s central effects contributing to the regulation of food intake, energy expenditure, and body weight (3).

Leptin stimulates ERK1/2 activation via SH2-containing protein tyrosine phosphatase 2 (SHP2) (Table 2 and Figure 1). SHP2 is a protein tyrosine phosphatase (PTP) that contains 2 SH2 domains located in its NH₂ terminus as well as a COOH-terminal PTP domain (130). This specific structure suggests that SHP2 is involved in regulating signals initiated by receptor tyrosine kinases (130). These phosphotyrosines act as docking sites for recruitment of SH2-containing proteins, including SHP2, to activate downstream signaling cascades (131). SHP2 promotes ERK1/2 activation in response to insulin and epidermal growth factor binding to their receptors (132, 133).

Leptin induces phosphorylation of the Tyr⁹⁸⁵ amino acid residue of the ObRb after JAK2 activation, thereby creating a binding site for the carboxyl-terminal SH2 domain of SHP2 (134). SHP2 itself becomes phosphorylated, recruits the adaptor protein growth factor receptor-bound protein-2 and induces JAK2-dependent activation of ERK1/2 (135). Leptin also activates the MAPK pathway independently of SHP2, probably via receptor activation leading to direct binding of growth factor receptor-bound protein-2 to JAK2 (136). Recently, it has been shown that young mice homozygous for a mutation at the site of the leptin receptor phosphorylation were slightly leaner than wild-type mice, although they still developed adult-onset or DIO (137). These phenotypes probably do not reflect the effect of this mutation on leptin-induced ERK activation but are consistent with the role of the mutation site as a binding site for the suppressor of cytokine signaling 3 (SOCS3), which is a negative regulator of leptin receptor signaling (137). In vitro studies have also demonstrated that ERK is required for the phosphorylation of S6 by the S6 kinase, which enhances cap-dependent translation and protein synthesis (138).

Other members of the MAPK family, p38 and JNK, are also activated by leptin in several cell types and present mainly peripheral actions (139–141), although the associated pathways have not been well characterized. In rat cardiomyocytes, leptin administration stimulates p38, which results in an increase in fatty acid oxidation, whereas inhibition of p38 activation prevents leptin-induced fatty acid oxidation (134). Similar to p38, JNK is not activated in response to leptin treatment in neuronal cells (135, 136) but confers an oncogenic potential to leptin’s action after its activation by promoting cancer cell survival and invasion (137).

F. Leptin and JAK2-independent signaling

You et al (137) observed that leptin may stimulate the MAPK and STAT3 pathways in cultured cells that are
genetically JAK2 deficient; however, the JAK2-independent signaling and its pathophysiological importance have not been verified in animals or humans. The src tyrosine kinase family members appear to be responsible for mediating leptin JAK2-independent signaling pathway (138). The JAK2-dependent and independent signaling pathways may synergistically mediate responses to leptin (286). Because in vivo leptin actions may differ from in vitro actions, studies of in vivo leptin signaling in humans are needed to study the JAK2-independent leptin signaling and implications.

IV. Leptin and Insulin Signaling

Insulin is a central hormone regulating carbohydrate and fat metabolism in the body (142). Insulin causes cells in the liver, muscle, and fat tissues to take up glucose from the blood, storing it as glycogen in the liver and muscle (142–144). Insulin binds to the extracellular portion of the α-subunits of the insulin receptor (143, 144). This, in turn, causes a conformational change in the insulin receptor that activates the kinase domain residing on the intracellular portion of the β-subunits (143, 144). The activated kinase domain autophosphorylates tyrosine residues on the C terminus of the receptor as well as tyrosine residues in the insulin-receptor substrate (IRS)-1 protein (144). The actions of insulin include 1) increasing or decreasing cellular intake of certain substances, most prominently increasing glucose uptake in muscle and adipose tissue (comprising about two-thirds of total body cells) (142, 144), 2) increasing DNA replication and protein synthesis via control of amino acid uptake (145), and 3) modification of the activity of numerous enzymes (146).

Evidence from in vivo and in vitro studies supports the hypothesis that leptin and insulin signaling networks may overlap on several levels. Intravenous infusion of leptin in mice has several effects on insulin-regulated processes, including increased glucose turnover, increased glucose uptake in skeletal muscle and brown adipose tissue, and decreased hepatic glycogen content (147–149). In vivo leptin administration has also been reported to stimulate insulin’s inhibitory effects on hepatic glucose output, while antagonizing insulin’s effect on glucokinase and phosphoenolpyruvate carboxykinase (PEPCK) expression, 2 key metabolic enzymes (150–152). In HepG2 human hepatoma cells, leptin has been shown to antagonize insulin-induced down-regulation of PEPCK expression, decrease insulin-stimulated tyrosine phosphorylation of IRS-1, and enhance IRS-1-associated PI3K activity (153). Also, in C2C12 muscle cells, leptin stimulates a non-IRS-1–associated PI3K and mimics insulin action on glucose transport and glycogen synthesis. In contrast, in OB-RβL-transfected HepG2 cells, leptin treatment resulted in the recruitment of p85 to IRS-2 but did not modulate the response to insulin (154). Interestingly, in Fao cells, leptin alone had no effects on the insulin signaling pathway, but leptin pretreatment transiently enhanced insulin-induced tyrosine phosphorylation and PI3K binding to IRS-1, while inhibiting these effects on IRS-2 (155). Also, this study demonstrated that treatment by leptin alone induces serine phosphorylation of Akt and glycogen synthase kinase 3 but to a lesser extent than treatment by insulin alone, and that the combination of these hormones did not result in an additive effect (155). These observations suggest complex interactions between the leptin and insulin signaling pathways that can potentially lead to differential modification of the metabolic and mitotic effects of insulin exerted through IRS-1 and IRS-2 and the downstream kinases that are activated. Together, these data point toward cell- and/or tissue-specific interactions between leptin and insulin signaling that are quite diverse and complex. Although the details of this interaction remain to be elucidated, we propose that leptin may contribute to some of the alterations in the metabolic and mitotic effects of insulin action that are involved in the development of insulin resistance, a characteristic manifestation of type 2 diabetes.

An example of the overlap between leptin and insulin resistance has been reported elsewhere (156). The JAK2/STAT3 pathway leads to increased transcription and expression of SOCS3. In fact, SOCS3 acts as a feedback inhibitor by attenuating ObR signaling, thereby playing a key role in leptin resistance, whereas it also attenuates insulin signaling, thereby playing a key role in insulin resistance (157). Apart from leptin, SOCS3 may be induced by other adipocytokines, including resistin (158), TNF-α (159), and IL-6 (160), which may act as amplifiers of SOCS3 expression, thereby further increasing both leptin resistance and insulin resistance.

In summary, it has been shown that a complex network of interacting signaling pathways appears to regulate food intake, energy balance, and body weight (161), suggesting that, in addition to signaling in the CNS, leptin exerts its metabolic effects by acting directly in peripheral tissues. The relative importance of this signaling for regulating adiposity and glucose homeostasis in the periphery remains to be explored. Furthermore, other important challenges include 1) the identification of different signaling pathways downstream of leptin that are integrated in the regulation of body weight, 2) the leptin-insulin cross talk in the CNS, 3) the elucidation of the molecular pathways responsible for leptin resistance/tolerance in obesity, and 4) the elucidation of the underlying mechanisms respon-
sible for the overlapping leptin and insulin resistance occurring in the brain in pathological states such as obesity and diabetes that are associated with insulin resistance in the periphery.

V. Leptin Resistance or Tolerance

The poor efficacy of endogenous leptin to induce weight reduction in obese individuals has given rise to the term of functional leptin resistance, which is based on the concept of insulin resistance in diabetes type 2, where reduced cellular and metabolic insulin responsiveness coexist with insulin hypersecretion. The lack of a precise definition of the term leptin resistance in a universal and quantifiable manner led the National Institutes of Health to conduct a workshop, Toward a Clinical Definition of Leptin Resistance (162). Leptin resistance or, as in our opinion is more correctly stated as tolerance, could be 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, although leptin’s inability to induce desired responses in specific situations results from multiple molecular, neural, environmental, and behavioral mechanisms (162).

The main physiological cause for leptin hypersecretion is diet-induced expansion of adipocytes. However, in DIO, the physiological function of progressive hyperleptinemia remains to be fully defined. It has been shown, however, that lipotoxicity in nonadipose tissues of congenitally aleptinemic obese rodents is far greater than in hyperleptinemic DIO rodents, leading to ectopic lipid deposition in liver, pancreatic islets, and heart and skeletal muscle and causing severe organ dysfunction and cell death with a disease cluster similar to metabolic syndrome (52, 163, 164). Increasing leptin levels in this condition appear to prevent lipotoxicity by minimizing ectopic lipid accumulation into nonadipocytes via leptin-induced fatty acid oxidation. In obesity, an expanding adipose compartment may initially improve whole-body insulin sensitivity, at least temporarily (165, 166). However, later in life, as energy storage capacity in adipocytes is exceeded during the period between the onset of overweight/obesity and the start of the metabolic syndrome, which is considered to be the result of failure to prevent organ damage inflicted by ectopic fatty acids and metabolic trauma (163, 167), leptin resistance develops.

The onset of leptin resistance varies among ObRb-expressing neurons. Diet-induced leptin resistance appears to manifest first in the ARC of the hypothalamus and later in other hypothalamic areas such as the VMH and dorsomedial hypothalamus (153). Interestingly, the impairment in the ObRb/Pi3K signaling pathway precedes that of the ObRb/JAK2/STAT3 pathway (154).

First, leptin resistance was thought to be due to leptin receptor mutations or mutations of other genes downstream of leptin, such as POMC and MC4R, which also produce an obese phenotype with associated neuroendocrine dysfunction (3). The instance of genetic mutation abrogating leptin receptor function is similar to classical hormone resistance/insensitivity syndromes (type A insulin resistance, GH insensitivity, etc.), in which genetic alterations of the hormone receptor prevent hormone action. Nonetheless, only a few cases of obesity in humans are due to monogenic syndromes; instead, most cases are multifactorial (155). Hence, the underlying mechanisms

Table 3. Potential Mechanisms of Underlying Leptin Resistance or Tolerance

<table>
<thead>
<tr>
<th>Leptin Resistance or Tolerance</th>
<th>Gene mutations</th>
<th>Impaired leptin signaling</th>
<th>Impaired leptin neural circuitry</th>
<th>Impaired BBB transport</th>
<th>Impaired ObRb trafficking</th>
<th>ER stress</th>
<th>Saturable nature of leptin pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin receptor</td>
<td>Molecules downstream of leptin receptor (POMC, MC4R)</td>
<td>SH2B1 ↓</td>
<td>SOCS3, PTP1B, SHP2 ↑</td>
<td>Altered synaptic plasticity</td>
<td>Axon guidance</td>
<td>ObRe–ObRa Antagonism</td>
<td>Triglycerides ↑</td>
</tr>
</tbody>
</table>

Leptin resistance occurs rarely due to monogenic mutations of leptin receptor or other genes downstream of leptin (POMC and MC4R). Leptin tolerance is usually multifactorial due to 1) impaired activation (down-regulation of SH2B1) or inhibition (up-regulation of SOCS3 or PTP1B) of leptin signaling; 2) impaired leptin-induced neural plasticity/circuitry, given that defects in MC4R and BDNF receptor signaling in the hypothalamus may modulate synaptic plasticity and axon guidance; 3) impaired leptin transport across the BBB because soluble leptin receptor isoform (ObRe) may antagonize the action of ObRa (which is abundantly expressed in brain microvessels constituting the BBB), thereby resulting in leptin’s endocytosis, whereas hypertriglyceridemia may also impair leptin transport; 4) impaired ObRb trafficking, as has been reported in relation to Bardet-Biedl syndrome proteins (responsible for promoting ObRb trafficking to the plasma membrane) impairment; 5) ER stress, which promotes the UPR, thereby inhibiting STAT3 phosphorylation; and 6) saturable nature of leptin signaling pathways.
responsible for leptin resistance are apparently multiple and probably include the following (Table 3): 1) impaired leptin receptor signaling (11, 127, 156–161, 163–167), 2) defective leptin transport across the BBB (168–172), 3) impaired ObRb trafficking (173, 174), 4) endoplasmic reticulum (ER) stress (21, 175–177), 5) saturable nature of leptin signaling pathways (19, 20, 178, 179), and 6) impaired leptin-induced neural plasticity/circuity (180–183).

A. Impaired activation of leptin receptor signaling/
inhibitors of leptin signaling

Leptin signaling is controlled by both positive (SH2B adaptor protein 1, SH2B1) and negative (SOCS3 and PTP1B) regulators. SH2B1 is an SH2 and pleckstrin homology domain-containing adaptor implicated in cell signal transduction and represents an important positive regulator of leptin sensitivity (115). SH2B1 binds to Tyr813 and enhances JAK2 stimulation, promoting leptin signaling pathways downstream of JAK2 (111). Moreover, SH2B1 binds directly to IRS-1 and IRS-2, inhibiting tyrosine dephosphorylation of IRS-1/2 and increasing PI3K pathway activation (115).

A large contributor to leptin resistance is the defect in negative feedback mechanisms such as SOCS3 and PTP1B, which dampen leptin receptor signaling and prevent overactivation of leptin signaling pathways (168, 169) (Figure 1). The JAK/STAT pathway is negatively regulated by members of the SOCS family, and leptin signaling via Tyr1138 and STAT3 induces SOCS3 expression (169, 170), which in turn switches off leptin signaling by binding to tyrosine residue Tyr985 and inhibiting phosphorylation/activation of JAK2 (171). Therefore, SOCS3 represents a pivotal feedback mechanism preventing the overactivation of leptin-signaling pathways. Overexpression of SOCS3 has been proposed as one of the main mechanisms for the onset of leptin resistance (169). Indeed, hypothalamic SOCS3 expression is significantly increased in several leptin-resistant animal models (169). Immunohistochemical studies suggest that the ARC is selectively leptin resistant in DIO mice, both male and female Tyr985 mice showed increased leptin sensitivity in female mice (173). However, in contrast to female l/l mice, both male and female Tyr985 mice showed increased susceptibility to high-fat diet (HFD)-induced obesity, supporting a positive action of signaling via Tyr985 in attenuating diet-induced impairment of energy metabolism. Also, because PTP1B is increased in leptin-resistant animals, a role for PTP1B in the onset of leptin resistance has been suggested (174). PTP1B is a ubiquitously expressed tyrosine phosphatase implicated in the negative regulation of leptin and insulin receptor signaling (156, 157, 163) (Figure 1). It has been shown that PTP1B deficiency in LeprB-expressing neurons results in reduced body weight and adiposity compared with wild-type controls and likely underlies the improved metabolic phenotype of global and brain-specific PTP1B-deficient models, suggesting that PTP1B may, independent of leptin signaling, contribute to the regulation of energy balance in mice (184). PTP1B represents another signaling molecule that may inhibit leptin signaling via JAK2 dephosphorylation (164). PTP1B-knockout mice are lean and have increased leptin sensitivity (164). Overexpression of the endogenous leptin enhancer SH2B1 has been shown to counteract PTP1B-mediated inhibition of leptin signaling in cultured cells (175). PTP1B expression is increased by HFD feeding and inflammation (176), but it is still unclear how PTP1B expression and activity are regulated after leptin stimulation or in case of DIO.

SHP2 has also recently been shown to play a critical role in leptin signaling by functioning in the hypothalamic control of energy balance and metabolism (177) (Table 2 and Figure 1). SHP2 appears to down-regulate the ObRb-JAK2/STAT3 pathway while promoting ERK1/2 activation by leptin (177, 178). It has been shown that a prominent phenotype of the mutant (CaMKIIα-Cre:Shp2flox/flox or CaSKO) mice was the development of early-onset obesity, with increased serum levels of leptin, insulin, glucose, and triglycerides (178). This study demonstrated that SHP2 down-regulates JAK2/STAT3 activation by leptin in the hypothalamus but that JAK2/STAT3 down-regulation is offset by the dominant SHP2 promotion of the leptin-stimulated ERK1/2 pathway, leading to induction rather than suppression of leptin resistance upon SHP2 deletion in the brain. These results suggest that a primary function of SHP2 in postmitotic forebrain neurons is to control energy balance and metabolism, representing a critical signaling component of leptin receptor ObRb in the hypothalamus (178). Hence, pharmaceutical augmentation of SHP2 activity in the brain might prove to be an efficient therapeutic strategy for alleviation of leptin resistance in obese patients.

B. Impaired leptin transport across the BBB

Leptin enters the brain by a saturable transport system (179). To reach the ObRb-expressing neurons in the brain, leptin has to cross the BBB (180). Leptin levels in the cerebrospinal fluid are decreased in cases of obesity, suggesting that defective leptin transport may contribute to leptin resistance (180). The short isoform of the leptin receptor, ObRa, abundantly expressed in brain microvessels constituting the BBB, plays a key role in leptin transport across the BBB (181). The soluble leptin receptor
isoform ObRe has been shown to antagonize ObRa action and leptin transport through inhibition of leptin surface binding and endocytosis (171). However, a distinct subpopulation of ARC neurons, which can be labeled by BBB-impermeable fluorescent tracers, has been shown to respond more rapidly and sensitively to circulating leptin compared with other hypothalamic ObRb neurons. These neurons might therefore make direct contact with the blood circulation by projections through the BBB (179, 180). In addition to hyperleptinemia, hypertriglyceridemia may also impair leptin transport (171). The exact clinical significance of these findings and/or any differential effects of ivc leptin administration in humans remain to be elucidated.

Further studies are needed to clarify the contribution of impaired leptin transport to the pathogenesis of leptin resistance. Because leptin does not appear to be able to cross the BBB easily, it may be possible to develop leptin agonists that cross the BBB more freely or, possibly, antagonists acting only peripherally. Such agents could possibly have beneficial effects on immune responses in autoimmune disease and/or on cardiovascular disease, without causing excess weight gain as would be expected from blocking central leptin action (182).

C. Impaired ObRb trafficking

Few ObRbs are located on the plasma membrane mediating leptin effects; most ObRbs are present in the trans-Golgi network and in small vesicles (173). It has been shown that specific proteins, named Bardet-Biedl syndrome proteins, are responsible in promoting ObRb trafficking to the plasma membrane (173). In mouse models, deletion of Bardet-Biedl syndrome proteins results in leptin resistance and obesity (174). Nonetheless, the importance of these proteins in humans with obesity has not been determined yet.

D. ER stress

ER homeostasis is regulated by balancing ER loading of nascent proteins with ER ability to fold these proteins. An imbalance may result in an overload of unfolded/misfolded proteins in the ER lumen, inducing ER stress (175). ER stress promotes the unfolded protein response (UPR) involving the activation of several UPR intracellular signaling pathways (176).

ER stress has recently been shown to play a role in the development of leptin resistance. Obese, diabetic humans and animals present higher levels of ER stress in the liver, adipose tissue, and pancreatic ß-cells (175), suggesting that ER capacity is directly related to leptin sensitivity (183, 184). Hence, ER stress reversal via treatment with chemical chaperones that decrease ER stress (176) could be a strategy to sensitize obese mice, and by extension humans, to leptin. Studies have shown that reducing ER function in mice creates ER stress, leading to blocked leptin action and hence leptin resistance, with a significant augmentation of obesity on a HFD (183). This suggests that ER stress inhibits STAT3 phosphorylation at an upstream step and provides a potential mechanism by which increased ER stress antagonizes STAT3-mediated leptin signaling. We have demonstrated that pretreatment with the ER stress inducers, tunicamycin and dithiothreitol, completely blocks leptin-stimulated STAT3 activation in human primary adipocytes and human peripheral blood mononuclear cells (PBMCs) in vitro, suggesting that increased ER stress in obese people may induce leptin resistance (21). ER stress may also act via other pathways. For example, overnutrition atypically activates the inhibitor of nuclear factor κB (NF-κB) kinase subunit β (IKKβ)/NF-κB, at least in part through elevated ER stress in the hypothalamus (185). Although forced activation of hypothalamic IKKβ/NF-κB interrupts central leptin signaling and actions, suppression of IKKβ significantly protects against obesity and leptin resistance (185). Because in vivo leptin actions may differ from in vitro actions, studies of in vivo leptin signaling in humans are needed to explore this hypothesis. Also, the cross talk between leptin and UPR signaling pathways needs to be fully clarified.

E. Saturable nature of leptin signaling pathways

To study leptin signaling in humans, we have recently performed for the first time in vivo experiments involving metreleptin administration in humans in doses that result in an increase of circulating levels of free leptin up to a peak level of 40 to 50 ng/mL (20). In long-term leptin administration trials in humans, we found that circulating free leptin levels did not result in weight loss, which indicates that free leptin levels in or above the ~40- to 50-ng/mL range may be clinically ineffective (20). Also, we observed that in vivo metreleptin administration (0.01 mg/kg for 30 minutes) induces phospho-STAT3 in both hAT and hPBMCs, but this signaling is saturable at a level of ~50 ng/mL metreleptin (20). These levels are higher than the putative threshold for saturating the BBB leptin transport system, which may explain the absence of clinical effect of high leptin levels in obese humans despite the dramatic effects seen when replacing leptin in subjects with very low concentrations of leptin (178, 179).

Consistent with this result, we have also observed in hAT ex vivo and human primary adipocytes in vitro and in mouse hypothalamic, muscle, and liver cell lines in vitro that leptin signaling pathways were saturable at a level of ~50 ng/mL (19), suggesting that no additional signaling effect may be observed at higher doses. Overall, we suggest...
that the saturable nature of leptin signaling pathways may play a key role in the development of leptin tolerance in obese humans.

F. Impaired leptin neural circuitry

It has been shown that leptin-deficient (ob/ob) mice differ from wild-type mice in terms of numbers of excitatory and inhibitory synapses and in terms of postsynaptic currents onto NPY and POMC neurons (181). Hence, when leptin was delivered systemically to ob/ob mice, the synaptic density rapidly normalized, an effect detectable within 6 hours, several hours before leptin’s effect on food intake (181). This study suggested that leptin-mediated plasticity in the ob/ob hypothalamus may underlie some of the hormone’s behavioral effects. Also, it has been reported that leptin changes brain structure, neuron excitability, and synaptic plasticity and regulates feeding circuits, suggesting that leptin may play a role in the development of the CNS (182). Indeed, leptin has been proposed to be a crucial regulator of both synaptic plasticity and axon guidance within the hypothalamus, suggesting fresh links between nutrition and neurodevelopment mediated by leptin, with potentially important implications for the physiology of energy balance and body weight homeostasis (183). Leptin’s anorexigenic and metabolic effects are mediated by a sophisticated network of neurons in the hypothalamus as well as other areas in the brain. Leptin resistance, hyperphagia and obesity are characteristic manifestations after defects in MC4R and BDNF receptor signaling in the paraventricular hypothalamus and the VMH, respectively (180). Therefore, leptin sensitivity may be impaired by genetic or environmental factors that modulate leptin neural circuitry. More studies are required to study in depth the leptin neurocircuitry by analyzing its anatomic, biochemical, and electrical components.

VI. Effects of Leptin on Glucose Metabolism in Animals and Humans

Leptin replacement is a promising therapeutic approach in several disease states characterized by lepthenia or hyperleptinemia, including congenital complete leptin deficiency, and states of energy deprivation, comprising anorexia nervosa or milder forms of hypothalamic amenorrhea, as well as syndromes of insulin resistance observed in conditions such as congenital or acquired lipodystrophy. Conversely, states of energy excess such as garden-variety obesity and diabetes type 2, which represent the vast majority of the obese and diabetic population, are associated with hyperleptinemia and are characterized by the absence of clinical effects of leptin, reflecting leptin tolerance or leptin resistance. Table 4 displays leptin administration effects in different disease states based on initial leptinemia. If the leptin resistance or tolerance obstacle is overcome, leptin treatment would become an effective therapeutic approach against common obesity and diabetes type 2.

A. States of leptin deficiency

1. Congenital leptin deficiency

Mice homozygous for a nonsense mutation in the ob gene, the ob/ob mice, have been studied for a long time and represent a commonly used model of obesity. The first demonstration of an effect of leptin on glucose homeostasis was reported in 1995 when these mice were treated with ip injections of leptin (2, 5, 6). Administration of leptin effectively decreased serum glucose and insulin concentrations in a dose-dependent manner, even at the lowest dose where no significant body weight loss was observed. Similarly, a single icv injection of leptin improved glucose tolerance as assessed by an ip glucose tolerance test (GTT) (186). In contrast, db/db mice, which have a mutation in the leptin receptor gene resulting in expression of a truncated and dysfunctional receptor, show no reduction in body weight or improvement in metabolic abnormalities in response to treatment with leptin (187). These observations collectively suggest that leptin may play an important role in glucose metabolism and that leptin administration may correct the metabolic disturbance in leptin-deficient states.

Congenital leptin deficiency is a rare autosomal recessive disorder, caused by mutations in the gene encoding for leptin. In 1997, 2 severely obese children who were members of the same highly consanguineous pedigree of Pakistani origin were reported to exhibit nearly undetectable concentrations of serum leptin (188). They had a homozygous frame-shift mutation involving the deletion of a single nucleotide in codon 133 of the leptin gene. Clinically, they exhibited profound obesity, hyperphagia, hyperinsulinemia, and hyperlipidemia as well as reproductive and immune dysfunction (189–191). Leptin replacement in 1 of these congenital leptin-deficient children decreased appetite, food intake, body weight, and serum insulin and cholesterol concentrations (192), and similar effects have been noted with leptin replacement in other unrelated individuals with leptin deficiency (193, 194). Since then, several other Pakistani children with leptin deficiency have been identified (195). We have studied another group of 4 adult patients of Turkish origin who carry a homozygous missense mutation (78, 191, 196, 197). Hyperinsulinemia was a common feature in these patients, and 1 of
them had diabetes mellitus (196). Leptin replacement dramatically reduced body weight and food intake in all patients. Leptin effectively decreased fasting and postprandial glycemia and normalized glycated hemoglobin (HbA1C) levels within 2 months of treatment in the diabetic patient (191). The other patients, who had normal glucose and insulin levels at baseline, maintained normal fasting glucose levels, but their insulin and C-peptide levels decreased significantly to less than half of their baseline values (191). Among them, only 1 adult male patient was submitted to meal tolerance tests before and after 1 week, 18 months, and 24 months of leptin replacement (198). The authors used deconvolution analysis of C-peptide kinetics, modified insulin kinetics modeling, and minimal glucose modeling to determine pancreatic insulin secretion, hepatic insulin extraction, and peripheral insulin sensitivity. After 1 week, leptin replacement increased hepatic insulin extraction by 2.4-fold (resulting in a 1.8-fold decrease of posthepatic insulin delivery), without significant changes in insulin secretion, and also increased insulin sensitivity by 10%. After 24 months, leptin replacement increased insulin sensitivity by at least 10-fold, with additional decreases in insulin secretion and hepatic insulin extraction (198).

In 1998, Clément et al (199) reported a homozygous mutation in the human leptin receptor gene that results in a truncated leptin receptor lacking both the transmembrane and the intracellular domains. Patients homozygous for this mutation showed early-onset morbid obesity, whereas their serum leptin concentrations were much higher than normal. These patients presented normal fasting glycemia and normal glucose tolerance despite their extreme obesity (199). More recently, Farooqi et al (200) sequenced the leptin receptor gene from subjects with early, severe obesity to determine the prevalence of pathologic mutations. Of the 300 subjects, 8 (3%) had nonsense or missense leptin receptor gene mutations; 7 were homozygous and 1 was heterozygous. Most subjects with the mutation presented normal fasting glycemia and normal glucose tolerance despite their extreme obesity (199). More recently, Farooqi et al (200) sequenced the leptin receptor gene from subjects with early, severe obesity to determine the prevalence of pathologic mutations. Of the 300 subjects, 8 (3%) had nonsense or missense leptin receptor gene mutations; 7 were homozygous and 1 was heterozygous. Most subjects with the mutation presented normal fasting glycemia and normal glucose tolerance despite their extreme obesity (199). More recently, Farooqi et al (200) sequenced the leptin receptor gene from subjects with early, severe obesity to determine the prevalence of pathologic mutations. Of the 300 subjects, 8 (3%) had nonsense or missense leptin receptor gene mutations; 7 were homozygous and 1 was heterozygous. Most subjects with the mutation presented normal fasting glycemia and normal glucose tolerance despite their extreme obesity (199). More recently, Farooqi et al (200) sequenced the leptin receptor gene from subjects with early, severe obesity to determine the prevalence of pathologic mutations. Of the 300 subjects, 8 (3%) had nonsense or missense leptin receptor gene mutations; 7 were homozygous and 1 was heterozygous. Most subjects with the mutation presented normal fasting glycemia and normal glucose tolerance despite their extreme obesity (199). More recently, Farooqi et al (200) sequenced the leptin receptor gene from subjects with early, severe obesity to determine the prevalence of pathologic mutations. Of the 300 subjects, 8 (3%) had nonsense or missense leptin receptor gene mutations; 7 were homozygous and 1 was heterozygous. Most subjects with the mutation presented normal fasting glycemia and normal glucose tolerance despite their extreme obesity (199). More recently, Farooqi et al (200) sequenced the leptin receptor gene from subjects with early, severe obesity to determine the prevalence of pathologic mutations. Of the 300 subjects, 8 (3%) had nonsense or missense leptin receptor gene mutations; 7 were homozygous and 1 was heterozygous. Most subjects with the mutation presented normal fasting glycemia and normal glucose tolerance despite their extreme obesity (199). More recently, Farooqi et al (200) sequenced the leptin receptor gene from subjects with early, severe obesity to determine the prevalence of pathologic mutations. Of the 300 subjects, 8 (3%) had nonsense or missense leptin receptor gene mutations; 7 were homozygous and 1 was heterozygous. Most subjects with the mutation presented normal fasting glycemia and normal glucose tolerance despite their extreme obesity (199).

### Table 4. Effects of Leptin Administration in Different Disease State on the Basis of Circulating Leptin Levels at Baseline

<table>
<thead>
<tr>
<th>Disease States Associated With Different Initial Leptin Levels</th>
<th>Therapeutic Approach</th>
<th>Main Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease states associated with undetectable initial leptin levels (&lt;=0.05 ng/mL)</td>
<td>Leptin</td>
<td>Significantly reduced food intake and body weight and improved metabolic derangements</td>
</tr>
<tr>
<td>Congenital leptin deficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease states associated with very low initial leptinemia (&lt;=5 ng/mL)</td>
<td>Leptin</td>
<td>Significantly improved insulin sensitivity and dyslipidemia</td>
</tr>
<tr>
<td>Congenital lipodystrophy syndromes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HALS</td>
<td></td>
<td>Significantly decreased central fat mass and improved insulin sensitivity but not dyslipidemia</td>
</tr>
<tr>
<td>HA</td>
<td></td>
<td>Recovery of menstruation; correction in the gonadal, thyroid, GH, and adrenal axes; improvements in bone metabolism and skeletal health</td>
</tr>
<tr>
<td>Diabetes mellitus type 1</td>
<td>Leptin and reduced insulin</td>
<td>Improved insulin sensitivity, glycemic control, and dyslipidemia in 2 patients</td>
</tr>
<tr>
<td>Disease states associated with moderately low initial leptinemia (~5 ng/mL)</td>
<td>Leptin</td>
<td>Ameliorated lipidemic profile but not glucose abnormalities</td>
</tr>
<tr>
<td>Lipodystrophy-associated diabetes type 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease states associated with initial hyperleptinemia (&gt;15 ng/mL)</td>
<td>Leptin monotherapy</td>
<td>No effect on weight reduction and glycemic control</td>
</tr>
<tr>
<td>Garden-variety obesity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garden-variety obesity</td>
<td>Leptin and pramlintide</td>
<td>Moderately diminished body weight</td>
</tr>
<tr>
<td>Obesity and diabetes type 2</td>
<td>Leptin</td>
<td>Did not alter body weight, marginal or no amelioration in glycemic control</td>
</tr>
</tbody>
</table>

* Adapted from Refs. 3, 4, 10, and 11.
betes than obesity when compared with ob/ob mice (201). The discrepancy between humans and mice is not yet explained, although mouse studies have shown that genetic background is an important modifier of the effects of leptin deficiency on insulin resistance and diabetes (202).

Leptin is currently available for life-long treatment of subjects with congenital leptin deficiency through a manufacturer-sponsored expanded-access program (203, 204). Figure 2 depicts the effect of recombinant leptin administration in congenital leptin deficiency.

2. Lipodystrophy

Lipodystrophy is a group of clinically heterogeneous acquired or inherited disorders characterized by complete or partial absence of sc fat (lipoatrophy) that can occur in conjunction with the pathological accumulation of adipose tissue (lipohypertrophy) in other distinct regions of the body (10, 204). Although congenital lipodystrophies are exceedingly rare, acquired lipodystrophies (especially those associated with HIV infection and its treatment) are more common. Interestingly, lipodystrophic patients exhibit insulin resistance, hyperglycemia, dyslipidemia, and hepatic steatosis (205). The severity of these metabolic derangements typically correlates with the degree of adipose tissue loss.

Several mouse models of lipodystrophy exist, and the metabolic profiles of these models are similar to those of human lipodystrophy (206). Among them, the lipodystrophic adipocyte fatty acid binding protein 2 sterol reg-

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**Figure 2.**

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Figure 2. The effect of recombinant leptin administration in leptin deficiency or leptin excess states. Recombinant leptin may have beneficial effects when administered in leptin-deficiency states, but minimal benefit and/or mostly adverse effects when administered in leptin-excess states. In congenital leptin deficiency and congenital lipodystrophy, recombinant leptin administration results in metabolic and neuroendocrine improvement; improvement seems to be more limited in HIV-associated acquired lipodystrophy syndrome, in which coadministration of pioglitazone, which acts by up-regulating endogenous adiponectin, may increase the metabolic benefit. On the other hand, recombinant leptin administration in type 2 diabetes mellitus and/or common obesity and normal or high circulating leptin levels has minimal or null metabolic effect, whereas it increases circulating leptin-binding protein and antileptin antibodies. Coadministration of pramlintide, an amylin analog targeting leptin tolerance, or leptin administration after weight loss, when leptin’s declined levels increase appetite, might prove to be of some benefit. Similarly, leptin up-regulation in animal models with NAFLD resulted in increased inflammation and fibrosis, thereby progression to NASH. Data on adverse effects, including renal impairment or T-cell lymphoma, observed mainly in a minority of leptin-deficiency patients, are currently inconclusive. Data on carcinogenic effect of leptin, mainly observed in hyperleptinemic individuals, are also inconclusive but may be tissue-specific; however, hypoleptinemia itself may be also implicated in carcinogenesis in hypoleptinemic individuals, and thus this aspect of leptin physiology remains to be fully elucidated. *, Only experimental data.
ulotary element-binding protein 1c (aP2-SREBP-1c) transgenic mice exhibit many typical features of human generalized lipodystrophy (207). These animals have marked insulin resistance, hyperglycemia, and dyslipidemia and very low serum concentrations of leptin due to the lack of sc adipose tissue. When these mice were treated with leptin, their metabolic abnormalities and diabetic phenotypes were almost normalized (207). Leptin treatment was associated with reduced food intake and weight loss, but inducing weight loss by restricting access to food did not lead to changes in insulin or glucose concentrations in transgenic mice, suggesting that the beneficial effects of leptin are separate from its effects on body weight (207). The icv administration of leptin was as effective as peripheral administration (208).

Another mouse model of lipodystrophy is the lipoatrophic A-ZIP/F-1 mouse model (209), which has a more severe phenotype with almost complete lack of adipose tissue, insulin resistance and diabetes, and low leptin levels. In this mouse model, administration of a high dose of leptin (30 μg/d) led to only moderate improvements in glucose and insulin concentrations (209). In contrast, transgenic overexpression of leptin in these mice leads to a lipoatrophic mouse model that has elevated leptin concentrations (LepTg+/+/A-ZIPTg/+). These mice have significantly fewer metabolic abnormalities when compared with the low-leptin A-ZIP/F-1 mice (210). The somewhat discrepant findings may be related to the difference in the achieved leptin concentrations with exogenous administration versus transgenic overexpression of leptin. In contrast to the former study in which plasma leptin concentration increased to 5 ng/mL (209), plasma leptin concentrations in the latter study were markedly elevated to 50 ng/mL (210). Collectively, these animal experiments demonstrated that exogenous leptin administration may overcome the diabetic abnormalities characteristic of lipodystrophy, and thus, these studies prompted therapeutic trials of leptin replacement in patients with lipodystrophy.

In humans, congenital lipodystrophy syndromes are rare; there have been fewer than 1000 described cases in the literature (203, 211). Lipodystrophic subjects have partial or total leptin deficiency (222). Leptin replacement dramatically improves dyslipidemia and insulin sensitivity and reduced HbA1C levels and intrahepatic fat content in various types of human lipodystrophy, notably those associated with severe hypoalbuminemia (fasting serum leptin less than 4–5 ng/mL in our laboratory) (213–216, 218–220, 319). Fasting blood glucose and HbA1C levels decreased markedly even in patients who are not fully responsive to other antihyperglycemic medications or high doses of insulin. Javor et al (216) also reported that 15 patients with lipoatrophic diabetes were able to decrease or even discontinue diabetic therapy and maintain normoglycemia after 12 months of leptin replacement. Petersen et al (214) performed a hyperinsulinemic-euglycemic clamp in 3 patients with lipoatrophic diabetes and showed remarkable improvement in insulin sensitivity after leptin treatment. This beneficial effect was seen both with hepatic insulin sensitivity and with whole-body insulin sensitivity. In conjunction with this, there was an 86% reduction in intrahepatic triglyceride content and a 33% reduction in muscle triglyceride content (214). Chong et al (218) recently published an open-label, uncontrolled prospective study in 48 patients with various acquired and inherited forms of lipodystrophy. Leptin replacement effectively decreased serum triglyceride concentrations by 59% and HbA1C levels by 1.5 percentage points within 1 year in these patients. The benefits of leptin replacement were sustained for up to 8 years of follow-up (218). Oral and Chan (220) collected several small, non-randomized, open-label trials in a composite study reporting on a total of more than 100 patients with severe lipodystrophy. Based on these studies, they reported that leptin treatment resulted in improvement in several metabolic parameters, including glycemic control, insulin sensitivity, plasma triglycerides, caloric intake, liver volume and lipid content, and intramyocellular lipid content. These beneficial responses were durable after 3 years of therapy (221).

However, lipodystrophy comprises a heterogeneous group of disorders with diverse circulating levels of leptin (222). Indeed, most patients with lipodystrophy may exhibit moderate hypoalbuminemia (fasting serum leptin ~5 ng/mL). Recent data have shown that although leptin treatment is effective in ameliorating lipid profiles, notably hypertriglyceridemia, it does not restore metabolic homeostasis in lipodystrophic subjects with moderate hypoalbuminemia (223), hence questioning the antidiabetic potential of leptin in lipodystrophic patients. Therefore, lipodystrophic subjects with moderate hypoalbuminemia are expected to respond poorly to leptin’s effect on glycemic control but may benefit from its hypolipidemic effect. This limitation of leptin treatment in the context of lipodystrophy associated with moderately low levels of circulating leptin highlights the need of developing better therapeutic approaches including combination therapy to treat metabolic derangements in these patients.

Leptin administration in replacement doses constitutes an important step forward in the understanding and treatment of various lipodystrophic syndromes, conditions characterized by a hypoalbuminemic state. Particularly, recombinant methionyl leptin administration in patients with lipodystrophy exhibiting severe hypoalbuminemia ameliorated hyperinsulinemia, insulin resistance, hyper-
glycemia, hypertriglyceridemia, and neuroendocrine abnormalities without significant adverse effects and provided the rationale for metreleptin treatment in these patients. It is important to underscore that the antidiabetic effect of leptin observed in these patients was achieved even after discontinuation of previously administered antidiabetic treatment, therefore excluding the possibility of a synergistic positive effect of antidiabetic medication and leptin on glucose and lipid abnormalities (213). However, there are several caveats. Existing studies are all open-label and uncontrolled; hence, a placebo-controlled trial is warranted, although the small number of patients and the lack of firm diagnostic criteria present hurdles to this endeavor (203). Well-designed, probably crossover, randomized, placebo-controlled trials would be needed to accurately assess and quantify efficacy as well as to allow for a comparison with the current standard of care. Furthermore, the side-effect profile of leptin has not yet been fully characterized. Deterioration of renal function, development of lymphomas, and other side effects have been reported in lipodystrophic subjects (223–225), but because these studies were not placebo controlled, it remains unknown whether the observed side effects are leptin-specific or random and/or represent the natural history of the underlying disease. Thus, although the optimal treatment regimen for this condition has not yet been fully determined, metreleptin is currently available for these subjects as part of an expanded access program, while an application for approval for this indication is under review by the FDA. Figure 2 depicts the effect of recombinant leptin administration in lipodystrophy.

3. HIV-associated lipodystrophy syndrome

Currently, the most prevalent type of lipodystrophy is an acquired form of the syndrome that occurs in HIV-infected individuals treated with highly active antiretroviral therapy (HAART) (224). HIV-associated lipodystrophy syndrome (HALS) affects an estimated 15% to 36% of HIV-infected patients and is associated with an increased risk of insulin resistance, diabetes mellitus, dyslipidemia, and cardiovascular disease (225). HALS has been associated with dyslipidemia including elevated serum low-density lipoprotein (LDL) cholesterol and triglyceride (TG) levels and lower high-density lipoprotein (HDL) cholesterol (226). HALS patients usually exhibit sc fat loss and increased abdominal fat. The impact of lipodystrophy on the psychosocial health in HIV-positive individuals, ranging from somatic discomfort to low self-esteem, stigmatization, and depression can be significant (227). The exact physiological mechanisms that lead to HALS are still not fully defined, but it has been suggested that protease inhibitors interfere with peroxisome prolif-erator-activated receptor-γ (PPARγ) action leading to an impairment of normal metabolic processes in the adipocyte (228). Nucleoside analogs are also associated with HALS, which is thought to be secondary to mitochondrial injury. There may also be direct effects of the HIV itself (224, 228).

In patients with HIV who are on HAART, leptin levels are mostly related to their overall fat mass, as also seen in individuals without HIV infection (229–231). Adiponectin levels, which are also low in patients with HIV, are mainly related to abnormal fat distribution rather than overall fat mass (232). Approximately 20% to 30% of individuals who have HALS manifest hyperadiponectinemia and hypoleptinemia, but these manifestations are less dramatic than those seen in individuals with congenital lipodystrophies (231). Nevertheless, both lower adiponectin and leptin levels have been associated with worse insulin resistance (231). Hence, we and others have investigated the role of leptin replacement in these patients. Nonetheless, generally it will be fair to expect a less dramatic response to leptin treatment in individuals suffering from HALS given that these patients do not manifest an absolute leptin deficiency comparable to congenital lipodystrophy (203).

In our initial randomized, placebo-controlled, crossover 2-month interventional study, we found that leptin in physiological doses improved metabolic disturbances in these patients (233). Leptin treatment was associated with a 15% decrease in central fat mass as well as significant improvements in insulin sensitivity and fasting insulin and glucose levels, albeit having no effects on LDL and TG (233). These results were confirmed by a longer independent study of open-label leptin treatment for 6 months (234). Leptin treatment was associated with a 32% decrease in visceral fat as well as significant improvements in fasting insulin, glucose levels, and lipid parameters including a decrease in LDL and non-HDL cholesterol and an increase in HDL by the sixth month (234). This study showed that whole-body insulin sensitivity was markedly improved and found that the leptin-induced increase in insulin sensitivity was especially pronounced in the liver but not skeletal muscle (234). These observations suggest that the benefits of leptin replacement are sustained, and even increased, for as long as treatment continues in patients with HALS. Although there are no head to head comparisons, studies performed to date indicate that leptin’s ability to improve insulin sensitivity is comparable to or better than that of other available medications, including metformin and thiazolidinediones (235, 236). However, the more pronounced effect of leptin on lipid metabolism in the study by Mulligan et al (234) needs to be interpreted cautiously given that this study was nonran-
domized and not placebo-controlled and its study participants continued to use their lipid-lowering regimens during leptin treatment. Recent results from a small randomized, double-blinded, placebo-controlled clinical trial indicate that leptin administration improves glycemia and non-HDL cholesterol levels to some extent but cannot ameliorate lipid kinetics in adults with HIV-associated dyslipidemic lipodystrophy, providing a mechanistic reasoning behind the inconsistent effect of leptin on lipid profiles (237). Specifically, metreleptin administration to hypoleptinemic patients with HIV-associated dyslipidemic lipodystrophy at doses that significantly elevate plasma leptin levels does not ameliorate the markedly abnormal fasting lipid kinetic defects characteristic of this condition, ie, accelerated lipolysis and adipocyte and hepatic reesterification, with blunted oxidative disposal of free fatty acids (237). Conversely, recombinant human GH and human GHRH, which present beneficial effects on lipid metabolism, have been shown to improve visceral fat, TG, and cholesterol levels but not glucose parameters in patients with HALS, presenting also an unclear impact on cardiovascular endpoint (238, 239).

Similar to leptin, hypoadiponectinemia is associated with insulin resistance, hypertriglyceridemia, and adipose tissue redistribution in patients with HALS (240–242). Adiponectin administration has been demonstrated in animals to improve insulin sensitivity and dyslipidemia (243, 244). Although adiponectin or leptin alone only partly alleviate insulin resistance in mouse models of lipodystrophy, the combined administration of both hormones fully normalizes insulin sensitivity (244). Given that no synthetic form of adiponectin is yet available for human use, animal studies as well as studies on medications that indirectly increase endogenous levels of adiponectin such as thiazolidinediones, have highlighted adiponectin’s therapeutic potential (245). We have found that pioglitazone, a thiazolidinedione that increases adiponectin levels, may have beneficial effects on HALS-associated insulin resistance. Recently, we performed a randomized, double-blinded pilot study on the combined administration of leptin and pioglitazone for 3 months in 9 HALS patients (246). Compared with pioglitazone alone, combined treatment reduced fasting serum insulin concentrations, increased adiponectin concentrations, improved insulin resistance (homeostasis model assessment [HOMA]), and attenuated postprandial glycemia in response to a mixed meal (246). These preliminary findings indicate that this combination could further improve glycemic control and needs to be replicated in larger and longer-term studies. If confirmed and proven to be superior to the current standard of care, the treatment of HALS in the future may involve leptin replacement in conjunction with pioglitazone and/or adiponectin. Figure 2 depicts the effect of recombinant leptin administration in HALS.

4. Hypothalamic amenorrhea

Hypothalamic amenorrhea (HA) is a disorder characterized by the cessation of menstrual cycles with anovulatory infertility and moderate to severe hypoleptinemia, usually caused by reduced food intake or chronic energy deficiency secondary to strenuous exercise (204). Leptin administration to lean women who are affected by this disorder and undertake strenuous exercise resulted in recuperation of reproductive outcomes, correction in the gonadal, thyroid, GH, and adrenal axes, and improvements in markers of bone formation (247–249). Nonetheless, larger clinical trials are needed to confirm the previous studies.

B. States of leptin excess

1. Common obese state

In contrast to the few obese subjects with congenital leptin deficiency, most obese individuals exhibit greater leptin concentrations than lean subjects due to their greater amount of fat mass and are unresponsive to exogenous leptin in terms of anorectic effects, body weight reduction, and glycemic control, due to leptin resistance as discussed in Introduction (22, 23) (Table 5). These results reduced expectations from leptin-based antiobesity therapeutic approaches.

Despite their shortcomings, mouse models with HFD-induced obesity may provide important insights into understanding the effects of leptin on glucose metabolism in obese humans because these mouse models are the closest to human obesity. Chronic infusion of leptin in C57BL/6J mice on HFD did not improve the glucose response to insulin during an insulin tolerance test, even though the body weight of mice effectively decreased compared with control mice (250). Moderate hyperleptinemia achieved by adenovirus gene therapy somewhat improved glucose tolerance in animals that had 4-fold greater leptin concentrations compared with control rats (251). Therefore, serum concentrations of leptin may be an important factor responsible for the restoration of aberrant glucose metabolism in HFD-induced obese animal models. Leptin administration in other mouse models of obesity has shown mixed results. Harris et al (252) found that leptin treatment improved insulin resistance and hyperglycemia in some strains of db/db mice, but the effects depended on sex and mode of administration (chronic or bolus ip administration). Other authors found no benefits of leptin administration in db/db mice (5). Leptin treatment in a mouse model
of obesity that is deficient in brown adipose tissue resulted in no improvement in glucose metabolism (253).

Subsequently, Heymsfield et al (22) conducted a randomized, double-blind, placebo-controlled trial to determine the relationship between increasing doses of exogenous leptin administration and weight loss in both lean and obese humans. The results of this trial indicated that daily administration of recombinant human methionyl leptin induced dose-dependent but modest weight loss in most but not all subjects. Only at pharmacological doses did leptin induce some consistent degree of weight loss, but even then, the effect was relatively mild (average weight loss was 7.1 kg after 24 weeks in the group receiving the highest dose of leptin). In addition, these investigators performed a standard oral GTT at baseline and after 24 weeks and found no indication that leptin administration af-

<table>
<thead>
<tr>
<th>Disease State</th>
<th>Estimated Prevalence</th>
<th>Clinical Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat loss associated with leptin deficiency</td>
<td>Possibly rare</td>
<td>Generalized fat wasting, impaired glucose tolerance, insulin resistance or type 2 diabetes, dyslipidemia, hepatic steatosis, acanthosis nigricans</td>
</tr>
<tr>
<td>Congenital generalized lipoatrophy</td>
<td>Rare</td>
<td>Fat wasting of the face, arms, legs, and buttocks; impaired glucose tolerance, insulin resistance, or type 2 diabetes; hypertriglyceridemia, hepatic steatosis</td>
</tr>
<tr>
<td>HAART-induced lipoatrophy</td>
<td>15%–36% of HIV-infected patients</td>
<td>Strenuous exercise, psychogenic stress, energy deficit, osteoporosis, neuroendocrine dysfunction with decreased GnRH pulsatility and estradiol levels, decreased thyroid and IGF-I levels, and increased GH levels</td>
</tr>
<tr>
<td>HA (functional)</td>
<td>3%–8.5% in women aged 13–44 y Up to 69% of trained female athletes</td>
<td></td>
</tr>
<tr>
<td>Anorexia nervosa</td>
<td>1%–3% of college-age girls</td>
<td>Disturbed body image, severe restriction of food intake, body weight loss, neuroendocrine dysfunction</td>
</tr>
<tr>
<td>Obesity as a manifestation of leptin deficiency</td>
<td>Possibly rare</td>
<td>Hyperphagia, early-onset morbid obesity</td>
</tr>
<tr>
<td>Complete congenital leptin deficiency</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>Heterozygous leptin deficiency</td>
<td>≤5%–6% in obese subjects</td>
<td>Garden-variety obesity with hypoleptinemia relative to fat mass, normal neuroendocrine function</td>
</tr>
<tr>
<td>Obesity associated with leptin resistance (involving leptin and molecular signaling pathways downstream of the leptin receptor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin receptor gene mutation</td>
<td>Rare</td>
<td>Phenotype similar to congenital leptin deficiency, hyperphagia but less remarkable hyperinsulinemia, hypogonadotropic hypogonadism, mild growth retardation, hypothalamic hypothyroidism, abnormal GH secretion</td>
</tr>
<tr>
<td>POMC mutations</td>
<td>Rare</td>
<td>Hyperphagia, early-onset obesity, hyperinsulinemia, hypogonadotropic hypogonadism, abnormal glucose homeostasis, hypoinsulinemia, hypocortisolemia</td>
</tr>
<tr>
<td>Prohormone convertase deficiency</td>
<td>Rare</td>
<td>Hyperphagia, early-onset obesity, hypogonadotropic hypogonadism, abnormal glucose homeostasis, hypoinsulinemia, hypocortisolemia</td>
</tr>
<tr>
<td>MC4R 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</td>
<td>Rare</td>
<td>Dysregulation of energy homeostasis with onset in childhood</td>
</tr>
<tr>
<td>Neurotropin receptor tropomyosin-related kinase B mutations</td>
<td>Rare</td>
<td>BDNF deficiency resulting in severe obesity, hyperphagia, developmental delay, and 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>
</tr>
</tbody>
</table>

*a Adapted from Refs. 3, 4, 10, and 11.*
fected glycemic control (22). Another study also demonstrated that weekly injection of 60 mg of pegylated recombinant human leptin did not lead to clinically significant weight loss after 8 weeks of treatment, despite serum leptin concentrations being as high as 3000 ng/mL, ie, 100-fold higher than physiological levels. Insulin sensitivity, assessed with the HOMA score, was also not significantly affected by leptin treatment (254). This has been confirmed in a recent study using the hyperinsulinemic-euglycemic clamp, where leptin treatment for 14 days did not have any effect on insulin sensitivity (281). These results were confirmed by others and illustrate that leptin administration is not an effective treatment for metabolic disturbances in common obesity, at least not unless leptin resistance can be overcome (256, 257). Nevertheless, in a manner equivalent to administration of insulin for the control of glycemia in type 2 diabetes with insulin resistance, the possibility exists that leptin administration might be able to overcome leptin resistance.

In leptin-based antiobesity approaches, another important issue to consider is that leptin levels drop dramatically with weight loss and remain low for up to 1 year after weight loss (258–260). This has been proposed to be associated with activation of mechanisms inducing weight regain, such as increased food intake and reduced energy expenditure (259) as seen in the case of lean subjects (261). Hence, studies have looked at the role of leptin replacement to physiological levels after weight loss. Small studies have shown an improvement in weight loss maintenance as well as a reversal of changes in thyroid hormones, energy expenditure, and neural activity centers involved in the regulatory, emotional, and cognitive control of food intake (259, 262). However, the fact that these studies were small and of sequential study design indicates that future randomized and controlled studies are required to define this potential use of leptin in more detail. This may be related to saturability of the leptin pathways at higher leptin concentrations, leading to absent response to increases beyond the saturation level but marked responses after weight loss when leptin levels are low (20). In this respect, studies of leptin administration in lean women showed a further reduction in fat mass without a reduction in lean body mass, indicating that lean subjects with low physiological leptin levels at baseline are sensitive to exogenously administered leptin, which decreases primarily fat mass while sparing lean mass and increasing bone mass (12).

Recent clinical data have shown that combination treatment with leptin and pramlintide, an amylin analog, modestly decreased body weight (−12%) in leptin-resistant obese individuals more than leptin or amylin alone, but the effect of the combination was rather additive not synergistic as had previously been suggested by experiments in rodents (264, 283). However, due to the relatively small period of treatment (20 weeks), it remains to be seen whether a longer-term clinical trial would result in further reduction or maintenance of body weight or may cause unanticipated beneficial or adverse effects. To address these issues, longer-term clinical trials have been initiated, and results are awaited with interest.

In summary, leptin monotherapy is not an effective therapeutic approach for obesity in most obese subjects (representing ~90% of the obese population) with hyperleptinemia (circulating leptin levels above 15 ng/mL). However, the efficacy of leptin monotherapy or combination therapy in obese subjects exhibiting hypoleptinemia or normoleptinemia remains unknown. Figure 2 depicts the effect of recombinant leptin administration in the common obese state.

2. Diabetes mellitus

a. Type 1 diabetes mellitus. Many patients with type 1 diabetes have low levels of leptin (265), and this has also been seen in animal models of type 1 diabetes mellitus (266). A decrease in leptin secretion may be related to a reduced adipose mass and reduced assimilation and storage of energy substrates in adipose tissue in insulin deficiency (266).

Hence, several studies have examined the effects of leptin administration on glucose metabolism in insulin-deficient animal models of diabetes mellitus. Subcutaneous infusion of leptin restored euglycemia and normalized peripheral insulin sensitivity in streptozotocin (STZ)-induced type 1 diabetic animals (267, 272). Central leptin infusion also restored euglycemia in STZ-induced diabetic animals (269–271). Similar effects of leptin were also demonstrated in other studies by using various animal models of type 1 diabetes (58, 272–274). Pair-feeding studies indicate that decreased food intake does not account by itself for the restoration of normoglycemia in leptin-treated type 1 diabetic animals (273). These effects of leptin were achieved through an insulinlike or insulin-sensitizing but insulin-independent mechanism mediated by the CNS (68). It has been suggested that leptin may act via activating intracellular signaling pathways that overlap with those of insulin and/or by other mechanisms (mainly suppression of glucagon) that remain to be fully elucidated. Indeed, leptin monotherapy or combination with low-dose insulin reversed the lethal catabolic state via suppression of hyperglucagonemia in nonobese diabetic mice with uncontrolled type 1 diabetes (58, 67). Moreover, leptin normalized HbA1c levels with far less glucose variability compared with insulin monotherapy (58). This recent promising work from the Unger laboratory (54, 55)
suggests that leptin administration in humans may present many advantages over insulin monotherapy for the treatment of diabetes type 1, characterized as a condition with relative leptin deficiency. In humans, significant reductions in circulating leptin levels have been documented in the newly diagnosed untreated human type 1 diabetic patients, suggesting that reduced leptin secretion might be a common feature in insulin-deficient diabetes (265). Insulin deficiency in type 1 diabetes leads to a state of increased lipolysis from adipocytes elevating circulating levels of free fatty acids and ketonemia. These metabolic products may reduce adipocyte’s ability to secrete leptin, which signals an energy deficit. In view of its beneficial effects in animals, it has been suggested that leptin replacement therapy may prove to be a useful adjunct therapy to restore euglycemia in type 1 diabetes (277). Indeed, 1 year of leptin therapy was effective in improving glycemic control and lipid profiles in 2 patients with type 1 diabetes and acquired generalized lipodystrophy (276). In these individuals, leptin therapy improved insulin sensitivity, although insulin was not completely discontinued but was significantly reduced (276). Leptin’s main therapeutic benefits in patients with type 1 diabetes may include its slow-acting glycemia-lowering effect preferred over the fast-acting glycemia-lowering effect that can trigger hypoglycemic events, its antisteatotic and antilipotoxic action in peripheral tissues performed over the steatosis promoted by insulin therapy that may cause cardiovascular disease, its suppressive effect on glucagon that could reduce the need for insulin, and its ability to restore insulin secretion in β-islets by reversing lipotoxicity (277). Nonetheless, larger clinical trials are needed to be performed to determine leptin’s safety and efficacy in reducing insulin requirements, hyperglycemia, glycemic fluctuations, and dyslipidemia in subjects with diabetes type 1.

b. Type 2 diabetes mellitus. The use of leptin in type 2 diabetes has generated interest in view of the potential insulin-independent activation of intracellular pathways involved in glucose metabolism. Hence, several studies have investigated the use of leptin in animal models of type 2 diabetes mellitus, and more recently, we and others published human studies (in this Section). Despite results from several animal studies demonstrating that leptin ameliorated insulin resistance, glycemic control, and lipid abnormalities in mice with diabetes type 2, the results of 2 clinical trials have shown that leptin treatment is largely ineffective in improving insulin resistance and diabetes in obese subjects with type 2 diabetes (20, 281). Given the fact that a non-negligible proportion of subjects with diabetes type 2 are not obese, it will be important to evaluate the antidiabetic effect of leptin in those subjects who exhibit normal or low levels of leptin.

Toyoshima et al (278) investigated the use of leptin in the MKR mouse, a diabetic mouse model with normal leptin levels and defects in IGF-I and insulin receptor signaling. They found an improvement in diabetes that was independent of the reduced food intake and was associated with reduced lipid stores in liver and muscle (278). Similarly, Kusakabe et al (279) generated a mouse model mimicking a combination of human type 1 and type 2 diabetes using low-dose STZ and HFD and examined the effect of leptin treatment. These animals exhibited hyperglycemia and hyperleptinemia and only mildly elevated serum insulin levels, suggestive of both insulin deficiency and insulin resistance. After leptin infusion, food intake and body weight decreased, glucose tolerance improved, and serum insulin levels decreased during an ip GTT. Pair feeding showed that the improved glycemic control was independent of the reduction in food intake (279). Finally, our group has investigated leptin therapy in a mouse model deficient in brown fat, which is hyperleptinemic and very sensitive to DIO (253). In these mice, treatment with ip leptin was not associated with any changes in insulin or glucose concentrations (253).

Human studies have also evaluated the use of leptin in obese diabetic patients. Recently, we conducted a double-blinded, placebo-controlled, randomized clinical trial to evaluate the effects of leptin in obese type 2 diabetic patients (20). Seventy-one obese subjects with diet-controlled type 2 diabetes mellitus were recruited and randomized to receive either metreleptin (20 mg/d) or placebo for 16 weeks. Metreleptin administration did not alter body weight or circulating inflammatory markers, but marginally reduced HbA1C (8.01 ± 0.93 to 7.96 ± 1.12, P = .03) (20). Furthermore, we have found that levels of leptin-binding protein and antibodies against metreleptin increased in response to metreleptin treatment, limiting circulating free leptin to ~50 ng/mL despite mean total leptin levels of ~1000 ng/mL in these subjects. Although these levels are higher than the putative threshold for saturating the BBB leptin transport system (280), circulating free leptin levels did not correlate with weight loss, indicating clinical ineffectiveness of free leptin levels in the 40- to 50-ng/mL range. Mittendorfer et al (281) also performed a study to evaluate the effects of exogenous leptin in obese type 2 diabetic patients. They conducted a randomized, placebo-controlled trial in obese subjects with newly diagnosed type 2 diabetes assigned to placebo or low-dose (30 mg/d) or high-dose (80 mg/d) metreleptin for 14 days and performed a hyperinsulinemic-euglycemic clamp in conjunction with stable isotope-labeled tracer infusions to evaluate multigorgan insulin sensitivity before and after leptin.
treatment. There were no significant differences between groups in body weight and body composition. Insulin sensitivity of the liver (suppression of glucose production), skeletal muscle (stimulation of glucose uptake), and adipose tissue (suppression of lipolysis) and basal substrate kinetics were not affected by leptin treatment, even though serum leptin concentrations increased 150-fold compared with baseline in the high-dose group (281). This is very different from leptin replacement in lipodystrophic patients, where the obtained leptin levels were near-physiological (214).

These findings provide more evidence of leptin resistance in the population of obese diabetic subjects. Because most patients with type 2 diabetes are overweight/obese and present hyperleptinemia with leptin resistance, leptin treatment is considered ineffective (20, 281). Targeting the molecular mechanisms of leptin resistance will be important in the treatment for obesity and diabetes type 2. For instance, it has been suggested that the combination of leptin treatment with leptin sensitizers could be a potentially feasible way to overcome leptin resistance. Amylin has been suggested to be one such sensitizer on the basis of animal experiments (282). Amylin may act with leptin to induce fat-specific weight reduction (274), and the amylin analog pramlintide has been used in conjunction with leptin in clinical trials presenting modest effects (277) and potential safety concerns (276). In a phase II clinical trial, the combination of an amylin analog and recombinant leptin produced significantly more weight loss than either treatment alone and in combination activate STAT3, AMPK, Akt, and ERK1/2 signaling pathways (21). These data indicate that leptin and amylin activate overlapping intracellular signaling pathways in humans and have additive, but not synergistic, effects (21).

However, leptin resistance or tolerance may or may not represent an insurmountable obstacle regarding the antidiabetic actions of leptin. Leptin resistance could selectively affect leptin signaling pathways related to appetite and body weight reduction and not those related to glycemic control. Although the ObRb/JAK2/STAT3 pathway is important for leptin-mediated reduction of appetite and body weight, it plays a minor role in leptin’s ability to improve euglycemia (285). Moreover, pharmacological inhibition of the hypothalamic PI3K signaling pathway impairs the insulin-sensitizing effects of leptin (286). Research aiming at identifying molecular mechanisms and medications that could up-regulate insulin-sensitizing signaling pathways downstream of leptin is awaited. Data from rodents have also indicated that low doses of leptin can ameliorate hyperglycemia without affecting food intake or body weight, underscoring that the pathways underlying the glucose-lowering actions of leptin are more sensitive than the ones responsible for its weight-reducing effects. Future clinical trials are needed to uncover the clear antidiabetic action of leptin in nonobese patients with diabetes type 2 who exhibit normo- or hypoleptinemia due to low adipose tissue mass in contrast to previous studies examining obese patients with type 2 diabetes. Results of larger studies on combination treatments or the development of specific leptin sensitizers that would enhance leptin’s efficacy are awaited with great interest. Figure 2 depicts the effect of recombinant leptin administration in type 2 diabetes.

3. Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD), which is considered to be the hepatic manifestation of insulin resistance syndrome, has emerged as a significant public health problem with worldwide distribution. The prevalence of NAFLD in the general population is 10% to 46% in the United States and 6% to 35% in the rest of the world, with a median of approximately 20% (287). The incidence of NAFLD in both adults and children is rising, in conjunction with the growing epidemics of obesity and type 2 diabetes mellitus. The histologic spectrum of NAFLD encompasses a wide spectrum of liver damage ranging from nonalcoholic simple steatosis (SS) to nonalcoholic steatohepatitis (NASH) and NASH-related cirrhosis with its complications. SS progresses to NASH in about 15% to 30% and in cirrhosis in less than 5% of cases, whereas NASH progresses to cirrhosis in 10% to 15% of cases over 10 years and in 25% to 30% of cases in the presence of advanced fibrosis (288).

As described in the section of leptin signaling (Section III), under normal conditions, leptin suppresses hepatic glucose production and de novo lipogenesis, whereas it induces fatty acid oxidation in hepatocytes, thereby providing an antisteatotic and insulin-sensitizing effect. On the other hand, hyperleptinemia has been proposed to play a role in the pathogenesis of NAFLD (156, 289). There is
Evidence that leptin acts as a proinflammatory and profibrogenic cytokine, thereby affecting liver fibrosis, a key feature of NASH. Xenobiotics-induced liver fibrosis was extremely diminished in ob/ob mice and Zucker (fa/fa) rats (290). Activated hepatic stellate cells (HSCs) express ObRb and possibly other ObR isoforms, whose activation leads to increased expression of proinflammatory and proangiogenic cytokines and growth factors, such as angiopoietin-1 and vascular endothelial growth factor, which affect hepatic inflammation and fibrosis (291). Moreover, leptin was shown to up-regulate collagen α1 in HSCs through the sequence p38 MAPK activation and SREPB-1c down-regulation (292). Furthermore, leptin may induce hepatic fibrogenesis by stimulating the production of tissue inhibitor of metalloproteinase-1 (293) and repressing matrix metalloproteinase-1 gene expression (294) in activated HSCs. Both these processes are mediated by the JAK/STAT and the JAK-mediated H2O2-dependent MAPK pathways. Inflammatory, fibrogenic, and proliferative leptin actions on HSCs may also be mediated via nicotinamide adenine dinucleotide phosphate oxidase, which acts downstream of JAK activation, but independently of STAT3; inhibition of nicotinamide adenine dinucleotide phosphate oxidase in HSCs resulted in a reduction of leptin-mediated up-regulation of fibrogenic markers (collagen α1 and α-smooth muscle actin) and of inflammatory mediators (monocyte chemotactic protein-1 and macrophage inflammatory proteins 1 and 2) and in a reduction of leptin-mediated HSC proliferation (295). Additionally, leptin facilitates proliferation and prevents apoptosis of HSCs (296), and it modulates all features of the activated phenotype of HSCs in a profibrogenic manner (myofibroblastic phenotype) (297). Once activated, HSCs contribute to further leptin expression (298), thereby possibly establishing a vicious cycle (156).

Data regarding the role of leptin on hepatic fibrosis via an effect on Kupffer or sinusoidal endothelial cells are currently limited. Leptin may promote hepatic fibrogenesis through up-regulation of TGF-β and connective tissue growth factor in isolated Kupffer cells; based on this finding, Kupffer cells-HSCs cross talk has been proposed for hepatic fibrosis (299). Leptin has also been reported to affect hepatic fibrosis through up-regulation of TGF-β in sinusoidal endothelial cells (290).

More recently, an up-regulation of CD14 (an endotoxin receptor recognizing bacterial lipopolysaccharide) in Kupffer cells was followed by hyperreactivity toward low-dose lipopolysaccharide in HFD steatotic mice but not in chow-fed control mice, which resulted in NASH progression (300). When recombinant leptin was administered in chow-fed mice, an up-regulation of CD14 in Kupffer cell was similarly observed resulting in hyperreactivity toward lipopolysaccharide, thereby inducing hepatic inflammation and fibrosis without steatosis. On the contrary, recombinant leptin administration to ob/ob mice did not up-regulate CD14 or induce inflammation and fibrosis, despite severe steatosis (300); these results demonstrated that leptin deficiency, apparently due to lack of its lipolytic effects, may lead to hepatic steatosis, but it is protective toward the progression of SS to NASH, whereas excess of this proinflammatory adipocytokine may favor hepatic inflammation and fibrosis.

In line with the aforementioned data, it was previously speculated that leptin under normal conditions may prevent liver steatosis, whereas its effect on quiescent HSCs and Kupffer and sinusoidal cells is minimal. However, prolonged hyperleptinemia, as observed in leptin tolerance states (ie, common obesity), may ultimately result in overexpression of SOCS3 and in activation of HSCs and Kupffer and sinusoidal cells. Overexpressed SOCS3 may aggravate both leptin tolerance and insulin resistance in hepatocytes and outweigh the primarily beneficial effect of leptin on hepatocytes. Activated HSCs and Kupffer and sinusoidal cells, which seem to display minimal or no leptin tolerance, may trigger the proinflammatory and profibrogenic cascade (156).

Although evidence for the inflammatory and fibrogenic role of leptin in NAFLD from in vivo and animal models is strong, relevant data from clinical studies in NAFLD patients are based mainly on cross-sectional studies, which cannot establish a causative relationship. Most authors have reported higher leptin levels in NAFLD (301, 302) or NASH (303, 304) patients than controls, in parallel with higher insulin resistance. However, other authors have reported similar leptin levels between NASH patients and controls (305, 306). Regarding liver histology, some authors have also reported that leptin levels were positively associated with hepatic fibrosis (303, 307, 308) or inflammation (303, 308). However, other authors reported no association between serum leptin levels and hepatic fibrosis or inflammation (304, 306).

Soluble leptin receptor (sOB-R) has been reported to be lower in the NAFLD patients than controls, as well as negatively associated with circulating leptin in one study, which might be attributed to counteracting sOB-R downregulation (301). However, other authors have reported higher sOB-R levels in morbidly obese patients with diabetes, which are positively associated with the stage of hepatic fibrosis (309), whereas others found no association of sOB-R levels with hepatic histology in children with NAFLD (308). More studies are needed to elucidate the interaction of leptin with its soluble receptor and its role, if any, in the pathogenesis of NAFLD. The cross-
sectional nature of the aforementioned clinical studies together with the heterogeneity within them render the drawing of any conclusion risky. Larger studies with better characterized and/or homogenous populations and carefully matched controls are of importance.

Prospective data regarding circulating leptin levels in NAFLD are limited. In a 3-year prospective study with paired biopsies, serum leptin levels were decreased more in patients with stable or improved disease compared with those with disease worsening; however, leptin could not independently predict disease or fibrosis progression (310). In a 7-year prospective study, leptin levels were higher in patients with than without NAFLD at baseline; however, leptin change was similar in those with disease remission or sustaining disease (311). The limitation of this study was its noninvasive nature (NAFLD was not biopsy-proven). Further prospective studies with paired biopsies and long-term follow-up are needed.

The effect of various therapeutic interventions on circulating leptin levels in patients with NAFLD are summarized in Table 6. Briefly, leptin levels remained essentially unaffected after treatment with pioglitazone (312), rosiglitazone (312), atorvastatin (313), ezetimibe (314), cysteamine (315), and ursodeoxycholic acid with or without vitamin E (316) but increased after short-term melatonin treatment (317) or decreased equally after metformin and lifestyle modifications or only lifestyle modifications (57).

Importantly, no study to date has investigated or is planned to investigate the effects of recombinant leptin administration on NAFLD patients with normal or high leptin levels. On the other hand, metreleptin administered in 10 NAFLD patients (8 with NASH) with lipodystrophy for a mean duration of 6.6 months resulted in improvement of hepatic steatosis and ballooning but not portal inflammation or fibrosis (63). These results are similar to Imajo et al (300) experimental model, indicating that recombinant leptin administration in NAFLD patients and lipodystrophy may improve steatosis but not fibrosis. Furthermore, there are 2 ongoing clinical trials having as primary endpoint the effect of recombinant leptin administration in hepatic histology in NAFLD patients with low leptin levels. In one of them (single-group, prospective, open-label), metreleptin (0.1 mg/kg/d once a day sc) in 10 adult male patients with NASH and low circulating leptin levels, but not lipodystrophy, was to be administered for 1 year (NCT00596934). In the other study, (single-group, prospective, open-label), metreleptin (0.1 mg/kg/d once a day sc) was planned to be administered for 1 year in 20 male or female patients with SS or NASH and lipodystrophy (NCT01679197).

Despite the lack of direct clinical evidence, leptin administration in NAFLD patients with hyperleptinemia might have adverse effect on liver fibrosis, thereby possibly worsening the prognosis of liver disease. If the results of the Imajo et al study (300) were translated into human pathophysiology, they might have 2 main implications: 1) leptin excess, which is usually observed in common forms of obesity, may be contributing toward NASH progression and 2) recombinant leptin administration for severe obesity may adversely affect the liver of obese individuals, because it may promote proinflammatory responses, whereas it has no lipolytic effects above a certain level due to its permissive role in controlling obesity (20). Considering that the prevalence of NAFLD in obese individuals is 50% to 95%, this becomes of crucial importance. Figure 2 depicts the effect of recombinant leptin administration in NAFLD.

C. Benefits, potential adverse effects, and challenges in relation to leptin replacement therapy

The benefits and potential adverse effects of recombinant leptin administration in leptin deficiency or leptin excess states are summarized in Table 3. Generally, leptin replacement therapy using an analog of leptin, metreleptin, also known as recombinant methionyl human leptin, has been proven effective for the treatment of congenital leptin deficiency, congenital and non-HIV–related acquired generalized lipodystrophy associated with severe hypoleptinemia, and HA (219, 220).

Metreleptin offers metabolic benefits such as improvements in insulin sensitivity, glucose tolerance, fasting glucose, glycosylated hemoglobin, hypertriglyceridemia, and liver function tests (219, 220). Patients with partial lipodystrophy, including those with PPARγ mutations (216), also gain metabolic benefits from leptin administration. In these subjects, leptin administration has been extensively studied in the context of open-label clinical trials (221).

Table 6. Effect of Various Therapeutic Interventions on Circulating Leptin Levels in Patients With NAFLD

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Intervention</th>
<th>Duration</th>
<th>Circulating Leptin Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>317</td>
<td>Melatonin 10 mg/d</td>
<td>28 d</td>
<td>Increased</td>
</tr>
<tr>
<td>275</td>
<td>Rosiglitazone 4 mg/d</td>
<td>6 mo</td>
<td>Unchanged</td>
</tr>
<tr>
<td>315</td>
<td>Enteric-coated cysteamine 600–2000 mg/d</td>
<td>6 mo</td>
<td>Unchanged</td>
</tr>
<tr>
<td>314</td>
<td>Ezetimibe 10 mg/d</td>
<td>2 y</td>
<td>Unchanged</td>
</tr>
<tr>
<td>316</td>
<td>Ursodeoxycholic acid 12–15 mg/kg/d ± Vitamin E</td>
<td>6 mo</td>
<td>Unchanged</td>
</tr>
<tr>
<td>313</td>
<td>Atorvastatin 10 mg/d</td>
<td>2 y</td>
<td>Unchanged</td>
</tr>
<tr>
<td>57</td>
<td>Metformin 1700 mg/d ± diet/exercise</td>
<td>6 mo</td>
<td>Decreased in either group</td>
</tr>
<tr>
<td>312</td>
<td>Pioglitazone 30 mg/d</td>
<td>1 y</td>
<td>Unchanged</td>
</tr>
</tbody>
</table>

Data are presented in publication date order.
HIV patients with HALS also exhibit significant improvements in their metabolic profile that are comparable to other treatment options such as metformin and thiazolidinediones (234). Leptin replacement therapy provides benefits beyond metabolic improvements: it ameliorates glomerular injury in generalized lipodystrophy (223), it has immunomodulatory effects in hypoleptinemic patients with severe lipodystrophy (222), it normalizes menstrual abnormalities in young women with lipodystrophy and polycystic ovary syndrome (224), and it improves the thyroid, GH, and adrenal axis abnormalities seen in women with HA (224).

Although open-label trials have shown benefits of metreleptin use, larger, randomized, placebo-controlled clinical trials are needed to conclusively demonstrate its efficacy and safety, particularly because severe adverse effects have been noted, including deterioration of renal function and proteinuric nephropathy (224) and occurrence of T-cell lymphomas (223) in a small number of patients with lipodystrophy. It is important to underscore that the open-label nature of the study design did not allow the investigators to establish or refute a causal link between metreleptin treatment and reported adverse effects. Similar adverse effects would not be entirely unexpected on the basis of the natural history of the disease, particularly in patients suffering from acquired lipodystrophy, which is traditionally thought to be due to autoimmune factors. Approximately 25% of cases with acquired generalized lipodystrophy are caused by autoimmune disease such as juvenile-onset dermatomyositis, rheumatoid arthritis, systemic lupus erythematosus, and Sjögren syndrome (219). Given the immunomodulatory effect of metreleptin, which clearly regulates the Th1/Th2 imbalance in leptin-deficient individuals (224), large, crossover, randomized, placebo-controlled clinical trials of sufficient duration are needed to study efficacy and adverse effects more tightly. Potential disadvantages of leptin therapy may include increased immune system function and inflammation and the generation of neutralizing autoantibodies against exogenous leptin (115, 190, 212, 219). Moreover, it has been reported that leptin accelerates autoimmune diabetes in the nonobese diabetic mouse model of diabetes type 1, probably by inducing proinflammatory cell responses (217).

It remains unknown whether similar adverse effects in response to leptin administration could potentially also be observed in disease states accompanied by hyperleptinemia due to leptin resistance such as garden-variety obesity. It also remains unknown whether similar side effects could be seen in response to higher than normal leptin levels, which are needed to effectively lower glycemia in conditions such as type 1 diabetes. In these conditions, potential side effects of leptin may become really prevalent due to the increased prevalence of the underlying disease. Common adverse events seen particularly in combination treatment with pramlintide/metreleptin in obese subjects included nausea and injection site erythema and were mild to moderate and decreasing over time (263). It has been proposed that leptin could possibly cause hypertension, impair endothelial function, promote platelet aggregation leading to thrombosis, increase immune function, foster inflammation and angiogenesis, and worsen diabetic complications and other concomitant diseases on the basis of preclinical studies, but no such side effects have been seen in the clinic (283). We have failed to observe effects in hypertension (255, 283), angiogenesis (263), or inflammation (20). Although it cannot be excluded that prolonged administration of leptin may cause hypertension, particularly in predisposed obese diabetic individuals in contrast to obese hypotensive subjects who present with leptin deficiency (255), we and others have not observed such side effects in our trials (20, 268). Moreover, leptin’s action in suppressing glucagon may place patients suffering from type 1 diabetes at increased risk for severe hypoglycemic episodes by impairing the counterregulatory response necessary to restore glycemia (283). Until now, these particular side effects have not been reported in leptin-deficient lipodystrophic or other patients treated with replacement doses of leptin. Other probable adverse effects may theoretically include sleep disorders, an early onset of puberty in children, and an increased risk of unplanned pregnancy, but again, they have not yet been observed in the clinic, whereas the potential teratogenic effects of leptin treatment need to be explored.

It is of paramount importance to discuss the potential carcinogenic adverse effects of leptin administration in patients with garden-variety obesity. It has been proposed that leptin may exert neoplastic effects via 2 mechanisms. First, leptin can act directly on cancer cells by stimulating receptor-mediated signaling pathways leading to tumor cell growth, migration, and invasion (193). Notably, this activity of leptin is commonly reinforced through entangled cross talk with multiple oncogenes, cytokines, and growth factors. Second, leptin may act indirectly by reducing tissue sensitivity to insulin, causing hyperinsulinemia and by regulating inflammatory responses with overproduction of cytokines such as IL-6, IL-12, and TNF-α (284) and influencing tumor angiogenesis, but we have not observed such effects of leptin in vivo (285). Leptin has also been proposed to play a role in hormone-dependent malignancies, such as breast and endometrial cancers, by activating the enzyme aromatase, an enzyme responsible for the transformation of androgens to estrogens (81). Expression of leptin receptors has been reported in numerous
cancer cell types including breast, colon, prostate, and thyroid (80). Leptin, through its receptor ObRb, may modulate growth and proliferation of cancer cells via activation of various growth and survival signaling pathways including canonical (JAK2/STAT3, PI3K/Akt, and MAPK/ERK1/2) and noncanonical (protein kinase C, JNK, and p38 MAPK) signaling pathways (80). For example, leptin has been demonstrated in vitro to stimulate JNK in human breast cancer cells in both a time- and a dose-dependent manner, with greater phosphorylated JNK levels after long-term exposure (80). In breast cancer, JNK activation by leptin leads to an up-regulation of matrix metalloproteinase-2 activity, which promotes cancer cell invasion (80). Leptin exerts proliferative and antiapoptotic effects on human colon cancer cells, which are mediated in part though JNK activation (80). It should be noted, however, that most in vitro studies have used extremely high leptin levels.

However, in contrast to many in vitro studies, epidemiological studies report inconsistent associations between serum leptin levels and risk of several malignancies. For example, in the case of colorectal cancer (CC), an obesity-associated cancer, some prospective studies found a positive association between serum leptin levels and CC risk (291), whereas others did not show any relationship. Moreover, some studies revealed that even hypoleptinemia was associated with CC risk, independently from BMI (293). Our group, which has focused on adipokines and cancer, has recently shown that hypoleptinemia, and not hyperleptinemia, was linked to pancreatic cancer independently from BMI and weight loss, to B cell chronic lymphocytic leukemia, and to low-risk myelodysplastic syndrome after adjustment for BMI and other risk factors (297) but these associations may reflect reverse causality. In addition, increasing serum leptin levels did not appear to increase substantially the risk of premenopausal breast cancer in situ, invasive pre- and postmenopausal breast cancer, endometrial cancer, prostate cancer, multiple myeloma, melanoma, and lung cancer (318–321). Thus, the possible association of leptin and malignancies in vitro appears to be not supported by published studies in humans. A possible association with leptin needs to be studied further with larger prospective, longitudinal, and mechanistic studies, which are needed to prove causality, provide further insights into both the mechanisms underlying the actions of this hormone and its potential role in cancer, and analyze its action in tumor progression if leptin is prescribed as an alternative or adjunct approach in patients with diabetes and malignancies in the future.

In conclusion, future larger trials using metreleptin are needed to systematically determine 1) the diagnostic criteria to be used as indicating hypoleptinemia and thus serve as inclusion criteria in ongoing and future trials, 2) the appropriate initial metreleptin dose, 3) how high the dose of metreleptin should be raised (based upon which algorithm) for the best metabolic response to be achieved or what dose would be needed to safely break through a possible resistance to metreleptin in certain individuals, 4) how patients on metreleptin should be monitored, and 5) whether the concomitant use of other leptin-sensitizing treatments could be useful therapeutic options in the treatment of obesity or to prevent metabolic adaptation induced by caloric restriction and to promote weight maintenance. Garden-variety obesity is a chronic disease with currently limited successful long-term therapy options, apart from bariatric surgery, which is both costly and risky. Combination therapy for obesity represents an encouraging step forward in the pharmacologic armamentarium. Finally, large randomized leptin administration studies are needed to obtain accurate information on potential adverse effects and long-term outcomes.

VII. Future Directions

Leptin is an adipocyte-secreted hormone that interacts with several organs that are involved in the regulation of energy homeostasis and metabolism. Although great progress has been made in terms of understanding leptin’s pathophysiological role, several fundamental questions, particularly regarding the effects of leptin on glucose metabolism, still remain to be answered. Much progress has been made with leptin treatment in patients with leptin deficiency and lipodystrophy, and such treatment does correct metabolic abnormalities. Results from these studies suggest that leptin could prove to be a potential antidiabetic agent in leptin-deficient states. However, formal double-blinded placebo-controlled phase III clinical trials are required to accurately determine placebo-subtracted efficacy and to assess comparative efficacy in relation to other effective therapies, and dose-finding studies are necessary to help guide appropriate treatment for these individuals. It is also unclear whether all patients with lipodystrophy would benefit from leptin therapy or whether only those with the most profound leptin deficiency are more likely to benefit. In contrast, the vast majority of obese individuals are hyperleptinemic and resistant or tolerant to exogenous leptin. Therefore, it is questionable whether leptin alone can be used to favorably modify glucose metabolism in garden-variety obesity and/or diabetestes. Hence, findings from ongoing and upcoming studies combining leptin with other antiobesity medications or sensitizers will be of particular interest.
Acknowledgments
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The Mantzoros laboratory is supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants 58785, 79929, 81913, and AG032030 and a VA Merit award (Grant 10684957). The Mantzoros Laboratory is also supported by a discretionary grant from Beth Israel Deaconess Medical Center.

All authors contributed to writing the manuscript. All authors read and approved the final manuscript.

Disclosure Summary: All authors state that there is no conflict of interest related to this paper.

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