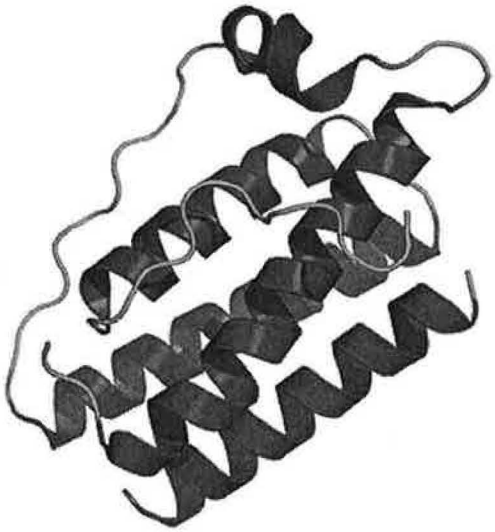
Internal Medicine Grand Rounds

April 25, 2008

LEPTIN THERAPY



THE PROMISE, THE DISAPPOINTMENT AND THE SILVER LINING

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This is to acknowledge that Abhimanyu Garg, M.D. has financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Garg will not be discussing "off-label" uses in his presentation.

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Special Interests:
Lipodystrophies and other disorders of adipose tissue
Progeroid syndromes
Lipoprotein disorders
Nutrition in diabetes and dyslipidemia
Regional obesity, insulin resistance and metabolic syndrome

Cover illustration: Three dimensional ribbon structure of leptin from 3dchem.com.

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Introduction:

Adipose tissue biology has become an important area of research, given the increasing prevalence of obesity and related metabolic complications all over the world. The discovery of the *ob* gene (1) and its product leptin in 1994 created tremendous interest in its role in human biology. Leptin is considered as an adipocyte-derived hormone, which is essential for the regulation of food intake and energy expenditure. Leptin is derived from *leptos* which means thin in Greek. Leptin is a member of class 1 cytokine superfamily and circulates as a 167 amino acid (16 kDa) protein. It is secreted by the adipocytes in proportion to the body fat stores (2). The *Lep* gene is one of the most extensively studied obesity genes. Homozygous *Lep* gene mutation, *ob*/*ob*, causes early-onset morbid obesity with diabetes in mice. In addition, affected mice exhibit hyperphagia, hypothermia, hypercorticosteronemia, decreased linear growth and infertility (3). The primary structure of leptin is highly conserved, and there is 84% homology in protein sequences between the mouse and human homologues (1). The *ob* mutation is a single base substitution (C→T at nucleotide position 428) that results in premature termination of the protein synthesis at codon 105 (1). Although *ob* trait is autosomal recessive, mice heterozygous for *lep* mutation (*ob*/+) are more obese and hypoleptinemic than the wild-type (+/+) littermates (4), indicating that the recessivity of this mutation is not complete.

Adipocytes represent the major source of circulating leptin, although a small amount of leptin is also synthesized in the placenta, gastric fundus, skeletal muscles and mammary epithelium. Leptin expression is influenced by the status of energy stores in the adipose tissue and obese people have higher serum leptin levels than the lean individuals. Subcutaneous adipose tissue secretes three times as much leptin as the omental adipose tissue (5). Fasting induces a fall in the mRNA of leptin in the adipose tissue and subsequently a decrease in circulating leptin levels. On the other hand, no acute rise in serum leptin level is seen after individual meals in humans (6). Insulin stimulates leptin production, but only after 24 to 48 hours. Circulating levels of leptin show a diurnal pattern, with the highest levels at midnight (50% increase over nadir value) and lowest levels in early morning hours. Pharmacological agonists of peroxisome proliferator-activated receptor γ (PPAR γ), thiazolidinediones (TZD) lower plasma leptin levels (7). There is a marked gender difference in serum leptin levels; adult women have approximately three times higher circulating leptin concentration compared to men.

Leptin exerts its actions through its interaction with leptin receptor (Ob-R), a member of the family of class 1-cytokine receptors located on cell surfaces. Multiple splice variants of Ob-R mRNA encode at least six leptin receptor isoforms (6, 8). Leptin receptor isoforms share an identical extracellular ligand-binding domain at the amino terminus but differ at the carboxy terminus. Only Ob-Rb (the long isoform) contains the intracellular motifs required for the activation of down stream signals like JAK (janus kinase)-STAT (signal transducers and activators of transcription) pathways. Leptin also affects ion channels, which may mediate its rapid non-transcriptional actions (9). Leptin receptors are highly expressed in the central nervous system, particularly in the hypothalamic nuclei involved in appetite regulation. Leptin receptors (Ob-Rb) are also expressed at a low level in several peripheral tissues including adipocytes, however their

role in leptin action is not very clear at present (6). In addition, soluble leptin receptor isoform functions as leptin binding proteins in the plasma. Soluble leptin receptors modulate serum leptin levels by delaying its clearance and determine the amount of free versus bound leptin in the serum. The free leptin is the form of leptin present in the cerebrospinal fluid and is presumed to be the biologically active form.

Leptin exerts its effects on energy balance mainly through its actions on the hypothalamus (10). Leptin activates neurons in the arcuate, ventromedial and dorsomedial hypothalamic nuclei and in the brainstem circuits implicated in the regulation of feeding behavior and energy balance (9, 11). Leptin decreases expression of orexigenic (appetite promoting) neuropeptides, neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the *medial* arcuate nucleus, while it increases the expression of anorexogenic (appetite suppressing) neuropeptides, α-melanocyte stimulating hormone (α-MSH) and cocaine-and-amphetamine regulated transcript (CART) in the *lateral* arcuate nucleus. Other possible mediators of leptin action in the brain include melanin concentrating hormone, corticotropin releasing hormone (CRH), cholecystokinin (CCK), glucagon-like peptide (GLP-1), urocortin, bombesin and serotonin. Apart from regulating food intake, these mediators are also involved in various other neuro-endocrine effects of leptin including its regulation of adrenocorticotropin (ACTH), thyroid stimulating hormone (TSH), gonadotropins and growth hormone (6).

While *in vitro* and some *in vivo* studies have demonstrated direct actions of leptin on peripheral tissues, the relative importance of these actions in humans remains to be proven. It has been suggested that leptin stimulates lipolysis and therefore may protect against ectopic adipogenesis (e.g. deposition of fat in the skeletal muscles, liver and β-cells in the pancreatic islets) (12). Other suggested peripheral actions of leptin include inhibition of insulin secretion by pancreatic β cells (through activation of ATP-sensitive K^+ channels in the pancreatic β- cells), control of blood pressure, regulation of immunity and hematopoiesis and angiogenesis (6). Unlike the well-established actions of leptin in the central nervous system, these peripheral actions of leptin remain controversial.

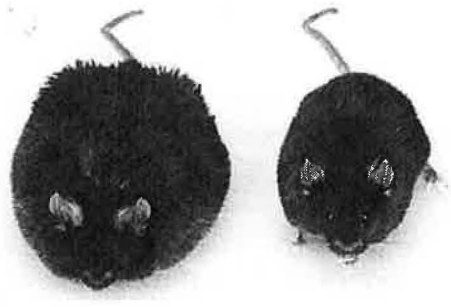


Fig. 1 Effect of leptin administration in genetically obese (ob/ob) mouse. The ob mouse on the left did not receive leptin and weighed ~67 g while the mouse on the right, who received daily injections of leptin for 4.5 wk, weighed 35 g. Normal mice weigh ~24 g. Photo by John Sholtis, The Rockefeller University, New York, N.Y. Copyright ©1995 Amgen Inc.

Daily intraperitoneal injections of either mouse or human recombinant OB protein reduced the body weight of ob/ob mice by 30 percent after 2 weeks of treatment (13). Continuing therapy for 4.5 weeks led to further near normalization of the body weight (Fig.1). Since leptin administration normalized all aspects of the obesity and diabetes syndrome and restored reproductive function in *ob/ob* mice by acting through leptin receptors in the central nervous system (14, 15), it was regarded as a signal crucial factor for energy balance, body adiposity and reproduction. These findings created tremendous enthusiasm and "The Promise" of therapeutic benefits of leptin therapy for treating human obesity.

Therapeutic Role of Leptin

Based on the marked benefit of leptin replacement therapy for ob/ob mouse, the initial studies in humans were carried out in obese patients. Since then, leptin therapy has been tried in hypoleptinemic patients with congenital leptin deficiency (due to leptin mutations), generalized and partial lipodystrophies (due to genetic defects as well as acquired, autoimmune disorders) and hypothalamic amenorrhea (due to excessive exercise and weight loss). The leptin therapy, however, still remains investigational and has not been approved by FDA.

A. Leptin Therapy for Obese

Amgen Inc. generated recombinant methionyl human leptin (now called metreleptin by Amylin Inc.) for clinical trials. The first study in obese and lean adults was reported by Heymsfield et al in 1999 (16). This trial had a complicated design (Fig. 2) and included both lean and obese subjects, continuous sc infusion of leptin in a dose escalating manner for up to 2 mg/kg body weight/ day as well as bolus sc injection once a day. For administering high dose of leptin (0.3 mg/kg/d), a highly concentrated preparation of metreleptin was developed. However, the group receiving this concentrated preparation experienced unacceptable reactions at the injection site and thus that preparation was discontinued and the enrollment in that group was halted. The data on continuous sc infusion of metreleptin and on the highly concentrated form were not reported in the paper by Heymsfield et al (16).

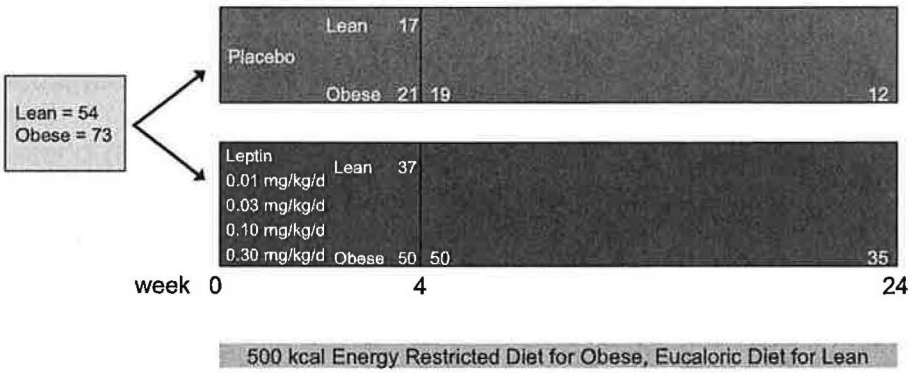
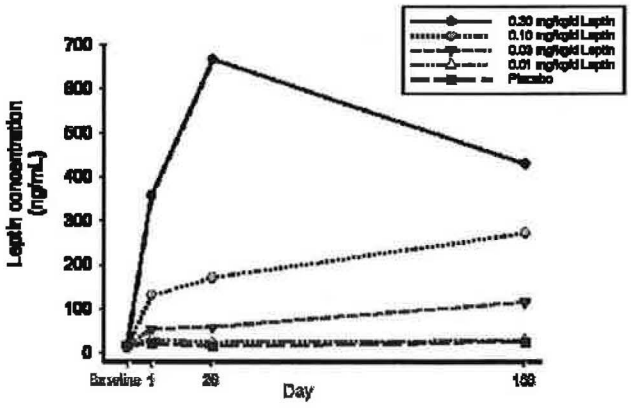


Fig. 2 The design of the leptin trial by Heymsfield at al (16). The numbers indicate the subjects who continued to participate in phase 1 (for 4 wk) and phase 2 (another 20 wk).



The lean subjects were randomized to receive leptin in various doses (0.01, 0.03, 0.10, 0.30 mg/kg/day) or placebo for only 4 wk and were placed on a eucaloric diet. The obese subjects were randomized to receive leptin in various doses (0.01, 0.03, 0.10, 0.30 mg/kg/day) or placebo initially for 4 wk but then continued the trial for additional 20 wk (Fig. 2).

Fig. 3 Maximum serum leptin levels during the study by Heymsfield et al (16).

The obese subjects were placed on an energy restricted (500 Kcal less than the weight maintenance energy) diet. During the trial, serum leptin levels were as high as 171-272 ng/mL and 480-667 ng/mL on the 0.10 mg/kg/day and 0.30 mg/kg/day dose of metreleptin, respectively (Fig. 3). The leptin levels achieved were thus more than 10 to 50 fold higher than those seen in normal healthy adult men and women in the US.

The investigators reported a dose dependent weight loss in the obese. The obese subjects on placebo, 0.01, 0.03, 0.10, 0.30 mg/kg/day of metreleptin lost a mean of 1.3, 0.7, 1.4, 2.4 and 7.1 kg of body weight, respectively (Fig. 4). The intent to treat analysis with last observation carried forward revealed a mean of 1.0, 0.7, 1.4, 2.1 and 3.3 kg of body weight loss in the various groups, respectively. The obese subjects mainly lost body fat and not lean body mass as measured by dual energy X-ray absorptiometry.

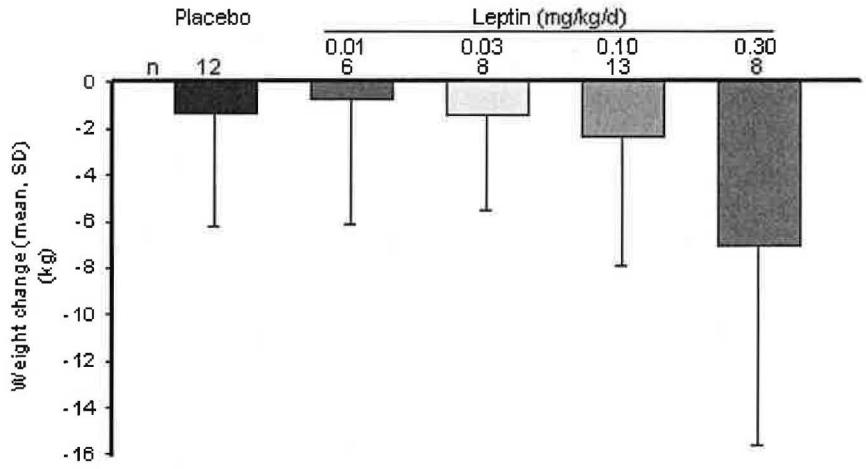


Fig. 4 Weight change (observed) during the study in obese subjects after 24 wk of leptin therapy or placebo. The n indicates the number of subject completing the phase 2.

Since this initial trial, only one other study has been reported about the efficacy of metreleptin in the obese subjects (17). In this randomized, double-blind, placebo-controlled, multicenter trial, 284 (188 F, 96M) obese (body mass index [BMI] 27-37 kg/m^2) subjects received either placebo or metreleptin (10 mg in morning, 10 mg in evening, or 10 mg twice daily) for a total of 12 wk. These doses of metreleptin were equivalent to approximately 0.11 or 0.22 mg/kg/d, quite close to the two high doses used by Heymsfield et al (16). Patients were advised to reduce energy intake by 500 kcal/d.

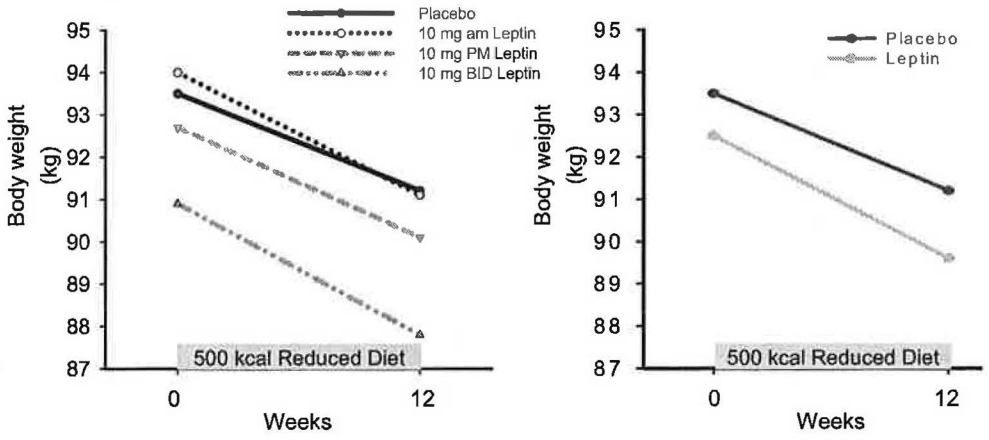


Fig. 5 Weight changes during the study by Zelissen et al (17). Left panel shows results with various schedules of administration of leptin and the right panel shows the combined results of all patients randomized to leptin therapy.

Unfortunately, the results were essentially negative and no significant change in body weight was observed with metreleptin compared to placebo (p=0.68). The mean weight loss in patients on placebo, 10 mg in morning, 10 mg in evening or 10 mg twice daily of metreleptin was 2.6, 2.8, 2.7 and 3.4 kg, respectively (Fig. 5). Those receiving metreleptin had more injection site reactions (83%) compared to those on placebo (36%) (Table 1). A total of 88% of the patients developed seroreactivity to metreleptin.

Most frequent adverse events

Table 1. Most frequent adverse events during the study by Zelissen et al (17).

	Placebo (all dose groups)	Leptin (all dose groups)
Injection-site reaction	26 (36)	172 (83)
Headache	7 (10)	34 (16)
Infection upper respiratory	15 (21)	33 (16)
Influenza-like symptoms	9 (12)	25 (12)
Pharyngitis	2 (3)	15 (7)
Back pain	4 (5)	11 (5)
Diarrhea	6 (8)	9 (4)
Dizziness	4 (5)	9 (4)
Nausea	5 (7)	9 (4)
Abdominal pain	3 (4)	9 (4)
Rhinitis	3 (4)	9 (4)

In addition to these trials, Amgen Inc. conducted other phase II studies in obese and a summary of that data essentially reveals no significant effects of various doses of metreleptin in reducing body weight in subjects with obesity or type 2 diabetes (Table 2). Some of the data in the Table includes subjects reported by Heymsfield et al (16) and Zelissen et al (17).

Table 2. Overview of completed Phase 2 studies with metreleptin therapy in obese subjects or patients with type 2 diabetes. Source Amylin Pharmaceutical Inc.

Study	Population/ Design	Duration (Week)	N (Total)	Metreleptin Dose (Administration)	Weight Loss (vs. placebo)	Statistical Significance
950272	Obese	24	256	0.01 - 2.0 mg/kg QD bolus SC infusion	-1.82 kg (bolus) +1.34 kg (infusion)	p = 0.378 p = 0.663
960240	Obese	33	30	0.3 - 2.0 mg/kg (SC infusion)	+2.4%	p = 0.287
970121	Obese	4	125	0.1 - 1.0 mg/kg (IV)	-1.7%	Not available
970164	Obese w/ dietary lead-in	12	284	10 mg QD or BID	+0.4% (BID)	p = 0.643
970213	Obese w/ dietary lead-in	28	228	10 mg BID	-1.0%	p = 0.381
970171	Type 2 DM	16	93 90	10 mg QD 10 mg BID	-0.43% -0.74%	p = 0.407 p = 0.167
970188	Type 2 DM	16	113	10 mg BID	-1.0%	p = 0.060
980219	Type 2 DM	24	66	10 mg BID	-1.2%	Not available
980236	Obese	52	267	10 - 20 mg QD	-0.5kg (10 mg)	p = 0.340

Hoffman La-Roche generated human ob protein, leptin, by recombinant technology and developed pegylated-ob (PEG-OB, conjugated with polyethylene and polypropylene) for clinical trials. The first trial was conducted in 30 obese men (mean

BMI 33.9 kg/m^2) (18). They were randomized to receive 20 mg PEG-OB (2 mL, 10 mg/mL) or placebo for 12 weeks. All subjects were prescribed a hypocaloric diet 500 kcal reduced from weight maintaining diet. The therapy led to sustained increase in concentrations of PEG-OB and leptin. However, while both the groups lost weight there was no significant difference in the weight loss between the two groups (Fig. 6). Interestingly, 24 hour energy expenditure as well as sleeping metabolic rate while subjects were in a respiratory chamber was also not different between the two groups.

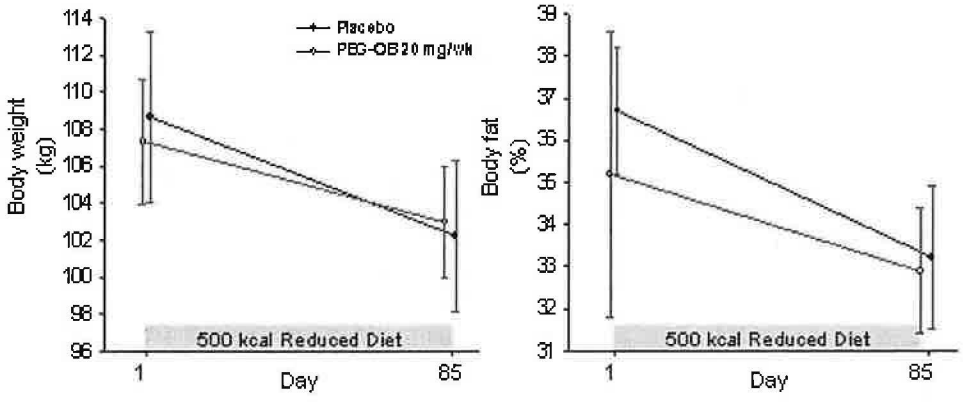


Fig. 6 Changes in body weight with PEG-OB therapy (20 mg per week) for 12 wk(18).

Following this negative trial, the same group of investigators reported two other trials with higher doses of PEG-OB, 60 mg or 80 mg per week (19, 20). The trial using 60 mg per wk of PEG-OB randomized 28 obese subjects (16F, 12M) with BMI from 28-39 kg/m^2, to placebo or PEG-OB for 8 wk. All subjects reduced energy intake by 800 kcal/d. There was no significant difference in the weight loss on placebo compared to PEG-OB, mean weight loss 3.8 kg vs. 4.8 kg, respectively (p=0.32) (Fig. 7). There were also no significant differences in the fasting glucose, insulin, FFA, triglycerides, CRP or sTNF-R levels (19).

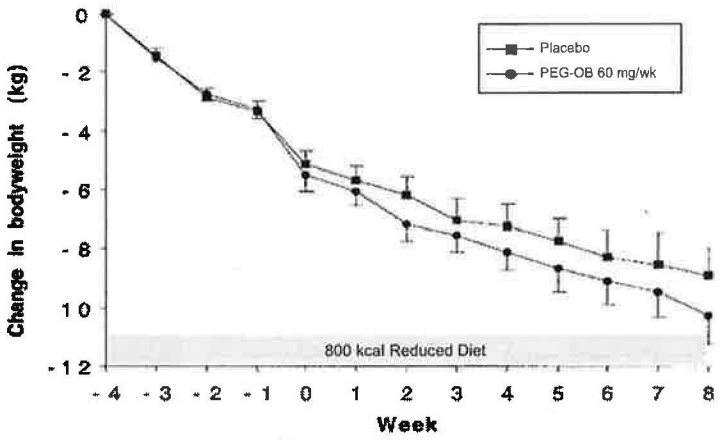


Fig. 7 Changes in body weight with PEG-Ob therapy (60 mg per wk). Patients were on a weight reducing diet, 800 kcal below the weight maintenance requirements diet 4 wk prior to being randomized (19).

In the other study (20), 24 obese men were randomized 1:1 to receive either placebo or metreleptin 80 mg per wk for a period of 6.5 wk. These subjects were also placed on a severely energy restricted diet of 500 kCal/d for 46 days. Compared to placebo, PEG-OB resulted in higher weight loss (mean 11.8 kg vs. 14.6 kg, respectively, p=0.027) (Fig. 8). Most of the weight loss was attributed to loss of body fat (74-79%). There was no difference in resting metabolic rate noted. PEG-OB led to reduction in appetite from 40 to 34 mm on the visual analogue scale whereas on placebo appetite increased from 38 to 48 mm (P=0.03).

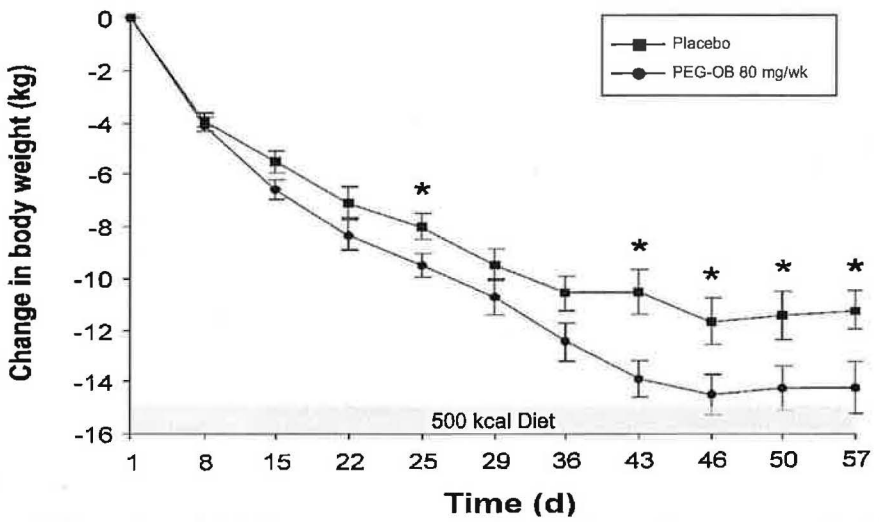


Fig. 8 Changes in body weight with PEG-OB therapy (80 mg per wk). Patients were on 500 kcal weight reducing diet (20).

More recently, based on the data in animal models of diet-induced obesity (DIO) investigators found that combination of pramlintide (an amylin analogue) and metreleptin can induce additional weight loss compared to metreleptin or pramlintide alone. In a 24 week, phase 2A, randomized, double-blind, active-drug controlled, multicenter study, 177 overweight or obese subjects were randomized in a ratio of 2:2:1 to receive pramlintide 360 µg/metreleptin 5 mg, pramlintide 360 µg/placebo or metreleptin 5 mg /placebo (Table 3). The weight loss achieved with metreleptin and pramlintide combination was significantly more than that achieved with metreleptin alone or pramlintide alone (Fig. 9). This preliminary study needs confirmation in further clinical trials.

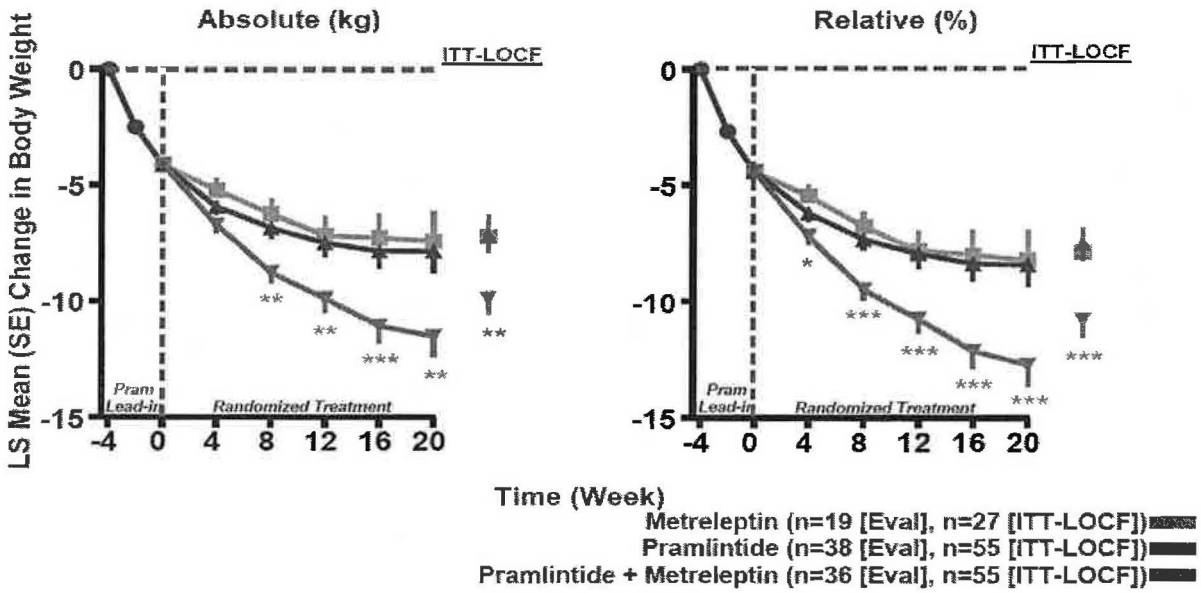
Table 3 Baseline demographics of patients randomized to various treatment regimen. Data from Amylin Inc. (on file).

Enrolled (N=177)	Metreleptin	Pramlintide	Metreleptin + Pramlintide	Non-Randomized
Enrolled (N)	27	56	56	38
Sex (% female)	63	63	63	63
Age (y)	40.5 ± 8.1	38.3 ± 9.1	38.5 ± 8.4	37.9 ± 7.5
Weight (kg)	93.8 ± 14.3	91.7 ± 11.1	93.9 ± 12.8	94.5 ± 16.0
BMI (kg/m^2)	32.0 ± 2.1	31.5 ± 2.0	32.0 ± 2.1	32.5 ± 1.9
Total Excess Body Weight (kg)	21.0 ± 7.2	19.2 ± 6.3	20.8 ± 6.9	22.4 ± 7.4

Because of the profound effects of leptin on appetite control, initial expectation was that most obese subjects might be deficient in leptin, like the *ob/ob* mice. However, with the exception of a very few individuals, most obese human beings have elevated plasma leptin levels (2). The higher leptin levels in obesity fail to limit further weight gain. Some investigators suggest that obesity may represent a leptin resistant state. Since increased energy stores would favor survival in periods of famine, it has been argued that the adipostatic aspect of leptin action may have been selected against during the course of evolution. Leptin resistance, perhaps mediated through saturable transport mechanisms across the blood brain barrier (21, 22) or defects in post-receptor signaling (23), would thus permit weight gain despite rising level of circulating leptin in obesity. Others have proposed that leptin's dominant role may be as a mediator of adaptation to fasting rather than as an anti-obesity hormone (6). Increasing serum leptin levels by

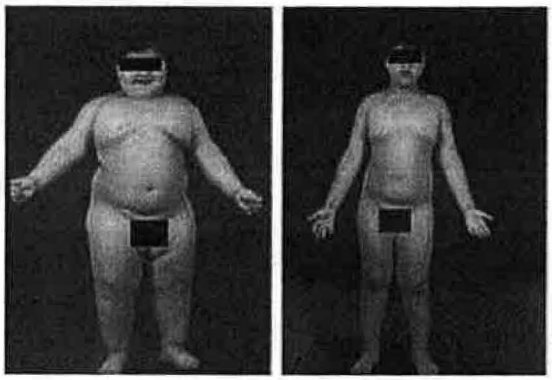
exogenous leptin therapy alone results in either no significant weight loss or only modest weight loss in obese subjects. Overall negative trials of leptin therapy in obesity or modest efficacy for weight loss are what brought "the disappointment" in the scientific community. Whether the combination of leptin with other satiety producing hormones such as pramlintide will become a therapeutic option remains to be determined.

Fig. 9 Body weight changes on various treatment regimen. Data from Amylin Inc.(on file). Square symbols indicate the group receiving metreleptin only, upright triangles, pramlintide and downward facing triangles pramlintide and metreleptin combination therapy.



B. Leptin in congenital leptin deficiency

Congenital leptin deficiency in humans was first described by Montague et al (24) in 1997 in two British children of Pakistani origin with severe childhood obesity and undetectable circulating leptin levels. They were found to be homozygous for a frameshift/premature stop mutation, c.398delG (Δ133G) in the leptin (*LEP*) gene. Extreme hyperphagia was noted, leading to marked obesity, and accompanied by metabolic, neuroendocrine and immune dysfunction. Farooqi et al (25) then reported the effects of leptin replacement therapy in the older child, a 9-year old girl who weighed 94.4 kg (> 99.9th percentile for age) before the initiation of leptin therapy. Twelve months of human recombinant leptin therapy at a dose (0.028 mg/kg lean mass) predicted to achieve 10% of predicted serum leptin levels based on age, gender and body fat mass resulted in a 16.4 kg weight loss. More than 95% of the weight loss was accounted for by loss of fat mass, and the total body fat content declined from 59% to 52%. A small decrease in lean mass and an increase in bone mass were also noted. Energy intake declined from about 1600 kcal/day to 1000 kcal/day, and while there was a decrease in basal energy expenditure consistent with weight loss, the total energy expenditure was almost unchanged as physical activity increased with amelioration of morbid obesity.



Fasting insulin and circulating free fatty acid levels decreased, but no significant change was noted in glucose or lipid levels which were normal at baseline.

Fig. 10 Effects of 4 years of leptin replacement therapy in a 4.5-year-old boy with congenital leptin deficiency (26). The left panel shows the subject before initiation of leptin therapy and right panel shows him after leptin therapy.

Subsequently, prolonged therapy for over 4 years in this child has been reported to have sustained beneficial effects on appetite, fat mass and hyperinsulinemia, besides normal initiation of puberty with menarche at age 12.1 years (26). Similar beneficial effects of leptin therapy were noted in two younger children (4.5 and 3.5 y) with congenital leptin deficiency with the same mutation in whom an increase in lean mass was also noted (26) (Fig. 10). Leptin therapy in these pre-pubertal children did not have any effect on gonadotropin levels, and did not result in precocious puberty, suggesting that leptin facilitated appropriately timed pubertal development. Further, there were improvements in circulating $CD4^+$ T cell number and function as assessed by their proliferative response and cytokine production in response to polyclonal stimulation (26).

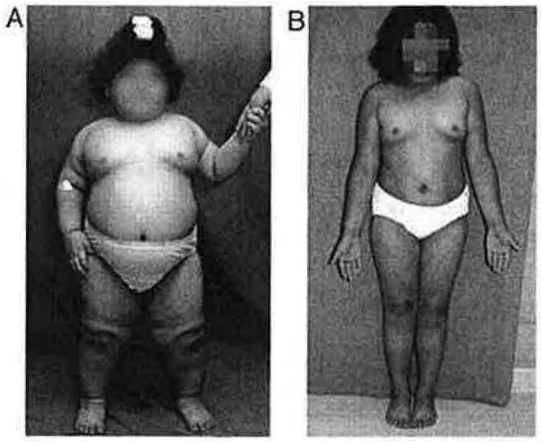


Fig. 11 Effects of 4 years of leptin replacement therapy in a 5-year-old girl with congenital leptin deficiency (27). Panel A before leptin therapy and panel B after leptin therapy.

Gibson et al (27) similarly administered leptin replacement therapy to a 5-year-old Canadian girl with congenital leptin deficiency, also of Pakistani origin, and with the same homozygous frameshift/ premature stop mutation. Four years of treatment resulted in sustained weight loss (BMI decreased from 43.4 to 24.2 kg/m^2) (Fig. 11 and 12), improvements in hyperinsulinemia and hyperlipidemia, and resolution of subclinical hypothyroidism.

Recently, functional magnetic resonance imaging (fMRI) studies in similar children starting leptin replacement therapy, has shown that leptin acts on neural circuits governing food intake to diminish perception of food reward while enhancing the response to satiety signals generated during food consumption (28). In the leptin deficient state, visual images of food elicited a high 'liking rating' and activation of striatal regions in both the fasting and fed state, while in the leptin replaced state, this response was seen only in the fasted state. Further studies are likely to shed more light on the mechanisms by which leptin regulates feeding behavior in humans. Currently, 8 children from 5 families of Pakistani origin with the frameshift/premature stop mutation, and one Egyptian child with a novel nonsense mutation have been identified with congenital leptin deficiency, of whom six are on leptin-replacement therapy (29).

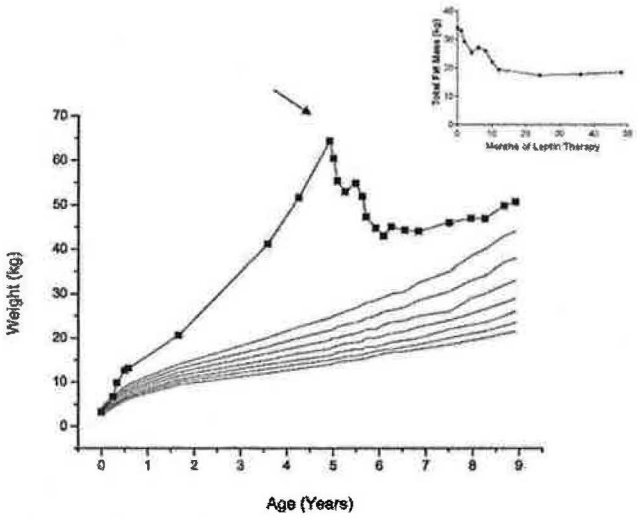


Fig. 12 Effect of leptin therapy on weight loss in a 5-year-old girl with congenital leptin deficiency. The arrow indicates the start of leptin therapy (27). Inset shows the changes in total fat mass.

Similar to children, adults with genetic congenital leptin deficiency also show good response to leptin-replacement therapy. A C105T missense mutation in the leptin gene has been identified in a large Turkish pedigree in association with morbid obesity and hypogonadism (30). Three siblings, (a female aged 40, another female aged 35 and a male aged 27 years) belonging to this pedigree have been treated with low dose physiological replacement of leptin (0.01 – 0.04 mg/kg body weight/d) for 18 months resulting in weight loss and improvement in metabolic parameters (31). The mean BMI decreased from about 51 to 27 kg/m^2, and was associated with reduction in serum triglycerides and increase in HDL cholesterol. One of the patients had diabetes, and following two months of leptin therapy, her plasma glucose and hemoglobin A1c levels normalized without any other hypoglycemic therapy. Further, there was resolution of hypogonadism in the male patient, and restoration of ovulatory cycles in the two female patients. Microanalysis of eating behavior in these patients revealed that food intake at 15 weeks was reduced by approximately 50%, and there were substantial changes in ratings of hunger and satiety before most meals (32). MRI revealed changes in tissue composition (increase in gray matter concentration) in brain areas containing neural circuits regulating hunger and satiety (33). Further, functional MRI studies showed that leptin replacement reduced brain activation in regions linked to hunger while enhancing activation in regions linked to satiety (34).

Thus, even though the total number of patients with congenital leptin deficiency is limited at this time, they respond dramatically to leptin replacement therapy. Congenital leptin deficiency therefore constitutes one condition which shows "Silver lining" response to leptin therapy. Whether subjects harboring heterozygous *LEP* mutations who have been found to have slightly low serum leptin levels and mild adiposity will also respond to leptin replacement therapy, remains to be determined.

C. Leptin and Lipodystrophy

The syndrome of lipodystrophy in humans encompasses a wide variety of genetic and acquired disorders characterized by selective loss of adipose tissue. Fat loss may be near total as in generalized lipodystrophies, or may be confined to certain areas only as in partial lipodystrophies. Both varieties may result from either genetic mutations or acquired causes as reviewed in several recent publications (35, 36). Despite their varying genotype and phenotype, most patients with lipodystrophy have similar metabolic complications due to marked insulin resistance. Further, circulating leptin levels are low, especially in patients with generalized lipodystrophies (37, 38), and may play an important role in the pathogenesis of metabolic complications.

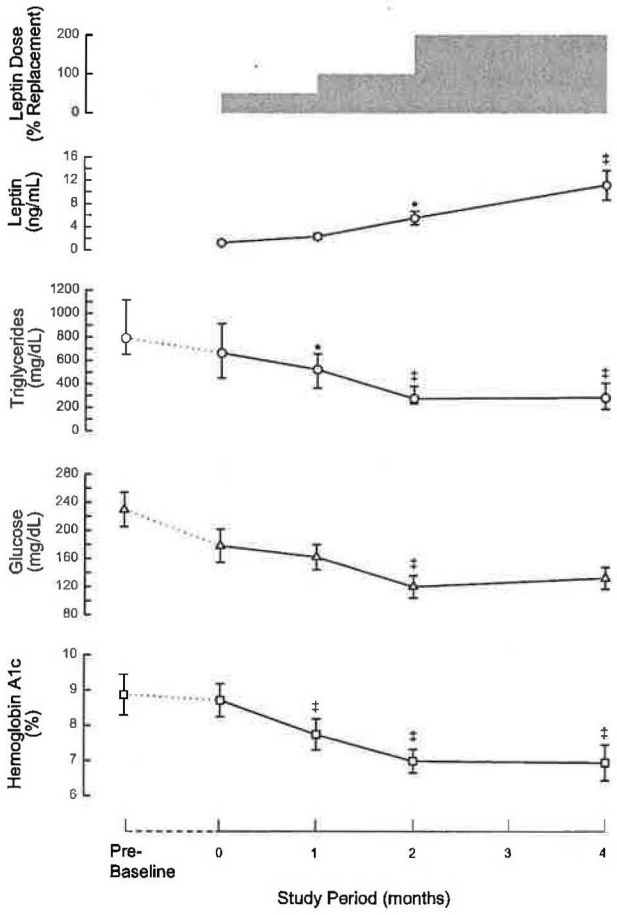


Figure 13: Effects of administration of recombinant methionyl human leptin administered over a period of 4 months on the levels of serum leptin, hemoglobin A1c, fasting plasma glucose and serum triglycerides in 9 patients with lipodystrophies. *: $p < 0.05$, ‡: $p < 0.001$ (data from Oral et al (42)).

Early investigations in mouse models of lipodystrophy provided clues to the utility of leptin therapy in lipodystrophy. Gavrilova et al (39) had shown the beneficial effect of adipose tissue transplantation on metabolic complications in the A-ZIP/F-1 lipodystrophic mice, but subsequent investigations showed that transplantation of fat from leptin deficient mice was not effective (40). It was clear that besides serving as a storehouse for excess energy, adipose tissue had an active secretory function involving the production of leptin, which regulated food intake and energy metabolism. This was more clearly shown by Shimomura and colleagues from the Brown and Goldstein lab in another mouse model of lipodystrophy, the n-SREBP1c mice which overexpress a truncated form of the transcription factor SREBP1 in adipose tissue (41). Continuous subcutaneous infusion of leptin for 12 days in these lipodystrophic mice resulted in marked improvement in hyperglycemia, hyperinsulinemia, hyperlipidemia and hepatic steatosis, an effect not observed in pair-fed mice. On Drs. Brown and Goldstein's advice, I proposed a similar study of leptin replacement therapy to Amgen Inc. and subsequently, the group at NIDDK, headed by Simeon Taylor joined us in developing a collaborative protocol.

Accordingly, a prospective open-label leptin-replacement trial was conducted in 9 lipodystrophic subjects at UT Southwestern Medical Center and the NIH. Four months of treatment with human recombinant leptin, administered as twice daily subcutaneous injections, resulted in marked improvement in metabolic complications (42). The fasting serum triglycerides decreased by nearly 60% and hemoglobin A1c declined by 1.9% (absolute value) (Fig. 13). There were improvements in glucose tolerance and insulin sensitivity, and reduction in liver volume. Self reported caloric intake decreased by 40% from an average of approximately 2600 kcal/day, and the mean weight loss was 3.6 kg. Leptin therapy was well tolerated with no significant adverse effects. Discontinuation of leptin therapy for a few days resulted in a steep increase in serum triglycerides and symptoms of pancreatitis in one of the patients despite adherence to the same low calorie diet.

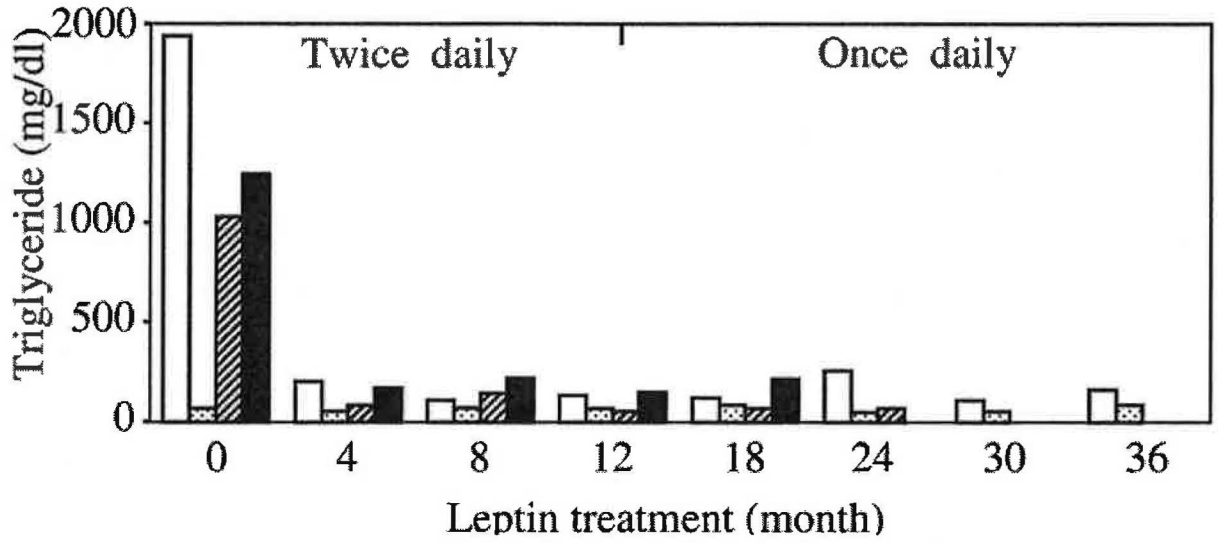


Fig. 14 Long term effects of leptin replacement therapy on serum triglyceride concentrations in patients with generalized lipodystrophy(47)

Continued therapy has been associated with sustained benefits and no serious side effects. Javor et al (45) reported significant and sustained improvement in glycemia, dyslipidemia and hepatic steatosis in 15 patients with generalized lipodystrophy treated with recombinant leptin for 12 months. Despite reduction or discontinuation of anti-diabetes medications, the fasting plasma glucose decreased from 205 ± 19 to 126 ± 11 mg/dL, and the hemoglobin A1c decreased from 9 ± 0.4% to 7.1 ± 0.5%. Similarly, serum triglycerides decreased by about 63% while total and LDL cholesterol decreased by about 40%. The mean weight loss and was about 4.4 kg, and a corresponding decrease in resting energy expenditure was also noted. Further, improvements in renal function in terms of reduction in proteinuria and hyperfiltration have also been reported by the NIH investigators (46). Recently, Japanese investigators have also reported similar sustained improvements in glycemic control, dyslipidemia and hepatic steatosis in 7 patients with generalized lipodystrophy who were followed for upto 36 months (47) (Fig. 14). Leptin replacement therapy was noted to be associated with improved insulin

sensitivity on glucose clamp studies, and improved insulin secretion on glucose tolerance tests in these patients. Improvements in peripheral glucose uptake and hepatic glucose suppression during euglycemic hyperinsulinemic clamp studies have been reported by us and other investigators as well (43, 44). Though limited by small numbers, these preliminary studies strongly suggest that leptin–replacement therapy is safe and efficacious in patients with generalized lipodystrophy. A randomized placebo controlled double-blind trial of leptin therapy in generalized lipodystrophy is nearing completion at UT Southwestern Medical Center.

Interestingly, leptin therapy was also associated with improvement in sex hormone profile. In female subjects, there was a decrease in serum free testosterone, increase in sex hormone binding globulin and restoration of normal menstrual cycles, while in male subjects, serum testosterone and sex hormone binding globulin levels increased (48). While these changes could be secondary to improvement in insulin sensitivity, there was also a more robust leuteinizing hormone (LH) response to LH-releasing hormone suggesting improved pituitary function. Serum IGF-1 levels also increased, but no changes in growth hormone, thyroid or adrenal hormones were noted. While there is some evidence that leptin may mediate some of the changes in pituitary hormone secretion during acute fasting (49, 50), the effect of chronic leptin therapy on pituitary function needs further investigation.

Leptin therapy has been shown to be modestly effective in patients with partial lipodystrophy as well. Park et al (51) reported a significant decline in fasting serum triglycerides, glucose and insulin in 6 patients with Familial Partial Lipodystrophy, Dunnigan (FPLD) variety who were treated with human recombinant leptin for 12 months. However, glucose tolerance and hemoglobin A1c did not change with therapy. It is possible that the longer duration of diabetes in these older subjects, or their higher baseline leptin levels may have been responsible for the less robust response in comparison with patients with generalized lipodystrophy. We are currently performing an open-label leptin replacement trial in FPLD subjects comparing the response between those with moderately low leptin levels to those with severely low leptin levels. Similarly, the benefit of leptin therapy in patients with HIV-associated lipodystrophy is also not clear. In a 2 month randomized double blind crossover study in 7 men with HIV associated lipodystrophy, significant decline in fasting serum insulin levels were noted, while glucose and lipid levels did not change (52). Larger, and more long term studies are needed before leptin therapy can be recommended to this group of patients. A randomized controlled trial is nearing completion at UT Southwestern Medical Center.

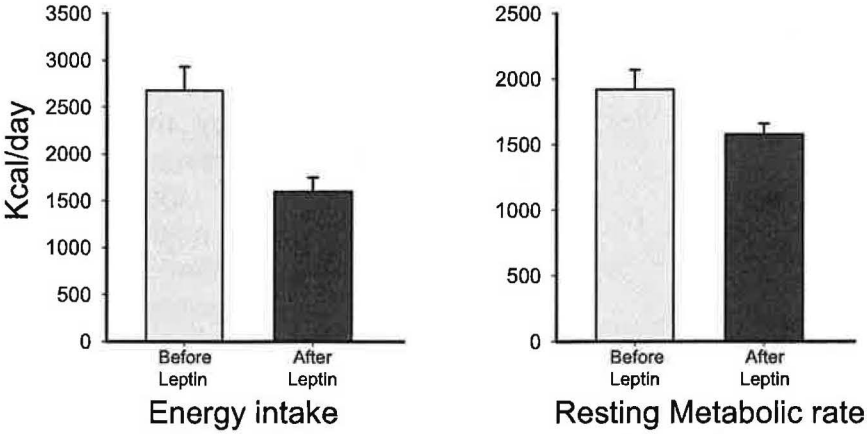


Fig. 15 Effects of metreleptin on energy intake and resting metabolic rate in patients with lipodystrophy. (data from Oral et al (42)).

The mechanism by which leptin therapy improves metabolic functions in lipodystrophic patients is not entirely clear. There is considerable debate about the relative importance of a central hypothalamic effect on regulation of energy intake versus a peripheral effect on energy metabolism in the liver and skeletal muscle. Leptin clearly has effects at both levels, and it remains to be determined which is more relevant to human lipodystrophic patients treated with leptin. As mentioned before, leptin therapy was associated with significant reductions in energy intake (42) suggesting its role in appetite regulation (Fig. 15). Detailed studies by McDuffie and colleagues (53) in 8 female subjects with generalized lipodystrophy before and after leptin replacement therapy showed decreased hunger and increased satiety in the leptin-replete state. Satiation, measured as the time to voluntary cessation of meal intake after a 12 hour fast, decreased by 53%, while satiety time, measured as the time to hunger after consumption of a standardized meal, increased by over 100% (Fig. 16). The authors concluded that leptin replacement lead to 'less caloric, shorter, more satiating meals, and longer lived satiety'.

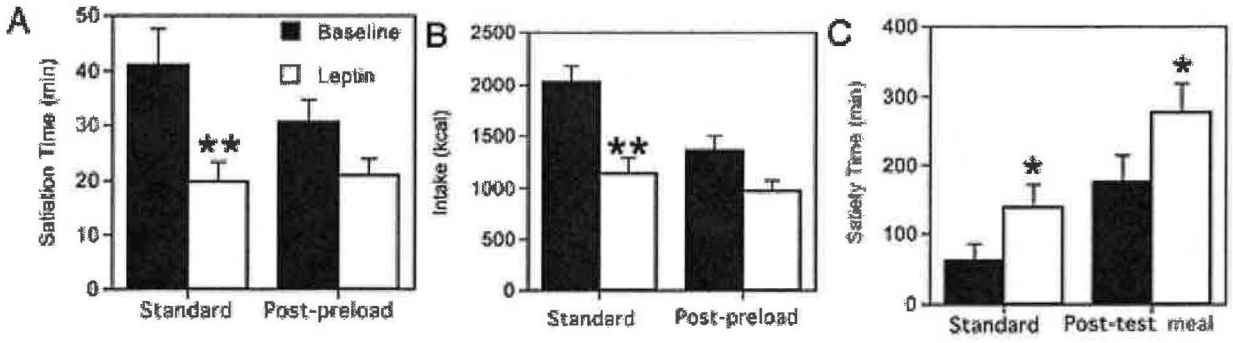


Figure 16: Effect of leptin-replacement therapy on satiety in patients with lipodystrophy (53)

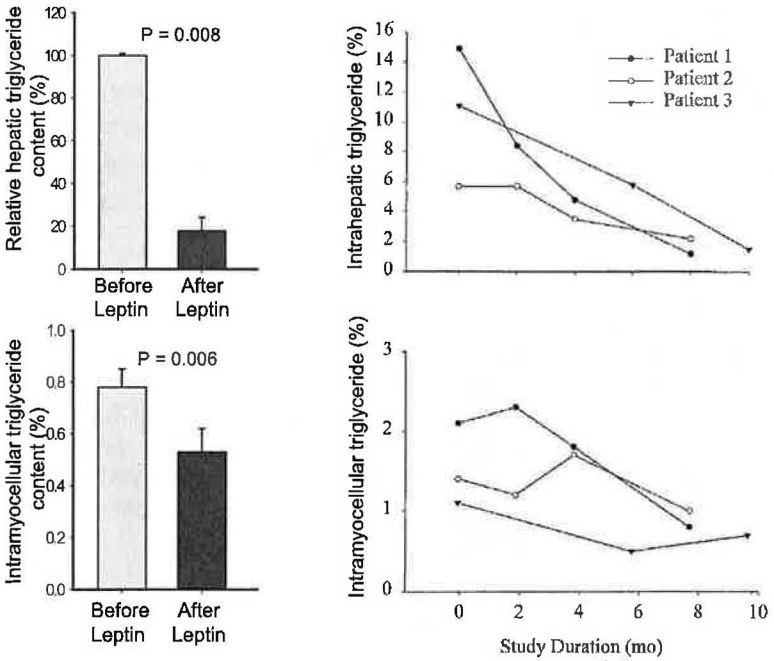


Figure 17 Effects of administration of recombinant methionyl human leptin on the levels of intrahepatic and intramyocellular triglyceride. Left panels from Petersen et al(43) and right panels from Simha et al (44) .

Besides this important effect, leptin may directly influence energy metabolism in peripheral tissues. It has been shown that leptin stimulates fatty acid oxidation by increasing the activity of AMP-activated protein kinase (54) which may be responsible for its anti-

steatotic effects. As mentioned before, leptin therapy was associated with reduction in hepatomegaly by 30-40% (42, 55), and histological studies have confirmed the improvement in steatosis and ballooning injury with a reduction in mean NASH activity score of 60% (55). Using magnetic resonance spectroscopy to quantitate hepatic fat, we and other investigators have shown a nearly 80% reduction in intrahepatic fat content (43, 44) (Fig. 17). Similarly, we have noted a 40% decline in intramyocellular lipid accumulation as well (44). These anti-steatotic effects of leptin may play an important role in its insulin sensitizing role.

Interestingly, leptin therapy has been reported to be of some benefit in subjects with other syndromes of extreme insulin resistance such as the Rabson-Mendenhall Syndrome (56). Recombinant leptin therapy for 10 months resulted in a 40-60% decrease in fasting serum glucose and insulin levels, and improved overall glucose control in two siblings with this rare disorder. Whether leptin has similar insulin–sensitizing effects in patients with the more common, but milder, insulin resistance syndrome remains to be determined.

D. Leptin and Hypothalamic Amenorrhea

In non-lipodystrophic women with hypothalamic amenorrhea secondary to exercise or weight loss, but with no eating disorders, leptin therapy (0.08 mg/kg/day) has been reported to improve reproductive hormone profile and menstrual function (57) (Fig. 18). Recombinant leptin therapy for 3 months is 8 such women with relative leptin deficiency increased LH levels, LH pulse frequency, estradiol levels, ovarian and follicular volume and the number of dominant follicles. 3 patients developed ovulatory menstrual cycles, and 2 showed pre-ovulatory follicular development. This was accompanied by weight loss of about 2.5 kg, and further studies are needed to determine the safety and efficacy of leptin therapy for secondary amenorrhea in women who already have a low body weight.

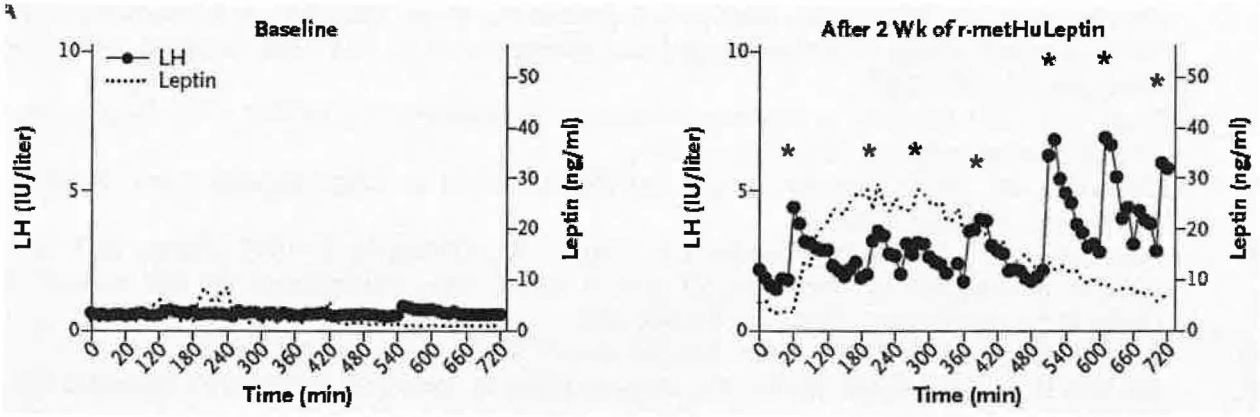


Fig 18. Serum LH and leptin levels in two patients with hypothalamic amenorrhea at baseline and after 2 weeks of metreleptin therapy (57).

Future of Leptin Therapy

Based on this review, leptin therapy certainly is indicated in patients with congenital leptin deficiency and these patients should be treated as early as possible. As far as patients with congenital generalized lipodystrophy are concerned, it is not clear yet at what age leptin replacement should be initiated. As more and more patients are treated, the safety and efficacy of leptin therapy in young children with CGL will be more evident. Whether response to leptin therapy is going to be similar in various types of CGL, type 1 due to AGPAT2 deficiency, type 2 due to BSCL2 mutations and type 3 due to CAV1 mutations, is not clear. Furthermore, the response of patients with partial lipodystrophies including HIV-infected patients with protease inhibitor induced lipodystrophy, who are moderately hypoleptinemic, to leptin therapy needs to be investigated. It is likely that leptin therapy may also be effective for patients with hepatic steatosis as well. The long term safety and efficacy of leptin therapy for these hypoleptinemic conditions remains to be determined.

The recent results of combination of metreleptin with pramlintide for weight loss in obese patients need to be confirmed. If confirmed, this can be a useful adjunct to diet and exercise in weight loss in obese. It is also possible that leptin therapy may have a role in preventing weight regain in obese patients who have undergone bariatric surgery, however, such studies need to be conducted. Finally, despite initial promise and then disappointment, it seems that we are encountering a "silver lining" phase of leptin therapy. Leptin therapy however still remains investigational and has not been approved by the FDA.

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