Brief Report

EFFECTS OF RECOMBINANT LEPTIN THERAPY IN A CHILD WITH CONGENITAL LEPTIN DEFICIENCY

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SEVERELY obese (*ob/ob*) mice are deficient in the adipocyte-derived hormone leptin, which acts on the hypothalamus to control appetite and energy expenditure.[1] The administration of leptin to these mice corrects their obesity by reducing their food intake and increasing their energy expenditure.[2-4] These mice also have hyperinsulinemia, corticosterone excess, and infertility, which also are reversed by treatment with leptin.[5] In humans, serum leptin concentrations, in general, correlate positively with indexes of obesity.[6,7] We previously described two cousins with severe, early-onset obesity and undetectable serum leptin concentrations who were homozygous for a frame-shift mutation in the leptin gene.[8] In this report, we describe the results of a trial of therapy with recombinant human leptin in the older of these children, a nine-year-old girl.

CASE REPORT

The patient had normal weight at birth but began gaining weight excessively at about four months of age (Fig. 1A). She had marked hyperphagia, was constantly hungry, demanded food continually, and was disruptive when denied food. As a result of her severe obesity, valgus deformities of the legs developed, for which she required bilateral proximal tibial osteotomies. In an attempt to improve her mobility, liposuction was performed to remove fat from her legs when she was six years old. She came from a highly consanguineous family of Pakistani origin; her parents were first cousins who were not severely obese. This study was approved by the Cambridge Local Research Ethics Committee in Cambridge, United Kingdom, and was undertaken with the informed consent of the parents and the assent of the child.

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METHODS

The patient was treated with recombinant methionyl leptin, administered subcutaneously once daily at 8 a.m. in a dose of 0.028 mg per kilogram of lean mass for 12 months. The dose was calculated to achieve a peak serum leptin concentration equivalent to 10 percent of the child's predicted normal serum leptin concentration (70 ng per milliliter), calculated on the basis of age, sex, and body composition.[9,10] Minor adjustments to the dose were made throughout the trial in response to changes in the patient's body composition.

A digital scale was installed in the patient's home, and daily weight measurements were taken. At base line and every two months thereafter, her height was measured with the same stadiometer and her body composition was measured by dual-energy x-ray absorptiometry (QDR 1000W, Hologic, Waltham, Mass.) to determine the bone mineral mass and the amounts of fat and lean soft tissue.[11] Spontaneous energy intake was measured with the use of a standardized test meal (containing 1670 kcal of energy) given at noon, after the patient had been fasting since breakfast. The amount of food consumed was measured covertly. The patient's sleeping metabolic rate was measured by whole-body indirect calorimetry, and her basal metabolic rate was estimated as the sleeping metabolic rate × 1.05.[12] The patient's total energy expenditure was measured with the use of doubly labeled water ($^2H^{18}O$) at base line and at 6 and 12 months.[13] The physical-activity level was calculated as the patient's total energy expenditure divided by her basal metabolic rate.

Plasma glucose and insulin and serum lipid concentrations, measured in samples obtained while the patient was fasting, and the serum thyrotropin and gonadotropin responses to combined stimulation with thyrotropin-releasing hormone and gonadotropin-releasing hormone were measured at base line and every two months thereafter. At base line and at 6 and 12 months, bone age was estimated on the basis of radiographs of the left hand and wrist with the use of the Radius Ulnar Score,[14] and serum insulin-like growth factor I and estradiol and fasting plasma concentrations of nonesterified fatty acids were measured. Twenty-four-hour urinary cortisol excretion was measured at base line and at 12 months. Spontaneous gonadotropin secretion was assessed at 12 months by the measurement of serum luteinizing hormone and follicle-stimulating hormone in samples obtained every 10 minutes for 6 hours during the day and for 12 hours overnight. Ultrasonography of the pelvis was performed at base line and at 6 and 12 months.

Leptin concentrations were measured with the use of a solid-phase sandwich enzyme-linked immunosorbent assay in serum samples taken before the injection of recombinant leptin and 1, 4, 8, 12, and 24 hours after injection on the first day of treatment with leptin and every four months thereafter. Serum was tested for antibodies to leptin with the use of a solid-phase radioimmunoassay, and the potential neutralizing effects of antileptin antibodies on leptin bioactivity were assessed in an in vitro bioassay developed by Amgen (Thousand Oaks, Calif.) that was based on the proliferation of 32D OBECA cells in the presence of leptin (Rich D: personal communication).

RESULTS

Clinical and Anthropometric Features

At base line, the patient was nine years old and weighed 94.4 kg (>99.9th percentile for age). Her height (140 cm) was at the 91st percentile (9th percentile when adjusted for bone age), and her predicted adult height was calculated to be from the 0.4th to the 25th percentile on the basis of the heights of her parents. On clinical examination, the patient was prepubertal and had no evidence of acanthosis nigricans; her temperature (36.5°C) and blood pressure (118/70 mm Hg) were normal.

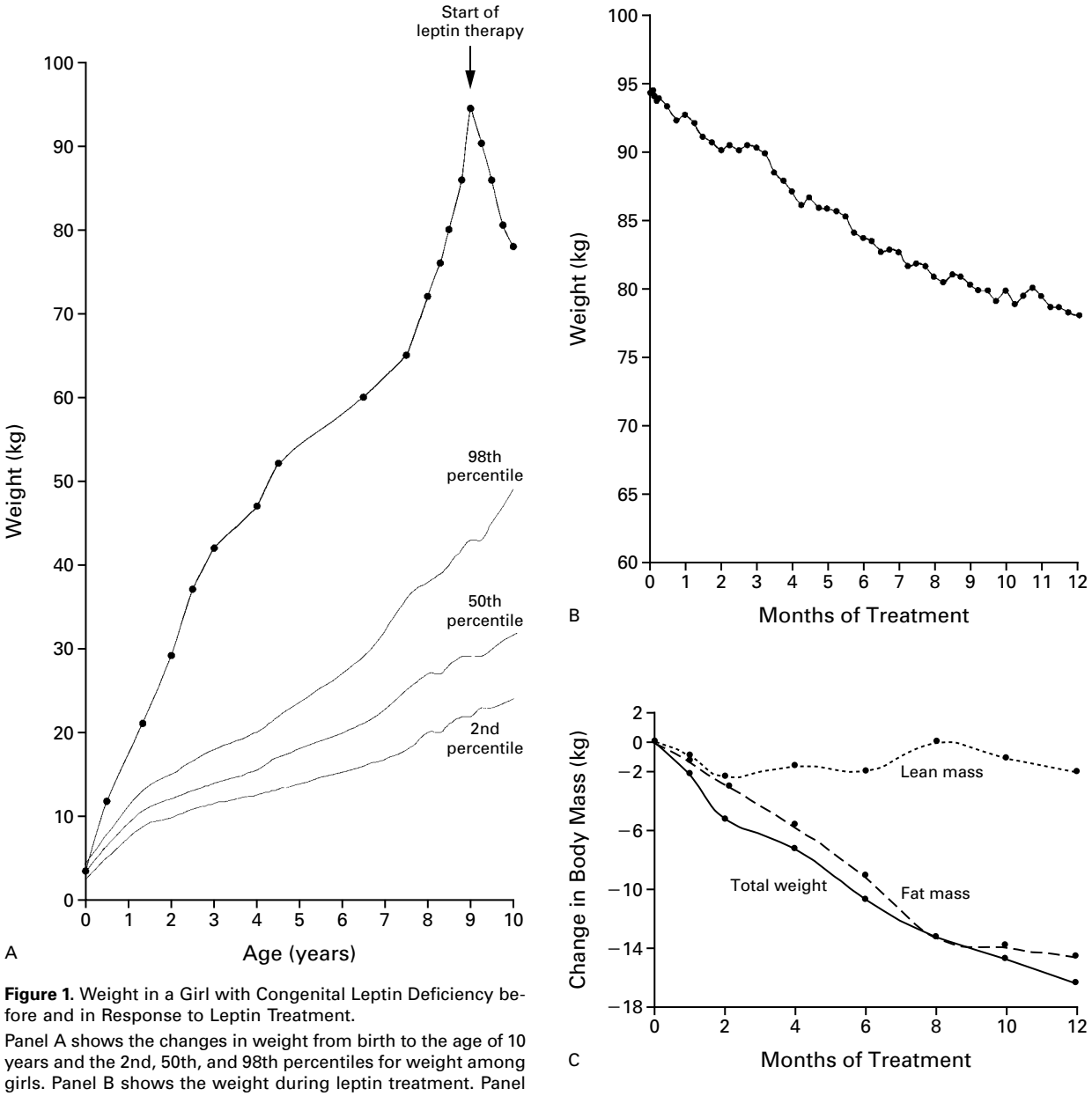

Figure 1. Weight in a Girl with Congenital Leptin Deficiency before and in Response to Leptin Treatment.

Panel A shows the changes in weight from birth to the age of 10 years and the 2nd, 50th, and 98th percentiles for weight among girls. Panel B shows the weight during leptin treatment. Panel C shows the changes in body composition during treatment.

The patient lost weight within two weeks after the initiation of leptin treatment. Weight loss continued over the 12-month period of treatment, during which she lost a total of 16.4 kg at a rate of approximately 1 to 2 kg per month (Fig. 1B). Her height after 12 months of therapy was 143 cm and remained at the 91st percentile. The injections of leptin were well tolerated, with no systemic or local reactions. There were no changes in blood pressure or basal temperature during treatment.

Body Composition

At base line, 59 percent of the patient's body weight (55.9 kg) was fat (normal range for age, 17 to 23 percent[15]). After 12 months of treatment, the amount of body fat decreased by 15.6 kg, accounting for 95 percent of the total weight loss (Fig. 1C); 52 percent of the patient's body weight was fat. Lean mass decreased by 0.82 kg, as a result of a decrease of 0.096 liter in body water. Bone mineral mass increased by 0.15 kg.

Energy Intake

At base line, the patient rapidly consumed almost all of the test meal, excluding foods she habitually disliked; her total energy intake was 1600 kcal. Moreover, she reported feeling hungry and requested additional food shortly afterward. Within seven days after the initiation of leptin treatment, a marked change in the patient's eating behavior was reported by her mother and observed by the investigating physician. At that time, the patient was eating quantities of food similar to those her siblings were consuming, at the same speed, and she no longer secretly sought food or demanded food between meals. Her energy intake at the first test meal after the initiation of treatment decreased by 42 percent, to 930 kcal, and her rate of food consumption decreased markedly. The reduction in her food intake was sustained throughout the study, with a mean energy consumption during therapy of 1000 kcal.

Energy Expenditure

At base line, the patient's basal metabolic rate (1840 kcal per day) and total energy expenditure (2960 kcal per day) were higher, in absolute terms, than those of a typical nine-year-old girl weighing 28 kg (normal values: basal metabolic rate, 1100 kcal per day; total energy expenditure, 1790 kcal per day).[16] However, when expressed per unit of lean mass, both her basal metabolic rate and her total energy expenditure were the same as the expected values (50 kcal and 80 kcal per day per kilogram of lean mass, respectively, both for the patient and for a normal nine-year-old girl).[16]

The patient's physical-activity level at base line was 1.6, similar to the mean (±SD) value of 1.7±0.2 for children of similar age.[17] In response to leptin treatment, the patient's basal metabolic rate decreased progressively to 1500 kcal per day at 12 months, an overall reduction of 18 percent. Her basal metabolic rate also decreased when adjustments were made for changes in body composition, because there was no significant change in lean mass. At 6 months, her total energy expenditure had decreased by 10 percent to 2650 kcal per day, but by 12 months it had returned to near the base-line value (2890 kcal per day). Her physical-activity level increased from 1.6 at base line to 1.9 at 12 months, which is consistent with the observed improvement in her mobility.

Metabolic and Endocrine Status

At base line, the patient was normoglycemic but had high plasma insulin and nonesterified fatty acid concentrations while fasting (Table 1). Her serum cholesterol and triglyceride concentrations were normal and did not change during treatment. Her plasma nonesterified fatty acid concentrations decreased, possibly because of the decrease in fat mass. The patient's thyroid, adrenal, and somatotropic function, as indicated by the serum concentration of insulin-like growth factor I, was normal at each evaluation. Dynamic tests of growth hormone secretion were not performed, because the child's growth velocity was normal. Her bone age was markedly advanced — at base line it was 12.5 years and at 12 months it was 13.4 years.

At base line, the patient's serum concentrations of estradiol, follicle-stimulating hormone, and luteinizing hormone were consistent with her prepubertal

TABLE 1. EFFECT OF LEPTIN TREATMENT ON METABOLIC AND ENDOCRINE FUNCTION IN A GIRL WITH LEPTIN DEFICIENCY.*

VARIABLE	TIME OF MEASUREMENT			NORMAL RANGE
	0 MONTHS	6 MONTHS	12 MONTHS	
Metabolic values (fasting)				
Plasma glucose (mg/dl)	78	90	85	75–115
Plasma insulin (μU/ml)	41	53	34	5–20
Serum cholesterol (mg/dl)	193	166	182	<200
Serum triglycerides (mg/dl)	115	89	133	<160
Plasma nonesterified fatty acids (mg/dl)	38	38	18	<18
Hormonal values				
Serum insulin-like growth factor I (U/ml)	19	24	28	7–50
Urinary cortisol excretion (μg/day)	62	—	55	10–100
Serum thyroxine (μg/dl)	5.8	7.1	7.6	4–11
Thyrotropin-releasing hormone stimulation test†				
Basal serum thyrotropin (mU/liter)	2.8	3.6	1.7	0.6–4.6
Peak serum thyrotropin (mU/liter)	9.8	7.4	4.9	
Gonadotropin-releasing hormone stimulation test‡				
Basal serum follicle-stimulating hormone (IU/liter)	0.3	2.8	2.9	
Peak serum follicle-stimulating hormone (IU/liter)	3.5	6.6	8.9	
Basal serum luteinizing hormone (IU/liter)	0.2	0.3	0.3	
Peak serum luteinizing hormone (IU/liter)	1.0	2.7	2.9	
Serum estradiol (pg/ml)	18.3	18.5	8.7	<20

*To convert the values for glucose to millimoles per liter, multiply by 0.056; to convert the values for insulin to picomoles per liter, multiply by 6; to convert the values for cholesterol to millimoles per liter, multiply by 0.026; to convert the values for triglycerides to millimoles per liter, multiply by 0.11; to convert the values for nonesterified fatty acids to nanomoles per liter, multiply by 0.039; to convert the values for urinary cortisol to nanomoles per day, multiply by 2.759; to convert the values for thyroxine to nanomoles per liter, multiply by 12.87; and to convert the values for estradiol to picomoles per liter, multiply by 3.67.

†The thyrotropin-releasing hormone stimulation test consisted of an intravenous injection of 200 μg of thyrotropin-releasing hormone, with serum measurements of thyrotropin at the time of the injection and 30 minutes later.

‡The gonadotropin-releasing hormone stimulation test consisted of an intravenous injection of 100 μg of gonadotropin-releasing hormone, with serum measurements of follicle-stimulating hormone and luteinizing hormone at the time of injection and 60 minutes later.

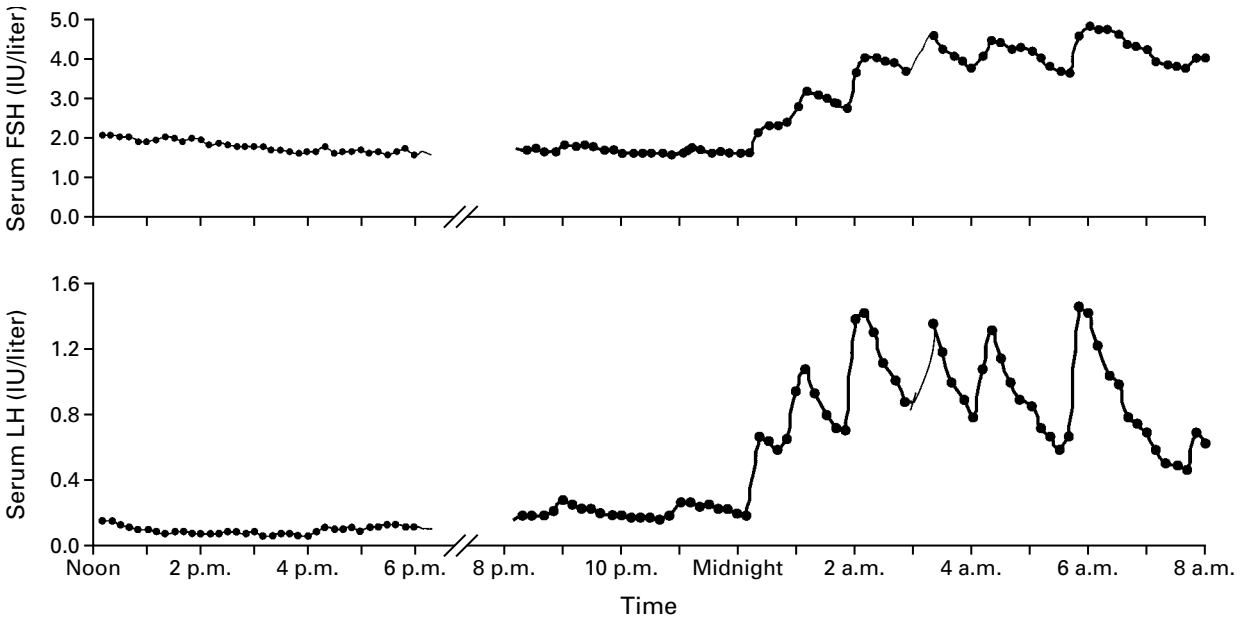

Figure 2. Serum Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) Concentrations in a Patient with Congenital Leptin Deficiency after 12 Months of Leptin Treatment.

Serum FSH and LH were measured at 10-minute intervals for 6 hours during the day and for 12 hours overnight.

Table 2. Pharmacokinetics of Leptin during Leptin Treatment.

Variable	Time of Measurement		
	0 months	8 months	10 months
Serum antileptin antibodies detected	No	Yes	Yes
Antibody titer	None	1:358	1:199
Serum leptin concentration (ng/ml)			
Before injection	<0.04	23.2	19.8
After injection			
1 hr	3.6	29.4	24.7
4 hr	5.3	81.0	38.0
8 hr	5.1	107.9	39.9
12 hr	4.1	66.8	31.0
24 hr	0.3	23.2	31.5

status (Table 1). Her basal and stimulated serum follicle-stimulating hormone and luteinizing hormone concentrations gradually increased during treatment, but her serum estradiol concentration remained in the prepubertal range. Pelvic ultrasonography showed a juvenile uterus and ovaries at each evaluation, and there was no development of secondary sexual characteristics during treatment. However, after 12 months of leptin treatment, the nocturnal pattern of gonadotropin secretion was pulsatile, which is consistent with early puberty (Fig. 2).

Pharmacokinetics of Leptin

Four hours after the first dose of leptin, the patient's peak serum leptin concentration was 5.3 ng per milliliter (Table 2). A low serum titer of nonneutralizing antileptin antibodies was detected after two months of therapy, which was not associated with any effect on weight loss or with other adverse events. The antibodies persisted thereafter, and their presence resulted in high serum leptin concentrations and a delay in the peak concentration after leptin administration.

DISCUSSION

The clinical features of congenital leptin deficiency, as previously described in this patient and her cousin, are marked hyperphagia, excessive weight gain in early life, and severe obesity.[8] The detailed evaluation we undertook before starting leptin therapy in this patient has provided further information on the role of leptin in human physiology. Although there are no normative data for a child of this weight, there was no evidence of substantial impairment in her basal or total energy expenditure, and her body temperature was normal, which is not the case in *ob/ob* mice, whose oxygen consumption, energy expenditure, and body temperature are low.[18] Thus, leptin may be less central to the regulation of energy expenditure in humans than in mice.

A further difference between *ob/ob* mice and all humans reported to have either leptin[8,19] or leptin-

receptor[20] mutations thus far relates to the consistently normal glucocorticoid concentrations in humans, in contrast to the marked excess in *ob/ob* mice.[21] Since glucocorticoids have profound effects on growth and insulin action, this difference between the species in the secretion of glucocorticoids may help to explain why leptin-deficient mice have impaired linear growth and severe insulin resistance,[22] whereas humans with leptin deficiency do not have growth retardation and have only moderate insulin resistance. Indeed, the patient and her affected cousin are both tall and have advanced bone ages. Bone age is a marker of skeletal maturation and is frequently advanced in obese children,[23] although rarely by more than three years.[24] Our patient's advanced bone age could not be attributed to excessive or premature secretion of adrenal or ovarian sex steroids.

Ob/ob mice have hypogonadotropic hypogonadism and are infertile.[25] It is difficult to confirm hypogonadotropic hypogonadism in a 9-year-old prepubertal girl, but a girl with a bone age of 12.5 years would usually have started to develop some secondary sexual characteristics. Strobel et al. found that two severely obese adults with congenital leptin deficiency did not undergo pubertal development and had low serum follicle-stimulating hormone and luteinizing hormone concentrations,[19] suggesting that hypogonadotropic hypogonadism is a feature of congenital leptin deficiency in humans.

Treatment of this nine-year-old patient with congenital leptin deficiency with recombinant leptin led to a sustained reduction in weight, predominantly as a result of a loss of fat, as is the case in *ob/ob* mice.[3] The weight loss during treatment indicated an average negative energy balance of approximately 400 kcal per day. The chief effect of leptin on energy balance was mediated by its suppressive effects on food intake. The patient's total energy expenditure was similar before and after 12 months of leptin therapy, with a reduction in her basal metabolic rate counterbalanced by an increase in her energy expenditure attributable to physical activity. Thus, the therapeutic effects of leptin were largely attributable to changes in energy intake.

The patient's basal and stimulated serum gonadotropin concentrations increased after 12 months of leptin treatment. The nocturnal pattern of gonadotropin secretion was pulsatile, which is a characteristic of early puberty.[26] Puberty might have begun spontaneously without leptin treatment, but this is unlikely given the fact that all adults with congenital leptin or leptin-receptor deficiency described so far have had severe hypogonadotropic hypogonadism.[19,20] Whether adequate serum leptin concentrations are required for normal pubertal development or, alternatively, whether leptin plays an active role in the initiation of puberty is not known.[27-29]

Antibodies to leptin were detected in serum after two months of treatment, and they persisted thereafter. Although the antibodies clearly interfered with measurements of serum leptin, they do not appear to have interfered with the response to treatment or to have been associated with any adverse effects; they did not neutralize the action of leptin in a bioassay.

In summary, the therapeutic response to leptin in this child with leptin deficiency confirms the importance of leptin in the regulation of body weight in humans and establishes an important role for this hormone in the regulation of appetite.

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