ORIGINAL ARTICLE

Recombinant Human Leptin in Women with Hypothalamic Amenorrhea

Corrine K. Welt, M.D., Jean L. Chan, M.D., John Bullen, B.A., Robyn Murphy, M.S., Patricia Smith, B.S., Alex M. DePaoli, M.D., Aspasia Karalis, B.A., and Christos S. Mantzoros, M.D., D.Sc.

ABSTRACT

BACKGROUND

Disruptions in hypothalamic–gonadal and other endocrine axes due to energy deficits are associated with low levels of the adipocyte-secreted hormone leptin and may result in hypothalamic amenorrhea. We hypothesized that exogenous recombinant leptin replacement would improve reproductive and neuroendocrine function in women with hypothalamic amenorrhea.

METHODS

Eight women with hypothalamic amenorrhea due to strenuous exercise or low weight were studied for one month before receiving recombinant human leptin and then while receiving treatment for up to three months. Six control subjects with hypothalamic amenorrhea received no treatment and were studied for a mean (±SD) of 8.5±8.1 months.

RESULTS

Luteinizing hormone (LH) pulsatility, body weight, ovarian variables, and hormone levels did not change significantly over time in the controls and during a one-month control period before recombinant leptin therapy in the treated subjects. In contrast, recombinant leptin treatment increased mean LH levels and LH pulse frequency after two weeks and increased maximal follicular diameter, the number of dominant follicles, ovarian volume, and estradiol levels over a period of three months. Three patients had an ovulatory menstrual cycle ($P<0.05$ for the comparison with an expected rate of spontaneous ovulation of 10 percent); two others had preovulatory follicular development and withdrawal bleeding during treatment ($P<0.05$). Recombinant leptin significantly increased levels of free triiodothyronine, free thyroxine, insulin-like growth factor 1, insulin-like growth factor–binding protein 3, bone alkaline phosphatase, and osteocalcin but not cortisol, corticotropin, or urinary N-telopeptide.

CONCLUSIONS

Leptin administration for the relative leptin deficiency in women with hypothalamic amenorrhea appears to improve reproductive, thyroid, and growth hormone axes and markers of bone formation, suggesting that leptin, a peripheral signal reflecting the adequacy of energy stores, is required for normal reproductive and neuroendocrine function.

From the Reproductive Endocrine Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School (C.K.W., P.S.); and the Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School (J.L.C., J.B., A.K., C.S.M.) — all in Boston; and Amgen, Thousand Oaks, Calif. (R.M., A.M.D.). Address reprint requests to Dr. Mantzoros at the Division of Endocrinology, Diabetes, and Metabolism, Beth Israel Deaconess Medical Center, 330 Brookline Ave., Stoneman 816, Boston, MA 02215, or at cmantzor@bidmc.harvard.edu.

Drs. Welt and Chan contributed equally to this article.

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Hypothalamic amenorrhea, either organic or functional,[1] is characterized by the absence of menstrual cycles, low estrogen levels, and low or normal levels of gonadotropins. It accounts for over 30 percent of cases of amenorrhea in women of reproductive age[2] and may lead to infertility and bone loss.[3] Functional hypothalamic amenorrhea occurs when a relative energy deficit (owing to weight loss, excessive exercise, or eating disorders) disrupts the secretion of hypothalamic gonadotropin-releasing hormone (GnRH) and other neuroendocrine axes.[4-7] However, the precise signal or signals indicating the availability of energy remain unknown.

Leptin, a hormone secreted by adipocytes that regulates energy homeostasis[8] and circulates at levels corresponding to fat mass and acute nutritional changes, is a prime candidate. As compared with controls matched for weight and body composition, women with hypothalamic amenorrhea have low leptin levels[9-11] and a striking absence of normal diurnal leptin variation.[12] Support for the concept that leptin is the critical link between sufficiency of energy stores and the integrity of the hypothalamic–pituitary–gonadal axis comes from reversal of infertility and delayed puberty in leptin-deficient rodents and humans receiving leptin treatment[13-15] and from our observation that leptin replacement normalizes starvation-induced decreases in reproductive hormones in lean men.[16]

To test the hypothesis that low leptin levels (i.e., relative leptin deficiency) cause reproductive and neuroendocrine dysfunction, we administered recombinant methionyl human leptin (r-metHuLeptin, Amgen) to women with hypothalamic amenorrhea in a prospective, open-label study to determine whether recombinant leptin would restore ovulation, correct hormonal abnormalities, and improve bone markers.

METHODS

SUBJECTS

Eligible subjects had had secondary hypothalamic amenorrhea for six months or more coincident with a period of increased exercise or low weight. All had stable weight (within 15 percent of ideal body weight for six months or more) and were otherwise healthy, without active eating disorders, and had not been taking medications, including estrogen, for at least three months. None of the women had hirsutism, acne, or ratios of luteinizing hormone (LH) to follicle-stimulating hormone (FSH) of more than 1.5, and all had normal thyrotropin and prolactin levels.

Fourteen women were enrolled. Eight received r-metHuLeptin, and six served as controls.

STUDY DESIGN

Subjects in the Active Treatment Group

Eight subjects were studied from 2002 to 2003 and provided written informed consent to participate in a prospective study of r-metHuLeptin. The protocol was approved by the institutional review boards of Beth Israel Deaconess Medical Center and Massachusetts General Hospital and was performed under an investigator-initiated investigational-new-drug application. The study was designed by the academic investigators; the data were held by the academic investigators and analyzed by the investigators and Amgen.

During a one-month observation period, subjects underwent weekly blood sampling for the determination of levels of leptin, LH, FSH, estradiol, progesterone, inhibin A, inhibin B, thyrotropin, free thyroxine (T_4), free triiodothyronine (T_3), corticotropin, cortisol, insulin-like growth factor 1 (IGF-1), IGF-binding protein 3, bone alkaline phosphatase, and osteocalcin. A urine sample was collected approximately two hours after the first morning voiding for determination of levels of cross-linked N-telopeptides of type I collagen. In all subjects, body composition (fat mass) and bone density were determined at the beginning and end of the initial one-month observation period with the use of bioelectrical impedance (RJL Systems) and dual-energy x-ray absorptiometry (Hologic QDR-4500). Subjects were subsequently admitted to the General Clinical Research Center of Beth Israel Deaconess Medical Center for 12 hours (7 p.m. to 7 a.m.) during which blood was sampled every 10 minutes for the measurement of leptin, LH (as a measure of GnRH secretion), thyrotropin, and corticotropin. The following morning, the resting metabolic rate was determined (DeltraTrac II Metabolic Monitor, SensorMedics) and transvaginal or transabdominal pelvic ultrasonography (with an ATL HDI 1500, 5-MH convex array transducer) was performed. After these studies, the subjects self-administered r-metHuLeptin (0.08 mg per kilogram of body weight per day) subcutaneously for two to three months, with 40 percent of the daily dose given at 8 a.m. and 60 percent at 8 p.m. to mimic the normal diurnal variation in leptin levels.[17] After two weeks,

Table 1. Characteristics of Subjects with Hypothalamic Amenorrhea.

Characteristic	Baseline in Subjects Receiving r-metHuLeptin (N=8)		Baseline in Controls (N=6)			Follow-up in Controls (N=6)	
	mean ±SD	*range*	*mean ±SD*	*range*	*P value**	*mean ±SD*	*P value†*
Age (yr)	24.8±5.4	19–33	33.4±3.5	27–38	0.01	34.1±3.7	0.59
Weight (kg)	54.6±4.7	48.2–62.3	55.7±5.1	50.6–63.0	0.61	56.1±6.4	0.75
Height (cm)	163.5±5.7	157.6–172.7	165.4±5.8	154.9–171.6	0.52	165.4±5.8	1.00
Body-mass index‡	20.5±2.0	18.8–24.4	20.3±1.5	18.5–22.1	0.95	20.5±2.0	0.94
Duration of hypothalamic amenorrhea (yr)	5.1±4.0	0.8–14.0	5.6±4.4	2.5–14.0	0.95	6.3±4.3	0.31
Leptin (ng/ml)	3.9±1.9	1.4–6.9	3.8±2.0	1.4–7.2	0.70	6.4±4.2	0.05
LH (IU/liter)§	1.8±1.2	0.2–4.4	3.5±4.1	0.5–10.6	1.00	2.8±2.0	0.60
FSH (IU/liter)§	6.2±2.2	2.9–9.6	6.9±1.6	5.0–9.2	0.52	8.0±2.3	0.25
Estradiol (pg/ml)§	38.5±21.1	<20–79	33.2±16.0	<20–61	0.69	29.7±16.5	0.71

* P values are for the comparison with the r-metHuLeptin–treated subjects at baseline.
† P values are for the comparison with baseline values in controls.
‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.
§ The normal ranges during the early follicular phase (days 1 through 6) are as follows: 1.0 to 8.2 IU per liter for LH, 2.8 to 8.9 IU per liter for FSH, and 20 to 142 pg per milliliter for estradiol.

subjects underwent another frequent-sampling study to assess the effect of leptin on hypothalamic–pituitary axes. Subjects were instructed not to change their diet and exercise level; adherence was assessed by means of daily exercise records and four-day food diaries filled out before each admission.

During treatment, subjects were seen weekly to obtain samples for the measurement of hormone and bone-marker levels and undergo pelvic ultrasonography; body composition was determined by means of bioelectrical impedance weekly and by means of dual-energy x-ray absorptiometry every other week. Bone density was determined by means of dual-energy x-ray absorptiometry every other week. If a dominant follicle (11 mm or greater)[18] was documented, subjects underwent ultrasonography and hormone measurements every other day and urinary levels of LH were measured daily. Ovulation was confirmed on the basis of one or more of the following: the growth of the dominant follicle (by 2 mm per day) from its preovulatory size (a follicle 18 mm in length or greater),[18] with subsequent collapse or appearance of internal echoes on ultrasonography; serum or urinary LH surge; and an increase in the progesterone level by more than 4 ng per milliliter. If subjects ovulated, the study was concluded at two months; otherwise, the dose of r-metHuLeptin was increased to 0.2 mg per kilogram per day (given in divided doses as described above) and given for a third month. Hormone levels, body composition, and bone density were reassessed at a follow-up visit one month after study completion.

Controls

Control subjects provided written informed consent to participate in two 12-hour blood-sampling studies (with samples obtained every 10 minutes) and ultrasound evaluations[19] in the General Clinical Research Center of Massachusetts General Hospital between 1982 and 1999. The two studies were separated by 1 to 24 months (mean [±SD], 8.5±8.1).

BIOCHEMICAL ANALYSIS

The following hormone levels were measured with the use of immunoassays: leptin (Linco Research); FSH and estradiol (Abbott Laboratories); progesterone, free T_4, free T_3, cortisol, IGF-1, and IGF-BP3 (Immulite, Diagnostic Products); thyrotropin and LH (Diagnostics Systems Laboratory); corticotropin (Nichols Institute Diagnostics); and inhibin A and inhibin B (Serotec). All samples from each subject (except inhibin B) were analyzed in duplicate in the same assay. The interassay and intraassay coefficients of variation were similar to those reported in previous studies[20,21] or by the manufacturer. The limit of detection for estradiol was 10 pg per

Table 2. Body Composition, Hormone Levels, and Bone-Marker Levels during r-metHuLeptin Treatment.*

Variable	Baseline (N=8)	Month 1 (N=8)	Month 2 (N=7)	Month 3 (N=5)	One-Month Follow-up (N=7)	Overall P Value†
Body composition						
Body weight (kg)	54.7±4.5	54.1±4.3‖§	54.0±3.6‡	52.2±3.5‡	53.6±3.8	<0.001
Fat mass						
kg	12.5±2.8	11.8±2.2‖§	11.1±2.0‡§	9.6±1.7‡	10.2±1.9	<0.001
%	22.4±3.7	21.4±3.2§	20.4±2.9‡¶	18.1±2.9‡	18.6±2.9	<0.001
Hormones						
Leptin (ng/ml)	3.4±1.5	9.7±4.1‡	20.6±15.7‡§	37.4±30.1‡§	9.4±10.1	<0.001
LH (IU/liter)	3.1±3.6	5.1±4.5‡¶	5.7±2.7‡§	6.7±4.2‡§	2.2±2.0	<0.001
FSH (IU/liter)	6.2±1.3	7.0±1.2	6.9±1.6	6.6±1.8	5.7±1.5	0.16
Estradiol (pg/ml)	26.9±7.7	44.1±25.7‖	54.4±20.9‡¶	71.2±22.8‡§	28.4±9.6	<0.001
Inhibin A (IU/ml)	0.89±0.4	1.15±0.5	1.66±1.2	1.85±1.8	0.88±0.5	0.37
Inhibin B (pg/ml)	99.7±46.8	136.3±54.3	126.1±35.1	145.0±55.5	110.7±53.2	0.54
Free T_3 (pg/ml)	1.90±0.2	1.99±0.3	2.23±0.4‡	2.59±0.4‡	2.23±0.4	<0.001
Free T_4 (ng/dl)	1.08±0.1	1.08±0.1	1.17±0.2‡	1.28±0.1‡	1.12±0.2	<0.001
Thyrotropin (μIU/ml)	2.48±1.3	2.90±1.1	3.06±1.3	4.52±2.2‡	2.63±1.8	0.056
Cortisol (μg/dl)	17.2±3.3	19.3±4.2¶	20.1±4.7¶	19.9±4.4	15.3±4.6	0.07
Corticotropin (pg/ml)	18.1±7.2	20.3±7.1	19.2±7.9	20.2±6.0	14.6±7.6	0.40
IGF-1 (ng/ml)	191.3±31.1	219.7±49.6‖	253.8±57.4‡¶	281.3±59.5‡§	212.4±44.8	<0.001
IGF-binding protein 3 (μg/ml)	4.46±0.6	4.44±0.8	4.80±0.9‖	5.20±0.3‡	4.51±1.0	<0.001
Bone markers						
Bone alkaline phosphatase (U/liter)	22.9±10.23	20.2±7.8§‖	23.2±8.6‡¶	24.8±12.3‡	26.8±11.1	<0.001
Osteocalcin (ng/ml)	17.5±13.7	21.2±15.3‖	22.0±13.0‡	22.3±18.2‡§	22.0±17.0	<0.001
Urinary N-telopeptides:creatinine	49.1±33.6	59.3±25.0	54.0±26.8	58.7±25.1	64.6±58.2	0.80

* During months 1 and 2 the dose of r-metHuLeptin was 0.08 mg per kilogram per day, and during month 3 the dose was 0.2 mg per kilogram per day. Values are the average over the specified period.
† Overall P values were derived by means of repeated-measures analysis of variance.
‡ P<0.001 for the comparison with baseline values.
§ P<0.001 for the comparison with follow-up values.
¶ P<0.01 for the comparison with follow-up values.
‖ P<0.01 for the comparison with baseline values.

milliliter. Hormone levels in the controls were measured in stored serum samples contemporaneously with hormone samples from treated subjects, except for LH pulsatility studies, which were conducted as noted above.

PULSATILITY ANALYSIS

A modified version of the Santen and Bardin algorithm was used to determine the characteristics of LH pulsatility,[22] and Cluster software (version 6.0) was used to determine the pulsatility of thyrotropin and corticotropin.[23] The assay's sensitivity as stated by the manufacturer and a coefficient of variation of 10.0 percent for thyrotropin and 8.0 percent for corticotropin (maximal interassay coefficient of variation, 9.2 percent and 7.8 percent, respectively) were used for analysis. Patterns of secretion of LH were classified as apulsatile in the absence of pulsations, low amplitude (less than 4 IU per liter), low frequency (fewer than 9 pulses per 24 hours), low amplitude and low frequency, or normal.[19]

STATISTICAL ANALYSIS

For the primary outcome (ovulation), an exact binomial distribution was used to test whether the rates of ovulation and dominant-follicle development exceeded 10 percent with r-metHuLeptin therapy, a generous rate in subjects with hypothalamic amenorrhea, who are not expected to have follicular growth or ovulation during such a short period without treatment.[24] Nonparametric Mann–Whitney or Wilcoxon signed-rank tests were used

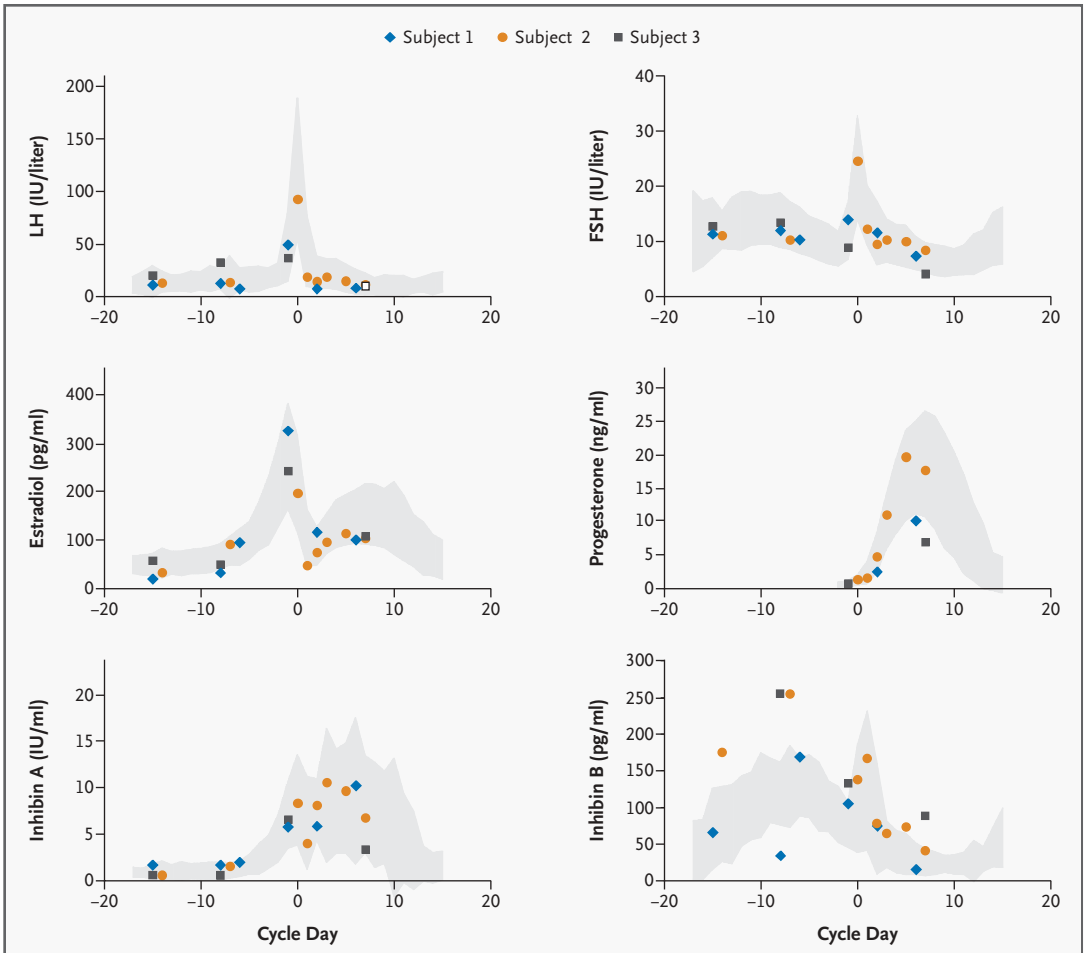


Figure 1. Levels of LH, FSH, Estradiol, Progesterone, Inhibin A, and Inhibin B in Three Subjects Who Ovulated during r-metHuLeptin Treatment.

The shaded areas represent 1 SD of the mean hormone levels in 44 women with normal cycles.[22] LH was measured with the use of a two-site, monoclonal, nonisotopic system (Abbott Laboratories), and LH and FSH values were expressed as equivalents of the Second International Reference Preparation 71/223 of human menopausal gonadotropin standards in order to compare them with data previously obtained with the use of these standards.

for the primary analysis and parametric t-tests were used for the secondary analysis to compare baseline characteristics of the two groups, ovarian, hormonal, and endometrial variables at the beginning of the one-month baseline period as compared with the end of the baseline period and at the end of the baseline period as compared with the maximal level during r-metHuLeptin treatment, and the pulsatility of LH, thyrotropin, and corticotropin and the metabolic rate at the end of the baseline period as compared with after two weeks of treatment. Similar results were obtained with the use of nonparametric and parametric testing except where noted. Changes in weekly hormone levels, body composition, and bone markers were evaluated with the use of a repeated-measures model, with an overall P value reported for change across the entire study. Post hoc tests were used to compare average values for each treatment month with baseline and follow-up values. Comparisons were declared statistically significant at an α level of less than 0.05. Values of leptin, LH, FSH, and estradiol were normalized logarithmically for analysis. Missing values were not imputed, since some subjects completed the study at month 2 according to the design. The primary analysis was conducted according to

Table 3. Reproductive Data during the One-Month Observation Period and during r-metHuLeptin Treatment.*

Variable	Beginning of Baseline	End of Baseline	Maximum during r-metHuLeptin Treatment
Ovulation (no. of subjects)	0	0	3†
Preovulatory follicle ≥18 mm (no. of subjects)	0	0	5†
Dominant follicle ≥11 mm (no. of subjects)	2	2	6†
Maximal follicular diameter (mm)	9.8±3.3	9.7±2.8	18.0±5.6‡
No. of dominant follicles	0.3±0.5	0.1±0.4	2.4±1.7‡
Ovarian volume (ml)	14.7±6.7	15.5±7.5	22.1±6.7‡
Endometrial thickness (mm)	4.9±2.0	4.3±1.8	7.7±2.0‡

* Plus–minus values are means ±SD.
† P<0.05 by the exact binomial test for the comparison with an expected rate of spontaneous ovulation of 10 percent.
‡ P<0.05 for the comparison with the end of baseline values.

the intention to treat and thus included all eight subjects who received r-metHuLeptin. A sensitivity analysis that excluded one subject who completed only one month of the study yielded similar results.

RESULTS

The baseline characteristics of controls and treated subjects are presented in Table 1. Controls were older but were otherwise similar to the treated subjects with respect to weight, the duration of amenorrhea, and hormone levels.

BODY COMPOSITION, LEPTIN LEVELS, AND METABOLIC RATE

In control subjects, leptin levels increased slightly between the initial and follow-up studies, but weight did not change significantly (Table 1). In treated subjects, leptin levels remained stable during the one-month observation period (3.9±1.9 ng per milliliter at the beginning of the baseline period and 3.0±2.0 ng per milliliter at the end of the baseline period, P=0.21) but increased appropriately with r-metHuLeptin treatment (Table 2). Body weight decreased slightly among treated subjects, primarily during month 3 (with its higher r-metHuLeptin dose), owing to a small but significant decrease in body fat and the absence of a change in lean mass on dual-energy x-ray absorptiometry. Similar results were obtained with the use of bioelectrical impedance (data not shown). Neither the change in the resting metabolic rate (from 1194.9± 148.5 to 1195.8±161.6 kcal per day, P=0.89) nor the change in food intake (from 1952.6± 614.5 to 1777.4±299.7 kcal per day, P=0.16) was significant after two weeks of r-metHuLeptin therapy.

REPRODUCTIVE DATA

Controls had no dominant follicles, spontaneous menstrual cycles, or withdrawal bleeding at any time during the study. During the observation period before the initiation of r-metHuLeptin therapy, treated subjects had no ovulation, preovulatory follicular development, or changes in ovarian variables; however, two subjects had a dominant follicle (Table 3). Two treated subjects completed the study at two months, after meeting the primary end point of ovulation, and five subjects continued to month 3. One subject withdrew after one month for reasons unrelated to the study.

Three of eight subjects had an ovulatory menstrual cycle during r-metHuLeptin therapy (P<0.05 for the comparison with an expected rate of spontaneous ovulation of 10 percent). Ovulation occurred 28, 35, and 58 days after the start of treatment in subjects who had had hypothalamic amenorrhea for 14 years, 6 years, and 9 months, respectively. Levels of LH, FSH, estradiol, and inhibin A (a protein secreted by granulosa cells of the dominant follicle and corpus luteum)[21] during the ovulatory cycle were within 1 SD of the mean for women with normal cycles, and progesterone levels were within 2 SD of the mean (Fig. 1).

Another two subjects who were treated with r-metHuLeptin had a preovulatory follicle (19.0 mm in one and 23.6 mm in the other) but did not ovulate. In these two women, estradiol levels peaked at 78 and 113 pg per milliliter but subsequently fell to 52 and 49 pg per milliliter, respectively, without a rise in the progesterone level. Both subjects had follicular regression and withdrawal bleeding. The sixth subject had her first dominant follicle in month 3, followed by growing and regressing follicles, and neither ovulated nor had withdrawal bleeding. The seventh subject did not have a dominant follicle and had the lowest leptin levels of any subject during treatment (maximum leptin level, 12.4 ng per milliliter). Overall, r-metHuLeptin significantly increased the maximal follicular diameter, number of dominant folli-

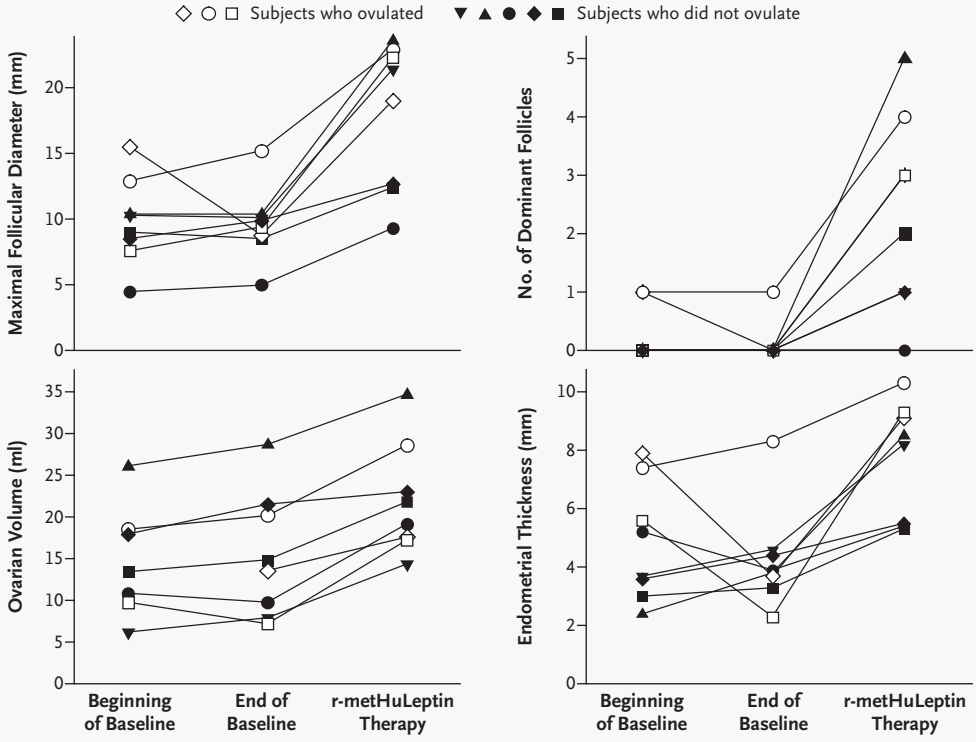


Figure 2. Follicular, Ovarian, and Endometrial Ultrasonographic Measurements at the Beginning and End of the One-Month Baseline Period and at Their Maximum during r-metHuLeptin Treatment.

Each symbol represents one subject.

cles, ovarian volume (during the follicular phase), and endometrial thickness (Table 3 and Fig. 2).

HORMONAL OUTCOMES

Reproductive Hormones

Control subjects had no significant changes between studies in mean hormone levels (Table 1) or LH pulsatility (mean levels changed from 2.8±2.1 to 2.2±1.2 IU per liter, P=0.35; pulse frequency changed from 5.6±2.6 to 3.1±3.3 pulses per 12 hours, P=0.14; and amplitude changed from 1.7±1.1 to 3.6±4.5 IU per liter, P=0.12). In agreement, LH-pulse patterns changed from low frequency or low frequency and low amplitude to normal in one subject, remained apulsatile in one subject, remained low frequency in two subjects, changed from normal to low frequency in one subject, and remained normal in one subject.

In contrast, two weeks of r-metHuLeptin increased mean LH levels (P=0.017) and pulse frequency (P=0.058 by nonparametric analysis and P=0.049 by parametric analysis) but not amplitude (P=0.58), with improvement or normalization of LH-pulse patterns in six of eight subjects (Fig. 3). In two subjects, one with a low-frequency pattern and one with a normal pattern, the patterns did not change after they received r-metHuLeptin; one of these subjects subsequently had a 12.4-mm follicle, and the other ovulated. LH and estradiol levels increased significantly in weekly measurements during r-metHuLeptin therapy and decreased to baseline after a one-month washout period (Table 2). The maximal level of inhibin B (a granulosa-cell protein secreted by the cohort of developing follicles)[21] in all subjects was at least 2 SD above the mean for women with regular cycles.

Thyroid Hormones during r-metHuLeptin Therapy

Baseline free T_3 and free T_4 levels were in the lower range of normal, increased within the normal range during month 2, and then declined toward baseline levels during follow-up (Table 2). From base-

line to week 2, mean thyrotropin levels (which changed from 2.7±1.9 to 2.6±1.5 μIU per milliliter, P=0.78), pulse frequency (4.6±1.8 to 3.3±1.5 pulses per 12 hours, P=0.14), and pulse amplitude (3.4±2.2 to 3.3±2.2 μIU per milliliter, P=0.89) did not change significantly, but the levels had a borderline increase over the three-month treatment period.

Cortisol and IGF Hormones during r-metHuLeptin Therapy

There was no significant change from baseline in cortisol or corticotropin levels during treatment (Table 2). After two weeks of r-metHuLeptin therapy, there was no significant change in corticotropin pulsatility (mean levels, from 12.6±3.4 to 13.0±3.21 pg per milliliter, P=0.67; frequency, from 5.6±1.8 to 6.4±0.9 pulses per 12 hours, P=0.31; and amplitude, from 16.4±6.5 to 17.4±5.2, P=0.67). IGF-1 increased significantly starting in month 1 and declined to baseline levels at follow-up, and IGF-BP3 increased during months 2 and 3 (Table 2).

BONE-MARKER OUTCOMES DURING r-metHuLeptin THERAPY

Markers of bone formation (levels of bone alkaline phosphatase and osteocalcin) increased significantly during r-metHuLeptin treatment, but urinary N-telopeptides, a marker of bone resorption, did not change significantly (Table 2). Total bone density did not change significantly during this short study (baseline, 1.08±0.11 g per square centimeter; month 1, 1.09±0.12 g per square centimeter; month 2, 1.11±0.12 g per square centimeter; and month 3, 1.17±0.08 g per square centimeter; P=0.11 for the overall comparison).

SAFETY OF r-metHuLeptin THERAPY

There appeared to be no adverse effects (including injection-site reactions) during therapy with r-metHuLeptin. Subjects reported a qualitative decrease in appetite, primarily during the third month, but otherwise felt well.

DISCUSSION

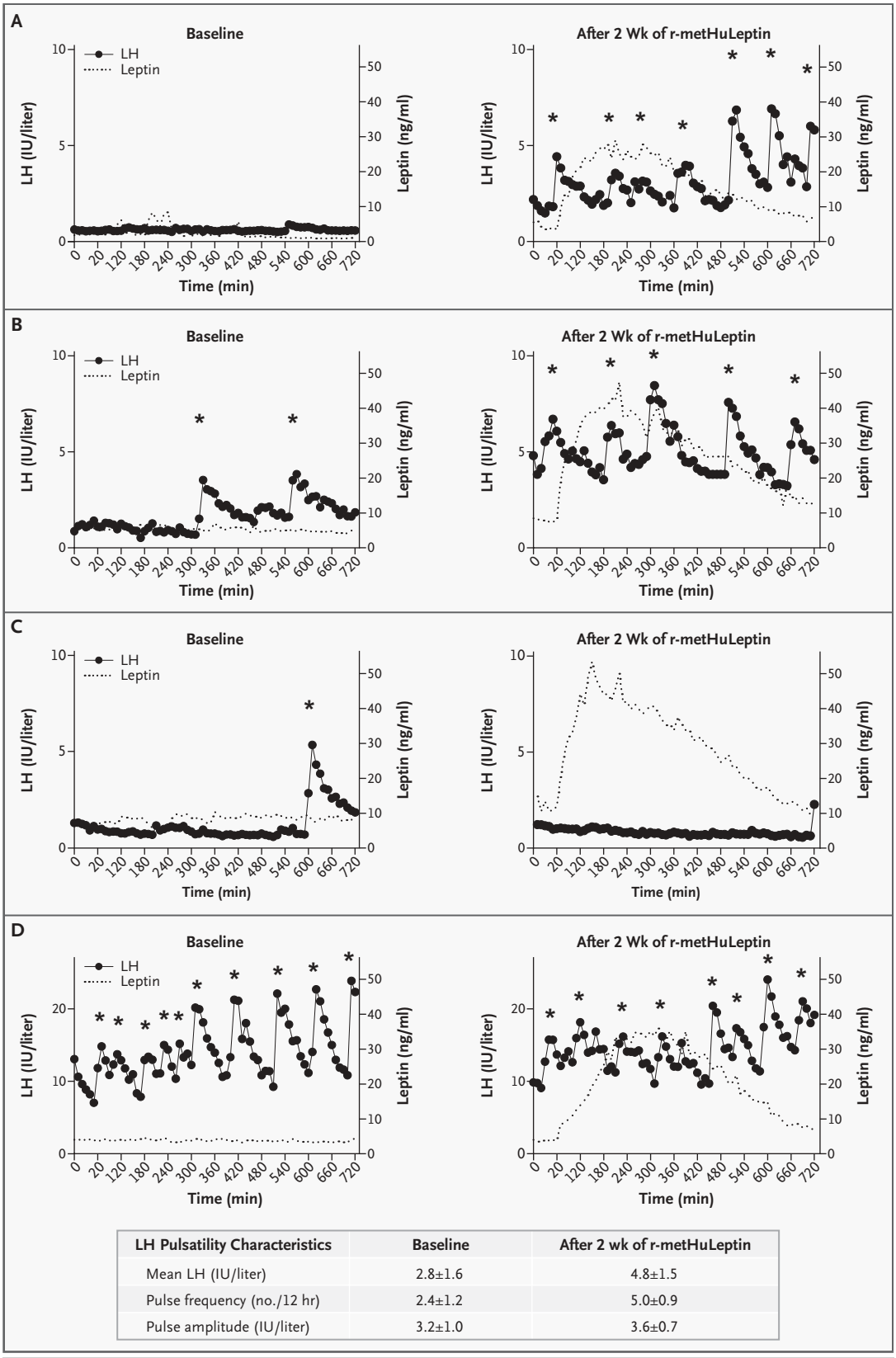
In women with hypothalamic amenorrhea, the administration of r-metHuLeptin in an effort to normalize the relative leptin deficiency results in follicular growth and ovulation and significantly increases levels of LH, estradiol, IGF-1, thyroid hormone, and bone-formation markers, indicating that low leptin levels may be responsible for reproductive and neuroendocrine abnormalities associated with this disorder. Leptin was originally identified as an antiobesity hormone[8,25] but is increasingly recognized as a hormonal mediator of the adaptation to energy deprivation. Studies in leptin-deficient mice[13] and humans[26-28] as well as short-term starvation experiments in rodents[14] and lean men[16] suggest that low leptin levels are probably responsible for alterations in reproductive, thyroid, and growth hormone axes.

We found that treatment with r-metHuLeptin improved reproductive function after only a few months, despite the fact that seven women had had amenorrhea for several years. The time to recovery was much shorter than that expected with the use of lifestyle modifications, in which the time to recovery lengthens in concert with the duration of amenorrhea.[24,29] Moreover, the improved reproductive function was not due to altered exercise patterns or to weight gain in these athletic subjects (who tend to have up to 40 percent less fat mass than controls of similar body weight).[30] Although leptin treatment has normalized bleeding patterns in women with lipodystrophy associated with leptin deficiency, improved insulin resistance may have accounted for improved menstrual cyclicity.[31]

In this prospective, interventional study of a group of women with hypothalamic amenorrhea, leptin administration led to normalization of levels of reproductive hormones, follicular development, and menstrual cyclicity. All women had follicular growth during r-metHuLeptin therapy and normalization of inhibin B levels, which reflects the number of growing ovarian follicles.[21] Follicular development was maximal during the third month of treatment in three subjects, suggesting that

Figure 3 (facing page). Representative Patterns of LH Pulsatile Characteristics at Baseline and after Two Weeks of r-metHuLeptin Therapy in Eight Subjects.

The dose of r-metHuLeptin was 0.08 mg per kilogram per day. Panel A shows an apulsatile pattern (two subjects) at baseline, which improved to a normal or low-frequency pattern during treatment. Panel B shows a low-frequency pattern (four subjects) before treatment, which improved to normal during treatment. Panel C shows a low-frequency pattern before and during treatment (one subject). Panel D shows a normal pattern before and during treatment (one subject). Pulses are indicated by asterisks. Leptin levels are depicted by the dotted lines. Data are expressed as means ±SD.



LH Pulsatility Characteristics	Baseline	After 2 wk of r-metHuLeptin
Mean LH (IU/liter)	2.8±1.6	4.8±1.5
Pulse frequency (no./12 hr)	2.4±1.2	5.0±0.9
Pulse amplitude (IU/liter)	3.2±1.0	3.6±0.7

r-metHuLeptin may sometimes have a delayed effect and that the rate of ovulatory response in this relatively short study may thus have been underestimated. The subject without dominant follicles had the lowest leptin levels, suggesting that a threshold level is required for normal follicular growth, but the small size of our study did not permit a detailed dose-finding analysis.

In contrast to the observation that dominant follicles are destined to ovulate in mature ovaries,[18] only 22 percent of dominant follicles in our subjects grew to a preovulatory size. This large number of growing follicles and pattern of follicular growth and regression are reminiscent of those seen in puberty, when ovaries contain multiple follicles (5 to 12 mm) that eventually regress, resulting in anovulatory cycles.[32] This perhaps explains the failure of some subjects to ovulate during the short treatment period. Furthermore, most subjects had GnRH-pulse patterns (low frequency, low amplitude, or both) typical of those seen during puberty, suggesting regression to a prepubertal or peripubertal state, and after only two weeks of therapy r-metHuLeptin increased GnRH-pulse frequency before the restoration of menstrual cycles. Thus, r-metHuLeptin treatment appears to recapitulate a pubertal pattern, and in agreement with other studies,[15,26,28,33] this finding suggests that leptin may have a role in the initiation of puberty. Whether once-daily treatment with r-metHuLeptin[15] or treatment in a pulsatile fashion imitating endogenous secretion would have similar or more beneficial effects in normalizing GnRH pulsatility, menstrual cycles, or both remains to be determined.

Chronic energy deprivation in women with hypothalamic amenorrhea is associated with other, more subtle but clinically relevant neuroendocrine abnormalities, including decreased thyroid-hormone levels, hyperactivity of the adrenal axis, and increased secretion of growth hormone and decreased secretion of IGF-1.[1,10,34,35] Treatment with r-metHuLeptin increased the levels of free T_3 and free T_4 (within normal ranges), consistent with findings of previous leptin-induced increases in thyroid hormone in fasting lean men,[16] children with leptin deficiency,[28] and lean and obese subjects during weight loss.[36] We found no significant effect of r-metHuLeptin on corticotropin or cortisol levels, similar to previous findings in humans.[16,28,31] It is important to note that levels of IGF-1 and IGF-BP3 increased during r-metHuLeptin therapy, suggesting that leptin may directly increase IGF-1 levels in the absence of changes in nutritional status, despite the relative resistance to growth hormone that is typically present in women with hypothalamic amenorrhea.[35]

The estrogen and IGF-1 deficiency and, possibly, the hypercortisolemia associated with hypothalamic amenorrhea contribute to bone loss, increasing the risk of stress fractures and osteoporosis.[3] In humans, the relationship between leptin and bone density has not been established,[37] and studies of leptin treatment in children with leptin deficiency[28] and women with lipoatrophy[38] have reported conflicting results. In this study, although it was too short to assess bone density, r-metHuLeptin increased markers of bone formation but not resorption. Whether this improvement in bone-formation markers is related to increasing levels of estradiol or IGF-1[39] or is a direct effect of leptin[37] and can translate into improved bone density remains to be determined.

Our findings help elucidate the pathophysiology of hypothalamic amenorrhea and may have therapeutic implications. In addition to diet and weight changes, estrogen is the current standard treatment but may have side effects and does not address the underlying infertility or associated neuroendocrine abnormalities. Further studies are warranted to determine the safety and efficacy of r-metHuLeptin, including the optimal dose and duration of treatment required to restore reproductive function without inducing an undesirable degree of weight loss in already lean subjects.

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