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Leptin treatment: Facts and expectations

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ABSTRACT

Leptin has key roles in the regulation of energy balance, body weight, metabolism, and endocrine function. Leptin levels are undetectable or very low in patients with lipodystrophy, hypothalamic amenorrhea, and congenital leptin deficiency (CLD) due to mutations in the leptin gene. For these patients, leptin replacement therapy with metreleptin (a recombinant leptin analog) has improved or normalized most of their phenotypes, including normalization of endocrine axes, decrease in insulin resistance, and improvement of lipid profile and hepatic steatosis. Remarkable weight loss has been observed in patients with CLD. Due to its effects, leptin therapy has also been evaluated in conditions where leptin levels are normal or high, such as common obesity, diabetes (types 1 and 2), and Rabson–Mendenhall syndrome. A better understanding of the physiological roles of leptin may lead to the development of leptin-based therapies for other prevalent disorders such as obesity-associated nonalcoholic fatty liver disease, depression and dementia.

1. Introduction

The adipose tissue functions as an endocrine organ, where hormones with cytokine-like actions, called adipokines, are synthesized and secreted [1]. Leptin is one of the most abundant and important adipokines. The most well-known effect of leptin is to regulate body weight and energy balance [2], but it also has fundamental roles in glucose and lipid homeostasis, reproduction, immunity, inflammation, bone physiology, and tissue remodeling. In its absence, severe and potentially lethal changes in body homeostasis occur [3].

Leptin deficiency is observed in specific conditions, such as lipodystrophy syndromes, hypothalamic amenorrhea, anorexia nervosa and congenital leptin deficiency (CLD) due

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CLD, congenital leptin deficiency; Cmax, peak serum concentration; CYP450, cytochrome P450; DXA, dual-energy X-ray absorptiometry; FGF21, fibroblast growth factor 21; FSH, follicle-stimulating hormone; GH, growth hormone; GnRH, gonadotropin-releasing hormone; HAART, highly active antiretroviral therapy; HbA1c, hemoglobin A1c; IGF-1, insulin-like factor-1; IGFBP, insulin-like growth factor-binding protein; LH, luteinizing hormone; LRT, leptin replacement therapy; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; Nf-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; SOCS3, suppressor of cytokine signaling-3; POMC, proopiomelanocortin; PTP1B, protein tyrosine phosphatase 1B; Tmax, time to the maximum concentration; TNFα, tumor necrosis factor α; TSH, thyroid stimulating hormone.

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Table 1 – Metreleptin drug facts.

Structure	146 amino acids (as in the mature human leptin), with an additional methionyl residue at the N-terminal end of the recombinant protein
Route and frequency of administration	Subcutaneous, once a day
Recommended starting dose for generalized lipodystrophy	0.06 mg/kg/day (if body weight ≤40 kg) 2.5 mg/day (males >40 kg) 5 mg/day (females >40 kg)
Maximum dose	0.13 mg/kg (if body weight ≤40 kg) 10 mg/day (if body weight >40 kg)
Cmax	4.0–4.3 hours
Tmax	4 hours (range 2–8 hours)
Volume of distribution	4–5 times plasma volume
Route of elimination	Renal
Half-life	3.8–4.7 hours
Most common adverse reactions (≥10%)	Headache, hypoglycemia, decreased weight, and abdominal pain
Contraindications	General obesity not associated with congenital leptin deficiency; hypersensitivity to metreleptin
Safety during pregnancy and nursing	Uncertain. During nursing, metreleptin therapy or nursing should be discontinued
Use in geriatric patients >65 years-old	Unclear; dose selection should be cautious, and start at the low end of the dosing range
Drug interactions	Potential to alter the formation of CYP450 enzymes

From Myalept package insert [11].
Cmax: peak serum leptin concentration.
Tmax: median time to the maximum concentration.

to mutations in the leptin gene. The clinical manifestations in these conditions may include increased insulin resistance, hyperglycemia, dyslipidemia, endocrine disruptions, and fatty liver disease. In addition, morbid obesity, impaired cognitive development, and potentially lethal T-cell hyporesponsiveness have been reported in patients with CLD [4].

The discovery of leptin in 1994 [5], and the observation that its replacement reverses morbid obesity in leptin-deficient mice [6,7] and in humans with CLD [8], led to speculation that it might be a powerful tool to treat common obesity, or to facilitate adherence to diet and avoid a decline in energy expenditure [9]. However, due to the leptin-resistant state that is observed in patients with common obesity, that was not the case. The effects of leptin replacement therapy (LRT) with recombinant human leptin have been extensively evaluated in humans with CLD, to whom LRT is the only available treatment. More recently, leptin treatment has been approved for the treatment of patients with generalized lipodystrophy [10]. Other possible therapeutic uses of leptin include the treatment of hypothalamic amenorrhea, partial forms of lipodystrophy, diabetes, neurodegenerative disorders, depression, and common obesity with relatively low levels of plasma leptin.

2. Metreleptin drug profile

The form of leptin that is currently available for human therapy is known as recombinant methionyl human leptin (metreleptin, Myalept®, Amylin Pharmaceuticals; recently acquired by Bristol-Myers Squibb, and subsequently by AstraZeneca plc), initially available as Leptin A-100 (when its patent was owned by Amgen). Metreleptin is the only pharmaceutical form of leptin, and is composed by the 146 amino acids of mature human leptin, with an additional methionyl residue at the N-terminal end of the recombinant protein. It is a nonglycosylated polypeptide with one disulfide bond between Cys-97 and Cys-147, and a molecular weight of approximately 16.15 kDa.

Myalept® has been recently approved by the FDA for the treatment of congenital or acquired generalized lipodystrophy (non-HIV-related), but not for the partial forms of the disease, for which safety and effectiveness have not been established yet. The recommended starting dose varies according to gender and body weight, to a maximum daily dose of 0.13 mg/kg if body weight ≤40 kg, and 10 mg/day if body weight >40 kg (Table 1). Metreleptin is administered once daily at the same time every day, subcutaneously [11]. Due to its short half-life, some researchers prefer to divide the dose into two subcutaneous injections, when treating patients with CLD. Patients need to be evaluated regularly, and doses, recalculated to avoid excessively rapid weight loss.

Pharmacokinetic studies have been conducted mostly on healthy individuals, and few patients with lipodystrophy (Table 1). Data indicate that renal clearance is the major route of elimination, with no apparent contribution of systemic metabolism or degradation. In the presence of anti-leptin antibodies, the clearance of metreleptin is expected to be delayed, and its biological effects, attenuated or completely neutralized [11].

The most commonly reported adverse reactions (≥10%) include headache, hypoglycemia, decreased weight, and abdominal pain. T-cell lymphoma has also been reported in patients with acquired generalized lipodystrophy being treated with metreleptin [12]. However, a causal relationship between metreleptin and the development and/or progression of lymphoma has not been established, since lymphoproliferative disorders, including lymphomas, have been reported in patients with familial partial lipodystrophy [13] and acquired generalized lipodystrophy [14] not treated with the drug. Therefore, doctors should consider the benefits and risks of treatment with metreleptin in patients with significant hematologic abnormalities and/or acquired generalized lipodystrophy. Due to the risk of hypoglycemia [12], dose

Table 2 – Effects of leptin replacement therapy in humans with congenital leptin deficiency due to mutations in the leptin gene.

Behavioral and anthropometric effects	↓ Body weight [16,20,26,37,46]
	↓ Total fat mass [16,20,26,37,46]
	↓ Food intake [16,31,37,38,48]
	↓ Hunger and in desire to eat [32,37,38,48]
	↑ Fullness after eating [37,38,48]
	↑ Physical activity [37]
	Attenuated decrease in energy expenditure after weight loss [34]
Metabolic effects	↓ Triglycerides [16,26,37,46]
	↑ HDL-cholesterol [16,26,37,46]
	↓ Plasma insulin, ↓ insulin secretion, ↑insulin hepatic extraction [28,42]
	↓ Plasma glucose, with resolution of type 2 diabetes in one patient [37]
	↑ Insulin sensitivity [28,42,46]
	↓ Liver fat content and in serum transaminases [46]
Endocrine effects	Reversal of hypogonadotropic hypogonadism [37,47]
	↑ Mean 24-hour serum cortisol and changes in cortisol circadian rhythm [37]
	↑ IGFBP-1 and IGFBP-2 [35,37]
	Resolution of subclinical hypothyroidism [20]
Immunological changes	↑ White blood cells count [20]
	Normalization of CD4 cells count (from reduced numbers), and of CD8 and B cells counts (from increased numbers) [16]
	Increase in T cell responsiveness [16,45]
	Switch from the secretion of predominantly Th2 to Th1 cytokines [16,45]
Neuroimaging changes	↑ Gray matter concentration in the anterior cingulate gyrus, parietal lobe, and medial cerebellum [40]
	↑ Gray matter concentration in the posterior half of the left thalamus (particularly the pulvinar nucleus), following treatment withholding/re-initiation [39]
	↓ Activation of regions linked to hunger (insula, parietal and temporal cortex) [30]
	↑ Activation of regions linked to inhibition and satiety (prefrontal cortex) [30]
	↑ Activation of posterior lobe of the cerebellum [30]

adjustment of insulin or insulin secretagogues may be necessary, if in use, and blood glucose levels should be closely monitored in those patients. Since autoimmune disorder progression has been observed in patients treated with metreleptin (autoimmune hepatitis and membranoproliferative glomerulonephritis) [12], benefits and risks should be weighed in patients with autoimmune disease. Hypersensitivity reactions (e.g., urticaria or generalized rash) have also been reported [15].

Anti-metreleptin antibodies have been identified in nearly all patients (>95%) treated with metreleptin from two NIH studies (NIH Studies 991265 and 20010769) and Study FHA101 (sponsor-initiated) [15], and which consequences have not been well characterized yet. Clinical trials have reported loss of metabolic control in the presence of antibodies against metreleptin [16,17], which could inhibit endogenous leptin action and cause loss of drug efficacy. Neutralizing activity was observed in 7 out of 741 patients treated (only part of those were tested for anti-leptin antibodies) [15]. In addition, in the package insert, the company recommends testing for anti-metreleptin antibodies with neutralizing activity in patients with severe infections or loss of efficacy during metreleptin treatment.

The contraindications for therapy with metreleptin include general obesity not associated with congenital leptin deficiency, and hypersensitivity to metreleptin. No adequate and well-controlled studies have been conducted with the drug in pregnant women. In nursing women, metreleptin therapy or nursing should be discontinued. No clinically meaningful differences have been observed regarding efficacy and safety of metreleptin between pediatric and adult patients. In the geriatric population, existing clinical trials have not included sufficient numbers of participants >65 years-old. However, due to decreased hepatic, renal and/or cardiac function, dose selection should be cautious and start at the low end of the dosing range [11].

No formal drug interaction studies have been performed. However, leptin is a cytokine that has the potential to alter the expression of cytochrome P450 (CYP450) enzymes. Therefore, caution is warranted when prescribing drugs metabolized by CYP450, such as oral contraceptives and drugs with a narrow therapeutic index [11]. Key facts about metreleptin are summarized in Table 1.

3. Leptin replacement therapy in patients with congenital leptin deficiency

Cases of CLD caused by mutations in the leptin gene are rare. To date, 8 different mutations leading to the leptin-deficient phenotype have been reported in a total of 34 individuals of Pakistani (n = 27) [16,18–22], Egyptian (n = 1) [23], Austrian (n = 1) [24], and Turkish origins (n = 5) [25–27]. The human model of leptin deficiency due to mutations in the leptin gene is currently the best human model that allows the understanding of the physiological effects of leptin in humans not previously exposed to endogenous leptin. Physiological doses of metreleptin have been evaluated in the treatment of CLD in patients of Turkish, Pakistani and Austrian background [8,16,20,26,28–48]. The effects of LRT in these patients are summarized in Table 2.

Our group has been following the treatment of the Turkish cohort for over 14 years. In these patients, treatment started at ages 5 (boy), 27 (male), 35 and 40 (females). The case of another girl from the same family had also been initially reported [25,27],

but she died of sepsis before treatment could be initiated. Treatment led to significant improvements in weight, endocrine function and behavior [37]. Leptin replacement was lifesaving, as eight members of this family with severe early-onset obesity, whom we presume to have been leptin-deficient, died during childhood due to infections and sepsis.

For patients with CLD, the recommended initial physiological dose of metreleptin is 0.02–0.04 mg/kg/day, which is calculated to achieve 10% predicted serum leptin concentration based on gender, age, and percentage of body fat (calculations based on pharmacodynamic and pharmacokinetic data from AMGEN, Thousand Oaks, CA). The administered dose remains the same if weight reduces or stabilizes. If weight increases over two consecutive month periods, the dose is increased to achieve 20%, and subsequently 50, 100, and 150% predicted serum leptin concentrations.

In the Turkish cohort of patients with CLD, treatment was initiated with metreleptin 0.02–0.04 mg/kg/day, administered subcutaneously once a day, at 6 pm. That time (i.e., in the evening) was chosen to mimic leptin's normal circadian rhythm, which peak occurs early in the morning, around 2 am [49]. In adults, this dose increases serum leptin to levels that are observed in healthy adult males with 20% body fat, or in healthy adult females with 30% body fat. The Turkish adults' initial mean dose was 4.1 ± 1.2 mg/day: 2.8 mg for oldest male, 4.2 mg for youngest female, and 5.3 mg for oldest female [37]. After 14 years of treatment, the most current dose is 1.95 ± 2.05 mg/day: 0.6 mg for the oldest male, 1.0 mg for the youngest female, and 5.0 mg for the oldest female. The child's initial dose was 1.36 mg/day [26], and the current dose is 1.2 mg/day. The oldest female is under a considerably higher dose, and significant weight regain occurred upon dose decreases. That patient had type 2 diabetes before treatment, which reversed after metreleptin initiation [26,37]. It is possible that her higher dose requirements are attributed to increased leptin resistance, in the context of common obesity combined with leptin deficiency. For the additional cases of CLD described by other investigators, the initial dose of metreleptin was calculated in the same manner, but some investigators prefer to divide the dose into two daily injections, or to administer a single injection in the morning. Efficacy does not seem to be different when a single injection is administered per day, but we have observed increased adherence with that regimen.

3.1. *Body composition, food intake and energy expenditure*

Leptin replacement therapy leads to significant decreases in body weight, body mass index (BMI) and fat mass. Our Turkish adult patients' mean BMI was 51.2 ± 2.5 kg/m^2 before treatment. After 18 months of treatment, the patients reached a stable mean BMI of 26.9 ± 2.1 kg/m^2, which remained fairly stable since then. The youngest male also lost a significant amount of weight, from a BMI of 39.6 kg/m^2 before treatment at age 5 (BMI Z Score 3.52), to 24.8 kg/m^2 at age 6 (BMI Z Score 3.03), 23.8 kg/m^2 at age 7 (BMI Z Score 2.46), 24.6 kg/m^2 at age 8 (BMI Z Score 2.25), 22.6 kg/m^2 at age 9 (1.85), 24.5 kg/m^2 at age 10 (BMI Z Score 2.01), 26.5 kg/m^2 at age 11 (BMI Z Score 2.06), and 27.0 kg/m^2 at age 12 (BMI Z Score 2.0). Most of the decrease in BMI was attributed to fat mass loss, as measured by dual-energy X-ray absorptiometry (DXA) [37]. Weight loss was concomitant to voluntary decrease in energy intake, with report of less hunger, less desire to eat, and greater fullness, both before and after the meals. Physical activity levels, measured by actigraphy, were also voluntarily increased [37].

Before treatment, energy expenditure and fat oxidation were comparable to those of age-, sex- and weight-matched controls. Leptin therapy did not increase energy expenditure, but it prevented the reduction in metabolic rate that is associated with weight loss [34]. Also, the same study showed that leptin therapy increased 24-h fat oxidation to levels higher than those of healthy controls under a 9- to 20-wk low-calorie diet. After adjusting fat oxidation for body fat content, lean mass, energy balance, and composition of the consumed diet, fat oxidation after leptin therapy was higher than predicted in the patients with CLD, and greater than in the controls. Similar results regarding changes in body weight and energy expenditure were reported in other cohorts, in which leptin therapy did not increase energy expenditure [8,16].

3.2. *Lipids and glucose metabolism*

In the Turkish cohort, leptin replacement normalized blood lipids (reducing triglycerides and increasing HDL), and reduced insulin levels and glucose. Also, glucose levels in the oldest patient, who had the diagnosis of type 2 diabetes before leptin therapy, decreased to normal levels, from 7.3 mmol/L before treatment, to 4.8 mmol/L after 18 months of leptin therapy [37]. Similar effects on triglycerides, HDL and insulin have been observed in the other patients as well [16,20].

Leptin regulates pancreatic β-cells function, by reducing the transcription of insulin, stimulating β-cell proliferation, inhibiting insulin secretion, and suppressing β-cell apoptosis [50]. By measuring glucose, insulin and C-peptide during meal tolerance tests and oral glucose tolerance tests, we showed that metreleptin increased insulin sensitivity by at least 5.7-fold, increased insulin hepatic extraction, and decreased insulin secretion [28]. Leptin withdrawal led to substantial weight gain, up to 10.0 kg after 6 weeks off-leptin, which determined an acute and transient increase in insulin sensitivity while off leptin, as the newly acquired adipose tissue absorbed glucose in excess [42].

3.3. *Liver steatosis*

In the Turkish cohort, liver enzymes and other biomarkers of liver function were normal before and after leptin replacement was initiated. In the Austrian patient, severe hepatic steatosis was reported before treatment. After 23 weeks of leptin replacement, liver fat content was reduced from 49.7% to 9.4%, with concomitant normalization of serum transaminases [46].

3.4. *Hypothalamic–pituitary–gonadal axis*

Leptin plays a crucial role in reproduction, regulating GnRH secretion in interaction with pro-opiomelanocortin (POMC), neuropeptide Y/Agouti-related protein (NPY/AgRP) and Kiss1 neurons in the arcuate nucleus [51]. Before treatment, the Turkish adults were hypogonadal: The adult male was prepubertal at age 27; the youngest female (age 35) had normal pubic

and axillary hair, small ovaries and borderline uterus volume, and diminished mammary tissue; the oldest female (age 40) had sparse pubic and axillary hair, small uterus and ovaries, and no mammary tissue. Both females had delayed spontaneous menarche between ages 29 and 35, with irregular menses. Gonadotropin responses to gonadotropin-releasing hormone (GnRH) stimulation were normal, compatible with hypogonadotropic hypogonadism [37].

After treatment, the most remarkable effect was the full development of secondary sexual characteristics [37], with the onset of regular ovulation and menstrual periods. In the adult male, testosterone and free testosterone levels reached normal values for adults, with the development of facial acne and facial, pubic and axillary hair, normal ejaculatory patterns, and growth of penis and testicles. The youngest male spontaneously entered puberty at age 12.

The effects of leptin therapy on raising basal and stimulated luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels to pubertal values, and on the initiation of nocturnal pulsatility have been also described in the Austrian patient, whose menarche occurred at age 16.3 years, 76 weeks after leptin was initiated [47].

3.5. *Hypothalamic–pituitary–adrenal axis and biomarkers of cardiovascular disease*

Our patients had normal levels of serum and urinary cortisol, differently to the hypercorticosteronemia that is observed in the *ob/ob* mice [37]. Serum cortisol levels were fully suppressed with 1 mg of dexamethasone [25]. During a 24-hour frequent blood sampling, leptin replacement increased the mean 24-hour levels of serum cortisol, from 111.46 ± 6.07 nmol/L to 164.71 ± 8.28 nmol/L, and altered its circadian rhythm, by decreasing the number of pulses, increasing their amplitude, increasing the morning peak, and increasing regularity [37]. In the patients of Pakistani origin, urinary and serum cortisol levels were within normal ranges as well, before and during leptin therapy [16].

Leptin exerts a sympatho-excitatory effect, contributing to obesity-induced hypertension. Besides, it contributes to the pathogenesis of cardiovascular disease by inducing a proinflammatory/prothrombogenic state, changing lipid metabolism, enhancing the generation of reactive oxygen species, and impairing vasorelaxation [52–54]. Levels of biomarkers of inflammation, coagulation, fibrinolysis and platelet aggregation were heterogeneous in the three adult Turkish patients. Leptin replacement led to changes in most of those biomarkers, but a pattern could not be established. However, leptin withdrawal tended to determine changes toward a decreased state of thrombogenesis and increased fibrinolysis [43], suggesting that the absence of leptin may protect against cardiovascular disease, even in a morbidly obese state, and that leptin excess leads to a proinflammatory state [55].

3.6. *Hypothalamic–pituitary somatotropic axis*

The adult patients' heights were within the family average, with no history of delayed or impaired growth. Growth hormone (GH) levels were undetectable, and responses to insulin-induced hypoglycemia and exercise tests were absent in the male (nadir glucose level, 2.05 mmol/L; GH of 0.1 μg/L in both tests) and in the younger adult female (nadir glucose level, 1.94 mmol/L; GH, 0.1 μg/L in the hypoglycemia test and 0.3 μg/L in the exercise tests). These results are probably attributed to obesity, given the absence of clinical features of growth hormone deficiency [25].

Before treatment, the youngest male's height was at the 50th percentile in the growth chart, with growth deceleration after treatment was initiated (from the 50th to the 10th percentile), possibly due to weight gain and inadequate dose adjustments. Doses were adjusted, and now the patient is between the 10th and the 25th percentile, within the targeted height. In patients of Pakistani origin, growth was normal before and during leptin therapy, with normal levels of insulin-like growth factor-1 (IGF-1) [16].

In the adults, all of the IGF-related parameters were within the normal range, except for a low postprandial insulin-like growth factor-binding protein-1 (IGFBP-1) [37]. Treatment did not change IGF-1, IGF-2, IGFBP-3 and IGFBP-6 levels, and increased IGFBP-1 and IGFBP-2 levels at the 18th month [37]. In three leptin-deficient patients of Pakistani origin, baseline mean IGFBP-2 levels were lower than those observed by us, which increased in two of the patients six months after leptin therapy. However, IGFBP-2 levels were not significantly different at baseline and post-therapy [35]. The increase in IGFBP-2 in some patients could be associated with the improvement of hepatic insulin sensitivity, but others suggest that leptin's effects on insulin resistance are independent of physiological levels of IGFBP-2 [56].

3.7. *Hypothalamic–pituitary–thyroid axis*

Leptin has a role in regulating thyroid stimulating hormone (TSH) secretion, and its absence may lead to thyroid dysfunction. Leptin has a highly organized circadian rhythm, with a pattern similar to that of TSH: a nadir in late morning and a peak in the early morning [57]. Thyroid function tests were normal for all our Turkish patients [43], but the leptin-deficient adult male had dysregulated patterns of TSH pulsatile and circadian rhythms [57]. Also, the girl who died before receiving treatment had subclinical hypothyroidism. Leptin replacement in the Turkish patients did not increase free T4 or T3 [43], as previously observed in three leptin-deficient children of Pakistani origin [16]. Subclinical hypothyroidism has been reported in other patients as well, which subsided upon leptin therapy initiation [20].

3.8. *Immunity*

T cell hyporesponsiveness has been reported in some patients with CLD [16], but not all [24,45]. In the Turkish child, lymphocyte proliferative responses to the mitogens phytohemaglutinin, concanavalin A, pokeweed, and tetanus and candida antigens were normal at baseline (except for tetanus). Two and six weeks after treatment, leptin enhanced T cell responsiveness, as shown by significant increases of responses to antigens, except for tetanus [45].

No changes in immune blood counts were observed in the Turkish adults after leptin therapy was initiated. Also, neither hypersensitivity reactions nor autoimmune diseases were detected. No hematologic abnormalities suggesting the development of lymphoma have been observed in nearly 14 years of leptin therapy.

Gibson et al. have reported that, in a Pakistani girl, white blood cell count doubled within the first month of therapy and remained elevated for the first 3 months, without any evidence of infection. Interestingly, the patient was asthmatic before therapy, and did not require hospital visits for asthma in the 12 months following therapy initiation [20]. Farooqi et al. reported that another child of Pakistani origin had a normal total lymphocyte number and a normal number of CD3 cells, with reduced CD4 cell number and increased CD8 and B cells, which normalized during leptin therapy [16].

3.9. *Brain structure and function*

Leptin has remarkable effects on brain structure and function. Eighteen months of leptin therapy increased gray matter concentration in three brain regions of the Turkish adults: anterior cingulate gyrus, parietal lobe, and medial cerebellum [40]. Annual withholding of replacement for several weeks reversed this effect in the anterior cingulate gyrus and medial cerebellum, and treatment re-initiation led to an unexpected increase in gray matter concentration in the posterior half of the left thalamus, particularly the pulvinar nucleus, which are areas implicated in neural circuits regulating hunger and satiation [39].

In functional studies, leptin replacement reduced activation of regions linked to hunger (insula, parietal and temporal cortex) and enhanced activation of regions linked to inhibition and satiety (prefrontal cortex), as well as the posterior lobe of the cerebellum, the brain region with the highest concentration of leptin receptors [30]. These results show that leptin has extra-hypothalamic effects in the regulation of food intake, reversibly altering neural structure and function, and modulating plasticity-dependent brain physiology in response to food cues [29].

In the Austrian patient, acute leptin therapy did not reduce activity in hunger-related regions, as we have previously reported [29]. In functional studies, the investigators observed activation in reward- and food-related areas (ventral striatum and the orbitofrontal cortex), and acute and long-term effects were observed in the hypothalamus, striatum, amygdala, orbitofrontal cortex, frontopolar cortex, and substantia nigra/ventral tegmental area [32].

The procognitive effects of leptin were observed in the youngest Turkish male patient, who showed improvements of several subtests within neuropsychological functioning tests [26].

4. Leptin replacement therapy in patients with lipodystrophy

Leptin replacement therapy is the treatment of choice for congenital generalized or acquired generalized lipodystrophy not associated with use of highly active antiretroviral therapy (HAART), recently approved by the FDA [10,58]. Administered as a subcutaneous injection once or twice a day, metreleptin reverses the metabolic abnormalities that are seen in lipodystrophy (reviewed in [58]). Significant reductions in fasting plasma glucose have been reported, as well as in fasting insulin, hemoglobin A1c (HbA1c), serum triglycerides, total cholesterol, and LDL-cholesterol. Also, significant improvements in overall liver health were reported, with significant reductions in mean liver volume, in alanine transaminase (ALT), and in aspartate transaminase (AST). Metreleptin has not been approved by the FDA for treating patients with partial forms of lipodystrophy, due to the lack of strong evidence supporting its safety and effectiveness in a more heterogeneous group. Similarly, it has been demonstrated that metreleptin improves some metabolic parameters and reduces visceral fat in patients with HIV/HAART-associated lipodystrophy syndrome [59,60], but its approval by the FDA depends on the conduction of large, randomized, placebo-controlled trials [61].

5. Leptin replacement therapy in patients with hypothalamic amenorrhea

Hypothalamic amenorrhea is a state of energy deprivation, usually associated with strenuous exercise or anorexia nervosa, in the absence of organic disease or ovarian failure. In those conditions, reduced leptin levels lead to impaired secretion of gonadotropin-releasing hormone and reproductive dysfunction. In all conditions (including non-athletic forms of hypothalamic amenorrhea), fat mass is decreased, determining substantial reductions in serum leptin levels, osteoporosis, and anovulation in women [62].

The effects of leptin replacement therapy in patients with hypothalamic amenorrhea have been demonstrated in open-label [63], and randomized double-blinded placebo-controlled trials [64,65], with improvement or normalization of the thyroid, somatotropic, adrenal and gonadal axes [64], and increases in bone mineral density and content at the lumbar spine level [65]. Also restoration of CD4+ T-cell count and of their proliferative potential has been observed [66], but without changes in angiogenesis factors [67].

6. Leptin replacement therapy in patients with common obesity

Although leptin replacement therapy determines impressive weight loss in patients with mutations in the leptin gene (and, to a lesser extent, in patients with lipodystrophy and hypothalamic amenorrhea), metreleptin has a very limited role in the treatment of patients with common obesity, due to the fact that these patients are hyperleptinemic and have central leptin resistance [68].

In the first trial to evaluate the effects of leptin in common obesity, considerable variability regarding weight loss was demonstrated with high doses of metreleptin (up to 0.3 mg/kg/day [mean −7.1 kg, SD 8.5]) [69], with larger response observed in patients receiving higher leptin doses. Similarly, absent response was observed in subsequent studies with obese individuals treated with leptin and advised to adhere to lifestyle modifications [70], and in the follow-up of patients submitted to Roux-en-Y gastric bypass [71]. In obese patients with type 2 diabetes, 20 mg/day of metreleptin did not alter body weight [72]. In a prospective, single-blinded study, the administration of leptin during maintenance of 10% weight loss did not prevent the increase in bone resorption and the decrease in bone formation that is associated with weight loss [73]. The addition

of pramlintide (an amylin-analog to the hormone secreted by the pancreas that elicits short-term satiety) to metreleptin therapy increased the amount of weight loss to 12.7% (vs. 8.2% with metreleptin alone) [74]. However, this study was suspended by Amylin Pharmaceuticals and Takeda Pharmaceuticals due to the development of anti-metreleptin antibodies.

Should leptin sensitization strategies with agents such as exendin-4 and fibroblast growth factor 21 prove to be effective in humans, metreleptin may become a therapeutic option in patients with common obesity, for either leading to weight loss, or for preventing weight regain [75]. Finally, clinical trials are needed to evaluate whether leptin therapy is effective for patients with relatively low leptin levels in the context of severe metabolic syndrome [76], or patients who are heterozygous for leptin-inactivating mutations [77].

7. Leptin replacement therapy in patients with type 1 and type 2 diabetes

Leptin therapy is effective in improving blood sugar levels in animals, by decreasing hepatic glucose production, increasing glucose uptake, inhibiting glucagon secretion, and modulating virally-induced autoreactive destruction of beta cells [78]. There is plenty of rationale for employing leptin as an adjuvant to insulin for type 1 diabetes, particularly because patients with that condition are relatively leptin-deficient [79]. However, clinical trials are still being undertaken (ClinicalTrials.gov identifier: NCT01268644).

In patients with type 2 diabetes, leptin therapy did not significantly affect body weight, body composition, insulin sensitivity and HbA1c [72,80], which may be attributed to insulin resistance. However, non-obese diabetic patients with normal or low leptin levels may benefit from this therapeutic approach, since they frequently have low adipose tissue mass and are more leptin-sensitive [79]. Several studies involving animals and humans have demonstrated that leptin may have a therapeutic role in lipodystrophic and nonlipodystrophic insulin-resistant and diabetic patients [81].

8. Leptin replacement therapy for other conditions

Metreleptin has been administered to patients with Rabson–Mendenhall syndrome, which is caused by mutations affecting the insulin receptor gene, leading to severe insulin resistance. The 1-year effects of metreleptin therapy were reported in five patients with that syndrome (the 10 year-effects were also reported in two of these patients), showing decreases in serum glucose, HbA1c, insulinemia (in the patients not on insulin), insulin dose, caloric intake and fat mass [82].

It has been demonstrated that leptin improved NAFLD (nonalcoholic fatty liver disease) in lipodystrophic patients [83]. A clinical trial has been conducted to assess this effect in non-lipodystrophic patients with nonalcoholic steatohepatitis (NASH) and relatively low circulating levels of leptin, but the results of this trial are not yet available (ClinicalTrials.gov identifier: NCT00596934).

Leptin deficiency is associated with depression in animals and humans [84], and leptin therapy has antidepressant-like behavioral effects in rodents [85]. Similarly, leptin deficiency has also been associated with dementia [86]. Given its remarkable neuroplastic effects, leptin may become a therapeutic tool in those conditions [87,88].

9. Future perspectives: Overcoming central leptin resistance

Leptin resistance contributes to obesity, type 2 diabetes and hypertension. The hypothalamus is the main brain area for leptin action to control food intake, energy expenditure and glucose homeostasis. There are two crucial intracellular factors that negatively control leptin signaling, and that play key roles in leptin resistance: 1) the suppressor of cytokine signaling-3 (SOCS3), and 2) the protein tyrosine phosphatase 1B (PTP1B). Mice with neuronal deficiency of SOCS3 or PTP1B display improved leptin and insulin sensitivity, and are resilient to body weight gain when exposed to high-fat diet [89].

Interestingly, cumulative evidence supports the concept that leptin resistance arises from hypothalamic neuroinflammation and neurodegeneration. Excessive caloric intake can cause chronic low-grade inflammation, affecting peripheral and central molecular pathways, which in turn leads to obesity, insulin resistance, glucose intolerance, and hypertension [90].

Evidence gathered for over a decade suggests that the adult hypothalamic neurogenesis plays a key role in the control of food intake and energy balance. In rodent studies, it was recently shown that high-fat diet induced hypothalamic inflammation, altered the hypothalamic neurogenesis and tipped the balance toward increased food intake, decreased energy expenditure, and increased insulin resistance. High calorie consumption led to hypothalamic astrogliosis and microglial activation, and caused a 15% reduction of proopiomelanocortin (POMC) neurons, which are activated by leptin to convey anorectic behavior and increased energy expenditure [91]. Thus, neuroinflammation could cause leptin resistance, altering the neuronal balance between orexigenic/anorexigenic neurons.

The nuclear factor kappa-light-chain-enhancer of activated B cells (Nf-κB), a transcriptional factor with well-established proinflammatory actions, seems to play a decisive role during high-fat diet induced hypothalamic leptin resistance [90]. By engineering brain knockout mice of key elements that participate in Nf-κB activation, it was demonstrated that this pathway is essential in triggering central leptin resistance [90]. Knockout mice of the Toll receptor accessory protein Myd88 [92] or the kinase IKKβ [93] were shown to prevent leptin resistance and body weight gain during high-fat diet induced obesity. Their phenotype showed resemblance to the neural SOCS3 knockout mice [94]. Thus, it seems that chronic overnutrition leads to central activation of the Nf-κB pathway, causes impaired intracellular signaling, decreases neurogenesis, and increases neurodegeneration [90]. In rodent studies, it was recently shown that inhibition of hypothalamic inflammation by targeting Toll-like receptor-4 or tumor necrosis factor α (TNFα) signaling proved to be successful in reversing diet-induced insulin resistance in the liver [95]. The latter results indicate that antiinflammatory interventions at the hypothalamic level

Table 3 – Potential uses of metreleptin.

Disorders associated with leptin deficiency	Generalized lipodystrophy [*] Partial lipodystrophy [58] Congenital leptin deficiency due to mutations in the leptin gene [4] Hypothalamic amenorrhea [62–67]
Disorders associated with normal or high serum leptin levels	Common obesity (patients with relative hypoleptinemia) [#] Common obesity for the prevention of weight regain [#] Type 1 diabetes [#] Type 2 diabetes [72,80] Rabson–Mendenhall syndrome [82] Nonalcoholic fatty liver disease [#] Neurodegenerative disorders and depression [#]

[*] Metreleptin is approved by the FDA only for non-HIV generalized lipodystrophy.
[#] Clinical trials are needed.

render beneficial metabolic-related outcomes both in the central nervous system and the periphery.

Presumably, overcoming leptin resistance is one of the most crucial challenges for employing leptin in anti-obesity treatments. Previous studies have shown that leptin resistance can be modulated by diet and exercise. New evidence has also shown that hypothalamic inflammation is a key etiological factor in the pathophysiology of obesity. Interestingly, in a recent rodent study, it was shown that the daily peripheral administration of a plant compound, ginsenoside Rb1, known for its antiinflammatory actions, decreased hypothalamic inflammation and reversed some of the outcomes associated to the obese phenotype [96].

Other pharmacotherapeutic approaches aimed at overcoming leptin resistance employed the combined administration of leptin and other weight loss-inducing hormones such as amylin [97], the long-acting glucagon-like peptide Exedin-4, or fibroblast growth factor 21 (FGF21) [75]. It appears that the efficacy of these three co-treatments is similar. In the future, antiinflammatory interventions and combined administration of leptin and weight-reducing hormones should be further investigated.

10. Conclusions

Metreleptin has profound metabolic effects in the regulation of food intake, energy expenditure, body weight, glucose and lipid metabolism, hypothalamic-pituitary axes (thyroid, adrenal, somatotropic and gonadotropic), immunity, and brain structure and function. Currently, it is approved for the FDA only for the treatment of patients with non-HIV generalized lipodystrophy, but its effects are potentially useful in patients with other conditions associated with low, normal or high levels of serum leptin (Table 3).

Continued and new studies on the effects of leptin replacement therapy alone, or in combination with other weight-reducing hormones or antiinflammatory drugs, are needed in preclinical and clinical experimental paradigms. These studies will certainly provide a better understanding of leptin's physiology. The translational potential of results obtained from animal and humans submitted to leptin replacement therapy is that, in the future, patients with CLD, lipodystrophy, hypothalamic amenorrhea, diabetes, common obesity, NAFLD, depression and dementia may benefit from novel leptin-based therapeutic strategies.

Author contributions

All the authors contributed in writing this review.

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Conflicts of interest

The authors declare no conflicts of interest.

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