Leptin treatment reduces body fat but does not affect lean body mass or the myostatin-follistatin-activin axis in lean hypoleptinemic women

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Brinkoetter M, Magkos F, Vamvini M, Mantzoros CS. Leptin treatment reduces body fat but does not affect lean body mass or the myostatin-follistatin-activin axis in lean hypoleptinemic women. Am J Physiol Endocrinol Metab 301: E99–E104, 2011. First published April 19, 2011; doi:10.1152/ajpendo.00146.2011.—Animal studies in vivo indicate that leptin treatment in extremely leptin-sensitive ob/ob mice reduces body weight exclusively by reducing fat mass and that it increases muscle mass by downregulating myostatin expression. Data from human trials are limited. Therefore, we aimed at characterizing the effects of leptin administration on fat mass, lean body mass, and circulating regulators of muscle growth in hypoleptinemic and presumably leptin-sensitive human subjects. In an open-label, single-arm trial, seven lean, strenuously exercising, amenorrheic women with low leptin concentrations (≤5 ng/ml) were given recombinant methionyl human leptin (metreleptin; 0.08 mg·kg−1·day−1) for 10 wk. In a separate randomized, double-blind, placebo-controlled trial, seven women were given metreleptin (initial dose: 0.08 mg·kg−1·day−1 for 3 mo, increased thereafter to 0.12 mg·kg−1·day−1 if menstruation did not occur), and six were given placebo for 9 mo. Metreleptin significantly reduced total body fat by an average of 18.6% after 10 wk (P < 0.001) in the single-arm trial and by 19.5% after 9 mo (placebo subtracted; P for interaction = 0.025, P for metreleptin = 0.004) in the placebo-controlled trial. There were no significant changes in lean body mass (P ≥ 0.33) or in serum concentrations of myostatin (P ≥ 0.35), follistatin (P ≥ 0.30), and activin A (P ≥ 0.20) whether in the 10-wk trial or the 9-mo trial. We conclude that metreleptin administration in lean hypoleptinemic women reduces fat mass exclusively and does not affect lean body mass or the myostatin-follistatin-activin axis.

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LEPTIN IS AN ADIPOCYTE-SECRETED HORMONE with pleiotropic effects on energy homeostasis and metabolism (7). Administration of leptin to obese leptin-deficient or wild-type animal models reduces body weight almost exclusively by reducing the amount of adipose tissue while sparing lean body mass (12). In obese human subjects, however, leptin treatment does not reduce body weight and fat mass unless it is given in high pharmacological doses, and even then it does not affect lean body mass (13, 15). This insensitivity to the weight-reducing effects of leptin could be due to leptin resistance (9) or leptin tolerance (24). On the contrary, recombinant methionyl human leptin (metreleptin; previously known as r-metHuLeptin) administered in the context of an uncontrolled study to lean, hypoleptinemic, and thus presumably leptin-sensitive women reduced body weight and fat mass (32), but its effects on lean body mass were not reported. Limited data from studies in other states of acquired hypoleptinemia in humans, e.g., in HIV-infected patients with lipodystrophy and hypoleptinemia (17, 25) and in healthy women during starvation-induced hypoleptinemia (3), indicate that metreleptin administration may also reduce lean body mass, although this effect may not always reach significance (P values ≤0.08). Inconsistent results have been reported in young patients with congenital leptin deficiency, since some studies indicate a decrease (10) and others an increase (8) in lean body mass in response to leptin treatment. Recent in vivo data in animals indicate that leptin treatment increases muscle mass and muscle fiber size of ob/ob mice, and this effect is associated with a leptin-induced reduction in protein expression levels of myostatin, which is a negative regulator of muscle growth (28). However, the ob/ob mouse model and humans with congenital leptin deficiency represent genetic conditions of complete leptin deficiency and extreme obesity. The effects of leptin treatment on lean body mass and circulating regulators of muscle growth in leptin-sensitive lean human subjects with acquired hypoleptinemia are not known.

Regulation of muscle mass in humans is complex and depends on the balance between protein degradation and protein synthesis, which in turn is affected by several nutritional, exercise, and hormonal factors (27). An important regulatory system is the myostatin-follistatin-activin axis, which, besides muscle mass, may also regulate fat accumulation in the body (26). Myostatin protein is produced by skeletal muscle, circulates in blood (11), and acts to limit muscle growth by signaling through the activin receptors (18, 21). Follistatin is an extracellular myostatin-binding protein that inhibits myostatin activity in vitro and promotes muscle growth in vivo (18, 19). Lack of myostatin activity is associated with hypoleptinemia but also increases leptin sensitivity (5) and leads to muscle hypertrophy, whereas lack of follistatin activity (and thus lack of inhibition of myostatin) leads to muscle cachexia (18). Recent genetic evidence in animals indicates that the effects of follistatin loss are evident even in myostatin-null mice, implying that myostatin cannot be the sole target for follistatin (19). Activin A is probably another ligand regulated by follistatin that functions with myostatin to limit muscle mass (19). Leptin treatment in leptin-deficient ob/ob mice reduces the levels of myostatin protein (28). Whether similar effects are present in humans remains to be investigated.

To examine the effects of leptin on both fat and lean body mass and, secondarily, the myostatin-follistatin-activin axis, we evaluated, in the context of two independent proof-of-concept studies, the effects of short- and long-term metreleptin...
administration in lean women with exercise-induced amenorrhea and hypoleptinemia, i.e., a leptin-deficient and thus leptin-sensitive state.

METHODS

Twenty-seven young, lean, strenuously exercising, amenorrheic (≥6 mo) women with leptin concentration <5 ng/ml were recruited from the community and participated in one of two studies designed to assess the effects of metreleptin on endocrine and reproductive function (6, 32). Seven of them did not complete the entire study and were not included in our analyses. Exclusion criteria included anemia, infectious diseases, renal or hepatic failure, type 1 or type 2 diabetes mellitus, cancer, lymphoma, malabsorption, alcoholism, smoking, drug abuse, use of glucocorticoids or other steroids, anti-seizure medications, or thyroid hormones, estrogen replacement, history of anorexia nervosa, depression, or other psychiatric disorders, evidence of polycystic ovary syndrome, congenital adrenal hyperplasia, abnormal prolactin levels or abnormal thyroid-stimulating hormone levels, history of anaphylaxis, pregnancy and lactation, and hypersensitivity to E. coli. Both studies were approved by the Beth Israel Deaconess Medical Center Institutional Review Board, and all subjects provided written informed consent.

Study 1: short-term single-arm protocol. Seven lean, strenuously exercising, amenorrheic women with hypoleptinemia (age 25 ± 6 yr, %body fat 22 ± 4%, screening leptin concentration 3.5 ± 1.7 ng/ml) were assigned to receive metreleptin (0.08 mg·kg⁻¹·day⁻¹, self-injected subcutaneously twice daily: 40% in the morning and 60% in the evening) for 10 wk of open-label treatment; metreleptin was supplied by Amgen (Thousand Oaks, CA). Subjects were evaluated at baseline and every 2 wk thereafter. On each occasion, they arrived at the Clinical Research Center in the evening. After a standardized dinner and an overnight fast, a blood sample was taken the next morning, and body composition (total body fat mass and bone-free lean mass) was assessed by dual energy X-ray absorptiometry (DEXA; QDR-4500; Hologic, Bedford, MA). For the duration of the study, subjects were instructed to maintain their normal exercise routine and dietary habits.

Study 2: long-term randomized placebo-controlled protocol. Thirteen lean, strenuously exercising, amenorrheic women with hypoleptinemia were randomly assigned, in a 1:1 ratio, to receive either metreleptin (n = 7; initial dose: 0.08 mg·kg⁻¹·day⁻¹ for 3 mo, increased thereafter to 0.12 mg·kg⁻¹·day⁻¹ if menstruation did not occur, self-injected subcutaneously between 1900 and 2300) or placebo (n = 6; same volume and timing as leptin) for 9 mo of double-blinded treatment; metreleptin and placebo were supplied by Amylin Pharmaceuticals (San Diego, CA). There were no significant differences between the placebo and metreleptin groups in baseline age (26 ± 3 and 27 ± 5 yr, respectively, P = 0.915), percent body fat (21 ± 4 and 24 ± 4%, respectively, P = 0.131), or screening leptin concentration (1.8 ± 0.7 and 2.7 ± 1.3 ng/ml, respectively, P = 0.209). Subjects were evaluated at baseline and every 3 mo thereafter. On each occasion, they arrived at the Clinical Research Center in the evening. After a standardized dinner and an overnight fast, a blood sample was taken the next morning, and body composition (total body fat mass and bone-free lean mass) was assessed by DEXA (QDR-4500; Hologic). For the duration of the study, subjects were instructed to maintain their normal exercise routine and dietary habits.

Statistical analysis. Statistical analyses were performed with PASW version 18.0 (SPSS, Chicago, IL). All data sets were tested for normality according to the Shapiro-Wilk procedure. Nonnormally distributed data were log-transformed for analysis and back-transformed for presentation as means and 95% confidence interval. Results for the remaining parameters are shown as means ± SD. The effect of leptin treatment was evaluated by using repeated-measures ANOVA with time as within-subjects factor in the single-arm protocol and repeated-measures ANOVA with time as within-subjects factor and treatment group (leptin vs. placebo) as between-subjects factor in the placebo-controlled protocol. A two-tailed P value <0.05 was considered significant.

RESULTS

Study 1: short-term single-arm metreleptin treatment. Metreleptin administration resulted in a significant increase in serum leptin concentration from baseline values of 3.1 ± 2.1 to 7.0 ± 1.6, 11.1 ± 6.6, 17.5 ± 12.7, 20.3 ± 15.8, and 40.0 ± 30.5 ng/ml after 2, 4, 6, 8, and 10 wk of treatment, respectively (P = 0.030). Body weight (P < 0.001), percent body fat (P < 0.001), and total body fat mass (P < 0.001) decreased significantly after initiation of metreleptin treatment, whereas total lean body mass did not change (P = 0.340) (Fig. 1). Myostatin (P = 0.353), follistatin (P = 0.301), and activin A (P = 0.535) concentrations in serum were not affected by 10 wk of metreleptin treatment (Fig. 2).

Study 2: long-term placebo-controlled metreleptin treatment. Serum leptin concentration increased significantly in the metreleptin-treated group (from baseline values of 4.2 ± 2.1 to 45.1 ± 25.8, 66.8 ± 30.3, and 68.1 ± 45.9 ng/ml after 3, 6, and 9 mo of treatment, respectively) but not in the placebo-treated group (from baseline values of 4.1 ± 2.3 to 3.6 ± 2.2, 3.1 ± 1.6, and 2.4 ± 1.2 ng/ml after 3, 6, and 9 mo of treatment, respectively (P for interaction = 0.008). There were significant interactions between time and group for percent body fat (P = 0.021) and total body fat mass (P = 0.025) and an almost significant interaction for body weight (P = 0.086). Within-group analysis indicated that percent body fat and total body fat mass decreased significantly among metreleptin-treated subjects (P for time = 0.004 and 0.004, respectively) but not among placebo-treated subjects (P for time = 0.876 and 0.575, respectively) (Fig. 1). On the other hand, total lean body mass did not change after metreleptin or placebo treatment (P for time = 0.330 and P for interaction = 0.409; Fig. 1). There were no significant effects of time (P ≥ 0.20), differences between treatment groups (P ≥ 0.45), or time-by-group interactions (P ≥ 0.62) for serum myostatin, follistatin, and activin A concentrations (Fig. 2).

DISCUSSION

We assessed longitudinal changes in total body fat and lean body mass as well as circulating regulators of muscle mass, i.e., myostatin, follistatin, and activin A, in young, hypoleptinemic, and apparently leptin-sensitive lean women during 10 wk (single-arm) and 9 mo (placebo-controlled) of metreleptin administration. We found that increasing leptin concentrations by exogenous leptin treatment in these hypoleptinemic women decreases body fat but does not affect lean body mass or serum myostatin, follistatin, and activin A concentrations. Therefore, the weight-reducing effect of metreleptin in these women is due exclusively to a reduction in fat mass, whereas lean body mass is preserved.
Our results are in sharp contrast to data from obese patients, in whom leptin administration does not affect body weight and fat mass unless it is given in extremely high pharmacological doses, and even then the effect is very mild (13, 15, 24). We found that the effect of metreleptin in our hypoleptinemic women was quite remarkable, with fat mass loss averaging 18.6% after 10 wk and 19.5% (placebo-subtracted) after 9 mo of treatment. This is consistent with animal data showing that leptin administration to leptin-sensitive animal models (ob/ob and wild-type mice) leads exclusively to fat loss (12) and indicates that our women were indeed leptin-sensitive. This notion is also supported by the fact that, as we reported elsewhere (6, 32), metreleptin treatment resulted in resumption of menses in ~70% of these women, with menstruation appearing at various stages during therapy (from 4 to 8 wk after initiation of treatment in the open label 10-wk study and from 4 to 32 wk in the randomized placebo-controlled 9-mo study). Together these observations suggest that metreleptin is efficacious in reducing fat mass when baseline leptin concentrations are low (i.e., lean hypoleptinemic women) but is ineffective in the face of elevated baseline leptin concentrations (i.e., in obese subjects).

We did not find any significant increase in lean body mass after metreleptin treatment, whether short or long term. Studies in HIV-infected lipoatrophic patients with hypoleptinemia (17, 25) and in healthy lean women during starvation-induced hypoleptinemia (3) reported trends for a reduction in lean body mass induced by metreleptin administration (P values ≤0.08). This would be an undesirable side effect of metreleptin treatment, especially among subjects who are of normal weight or underweight to begin with. We tested this possibility by monitoring changes in lean body mass in the short term (every 2 wk during a 10-wk open-label leptin administration trial) and the long term (every 3 mo during a 9-mo placebo-controlled trial) in young, lean, hypoleptinemic women and found no effect of metreleptin administration on lean body mass. It is possible that the substantial reduction in ectopic fat induced by metreleptin treatment in lipoatrophic HIV patients (17, 25) could be responsible for the observed trends for reduced lean mass, because ectopic fat depots (e.g., within liver or muscle) are inaccurately assessed by DEXA as components of lean body mass (30). Likewise, the considerable body fluid shifts during acute starvation (3) would make it difficult to reliably assess lean body mass with DEXA. Nevertheless, we cannot exclude a priori the possibility that a lean mass-reducing effect of metreleptin administration was not apparent in the present study because our subjects were strenuous exercisers, and regular exercise may help preserve lean body mass during conditions of weight reduction (1, 2). However, the randomized and placebo-controlled nature of our long-term study ascertains that there is no statistically significant differential effect of metreleptin vs. placebo on lean body mass in these women.

We also found no changes in serum concentrations of myostatin, follistatin, or activin A in serum in response to metreleptin treatment, whether in the short or the long term. Myostatin, a member of the transforming growth factor-β superfamily, is a negative regulator of skeletal muscle growth (18). Genetic loss of myostatin function is associated with muscle hypertrophy in mice and humans (21, 29) in an apparently dose-dependent manner (22), whereas myostatin excess is associated with muscle cachexia (34). Furthermore, most studies in humans indicate that myostatin concentrations in serum correlate inversely with lean body mass and that myostatin gene expression and circulating concentrations are greater in conditions of muscle wasting (e.g., due to disease or aging) (11, 20, 33) and lower in conditions stimulating muscle growth (e.g., after resistance exercise) (31). However, others fail to reproduce these observations (4, 16). Myostatin is expressed uniquely in the human skeletal muscle and is secreted into plasma (11), where it circulates as a latent complex with proteins such as the myostatin propeptide, follistatin, and the follistatin-related gene, which bind and inhibit its activity (14, 18). Recently, it was reported that activin A may also function along with myostatin to...
limit muscle growth and that follistatin is as major extracellular protein that binds both myostatin and activin A, thereby inhibiting their activity and promoting muscle accretion (19). A previous study in leptin-deficient and thus extremely leptin-sensitive ob/ob mice reported that leptin treatment increased muscle mass and reduced myostatin protein levels (28). Our findings do not support these results and indicate that metreleptin administration in lean hypoleptinemic women does not alter the myostatin-follistatin-activin axis, in agreement with the absence of change in lean body mass. This holds true regardless of several other metreleptin-induced changes in some systemic regulators of muscle metabolism (e.g., cortisol, insulin-like growth factor axis) that we reported previously in these women (6, 32).

This is the first study to report the effects of leptin on circulating regulators of muscle mass. We prospectively monitored lean leptin-sensitive women with acquired hypoleptinemia and made frequent measurements during both a short-term (every 2 wk for 10 wk), single-arm, open-label trial and a long-term (every 3 mo for 9 mo), placebo-controlled, double-blinded trial and found no changes in lean body mass and the myostatin-follistatin-activin axis, in agreement with the absence of change in lean body mass. This holds true regardless of several other metreleptin-induced changes in some systemic regulators of muscle metabolism (e.g., cortisol, insulin-like growth factor axis) that we reported previously in these women (6, 32).

In summary, we measured total body fat mass and lean body mass and circulating levels of regulators of muscle mass in lean hypoleptinemic women during 10 wk of open-label treatment and 9 mo of double-blinded treatment with exogenous leptin. We found that metreleptin does not affect lean body mass or the concentrations of myostatin, follistatin, and activin A in serum. Thus, similar to studies in animals (12), the weight-

Fig. 2. Effect of metreleptin administration for 10 wk (single-arm protocol; right) and 9 mo (randomized placebo-controlled protocol; left) on serum myostatin (top), follistatin (middle), and activin A (bottom) concentrations in lean hypoleptinemic women. Values are shown as means with 95% confidence interval.
reducing effect of metreleptin in these leptin-sensitive women is due entirely to a considerable reduction in fat mass. The mechanisms, central or peripheral, for this targeted body fat-reducing effect of leptin are not presently clear. Elucidating the mechanisms underlying leptin sensitivity and exploiting them to induce weight loss in obese subjects would be of major pathophysiological and therapeutic significance and could potentially provide tangible benefits to those who strive to lose weight, especially given that the expected weight loss will be, unlike other treatments, due almost exclusively to loss of adipose tissue.

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DISCLOSURES

The authors have nothing to disclose.

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