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The long-term effect of recombinant methionyl human leptin therapy on hyperandrogenism and menstrual function in female and pituitary function in male and female hypoleptinemic lipodystrophic patients

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Abstract

Lipodystrophy patients are hypoleptinemic and insulin resistant. Women have enlarged polycystic ovaries, hyperandrogenism, and amenorrhea. We have determined the role of correction of hypoleptinemia on these metabolic and neuroendocrine parameters.

Ten females and 4 males with generalized lipodystrophy were treated with recombinant methionyl human leptin (r-metHuLeptin) in physiologic doses in an open-labeled study for a period of 12 and 8 months, respectively.

In the female group, serum free testosterone decreased from 39.6 ± 11 to 18.9 ± 4.5 ng/dL ($P < 0.01$) and serum sex hormone binding globulin increased from 14 ± 2.5 to 25 ± 4.8 nmol/L ($P < 0.02$). Luteinizing hormone (LH) responses to LH releasing hormone were more robust after therapy and significantly changed in the youngest group of 3 female patients ($P < 0.01$). Ovarian ultrasound showed a polycystic ovarian disease pattern in all patients and did not change after therapy. Eight of the 10 patients had amenorrhea prior to therapy and all 8 developed normal menses after therapy.

In the male group, serum testosterone tended to increase from 433 ± 110 to 725 ± 184 ng/dL ($P = 0.1$) and sex hormone binding globulin also increased from 18.25 ± 2.6 to 27 ± 1.7 nmol/L ($P < 0.04$) following r-metHuLeptin therapy. Serum LH response to LH releasing hormone did not show significant changes. Five additional hypoleptinemic male subjects with minimal metabolic abnormalities underwent normal pubertal development without receiving r-metHuLeptin therapy.

In both genders, insulin-like growth factor increased significantly and there were no differences in growth hormone, thyroid, or adrenal hormone levels following r-metHuLeptin therapy.

Glycemic parameters significantly improved after r-metHuLeptin therapy in both groups. Hypoglycemic medications were discontinued in 7 of 12 patients and dramatically reduced in 5 patients.

r-metHuLeptin therapy plays an important role in insulin sensitivity. In females, it plays an additional role in normalizing menstrual function. This is likely to occur both from increasing insulin sensitivity and from restoring LH pulsatility. The persistent hypoleptinemic state in these subjects did not inhibit pubertal development.

1. Introduction

The hyperinsulinemic state associated with severe insulin resistance leads to 2 main forms of clinical expression. These are the hyperandrogenic features in females, which are also shared by the common forms of polycystic ovarian syndrome (PCOS), and the glucose and lipid disorders that characterize the so-called metabolic syndrome in both male and female patients.

Adipocytes produce important humoral factors such as leptin that affect both insulin resistance and hypothalamic-pituitary function [1-3]. Most of the available information regarding the physiologic role of leptin has been derived from rodent studies. In humans, there were several correlative studies of serum leptin concentrations to a variety of regulatory events. Recombinant methionyl human leptin (r-metHuLeptin) therapy has been studied in 2 hypoleptinemic states in patients. The first is congenital

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leptin deficiency, analogous to the *ob/ob* mouse, where the main phenotypic characteristic is obesity. Recombinant leptin induces pulsatile luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion at the time of puberty [4]. The second condition is severe insulin-resistant lipodystrophy [2,3], which is analogous to lipodystrophic rodent models, where the main phenotypic characteristic is insulin resistance [5-7]. Administration of r-metHuLeptin therapy in this hypoleptinemic state gives us the opportunity to study the direct effects of leptin on insulin resistance and on neuroendocrine function.

In the present study, we have (a) extended our previous studies of lipodystrophy [3] and investigated the role of recombinant leptin therapy on the hyperandrogenic state and menstrual dysfunction of patients up to 1 year of treatment; (b) evaluated the effect of r-metHuLeptin on the growth hormone (GH) and insulin-like growth factor 1 (IGF-1) axis; (c) evaluated the pituitary-adrenal and thyroid axis over a 1-year period of r-metHuLeptin therapy; and (4) evaluated the effect of r-metHuLeptin therapy on the pituitary gonadal axis in a few male subjects to complement recent studies in male normal volunteers [8].

2. Materials and methods

2.1. Patients

We studied 14 patients with generalized lipodystrophy who were all characterized by having decreased levels of plasma leptin. Of these patients, 10 were female and 4 were male. Nine patients had congenital generalized lipodystrophy and 5 had acquired generalized lipodystrophy. Female patients received r-metHuLeptin therapy for a period of 12 months and male patients for a period of 8 months. Individual patients are designated in the tables by numbers that can be equated to previous publications [2,3].

We also included baseline data on 5 male lipodystrophic patients. These male patients did not receive leptin therapy due to their minimal metabolic abnormalities.

2.2. Study design

A more detailed description of this prospective open-label study was published previously [2]. Amgen, Inc (Thousand Oaks, CA) provided r-metHuLeptin for the trial. Response of each patient was compared with their baseline state. The study was approved by the institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases. Informed written consent was obtained. Patients were evaluated as inpatients before treatment and again after 4, 8, and 12 months of r-metHuLeptin therapy. All patients were on stable doses of concomitant medications for at least 6 weeks before starting leptin. During the study, hypoglycemic drugs were reduced or discontinued as needed.

r-metHuLeptin was administered sc every 12 hours in doses to achieve physiologic concentrations of serum leptin. The physiologic replacement dose was estimated to be 0.03 mg/kg/d for females under 18 years and 0.04 mg/kg/d for adult females. For all males, the dose was 0.02 mg/kg. Patients were treated with 50% of the replacement dose for the first month, 100% of the replacement dose the next month, and starting with the third month 200% of the replacement dose through the remainder of the study.

Hormone concentrations were determined at 0, 4, 8, and 12 months of r-metHuLeptin therapy. Hormone stimulation tests were performed before r-metHuLeptin therapy and after 4 months of therapy. The hormonal analyses were timed based on the length of therapy, not on any other parameter such as a particular period in the menstrual cycle.

2.3. Hormone assays

Serum leptin levels were determined by immunoassays using a commercial kit (Linco Research, Inc, St. Charles,

Table 1
Baseline characteristics of lipodystrophic patients

Patient	Age/sex	Type	Gene	Leptin (ng/mL)	BMI (kg/m^2)	Total fat (%)
NIH 1	17/F	AGL		1.48	14.6	6.4
NIH 2	17/F	CGL	AGPAT2	2.49	24.2	7.1
NIH 3	27/F	AGL		1.74	20.4	10.9
NIH 4	17/F	CGL	AGPAT2	1.22	21.4	8.8
NIH 5	15/F	CGL	AGPAT2	1.55	25.4	8.6
NIH 6	36/F	CGL	AGPAT2	0.5	22.2	7.9
NIH 8	40/F	CGL	AGPAT2	1.41	18.1	8.6
NIH 13	12/F	CGL	Seipin gene	0.8	23	8.6
NIH 19	30/F	AGL		1.26	19.3	7.8
NIH 20	22/F	CGL	AGPAT2	1.55	25.2	8.6
NIH 11	13/M	CGL	AGPAT2	1.31	23.6	8.3
NIH 14	35/M	AGL		1.09	26.9	7.8
NIH 15	68/M	AGL		0.5	22.6	6.8
NIH 24	17/M	CGL	Seipin gene	0.87	24.1	6.7
Mean ± SEM				1.3 ± 0.1	22 ± 0.9	8 ± 0.3

The NIH number refers to the same patients that have appeared in previous publications [2,3,10]. AGL indicates acquired generalized lipodystrophy; CGL, congenital generalized lipodystrophy.

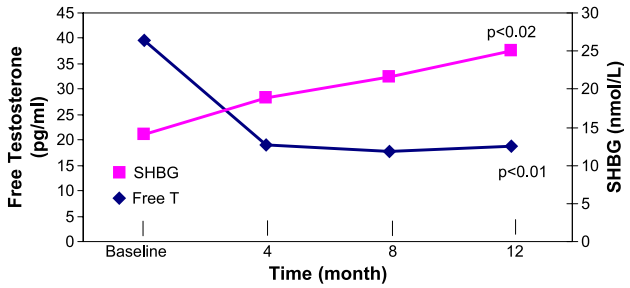

Fig. 1. Mean changes in 10 female patients in free T (reference range, 3-19 pg/mL) and SHBG (reference range, 20-130 nmol/L) as a function of r-metHuLeptin therapy.

MO). Serum FSH and LH concentrations were assayed using a microparticle enzyme immunoassay on an AxSYM system (Abbott Diagnostics, Abbott Park, IL). Estradiol (E2) and testosterone were determined at the Mayo Medical Laboratories (Rochester, MN) via a chemiluminometric immunoassay. Free testosterone (free T) was assayed by equilibrium dialysis at Mayo Medical Laboratories [9]. Sex hormone binding globulin (SHBG) was also measured at the Mayo Medical Laboratories using Immulite solid-phase, two-site chemiluminescent immunoassay (Diagnostic Products, Los Angeles, CA). Serum thyroid stimulating hormone (TSH) levels were measured with a two-site chemiluminescent immunometric assay on DPC Immulite 2000 equipment (Diagnostic Products) and serum adrenocorticotropic hormone (ACTH) concentrations were determined with an immunochemiluminometric assay on an Advantage chemiluminescent system (Nichols Institute Diagnostics, San Juan Capistrano, CA). Serum triiodothyronine (T3) and cortisol levels were also determined via competitive immunoassays, namely, via chemiluminescent immunometric assay on DPC Immulite 2000 equipment (Diagnostic Products). Free T4 was measured with an electrochemiluminescent competitive immunoassay on Elecsys 2010 equipment (Roche Diagnostics, Indianapolis, IN) [3].

Androstenedione (A4) and dehydroepiandrosterone were determined by radioimmunoassay at the Mayo Medical Laboratories (Diagnostic System Laboratories, Inc, Webster, TX).

Growth hormone was determined by immunometric assay using an Immulite 2000 analyzer. IGF-1 was determined via a two-site chemiluminescence immunoassay.

Table 2
Effect of r-metHuLeptin therapy on gonadotropin secretion in female patients

Gonadotropins	Baseline	4 mo	8 mo	12 mo
LH (U/L)	6.4 ± 1.9	8.6 ± 2.2	5.2 ± 1.1	6.7 ± 1.5
FSH (U/L)	5.2 ± 0.7	5.8 ± 1	4.9 ± 0.6	5 ± 0.6
LH/FSH	1.3 ± 0.3	1.8 ± 0.5	1.1 ± 0.1	1.3 ± 0.2

Levels of LH, FSH, and LH/FSH ratio are expressed as means ± SEM of the 10 female patients.

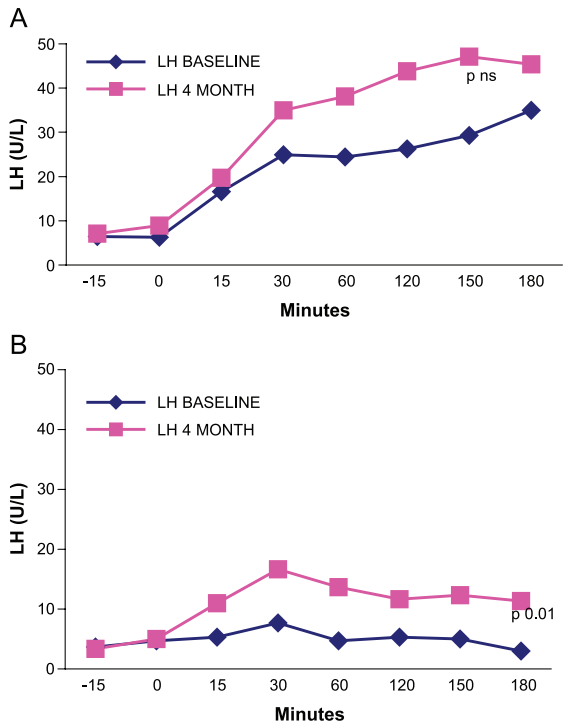

Fig. 2. (A) Response in LH to administration of LHRH in 10 female patients at baseline and at 4 months of r-metHuLeptin therapy. (B) Three youngest patients (NIH 4, 5, and 13, ages 17, 14, and 12, respectively).

2.4. Procedures

LH releasing hormone (LHRH) tests were performed after an overnight fast and before administration of any medication. LHRH (100 μg Factrel, Ayerst Pharmaceuticals, Philadelphia, PA) was administered as iv boluses. Blood samples were drawn at −15, 0, 15, 30, 60, 120, 150, and 180 minutes for LH and FSH.

Percentage fat (%) was determined using whole-body dual-energy x-ray absorptiometry (QDR 4500, Hologic, Inc, Bedford, MA) [3,10].

Pelvic ultrasounds were performed using ATL HDI-5000 (Siemens, Malvern, PA).

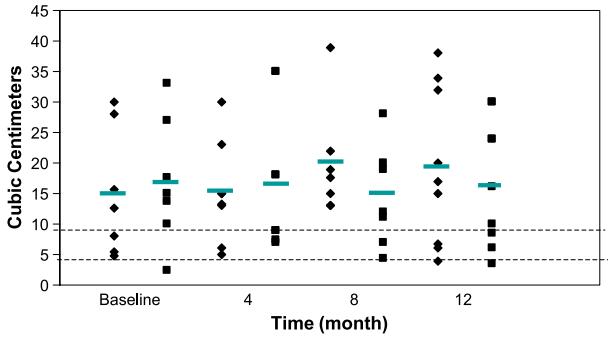

Fig. 3. Ovarian size (cm^3) as a function of time on r-metHuLeptin therapy. Filled diamonds, right ovaries; filled squares, left ovaries; dashed line, reference range of ovarian size [29]; solid line, mean values of ovarian size in our patients.

Table 3
Menstrual function before and after r-metHuLeptin therapy

Patient	Baseline menstrual status	12 mo menstrual status
NIH 1	Menses once a year	Regular menses
NIH 2	Primary amenorrhea	Regular menses
NIH 3	Regular menses	Regular menses
NIH 4	Primary amenorrhea	Regular menses
NIH 5	Primary amenorrhea	Regular menses
NIH 6	Hysterectomy without oopherectomy	Hysterectomy without oopherectomy
NIH 8	Irregular menses	Regular menses
NIH 13	Primary amenorrhea	Regular menses
NIH 19	Irregular menses	Regular menses
NIH 20	Irregular menses	Regular menses

2.5. Statistical analyses

Measurements are presented as means ± SE. To compare study variables during various study periods, ANOVA with repeated measures was used. Paired *t* test was employed to compare results wherever applicable. Data not normally distributed were $\log_{10}$ transformed prior to ANOVA analysis. $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Baseline characteristics for female patients

We studied 10 female patients with generalized lipodystrophy. Seven had congenital generalized lipodystrophy or the Seip-Berardinelli syndrome [generalized fat loss from birth in association with other clinical criteria (OMIM 269700)[1]]. Three had acquired generalized lipodystrophy with a history of fat loss in childhood.

The age range was 12 to 40 years, and all had reproductive organs, except one (NIH 6) who had a hysterectomy prior to the study. The mean serum leptin level was 1.4 ng/mL (range, 0.5-2.5 ng/dL), the mean of the body mass index (BMI) was 21.4 kg/m^2 (range, 14.6-25.4 kg/m^2), and the mean of percentage total body fat was 8.5% (range, 7.1-10.9%; Table 1).

3.2. Effect of r-metHuLeptin therapy on free T, SHBG, total testosterone, and estradiol

The mean baseline value of free T was 39.6 ± 11 pg/mL, which is approximately double the upper limit of the reference range. Six of 10 patients had values above the upper limit of normal. At 4 months of recombinant leptin therapy, the mean plasma level was reduced by 52% and this was maintained through the 12-month period ($P < 0.01$; Fig. 1).

[1] This 6-digit number is the entry number in OMIM (Online Mendelian Inheritance in Man), a continuously updated electronic catalogue of human genes and genetic disorders.

Table 4
Effect of r-metHuLeptin therapy on testosterone (ng/dL) in male lipodystrophy patients

Patients	Baseline	4 mo	8 mo
NIH 11	410	714	1044
NIH 14	510	702	954
NIH 15	144	235	222
NIH 24	669	569	682
Mean ± SEM	433 ± 110	555 ± 111	725 ± 184

At baseline, SHBG was 14.03 ± 2.5 nmol/L and increased in a linear fashion over the 12-month period ($P < 0.02$; Fig. 1).

The total testosterone, which represents both free T and bound testosterone, was significantly reduced from baseline (mean, 92 ± 30 ng/dL) to 4 months (mean, 50.3 ± 9 ng/dL) and the trend maintained, thereafter, up to 12 months (mean, 54.8 ± 8.8 ng/dL).

As mentioned previously, the estradiol samples were not collected at a defined point in the menstrual cycle, which may account for the variability seen. There was a trend to increase estradiol concentration during the period of r-metHuLeptin therapy (baseline mean, 44.5 ± 10 pg/dL; 12 months mean, 73.9 ± 25 pg/dL), but the changes were not statistically significant.

The mean baseline plasma level of androstenedione was normal at 256 ± 7 ng/dL (reference range, 30-310 ng/dL) and the mean plasma level of dehydroepiandrosterone was 1.38 ± 0.54 μg/mL (0.35-4.30 ng/dL). Neither significantly changed over the 12-month period of r-metHuLeptin therapy.

3.3. Effect of r-metHuLeptin therapy on gonadotropins

The mean baseline LH was 6.4 ± 1.9 U/L, FSH 5.2 ± 0.7 U/L, and the ratio of LH/FSH was 1.3 ± 0.3; there was

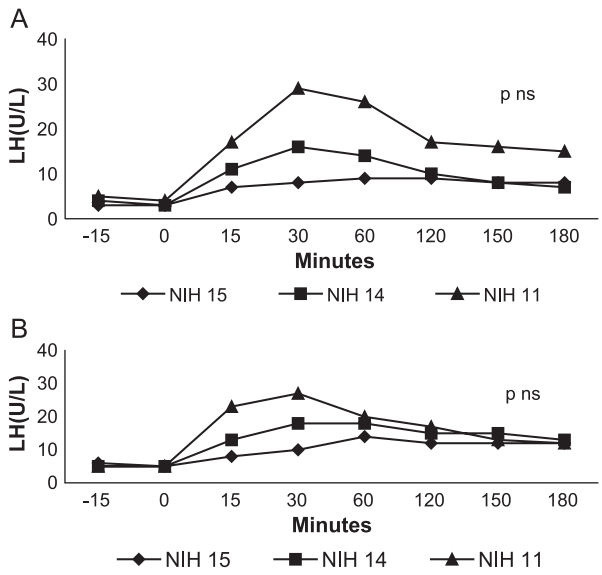

Fig. 4. Response in LH to administration of LHRH in 3 male patients. (A) Baseline response. (B) Response after 4 months of r-metHuLeptin therapy.

Table 5
Treatment regimens before and after r-metHuLeptin therapy

	Treatment regimen[a]	
Patients	Baseline	12 mo
NIH 1	Metformin 1000 mg/d	None
NIH 2	Insulin 386 U/d	None
NIH 3	Insulin 70 U/d, metformin 1000 mg/d	Metformin 1000 mg/d
NIH 4	Insulin 520 U/d	None
NIH 5	Insulin 750 U/d	None
NIH 6	Metformin 2550 mg/d	None
NIH 8	Insulin 160 U/d, pioglitazone 15 mg/d	Insulin 40 U/d, pioglitazone 30 mg/d
NIH 11	Metformin 2550 mg/d	None
NIH 13	None	None
NIH 14	Insulin 120 U/d	Insulin 20 U/d
NIH 15	Insulin 200 U/d	Insulin 60 U/d
NIH 19	Insulin 500 U/d, metformin 2450 mg/d	Insulin 40 U/d, metformin 2000 mg/d
NIH 20	Insulin 520 U/d, metformin 850 mg/d	None
NIH 24	None	None

[a] None of these patients were using other medications such as estrogen or progestational agents.

no significant change over the 12-month period. Six of 10 patients maintained LH/FSH ratio below 2 during the 12-month period (Table 2).

A LHRH test was performed in the 10 patients before r-metHuLeptin therapy and after 4 months of therapy. The LH response to LHRH was 4-fold increased from baseline to the peak value. At 4 months after treatment, the mean increment was more robust but did not achieve statistical significance (Fig. 2A). The FSH response to LHRH did not change significantly after 4 months of r-metHuLeptin therapy (data not shown). When the 3 youngest patients were considered separately (ages 12, 14, and 17), the LH response to LHRH administration was markedly attenuated prior to therapy, but after 4 months of r-metHuLeptin administration these responses were significantly increased ($P < 0.01$; Fig. 2B).

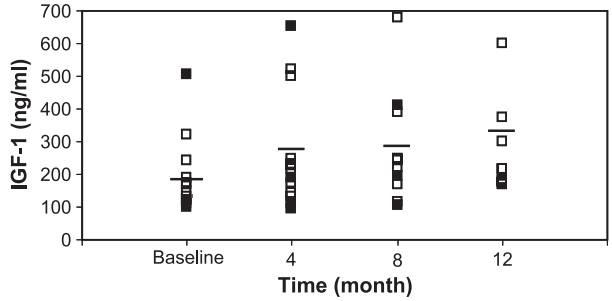

Fig. 6. IGF-1 as a function of time on r-metHuLeptin therapy. Open symbols, female patients; solid symbols, male patients; solid lines, mean values of IGF-1. The increment of IGF-1 analyzed by ANOVA in the female group up until 12 months of treatment with r-metHuLeptin therapy was statistically significant ($P < 0.02$). Statistical analysis of the females and males as a group up until 8 months of r-metHuLeptin therapy was also significant ($P < 0.03$).

3.4. Ovarian size and menstrual function

Ovarian ultrasound of the 10 females revealed a pattern consistent with PCOS. The mean ovarian volume was greater than 15 cm^3 (right) and 17 cm^3 (left) at baseline, and there was no significant change following r-metHuLeptin therapy (Fig. 3).

Prior to r-metHuLeptin therapy, 8 of the 10 females had grossly irregular menses or primary amenorrhea (NIH 1, 2, 4, 5, 8, 13, 19, and 20). Following r-metHuLeptin therapy, all 8 achieved regular monthly menstrual cycles. One patient (NIH 3) had normal menses prior to r-metHuLeptin therapy and 1 (NIH 6) had a hysterectomy prior to therapy (Table 3).

3.5. Baseline characteristics and effect of r-metHuLeptin therapy in male subjects

We studied gonadotropins and testosterone levels in 4 men, 2 of whom had congenital generalized lipodystrophy and 2 had acquired generalized lipodystrophy. The age range was 13 to 68 years. The mean serum level of leptin was 0.94 ± 0.17 ng/mL (range, 0.5-1.31 ng/mL), the mean BMI was 24.3 kg/m^2 (range, 22.6-26.9 kg/m^2), and the mean percentage fat was 7.4 ± 0.39% (range, 6.7-8.3%; Table 1).

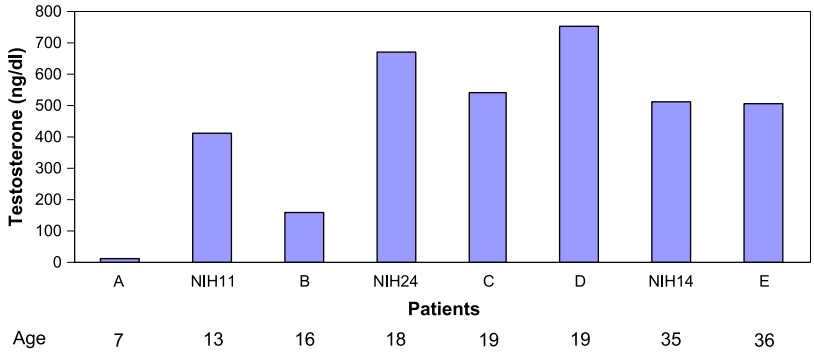

Fig. 5. Serum testosterone concentration of 8 male lipodystrophic patients. Reference range of testosterone in males is 240 to 950 ng/dL. Columns, 5 patients untreated with leptin (A-E) and 3 patients subsequently treated with leptin (NIH 11, 14, and 24). Testosterone values (y axis) are plotted as a function of chronological age. Patient NIH 15 is not included because there was no information about the relationship between his lipodystrophy and pubertal development.

Table 6
Effects of r-metHuLeptin therapy on thyroid and adrenal axis

Data[a]	Baseline	4 mo	8 mo	12 mo	P
TSH (μU/mL)	1.37 ± 0.1	1.24 ± 0.3	1.22 ± 0.3	1.31 ± 0.5	NS
T3 (nmol/L)	124 ± 10	113.6 ± 7	128 ± 8	126.1 ± 11.5	NS
Free T4 (pmol/L)	1.05 ± 0.04	1.03 ± 0.04	1.04 ± 0.05	1.04 ± 0.04	NS
ACTH (pg/mL)	35.4 ± 4.5	34.6 ± 6.3			NS
Cortisol (nmol/L)	18.2 ± 2.4	15.8 ± 1.2			NS

[a] Mean of 14 patients (10 females and 4 males).

The mean baseline testosterone level was 433.2 ± 110 ng/dL (range, 144-669 ng/dL); after 8 months of r-metHuLeptin therapy, it increased to 725 ± 184.6 ng/dL (range, 222-1044 ng/dL; Table 4). In contrast to the female patients, the male group showed a trend to increase testosterone under treatment.

Three of these patients were studied by LHRH stimulation test. The LH response to LHRH was 4-fold increased from baseline, and an almost identical response was seen after 4 months of r-metHuLeptin therapy (Fig. 4A and B).

3.6. Pubertal stage and serum testosterone in male lipodystrophic patients untreated with r-metHuLeptin therapy

Eight patients who were hypoleptinemic from early childhood were studied (3 from Tables 1 and 5 additional patients who did not receive r-metHuLeptin therapy). The baseline characteristics for this group of 8 patients were indistinguishable from the group shown in Table 1. Six of them had congenital generalized lipodystrophy and 2 had acquired generalized lipodystrophy.

All 8 patients underwent appropriate pubertal development (ie, Tanner stage progression), unrelated to r-metHuLeptin therapy. Further, each patient had an appropriately normal serum testosterone level for their chronological age. Note that 1 prepubertal patient at age 7 had an appropriately low testosterone level (Fig. 5).

Patient NIH 11 is especially instructive; he had begun pubertal development at age 10 and his testosterone concentration was 177 ng/mL and progressively increased through puberty into the normal adult range.

3.7. Changes in GH, IGF-1, and glycemic parameters as a function of r-metHuLeptin therapy

In the female patients, the mean baseline IGF-1 concentration was 125 ± 24 ng/dL, and there was an approximately 73% increase over the 12 months of r-metHuLeptin therapy ($P < 0.02$; Fig. 6). The corresponding baseline GH was 0.62 ± 0.22 ng/mL, but there was no further significant change after r-metHuLeptin therapy.

When we analyzed females and males as a group, the mean baseline IGF-1 was 178 ± 30 ng/mL. There was an approximately 53% increase over the 8 months of r-metHuLeptin therapy ($P < 0.03$). Again, GH concentration did not change.

In the female group, the mean baseline fasting blood glucose was 182.7 ± 23.8 mg/dL; 12 months after r-metHuLeptin therapy, it reduces to 112.7 ± 8.4 mg/dL ($P < 0.006$). The mean of the hemoglobin A1c was 8.43 ± 0.29%, reducing to 6.5 ± 0.39% ($P < 0.0003$) at 12 months. In the male group, changes in glycemia and hemoglobin A1c were similar to the female group. Hypoglycemic medication (insulin or insulin sensitizers) were discontinued in 7 of 12 patients and dramatically reduced in 5 patients (Table 5). Two patients were without medication prior to r-metHuLeptin therapy.

The mechanism by which leptin induces increased insulin sensitivity is unclear. Although there is reduced food intake initially, this does not appear to explain the change in insulin sensitivity [2,10].

3.8. Effect of r-metHuLeptin therapy on the thyroid and adrenal axis

We evaluated the effect of r-metHuLeptin therapy on the thyroid and adrenal axis in both males and females. The mean TSH was 1.48 ± 0.41 μU/mL, T3 was 126.6 ± 13.7 nmol/L, and free T4 was 1.03 ± 0.05 pmol/L. There was no significant change over the 12-month period for the females and 8-month period for the males.

Cortisol mean was 17.3 ± 3.13 nmol/L and ACTH mean was 34.2 ± 5.34 pg/mL. There was no significant change over the 12-month period for females and 8-month period for males (Table 6).

4. Discussion

Three human conditions have now been treated with r-metHuLeptin therapy. The first is congenital leptin deficiency, characterized by obesity, where leptin intervention induces satiety and marked weight reduction [4]. The second is acquired and congenital forms of lipodystrophy, characterized by insulin resistance [2], and the third is the Rabson-Mendenhall syndrome with a presumed mutation in the insulin receptor and also characterized by insulin resistance [11]. In the 2 latter conditions, r-metHuLeptin therapy improves insulin sensitivity.

4.1. Mechanism of r-metHuLeptin therapy on insulin sensitivity and on the gonadotropin-ovarian axis

In the present study, we have extended r-metHuLeptin therapy over a period of 12 months in 10 severely

hypoleptinemic female lipodystrophic patients to study pituitary-ovarian function. The most dramatic clinical findings are the decrease in serum androgen levels and the induction of normal menses.

The reduction of free T and the rise in SHBG are most consistent with the concomitant improvement in insulin sensitivity shown here by improved blood glucose, hemoglobin A1c values, and reduction in antidiabetic medications. These changes in glucose and other metabolic parameters are consistent with our previous observations [2,3,11].[2] Further, the reduction in androgen levels, increased insulin sensitivity, and normalization of menses is consistent with the emerging literature relating improved insulin sensitivity to normalization of menstrual function in the common forms of PCOS [12,13]. Thus, syndromic forms of insulin resistance provided the first information about the link of hyperinsulinism to excess ovarian androgen production [14-16]. Although it is known that hyperinsulinism directs ovarian steroid toward excess androgen production by *P*450c17α increased activity in the theca cell [13], the signal transduction system that confers these changes is unclear [17].

R-metHuLeptin administration has 2 major therapeutic effects in patients. The first is to decrease food intake and induce weight loss in congenital leptin deficiency and the second is to improve insulin sensitivity and associated metabolic parameters in hypoleptinemic lipodystrophic patients [2]. These effects of leptin in both animal models and patients, however, appear to be independent of leptin effect on the hypothalamic pituitary axis. Although it is possible that the fall in androgen levels seen in our patients affected gonadotropin regulation, it is more likely that leptin has an independent effect on the gonadotropin-ovarian axis.

Although there was no change in the basal LH, FSH, or LH/FSH ratio from the pretherapy period through the 12 months of r-metHuLeptin therapy in the female patients as a group, there was a 4-fold increase in LH response to LHRH prior to leptin therapy and a more robust increase after leptin administration (changes not statistically significant). This was most dramatic in the 3 youngest adolescent patients; in these patients, there was essentially a flat response to LHRH stimulation prior to r-metHuLeptin therapy and a significantly increased response following therapy.

The major change following leptin administration, however, is normalization of menses without a change in ovarian size. This is most consistent with the effect of leptin to regularize normal pulsatile LH secretion, allowing for the full agonistic effect of the gonadotropin on the ovary [8]. Although hypoleptinemic lipodystrophic patients are usually infertile, this is not invariably true, as rarely these patients conceive [18,19].

[2] A more detailed article of long-term changes in metabolic parameters is in preparation.

The effect of hyperinsulinemia on gonadal function in males and females is different. In contrast to females, hyperinsulinemia increases androgen production in males. It would appear, however, that the leptin effect on LH pulsatility is important in both males and females. Recently, Mantzoros and colleagues have presented data on normal male volunteers and demonstrated that the reduction in testosterone induced by starvation was abrogated by leptin administration [8]. Thus, leptin administration not only prevented the decrease in testosterone concentration but also maintained the pulsatile secretion. Our data suggesting that recombinant leptin administration augments testosterone secretion in males are consistent with these findings.

4.2. Role of r-metHuLeptin therapy on pubertal development

The effects of exogenous leptin administration on sexual maturation in rodents have produced mixed results. Peripheral leptin administration induced puberty in normal female mice [20,21] and prematurely increased serum LH in female rats [22]. Leptin administered intracerebroventricularly also produced an increase in LH mediated by an increased in gonadotropin-releasing hormone. Infusion of leptin into the hypothalamus increases the release of gonadotropin-releasing hormone in fasted but not in fed female rats [23,24].

Nevertheless, other studies show that leptin administered intracerebroventricularly or peripherally to mice or rats reduces food intake and pubertal growth but does not accelerate puberty compared with the control animals [25].

The role of leptin in puberty remains unclear in both animal models and humans. In our experience, leptin does not induce pubertal changes (ie, Tanner stage progression) when administered to prepubertal females, but it plays a critical role in inducing menses and presumably ovulation. This is also consistent with studies in congenital leptin deficiency. In the male subjects, however, it is unclear what role leptin plays in the development of the gonadotropin-testicular axis.

We have studied a few male subjects with lipodystrophy and severe hypoleptinemia and found that r-metHuLeptin administration either maintains testosterone levels or increases them and maintains a constant response to LHRH. It is also of note that these hypoleptinemic males undergo normal pubertal development and testosterone production even prior to leptin therapy. Up until recently, most of the information about leptin action in humans came from correlative data. Thus, the hypothesis that a leptin surge prior to puberty induces testosterone production in males was based on this type of information [26]. In our male patients, however, puberty and testosterone production ensues in spite of consistent very low leptin levels (as evidenced by progressive Tanner stage development). Further, it seems clear that r-metHuLeptin administration does not induce LH secretion prior to appropriate pubertal timing [4,11].

4.3. Role of r-metHuLeptin therapy on IGF-1

We find that mean IGF-1 concentration is increased in the 10 female patients over the course of 1 year of r-metHuLeptin therapy. When the female and male patients are considered as a group, the IGF-1 values are increased after 8 months of r-metHuLeptin therapy. We attribute this change to improved insulin action or increased insulin sensitivity [27]. Under the conditions of this study, however, we were unable to demonstrate a significant change in GH concentration.

5. Conclusions

Our conclusions are conditioned by the fact that our study is small, not randomized, and in a rare disease. The experimental model, however, offers the opportunity to study a long-standing hypoleptinemic group of patients and to use r-metHuLeptin to correct their leptin deficiency. Under these circumstances, there is an increase in insulin sensitivity and improvement in metabolic status. Because increased insulin sensitivity could explain the decreased androgen levels in female patients, we cannot be certain that there are additional effects on the pituitary-ovarian axis. Taken together with other studies in humans who are not insulin resistant, we conclude that leptin has important effects on gonadotropin pulse generation and that this accounts for changes in menstrual function and probably ovulation. It is also of note that agents that increase insulin sensitivity improved ovulatory function in women with common forms of PCOS, but these agents combined with centrally acting agents such as clomiphene produce a much greater effect [28]. In males, the evidence suggests that leptin also has important effects in gonadotropin pulse generation, but in our patients there is no clear functional consequence with respect to pituitary testicular function.

Although leptin also affects pulsatility of other pituitary hormones such as TSH, this appears less important as thyroid hormones, corticosteroids, and GH are normal and unchanged by recombinant leptin therapy. It is likely that the increase in IGF-1 is related to improvement in insulin sensitivity.

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