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RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED STUDY of LEPTIN ADMINISTRATION after GASTRIC BYPASS

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Abstract

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 pre-surgical 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.

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

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CONFLICTS OF INTEREST

The rest of the authors have nothing to disclose.

Keywords

leptin; gastric bypass; bariatric surgery; obesity

INTRODUCTION

Weight loss from surgical and non-surgical 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).

Since 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 post-obese 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 pre-weight 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 approximately 38% of total body weight at one 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 non-surgical 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 pre-surgical weight to current weight between 18% and 45% and had a current BMI of 28 – 50 kg/m^2 were invited to participate. In order 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; more than 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 metreleptin (Group L-P). Metreleptin, 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 pre-surgical 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 amount 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 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 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 hours 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 minutes 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-iodinated human leptin tracer (sensitivity 7.8 pg/ml; 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 urine 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 resting energy expenditure. Thirteen 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 with 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 *vs.* placebo), assignment stratum (low *vs.* high post RYGB weight loss) and group-by-stratum interaction as fixed effects and the baseline level of the outcome measure as a continuous covariate. Post hoc comparisons between groups within stratum were conducted in the presence of a significant overall F-test. Prior to analysis of the cross-over design, the main effect of period (ignoring treatment) was tested to exclude possible secular trends in the two-period design ($P = 0.72$), and the main effect of treatment sequence in those receiving placebo was tested to exclude possible carry-over effects from the earlier leptin treatment ($P = 0.68$). Since the carry-over and period effects were considered very modest, repeated measures analysis of the within-subject difference between change during the placebo period *vs.* change during the leptin-treated period were assessed with general linear models for repeated measures. Secondary analyses to estimate the association between change in leptin per kilogram of fat mass and weight change during the first period were estimated with Pearson correlation. Results are expressed as means with standard errors. Primary outcomes were assessed with intention-to-

treat analyses with two-tailed alpha levels of 0.05; post-hoc comparisons of pair-wise differences were assessed with Bonferroni correction. No adjustment for multiplicity was made for the analysis of secondary measures as they were conducted to explore various alternative mechanisms and not test hypotheses concerning leptin treatment efficacy.

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 7 subjects who did not complete the study, 1 started a weight loss program, 1 started weight loss medication, 1 underwent liposuction, 1 was removed from the study due to an unrelated medical condition and 1 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 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 post-operative 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, $P = 0.03$ for the group by time interaction). No changes were observed in salivary or urine free cortisol levels in either group. REE did not change in either group. While respiratory quotients increased in both groups from baseline to week 16, there was no difference between the groups (Table 2).

DISCUSSION

The main objective of this study was to determine if the plateau in weight loss that occurs after RYGB can be overcome by administering replacement doses of leptin to subjects

deemed relatively leptin insufficient or hypoleptinemic. Obese individuals are usually hyperleptinemic and appear to be resistant to the weight loss effects of exogenous leptin administration that elevate circulating concentrations to within the physiological range. In order to undergo weight loss, such individuals require doses of leptin that produce 10-fold elevations of plasma leptin concentrations that are associated with intolerable injection site reactions (2). Lack of weight loss has also been noted when leptin is administered to obese patients with type 2 diabetes (20, 21). In contrast, leptin administration to congenital and acquired leptininsufficient humans and animals that restore circulating concentrations to within physiological range reduces weight, increases energy expenditure, decreases energy intake and fat mass, and increases activity of the hypothalamic-pituitary-thyroid axis and sympathetic nervous system (3, 4, 6, 10, 22–24). We therefore hypothesized that patients post RYGB who are hypoleptinemic relative to BMI-matched controls who had not undergone weight loss would exhibit further weight loss and changes in neuroendocrine function when circulating leptin levels are restored to high physiologic range. Contrary to our hypothesis, however, we did not observe a 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) 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 ($P < 0.0001$) (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 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 (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 bioinactive form, reverse T3 (15, 25). We have previously shown that one 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 hypoleptinemic 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 corticotrophin releasing factor (37). In a 6-month study of leptin deficient adults after leptin replacement and weight loss, there was higher 24 hr 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 non-significant 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 hour 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 non-weight 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 post-operative 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.

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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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Bariatrics, Inc., GI Dynamics, High Point Pharmaceuticals LLC, Medical University of South Carolina (MUSC), Novo Nordisk. He is on the advisory board of Amylin Pharmaceuticals Inc., Ethicon Endo-Surgery Inc., GlaxoSmithKline Consumer Healthcare LP, Novo Nordisk, Orexigen Therapeutics Inc., VIVUS Inc., Takeda Pharmaceuticals, Zafgen Inc. Dr. Aronne is on the speakers bureau for VIVUS Inc. and has ownership interest in Cardiometabolic Support Network, LLC, Myos Corporation. Amylin Pharmaceuticals Inc. generously supplied placebo and leptin for this trial.

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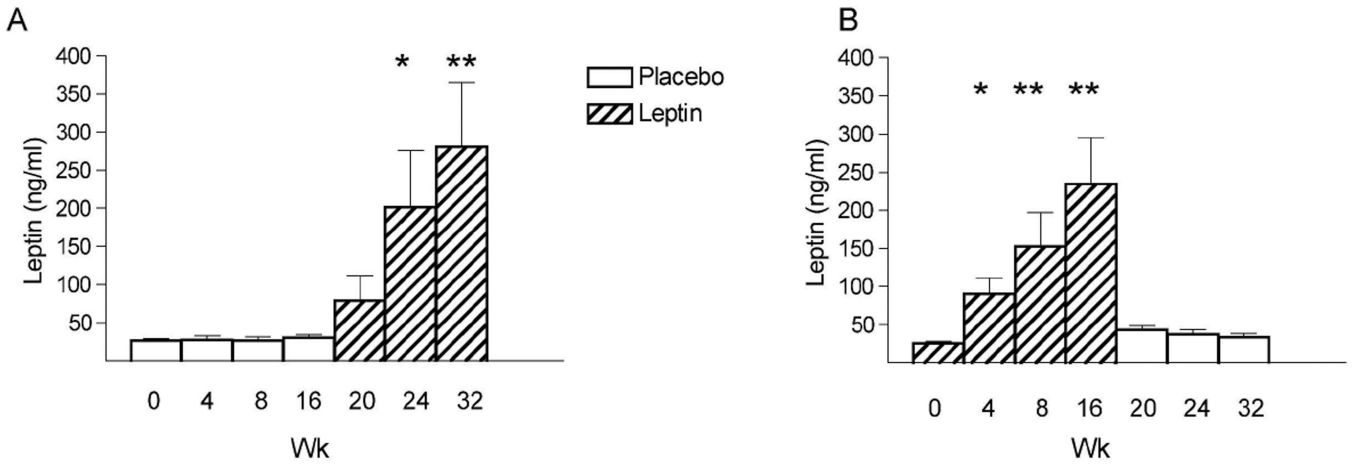
WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Obese individuals have high levels of leptin in the peripheral circulation and are resistant to the weight reducing effect of leptin administration at physiological doses.
- Although Roux-en-Y gastric bypass surgery (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.

WHAT THIS STUDY ADDS

- Leptin administration to women who have reached a weight plateau following Roux-en-Y Gastric Bypass and are relatively leptin 'insufficient' did not lead to further weight reduction.
- Leptin administration did not lead to changes in percent fat mass, resting energy expenditure, thyroid hormones, or cortisol levels either.
- Leptin resistance may continue to impact an individual's ability to continue to lose weight, even at levels considered to be relatively 'insufficient'.



Group	Wk 0	Wk 4	Wk 8	Wk 16	Wk 20	Wk 24	Wk 32
P-L	26 ± 2.8	27 ± 5.7	26 ± 5.1	30 ± 4.5	79 ± 33	202 ± 74*	281 ± 84**
L-P	25 ± 2.5	90 ± 21*	152 ±45**	234 ± 61**	43 ± 5.6	37 ± 6.1	33 ± 5.5

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.

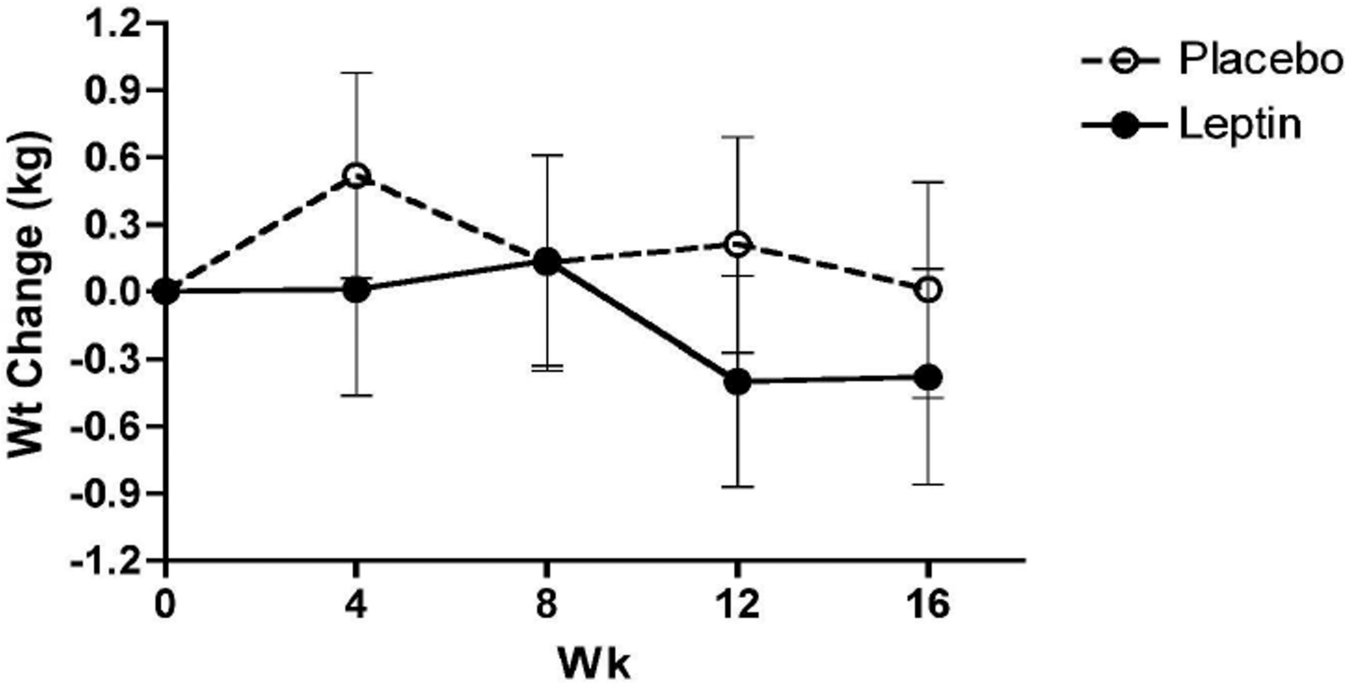


Figure 2.
Change in body weight during 16 weeks of either placebo or leptin administration.

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)	85.7 ± 1.6	88.0 ± 1.4	0.29
Leptin/kg FM (ng/ml/kg)	0.70 ± 0.06	0.67 ± 0.06	0.79
Leptin (ng/ml)[A]	26.1 ± 2.8	25.1 ± 2.8	0.82

Data are presented as mean ± SEM.

[A] n=9 (each group).

TABLE 2

Changes in body composition, energy expenditure and endocrine function

	Placebo		Leptin		*P* value
Parameter	**Wk 0**	**Wk 16**	**Wk 0**	**Wk 16**	
Wt (kg)	90.9±4.1	91.1±5.1	90.3±3.9	87.1±3.6	0.64
Waist Circ (cm)	40.0±0.8	38.7±1.4D	40.1±1.0	38.5±1.2	0.52
Total Adipose Tissue (kg)[A]	39.9±3.0	39.0±3.6[D, E]	35.9±2.9	34.0±2.9[E]	0.06
Fat Mass (%)[A]	42.6±1.4	41.5±1.8[D, E]	40.9±1.6	39.0±2.0[E]	0.02
Fat Free Mass (kg)[A]	50.7±24.0	51.8±32.8	51.8±22.6	50.3±23.6	0.40
REE (cal)[B]	1342±70	1352±58	1388±77	1325±69	0.78
REE/kg FFM[B]	27.0±1.6	25.4±3.2	26.6±1.5	27.3±1.6	0.25
R/Q[B]	0.76±0.01	0.79±0.01[D]	0.77±0.01	0.80±0.01[D]	0.57
Free T3 (pg/ml)[C]	2.48±0.13	2.65±0.14	2.54±0.15	2.67±0.15	0.84
Free T4 (ng/dl)[C]	1.13±0.03	1.11±0.04	1.13±0.06	1.10±0.05	0.67
TSH (μIU/ml)[C]	1.41±0.23	1.11±0.19[D, E]	1.21±0.12	1.29±0.15[E]	0.03
AM cortisol (μg/dl)	0.77± 0.09	1.01± 0.09	0.70± 0.09	0.76± 0.10	0.32
PM cortisol (μg/dl)	0.15± 0.11	0.40±0.11	0.14± 0.13	0.37± 0.13	0.90
24h urine cortisol (ug/g creatinine)	18.8± 2.7	26.5± 2.5	18.1± 2.8	21.3± 2.8	0.43

Data are presented as mean ± SEM.

[A] n=9 (each group).

[B] n=10 (placebo), n=12 (leptin).

[C] n=8 (each group) *P* value is indicated for group*time effect.

[D] $P < 0.05$ vs. week 0.

[E] $P < 0.05$ between groups.

All comparisons are based on linear mixed model estimates with baseline value entered as a continuous covariate.