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Special issue:
Leptin and adiponectin : the Yin and Yang of the adipocyte

Therapeutic use of recombinant methionyl human leptin

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Abstract

Recombinant methionyl human leptin (r-metHuLeptin) was first used as a replacement therapy in patients bearing inactivating mutations in the leptin gene. In this indication, it was shown since 1999 to be very efficient in inducing a dramatic weight loss in rare children and adults with severe obesity due to the lack of leptin. These first clinical trials clearly showed that r-metHuLeptin acted centrally to reduce food intake, inducing loss of fat mass, and to correct metabolic alterations, immune and neuro-endocrine defects. A few years later, r-metHuLeptin was also shown to reverse the metabolic complications associated with lipodystrophic syndromes, due to primary defects in fat storage, which induce leptin deficiency. The beneficial effects, which could be mediated by central and/or peripheral mechanisms, are thought to mainly involve the lowering effects of leptin on ectopic lipid storage, in particular in liver and muscles, reducing insulin resistance. Interestingly, r-metHuLeptin therapy also reversed the hypothalamic-pituitary-gonadal axis dysfunctions associated with hypothalamic amenorrhea.

However, if r-metHuLeptin treatment has been shown to be dramatically efficient in leptin deficient states, its very limited effect in inducing weight loss in common obese patients revealed that, in patients with adequate leptin secretion, mechanisms of leptin resistance and leptin tolerance prevent r-metHuLeptin from inducing any additional effects.

This review will present the current data about the effects of r-metHuLeptin therapy in humans, and discuss the recent perspectives of this therapy in new indications.

Introduction

The discovery of leptin in 1994 [1] rapidly allowed the production of r-metHuLeptin (Amgen, Thousand Oaks, CA, USA then Amylin, San Diego, CA, USA), a recombinant analog of human leptin composed by the 146 aminoacids of mature human leptin with an additional methionyl residue at the N-terminal end of the recombinant protein. R-metHuLeptin is the only pharmaceutical form of leptin at present. An efficient replacement therapy using recombinant methionyl human leptin (r-metHuLeptin), in rare patients with primary leptin deficiency due to inactivating mutations in the leptin gene [2] or to lipodystrophic syndromes [3]. Beside the treatment of these rare conditions, r-metHuLeptin also generated great hopes for the treatment of common obesity, but clinical trials using pharmacological doses of r-metHuLeptin showed a very limited effect in inducing weight loss in most of obese patients [4]. Since then, the better understanding of leptin involvement in metabolic regulations allowed to consider new indications for the use of r-metHuLeptin as a therapeutic agent, alone or in combination. In addition to its major role in the regulation of energy homeostasis and cellular triglyceride storage, leptin also appeared as a regulator of endocrine and immune functions.

1- Replacement therapy with r-metHuLeptin

1.1 Leptin deficiency syndromes linked to inactivating mutations in the leptin gene

Patients with congenital leptin deficiency due to inactivating homozygous mutations in the leptin gene are mainly characterized by a very severe obesity with marked hyperphagia [5]. Leptin replacement was shown in 1995 to induce dramatic weight loss in *ob/ob* mice [6,7], and the first results of r-metHuLeptin therapy in humans with congenital leptin deficiency were reported in 1999 [2].

R-metHuLeptin was administrated in various doses. In affected children from the first reported family, r-metHuLeptin therapy has been initially designed to produce plasma leptin levels at only 10% of those predicted by height and weight (i.e., approximately 0.028 mg/kg of lean body mass, administrated subcutaneously once daily at 8 a.m.) [2]. However, if the clinical response was not sufficient, r-metHuLeptin doses were increased to achieve 20 to 150% of the predicted leptin concentration [8]. In three adults from another family, r-metHuLeptin therapy started at doses of 0.02 to 0.04 mg/kg/d through a once daily injection in the evening, and doses were subsequently adjusted, on the basis of the clinical responses, to 0.01 to 0.04 mg/kg/d (0.3 to 3.6 mg/d) [9-11].

In these patients, r-metHuLeptin reduced food intake via neural circuits that diminish the perception of food reward and enhance the response to satiety signals [12-14], leading to a sustained loss of weight, mainly due to the loss of fat mass, both in

children [2,15] and in adults [9]. In contrast to other situations of weight loss, the leptin replacement-induced reduction of fat mass was not associated with a decrease in basal metabolic rate or in fat oxidation [16].

Altered glucose and lipid homeostasis linked to severe obesity were also reversed after r-metHuLeptin therapy [8,9,15]. Insulin resistance, as assessed by hyperinsulinemia, decreased in all patients, in parallel with the gradual loss of fat mass, and an adult woman with diabetes achieved normal HbA1c levels after r-metHuLeptin therapy, without any other antidiabetic agent. Metabolic investigations, using standardized meal tests, have shown that r-metHuLeptin acutely increases hepatic extraction of insulin. However, a long-term (24 months) therapy resulted in a 2-fold decrease in insulin secretion and delivery, with a 10-fold increase in insulin sensitivity [17]. Although the pathophysiological mechanisms were not fully understood, a short interruption in the treatment, in the context of rapid weight gain, has been shown to further increase insulin sensitivity in genetically leptin-deficient adults [18]. In accordance, adiponectin plasma levels increased during r-metHuLeptin therapy, but were even higher after a 7 week-interruption period [11]. Regarding lipid profile, serum LDL-cholesterol and triglycerides decreased while HDL-cholesterol increased under r-metHuLeptin treatment [8,9,15].

Complete leptin deficiency is also associated with several neuro-endocrine defects. Hypogonadotropic hypogonadism is a common feature in congenital leptin deficiency, although spontaneous puberty occurred in some patients. r-metHuLeptin therapy led to normal maturation of the hypothalamic-pituitary reproductive axis in adults [9], and allowed appropriately timed pubertal development in treated children [8]. R-metHuLeptin administration did not cause precocious activation of the hypothalamic-pituitary gonadal axis in treated children, showing that leptin acts as a permissive factor for the development of puberty. In addition, the physiological synchronized circadian rhythmicity of leptin and thyrotropin secretion is impaired in leptin-deficient subjects [19], and central hypothyroidism has been diagnosed in some, but not all cases of congenital leptin deficiency [8,15,20]. R-metHuLeptin therapy has improved thyroid function tests in affected patients [8,15]. Although the hypothalamic-pituitary adrenal axis does not seem to be severely affected in these patients, it has been observed that r-metHuLeptin therapy improved the dynamics of cortisol secretion [9]. Growth was not significantly impaired in untreated affected children, and age-related insulin-like growth factor (IGF)-1 levels were in the normal ranges in all patients [8,9]. IGF binding protein (IGFBP)-1 levels were low at baseline, and increased during r-metHuLeptin therapy, but this could be secondary to weight loss and/or decreased insulinemia [9]. Serum IGFBP-2 was also increased by r-metHuLeptin therapy, which could participate in the improvement of hepatic insulin sensitivity [21].

CD4+ T cell lymphopenia and hyporesponsiveness, which are observed in congenital leptin deficiency, were dramatically improved by r-metHuLeptin therapy [8,9,15]. The

secretion of interferon γ , a Th1 pro-inflammatory cytokine, which was completely suppressed in non-treated patients, was significantly increased. R-metHuLeptin also increased the secretion of Th2/regulatory cytokines such as interleukin 4 and 10. In addition, under r-metHuLeptin treatment, basal oversecretion of transforming growth factor β decreased to normal levels [8]. Clinically, the improvement in asthma symptoms and the decrease of recurrent infectious episodes under r-metHuLeptin therapy could probably be related, at least partially, to this switch from a predominant Th2 to a Th1 cytokine response [15]. However, this immune reconstitution could also enhance the production of antibodies to r-metHuLeptin, which developed in all patients after about 6 weeks of treatment, and were capable of transient neutralizing leptin activity [8].

Bone mineral density was not shown to be significantly altered in patients with congenital leptin deficiency, both before and after r-metHuLeptin treatment, although one of the adult patients had osteoporosis at baseline, possibly secondary to hypogonadism [10].

A total of 20 patients with congenital leptin deficiency due to distinct mutations in the leptin gene have been identified. Most of these patients are currently on r-metHuLeptin therapy with remarkable beneficial effects on body composition and weight, endocrine functions and immunity [10]. Therefore, antibodies produced against r-metHuLeptin do not seem to induce any long-term significant inhibition of therapeutic activity.

1.2 Lipodystrophic syndromes with leptin deficiency

Monogenic and acquired forms of lipodystrophic syndromes are rare disorders associated with severe metabolic complications such as insulin resistant diabetes, marked hypertriglyceridemia and liver steatosis. In these diseases, fat has a very limited ability to store excess caloric intake as triglycerides, and also shows profound defects in its endocrine functions, including leptin deficiency. Triglycerides, fatty acids and their derivatives are ectopically stored in the liver and in cardiac and skeletal muscles, where they inhibit several insulin-stimulated signalling pathways. Recent advances in molecular genetics of lipodystrophic syndromes have pointed out that several primary defects in adipose lipid droplet formation, maintenance and metabolic regulation play an important role in their pathophysiology [22,23].

Several reports have described the sustained beneficial effects of r-metHuLeptin substitution in several different forms of lipodystrophic syndromes with severe metabolic complications.

1.2.1 Genetic or acquired lipodystrophic syndromes (excluding HIV infection)

In lipoatrophic mice with a primary defect in adipocyte differentiation, which recapitulates the clinical and biological findings observed in human lipodystrophies, including defects in leptin secretion, treatment with low doses of recombinant leptin was able to restore insulin sensitivity, independently, at least partly, of caloric restriction [24]. In addition, transplantation of fat from control, but not from *ob/ob* leptin-deficient animals, was able to reverse the metabolic alterations [25], supporting the hypothesis that fat loss, and particularly fat loss-associated leptin deficiency, plays a major role in the metabolic complications of lipodystrophies.

Subsequently, three open-label trials in patients with lipodystrophic syndromes associated with a severe leptin deficiency have shown that a short-term r-metHuLeptin therapy, although it did not reverse the lipoatrophy itself, has important beneficial effects on glucose homeostasis and dyslipidemia, improving hepatic and muscular insulin resistance with a decrease in liver steatosis and muscle lipid content [3,26,27]. In the first study, r-metHuLeptin was administered at increasing doses up to 0.08 mg/kg/d, in two daily subcutaneous injections, for four months, in nine women with congenital (n=5) or acquired (n=3) generalized lipodystrophic syndromes, and one woman with familial partial lipodystrophy [3]. R-metHuLeptin was well tolerated, but induced a major decrease in the daily caloric intake (on average, 2680 to 1600 kcal/day) with a mean body weight loss of 3.6 kg. Fifty to 65% of the weight loss was attributed to the decrease in liver volume (which reached on average -28%). Resting metabolic rate also decreased, in contrast to what was observed in patients with inactivating mutations in the leptin gene [16]. Fasting triglycerides (mean at baseline, 14 g/l) decreased by a mean 60%, and mean HbA1c in the eight patients with diabetes (9.1% at baseline) by 1.9 pt. The rate of glucose disappearance during an insulin tolerance test, which doubled after r-metHuLeptin therapy, indicated an improvement in whole-body insulin sensitivity. In accordance with results obtained in pair-feed lipodystrophic mice [24], serum triglycerides and insulin rapidly increased after treatment withdrawal, despite a constant food intake, in one patient [3], indicating that the metabolic effects of r-metHuLeptin were, at least partly, independent of caloric restriction. Using hyperinsulinemic euglycemic clamp with isotopes infusion and nuclear magnetic resonance spectrometry, Petersen et al [26] showed in three of these nine patients that r-metHuLeptin therapy improved the severe hepatic and muscular insulin resistance which characterized these patients: a fourfold increase in the rate of glucose infusion rate and a twofold decrease in hepatic glucose production were observed during the hyperinsulinemic euglycemic clamp. However, regarding the insulin resistance at the adipose tissue level, r-metHuLeptin therapy did not significantly decrease the glycerol turnover. These metabolic changes were associated with a dramatic decrease in hepatic triglyceride content (-85%), and an approximately 30% reduction in intramyocellular triglyceride and fatty acylCoA contents [26]. These results were confirmed in three other patients by Simha et al [27].

These studies suggest that the beneficial metabolic effects of r-metHuLeptin therapy could be due predominantly due its anti-steatotic actions, previously observed in liver, islet cells, skeletal muscle and heart [28]. Indeed, accumulation of fatty-acid-derived metabolites inhibits insulin signalling, causing insulin resistance in skeletal muscle and liver [29]. Interestingly, it has been shown that leptin, directly and/or through the sympathetic nervous system, activates adenosine monophosphate-activated kinase (AMPK) in muscle, which in turn activates fatty acid oxidation [28,30]. However Petersen et al did not evidenced any alteration in the fasting respiratory quotient after r-metHuLeptin therapy in generalized lipoatrophic patients, and proposed that intrahepatic and intramyocellular lipid lowering effects were mostly mediated by a reduction in energy intake [26]. Another hypothesis, although not precisely investigated in patients, is that the repression of stearyl-CoA desaturase-1 by leptin could contribute to decreased lipid synthesis, explaining the antisteatotic effects of leptin in the liver [31,32]. It is still unknown if the therapeutic benefits of r-metHuLeptin are due to its central and/or peripheral effects.

Hyperphagia is one of the symptoms of lipodystrophic syndromes, probably due to low endogenous circulating levels of leptin, which are decreased in proportion to the reduced subcutaneous fat stores [33]. Studying eight subjects with congenital or acquired generalized, or partial lipoatrophy, Mc Duffie et al [34] clearly showed that a four-month r-metHuLeptin therapy, at doses up to 0.08 mg/kg/d, did not modified the relative macronutrient composition of consumed food, significantly increased by twofold the satiety time defined as the length of time subjects remained sated after ingestion of a standardized preload meal. In addition, satiation, i.e the time to voluntary cessation of eating from a standardized meal after a 12-h fast, was decreased. Thus, eating behaviour induced by r-metHuLeptin therapy resulted in less caloric, shorter, more satiating meals and longer-lived satiety. This effect may be mediated at least in part through a decreased fasting ghrelin concentration, a peptide produced mainly by the stomach and implicated in the short-term control of hunger onset, which is increased at baseline in patients with lipodystrophies [34]. Of note, the unique patient with partial lipodystrophy investigated in this study experienced minor changes in satiety and satiation compared to patients with generalized lipoatrophy [34].

Other open-label studies have reported that metabolic improvements are sustained during long-term (12 months) r-metHuLeptin therapy with usual replacement doses in two [35] then fifteen [36] patients with generalized lipoatrophy, diabetes, and low leptin levels. In the study from Javor et al [36], weight loss was stabilized after four months of treatment, achieving a total of 4.4 kg on average (BMI decreasing from 21.5 to 20 kg/m²), due to dramatic reduction of appetite whereas resting energy rate was decreased. Improvement of glycemic control (mean decrease in HbA1c of 1.9 pt) was obtained although most patients were able to either discontinue insulin therapy (6 of 9 patients), decrease mean insulin doses by a mean 65% (3 of 9 patients), or discontinue oral antidiabetic therapy (6 of 8 patients), due to euglycemia [36].

Important reductions were observed in triglycerides (from a mean 13.8 to 5.16 g/l), total and LDL-cholesterol (from a mean 2.84 to 1.67g/l, and 1.39 to 0.85 g/l, respectively) but HDL-cholesterol was unchanged. A progressive effect of r-metHuLeptin was evidenced on liver volume (from a mean 3,663 to 2,190 cm³ after 12 months of therapy), due to a decrease in steatosis, as proved in several patients by histological analysis [37], which also showed a reduction by a mean 60% of nonalcoholic steatohepatitis activity, although fibrosis was unchanged.

One patient stopped the treatment after 8 months, due to worsening proteinuria with diagnosis of membranoproliferative glomerulonephritis [36]. However, considering the results obtained in the other 15 patients, a significant reduction in proteinuria and hyperfiltration was demonstrated [38], and confirmed in other studies [31,39] suggesting that r-metHuLeptin is not harmful for the kidney.

One of the patients from this study, diagnosed with acquired generalized lipodystrophy, had diffuse lymphadenopathy at baseline, which aggravated during r-metHuLeptin treatment, although metabolic parameters improved. Histological analysis revealed the presence of a T-cell lymphoma, and the treatment was discontinued after 8 months [36]. Another case of T cell lymphoma was diagnosed under r-metHuLeptin therapy in a patient with acquired lipodystrophy [31]. The responsibility of r-metHuLeptin is far to be proven, and T cell lymphoma may occur in acquired lipodystrophies in the absence of such therapy [40]. However, r-metHuLeptin therapy should probably be avoided in patients with both acquired lipodystrophy and lymphadenopathy.

Finally, in this long-term evaluation study of r-metHuLeptin replacement therapy, seven patients developed binding antibodies directed against leptin, but without evidence of neutralizing activity [36].

Long-term r-metHuLeptin-replacement therapy was subsequently reported in seven Japanese patients with generalized lipodystrophy [39] and confirmed the previous results depicted above. In addition, benefits on glucose and triglycerides levels were achieved as soon as 7 days after treatment initiation, despite the use of very low initial doses of r-metHuLeptin (0.01-0.02 mg/kg/d). Once-daily injection was sufficient to control metabolic homeostasis. Insulin secretion, assessed from insulin response during OGTT, was rapidly and dramatically improved in the two patients with acquired lipodystrophies, whereas it remains depressed in congenital generalized lipodystrophies due to seipin mutations or of unknown cause. Non neutralizing antileptin antibodies were detected in two patients [39].

Immune modulatory effects of r-metHuLeptin have been investigated by Oral et al using peripheral blood mononuclear cells (PBMC) from ten patients with lipodystrophic syndromes during a four to eight-month replacement therapy [41]. R-metHuLeptin induced a significant increase in the absolute number of T lymphocytes, with a modest increase in activated (CD3+ CD25+) T cells. Stimulated TNF α production by patients' PBMC, which was low at baseline, was normalized by the treatment. The authors did not note any changes in the biological markers of auto-

immunity during therapy. Whether r-metHuLeptin therapy could exacerbate autoimmune diseases, frequently associated with lipodystrophic syndromes, remains to be established.

The precise impact of r-metHuLeptin therapy on body composition at short and long-term (12 months) has been investigated in 14 lipodystrophic patients by Moran et al [42]. As previously observed, r-metHuLeptin therapy decreased calorie intake by approximately 45% and resting energy expenditure by about 11% at the four-month time point, but remained stable thereafter. The same trend was observed for weight (mean -3kg) and fat mass (mean -0.4 kg). In contrast to what was reported in patients with inactivating mutations in the leptin gene, r-metHuLeptin also decreased lean body mass (on average by 2.9 kg) by the fourth month of therapy, without any significance difference in the 4- to 12- month values. There was no change in bone mineral content or density, calcium, phosphorus, vitamin D, or markers of bone formation and resorption during r-metHuLeptin replacement, as previously observed by Simha et al in two women with generalized lipodystrophy [43]. Bone density was also unchanged for up to 36 months of r-metHuLeptin therapy in seven lipodystrophic patients studied by Ebihara et al [39].

These results have to be interpreted together with those regarding other endocrine changes. Indeed, a four-month r-metHuLeptin therapy in seven women with lipodystrophy has been shown to restore a normal response to growth hormone-releasing hormone (GHRH) stimulation and to improve the hypothalamic-pituitary gonadal axis functions, with correction of the attenuated response of luteinizing hormone (LH) to LHRH. R-metHuLeptin also increased estradiol and decreased testosterone serum levels, and improved menstrual abnormalities, whereas thyroid and adrenal functions appeared normal at baseline and were unchanged [44]. The beneficial effect of r-metHuLeptin on the gonadal axis has been confirmed in ten, then 23 hypoleptinemic lipodystrophic women [45,46], the latter trial having used higher doses of r-metHuLeptin (up to 0.24 mg/kg/d, adapted to metabolic control) [46]. Most of the patients had polycystic ovary syndrome with menstrual irregularities at baseline, and almost all had recovered normal menses after therapy [46]. Serum testosterone decreased and sex-hormone binding globulin (SHBG) increased after 1 yr of treatment, by about two-fold, although basal levels of gonadotropins and ovarian size were not significantly modified [45,46], in favour of a predominant role of higher insulin sensitivity in improved ovarian functions. However, a qualitative improvement in gonadotrophin pulsatility in response to r-metHuLeptin cannot be ruled out. In accordance with results from Javor et al [36], IGF1 levels significantly increased in response to 1 yr- therapy [45,46]. As observed in patients with primary congenital defects in leptin, r-metHuLeptin treatment did not induced LH secretion prior to appropriate pubertal timing, but had a permissive effect, which was evidenced by an increase in the LH response to LHRH in three adolescents [45].

R-metHuLeptin has also been administered to children with congenital lipodystrophy and leptin deficiency, before the onset of diabetes [47]. Seven children (of 2.4 to 13.6 years-old) were treated for four months as part of an open-label trial. R-metHuLeptin did not induce any significant changes in weight, fat mass or puberty status, but clearly reduced triglycerides level (by a mean 63%), liver volume (- 20.3%) and increased insulin sensitivity by 30%, through an increase in peripheral glucose uptake, as assessed by euglycemic hyperinsulinemic clamps [47]. However, in five of eight children treated for 28 months, a partial or total resistance to r-metHuLeptin therapy was evidenced, which was associated with the early appearance of binding antileptin antibodies, exhibiting an *in vitro* neutralizing activity in two patients [48].

The efficacy of r-metHuLeptin replacement therapy to improve metabolic alterations seems to depend on the clinical forms of lipodystrophy. In six women with Dunnigan-type familial partial lipodystrophy (FPLD2), with hypoleptinemia (mean 3.8 ng/ml), dyslipidemia and diabetes, 0.08 mg/kg/d of r-metHuLeptin over 12 months induced moderate effects on weight loss (62.1 to 60.2 kg on average), with non-significant reductions in resting energy expenditure, percent body fat and liver volume [49]. Despite a decrease in fasting glycemia and an improvement in insulin sensitivity (assessed by the insulin tolerance test, ITT), HbA1c remained elevated (8.4 to 8%, non significant). Oral glucose tolerance tests showed that impaired glucose tolerance and insulin response were not improved by the treatment. These disappointing results could be due to depressed endogenous insulin secretion in these patients [49]. However, r-metHuLeptin was significantly efficient in reducing hypertriglyceridemia [49].

A patient with FPLD3 and low leptin levels due to PPARG mutation, was treated with r-metHuLeptin, with relatively high doses (up to 0.12 mg/kg/d) [50]. In contrast to results obtained in FPLD2, dramatic improvements in both glucose homeostasis and triglyceridemia were observed at 18 months, with increased insulin sensitivity (assessed by ITT) but also increased insulin secretion in response to an oral glucose tolerance test. Therefore, effects of r-metHuLeptin on β -cell function, which was only investigated during OGTT in rare studies, remains unclear. No improvement in insulin secretion was observed in six women with FPLD2 [49], in six patients with generalized lipodystrophy [36], and in five patients with congenital lipodystrophy due to seipin mutations [39]. In addition, as noted below, the severe fasting insulinopenia observed in two cases of acquired lipodystrophy associated with type 1 diabetes was not reversed by r-metHuLeptin, although an improvement in insulin sensitivity and a decrease in serum triglycerides and liver steatosis were observed [51]. However, insulin secretion was dramatically improved after r-metHuLeptin therapy at least in a patient with FPLD3 and in two patients with acquired generalized lipodystrophy [39]. Therefore, further studies are needed with precise metabolic investigations to know if r-metHuLeptin therapy is able to improve relatively preserved insulin secretion in lipodystrophic patients.

Recently, the group of P. Gorden reported their 8-yr experience of r-metHuLeptin replacement therapy in 48 patients with acquired or inherited, generalized or partial lipodystrophies with metabolic alterations [31]. They confirmed the significant and sustained improvements in glycemic control and dyslipidemia. Some patients showed an apparent secondary refractoriness to r-metHuLeptin, which was thought to be caused by a non-compliance to treatment. Therapeutic doses have been adapted to the metabolic responses, and the maximal dose (0.24 mg/kg/d) was still less than previously used in obesity studies [4]. E Oral and J Chan collected the published clinical experience of r-metHuLeptin therapy in lipodystrophies not related to HIV infection [52]. Although all the trials were all open-labelled, it can be conclude, from over 100 patients treated, that r-metHuLeptin is generally safe and efficient in treating the metabolic complications of lipodystrophies, and that the degree of therapeutic response is related to the severity of metabolic abnormalities [52].

From these studies, and from unpublished experiences of other teams [53] including ours, it appears that the magnitude of the response to r-metHuLeptin is probably determined, in part, by the magnitude of fat loss (on average, treatment response are greater in patients with generalized lipoatrophy than in those with partial lipoatrophy), and by the magnitude of metabolic abnormalities at baseline (on average, treatment response are greater in patients with markedly elevated HbA1c and/or triglyceride levels at baseline).

If r-metHuLeptin is very well-tolerated, its immune modulatory effects remains a subject of concerns since it could, in theory, exacerbate auto-immune conditions, frequently associated with acquired forms of lipodystrophy, and/or induce a secondary resistance to treatment due to the appearance of anti leptin antibodies. In addition, two cases of T cell lymphomas were described under r-metHuLeptin replacement therapy. Even if proliferative lymphoid cells disorders can be associated with acquired forms of lipodystrophy in the absence of leptin therapy [40] and personal observations], it is unknown if r-metHuLeptin could have played a promoting role in these two cases.

1.2.2 Lipodystrophic syndromes linked to HIV infection and/or antiretroviral treatment

HIV-associated lipodystrophic syndrome is a collective term for a very heterogeneous set of conditions frequently observed in, and unique to, HIV-infected patients receiving antiretroviral therapy. Briefly, the clinical presentation of HIV-associated lipodystrophic syndrome varies from generalized lipoatrophy to “lipohypertrophy” (with buffalo humps and abdominal obesity), with mixed, intermediate forms in between that resemble partial lipodystrophies. The severity of metabolic complications (i.e. dyslipidemia, insulin resistance with altered glucose tolerance, liver steatosis, cardiovascular diseases) is also highly variable from one form to another, and from one patient to another [54].

Pathophysiological mechanisms of HIV-associated lipodystrophic syndromes are also complex, multiple, and incompletely deciphered. Generally, it seems that the previously used antiretroviral treatments, particularly nucleoside reverse transcriptase inhibitors such as stavudine or zidovudine, had the potential to induce severe lipoatrophy that only partially reversed after switching to newer agents. These treatments are not used anymore in industrialized countries and the incidence of the more severe forms of lipoatrophy has decreased, whereas partial lipodystrophies with abdominal and/or cervical accumulation of fat and metabolic complications develop still frequently. Other factors, like the use of some HIV protease inhibitors or other classes of antiretroviral therapies, but also virally-mediated mechanisms as immune deficiency and low grade inflammatory state, the responses to the therapies, and the aging process, could interfere with adipose tissue distribution, insulin sensitivity and lipid metabolism [54,55].

In contrast to the rare (genetic, idiopathic, or auto-immune-related) forms of lipodystrophy in which a marked and sustained effect of r-metHuLeptin has been well documented in long-term studies, it is presently unknown whether r-metHuLeptin may also have significant long-term therapeutic potential in HIV-associated lipodystrophic syndrome. Indeed, three short-term trials of r-metHuLeptin therapy in patients with HIV-associated lipodystrophy with low leptin levels and hypertriglyceridemia at baseline have provided mixed results [56-58].

In the first study from the group of Mantzoros [56], seven HIV-infected men with highly active antiretroviral therapy (HAART)-associated lipoatrophy, hypoleptinemia (< 3 ng/ml) and moderate hypertriglyceridemia, were treated by r-metHuLeptin (with low doses, i.e. 0.04 mg/kg/d) for two months through a placebo-controlled crossover study. Interestingly, despite a 1 month $\frac{1}{2}$ or a 2 month- therapy, two subjects were withdrawn from the study due to hypertriglyceridemia, reaching values greater than 1000 mg/dl. Multivariate statistical analysis showed that this short-term r-metHuLeptin therapy significantly decreased body fat, mainly due to a decline in trunk fat, and tended to decrease, independently to other factors, insulinemia and the insulin resistance index HOMA-IR ($p= 0.07$ and 0.06 , respectively). R-metHuLeptin also increased HDL-cholesterol levels, at least partially through the decrease in visceral fat mass. R-metHuLeptin was well-tolerated, without any side effects, and without any alteration in HIV control, but had no significant effect on liver volume, percentage of liver fat, blood pressure, inflammatory markers (CRP, IL6, TNF α), serum adiponectin, fasting glucose or triglycerides. The insulin-like growth hormone (IGF) axis (i.e. serum IGF1, IGF2, IGF-binding proteins 1 to 4) was not altered by the therapy [59].

Another study directed by CS. Mantzoros [58] studied the therapeutic combination of pioglitazone and r-metHuLeptin in HIV-infected lipoatrophic hypoleptinemic patients. Five patients receiving the combination therapy were compared to four patients treated by pioglitazone alone, in a double-blinded fashion. R-metHuLeptin therapy, administered for 3 months at a 0.04 mg/kg/d dose, significantly reduced fasting insulinemia and HOMA-IR, as well as postprandial glycemia, and increased serum

adiponectin, but did not affect body mass index, body fat mass and distribution, or serum lipid profile.

Mulligan et al [57] performed an open-label, 6-month pilot study of r-metHuLeptin treatment, at 0.02 then 0.06 mg/kg/d, in eight HIV-infected men with the same inclusion criteria (lipoatrophy, hypoleptinemia, moderate hypertriglyceridemia). Visceral fat significantly decreased after 6 months of therapy (on average, - 32%), with decrease in the trunk-to-limb fat ratio, and loss of body weight (-2 kg on average). Fasting total, LDL-cholesterol and triglycerides decreased. Metabolic investigations, with euglycemic hyperinsulinemic clamps and stable isotope tracer studies, showed that r-metHuLeptin improved the ability of insulin to suppress both endogenous hepatic glucose production (by decreasing both glycogenolysis and gluconeogenesis) and adipose tissue lipolysis (as assessed by a decrease in serum free fatty acids). However, skeletal muscle insulin sensitivity (evaluated by the insulin-mediated whole-body glucose uptake) was not significantly improved by r-metHuLeptin therapy.

Taken together, these trials, which included only a few patients (a total of less than 25 patients) with HIV-associated lipoatrophy, consistently showed that r-metHuLeptin did not aggravate lipoatrophy, and improved hepatic insulin sensitivity. A significant decrease in hypertriglyceridemia was observed in only one study, using a longer duration and higher doses of r-metHuLeptin than the two other trials. The effects of r-metHuLeptin in the most frequent forms of HIV-associated lipodystrophy, i.e. the mixed or lipohypertrophic forms are still unknown. Larger studies are needed to evaluate the long term effect of r-metHuLeptin in HIV-associated lipodystrophic syndromes.

1.3 Hypothalamic amenorrhea

Hypothalamic amenorrhea is due to dysfunction of the hypothalamic-pituitary-gonadal axis in the absence of organic disease. Its clinical spectrum ranges from hormonal abnormalities in strenuously exercising athletes to anorexia nervosa. Affected subjects are hypoleptinemic, which might contribute to neuroendocrine dysfunctions with subsequent anovulation in women, and osteoporosis. In an open-label interventional study [60], three months of r-metHuLeptin treatment with replacement doses in women with amenorrhea improved or normalized the gonadal, thyroid and, to a less degree, growth hormone axes. A recent extension of this pilot study confirmed these results. A randomized double blinded placebo-controlled trial [61] of r-metHuLeptin with replacement doses (0.04 to 0.12 mg/kg/d) over 36 weeks, in 20 women with amenorrhea for a mean duration of 4 or 5 years, showed that r-metHuLeptin therapy resulted in recovery of menstruation (7 of 10 patients), and corrected the abnormalities of the gonadal, thyroid and adrenal axes. However r-metHuLeptin also induced a progressive loss of total body fat mass (of about 2 kg of fat on average), without changes in patients' exercise patterns and eating habits.

Those results are very encouraging for the treatment hypothalamic amenorrhea and the perspective of pregnancy for these patients. However, the risk of weight loss has to be considered, and further studies should be performed to look forward the long-term safety of this therapeutic approach.

As far as bone remodeling is concerned, short-term r-metHuLeptin therapy in these women has been shown to increase circulating levels of bone formation markers [60]. A long-term r-metHuLeptin treatment also showed an increase in bone mineral density and content at the lumbar spine level [62]. However, these effects, which have not been observed in other cases of r-metHuLeptin therapy, could have been linked to other neuroendocrine improvements. Before r-metHuLeptin could be proposed for the treatment of osteoporosis, more studies are required to elucidate the mechanisms underlying the effects of leptin on bone remodelling in humans.

2- Leptin therapy in common obesity

The discovery of leptin in 1994 [1] generated great hopes for the treatment of common obesity. However, subsequent clinical trials using pharmacological doses of r-metHuLeptin were disappointing, showing a very limited effect in inducing weight loss in obese patients [4]. Indeed, the vast majority of obese humans have high circulating leptin levels and are either resistant or tolerant to its weight-reducing effects [63]. The precise mechanisms underlying leptin resistance or tolerance in obesity are still not fully understood.

Recent studies suggested that leptin sensitivity is directly related to endoplasmic reticulum (ER) capacity: ER stress blocks leptin action and generates leptin resistance in mice, suggesting that ER stress provides a potential mechanism for the development of leptin resistance [64]. This data has been recently confirmed in human primary adipocytes in vitro [65], indicating that ER stress may also induce leptin resistance in humans [66] and suggesting that improving ER stress could be used as a strategy to sensitize not only obese mice but also humans to leptin.

Strategies to address leptin resistance in common obesity have included the use of high pharmacological doses of r-metHuLeptin, and/or its coadministration with presumed leptin sensitizers.

An early trial with high doses of r-metHuLeptin (up to 0.3mg/kg/d) resulted in a weight loss which was non significant after 24 weeks of treatment. Indeed, there was considerable variability in the amount of weight lost by individual subjects (mean -7.1kg; SD 8.5) [4]. Moreover, these doses induced skin irritation and swelling at the injection site in 62% of patients and headache in half of the patients.

In a recent double-blinded, placebo-controlled, randomized trial, r-metHuLeptin administration at a dose of 10 mg twice daily (20 mg/d) for 16 weeks did not alter body weight or circulating inflammatory markers in obese patients with type 2

diabetes [65]. In this trial, r-metHuLeptin therapy was associated with an increased leptin binding protein that may decrease circulating free leptin levels.

Recently, amylin, a hormone secreted by the pancreas that also contributes to the regulation of energy homeostasis through hindbrain signals of short-term satiety, was proposed to improve r-metHuLeptin responsiveness in diet-induced obesity [67]. A recent double-blinded randomized study conducted by Amylin Pharmaceuticals, Inc. found that overweight and obese participants lost significantly more weight on the combination of r-metHuLeptin and pramlintide, an analog of amylin (mean weight loss of 12.7%), than on treatment with either agent alone (mean 8.2% for r-metHuLeptin and 8.4% for pramlintide) [68]. This weight loss corresponds to 67 % (\pm 4%) of patients' excess body weight by week 20 (mean initial BMI=32 kg/m²). The greater reduction in body weight was significant as early as week 4, and weight loss continued throughout the 24 weeks of treatment, without evidence of a plateau and with a good tolerance.

Despite those very promising weight-loss results and improvements in metabolic markers in overweight and mildly obese subjects, progress in the development of pramlintide/r-metHuLeptin combination therapy has since stalled, with Amylin Pharmaceuticals and Takeda Pharmaceuticals stating on 16th March 2011 that the "clinical study was voluntarily halted to investigate a new antibody-related laboratory finding with metreleptin treatment in two patients who participated in a previously completed clinical study of obesity" (Press Release, Amylin and Takeda Voluntarily Suspend Clinical Activities in Obesity Trial, 3/16/11). These findings need to be fully resolved before further development of recombinant human leptin, because antibody formation in that study was observed with or without combination of amylin.

A more promising area of clinical interest is the potential role of r-metHuLeptin treatment in weight loss maintenance. It has been proposed that falling leptin levels due to weight loss activate neuroendocrine mechanisms that may drive patients to regain weight. These mechanisms may include increasing energy intake, by increasing hunger, and decreasing energy expenditure, by decreasing thyroid hormone levels and subsequently slowing metabolism [69]. Thus, r-metHuLeptin therapy may avoid these neuroendocrine deleterious responses and prevent "yo-yo" dieting commonly seen in clinical practice. This is currently being investigated and, if successful, may have major implications in weight loss management.

3- Leptin and diabetes

3.1 Type 1 diabetes

If the intensification of insulin treatment in type 1 diabetes reduced in a major way the degenerative complications by improving blood sugar control, it does not restore the metabolic homeostasis compromised by the destruction of the beta-pancreatic islets. Several studies in insulin-deficient rodents suggested that the administration of leptin could treat type 1 diabetes or improve the effect of insulin therapy [70-73], and has a more favorable effect than insulin on lipid and cholesterol metabolism. These effects were not only connected to the appetite-suppressing effect of leptin. Indeed, it has been shown that leptin, in an insulin and nutrition-independent manner, decreased the hepatic glucose output and increased the peripheral glucose utilization [70]. In addition, leptin inhibits the oversecretion of glucagon associated with insulin deficiency, stimulates insulin signaling, activates the muscular IGF1 receptor [72], and reduces the amount of liver triglycerides [73] in insulin-deficient animals. A recent study evidenced that high doses of leptin could also modulate the autoreactive destruction of beta cells in a virally induced rodent model of type 1 diabetes, and prevent hyperglycaemia [74].

The dramatic reduction in adipose tissue observed in animal models of insulin-deficient diabetes makes a good rationale for restoring leptin levels as in lipoatrophic models. Thus, what would be the effect of r-metHuLeptin treatment in type 1 diabetes in humans?

Only one study has reported the effects of r-metHuLeptin therapy in two patients with type 1 diabetes associated with acquired lipodystrophy, which represents an increased risk for autoimmune disorders [51]. R-metHuLeptin (0.08 mg/kg/d) improved the glycaemic control, allowing a decrease in the insulin requirements (3.3–5 U/kg/d to 1 U/kg/d), and lowered circulating triglycerides and liver enzymes after 12 months of therapy. This study showed that r-metHuLeptin reduced insulin resistance and ectopic fat in these patients but had no effect on fasting insulin levels [51].

A phase 1 Interventional study is now running to study the effects of r-metHuLeptin administration in Patients with Type 1 Diabetes Mellitus and will end in 2013 (Principal investigator: A. Garg, Ut Southwestern Medical Center).

3.2 Type 2 diabetes

Type 2 diabetes occurs mainly in subjects with centrally-distributed fat accumulation and is characterized by a decrease in insulin action and an insulin secretory defect. A mouse model mimicking human type 2 diabetes with increased adiposity using low-dose streptozotocin, a toxic pancreatic beta cell drug, and high-fat diet has been treated with recombinant mouse leptin for 14 days using a mini-osmotic pump [75]. Continuous leptin infusion reduced food intake, body weight and insulin resistance. It improved glucose and lipid metabolism independently of the food intake reduction and it reduced liver and skeletal muscle triacylglycerol contents. In order to circumvent peripheral leptin resistance in models of obesity and type 2 diabetes,

application of one time central leptin gene therapy to enhance leptin supply locally in the hypothalamus, has been proposed and offers an efficient and durable antidiabetic effect (review in [76]). However its implementation in humans is not possible in the next future.

The effects of r-metHuLeptin therapy have been evaluated in obese subjects with type 2 diabetes in a double-blinded, placebo-controlled, randomized trial [65]. Subjects were hyperleptinaemic. As mentioned in the paragraph 2., the treatment did not affect body composition. After 16 weeks of treatment, r-metHuLeptin reduced HbA1c marginally but significantly, by 0.1-0.2 %. Long term trials are needed to better evaluate whether r-metHuLeptin therapy may be useful in the therapeutic strategy of type 2 diabetes.

3.3 Diabetes due to extreme insulin resistance

The Rabson-Mendenhall syndrome is one of the most severe forms of insulin resistance, caused by a mutation affecting both alleles of the insulin receptor gene [77]. As the administration of r-metHuLeptin has been shown to improve insulin stimulated hepatic and peripheral glucose metabolism in severely insulin resistant lipodystrophic patients, one study has evaluated r-metHuLeptin therapy in this syndrome of poor prognosis for which treatment options are very limited.

A 11 yr-old girl and her 13 yr-old brother presenting with a phenotype consistent with the diagnosis of Rabson-Mendenhall syndrome, i.e. severe insulin resistance with acanthosis nigricans and diabetes, growth retardation, but normal triglyceridemia and no liver steatosis, were treated with r-metHuLeptin. Unfortunately, the genotype of these patients was not available. Their baseline leptin serum levels were appropriate for fat mass. R-metHuLeptin was administrated twice daily, in addition to their previous antidiabetic therapy, including metformin and rosiglitazone in both patients, and 300U of daily insulin in the male sibling. After gradual increase, the maximal r-metHuLeptin administration doses were stable at 0.09 mg/kg/d for the female and 0.06 mg/kg/d for the male patient, from the sixth month to the last month (10th month) of treatment [78].

After 10 month of treatment, a 40-60% decrease in fasting serum glucose levels and a 15% decrease in HbA1c were observed, concomitantly with a 30% reduction in insulin doses in the male sibling. Fasting insulinemia, which was extremely high at baseline (194 to 268 U/ml), was reduced by 80% in the female patient, who was not treated with exogenous insulin, and glucose levels decreased by 40% as compared with the pretherapeutic levels during insulin tolerance tests, showing that the pharmacological doses of r-metHuLeptin had improved insulin sensitivity. R-metHuLeptin induced a 11% reduction in the mean caloric intake, resulting in a modest loss of fat mass, with lean body mass and resting energy expenditure remaining stable. All these metabolic effects were reversed after treatment withdrawal. Importantly, r-metHuLeptin therapy did not impair the growth and

development of the siblings, and did not induce puberty in the prepubertal sister, consistent with the permissive effect of leptin on gonadotrophin secretion once puberty occurs only [78]. Long-term effects of r-metHuLeptin have not been reported in these children.

4- Other indications for recombinant human leptin therapy?

4.1 Obesity with low leptin serum level

Anthropometric studies were performed by Farooqi et al. in 13 heterozygous subjects with leptin inactivating mutations from the same family, compared to three carriers of the homozygous wild-type allele, and to 96 ethnically-matched controls [79]. Heterozygote carriers of leptin inactivating mutations had significantly lower leptin serum levels than expected for percent body fat, and higher percentage of body fat than predicted from their weight and height. These results suggest that, if increasing leptin levels above a minimal threshold did not significantly alter the energy balance in common obese patients, the sensitivity to serum leptin changes in the low range values could have interesting therapeutic effect in obese subjects with relatively low levels of circulating leptin [79]. However, results of r-metHuLeptin therapy trials in these patients are not available for the moment.

4.2 Depression and dementia

The article by Yamada et al. [80] challenges some conventional views of leptin with evidence that the lack of leptin action in the hippocampus is associated with depressive behaviour in mice. Human studies show links between depression, adiposity, and proinflammatory states [81], and a recent study of r-metHuLeptin replacement therapy in three patients showed *in vivo* evidence of remarkably plastic, reversible and regional specific effects of leptin on human brain morphology (assessed by MRI), affecting in particular the gray matter concentration in areas involved in food intake [82].

Regarding dementia, several studies in cells and in mice suggest that leptin could be protective against Alzheimer disease, in particular through activation of AMPK and the sirtuins, leading to a reduction in the phosphorylation of protein tau and the production of β amyloid substance in neurons [83,84]. In humans, some reports have shown a negative correlation between circulating leptin levels and risk of Alzheimer disease [85,86] or cognitive decline [87]. Interestingly, a longitudinal study of 785 cognitively-normal persons with a mean age of 79 showed that higher circulating levels of leptin were associated with a reduced incidence of dementia and Alzheimer disease, during a median follow-up of 8.3 years [88]. Taken together, these data open favorably the way for clinical trials of recombinant human leptin as a novel therapy for Alzheimer dementia [89].

Conclusion

Administering r-metHuLeptin to correct overt hypoleptinemia significantly improves the metabolic abnormalities in patients bearing inactivating mutations in the leptin gene or lipodystrophic syndromes. R-metHuLeptin therapy have shown significant, albeit less pronounced effect in hypoleptinemic patients with human immunodeficiency virus–induced lipodystrophy and metabolic syndrome. In contrast, obese hyperleptinemic subjects with or without type 2 diabetes do not respond to exogenously administered r-metHuLeptin.

Although r-metHuLeptin allows important metabolic improvements in patients with lipodystrophic syndromes, it is not widely available for the moment. In Europe, the pharmaceutical firm Amylin (San Diego, CA, USA) supports several compassionate programs. This restricted access is an important limit to its clinical use, and, if an expanded FDA-authorized access program is developed in USA, its approval as an orphan drug in Europe is urgently needed.

References

- [1] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, J.M. Friedman, Positional cloning of the mouse obese gene and its human homologue, *Nature* 372 (1994) 425-432.
- [2] I.S. Farooqi, S.A. Jebb, G. Langmack, E. Lawrence, C.H. Cheetham, A.M. Prentice, I.A. Hughes, M.A. McCamish, S. O'Rahilly, Effects of recombinant leptin therapy in a child with congenital leptin deficiency, *N. Engl. J. Med.* 341 (1999) 879-884.
- [3] E.A. Oral, V. Simha, E. Ruiz, A. Andewelt, A. Premkumar, P. Snell, A.J. Wagner, A.M. DePaoli, M.L. Reitman, S.I. Taylor, P. Gorden, A. Garg, Leptin-replacement therapy for lipodystrophy, *N. Engl. J. Med.* 346 (2002) 570-578.
- [4] S.B. Heymsfield, A.S. Greenberg, K. Fujioka, R.M. Dixon, R. Kushner, T. Hunt, J.A. Lubina, J. Patane, B. Self, P. Hunt, M. McCamish, Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial, *JAMA, J. Am. Med. Assoc.* 282 (1999) 1568-1575.
- [5] C.T. Montague, I.S. Farooqi, J.P. Whitehead, M.A. Soos, H. Rau, N.J. Wareham, C.P. Sewter, J.E. Digby, S.N. Mohammed, J.A. Hurst, C.H. Cheetham, A.R. Earley, A.H. Barnett, J.B. Prins, S. O'Rahilly, Congenital leptin deficiency is associated with severe early-onset obesity in humans, *Nature* 387 (1997) 903-908.
- [6] J.L. Halaas, K.S. Gajiwala, M. Maffei, S.L. Cohen, B.T. Chait, D. Rabinowitz, R.L. Lallone, S.K. Burley, J.M. Friedman, Weight-reducing effects of the plasma protein encoded by the obese gene, *Science* 269 (1995) 543-546.
- [7] M.A. Pelleymounter, M.J. Cullen, M.B. Baker, R. Hecht, D. Winters, T. Boone, F. Collins, Effects of the obese gene product on body weight regulation in ob/ob mice, *Science* 269 (1995) 540-543.
- [8] I.S. Farooqi, Leptin and the onset of puberty: insights from rodent and human genetics, *Semin. Reprod. Med.* 20 (2002) 139-144.

- [9] J. Licinio, S. Caglayan, M. Ozata, B.O. Yildiz, P.B. de Miranda, F. O'Kirwan, R. Whitby, L. Liang, P. Cohen, S. Bhasin, R.M. Krauss, J.D. Veldhuis, A.J. Wagner, A.M. DePaoli, S.M. McCann, M.L. Wong, Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults, *Proc. Natl. Acad. Sci. U. S. A.* 101 (2004) 4531-4536.
- [10] G. Paz-Filho, M.L. Wong, J. Licinio, Ten years of leptin replacement therapy, *Obes. Rev.* 12 (2011) e315-323.
- [11] V.P. Andreev, R.C. Dwivedi, G. Paz-Filho, O.V. Krokhin, M.L. Wong, J.A. Wilkins, J. Licinio, Dynamics of plasma proteome during leptin-replacement therapy in genetically based leptin deficiency, *Pharmacogenomics J.* 11 (2011) 174-190.
- [12] I.S. Farooqi, E. Bullmore, J. Keogh, J. Gillard, S. O'Rahilly, P.C. Fletcher, Leptin regulates striatal regions and human eating behavior, *Science* 317 (2007) 1355.
- [13] K. Baicy, E.D. London, J. Monterosso, M.L. Wong, T. Delibasi, A. Sharma, J. Licinio, Leptin replacement alters brain response to food cues in genetically leptin-deficient adults, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 18276-18279.
- [14] S. Frank, M. Heni, A. Moss, J. von Schnurbein, A. Fritsche, H.U. Haring, S. Farooqi, H. Preissl, M. Wabitsch, Leptin therapy in a congenital leptin-deficient patient leads to acute and long-term changes in homeostatic, reward, and food-related brain areas, *J. Clin. Endocrinol. Metab.* 96 (2011) E1283-1287.
- [15] W.T. Gibson, I.S. Farooqi, M. Moreau, A.M. DePaoli, E. Lawrence, S. O'Rahilly, R.A. Trussell, Congenital leptin deficiency due to homozygosity for the Delta133G mutation: report of another case and evaluation of response to four years of leptin therapy, *J. Clin. Endocrinol. Metab.* 89 (2004) 4821-4826.
- [16] J.E. Galgani, F.L. Greenway, S. Caglayan, M.L. Wong, J. Licinio, E. Ravussin, Leptin replacement prevents weight loss-induced metabolic adaptation in congenital leptin-deficient patients, *J. Clin. Endocrinol. Metab.* 95 (2010) 851-855.
- [17] V.P. Andreev, G. Paz-Filho, M.L. Wong, J. Licinio, Deconvolution of insulin secretion, insulin hepatic extraction post-hepatic delivery rates and sensitivity during 24-hour standardized meals: time course of glucose homeostasis in leptin replacement treatment, *Horm. Metab. Res.* 41 (2009) 142-151.
- [18] G. Paz-Filho, K. Esposito, B. Hurwitz, A. Sharma, C. Dong, V. Andreev, T. Delibasi, H. Erol, A. Ayala, M.L. Wong, J. Licinio, Changes in insulin sensitivity during leptin replacement therapy in leptin-deficient patients, *Am. J. Physiol. Endocrinol. Metab.* 295 (2008) E1401-1408.
- [19] C.S. Mantzoros, M. Ozata, A.B. Negrao, M.A. Suchard, M. Ziotopoulou, S. Caglayan, R.M. Elashoff, R.J. Cogswell, P. Negro, V. Liberty, M.L. Wong, J. Veldhuis, I.C. Ozdemir, P.W. Gold, J.S. Flier, J. Licinio, Synchronicity of frequently sampled thyrotropin (TSH) and leptin concentrations in healthy adults and leptin-deficient subjects: evidence for possible partial TSH regulation by leptin in humans, *J. Clin. Endocrinol. Metab.* 86 (2001) 3284-3291.
- [20] G. Paz-Filho, T. Delibasi, H.K. Erol, M.L. Wong, J. Licinio, Congenital leptin deficiency and thyroid function, *Thyroid Res.* 2 (2009) 11.
- [21] K. Hedbacker, K. Birsoy, R.W. Wysocki, E. Asilmaz, R.S. Ahima, I.S. Farooqi, J.M. Friedman, Antidiabetic effects of IGFBP2, a leptin-regulated gene, *Cell Metab.* 11 (2010) 11-22.
- [22] A. Garg, Lipodystrophies: genetic and acquired body fat disorders, *J. Clin. Endocrinol. Metab.* 96 (2011) 3313-3325.
- [23] C. Vigouroux, M. Caron-Debarle, C. Le Dour, J. Magre, J. Capeau, Molecular mechanisms of human lipodystrophies: from adipocyte lipid droplet to oxidative stress and lipotoxicity, *Int. J. Biochem. Cell Biol.* 43 (2011) 862-876.

- [24] I. Shimomura, R.E. Hammer, S. Ikemoto, M.S. Brown, J.L. Goldstein, Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy, *Nature* 401 (1999) 73-76.
- [25] C. Colombo, J.J. Cutson, T. Yamauchi, C. Vinson, T. Kadowaki, O. Gavrilova, M.L. Reitman, Transplantation of adipose tissue lacking leptin is unable to reverse the metabolic abnormalities associated with lipoatrophy, *Diabetes* 51 (2002) 2727-2733.
- [26] K.F. Petersen, E.A. Oral, S. Dufour, D. Befroy, C. Ariyan, C. Yu, G.W. Cline, A.M. DePaoli, S.I. Taylor, P. Gorden, G.I. Shulman, Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy, *J. Clin. Invest.* 109 (2002) 1345-1350.
- [27] V. Simha, L.S. Szczepaniak, A.J. Wagner, A.M. DePaoli, A. Garg, Effect of leptin replacement on intrahepatic and intramyocellular lipid content in patients with generalized lipodystrophy, *Diabetes Care* 26 (2003) 30-35.
- [28] R.H. Unger, G.O. Clark, P.E. Scherer, L. Orci, Lipid homeostasis, lipotoxicity and the metabolic syndrome, *Biochim. Biophys. Acta* 1801 (2010) 209-214.
- [29] G.I. Shulman, Cellular mechanisms of insulin resistance, *J. Clin. Invest.* 106 (2000) 171-176.
- [30] Y. Minokoshi, Y.B. Kim, O.D. Peroni, L.G. Fryer, C. Muller, D. Carling, B.B. Kahn, Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase, *Nature* 415 (2002) 339-343.
- [31] A.Y. Chong, B.C. Lupsa, E.K. Cochran, P. Gorden, Efficacy of leptin therapy in the different forms of human lipodystrophy, *Diabetologia* 53 (2010) 27-35.
- [32] P. Cohen, J.M. Friedman, Leptin and the control of metabolism: role for stearoyl-CoA desaturase-1 (SCD-1), *J. Nutr.* 134 (2004) 2455S-2463S.
- [33] W.A. Haque, I. Shimomura, Y. Matsuzawa, A. Garg, Serum adiponectin and leptin levels in patients with lipodystrophies, *J. Clin. Endocrinol. Metab.* 87 (2002) 2395.
- [34] J.R. McDuffie, P.A. Riggs, K.A. Calis, R.J. Freedman, E.A. Oral, A.M. DePaoli, J.A. Yanovski, Effects of exogenous leptin on satiety and satiation in patients with lipodystrophy and leptin insufficiency, *J. Clin. Endocrinol. Metab.* 89 (2004) 4258-4263.
- [35] K. Ebihara, H. Masuzaki, K. Nakao, Long-term leptin-replacement therapy for lipoatrophic diabetes, *N. Engl. J. Med.* 351 (2004) 615-616.
- [36] E.D. Javor, E.K. Cochran, C. Musso, J.R. Young, A.M. DePaoli, P. Gorden, Long-term efficacy of leptin replacement in patients with generalized lipodystrophy, *Diabetes* 54 (2005) 1994-2002.
- [37] E.D. Javor, M.G. Ghany, E.K. Cochran, E.A. Oral, A.M. DePaoli, A. Premkumar, D.E. Kleiner, P. Gorden, Leptin reverses nonalcoholic steatohepatitis in patients with severe lipodystrophy, *Hepatology* 41 (2005) 753-760.
- [38] E.D. Javor, S.A. Moran, J.R. Young, E.K. Cochran, A.M. DePaoli, E.A. Oral, M.A. Turman, P.R. Blackett, D.B. Savage, S. O'Rahilly, J.E. Balow, P. Gorden, Proteinuric nephropathy in acquired and congenital generalized lipodystrophy: baseline characteristics and course during recombinant leptin therapy, *J. Clin. Endocrinol. Metab.* 89 (2004) 3199-3207.
- [39] K. Ebihara, T. Kusakabe, M. Hirata, H. Masuzaki, F. Miyanaga, N. Kobayashi, T. Tanaka, H. Chusho, T. Miyazawa, T. Hayashi, K. Hosoda, Y. Ogawa, A.M. DePaoli, M. Fukushima, K. Nakao, Efficacy and safety of leptin-replacement therapy and possible mechanisms of leptin actions in patients with generalized lipodystrophy, *J. Clin. Endocrinol. Metab.* 92 (2007) 532-541.
- [40] J.A. Yiannias, D.J. DiCaudo, E. Maskin, Peripheral T-cell lymphoma presenting as lipoatrophy and nodules, *Int. J. Dermatol.* 45 (2006) 1415-1419.

- [41] E.A. Oral, E.D. Javor, L. Ding, G. Uzel, E.K. Cochran, J.R. Young, A.M. DePaoli, S.M. Holland, P. Gorden, Leptin replacement therapy modulates circulating lymphocyte subsets and cytokine responsiveness in severe lipodystrophy, *J. Clin. Endocrinol. Metab.* 91 (2006) 621-628.
- [42] S.A. Moran, N. Patten, J.R. Young, E. Cochran, N. Sebring, J. Reynolds, A. Premkumar, A.M. Depaoli, M.C. Skarulis, E.A. Oral, P. Gorden, Changes in body composition in patients with severe lipodystrophy after leptin replacement therapy, *Metab., Clin. Exp.* 53 (2004) 513-519.
- [43] V. Simha, J.E. Zerwekh, K. Sakhaee, A. Garg, Effect of subcutaneous leptin replacement therapy on bone metabolism in patients with generalized lipodystrophy, *J. Clin. Endocrinol. Metab.* 87 (2002) 4942-4945.
- [44] E.A. Oral, E. Ruiz, A. Andewelt, N. Sebring, A.J. Wagner, A.M. Depaoli, P. Gorden, Effect of leptin replacement on pituitary hormone regulation in patients with severe lipodystrophy, *J. Clin. Endocrinol. Metab.* 87 (2002) 3110-3117.
- [45] C. Musso, E. Cochran, E. Javor, J. Young, A.M. Depaoli, P. Gorden, The long-term effect of recombinant methionyl human leptin therapy on hyperandrogenism and menstrual function in female and pituitary function in male and female hypoleptinemic lipodystrophic patients, *Metab., Clin. Exp.* 54 (2005) 255-263.
- [46] A.O. Lungu, E. Safar Zadeh, A. Goodling, E. Cochran, P. Gorden, Insulin Resistance Is a Sufficient Basis for Hyperandrogenism in Lipodystrophic Women with Polycystic Ovarian Syndrome, *J. Clin. Endocrinol. Metab.* (2011)
- [47] J. Beltrand, M. Beregszaszi, D. Chevenne, G. Sebag, M. De Kerdanet, F. Huet, M. Polak, N. Tubiana-Rufi, D. Lacombe, A.M. De Paoli, C. Levy-Marchal, Metabolic correction induced by leptin replacement treatment in young children with Berardinelli-Seip congenital lipodystrophy, *Pediatrics* 120 (2007) e291-296.
- [48] J. Beltrand, N. Lahlou, T. Le Charpentier, G. Sebag, S. Leka, M. Polak, N. Tubiana-Rufi, D. Lacombe, M. de Kerdanet, F. Huet, J.J. Robert, D. Chevenne, P. Gressens, C. Levy-Marchal, Resistance to leptin-replacement therapy in Berardinelli-Seip congenital lipodystrophy: an immunological origin, *Eur. J. Endocrinol.* 162 (2010) 1083-1091.
- [49] J.Y. Park, E.D. Javor, E.K. Cochran, A.M. DePaoli, P. Gorden, Long-term efficacy of leptin replacement in patients with Dunnigan-type familial partial lipodystrophy, *Metab., Clin. Exp.* 56 (2007) 508-516.
- [50] J.M. Guettier, J.Y. Park, E.K. Cochran, C. Poitou, A. Basdevant, M. Meier, K. Clement, J. Magre, P. Gorden, Leptin therapy for partial lipodystrophy linked to a PPAR-gamma mutation, *Clin. Endocrinol. (Oxf)* 68 (2008) 547-554.
- [51] J.Y. Park, A.Y. Chong, E.K. Cochran, D.E. Kleiner, M.J. Haller, D.A. Schatz, P. Gorden, Type 1 diabetes associated with acquired generalized lipodystrophy and insulin resistance: the effect of long-term leptin therapy, *J. Clin. Endocrinol. Metab.* 93 (2008) 26-31.
- [52] E.A. Oral, J.L. Chan, Rationale for leptin-replacement therapy for severe lipodystrophy, *Endocr. Pract.* 16 (2010) 324-333.
- [53] D.B. Savage, S. O'Rahilly, Leptin therapy in lipodystrophy, *Diabetologia* 53 (2010) 7-9.
- [54] M. Caron-Debarle, C. Lagathu, F. Boccara, C. Vigouroux, J. Capeau, HIV-associated lipodystrophy: from fat injury to premature aging, *Trends Mol. Med.* 16 (2010) 218-229.
- [55] F. Villarroya, P. Domingo, M. Giralt, Drug-induced lipotoxicity: lipodystrophy associated with HIV-1 infection and antiretroviral treatment, *Biochim. Biophys. Acta* 1801 (2010) 392-399.

- [56] J.H. Lee, J.L. Chan, E. Sourlas, V. Raptopoulos, C.S. Mantzoros, Recombinant methionyl human leptin therapy in replacement doses improves insulin resistance and metabolic profile in patients with lipoatrophy and metabolic syndrome induced by the highly active antiretroviral therapy, *J. Clin. Endocrinol. Metab.* 91 (2006) 2605-2611.
- [57] K. Mulligan, H. Khatami, J.M. Schwarz, G.K. Sakkas, A.M. DePaoli, V.W. Tai, M.J. Wen, G.A. Lee, C. Grunfeld, M. Schambelan, The effects of recombinant human leptin on visceral fat, dyslipidemia, and insulin resistance in patients with human immunodeficiency virus-associated lipoatrophy and hypoleptinemia, *J. Clin. Endocrinol. Metab.* 94 (2009) 1137-1144.
- [58] F. Magkos, A. Brennan, L. Sweeney, E.S. Kang, J. Doweiko, A.W. Karchmer, C.S. Mantzoros, Leptin replacement improves postprandial glycemia and insulin sensitivity in human immunodeficiency virus-infected lipoatrophic men treated with pioglitazone: a pilot study, *Metab., Clin. Exp.* 60 (2011) 1045-1049.
- [59] A.M. Brennan, J.H. Lee, S. Tsiodras, J.L. Chan, J. Doweiko, S.N. Chimienti, S.G. Wadhwa, A.W. Karchmer, C.S. Mantzoros, r-metHuLeptin improves highly active antiretroviral therapy-induced lipoatrophy and the metabolic syndrome, but not through altering circulating IGF and IGF-binding protein levels: observational and interventional studies in humans, *Eur. J. Endocrinol.* 160 (2009) 173-176.
- [60] C.K. Welt, J.L. Chan, J. Bullen, R. Murphy, P. Smith, A.M. DePaoli, A. Karalis, C.S. Mantzoros, Recombinant human leptin in women with hypothalamic amenorrhea, *N. Engl. J. Med.* 351 (2004) 987-997.
- [61] S.H. Chou, J.P. Chamberland, X. Liu, G. Matarese, C. Gao, R. Stefanakis, M.T. Brinkoetter, H. Gong, K. Arampatzi, C.S. Mantzoros, Leptin is an effective treatment for hypothalamic amenorrhea, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 6585-6590.
- [62] E. Sienkiewicz, F. Magkos, K.N. Aronis, M. Brinkoetter, J.P. Chamberland, S. Chou, K.M. Arampatzi, C. Gao, A. Koniaris, C.S. Mantzoros, Long-term metreleptin treatment increases bone mineral density and content at the lumbar spine of lean hypoleptinemic women, *Metab., Clin. Exp.* 60 (2011) 1211-1221.
- [63] M.G. Myers, M.A. Cowley, H. Munzberg, Mechanisms of leptin action and leptin resistance, *Annu. Rev. Physiol.* 70 (2008) 537-556.
- [64] J.C. Won, P.G. Jang, C. Namkoong, E.H. Koh, S.K. Kim, J.Y. Park, K.U. Lee, M.S. Kim, Central administration of an endoplasmic reticulum stress inducer inhibits the anorexigenic effects of leptin and insulin, *Obesity* 17 (2009) 1861-1865.
- [65] H.S. Moon, G. Matarese, A.M. Brennan, J.P. Chamberland, X. Liu, C.G. Fiorenza, G.H. Mylvaganam, L. Abanni, F. Carbone, C.J. Williams, A.M. De Paoli, B.E. Schneider, C.S. Mantzoros, Efficacy of metreleptin in obese patients with type 2 diabetes: cellular and molecular pathways underlying leptin tolerance, *Diabetes* 60 (2011) 1647-1656.
- [66] L. Ozcan, A.S. Ergin, A. Lu, J. Chung, S. Sarkar, D. Nie, M.G. Myers, Jr., U. Ozcan, Endoplasmic reticulum stress plays a central role in development of leptin resistance, *Cell Metab.* 9 (2009) 35-51.
- [67] J.D. Roth, B.L. Roland, R.L. Cole, J.L. Trevaskis, C. Weyer, J.E. Koda, C.M. Anderson, D.G. Parkes, A.D. Baron, Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 7257-7262.
- [68] E. Ravussin, S.R. Smith, J.A. Mitchell, R. Shringarpure, K. Shan, H. Maier, J.E. Koda, C. Weyer, Enhanced weight loss with pramlintide/metreleptin: an integrated neurohormonal approach to obesity pharmacotherapy, *Obesity* 17 (2009) 1736-1743.
- [69] M. Rosenbaum, R. Goldsmith, D. Bloomfield, A. Magnano, L. Weimer, S. Heymsfield, D. Gallagher, L. Mayer, E. Murphy, R.L. Leibel, Low-dose leptin reverses skeletal

- muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight, *J. Clin. Invest.* 115 (2005) 3579-3586.
- [70] N. Chinookoswong, J.L. Wang, Z.Q. Shi, Leptin restores euglycemia and normalizes glucose turnover in insulin-deficient diabetes in the rat, *Diabetes* 48 (1999) 1487-1492.
- [71] F. Miyanaga, Y. Ogawa, K. Ebihara, S. Hidaka, T. Tanaka, S. Hayashi, H. Masuzaki, K. Nakao, Leptin as an adjunct of insulin therapy in insulin-deficient diabetes, *Diabetologia* 46 (2003) 1329-1337.
- [72] X. Yu, B.H. Park, M.Y. Wang, Z.V. Wang, R.H. Unger, Making insulin-deficient type 1 diabetic rodents thrive without insulin, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 14070-14075.
- [73] M.Y. Wang, L. Chen, G.O. Clark, Y. Lee, R.D. Stevens, O.R. Ilkayeva, B.R. Wenner, J.R. Bain, M.J. Charron, C.B. Newgard, R.H. Unger, Leptin therapy in insulin-deficient type I diabetes, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010) 4813-4819.
- [74] A.J. Kruger, C. Yang, K.L. Lipson, S.C. Pino, J.H. Leif, C.M. Hogan, B.J. Whalen, D.L. Guberski, Y. Lee, R.H. Unger, D.L. Greiner, A.A. Rossini, R. Bortell, Leptin treatment confers clinical benefit at multiple stages of virally induced type 1 diabetes in BB rats, *Autoimmunity* 44 (2011) 137-148.
- [75] T. Kusakabe, H. Tanioka, K. Ebihara, M. Hirata, L. Miyamoto, F. Miyanaga, H. Hige, D. Aotani, T. Fujisawa, H. Masuzaki, K. Hosoda, K. Nakao, Beneficial effects of leptin on glycaemic and lipid control in a mouse model of type 2 diabetes with increased adiposity induced by streptozotocin and a high-fat diet, *Diabetologia* 52 (2009) 675-683.
- [76] S.P. Kalra, Central leptin gene therapy ameliorates diabetes type 1 and 2 through two independent hypothalamic relays; a benefit beyond weight and appetite regulation, *Peptides* 30 (2009) 1957-1963.
- [77] D. Accili, A. Cama, F. Barbetti, H. Kadowaki, T. Kadowaki, S.I. Taylor, Insulin resistance due to mutations of the insulin receptor gene: an overview, *J. Endocrinol. Invest.* 15 (1992) 857-864.
- [78] E. Cochran, J.R. Young, N. Sebring, A. DePaoli, E.A. Oral, P. Gorden, Efficacy of recombinant methionyl human leptin therapy for the extreme insulin resistance of the Rabson-Mendenhall syndrome, *J. Clin. Endocrinol. Metab.* 89 (2004) 1548-1554.
- [79] I.S. Farooqi, J.M. Keogh, S. Kamath, S. Jones, W.T. Gibson, R. Trussell, S.A. Jebb, G.Y. Lip, S. O'Rahilly, Partial leptin deficiency and human adiposity, *Nature* 414 (2001) 34-35.
- [80] N. Yamada, G. Katsuura, Y. Ochi, K. Ebihara, T. Kusakabe, K. Hosoda, K. Nakao, Impaired CNS leptin action is implicated in depression associated with obesity, *Endocrinology* 152 (2011) 2634-2643.
- [81] G.E. Miller, K.E. Freedland, R.M. Carney, C.A. Stetler, W.A. Banks, Pathways linking depression, adiposity, and inflammatory markers in healthy young adults, *Brain. Behav. Immun.* 17 (2003) 276-285.
- [82] E.D. London, S.M. Berman, S. Chakrapani, T. Delibasi, J. Monterosso, H.K. Erol, G. Paz-Filho, M.L. Wong, J. Licinio, Short-term plasticity of gray matter associated with leptin deficiency and replacement, *J. Clin. Endocrinol. Metab.* 96 (2011) E1212-1220.
- [83] N. Tezapsidis, J.M. Johnston, M.A. Smith, J.W. Ashford, G. Casadesus, N.K. Robakis, B. Wolozin, G. Perry, X. Zhu, S.J. Greco, S. Sarkar, Leptin: a novel therapeutic strategy for Alzheimer's disease, *J. Alzheimers Dis.* 16 (2009) 731-740.
- [84] S.J. Greco, A. Hamzelou, J.M. Johnston, M.A. Smith, J.W. Ashford, N. Tezapsidis, Leptin boosts cellular metabolism by activating AMPK and the sirtuins to reduce tau

- phosphorylation and beta-amyloid in neurons, *Biochem. Biophys. Res. Commun.* 414 (2011) 170-174.
- [85] T. Olsson, B. Nasman, S. Rasmuson, B. Ahren, Dual relation between leptin and cortisol in humans is disturbed in Alzheimer's disease, *Biol. Psychiatry* 44 (1998) 374-376.
- [86] D.A. Power, J. Noel, R. Collins, D. O'Neill, Circulating leptin levels and weight loss in Alzheimer's disease patients, *Dement. Geriatr. Cogn. Disord.* 12 (2001) 167-170.
- [87] K.F. Holden, K. Lindquist, F.A. Tylavsky, C. Rosano, T.B. Harris, K. Yaffe, Serum leptin level and cognition in the elderly: Findings from the Health ABC Study, *Neurobiol. Aging* 30 (2009) 1483-1489.
- [88] W. Lieb, A.S. Beiser, R.S. Vasan, Z.S. Tan, R. Au, T.B. Harris, R. Roubenoff, S. Auerbach, C. DeCarli, P.A. Wolf, S. Seshadri, Association of plasma leptin levels with incident Alzheimer disease and MRI measures of brain aging, *JAMA, J. Am. Med. Assoc.* 302 (2009) 2565-2572.
- [89] J.M. Johnston, S.J. Greco, A. Hamzelou, J.W. Ashford, N. Tezapsidis, Repositioning leptin as a therapy for Alzheimer's disease, *Therapy* 8 (2011) 481-490.