

## 20 YEARS OF LEPTIN

# Leptin in common obesity and associated disorders of metabolism

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## Abstract

The molecular mechanisms of body weight and body composition regulation have long been a research focus in the hopes of identifying tractable pathways for therapeutic interventions for obesity and diabetes, as well as related disorders such as nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH) and polycystic ovary syndrome. The metabolic consequences of obesity and type 2 diabetes (T2D) were already a focus of the world's attention in 1994 when the discovery of leptin generated enormous enthusiasm for the potential to treat common (non-monogenic) obesity and its associated metabolic disorders with an adipokine hormone that regulated body weight as well as lipid and carbohydrate metabolism. Recombinant human leptin and many leptin analogs were developed and studied in animals and a few in human clinical trials. Overall, the opportunity for leptin as a therapeutic in unselected patients with obesity and T2D has not been substantiated in clinical trials. The potential for combination therapy suggested by clinical studies with leptin and pramlintide supports a path toward obesity treatment through the leptin pathway. The profound metabolic benefits seen with leptin in numerous forms of leptin deficiency, including lipodystrophy, provide hope for the opportunity to identify selected subsets of patients who could benefit from leptin treatment. This review provides a comprehensive overview of the clinical data on a subset of the potential utilities of leptin, specifically as a therapeutic for general or common obesity and its metabolic consequences including T2D and NAFLD/NASH.

## Key Words

- ▶ leptin
- ▶ obesity
- ▶ leptin resistance
- ▶ type 2 diabetes
- ▶ recombinant leptin
- ▶ lipodystrophy
- ▶ nonalcoholic fatty liver disease
- ▶ nonalcoholic steatohepatitis

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## Introduction

Obesity had been considered a disease associated with behavior, an inappropriate consumption of food coupled with relative inactivity. Without a molecular mechanism to explain the pathology, obesity was merely a lack of willpower. The discovery of leptin in 1994 (Zhang *et al.* 1994) as an adipocytokine regulator of food intake, body weight, and fat mass, as well as an important regulator of the immune and neuroendocrine systems, unleashed enormous excitement in the study of obesity as a disease with a potential molecular mechanism, as well as many

new related fields of research. The opportunity to dissect molecular pathways of feeding behavior, as well as nutrient-sensitive metabolic and neuroendocrine regulation, was opened.

## Leptin's discovery and early promise

Leptin will forever be known as a potent regulator of feeding behavior and body weight based on the elegant studies carried out in the *ob/ob* and *db/db* mouse models

of obesity, which led to its discovery (Coleman 1973, Friedman *et al.* 1991, Bahary *et al.* 1993, Zhang *et al.* 1994), and the profoundly influential studies in *ob/ob* humans that stimulated interest in leptin as a rational therapy for obesity and its many associated disorders (Farooqi *et al.* 2002; Fig. 1). As it is often the case, the initial phenotype ascribed to the molecule becomes a dominant feature in the study and understanding of the actions of the molecule. The role of leptin in feeding and body weight regulation, as well as metabolism, quickly became a primary focus of this study, based on the remarkable activities of leptin in the *ob/ob* model of hyperphagic obesity. A cautionary note regarding the translatability of leptin alone as a therapeutic for common obesity was identified in rodent studies evaluating leptin administration to diet-induced obesity (DIO) mice with already elevated levels of leptin. These studies did not result in the same type of body weight loss (El-Haschimi *et al.* 2000) as

in the *ob/ob* mice and identified a caution for the potential utility for simple obesity in humans.

The first reported clinical study demonstrating the biological potential of leptin as a human therapeutic for obesity and possibly diabetes was the replacement of leptin in two morbidly obese children who lacked leptin activity due to an *ob/ob* mutation (Montague *et al.* 1997). Leptin replacement in these children with r-metHuLeptin (Amgen, Inc., Thousand Oaks, CA, USA) provided data supporting the potential for the use of leptin in obese humans. The caveat for these observations was the recognition that leptin levels were elevated in the general obese population (Considine *et al.* 1996) in proportion to fat mass, with women having higher levels than men (Rosenbaum *et al.* 1996). Many important subsequent studies of *ob/ob* patients have provided important insights into the biology and potential utility of leptin (Farooqi *et al.* 2002) beyond obesity.



**Figure 1**

The remarkable impact of r-metHuLeptin on body weight in children with congenital leptin deficiency. (A) Weights of child A compared with normal centiles for girls and of child B and child C compared with normal centiles for boys. Arrows indicate the start of r-metHuLeptin therapy. (B) Clinical photographs of child B before (height, 107 cm) and 24 months after r-metHuLeptin therapy (height, 124 cm). Reproduced from Farooqi IS,

Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jeff SA, Perna F, Fontana S, *et al.* 2002 Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *Journal of Clinical Investigation* 110 1093–1103, with permission from the American Society for Clinical Investigation. Copyright 2002.

The primary source of leptin production is adipose tissue. Accordingly, studies of leptin in states of 'relative leptin deficiency', specifically in patients with generalized or partial loss of body fat (e.g. lipodystrophy), provide further evidence for leptin's potential therapeutic value in metabolic disease. Studies on patients with lipodystrophy were initiated in parallel to the observations of the remarkable effects of leptin in *ob/ob* patients; these studies in multiple forms of lipodystrophy across multiple centers globally play a significant role in supporting leptin's potential to improve profound metabolic disturbances including severe insulin resistance, type 2 diabetes (T2D), hypertriglyceridemia, and nonalcoholic fatty liver disease (NAFLD).

Together, the observations of therapeutic utility of leptin in the *ob/ob* (absolutely leptin deficient) and lipodystrophic (being relatively leptin deficient) mice and humans provide insights into how leptin might have applications for broader populations of patients with metabolic diseases. Supported by these findings, numerous obesity studies evaluating different forms of leptin have been carried out either as monotherapy or in combination with agents to enable the activity of leptin. Recombinant leptin has also been studied in patients with T2D to assess both weight loss and metabolic effects independent of weight loss. These studies have underscored the challenges of defining effective therapeutic applications of leptin in common metabolic disorders.

### Rationale for leptin treatment in simple obesity

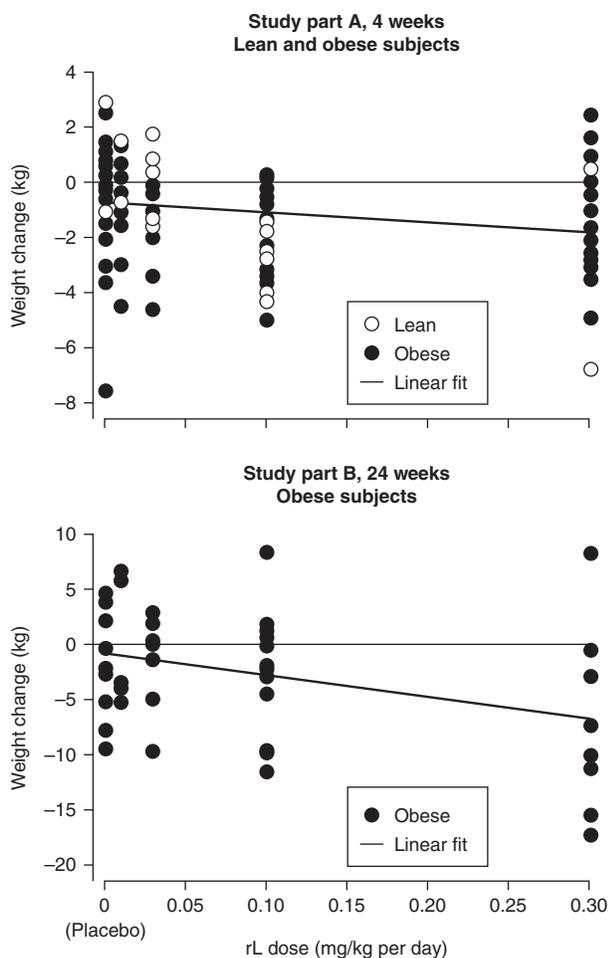
Soon after the discovery of leptin, assays to detect leptin were developed to measure its circulating levels in humans with a broad range of BMI and fat masses. Leptin levels in normal humans were identified to have a circadian rhythm and response to fasting and feeding (Schoeller *et al.* 1997). Furthermore, women had substantially higher levels of leptin, even when adjusted for fat mass (Rosenbaum *et al.* 1996). Unlike profoundly obese *ob/ob* mice and humans who have no leptin, typical obese humans had significantly higher leptin levels compared with normal-weight individuals (Considine *et al.* 1996). This observation, coupled with the underlying belief that leptin should function to regulate body weight at any level of fat mass, led to the hypothesis of 'leptin resistance' (Frederich *et al.* 1995). This concept of hormone resistance resembles that applied to insulin and insulin resistance seen in obesity leading to T2D and treated with high doses of insulin to

overcome the insulin resistance. This similarity to insulin resistance provided the rationale to use pharmacological doses of leptin to overcome leptin resistance and to treat obesity. In the 1990s, many groups worked to unlock the potential of leptin to reduce body weight, specifically fat mass in the obese, through the pharmacologic application of leptin.

### Interventional clinical trials with leptin in simple obesity

The first clinical trial studying common polygenic or simple obesity, as opposed to monogenic obesity, with leptin (Heymsfield *et al.* 1999) utilized daily s.c. recombinant methionyl human leptin (aka rL, r-methLeptin, A-100, or metreleptin, Amgen, Inc., Amylin Pharmaceuticals (San Diego, CA, USA), Bristol Myers Squibb (New York, NY, USA), and now AstraZeneca). This double-blind, placebo-controlled evaluation of leptin in both lean (BMI 20–27.5 kg/m<sup>2</sup>) and obese (BMI 27–36 kg/m<sup>2</sup>) individuals had two components: part A assessed the response of normal-weight and obese individuals for 4 weeks and then part B assessed only obese individuals for an additional 20 weeks. Once-daily s.c. dose groups comprised placebo, 0.01, 0.03, 0.10, and 0.30 mg/kg body weight. The concentration of leptin administered was 5 mg/ml and thus the volume of dosing in the higher dose groups was substantial (e.g. a 100-kg individual in the 0.3 mg/kg dose group would receive 30 mg of leptin in 6 ml provided as three 2 ml injections daily).

A little-appreciated outcome of the study was the weight loss in the lean group over the first 4 weeks of the trial. Despite not being asked to reduce their food intake or body weight, the normal-weight participants in the 0.1 and 0.3 mg/kg dose groups appear to have lost weight after 4 weeks of treatment (Fig. 2), suggesting a responsiveness of relatively lean humans to the weight-loss effects of leptin. The mean weight changes in the obese group increased as the dose of leptin was increased and ranged from –0.7 (5.4) kg for the 0.01 mg/kg group (*n*=6) to –7.1 (8.5) kg for the 0.30 mg/kg (*n*=8) group over 24 weeks (Fig. 2). This amount of weight loss was less than would have been anticipated if the obese patients were sensitive to the weight-reducing effects of leptin seen in leptin-responsive models. At the two highest dose groups, the composition of weight loss was reported to be 95% fat loss. No clinically significant adverse events were identified in the study, although mild to moderate injection-site reactions were noted; the volume of injection may have played a role.



**Figure 2**

Relationship between r-metHuLeptin (rL) dose and body weight change. Reproduced, with permission, from Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P *et al.* 1999 Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *Journal of the American Medical Association* **282** 1568–1575. Copyright (1999) American Medical Association. All rights reserved.

Given that leptin secretion in humans seems to follow a circadian rhythm (Sinha *et al.* 1996), it was important to address the potential importance of delivering leptin in a time-appropriate pattern to enable a therapeutic response. Zelissen *et al.* (2005) conducted a clinical trial evaluating the weight-loss potential of a dietary restriction of 500 kcal coupled with 10 mg (2 ml) of recombinant leptin administered in the morning, evening, or both morning and evening for 12 weeks after a 3-week lead-in. These doses represent  $\sim 0.1$  mg/kg per day for the morning or evening doses and  $\sim 0.2$  mg/kg per day for the morning and evening dosing. The study did not demonstrate any significant weight loss difference between active and placebo groups. No adverse events of significance

were reported; injection-site reactions were more common in patients receiving recombinant leptin (83%) vs placebo (36%).

Although other clinical trials in general obesity with recombinant leptin as monotherapy have examined concomitant diet restriction, route of delivery (to perhaps circumvent the blood-brain barrier to overcome leptin resistance), and responder subsets (obese individuals with relatively low leptin levels), results of these trials have yet to be published.

Two engineered forms of leptin designed to be dosed on a weekly basis, instead of daily, were A-200 or Fc-leptin (Amgen, Inc.) and pegylated human recombinant leptin (PEG-OB) (F. Hoffman-La Roche Ltd, Basel, Switzerland). These molecules were studied in general obese and overweight patients.

The only public sources of data available for once-weekly Fc-leptin in clinical studies are an abstract and oral presentation (Bartness 2001). The primary objectives of the study presented were safety and efficacy of Fc-leptin dosed weekly for 24 weeks in men and women with simple obesity. As seen with daily recombinant leptin (r-metHu-leptin), Fc-leptin demonstrated increasing body weight reduction with increasing dose of Fc-leptin, but the magnitude of weight loss relative to placebo was once again disappointing. No safety or tolerability issues were reported. Further details of the study have not been published or discussed.

A pegylated form of leptin, PEG-OB, was studied in obese men (Hukshorn *et al.* 2000, Westerterp-Plantenga *et al.* 2001) using weekly s.c. doses of 20 mg PEG-OB (2 ml injection) in conjunction with moderate energy restriction (2 MJ/day). The rationale for dose selection for this study is not clear. This pilot 12-week clinical trial in 30 obese men (BMI  $34.2 \pm 3.6$  kg/m<sup>2</sup>) demonstrated no statistically significant body weight differential over the course of the trial compared with placebo. Curiously, there was a non-statistically significantly greater weight loss in the placebo group vs the PEG-OB group ( $-6.4$  vs  $-4.3$  kg).

A subsequent weekly PEG-OB trial using a higher dose of 60 mg (6 ml injected)/week coupled with a 3600 kJ/day energy deficit for 4 weeks followed by 8 weeks of drug treatment in 28 obese individuals (16 women and 12 men with a BMI range of 27.7–38.7 kg/m<sup>2</sup>) demonstrated a non-significant difference in body weight loss in the PEG-OB group compared with placebo group ( $-4.8$  PEG-OB vs  $-3.8$  kg placebo) (Hukshorn *et al.* 2002). The 4-week lead-in phase resulted in a significant 5.3-kg weight loss in the 28 individuals entering the treatment phase of the trial. Given the small size of the study, a meaningful analysis of

subsets (e.g. male vs female) in this study was not possible. In addition to body weight, no differential changes were noted in metabolic profile (glucose, insulin, Homeostatic Model Assessment-Insulin Resistance (HOMA-IR), free fatty acids, triglycerides, and total cholesterol) or inflammatory markers (C-reactive protein and soluble tumor necrosis factor alpha receptor concentrations) in these otherwise healthy obese and overweight individuals.

The potential for leptin to enable weight loss when coupled to a very low-calorie diet as an adjunctive intervention was evaluated in a study (Hukshorn *et al.* 2003) using PEG-OB at a dose of 80 mg weekly (presumably 8 ml injected) vs a matched placebo. This small clinical trial carried out in 24 overweight men (BMI 28.8 kg/m<sup>2</sup>) during a semi-starvation energy deficit (2.1 MJ/day) diet for 46 days demonstrated that PEG-OB treatment at 80 mg weekly led to a significant additional weight loss of 2.8 kg. Although modest, this study supports a weight-loss benefit in this population.

Overall, the available clinical data with daily and weekly forms of leptin demonstrate an increasing amount of weight loss with increasing doses of leptin in interventional studies on individuals with moderate to significant concomitant dietary energy restriction. The feasibility of the dosing volumes required to achieve the doses used in these trials seems challenging. Clearly, the magnitude of weight loss reported in these unselected patients is of little clinical importance in regards to the desired metabolic and other benefits sought with weight loss in obese patients. These observations once again bring forward the question of leptin resistance, if one is to assume that the underlying action of leptin is to regulate food intake (both up in the absence of leptin and down in the presence of higher leptin levels), as well as perhaps energy expenditure, in order to control body weight (maintain a 'set-point').

### Leptin for weight-loss maintenance in obesity

The typical approach to the treatment of obesity and its associated metabolic disturbances is focused on caloric restriction and increased energy expenditure. Many noninvasive approaches (very low-calorie diet, behavioral therapy, etc.) have demonstrated reasonable success to enable improvements in compliance with a weight-loss program, thus leading to meaningful weight reduction; more invasive procedures (gastric bypass) lead to greater weight loss. Unfortunately these approaches are often associated with weight regain after the active weight-loss phase (Holden *et al.* 1992). An alternative approach to

the treatment of obesity is to initiate therapy after the active phase of weight loss in order to mitigate the counter-regulatory response of the body to regain weight, increase appetite, and reduce energy expenditure. This type of post-weight-loss intervention would enable weight-loss maintenance.

Study of the response of endogenous leptin during different types of caloric restriction (Wadden *et al.* 1998) demonstrated a significant fall in leptin levels with caloric deprivation. A number of pilot clinical trials with recombinant leptin have studied the potential to mitigate the tendency of weight regain after caloric restriction or weight loss. These interventional studies have provided a reasonably consistent view that some of the counter-regulatory responses to caloric restriction might be modified by leptin, including changes in skeletal muscle, autonomic and neuroendocrine adaptation to maintenance of reduced body weight (Rosenbaum *et al.* 2005), and reproductive hormonal regulation (Welt *et al.* 2004), while other potential mediators of weight regain were not consistently impacted (cortisol, growth hormone, and thyroid axes) (Rosenbaum *et al.* 2002, Chan *et al.* 2003, Schurgin *et al.* 2004, Gavrilu *et al.* 2005, Shetty *et al.* 2011).

Korner *et al.* conducted a pilot study in 27 women who were at least 18 months post Roux-en-Y gastric bypass and weight stable, and had a relatively low leptin level compared with weight-matched controls. The investigators used a crossover design with a relatively modest dose of recombinant leptin (0.05 mg/kg twice daily) intended to achieve a low pharmacologic level of leptin (approximately tenfold endogenous levels). No difference in the weight change was observed between the groups at the end of 8 weeks of treatment. Furthermore, no changes were noted in either thyroid hormone or cortisol levels (Korner *et al.* 2013).

At this point, scant data exist to support the claim that weight loss alone reduces 'leptin resistance' in unselected obese patients and, thus, response to exogenous leptin to allow weight maintenance at a reduced body weight. Of further note, gastric bypass, particularly the Roux-en-Y procedure, induces substantial changes in gut hormones that might alter the activity of leptin (Peterli *et al.* 2012). Lastly, it should be pointed out that the post-gastric bypass study investigated the action of leptin in women only.

### Leptin treatment for T2D

T2D is the most significant metabolic disorder associated with obesity and has increased in prevalence along with obesity worldwide. The clear consensus initial treatment

approach to T2D is weight loss. Leptin has been envisioned as a potential therapeutic for T2D, both for its impact on food intake and body weight in multiple animal models and for having demonstrated potential independent mechanisms to improve insulin action (Kalra 2009).

The first clinical trial to assess the potential activity of leptin in obese humans with T2D was a single-center short-term (2 weeks) pilot study with recombinant leptin (low dose of 20 mg/day; high dose of 80 mg/day) in patients with T2D naïve to diabetes medication. The objective of the study was to determine whether recombinant leptin had effects independent of weight loss on insulin action. Insulin action across multiple organs was evaluated with a hyperinsulinemic–euglycemic clamp coupled with stable isotopically labeled tracer infusions. Although the recombinant leptin led to three- to 150-fold increases in leptin levels, no improvement was noted in insulin-mediated glucose disposal nor in insulin-mediated suppression of glycerol or palmitate rates of appearance (Mittendorfer *et al.* 2011).

A second clinical trial designed to evaluate the longer term impact of recombinant leptin in T2D patients naïve to diabetes medications was conducted, but never published in its entirety. The trial assessed recombinant leptin and placebo injected daily for 16 weeks with HbA1c as the primary outcome measure and body weight change as a principle secondary measure. In the subset of patients published (Moon *et al.* 2011), the HbA1c reduction with recombinant leptin 10 mg/kg twice daily in treated patients was greater than in placebo patients but not clinically relevant, and no body weight loss was reported in this subgroup.

Although no other interventional studies in obese patients with T2D have been reported, a pilot study analyzing glucose and lipid metabolism in muscle and adipose tissues with acute elevation of leptin using sterilized recombinant human leptin (Sigma–Aldrich) in lean men found no difference in systemic, skeletal, muscle, or adipose tissue glucose metabolism (Wolsk *et al.* 2011). The investigators did note increased activation of STAT proteins (17-fold) and AMP-activated protein kinase (1.43-fold) after 1 h of leptin infusion and subsequent increase in systemic palmitate and fat oxidation. This evidence of leptin signaling in lean men may indicate responsiveness to high physiological levels of leptin and help to explain the acute activity of leptin in a leptin-responsive group. It is also interesting to consider that chronically increased activation of STAT3 may contribute to the induction of leptin resistance. (El-Haschimi *et al.* 2000).

## Combination pharmacotherapy studies with leptin to overcome leptin resistance

Based on the lack of significant response to leptin as monotherapy both in DIO mice and humans and the recognition of the redundancy of the neuro-regulatory system involved in body weight regulation, a substantial interest has arisen in the identification of agents known to induce weight loss which might synergize with leptin. Conceptually, this potential synergy would incorporate a leptin sensitizer to overcome leptin resistance (Myers *et al.* 2012), thus enabling the expected weight-reducing activity and metabolic benefits of leptin.

An early study with weekly Fc-leptin designed to assess the potential for synergistic weight loss with caffeine and ephedrine was published recently (Liu *et al.* 2013). The clinical trial evaluated 90 obese individuals spread across three treatment groups: 20 mg Fc-leptin subcutaneously once per week (L), 200 mg caffeine and 20 mg ephedrine three times per day orally (CE), and the combination of the two (LCE). At the end of 24 weeks, significant weight loss was noted with CE ( $-5.9 \pm 2.4\%$ ) and LCE ( $-6.5 \pm 1.1\%$ ), but not with L alone nor with LCE. The study was too small to assess responder subsets. The authors note that only the LCE group significantly reduced visceral fat mass ( $-11.0 \pm 3.3\%$ ,  $P < 0.05$ ), an intriguing finding suggesting that leptin may have preferential effects on specific metabolically sensitive fat depots.

The quest to identify leptin sensitizers or at least agents that synergize with leptin in regards to body weight loss was intense. One group of investigators at Amylin Pharmaceuticals systematically evaluated potential combinations of hormones with leptin in DIO rats and observed amylin's apparent synergy with respect to fat-specific weight loss (Trevaskis *et al.* 2010). Subsequently, this finding was extended to DIO mice (Kusakabe *et al.* 2012). These observations provided the rationale for a clinical trial testing the hypothesis that amylin or pramlintide (Amylin Pharmaceuticals) when administered in combination with recombinant leptin (metreleptin) could synergize with leptin in the treatment of obesity and metabolic disease in obese patients.

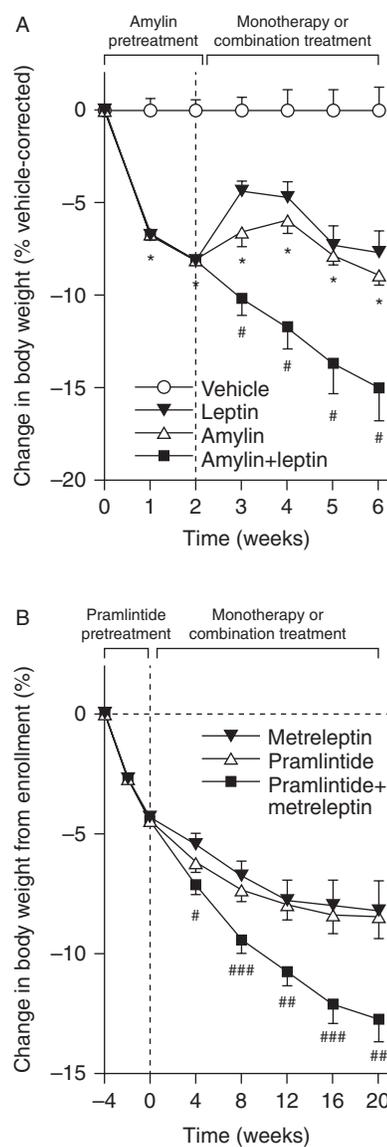
This hypothesis was tested in a 24-week randomized, double-blinded, active drug-controlled clinical trial of 177 overweight and obese males (age 18–55) and females (age 18–45) with a BMI being 27–35 kg/m<sup>2</sup>. Recombinant leptin and pramlintide (or their matching placebos) were given twice daily as separate s. c. injections. During the 4-week lead-in, the subjects were instructed to follow a 40% caloric-deficit diet (~550–1150 kcal/day deficit based

on weight, height, gender, activity level, and age) and started first on 180 and then 360  $\mu\text{g}$  of pramlintide twice daily with a target weight loss of 2–8%. At the completion of the lead-in, 21% of the subjects withdrew (9% due to insufficient weight loss, 3% due to adverse events, and 10% for ‘other’ reasons). The 139 subjects completing the 4-week lead-in lost a mean of 4.3%. During the double-blind treatment phase of the study, the subjects were instructed to follow a 20% caloric-deficit diet. Weight loss in the monotherapy completer groups with recombinant leptin and pramlintide stabilized at  $\sim 16$  weeks and was  $8.2 \pm 1.3$  and  $8.4 \pm 0.9\%$  from the initial baseline respectively ( $\sim 4\%$  loss from initiation of the combination portion of the study). The recombinant leptin and pramlintide combination completers group demonstrated a mean weight loss of  $12.7 \pm 0.9\%$  from initial baseline and had not yet been stabilized (Fig. 3). Of note, the intent-to-treat analysis using the last observation carried forward demonstrated mean weight losses from baseline of  $7.9 \pm 1.0$  and  $7.5 \pm 0.8\%$  for recombinant leptin and pramlintide monotherapy, respectively, and a weight loss of  $10.8 \pm 0.8\%$  for the combination, with  $P < 0.05$  when compared with the monotherapies (Roth *et al.* 2008, Chan *et al.* 2009, Ravussin *et al.* 2009).

Unfortunately, the confirmatory clinical trial (NCT01235741) was discontinued before completion of enrollment due to the identification of potentially neutralizing anti-drug antibodies in previously exposed patients (ClinicalTrials.gov March 4, 2014). Overall, these data provide promise for combination therapy for general obesity or at least a subset of obese patients.

### Leptin for nonalcoholic steatohepatitis

NAFLD and nonalcoholic steatohepatitis (NASH) are a spectrum of common disorders of the liver which are thought to be related to inappropriate accumulation/deposition of ‘fat’ in the liver. NAFLD and NASH are associated with obesity (particularly visceral fat accumulation), insulin resistance, and diabetes. In addition, the persistence and progression of NASH have been recognized as a significant risk for the development of cirrhosis and hepatic decompensation leading to liver transplant or death (Schwenger & Allard 2014). There is currently no effective treatment of NASH, but weight loss along with treatment of the underlying insulin resistance and diabetes has been the mainstay early in the disease course. The desirable attributes of potential therapies for NAFLD and NASH would include removal of inappropriate fat (triglyceride and perhaps related molecules,



**Figure 3**

Weight-loss effect of combined amylin and leptin in DIO rats and overweight/obese humans. (A) Change in body weight for DIO rats pretreated for 14 days with amylin and then maintained on amylin (open triangles) or switched to either leptin monotherapy or amylin + leptin combination therapy for an additional 28 days. (B) Change in body weight for 93 evaluable human subjects pretreated with pramlintide for 4 weeks and then treated with pramlintide, metreleptin, or pramlintide + metreleptin combination. Mean  $\pm$  S.E.M. (A) and LS mean  $\pm$  S.E.M. (B): \* $P < 0.05$  vs vehicle controls; # $P < 0.01$ , ## $P < 0.01$ , and ### $P < 0.001$  vs monotherapies.

e.g. diacylglycerol) from the liver, presumably resulting in decreased inflammation and fibrosis.

NAFLD and NASH are a significant component of lipodystrophy, a group of disorders characterized by relative leptin deficiency and abnormal amounts and distributions of adipose tissue leading to abnormal

deposition of fat in the liver (Garg 2011, Safar Zadeh *et al.* 2013). Recombinant leptin has been studied in patients with lipodystrophy and NASH diagnosed by biopsy and histological assessment. After treatment with recombinant leptin for a mean of 6.6 months, repeat histological examinations showed significant improvements in steatosis ( $P=0.006$ ) and ballooning injury ( $P=0.005$ ), with a reduction in mean NASH activity by 60% ( $P=0.002$ ). Fibrosis was unchanged over this short interval (Javor *et al.* 2005).

These findings provided the rationale to evaluate the potential for recombinant leptin in NAFLD and NASH in patients without significant lipodystrophy. A single-center pilot study analyzed ten men with biopsy-proven NASH, treated with 0.1 mg/kg recombinant leptin over 1 year to assess changes in liver histopathology, as well as changes in magnetic resonance spectroscopy of the liver. The study has been identified as completed, but not yet reported. (ClinicalTrials.gov Identifier: NCT00596934 March 9, 2014).

The potential application of leptin in patients with NAFLD and NASH will require further understanding of leptin's role in lipid handling, inflammation, and fibrosis in the liver along with identification of the potential subsets of patients who might benefit. One such population of patients might be those with a particular sensitivity to steatosis at a relatively low BMI.

### Safety and tolerability of leptin in common metabolic disorders

It is difficult to critically assess the overall safety and tolerability of leptin, given the diverse populations studied to date and the limited published data. Up to this point, the adverse events most common among the reported studies are injection-site reactions; overall, these have been mild to moderate and self-limited. Based on the clinical data reported for patients with generalized lipodystrophy, the potential for development of T-cell lymphoma was identified with or without metreleptin treatment and has been called out as a potential risk of metreleptin (Nainggolan 2014).

Notably, animal studies may help to identify potential target organ toxicities that could affect the metabolic disorders that leptin is potentially able to treat. Non-clinical studies have implicated the action of leptin in a number of pathways that could contribute to a safety concern in the clinic. Specifically, hypertension studies on rodents have implicated leptin as a potential mediator of obesity-associated hypertension (Hall *et al.* 2010). To this

point, increases in blood pressure have not been noted in any of the clinical trials reported. Furthermore, an acute study with pharmacological doses of recombinant leptin (0.2 mg/kg) in normal subjects demonstrated no impact on blood pressure or vascular reactivity (Brook *et al.* 2007).

Another potential risk identified in animals is whether leptin, as an adipocytokine, might exhibit proinflammatory activity (Loffreda *et al.* 1998). This concern has been specifically studied in the clinic with recombinant leptin in two studies to assess changes in inflammatory mediators before and after leptin treatment. Collectively, the data do not demonstrate proinflammatory activity of leptin (Canavan *et al.* 2005, Chan *et al.* 2005). Many other findings in animals have been or will need to be addressed, but are beyond the scope of this review.

A concern that has come to light in the clinic and that needs further consideration is the development of anti-drug antibodies that could cross-react with endogenous leptin and thus cause an effective leptin-deficient state, leading to loss of efficacy and infection (FDA Leptin Advisory Panel 2013). As noted earlier, the confirmatory study with metreleptin and pramlintide was stopped due to antibody formation. Development of anti-drug antibodies following leptin administration will need further study to understand the potential risk of leptin as a therapeutic in broader populations.

### Discussion

The identification of leptin (*ob/ob* gene) and its receptors has opened a significant door to our understanding of molecular pathways in the CNS and periphery that regulate feeding, body weight, metabolism, neuroendocrine regulation, and immune function. The larger promise of leptin after its discovery was for the treatment of general obesity and T2D. The studies outlined earlier, in unselected obese and T2D populations, with daily and weekly dosing of leptin as monotherapy, have clearly demonstrated no significant therapeutic activity acutely or chronically (through 24 weeks) capable of delivering clinically important weight loss or metabolic improvement (insulin sensitization; improvement in glucose or lipids). What remains to be determined is whether identifiable subsets of patients ('leptin low' or *ob* gene heterozygotes) with apparent general obesity or severe insulin resistance (ethnic-specific sensitivities to changes in fat distribution, although not currently considered lipodystrophy) might respond with clinically important weight loss and/or metabolic improvements to leptin monotherapy.

Based on nonclinical observations that leptin alone in DIO animals and obese humans did not demonstrate the dramatic weight-reducing benefits seen in *ob/ob* mice and humans, it seems that either a resistance to the weight-reducing actions of leptin exists in DIO mice and obese humans, or that leptin is not actively functioning to reduce body weight in the obese state. The opportunity to potentiate or enable the weight-reducing activities of leptin has led to significant efforts to combine leptin with other interventions or agents known to induce weight loss in order to overcome 'leptin resistance'. In essence, could weight loss alone lead to a return of leptin's actions to alter food seeking and weight loss/maintenance? Inducing weight loss through significant dietary caloric restriction or bariatric surgery followed by leptin treatment has demonstrated mixed results, as noted earlier, but none clinically significant. A more successful approach to unlocking leptin's therapeutic potential in simple obesity (and perhaps T2D) has focused on using pramlintide in combination with metreleptin to overcome 'leptin resistance'. The pilot study demonstrating a clinically significant 12% weight loss with this combination was intriguing, but was not placebo controlled. The confirmatory study was unfortunately discontinued due to the identification of apparent neutralizing antibodies. There will certainly be continued interest in developing interventions or combination therapies with leptin to enable body weight/fat loss.

The opportunity to impact other common metabolic disorders associated with obesity, specifically T2D, severe insulin resistance, NASH, and perhaps polycystic ovary syndrome as well as possibly T1D (Wang *et al.* 2010) will require further study. Given leptin's pleiotropic activities, careful experimental design will need to separate the weight loss-requiring and weight loss-independent actions of leptin to better aim the therapy at disease states that would be responsive. With the identification of leptin's effect in severe insulin-resistant normal-weight humans (Cochran *et al.* 2004), it would not be surprising to see the identification of a subset of patients with T2D and normal weight or modest overweight with severe insulin resistance, high triglycerides, and NAFLD/NASH who respond well to leptin.

Notably, much of what we have learned about the activity of leptin in humans, as well as the data supporting the initial approval of recombinant leptin were developed by a critical set of investigator-initiated clinical trials, both completed and ongoing. These studies have demonstrated significant beneficial effects of leptin administration in disorders of leptin deficiency (*ob/ob* and generalized

lipodystrophy) and have led to the recent approval of recombinant leptin (metreleptin) in Japan for the treatment of lipodystrophy (Chou & Perry 2013) and in the USA for the treatment of generalized lipodystrophy. Fewer trials have evaluated patients with partial lipodystrophy, both non-HIV associated (Simha *et al.* 2012) and HIV associated (Mulligan *et al.* 2009, Magkos *et al.* 2011), but, overall, these trials have shown benefit, albeit less dramatic than that seen in generalized lipodystrophy. The next steps for leptin will likely be in the hands of the broader scientific and clinical investigative community to further define its biology and clinical applications.

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