Randomized Double-Blind Placebo-Controlled Study of Leptin Administration after Gastric Bypass

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Objective: Obese individuals have high levels of circulating leptin and are resistant to the weight-reducing effect of leptin administration at physiological doses. Although Roux-en-Y gastric bypass (RYGB) is an effective weight loss procedure, there is a plateau in weight loss and most individuals remain obese. This plateau may be partly due to the decline in leptin resulting in a state of relative leptin insufficiency. The main objective of this study was to determine whether leptin administration to post-RYGB patients would promote further weight reduction.

Design and Methods: This was a randomized, double-blind, placebo-controlled cross-over study of 27 women who were at least 18 months post-RYGB and lost on average 30.8% of their presurgical body weight. Subjects received either leptin or placebo via subcutaneous injection twice daily for 16 weeks, then crossed over to receive the alternate treatment for 16 weeks.

Results: Weight change after 16 weeks of placebo was not significantly different from that after 16 weeks of leptin. No changes were observed in percent fat mass, resting energy expenditure, thyroid hormones, or cortisol levels.

Conclusion: Contrary to our hypothesis, we did not observe a significant effect of leptin treatment on body weight in women with relative hypoleptinemia after RYGB.

Introduction

Weight loss from surgical and nonsurgical obesity treatment is associated with a weight plateau despite an individual’s continued attempt at weight reduction, and is frequently followed by weight regain. Decreased energy expenditure, difficulty with diet and exercise compliance, and alterations in hormones involved in body weight regulation are all contributing factors. Leptin is a critical afferent component of a regulatory loop linking fat mass to food intake and energy expenditure. Leptin also modulates the activity of other hormones involved in body weight regulation, including thyroid hormones, catecholamines, cortisol, and insulin (1). Because leptin is secreted by adipocytes, individuals with greater fat mass have higher levels of leptin than leaner individuals. While physiologic replacement doses of leptin cause fat loss in obese humans with congenital leptin deficiency, high pharmacologic levels of leptin are required to induce weight loss in otherwise healthy obese individuals (2–6). This suggests that those with common forms of obesity are in a state of relative “leptin resistance” with regard to the ability of exogenous leptin to reduce body weight. Following weight loss, however, leptin levels decline out of proportion to the reduction in fat mass, indicating that caloric restriction produces a dissociation of circulating leptin concentrations and body fat content (7–11). Studies demonstrate persistence of relative hypoleptinemia in the postobese state after bariatric surgery (12) or caloric restriction (13) when levels are compared...
to BMI-matched controls or adjusted for fat mass. Many of the metabolic, autonomic and neurohormonal changes that occur with caloric restriction and are likely to promote weight regain are mediated in part by a decrease in circulating concentrations of leptin (14). Administration of replacement doses of leptin that restore circulating concentrations to preweight loss levels reverses many of these changes (2,15). The weight-reduced state is, therefore, considered a condition of relative leptin insufficiency that may contribute to the plateau in weight loss or weight regain commonly seen in those trying to achieve or maintain a reduced body weight.

Unlike diet therapy alone, Roux-en-Y gastric bypass (RYGB) surgery results in a reduction of ~38% of total body weight at 1 year that is mostly maintained over the long-term (16). Weight loss results from a restriction to nutrient flow and changes in gut hormone secretion that favor a state of reduced weight (17). Despite significant weight reduction, most individuals experience a weight loss plateau with a body mass index (BMI) still within the obese range (18). We have previously shown that plasma leptin levels are significantly lower in weight-stable individuals post-RYGB compared with BMI matched nonsurgical controls and that the decrease in leptin correlates with percent change in body weight (12,17). It is possible that individuals who have lost weight after RYGB are in a state of relative leptin insufficiency and would, therefore, respond to supplementation of leptin at a dose that would not normally cause a significant change in body weight in an obese individual. The main objective of this randomized, prospective, double-blind, placebo-controlled study was to determine whether leptin administration to a group of post-RYGB patients who reached a stable state of weight loss would promote further weight reduction. We further sought to establish whether leptin administration in this weight-reduced state would be associated with changes in resting energy expenditure (REE), body composition, appetite, and levels of circulating hormones involved in weight regulation.

Methods

Women between the ages of 25 and 65 years who were at least 18 months post-RYGB, had a percent total weight loss from highest presurgical weight to current weight between 18 and 45% and had a current BMI of 28-50 kg m\(^{-2}\) were invited to participate. To determine if a potential subject was hypoleptinemic, leptin levels were correlated with BMI from a control cohort of 55 obese, non-weight-reduced women who had participated in previous studies from our group (JK, unpublished data). Given differences in leptin values performed in various assays (19), it was important to use values from control subjects that were determined by assays in our own laboratory. The following regression equation was generated to calculate leptin levels from a non-weight-reduced cohort: (0.991 × BMI) – 3.37. If the leptin level of a potential subject was less than the level predicted from this equation, then that individual was considered hypoleptinemic and eligible for enrollment. Women were excluded if they had any of the following: type 1 or type 2 diabetes; untreated hypertension; coronary artery, cerebrovascular, renal, hepatic, neurologic or untreated thyroid disease; alcohol dependence; chronic tobacco or opiate use; weight altering medication use; history of plastic surgery (excluding facial) or liposuction; >3% self-reported change in weight over the last 3 months; or, unwillingness to maintain the same level of physical activity throughout the study. Women who were pregnant, nursing or premenopausal and not using contraception were excluded from the study. This study is in accordance with the guidelines of the Declaration of Helsinki and was approved by the Columbia University Institutional Review Board. All subjects provided written informed consent.

Subjects who met entry criteria were scheduled for a second visit 4-6 weeks after screening. If weight was within 3% of screening weight, enrollment occurred and subjects entered a 2 week single-blind placebo run-in period, after which they were randomized to receive either placebo (Group P-L) or recombinant human mettleptin (Group L-P). Mettleptin, referred to as “leptin”, and placebo were generously donated by Amylin Pharmaceuticals (San Diego, CA). Randomization was stratified into two groups: those whose weight loss from the highest presurgical weight was 18-34.9% of total body weight (“low”) or those whose weight loss was 35-50% of total body weight (“high”). This stratification was to ensure that the P-L and L-P groups consisted of subjects who had undergone similar amounts of weight loss after bypass, as the degree of weight loss could conceivably affect the response to leptin. After 16 weeks, subjects were crossed over to receive either placebo or leptin for the remaining 16 weeks without a washout period. The dose of leptin (0.05 mg kg\(^{-1}\) body weight self-administered via subcutaneous injection twice daily) was expected to raise maximum plasma leptin levels to high physiologic/low pharmacologic levels, yet would not be expected to cause clinically significant weight loss in a person who had not undergone weight reduction (2). In the case of intolerable injection site reactions, a dose reduction to 0.03 mg kg\(^{-1}\) twice daily was allowed. Subjects returned all vials and received another month’s supply every 4 weeks, during which weight and adverse effects were monitored. Venous blood was collected at regular intervals to monitor safety labs and quantify trough levels (12 h post dose) of leptin.

In addition to blood collection, body composition analysis and REE were performed at weeks 0 (after the run-in period) and 16 (prior to cross-over treatment). Body composition was assessed using dual X-ray absorptiometry (DXA, Hologic QDR 4500A, Waltham, MA; APEX 3.2 software). REE was measured by indirect hood calorimetry (Parvo Medics System – True Max 2400). Venous blood samples were collected in EDTA tubes that were centrifuged for 15 min at 4°C and stored at –80°C until assayed in duplicate. Leptin was measured by RIA (LINCO Research Inc., St. Charles, MO) using a \(^{125}\)I-labeled human leptin tracer (sensitivity 7.8 pg mL\(^{-1}\)); intra-assay and interassay coefficients of variation <5%. Plasma was diluted as necessary to obtain readings within the assay range. Thyroid hormones were measured by Immulite Analyzer (Siemens, Los Angeles, CA). Salivary cortisol was measured by ELISA (Minneapolis, MN) and urinary free cortisol by liquid chromatography/mass spectrometry.

The primary outcome was body weight. Secondary outcomes were changes in anthropometry, including waist circumference, hip circumference and BMI, as well as changes in thyroid function, cortisol production and REE. Thirty subjects in each group provided a power of 80% to detect an 8 kg difference in body weight (as achieved with high dose leptin in obese subjects (2) assuming a 5% type I error rate and a standard deviation of 7 kg). Distributions of continuous variables were assessed for normality with Kolmogorov-Smirnov test and Q-Q plots; no measure required transformation. Baseline differences between randomized groups in categorical variables were assessed by Fisher’s Exact test; continuous variables were assessed with Student’s \(t\) test. Outcomes were based on a modified intent-to-treat analysis in which all randomized subjects who had at least one post randomization visit were included in the analysis. Between-group differences in change from baseline during the first 16-week period were assessed with general linear models with treatment group (leptin...
TABLE 1 Baseline characteristics

| Parameter              | Group P-L (n=13) | Group L-P (n=14) | P value |
|------------------------|------------------|------------------|---------|
| Age (y)                | 42.8 ± 2.4       | 51.1 ± 1.7       | 0.01    |
| Pre-RYGB BMI (kg m⁻²)  | 49.0 ± 1.6       | 48.5 ± 1.7       | 0.82    |
| Post-op Period (mo)    | 41.7 ± 6.5       | 67.1 ± 7.0       | 0.02    |
| Wt loss (%)            | 30.9 ± 2.0       | 30.8 ± 1.9       | 0.97    |
| Wt (kg)                | 91.9 ± 3.8       | 89.6 ± 3.9       | 0.69    |
| BMI (kg m⁻²)           | 34.9 ± 0.9       | 34.4 ± 1.3       | 0.79    |
| Glucose (mg dL⁻¹)      | 85.7 ± 1.6       | 88.0 ± 1.4       | 0.29    |
| Leptin/kg FM (ng mL⁻¹) | 0.70 ± 0.06      | 0.67 ± 0.06      | 0.79    |
| Leptin (ng mL⁻¹)       | 26.1 ± 2.8       | 25.1 ± 2.8       | 0.82    |

Data are presented as mean ± SEM. *n=9 (each group).

Results

Sixty-nine subjects were screened for participation; 35 met enrollment criteria. Eight subjects failed to have at least one follow-up visit post randomization and were excluded from the analysis. Of the remaining 27 subjects, 23 completed the first 16 weeks and 20 completed the entire 32 week study. Of the seven subjects who did not complete the study, one started a weight loss program, one started weight loss medication, one underwent liposuction, one was removed from the study due to an unrelated medical condition, and one was lost to follow-up. Two subjects withdrew due to side effects that were described as worsening chronic fatigue, flu-like symptoms and lower extremity edema that persisted despite a reduction in medication dose. After unblinding, both subjects were found to have been administering placebo at the time of withdrawal from the study. No other subjects required a dose reduction.

Baseline characteristics of the study population were similar between groups with the exception of age and duration of post-operative period, which was a mean of 54.9 ± 5.3 months (Table 1). Mean percent weight loss from highest pre-operative weight to weight at time of screening visit was 30.8 ± 1.4%, with a range of 18.2-44.7%, and mean BMI was 34.7 ± 0.8 kg m⁻², with a range of 28.4-41.7 kg m⁻².

Plasma leptin levels were significantly higher during the periods of leptin therapy and progressively rose during the course of treatment (Figure 1; P < 0.001). Leptin administration did not result in a decrease in body weight when compared to placebo (Figure 2). Mean

![Figure 1](https://www.obesityjournal.org/figures/fig1.jpg)

**FIGURE 1** Plasma leptin levels during the course of study. A) Group P-L, received placebo followed by leptin; and B) Group L-P, received leptin followed by placebo. Actual leptin values are indicated in the table as mean ± SEM. *P < 0.01; **P < 0.001 compared with Wk 0 within group.
weight change after 16 weeks of placebo treatment was 0.02 ± 0.76 kg (range −10.2 to 5.1 kg) and −0.39 ± 0.35 kg (range −3.6 to 2.4 kg) during leptin treatment. Weight change after leptin treatment was not affected by either baseline leptin values \((P = 0.94)\), percent of weight loss after surgery \((P = 0.48)\), or duration of the postoperative period at the time of the study \((P = 0.36)\). Percent fat mass change in the two groups did not differ when raw scores were tested, but adjustment for percent fat mass prior to treatment unmasked a statistically significant decline in fat mass in the placebo treated group (Table 2; \(P = 0.02\)), while the leptin group was unchanged.

Thyroid hormone levels remained the same throughout the study; however, the placebo group exhibited a decrease in TSH (Table 2).
significant effect of leptin treatment on body weight, the primary endpoint of this study. Fat mass was also unchanged after leptin treatment.

It may not be possible to generalize the effect of leptin after RYGB with the effect after simple caloric restriction even though other studies assessing leptin treatment following weight loss (25,26) found results similar to ours. Major differences between subjects in studies of caloric restriction and our population include the greater degree of weight loss achieved following RYGB in our subjects and the longer period of maintenance of weight loss, both of which may alter the relationship between leptin signaling and body weight. We specifically chose to study this population because of the maintained weight loss and the profound effects of this procedure on gut hormone secretion and gastrointestinal anatomy. Although considered a long-acting adiposity hormone, leptin may also regulate body weight through influencing the action of short-acting anorectic hormones from the gut that control eating behavior at individual meals (27) and by potentiating the intake suppressive effects of gastric distension (28,29). Leptin has been shown to enhance the anorexia and weight loss induced by GLP-1 (30). We postulated that the marked augmentation of postprandial GLP-1 secretion after RYGB (17) would make these individuals uniquely sensitive to leptin administration. It is possible that our subjects have reached a new steady state of energy balance where further weight loss would have to be achieved through additional leptin-independent mechanisms, or that leptin resistance was not ameliorated by RYGB-induced weight loss.

The mean leptin level in our post-RYGB group (21.6 ± 2.0 ng mL⁻¹) was 35% lower than our historical control cohort of 26 women with a similar mean body weight of 94.9 ± 2.8 kg and a mean leptin concentration of 33.5 ± 2.5 ng mL⁻¹ (P < 0.0001) (12). This reduced level of leptin is certainly within the range of decrease observed in studies that show reversal of neuroendocrine adaptations with leptin replacement after calorie restriction (15). Even when taking into account differences in leptin assays (19), levels in our subjects were below 50 ng mL⁻¹ which appears to be the level at which leptin signaling pathways are saturated (20,31). While leptin administration resulted in levels within the targeted high physiologic range, twice daily administration does not mimic the normal pulsatile and circadian secretion of leptin, which may be required for effective weight loss. In some individuals there was a progressive rise in leptin levels that reached pharmacologic concentrations that has been noted in other studies and is consistent with the development of non-neutralizing antibodies (2). Other concerns with achieving very high levels of leptin include the development of tachyphylaxis or resistance to the action of leptin, either through alteration of blood-brain barrier leptin transport, induction of cellular processes in leptin receptor positive neurons that inhibit leptin signaling, or alterations in neural function (32). It seems unlikely that resistance was induced during this short time period as we should have detected initial weight loss before the resistance developed. Furthermore, Heymsfield et al. observed weight loss over a 24-week period in obese individuals using a higher dose of leptin that produced a mean serum leptin concentration of 480 ng mL⁻¹ (2). A limitation inherent to this small outpatient study is that caloric intake and physical activity were not closely monitored. Thus, variability in energy intake and expenditure between subjects may have masked changes in body weight that were smaller than anticipated.

Resting energy expenditure decreases with weight loss. This metabolic adaptation likely presents a barrier to continuous weight loss or weight maintenance (33,34). Johannsen et al. showed that severely obese individuals who lost on average 38% of initial body weight through diet restriction and vigorous exercise exhibited a decline in resting metabolic rate and leptin levels out of proportion to the decrease in body mass (34). Rosenbaum et al. showed that the decrease in total energy expenditure associated with a 10% reduction of total body weight was primarily due to a decrease in non-REE that was partially reversed after leptin replacement (15). Similarly, REE was unaffected by leptin administered during an energy restricted diet (35). We also did not observe a change in REE during leptin treatment; however, measurements of total energy expenditure or sympathetic activity were not performed.

Thyroid hormones are subject to physiologic regulation during the transition from the fed to the fasted or weight-reduced state. Weight loss is associated with small but significant decreases in circulating T3 and increases in its bioactive form, reverse T3 (15,25). We have previously shown that 1 year after RYGB levels of free T3 are reduced compared to baseline values, while free T4 and TSH remain the same (17). Administration of leptin blunts the decrease in thyroid hormone levels in fasted or calorie-restricted weight-reduced adults in some (15,25) but not all (14,26) studies, increases levels of free T3 and free T4 in children with leptin deficiency (23) and in hypoestrogenic women with hypothalamic amenorrhea (24). We were unable to demonstrate any effect on thyroid hormone levels in this study. It is possible that the thyroid hormone axis has re-equilibrated years after bypass. It would be of interest to follow patients prospectively to determine if levels of free T3 return to baseline after a prolonged period of weight maintenance.

Glucocorticoids play a role in the neuroendocrine control of food intake and energy expenditure (36). Obesity, particularly abdominal obesity, is associated with hyperactivity of the hypothalamic-pituitary-adrenal axis (36). Weight reduction by starvation has been shown to elevate plasma cortisol, but studies assessing the effect of weight loss by very low calorie diet have led to conflicting results regarding morning plasma cortisol levels and the stimulated cortisol response to corticotropin releasing factor (37). In a 6-month study of leptin deficient adults after leptin replacement and weight loss, there was higher 24 h mean concentration of cortisol with a greater morning rise (38), and in a 3-month study of leptin replacement in women with hypothalamic amenorrhea there was a nonsignificant rise in serum cortisol (24). In the absence of further weight loss, we were unable to demonstrate any effect of leptin administration on salivary and urine cortisol measurements. A limitation to our study is that 24 h urinary cortisol secretion or ACTH pulsatility were not assessed.

Our results do not negate the possibility that post-RYGB adults are more leptin responsive than the nonweight reduced obese, as we have only tested some of the pleiotropic effects of leptin. It is possible that our “read-out” of leptin action was not sensitive enough or did not examine the appropriate pathways for which leptin responsiveness may have been regained. Furthermore, our subjects had a longstanding period of maintenance at a reduced weight with a range of 28-82 months. Although the change in weight was not affected by the duration of weight loss in this study, the results may not have been the same if leptin was administered during an earlier postoperative period. Given the demonstration of neuronal plasticity within the arcuate nucleus of the hypothalamus in rodents (39), a chronic decrease in fat stores and relatively low leptin levels may over time be perceived by the central nervous system as leptin sufficient. It is also possible that
measurement of peripheral leptin levels may not accurately reflect alterations in transport across the blood–brain barrier and resultant changes in central nervous system leptin concentrations. Hormonal changes unique to RYGB also preclude extrapolating these results to diet-induced weight loss. In addition to the postprandial increases in the anorectic peptides GLP-1 and PYY after RYGB, circulating levels of the orexigenic peptide, ghrelin, tend not to increase. The interplay of these peptides with leptin responsive pathways could be quite different between surgery and simple caloric restriction. Anorexigenic pathways stimulated by insulin and amylin may also be required for further weight loss. The possibility still exists that there is a subpopulation of people who respond to exogenous leptin. For example, it has recently been shown that individuals carrying the 1251L allele of the MC4R are predisposed to better metabolic status and more weight loss after RYGB (40). It is conceivable that a subpopulation could be identified that would respond better to leptin as a potential weight loss enhancing agent. As with therapies for other diseases, identification of the optimal treatment depends in part on the selection of therapy administered in the appropriate setting for a given individual.

Acknowledgment

The authors gratefully acknowledge the participants of this study. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Metreleptin was generously provided by Amylin Pharmaceuticals, Inc (San Diego, CA). R.C. assisted with data collection, analysis and writing of the manuscript. J.K. conceived of the study, assisted with data analysis and the writing of the manuscript. G.F. assisted with data collection, and coordinated and conducted patient visits. D.J.M. conducted statistical analyses. W.K. provided nutritional guidance to the study subjects. I.C. performed hormone assays for the study. L.J.A. conceived of the study and edited the manuscript. J.K., R.C., D.J.M. and L.J.A. had final approval of the submitted and published versions.

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