

# Congenital leptin deficiency: diagnosis and effects of leptin replacement therapy

Deficiência congênita de leptina: diagnóstico e efeitos da terapia de reposição

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## SUMMARY

To describe our 10-year experience in treating leptin-deficient humans. Three adults and one boy presented with childhood-onset morbid obesity, hypogonadism and family history of obesity and early death. Serum leptin was inappropriately low. A recessive C105T leptin gene mutation was identified. Metabolic and endocrine assessments were conducted, before and while on and off leptin. The adults' body mass index decreased from  $51.2 \pm 2.5$  to  $29.5 \pm 2.8$  kg/m<sup>2</sup>. Serum lipids normalized, insulin resistance decreased, and one of the initially diabetic females became normoglycemic. Hypogonadotropic hypogonadism was reversed, and other changes were observed in the adrenal, sympathetic, somatotrophic and thyroid functions. Leptin replacement therapy reverses endocrine and metabolic alterations associated with leptin deficiency. Some of these results may be extrapolated to other diseases. Arq Bras Endocrinol Metab. 2010;54(8):690-7

## SUMÁRIO

Descrever nossa experiência de 10 anos tratando pacientes deficientes em leptina. Três adultos e um menino apresentaram obesidade mórbida com início na infância, hipogonadismo e história familiar de obesidade e morte precoce. A leptina sérica era inapropriadamente baixa. A mutação recessiva C105T no gene da leptina foi identificada. Avaliações metabólicas e endócrinas foram realizadas antes e durante o tratamento. O índice de massa corporal dos adultos baixou de  $51,2 \pm 2,5$  para  $29,5 \pm 2,8$  kg/m<sup>2</sup>. Houve normalização dos lipídios séricos, a resistência insulínica diminuiu e a paciente que era diabética se tornou normoglicêmica. O hipogonadismo hipogonadotrópico foi revertido e outras alterações foram observadas nas funções adrenal, simpática, somatotrópica e tireoidiana. A reposição de leptina reverte as alterações endócrinas e metabólicas associadas com a deficiência de leptina. Alguns desses resultados podem ser extrapolados para outras doenças. Arq Bras Endocrinol Metab. 2010;54(8):690-7

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## INTRODUCTION

Leptin is a hormone produced mainly by the white adipose tissue, with multiple actions in the endocrine and immune systems, including glucose homeostasis, reproduction, bone formation, tissue remodeling and inflammation (1). Leptin is a key regulator of energy homeostasis, by regulating energy intake and expenditure through its actions on the arcuate nucleus of the hypothalamus (2,3). Leptin levels are positively correlated with fat mass, being increased in obesity (4,5).

Among mice that are leptin-deficient (*ob/ob*) or leptin-resistant (*db/db*), blunted metabolic rate and hyperphagia are characteristic features and obesity is a hallmark feature (6-10). In particular, *ob/ob* mice also have cellular immune deficiency, hypogonadotropic hypogonadism, hypercortisolism, decreased levels of growth hormone, type 2 diabetes and central hypothyroidism. This phenotype is prevented or even reversed with the administration of exogenous leptin.

In humans, leptin deficiency is observed in lipodystrophy syndromes, which present with low or

absent leptin levels due to abnormal deposition and distribution of adipose tissue. In addition, congenital human leptin deficiency may also be caused by mutations in the leptin gene.

These forms of leptin deficiency due to mutations in the leptin gene are extremely rare, with 20 patients having been identified in the world to date. These are patients of Pakistani (n = 12) (8,11,12), Turkish (n = 5) (7,13,14), Egyptian (n = 2) (15) and Austrian (n = 1) (16) background. The evaluation of those patients before and during leptin replacement therapy has unveiled the importance of leptin in the homeostasis of several systems, such as the brain, immunity and glucose metabolism.

In this manuscript, we have summarized the diagnostic approach and the phenotypic findings of five patients from a unique Turkish leptin-deficient family with a mutation in the leptin gene. Moreover, we describe the endocrine and metabolic effects of leptin replacement therapy in these patients.

## PATIENTS AND METHODS

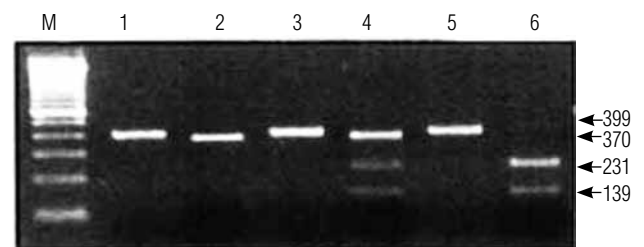
In 1998, investigators from the Gulhane Medical School in Ankara, Turkey and from the Cochin Institute of Molecular Genetics, University of Paris VII identified two adults (male A, 22-year-old; female B, 34-year-old) and one 6-year-old girl from Turkey, who presented with morbid obesity starting at early childhood, hypogonadotropic hypogonadism (in the adults), and extremely and inappropriately low serum leptin levels (0.9 ng/mL for the adult male, 1.6 ng/mL for the adult female, and 1.1 ng/mL for the girl). Due to this unique phenotype, the diagnosis of leptin deficiency was suspected.

Subsequently, a Mendelian recessive leptin gene mutation was identified, consisting of a C→T substitution in codon 105 of the leptin gene, resulting in an Arg→Trp replacement in the mature protein. That substitution abolishes an *MspI* restriction site, allowing rapid PCR screening for the mutation (7). This is the same mutation that is observed in the *ob/ob* mouse where it causes a premature stop codon.

For the identification of the mutation, the coding region of exon 3 is amplified by PCR. Primers derived from the human sequence (GenBank accession no. NM\_000230.2) were used. The sense primer (5'-CAGTCAGTCTCCTCCAAACA-3') corresponded to nucleotides 202-221, and the antisense primer

(5'-CTTAACGTAGTCCTTGCAGG-3') was complementary to nucleotides 580-600.

Subsequently, a restriction enzyme assay is performed with DNA of the PCR-amplified coding region of exon 3, digested by *MspI*. Since the mutation causes the disappearance of one of the two *MspI* sites, patients that are homozygous for the mutation will show only two bands of 29 and 370 bp whereas patients without the mutation will have three bands of 29, 139, and 231 bp in the agarose gel. With regard to heterozygous patients, they will display 4 bands of 29, 139, 231 and 370 bp (Figure 1). That particular mutation leads to the synthesis of a truncated protein that is not secreted into the medium, as evaluated in early functional studies (7).

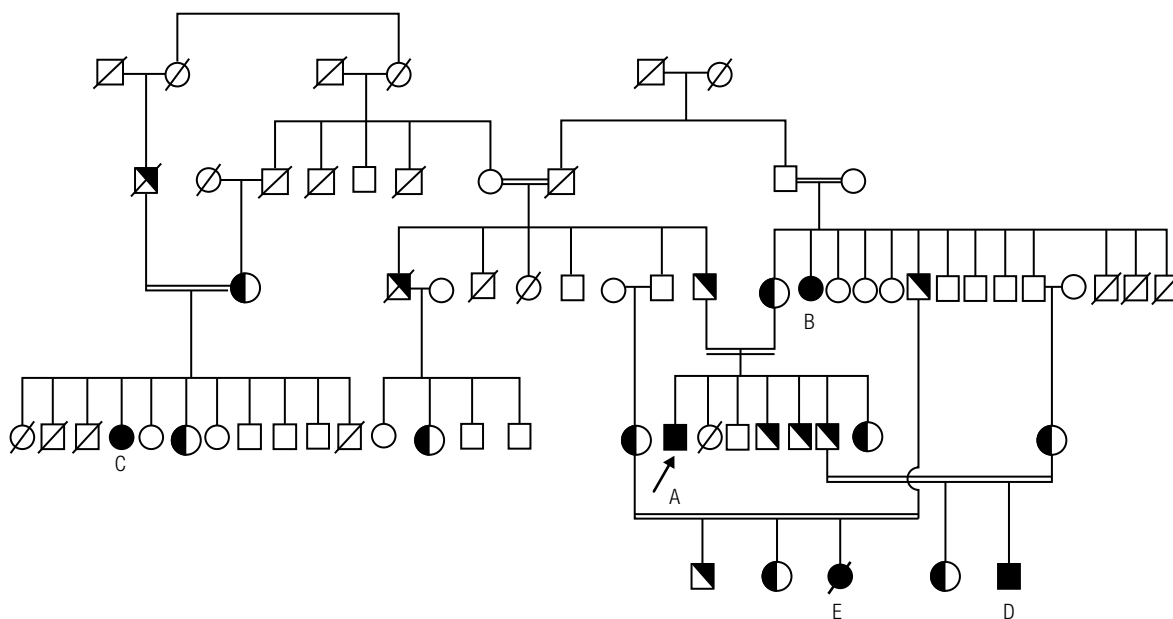


Patients 1 and 2 are homozygous, patients 3 and 4 are heterozygous, and patients 5 and 6 do not carry the C105T mutation of the leptin gene. Samples 2, 4 and 6 were submitted to *MspI* digestion.

**Figure 1.** Agarose gel of homozygous, heterozygous and wild-type patients for the C105T mutation of the leptin gene. Adapted from (7) with permission.

In 1999, in collaboration with Ozata, we identified in this extended family a new adult homozygous female (patient C, 30-year-old) (13), who was severely obese and amenorrheic (6). Subsequently, a 5-year-old boy (patient D) from the same pedigree was diagnosed with leptin deficiency. In this family, all but one wild-type and heterozygous individual had normal body weight, or were overweight. One heterozygous male individual (patients D's father) is obese (BMI of 32 kg/m<sup>2</sup>), dyslipidemic (total cholesterol = 226 mg/dL, triglycerides = 340 mg/dL, HDL = 30 mg/dL and LDL = 128 mg/dL) and has been recently diagnosed with type 2 diabetes in two oral glucose tolerance tests (95 mg/dL at baseline and 206 mg/dL 2h after 75 g of glucose).

Eight members of this family, whom we presume to have been leptin-deficient, had severe obesity and died during childhood due to infections. This number includes the 6 year-old girl, who died before the initiation of treatment. The pedigree chart is illustrated in figure 2.



**Figure 2.** Pedigree chart of the Turkish cohort. Patient A: 34-year-old male; patient B: 44-year-old female; patient C: 49-year-old female; patient D: 10-year-old boy (actual ages); patient E: 9-year-old deceased girl.

### LEPTIN REPLACEMENT THERAPY

At the diagnosis, clinical assessments of the endocrine, sympathetic, and immune functions were performed. Subsequently, over 10 years, we evaluated the effect of leptin replacement therapy with daily injection of recombinant methionyl human leptin (r-metHuLeptin, Amylin Pharmaceuticals, San Diego, USA). In addition, we assessed the effects of brief periods of leptin withdrawal and reinstatement.

Treatment with r-metHuLeptin was initiated at ages 5 (boy – patient D), 27 (adult male – patient A), 30 and 40 (females B and C). Leptin replacement therapy is undertaken at low physiological doses, starting at 0.02-0.04 mg/kg/day given subcutaneously at 6 pm. A daily subcutaneous injection in the evening was chosen to mimic leptin’s normal circadian rhythm, which peak occurs at night (17). For the adults, this dose increases serum leptin to levels that are normally observed in adult males with 20% body fat, or in adult females with 30% body fat. The child’s initial dose was calculated to increase the peak serum leptin to 70 ng/mL (18,19). Subsequently, doses were recalculated and scaled-down, avoiding excessively rapid weight loss.

The adults’ initial mean dose was  $4.1 \pm 1.2$  mg/day: 2.8 mg for male patient A, 4.2 mg for female patient B, and 5.3 mg for female patient C. The current dose is  $1.4 \pm 1.9$  mg/day: 0.3 mg for A, 0.45 mg for B,

and 3.6 mg for C. The child’s initial dose was 1.36 mg/day, and the current dose is 0.95 mg. More significant decreases in leptin dose for patient C resulted in weight gain possibly because that patient presents higher leptin resistance, associated with common obesity.

### PHENOTYPE BEFORE AND DURING TREATMENT

#### Body composition, food intake and energy expenditure

The most evident phenotypic finding in our patients was morbid obesity. The adults’ initial mean body mass index (BMI) was  $51.2 \pm 2.5$  kg/m<sup>2</sup> (51.4, 46.7, and 55.4 kg/m<sup>2</sup>, for patients A, B, and C respectively). After 18 months of treatment, the patients reached a stable mean BMI of  $26.9 \pm 2.1$  kg/m<sup>2</sup> (24.8, 26.1, and 31.3 kg/m<sup>2</sup> for A, B, and C respectively) (Figures 3 and 4). Since early 2003, the patients’ weight has remained fairly stable, and the latest BMI (as of March 2010) was  $29.5 \pm 2.8$  kg/m<sup>2</sup>. The boy also lost a significant amount of weight, going from a BMI of 39.6 kg/m<sup>2</sup> before treatment at age 5, to 22.6 kg/m<sup>2</sup> at age 9 (Figure 5). Most of the decrease in BMI was attributed to fat mass loss, as measured by DXA (13). In 2001, the initial total body fat percentages were 43.7, 45.7, and 49.9% for patients A, B, and C, respectively. In 2007, those percentages were equal to 7.0, 36.4, and 45.0%, respectively for patients A, B, and C.

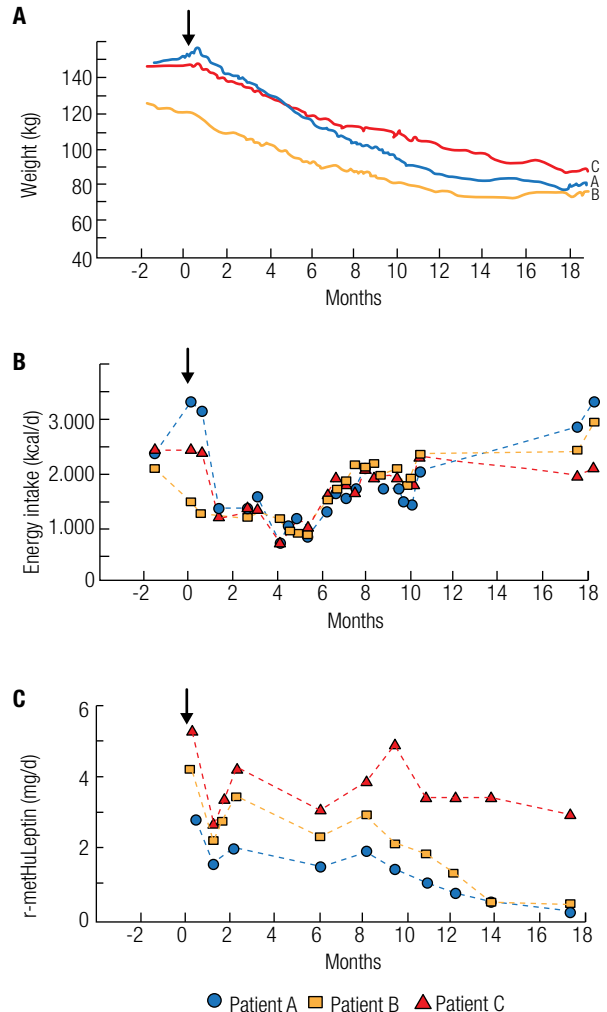


From left to the right: patients C, B and A (blurred faces), before (a) and 18 months after r-metHuLeptin treatment (b).

**Figure 3.** Adult patients before (A) and 18 months after treatment (B). Adapted from (13) with permission.

Before treatment, patient A had low bone mineral density (BMD) at the lumbar spine (BMD of L2–L4, 0.924 g/cm<sup>2</sup>; T-score -1.96; Z-score -2.36), and the females had normal BMDs at all sites. After 6 years, the male's BMD at the lumbar spine increased by 11% (BMD of L2–L4, 1.042 g/cm<sup>2</sup>; T-score -1.5; Z-score -1.1). The females' BMDs remained within normal range, without significant changes.

Weight loss was achieved without any instruction on dietary changes or on increase in physical activity. In the adults, leptin replacement decreased energy intake from 2,384 ± 946 kcal/day to 1,179 ± 790 kcal/day (Figure 4). In addition, leptin replacement changed the macronutrient content of the patients' diet, with an increase in carbohydrate and a decrease in fat consumption (20). In the child, after two years, caloric intake decreased from 2,709 ± 370 to 2,194 ± 292 kcal/day, which is 106% of the recommended caloric intake for a boy this age, height and weight (14). Activity levels were measured in the adults by actigraphy, which showed progressive and linear increases (13).

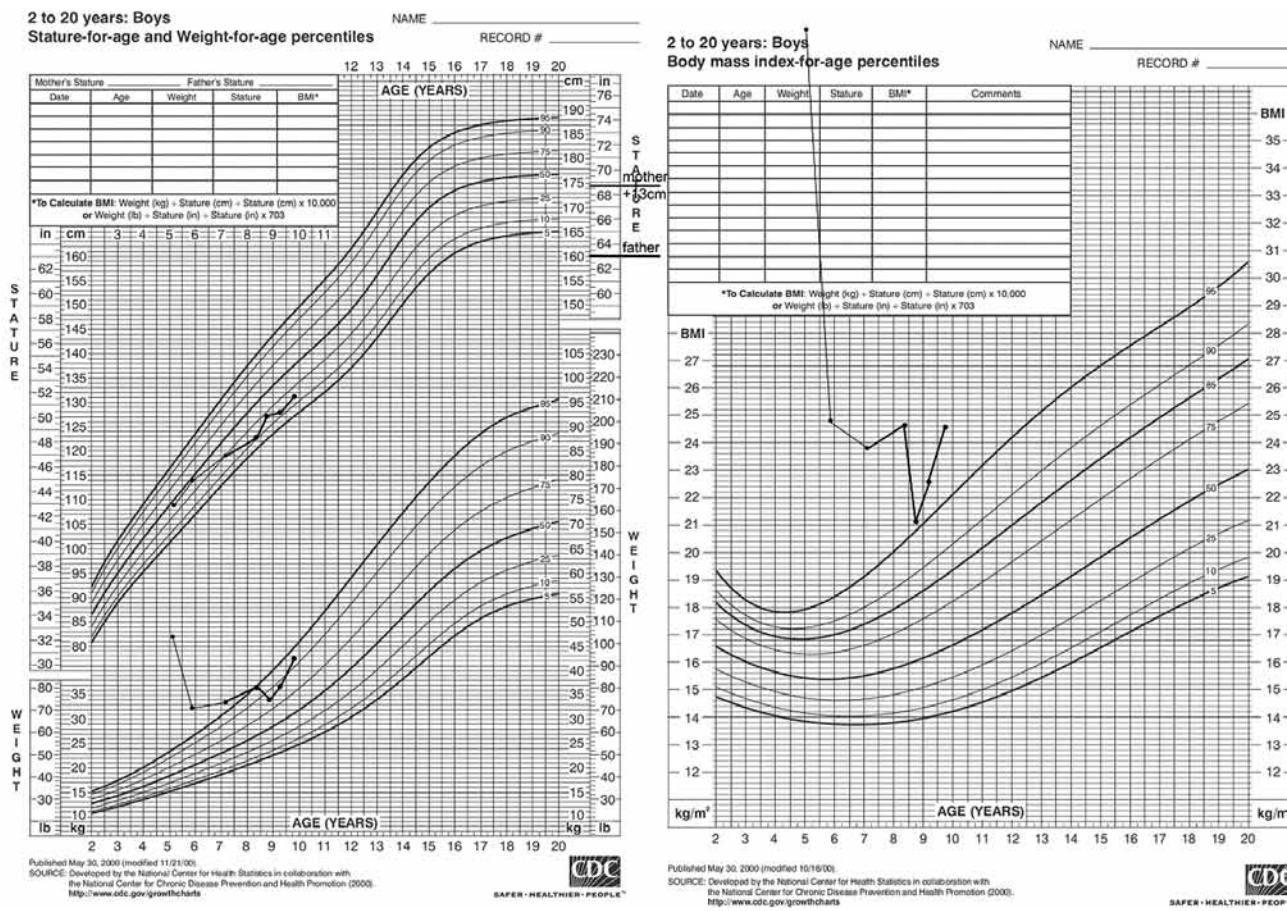


**Figure 4.** Weights (A), energy intake (B), and r-metHuLeptin dose (C) over the first 18 months of treatment. Adapted from (13) with permission.

Before treatment, 24-h energy expenditure and 24-h fat oxidation in the adults were comparable to those of age-, sex- and weight-matched controls. During treatment, the weight loss-associated decrease in energy expenditure was less pronounced in our patients than in controls under a weight loss program. In addition, fat oxidation was also higher in our patients. Therefore, leptin replacement prevented the reduction in metabolic rate that is associated with weight loss (21).

### Lipid and glucose metabolism

Before treatment, all patients had low HDL-cholesterol and normal or high triglycerides and insulin (Table 1). In addition, the older female was diagnosed with type 2 diabetes (6,13). Leptin replacement normalized serum lipid, glucose and insulin levels, leading to the resolution of type 2 diabetes in patient C (13). These changes are sustained until the present time.



**Figure 5.** Patient's D growth and body mass index charts. The patient experienced substantial weight gain in the last evaluation due to a brief period of shortage of supplies.

**Table 1.** Serum lipids, glucose and insulin before and after treatment

	Total cholesterol (mg/dL)		HDL-c (mg/dL)		LDL-c (mg/dL)		Triglycerides (mg/dL)		Insulin (uU/mL)		Glucose ng/dL	
	Before	After#	Before	After#	Before	After*	Before	After#	Before	After#	Before	After#
A	137	96	29.9	53.3	82	29	125	69	4.8	1.8	91	78
B	115	110	36.8	51.2	62	49	79	47	3.8	1.8	88	84
C	181	125	28.6	38.4	103	64	247	115	7.5	3.1	131	86
D*	166	155	36	65	87	66	216	120	21	7	79	87

\* After 2 years; # After 18 months.

By measuring glucose, insulin and C-peptide and mathematically modeling glucose homeostasis, during a 24-h period of intensive blood sampling (every 7 minutes; 207 samples in total/24h), with standardized meals used as tolerance tests, we observed that leptin replacement increased insulin sensitivity by at least 5.7-fold, decreased pancreatic insulin secretion calculated by deconvolution of plasma C-peptide levels and also decreased hepatic extraction of insulin (22) estimated

as the instantaneous differences between insulin secretion and post hepatic delivery rates.

During the 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> year of treatment, the adults were submitted to brief periods of leptin withdrawal during 6 weeks, leading to substantial weight gain (5.6 ± 3.8 kg for patient A, 5.4 ± 0.9 kg for patient B, and 6.0 ± 2.2 kg for patient C). This weight gain determined an acute and transient increase in insulin sensitivity (measured by euglycemic hyperinsulinemic

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clamps) while off leptin, as the newly acquired adipose tissue absorbed glucose in excess (23).

### Gonadal and reproductive function

Before treatment, the adults were hypogonadic. The adult male was prepuberal: no beard, bilateral gynecomastia, scanty pubic and axillary hair, small penis, small testis, and azoospermia. Patient C had spontaneous menarche at age 35, and had scanty pubic and axillary hair, small uterus and ovaries, and no mammary tissue. The younger female had spontaneous menarche at age 29, with normal pubic and axillary hair, small ovaries and borderline uterus, and diminished mammary tissue. Gonadotropin responses to GnRH stimulation were normal, which favors the diagnosis of hypogonadotropic hypogonadism (Table 2).

After treatment, menstrual periods became regular in both patients, with serial midluteal phase progesterone measurements > 10 ng/mL, which are indicative of ovulation. The male adult's testosterone and free testosterone levels reached normal values for adults. All adults fully developed secondary sexual characteristics and developed normal sexual function (13).

### Adrenals and sympathetic tone

Contrary to *ob/ob* mice, leptin-deficient humans do not present with hypercortisolemia. Our patients had normal levels of free urinary cortisol. Serum cortisol levels were 11.5, 22.3, and 26.4 µg/dL for patients A, B, and C respectively. Those levels were suppressed to less

than 5 µg/dL with 1 mg of dexamethasone (6). Patient A was submitted to frequent blood sampling over 24 hours, which showed that leptin replacement increases the mean 24-hour levels of serum cortisol, from  $4.04 \pm 0.22$  µg/dL to  $5.97 \pm 0.30$  µg/dL. Leptin also altered the circadian rhythms of cortisol, by decreasing the number of pulses from 25 to 19, increasing their amplitude, increasing the morning peak, and increasing regularity (13).

Low sympathetic tone was shown in the adults (patients A, B, and C) and in the girl (patient E), evaluated by cold pressor response tests, by orthostatic hypotension tests, and by skin response tests. Results in the leptin-deficient patients (Table 3) were significantly different from 15 age- and sex-matched healthy controls, in which systolic cold pressor response was  $10.6 \pm 0.37$  mmHg ( $z = -4.74$ ;  $P < 0.001$ ) and diastolic cold pressor response was  $12.0 \pm 0.38$  mmHg ( $z = -4.754$ ;  $P < 0.001$ ). Sympathetic function was normal in heterozygous and wild-type subjects (6).

In patient A, there were no aldosterone and renin responses to the postural test: supine position: aldosterone < 10 pg/mL; renin, 0.3 ng/mL; upright position: aldosterone < 10 pg/mL; renin, 0.3 ng/mL. In patient B, responses to the postural test were adequate: supine position: aldosterone 50.3 pg/mL; renin 1.4 ng/mL; upright position: aldosterone 92.3 pg/mL; renin 4.5 ng/mL. In patient C, the response was also adequate: supine position: aldosterone 25 pg/mL; renin, 0.3 ng/mL; upright position: aldosterone 127.5 pg/mL; renin, 4.8 ng/mL.

**Table 2.** Sex hormones before treatment

	FSH		LH		Estrogen	Total testosterone
	Before GnRH	Peak after GnRH	Before GnRH	Peak after GnRH		
A	9	18.4	4.4	23.2	80.8	80.0
B	6	9.6	5.3	18.4	53.0	53.0
C	2.6	5.1	1.6	14.7	31.0	31.0

Normal reference ranges for FSH (IU/L): 1.4-18.1; LH (IU/L): 1.5-9.3; estrogen (pg/mL): 21-76; total testosterone (ng/mL): 241-827 (male reference range).

**Table 3.** Cold pressor blood pressure and orthostatic hypotension tests in leptin-deficient humans before treatment.

	Cold pressor blood pressure response		Orthostatic hypotension test	
	Systolic	Diastolic	Systolic and diastolic pressure before	Systolic and diastolic pressure after
A	$7.2 \pm 0.16$	$6.95 \pm 0.12$	$132.5 \pm 2.88 / 95.0 \pm 4.08$	$91.2 \pm 2.5 / 61.2 \pm 2.5$
B	$7.22 \pm 0.17$	$7.42 \pm 0.17$	$171.2 \pm 3.5 / 108.7 \pm 2.9$	$143.2 \pm 5.3 / 80.0 \pm 4.1$
C	$7.62 \pm 0.12$	$7.2 \pm 0.13$	$123.7 \pm 2.9 / 72.5 \pm 2.8$	$88.7 \pm 4.7 / 61.7 \pm 2.3$
E	$7.57 \pm 0.17$	$7.05 \pm 0.13$	$113.7 \pm 3.5 / 70.5 \pm 4.2$	$86.7 \pm 2.2 / 62.7 \pm 2.2$

Results in mmHg.

## Somatotropic axis

The adult patients had no history of delayed or impaired growth, and their heights were within the family mean height. Growth hormone levels were  $\leq 0.01$  ng/mL, and responses to insulin-induced hypoglycemia and exercise tests were absent in the male (nadir glucose level, 37 mg/dL; GH of 0.1 ng/mL in both tests) and in the younger adult female (nadir glucose level, 35 mg/dL; GH, 0.1 ng/mL in the hypoglycemia test and 0.3 ng/mL in the exercise tests). Given the absence of clinical features of growth hormone deficiency, these results are probably attributed to obesity (6). Before treatment, the boy's height was at the 50<sup>th</sup> percentile in the growth chart. Weight gain and growth deceleration was observed over 2 years (from the 50<sup>th</sup> to the 10<sup>th</sup> percentile), possibly due to inadequate dose adjustments. After leptin dose increases, the child is now between the 10<sup>th</sup> and the 25<sup>th</sup> percentile, within the targeted height (Figure 5).

In the adults, all of the IGF-related parameters were within normal range, except for postprandial IGFBP-1 (13). After 18 months of leptin replacement therapy (Table 4), we observed significant increases in pre and postprandial IGFBP-1 levels. We also observed significant increases in mean IGFBP-2, possibly attributed to the decrease in insulin levels (24).

**Table 4.** Mean insulin-like growth factors and insulin-like growth factor binding globulins, before and after treatment

	Before	18 months after
IGF-I	141 $\pm$ 14	154 $\pm$ 16
IGF-II	484 $\pm$ 17	487 $\pm$ 17
IGFBP-1 (fasting)	21.1 $\pm$ 1.3	140 $\pm$ 42
IGFBP-1 (postprandial)	3.7 $\pm$ 0.5	18.1 $\pm$ 2.6
IGFBP-2	528 $\pm$ 54	936 $\pm$ 53
IGFBP-3	1606 $\pm$ 96	1633 $\pm$ 82
IGFBP-6	299 $\pm$ 12	282 $\pm$ 12

Reference range values: IGF-I: 120-400 ng/mL; IGF-II: 290-730 ng/mL; IGFBP-1 (fasting): 13-120 ng/mL; IGFBP-1 (postprandial): 10-30 ng/mL; IGFBP-2: 360-1020 ng/mL; IGFBP-3: 1500-3600 ng/mL; IGFBP-6: 100-340 ng/mL.

## Thyroid function

It is known that leptin has a circadian rhythm that is highly organized (18), and with a pattern similar to that of TSH: nadir in late morning and peak in the early morning (19). Although thyroid function tests were normal for all adults and for the boy (17), the leptin-deficient adult male (patient A) presented dysregulated patterns

of TSH pulsatile and circadian rhythms (25). Those data confirm that leptin has a role in regulating TSH secretion in humans (26), and its absence may lead to thyroid dysfunction. Leptin replacement did not increase free T4 or T3 (17), as previously observed in other studies (12).

## DISCUSSION

Leptin replacement is currently the only successful hormonal treatment for a monogenic form of human obesity. In a cohort of Turkish leptin-deficient patients, our 10-year experience of leptin replacement therapy showed that treatment leads to substantial effects on body composition, food intake and energy expenditure, lipid and glucose metabolism, sympathetic tone, and gonadal, adrenal, somatotrophic and thyroid functions. Additional results also suggest that leptin has important roles on non-endocrine parameters, such as inflammation and coagulation (27), immunity (11,28), and brain structure and function (14,29-33).

Although leptin therapy has proven ineffective in treating patients with common and complex obesity, patients with severe forms of obesity and metabolic syndrome may present relatively low levels of leptin, corrected by adipose tissue mass (34). It is also known that specific polymorphisms in the leptin gene are associated with higher or lower leptin levels (35,36). Therefore, obese patients with relatively low leptin levels, or with polymorphisms associated with lower leptin, might benefit from leptin therapy.

Although leptin deficiency is an extremely rare disease, our results provide new insight into the long-term effects of leptin in humans through a unique model of obesity presenting with leptin deficiency, without leptin resistance. By understanding the physiology of leptin, we will be able to design future studies that will evaluate the effects of leptin in other diseases, such as common obesity, lipodystrophy syndromes, diabetes, hypothalamic amenorrhea, anorexia nervosa, mood and cognitive disorders, immune deficiencies, and lipotoxicity.

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