Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults

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Genetic mutations in the leptin pathway can be a cause of human obesity. It is still unknown whether leptin can be effective in the treatment of fully established morbid obesity and its endocrine and metabolic consequences in adults. To test the hypothesis that leptin has a key role in metabolic and endocrine regulation in adults, we examined the effects of human leptin replacement in the only three adults identified to date who have genetically based leptin deficiency. We treated these three morbidly obese homozygous leptin-deficient adult patients with recombinant human leptin at low, physiological replacement doses in the range of 0.01–0.04 mg/kg for 18 months. Patients were hypogonadal, and one of them also had type 2 diabetes mellitus. We chose the doses of recombinant methionyl human leptin that would achieve normal leptin concentrations and administered them daily in the evening to model the normal circadian variation in endogenous leptin. The mean body mass index dropped from 51.2 ± 2.5 (mean ± SEM) at baseline to 26.9 ± 2.1 kg/m^2 after 18 months of treatment, mainly because of loss of fat mass. We document here that leptin replacement therapy in leptin-deficient adults with established morbid obesity results in profound weight loss, increased physical activity, changes in endocrine function and metabolism, including resolution of type 2 diabetes mellitus and hypogonadism, and beneficial effects on ingestive and noningestive behavior. These results highlight the role of the leptin pathway in adults with key effects on the regulation of body weight, gonadal function, and behavior.

The increasing rates of obesity and consequent morbidity represent a major epidemic worldwide and threaten to bankrupt health care systems (1–3). While prevention is of great importance, it is medically relevant to identify biological pathways with the potential to treat obesity and related disorders, particularly in adults with fully established obesity and comorbid conditions, such as type 2 diabetes mellitus. Leptin, the product of the *ob* gene, plays a central role in the regulation of food intake and energy expenditure (4). Mutations in the leptin pathway can be a cause of human obesity (5–7). In children with complete leptin deficiency and who are still in the process of gaining weight and developing obesity, leptin replacement therapy can lead to substantial weight reduction (8, 9).

It is still unknown whether the leptin pathway is relevant to the treatment of established morbid obesity and its endocrine and metabolic consequences in adults. We addressed this question by treating three homozygous leptin-deficient adults with morbid obesity. Morbid obesity had been fully established for two to four decades in those patients, and they had been at a stable (but very high) weight for >10 years. They were hypogonadal, and one of them had type 2 diabetes mellitus. We report here the results of the first 18 months of replacement therapy with recombinant human leptin, showing that leptin is highly effective in dramatically reducing body weight in leptin-deficient morbidly obese adults with stable body weight. Moreover, leptin treatment ameliorated type 2 diabetes mellitus and resolved hypogonadism. This study has three unique features: it represents the only opportunity to study the effects of leptin in leptin-naive adults, it describes hormone replacement treatment of a genetic form of obesity in adults, and it addresses the effects of leptin replacement in the only individual identified with leptin-deficiency who has a diagnosis of type 2 diabetes mellitus.

We have previously demonstrated that genetically based leptin deficiency due to a nonconservative missense leptin gene mutation (Cys-to-Thr in codon 105) in a highly consanguineous extended Turkish pedigree is associated with morbid obesity and hypogonadism (7, 10). We prospectively studied the effects of leptin replacement therapy with recombinant methionyl human leptin (r-metHuLeptin) in three adult homozygous patients from this family who are the only three adult leptin-naive individuals identified in the world so far. This work was done to test the hypotheses: (*i*) that the leptin pathway is relevant for the regulation of body weight in fully established morbid obesity in adults whose food intake is in the normal range and whose body weight has reached stable plateaus in the morbid obesity range, (*ii*) that leptin treatment resulting in weight loss would have an effect on type 2 diabetes mellitus, and (*iii*) that leptin replacement will improve gonadal function.

To achieve those goals, we assessed a variety of parameters throughout the course of leptin replacement in these patients. Measures included indices of endocrine function, such as 24-h plasma concentrations of frequently sampled leptin, luteinizing hormone (LH), testosterone (T), and cortisol, as well as measures of other hormones related to body weight regulation, including ghrelin and adiponectin (11–17). Because nutritional intake is well known to modulate the insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) (18), we measured IGFs and IGFBPs through the course of leptin replacement. We also assessed body composition, lipid profiles, and indices of glucose metabolism.

Abbreviations: ApEn, approximate entropy; BMI, body mass index; CV, coefficient of variation; IGF, insulin-like growth factor; IGFBP, IGF-binding proteins; LH, luteinizing hormone; r-metHuLeptin, recombinant methionyl human leptin; T, testosterone; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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Methods

Patients were recruited in Turkey and admitted for a period of 18 months to the National Center for Research Resources-supported General Clinical Research Center at the University of California at Los Angeles, where the study took place under protocols approved by the Food and Drug Administration and by the University of California at Los Angeles Institutional Review Boards and the Central Ethical Committee of the Turkish Ministry of Health. The study started with a 3-month baseline period at the General Clinical Research Center. This extended baseline period was designed to avoid the confounding factors of international travel, time-zone differences, and potential changes in food choice. After completion of the 3-month baseline period, patients received r-metHuLeptin s.c., once daily in the evening (18:00–20:00) at low physiological replacement doses in the range of 0.01–0.04 mg/kg for 18 months. Doses were started at the higher level and titrated down during the course of weight loss. The drug was generously provided by Amgen Biologicals. The amount of drug administered was adjusted downward as subjects lost weight, and the dose was further reduced when normal BMI was achieved in order to avoid excessive weight loss. The doses of r-metHuLeptin were designed to achieve a normal leptin concentration based on body fat of 30% in females and 20% in males (Amgen data on file). We chose daily evening administration to model the normal circadian variation in endogenous leptin, which is characterized by a pulsatile circadian rhythm with marked nocturnal rise (19).

Diet, Activity, and Body Composition. Patients were allowed to eat ad libitum so that the effects of leptin on food intake and nutrient choice could be documented. Daily weight measurements were taken at the General Clinical Research Center. Food records were obtained at regular intervals to assess dietary intake and analyzed by using NUTRITIONIST PRO (First DataBank, San Bruno, CA), a nutrition analysis software. Level of physical activity was likewise not structured by the investigators. Twenty-four-hour physical activity levels were assessed by an accelerometer-based activity monitor (Actiwatch, Mini-Mitter, Sunriver, OR). Food intake and activity level were structured only during the 24-h blood collections and the preceding 48-h acclimatization periods. Body composition was measured by dual energy x-ray absorptiometry (DEXA) scanning (Hologic QDR 4500, Waltham, MA). The DEXA scanner was calibrated by using a soft-tissue phantom before each measurement.

Twenty-Four-Hour Blood Collection. At baseline and 6 months after initiation of treatment, blood was collected from the male patient every 7 min through an indwelling antecubital catheter for measurement of leptin, LH, T, and cortisol. This generated 1,656 endocrine data points from eight time series of 207 points each (four series at baseline and four posttreatment). The patient was acclimated to the room where blood was collected for 48 h before sample collection. During the acclimatization and blood collection period, the patient was on an isocaloric diet, with four daily meals: breakfast (08:30, 20% of calories), lunch (12:30, 35% of calories), dinner (18:00, 35% of calories), and evening snack (21:00, 10% of calories). Macronutrient content of isocaloric diet was 20% protein, 55% carbohydrates, and 25% fat. Blood samples were collected in prechilled EDTA-containing tubes and centrifuged every 2 h. Plasma was then aliquotted, immediately frozen in dry ice, and stored at −80°C until assays were performed.

Methods for the measurement of hormones, IGF and IGFBP, lipids, insulin sensitivity, and 24-h endocrine data analysis can be found in *Supporting Methods*, which is published as supporting information on the PNAS web site.

Behavior. Patients were assessed by means of the Hamilton scales of anxiety and depression administered to them in Turkish (19, 20).

Fig. 1. Patients are shown here at baseline and after leptin replacement. The older female patient (patient C) is on the left, and the younger female patient (patient B) is next to her. The male patient (patient A) is on the far right. For comparison purposes, two research nurses whose weights have been stable during this period are shown in the center. Patients' faces have been blurred to maintain confidentiality.

Results

At baseline, male patient A was 27 years old, and his body mass index (BMI) was 51.4 kg/m^2. Female patients B and C were 35 and 40 years old with a BMI of 46.7 and 55.4 kg/m^2, respectively. Weight loss was observed within 1 week after the initiation of treatment. Patients' BMI dropped continuously throughout the study (Figs. 1 and 2*a*) on physiological (low) doses of r-metHuLeptin replacement treatment (Fig. 2*c*). Leptin injections were very well tolerated; one of the two female patients had a mild skin reaction at the site of injection for 1 week, which resolved spontaneously. Patients have been otherwise totally free of side effects or adverse reactions.

Food Intake and Body Weight. The effects of leptin replacement on food intake and body weight are shown in Figs. 1 and 2. At baseline the male patient gained weight and increased food intake after arrival in the United States. In contrast, one female patient maintained stable levels of body weight and food intake, whereas the other decreased food intake and lost weight. The mean BMI dropped from 51.2 ± 2.5 kg/m^2 (mean ± SEM) at baseline to 36.5 ± 2.3 kg/m^2 after 6 months; BMI was 28.9 ± 3.2 kg/m^2 after 12 months of treatment (Fig. 2*a*) and 26.9 ± 2.1 after 18 months of treatment. The weight losses of patients A, B, and C after 18 months of treatment were 76.2, 47.5, and 60.0 kg, respectively, corresponding to 53.8%, 43.5%, and 44.5% of their body weight, respectively.

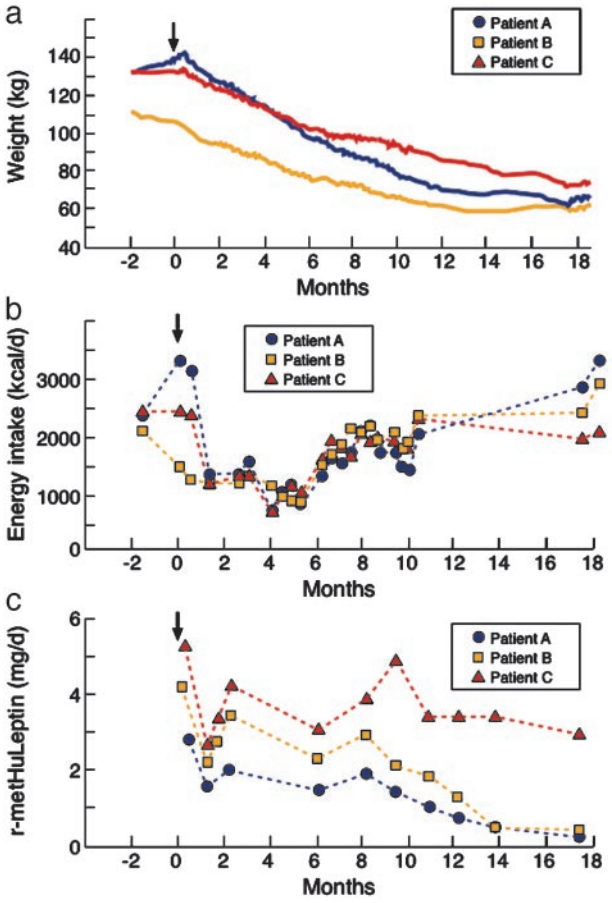

Fig. 2. Effects of leptin replacement in adults. (*a*) Effects of r-metHuLeptin on weight in three leptin-deficient adults. Arrow indicates the start of r-metHuLeptin therapy. (*b*) Effects of r-metHuLeptin on energy intake in leptin-deficient adults. (*c*) Dose of s.c. r-metHuLeptin used in the three leptin-deficient adults.

Note that patient A lost over half of his body weight without any type of structured dietary or physical activity interventions. After leptin replacement was initiated, all patients had an initial marked reduction in food intake. The mean daily caloric intake dropped 49% from 2,330 ± 322 kcal/day at baseline to 1,180 ± 52 kcal/day at week 2 following r-metHuLeptin administration. After leptin replacement, daily caloric intake decreased, reaching a nadir at 4–6 months; thereafter, it increased progressively (Fig. 2*b*). Parallel to the decrease in body weight, mean activity counts given by Actiwatch during the day were increased progressively and linearly in all patients throughout the study (Fig. 3).

Body Composition. As shown in Table 1, patients lost fat preferentially. A proportionately smaller decrease in fat-free body mass also

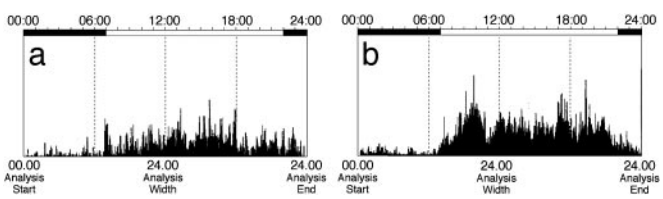

Fig. 3. Levels of 24-h physical activity measured in patient C during the course of leptin replacement. Representative days at baseline are shown in *a* and at 12 months after leptin in *b*.

Table 1. Effects of leptin replacement therapy on body composition

Measurement	Patient	Months of treatment: 0 (baseline)	3	6	18	Significance*
Body weight, kg	A	134.1	118.4	99.7	64.3	
	B	108.4	89.2	77.9	60.4	$P < 0.05$
	C	132.4	116.6	101.3	74.3	
Fat mass, kg	A	58.6	50.8	33.1	6.5	
	B	52.0	41.2	27.8	14.4	$P < 0.02$
	C	64.9	54.9	44.6	25.8	
Fat-free mass, kg	A	75.5	67.6	63.6	57.8	
	B	56.4	48.0	50.1	46.0	$P < 0.03$
	C	67.5	61.7	56.7	48.5	
Body fat, %	A	43.8	43.6	34.2	10.1	
	B	47.9	46.2	36.2	23.8	$P < 0.05$
	C	49.5	47.1	44.0	34.7	

*Paired comparisons from month 0 to month 18 for mean of the three patients.

occurred. The loss of fat-free mass was substantially smaller than that of fat. At 18 months after treatment, patient A had 11% of his initial fat mass and 77% of his lean body mass. Those figures were 28% and 82% for patient B and 40% and 72% for patient C, respectively. All these changes are statistically significant.

Clinical Endocrine Function. At baseline, before leptin replacement, patient A had clinical features of hypogonadism, as previously reported (10). His baseline measurements of testosterone and free testosterone were below the normal range for his age (10). After 6 months of treatment he reported improvement in muscle strength, sense of well-being, and energy. Additionally, during the course of leptin replacement, we documented increased facial hair, onset of facial acne, development of pubic and axillary hair, growth of penis and testicles, and normal ejaculatory patterns. Patients B and C had at baseline regular menstrual periods characterized by a luteal phase defect with low midluteal phase progesterone levels (10). After leptin replacement both patients had regular menstrual periods that were associated with serial midluteal phase progesterone measurements >10 ng/ml, which are indicative of ovulation.

As shown in Table 2, at baseline, patients A and B had normal glucose and insulin levels. They also had normal glucose tolerance after a 2-h, 75-g standard oral glucose tolerance test; patient C had a clinical diagnosis of type 2 diabetes mellitus. In the context of no other treatment, her fasting and postprandial glucose values decreased after 2 months of treatment, and her hemoglobin A1c levels, a measure of diabetes control, are in the normal range at the present time. The other two patients maintained normal fasting glucose levels but their insulin and C-peptide values decreased significantly to less than half of their original values. This decrease is indicative of a marked decrease in insulin resistance.

Twenty-Four-Hour Endocrine Rhythms. Twenty-four-hour average concentrations of leptin, LH, T, and cortisol significantly increased ($P < 0.0001$) from a baseline of 0.77 ± 0.01 ng/ml, 0.75 ± 0.04 milliunits/ml, 2.61 ± 0.06 ng/ml, and 4.04 ± 0.22 μg/dl, to 12.67 ± 0.83 ng/ml, 2.75 ± 0.07 milliunits/ml, 7.50 ± 0.07 ng/ml, and 5.97 ± 0.30 μg/dl, respectively, 6 months after leptin replacement (see Table 3, which is published as supporting information on the PNAS web site, and Fig. 4). In contrast, the relative increment of LH, T, and cortisol significantly decreased ($P < 0.002$) from 20.9 ± 2.16%, 11.0 ± 0.8%, and 15.9 ± 1.3% to 12.2 ± 1.0%, 6.5 ± 0.4%, and 11.1 ± 0.8%, respectively, 6 months after leptin replacement. For LH and T there were no changes in the number of pulses (29 pulses per 24 h for LH before treatment and 28 pulses per 24 h for T before treatment vs. 29 and 30 after treatment, respectively), which had the same concentration-independent features before or after leptin replacement. Pulse heights for leptin, LH, T, and cortisol were

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Table 2. Effects of leptin replacement on metabolic parameters

Parameter	Patient	Months of treatment 0	2	4	6	8	18
Fasting glucose, mg/dl	A	91	89	88	85	75	78
	B	88	93	82	89	81	84
	C	131	143	120	116	105	86
Fasting insulin, microunits/ml*	A	4.8	5.2	4.0	3.1	2.3	1.8
	B	3.8	4.4	3.2	4.0	2.1	1.8
	C	7.5	14	7.7	4.4	4.9	3.1
Fasting C-peptide, ng/ml*	A	2.2	2.5	1.7	1.2	0.8	0.5
	B	2.0	1.6	1.0	1.4	0.5	0.6
	C	4.0	4.0	4.3	2.6	2.1	1.2
Total cholesterol, mg/dl	A	137	124	107	111	114	96
	B	115	117	106	109	99	110
	C	181	160	134	147	125	125
HOMA-IR†	A	1.08	1.14	0.87	0.65	0.43	0.35
	B	0.83	1.01	0.65	0.89	0.42	0.37
	C	2.43	4.94	2.28	1.26	1.30	0.66
Triglycerides, mg/dl	A	125	82	67	72	60	69
	B	79	66	53	57	27	47
	C	247	225	165	148	127	115
HDL cholesterol, mg/dl	A	29.9	45.7	33.1	29.9	32.7	53.3
	B	36.8	36.8	37.6	40.3	44.2	51.2
	C	28.6	27.5	24.5	31.3	32.7	38.4
LDL cholesterol, mg/dl	A	82	62	61	67	69	29
	B	62	67	58	57	49	49
	C	103	88	77	86	67	64
Apolipoprotein A1, mg/dl	A	88	98	71	74	76	82
	B	81	83	81	90	83	94
	C	88	100	81	85	82	89
Apolipoprotein B, mg/dl	A	83	68	54	59	61	50
	B	57	58	54	54	42	47
	C	114	113	89	80	67	68

*$P < 0.05$ for paired comparisons from month 0 to month 18 for mean of the three patients.

†HOMA-IR = fasting insulin (microunits/ml) × fasting glucose (mmol/liter)/22.5.

higher in the postleptin series (increased from 0.87 ± 0.02 ng/ml, 0.88 ± 0.13 milliunits/ml, 3.10 ± 0.12 ng/ml, and 4.85 μg/dl at baseline, to 13.7 ± 3.1 ng/ml, 3.20 ± 0.24 milliunits/ml, 8.12 ± 0.17 ng/ml, and 6.25 ± 1.11 μg/dl 6 months after leptin replacement, for leptin, LH, T, and cortisol, respectively). The ApEn of LH and T increased from a baseline of 0.532 and 0.632 to 0.641 and 0.799, respectively, after leptin replacement. The LH–T cross-ApEn was 0.545 at baseline and increased to 0.669 after treatment, indicating decreased synchronicity (or increased irregularity) of the LH–T relationship across the 24-h period, 6 months posttreatment.

In addition, 6 months of leptin replacement resulted in higher 24-h mean concentrations of cortisol (from 4.04 ± 0.22 to 5.97 ± 0.30 μg/dl), which were associated with fewer pulses (25 vs. 19) of greater height (from 4.85 to 6.25 ± 1.11 μg/dl), including a greater morning rise (Fig. 4). This was associated with a decrease from baseline to posttreatment in relative increment (15.9 ± 1.3 vs. 11.1 ± 0.8%) and ApEn (0.441 vs. 0.364).

Ghrelin and Adiponectin. The levels of these peptides across the treatment period are listed in Table 4, which is published as supporting information on the PNAS web site, and indicate no unexpected abnormalities in these fat- and appetite-modulating hormones. As C-peptide is only minimally extracted by the liver, it is a better marker of pancreatic insulin secretion than peripheral insulin levels, which undergo large and variable first-pass rates of hepatic extraction. We examined the relation of fasting adiponectin and C-peptide concentrations. As patients lost weight, fasting adiponectin levels increased, exhibiting significant negative correlations with the levels of fasting C-peptide ($r = -0.53$; $P < 0.04$; see Fig. 5, which is published as supporting information on the PNAS web site) and also with the insulin sensitivity index derived by using the Homeostatic Model Assessment model ($r = -0.52$; $P < 0.04$).

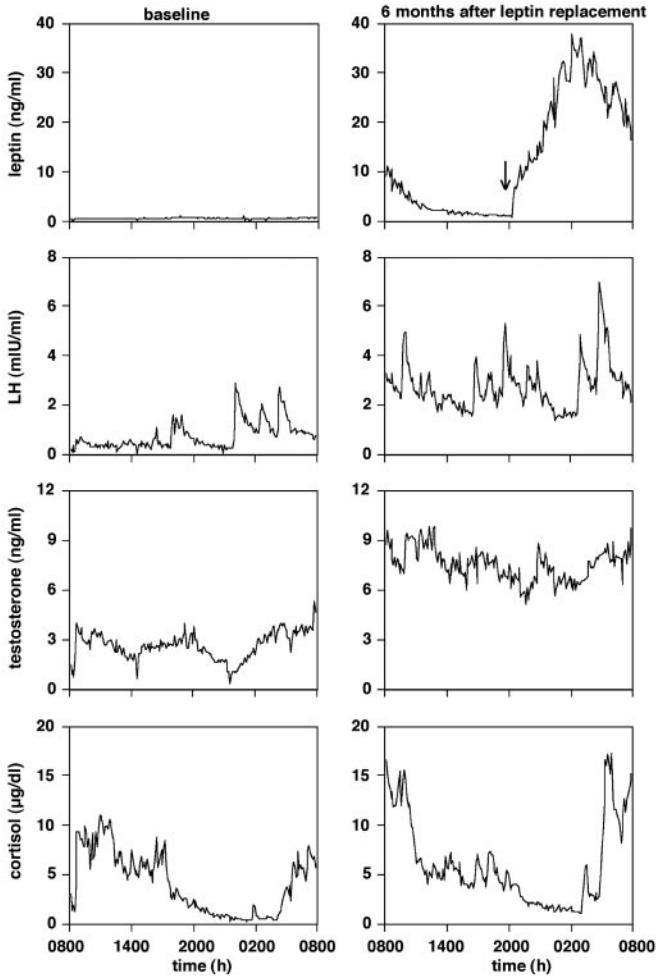

Fig. 4. Measurements of intensively collected samples of plasma hormone concentrations over the course of 24 h in the male patient, studied at baseline before and after 6 months daily leptin replacement by s.c. injection in the evening (arrow shows time of daily leptin injection). Each time series has 207 samples collected every 7 min, after 2 days of acclimatization in the research suite; meals were standardized.

IGF Axis. The levels of IGF-I, IGF-II, and IGFBP-1, -2, -3, and -6 are shown in Table 5, which is published as supporting information on the PNAS web site. All of the IGF-related parameters were within the normal range before initiation of therapy except the postprandial IGFBP-1, which was below the lower limit of normal. Leptin treatment resulted in elevations of IGFBP-2 and IGFBP-1, which were evident as soon as 2 months of therapy (with a 50% increase in IGFBP-2 and fasting IGFBP-1, as well as a doubling of postprandial IGFBP-1). By 18 months of therapy, IGFBP-1 and IGFBP-2 levels were dramatically increased (by 7-fold and 2-fold, respectively, significant at the 10^{-4} level) and were at or above the upper limit of normal. Serum levels of IGF-I, IGF-II, IGFBP-3, and IGFBP-6 were not changed in response to treatment.

Lipids. The main lipoprotein abnormality at baseline in all three subjects was reduced HDL cholesterol (0.74–0.95 mmol/liter). Plasma LDL cholesterol and triglyceride levels were low normal except for baseline triglycerides of 2.8 mmol/liter in patient C. During the course of leptin treatment, triglyceride levels dropped 49–66%, with smaller relative reductions in LDL cholesterol (21–

65%) and apolipoprotein B (18–40%). On the other hand, there were increases in HDL cholesterol (34–78%) although most of the increase occurred between 8 and 18 months. Interestingly, levels of apolipoprotein A1, the main HDL protein component, changed minimally over the course of treatment.

Behavior. The patients did not score at baseline or after treatment on the Hamilton scale of anxiety and depression. Nevertheless, the most immediate change noted after initiation of leptin treatment was in ingestive and noningestive behavior. Ingestive behavior changed, as noted in Fig. 2*a*. Noningestive behavior of all three patients was consistently observed to change from very docile and infantile to assertive and adult-like, within 2 weeks of the onset of leptin treatment, before weight loss occurred. This was a subjective but universal observation made by the treatment staff, which included highly experienced clinical research psychiatrists.

Discussion

We show here that leptin replacement therapy in leptin-deficient adults with established morbid obesity results in profound weight loss, changes in behavior, energy intake, increased physical activity, and resolution of type 2 diabetes mellitus and hypogonadism. These results indicate that the leptin pathway has central effects that are highly relevant for the regulation of body weight in adults. Previous studies had shown that leptin treatment can improve glucose homeostasis in patients with lipodystrophy (21) and in children treated with leptin on a long-term basis (8, 9). In those children, leptin replacement reversed the effects of leptin deficiency, leading to puberty at an appropriate age (8, 9). What such previous work had not elucidated, and which is documented here, is that leptin replacement is effective after morbid obesity is fully established at a stable level for several decades; that it can not only reverse obesity but also resolve its complications, such as diabetes mellitus; and that it can induce puberty even a decade or longer after the normal age for pubertal development.

At baseline, our patients were not eating as voraciously as they reportedly had during their childhood. Their pattern of food intake was stable at the 1,800–3,000 kcal/d range. The onset of leptin replacement was associated with marked decrease in food intake. After reaching a nadir at months 4–6 after onset of leptin administration, the patients gradually started to increase their food intake toward baseline levels. In parallel, their level of physical activity increased. It is therefore reasonable to presume that the initial weight loss was due to reduced food intake, but that over the course of the study period continued weight loss was achieved by progressively increased levels of physical activity above baseline levels, in the context of food intake that increased from its nadir but did not substantially exceed baseline. We conclude that the profound effects of leptin on body weight were caused by the combination of an initial reduction in food intake followed by a progressive increase in the amount of physical activity. We hypothesize that weight loss in the non-leptin-deficient obese might likewise be more successful in the context of interventions that initially decrease food intake but that subsequently increase physical activity without raising food intake from baseline levels.

In this study, body weight loss was preferentially due to loss of fat, although loss of muscle also occurred, to a much smaller degree. An advantage of intervening at the leptin-signaling level might be such a preferential loss of body fat.

Analysis of 1,656 endocrine data points showed that, in parallel with clinical resolution of hypogonadism, there were complex changes in intensively sampled hormone profiles. First, we show that once-a-day leptin administration in the evening mimics the nocturnal rise of leptin, resulting in a pattern that has similarities with the endogenous situation. However, some differences were also observed. First, mean 24-h plasma concentrations of leptin increased 16-fold from 0.77 ± 0.01 to 12.67 ± 0.83 ng/ml. The level of regularity of plasma leptin concentrations increased markedly after treatment as documented by a decrease in ApEn from 0.772 to 0.268. This regularity is due to the fact that the leptin profile after treatment was driven by an organized pattern of once-a-day exogenous administration. After leptin levels increased chronically in the context of leptin replacement, we documented complex changes in the dynamics of the other endocrine systems studied intensively. There were increases in the 24-h average concentrations of LH, T, and cortisol. Even though the absolute concentrations of those hormones increased, their relative increment decreased. This observation indicates that after leptin treatment there is an increase in the plasma concentrations of LH, T, and cortisol, but that those increased levels vary less as a percentage of their values from one time point to the next and that such variation occurs more irregularly (as documented by increased ApEn of LH and T after treatment). For LH and T there were no changes in the number of pulses, which had the same concentration-independent features before and after leptin replacement. Pulse heights were significantly higher in the postleptin series, which explains the effects of treatment leading to increased mean 24-h values. These results indicate that leptin administration to an adult hypogonadic male had a profound effect on the dynamics of sex hormone concentrations while concurrently causing the onset of puberty at age 27. It can be concluded that puberty was delayed in this case because of the lack of leptin, and that leptin administration led to maturation of the male reproductive axis. This confirms previous preclinical data on leptin's ability by activation of nitric oxide synthase to stimulate the release of luteinizing hormone-releasing hormone from the hypothalamus and LH from the pituitary both *in vitro* and *in vivo* (22–24).

Cortisol dynamics was characterized by higher 24-h mean concentrations after leptin replacement, with fewer pulses, of greater height, including a greater morning rise. ApEn and relative increment decreased after treatment. In the absence of leptin, cortisol dynamics is characterized by a higher number of smaller peaks, with smaller morning rise, increased relative variability, and increased pattern irregularity. These data suggest that leptin has a role in organizing the dynamics of human hypothalamic–pituitary–adrenal (HPA) function. This was not detected by Mantzoros *et al*. (25), who studied the effects of short-term leptin replacement after fasting in healthy volunteers. The acute nature of their studies may have precluded them from identifying the impact of leptin on HPA function. Such an effect of leptin is further supported by our previous observation of a very strong association between the minute-to-minute variability of frequently sampled corticotropin/cortisol and leptin (26). Based on our results, we propose that leptin contributes to regulate the dynamics of human HPA function.

Ghrelin and adiponectin levels were within a range that is commensurate with the changes in our patients' weight. These data indicate that the profound phenotypic changes observed at baseline in our patients were due to the direct effects of leptin without unexpected abnormalities in these other peptides that are related to fat mass and food intake. The obese are known to have increased insulin resistance and decreased adiponectin levels (12), which increase with weight loss (27). It is thought that adiponectin can decrease the insulin resistance of obesity (28–30). We documented here a statistically significant negative association between the fasting levels of adiponectin and C-peptide (a marker of insulin secretion) during the course of weight loss; we also show a significant negative association between leptin and Homeostatic Model Assessment of insulin resistance. It is not possible in this type of patient-oriented research to fully ascertain whether changes in glucose homeostasis are (*i*) a direct effect of leptin, (*ii*) mediated solely by the weight loss, (*iii*) associated with increased levels of adiponectin, or (*iv*) the combined effect of these (and other) factors.

All of the IGF-related parameters in our patients were within the normal range before initiation of therapy except the postprandial IGFBP-1, which was suppressed. Compared with those of severely

obese individuals, however, these IGFBP-1 levels are not unusual; in fact, the fasting IGFBP-1 levels in untreated leptin-deficient patients are higher than in other obese individuals with lower BMI (31). By 18 months of therapy, IGFBP-1 and -2 levels were dramatically increased (by 7-fold and 2-fold, respectively) and were at or above the upper limit of normal. These changes were far more sensitive and dramatic than the changes in insulin levels or other metabolic parameters and were more pronounced than those commonly observed in other forms of weight loss (32). Unlike in other forms of weight loss, IGF-I and IGFBP-3 were unchanged. IGF-I treatment leads to marked suppression of leptin levels in both rodents and humans (33, 34). Our findings suggest that long-term leptin treatment of leptin-deficient individuals is associated with a unique set of changes in the IGF axis profile.

The principal lipoprotein abnormality in these obese leptin-deficient subjects was reduced HDL cholesterol, a common correlate of excess adiposity. Leptin treatment *per se* did not reverse this abnormality, and substantial increases were observed only after 18 months, consistent with evidence that increases in HDL with weight reduction are generally not observed during the active stages of weight loss. The lack of increase in apolipoprotein A1 suggests that there was a change in HDL composition with treatment. Reductions in triglycerides, LDL cholesterol, and apolipoprotein B during the course of treatment are also consistent with the effects of reduced adiposity. One might speculate that the levels of triglycerides at baseline were relatively low in relation to adiposity, perhaps reflecting an effect of leptin deficiency *per se*.

Of note, noningestive behavior changed dramatically within 2 weeks of initiation of leptin replacement, before weight loss occurred. That change was not reflected in psychometric scales used to quantify depression or anxiety but consisted instead of a change in behavior and interpersonal attitudes from a baseline of infantile and docile to assertive and adult-like. The locus for this event is most probably central. It had been our original intention to conduct positron-emission tomography scans at baseline and longitudinally during treatment; however, patients did not fit into the equipment because of their morbid obesity. The findings presented here further support a role for leptin in the regulation of behaviors and interpersonal attitudes that go beyond hunger, satiety, and food intake. Future studies should explore the behavioral effects of leptin and its neurobiological substrates.

These three genetically related individuals share the same mutation in the leptin gene; however, their phenotypes and response to treatment were not uniform. Male patient A had rates of weight and percent fat loss that were greater than those of female patients B and C. Of those two women, only one developed type 2 diabetes mellitus. The other two patients had fasting glucose and insulin levels that were relatively low for the severity and duration of morbid obesity. Moreover, the diabetic patient lost body weight and body fat more slowly than the other patients (see Fig. 2 and Table 1). All three patients lacked some of the biochemical sequelae of obesity, such as hyperlipidemia and hypertension. Two important points are evident here. First, it appears that obesity in the absence of leptin is associated with a different phenotype than that of obesity associated with hyperleptinemia. In the former, there is hypogonadism but not pronounced cardiovascular and metabolic morbidity. The latter is characterized by metabolic and cardiovascular sequelae in the context of normal reproductive function. The hypothesis that the cardiovascular and metabolic complications of obesity are mediated at least partially by leptin should therefore be tested. Second, in the light of the diversity of the phenotype among the three patients, it is evident that the effects of other, nonshared gene alleles as well as the environment partially affect the leptin-deficiency phenotype and have an impact on the pharmacogenetics of obesity.

This study demonstrates that in a common and complex disorder of gene–environment interactions, such as obesity, genetic substrate is of crucial importance to the outcome of treatment. While in the presence of a leptin gene mutation, leptin treatment of obesity in adults was extraordinarily successful; in the absence of such a mutation, the same intervention was not markedly effective (35). The genetic heterogeneity of the obesity phenotype therefore results in heterogeneity of treatment response. The dissection of complex phenotypes at the genetic and pharmacogenetic levels will facilitate tailored approaches to therapeutics. Moreover, insights gained from rare cases caused by single-gene mutations may shed new light into the biology of underlying disease processes, leading to new therapeutic insights that might be applicable to patients in whom the phenotype is the result of the interaction of environmental factors and multiple susceptibility genes.

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