

# Translational Research of Leptin in Lipodystrophy and Its Related Diseases

Ken Ebihara and Kazuwa Nakao

**Abstract** Leptin, an adipocyte-derived hormone, plays crucial roles in the regulation of energy expenditure and food intake. Through analyses of leptin transgenic mice, we have demonstrated that leptin has pleiotropic effects such as regulation of insulin sensitivity and lipid metabolism. Lipodystrophy is a disease characterized by a lack of adipose tissue, which leads to metabolic disorders including insulin resistant diabetes, hypertriglyceridemia, and fatty liver. We demonstrated that leptin deficiency plays an important role in the pathogenesis of metabolic disorders in lipodystrophy. We also demonstrated the efficacy of leptin replacement therapy in lipodystrophy. Leptin improves insulin sensitivity at least partly by cancellation of lipotoxicity in the liver and skeletal muscle. It is also possible that leptin improves insulin secretion by cancellation of lipotoxicity in pancreatic beta cells. Using animal models, we demonstrated that leptin activates hepatic AMP-activated protein kinase (AMPK), and hepatic AMPK activation is involved in the therapeutic effects of leptin. To elucidate the pathogenic mechanism of hyperphagia in lipodystrophy, we measured food-related neural activity by fMRI and investigated subjective feelings of appetite. We found insufficiency of postprandial suppression of food-related neural activity and formation of satiety feelings in patients with lipodystrophy, which might be largely due to leptin deficiency. In March 2013, marketing and manufacturing approval was granted for metreleptin for the treatment of lipodystrophy in Japan on the basis of the results of our investigator-initiated trial. This is the first global approval of leptin formulation. Leptin has potential as a drug for the treatment of more common metabolic diseases including diabetes, hyperlipidemia, and fatty liver.

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## Introduction

Leptin, an adipocyte-derived hormone originally identified from hereditary obese mice (ob/ob mice) [1], plays crucial physiologic roles in the regulation of energy expenditure and food intake [2–6]. In obese animals and subjects, plasma leptin concentrations are increased in proportion to the degree of adiposity [7–9], indicating that leptin is a satiety signal communicating the size of adipose stores to the brain [10–12] and that leptin resistance is related to obesity [7, 13–15]. Leptin deficiency in human subjects is associated with morbid obesity with insulin resistance, indicating the physiological role of leptin in both animal models and humans [16, 17]. Leptin is implicated in a number of manifestations seen in obese animal models [11, 18–21]. In this chapter, we introduce our past basic and clinical studies for clinical application in lipodystrophy and its related diseases.

## Transgenic Mice Overexpressing Leptin

To explore the clinical implications of leptin *in vivo*, we generated leptin transgenic (LepTg) mice displaying elevated plasma leptin concentrations comparable to those seen in obese subjects [22]. A fusion gene comprising the human SAP promoter upstream of the mouse leptin cDNA coding sequences was designed to target hormone expression to the liver [23, 24]. Overexpression of leptin in the liver resulted in the complete disappearance of both white adipose tissues in mice [22]. Such a phenotype did not occur when transgene expression was targeted to adipose tissue, the endogenous site of leptin production, using adipocyte-specific promoters [25]. The hyperleptinemia seen in these LepTg mice provides a unique experimental system in which the long-term effects of leptin are investigated *in vivo* [18–22]. LepTg mice exhibit augmented glucose metabolism and increased insulin sensitivity of both skeletal muscle and liver [22], supporting the concept that leptin acts as an antidiabetic hormone *in vivo* [26–28]. These studies suggest the potential usefulness of leptin treatment of diabetes and obesity.

## Crossbreeding Experiment of LepTg Mice with A-ZIPTg Mice

Lipodystrophy is a disease characterized by a lack of adipose tissue. It can be developed by a genetic abnormality, immune disorder, viral infection, or drugs. Irrespective of the etiology, loss of adipose tissue leads to severe insulin-resistant

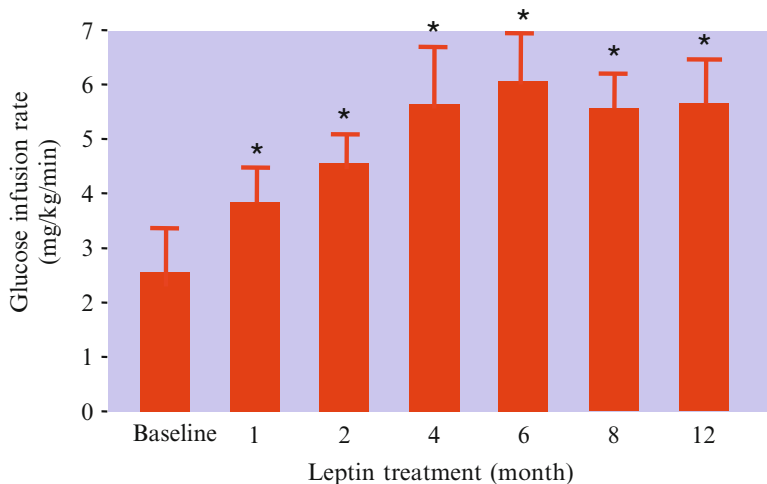
diabetes, hypertriglyceridemia, and fatty liver [29]. The precise mechanism by which this paucity of fat results in these metabolic disorders remains to be elucidated. Plasma leptin concentrations are markedly reduced in patients with lipodystrophy and in rodent models of lipodystrophy [30–33]. Given leptin's antidiabetic action, leptin deficiency may play a role in the pathogenesis of metabolic disorders in lipodystrophy; thus, leptin may be a drug for patients with lipodystrophy.

A mouse model of generalized lipodystrophy (A-ZIPTg mice) was generated by expressing in adipose tissue a protein that inactivates basic zipper transcription factors [32]. To assess the pathophysiological role and therapeutic potential of leptin in metabolic disorders associated with lipodystrophy, we crossed LepTg mice and A-ZIPTg mice to produce doubly transgenic (LepTg/A-ZIPTg) mice virtually lacking adipose tissue and expressing approximately tenfold higher levels of leptin than normal controls [34]. LepTg/A-ZIPTg mice were hypophagic in comparison with A-ZIPTg mice and exhibited decreased hepatic steatosis. Glucose and insulin tolerance tests displayed increased insulin sensitivity and normal glucose tolerance in LepTg/A-ZIPTg mice, which was comparable to LepTg mice. Pair-feeding experiments demonstrated that the effects of leptin were not solely due to decreased food intake. These results demonstrate that leptin can improve insulin resistance and diabetic manifestations in a mouse model of severe systemic lipodystrophy, indicating that leptin is therapeutically useful in the treatment of lipotrophic diabetes [34].

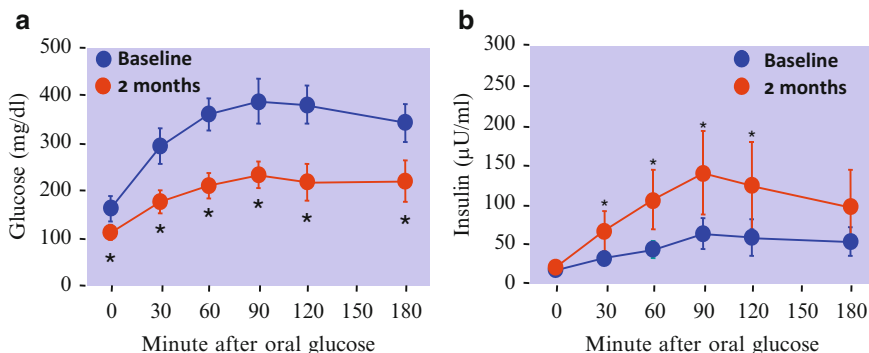
## Leptin Replacement Therapy in Japanese Patients with Lipodystrophy

Four-month leptin replacement therapy has been reported to improve glucose and lipid metabolism in lipodystrophy patients in the USA [35]. To elucidate the efficacy, safety, and mechanisms underlying leptin replacement therapy in Asian patients with lipodystrophy, we treated seven Japanese patients, two acquired and five congenital types, with a physiological replacement dose of leptin [36, 37]. Leptin replacement therapy dramatically improved fasting glucose levels (mean  $\pm$  SE, 172  $\pm$  20 to 120  $\pm$  12 mg/dl,  $P < 0.05$ ) and triglyceride levels (mean  $\pm$  SE, 700  $\pm$  272 to 260  $\pm$  98 mg/dl,  $P < 0.05$ ) within 1 week. By 2 months, six of seven patients were able to discontinue all antidiabetic drugs, including insulin.

To investigate the underlying mechanism of metabolic improvement by leptin, we evaluated insulin sensitivity using a hyperinsulinemic euglycemic glucose clamp study in human patients (Fig. 1). The glucose infusion rate as an index of insulin sensitivity was distinctly low at baseline but was improved month by month after the initiation of leptin therapy. We also evaluated the glucose tolerance and the ability of insulin secretion with the oral glucose tolerance test (Fig. 2). After 2 months of leptin therapy, the glucose level was dramatically improved and, at the same time, the ability of insulin secretion was also clearly improved. In lipodystrophy, triglyceride accumulates excessively in the cells of non-adipose tissues including the liver and

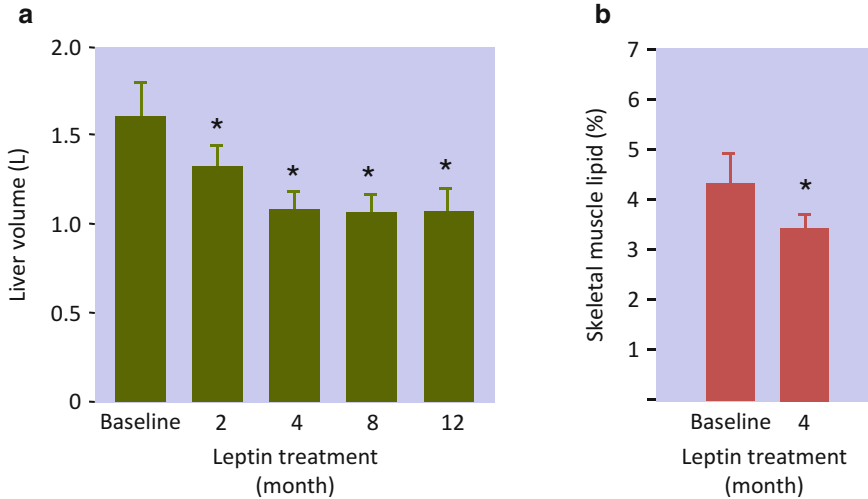


**Fig. 1** Glucose infusion rate in a hyperinsulinemic euglycemic glucose clamp study in 10 patients with generalized lipodystrophy. Insulin sensitivity was distinctly low at baseline but improved month by month after the initiation of leptin therapy. \* $p < 0.05$  vs baseline



**Fig. 2** 75 g glucose tolerance test in 10 patients with generalized lipodystrophy at baseline and 2 months after the initiation of leptin replacement therapy. (a) Plasma glucose. (b) Insulin levels before and after an oral glucose load. Leptin replacement therapy improved glucose tolerance and insulin secretion. \* $p < 0.05$  vs baseline

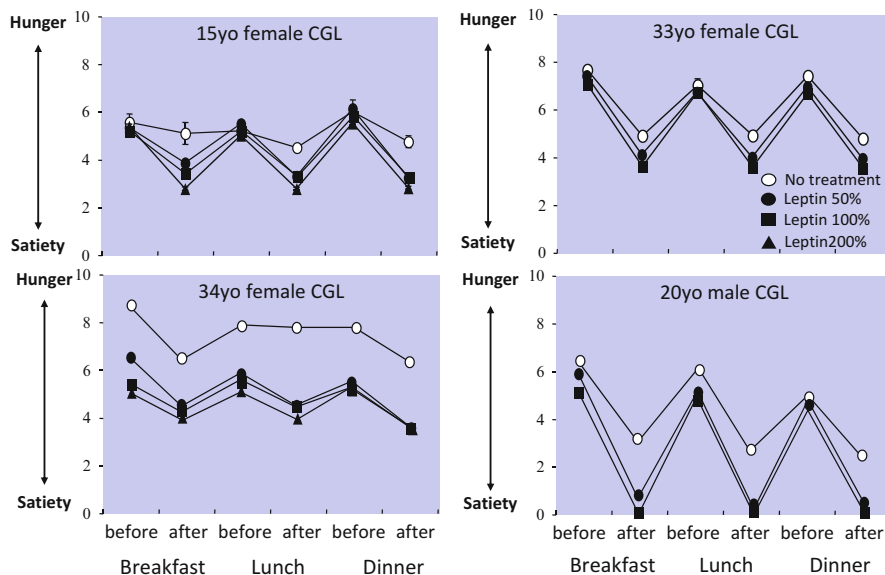
skeletal muscle [38]. In the liver, the amount of triglyceride accumulation is known to be correlated with the severity of insulin resistance [39]. Triglyceride accumulation in skeletal muscle also leads to insulin resistance [39]. In pancreatic beta cells, triglyceride accumulation is known to impair insulin secretion [39]. Cellular dysfunction caused by ectopic fat deposition has been referred as “lipotoxicity” [40]. To investigate the cancellation of lipotoxicity as a potential mechanism by which



**Fig. 3** Ectopic fat deposition in insulin-target tissues. **(a)** Liver volume calculated by CT in 10 patients with generalized lipodystrophy before and after the initiation of leptin replacement therapy. **(b)** Skeletal muscle lipid content estimated by MRI before and 4 months after the initiation of leptin replacement therapy. \* $p < 0.05$  vs baseline

leptin improves insulin sensitivity and insulin secretion, we evaluated the tissue lipid content in the liver and skeletal muscle in patients with lipodystrophy (Fig. 3). Liver volume as an index of fatty liver was calculated by computed tomography (CT). Leptin therapy effectively decreased liver volume. Skeletal muscle lipid content was estimated by magnetic resonance imaging (MRI). Skeletal muscle lipid content was also significantly decreased after 4 months of leptin therapy. These results suggest that leptin improves insulin sensitivity at least partly by cancellation of lipotoxicity in the liver and skeletal muscle. Although we did not evaluate lipid content in pancreatic beta cells in this study, it is also possible that leptin improves insulin secretion by cancellation of lipotoxicity in beta cells.

We also evaluated subjective feelings of appetite with a 100 mm visual analog scale [41] in patients before and after the initiation of leptin therapy. On this assessment, participants were instructed to rate how hungry they were by marking on a scale before and after each meal. A higher score indicated a greater extent of hunger. As shown in Fig. 4, the self-reported hunger scores before meals were not different between before and after the initiation of leptin therapy in most patients. In contrast, after meals, the score was effectively suppressed after leptin treatment in most patients. These results indicate that leptin reinforces the formation of satiety feelings after a meal.



**Fig. 4** Subjective appetite feelings evaluated with a 100 mm visual analog scale in representative patients with generalized lipodystrophy with or without leptin treatment. Patients were treated at 50 %, 100 %, and 200 % of the replacement dose. *CGL* congenital generalized lipodystrophy

## Significance of Hepatic AMPK in the Metabolic Action of Leptin

Leptin effectively improves insulin sensitivity accompanied by dramatic reduction of fat content in the liver and skeletal muscle in patients with lipodystrophy [35, 37, 38]. Using rodent models, it was demonstrated that leptin activates 5'-AMP-activated protein kinase (AMPK) in the skeletal muscle through both central and direct pathways [42]. AMPK is a heterotrimeric enzyme that is conserved from yeast to humans and functions as a “fuel gauge” to monitor the status of cellular energy. AMPK potently stimulates fatty acid oxidation by inhibiting the activity of acetyl-CoA carboxylase. Thus, AMPK activation by leptin is a plausible mechanism by which leptin reduces ectopic fat in the skeletal muscle. In addition to the skeletal muscle, recent studies have shown the physiological significance of AMPK in the liver [43, 44]. However, the effect of leptin on hepatic AMPK activity remained to be determined. The role of AMPK in the pathogenesis of metabolic abnormalities in lipodystrophy also remained unclear. We investigated the effect of leptin on hepatic AMPK activities and the pathophysiological role of AMPK in A-ZIPTg mice, a mouse model of generalized lipodystrophy [45].

We demonstrated that leptin activates hepatic AMPK through the central nervous system and alpha-adrenergic sympathetic nerves. AMPK activities were decreased in the fatty liver of A-ZIP/F-1 mice, and leptin administration increased AMPK

activities in the liver as well as in skeletal muscle with a significant reduction in triglyceride content. Activation of hepatic AMPK with A769662 also led to a decrease in hepatic triglyceride content and blood glucose levels in A-ZIP/F-1 mice. These results indicate that downregulation of hepatic AMPK activities plays a pathophysiological role in the metabolic disturbances of lipodystrophy, and that hepatic AMPK activation is involved in the therapeutic effects of leptin.

## **fMRI Analysis of Food-Related Brain Activity in Patients with Lipodystrophy**

Lipodystrophic patients also exhibit eating disorders, which makes diet therapy difficult [46]. Leptin replacement therapy was shown to suppress appetite in lipodystrophic patients [46, 47]. However, there is no report on the comparison of eating behaviors between healthy subjects and patients with lipodystrophy. Therefore, the pathophysiological role of leptin in eating disorders in patients with lipodystrophy remains unclear. From experimental studies in human and animals, it has long been established that leptin suppresses energy intake mainly by acting on the hypothalamus [11, 48]. However, there is little information about how the neural networks including the hypothalamus are influenced by leptin signals. Recently the advent of functional neuroimaging techniques such as functional magnetic resonance imaging (fMRI) has been providing novel insights into homeostatic and hedonic aspects of human eating behavior. fMRI measurements of food-related neural activity in congenital leptin-deficient patients were reported [49]. These analyses revealed that leptin treatment modulates neural activity in reward and food-related areas such as the ventral striatum and orbitofrontal cortex.

To reveal the pathogenic mechanism of eating disorders in lipodystrophic patients, we measured food-related neural activity by fMRI scans and investigated subjective feelings of appetite under both fasting and postprandial conditions in patients and age- and sex-matched healthy subjects [51]. In addition, we performed the same sequential analyses in the same patients with leptin replacement therapy [51]. Although there was little difference in the enhancement of neural activity by food stimuli between patients and controls under fasting, postprandial suppression of neural activity was insufficient in many regions of interest including the amygdala, insula, nucleus accumbens, caudate, putamen, and globus pallidus in patients compared with controls. Leptin treatment effectively suppressed postprandial neural activity in many of these regions of interest, whereas it showed little effect under fasting in patients. Consistent with these results, postprandial formation of satiety feelings was insufficient in patients compared with controls, which was effectively reinforced by leptin treatment. These results demonstrate the insufficiency of postprandial suppression of food-related neural activity and formation of satiety feelings in patients with lipodystrophy, which might be largely due to leptin deficiency. This study also demonstrated that leptin has little involvement in the regulation of neural activity and eating behavior under fasting, whereas leptin plays a significant role in

these regulations under postprandial conditions. We found that leptin suppressed neural activity in regions involved in the reward system such as the amygdala, hippocampus, NcA, and caudate. Further study is needed to elucidate the role of the reward system on appetite regulation by leptin.

## Conclusions

Metreleptin is an analog of human leptin originally developed by Amgen and has been used for treatment in patients with lipodystrophy also in Japan. In July 2012, Shionogi originally filed a New Drug Application (NDA), which was based on the results of our investigator-initiated trial conducted by the Kyoto University Graduate School of Medicine with assistance from the Translational Research Center (the current Institute for Advancement of Clinical and Translational Science) at Kyoto University Hospital. In March 2013, marketing and manufacturing approval was granted by the Japanese Ministry of Health, Labour and Welfare for subcutaneous metreleptin for the treatment of lipodystrophy. This is the first global approval of leptin formulation [51]. In patients with lipodystrophy, leptin improved insulin resistance, hypertriglyceridemia, and fatty liver. We have demonstrated the therapeutic usefulness of leptin in insulin-deficient diabetes, non-obese type 2 diabetes, type 2 diabetes with mild obesity, hypertriglyceridemia, and non-alcoholic fatty liver [45, 52–56]. Leptin has potential as a drug for the treatment of more common metabolic diseases including diabetes, hyperlipidemia, and fatty liver.

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