Augmenting Leptin Circadian Rhythm Following a Weight Reduction in Diet-Induced Obese Rats: Short- and Long-Term Effects

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The current study sought to examine whether leptin injections following a weight reduction in diet-induced obese rats would reduce both the enhanced food intake and body weight (BW) regain observed during the refeeding phase. Female Wistar rats (n = 100, 20 per group) were divided into 5 groups: (1) LEP rats were fed a high-fat (HF) diet (35% wt/wt) for 8 weeks to induce obesity and were then food-restricted (50% ad libitum) with a fortified high-fat diet for 2 weeks to induce a 20% BW loss. These rats were then refed the HF diet ad libtum for another 11 weeks. They were given leptin injections (200 μg/kg BW, twice daily, intraperitoneally) for 19 days concomitant with the onset of refeeding. (2) SAL rats were treated in the same manner as LEP rats except that they were given saline injections; (3) PF rats were treated like SAL rats except that they were pair-fed with the LEP rats; (4) HFC rats were fed HF diet ad libitum; and (5) LFC rats were fed a low-fat (LF) diet (AIN-93M) ad libitum. Ten rats from each group were killed after leptin treatment and at the end of the study. Food and caloric intakes were monitored, and body composition and plasma glucose, insulin, and leptin levels were assessed at death. Leptin injections after a weight reduction briefly reduced energy intake during the first week only. After 19 days of treatment and to the end of the study, LEP and SAL rats were similar in energy intake, BW (LEP: 393 ± 11.2 g, SAL: 371 ± 14.1; difference not significant [NS]) and total body fat percent (LEP: 19.3 ± 1.5, SAL: 17.6 ± 1.5; NS). Leptin treatment induced hyperinsulinemia and insulin resistance. All of the metabolic abnormalities observed at the end of treatment period disappeared at the end of the study (8 weeks post-leptin injection). We conclude that bolus leptin injections to manipulate leptin circadian rhythm in diet-induced obese rats after a weight reduction caused temporary insulin resistance and hyperinsulinemia, and were ineffective in influencing food intake, BW, and fat content. Leptin resistance was evident following 1 week of treatment in this study. Leptin treatment had no effect on body fat content both short-term and long-term. Exogenous leptin treatment may, in the long run, increase leptin resistance in diet-induced obese animals. Hence, long-term leptin treatment may not be beneficial to obese individuals consuming a HF diet.

OVERWEIGHT and obesity are problems of epidemic proportions. According to the World Health Organization, more than 300 million people are obese worldwide.[1] In the United States, the Centers for Disease Control and Prevention recently released a report estimating that more than 64.5% of all adults are overweight and that 31% are obese.[2] Because obesity is associated with increased incidence of many chronic diseases,[3] weight loss has become an important health goal for the American population. Among American men and women, the prevalences of attempting to lose and maintain weight were 28.8% and 35.1% among men and 43.6% and 34.4% among women, respectively.[4] However, long-term maintenance of weight loss is difficult. Studies have reported that more than 30% of weight lost is regained within 1 year, with more weight being regained in subsequent years.[5] Repetition of this pattern, called weight cycling or yo-yo dieting, is a very common phenomenon in the United States.

In animal models, weight regain is typically enhanced in the initial weeks of refeeding following a weight reduction.[6,7] The rate of weight regain following weight reduction can be separated into 2 phases: (1) the critical period—defined as the first 2 to 3 weeks of initial weight regain, it is characterized by both an enhanced food intake and rate of weight gain compared to controls; and (2) the return phase—defined as the rate of weight regain which returns to that of the control, noncycling rats. With this in mind, we believed that if the rate of weight regain during the "critical" period is reduced, we may be able to alter both the final body weight and body composition of the rats.

Exogenous leptin administration is known to reduce food intake, increase energy expenditure, and therefore induces a reduction in body weight (BW) in animals.[8] In addition, because leptin also increases both fat lipolysis and oxidation while preserving lean body mass,[9] administering leptin during the critical period may also aid in attaining a more desirable body composition, as well as in altering final BW.

While exogenous leptin treatment has been viewed as a possible remedy for obesity in some populations, there is evidence against this. It may actually accelerate the onset of leptin resistance. In our previous study, a leptin infusion with implanted miniosmotic pumps for 2 weeks post-weight reduction reduced body fat content but had no effects on BW.[10] Martin et al[11] demonstrated in Long-Evans rats that leptin resistance was exhibited during the final 2 weeks of the study in a 28-day administration paradigm using a leptin infusion (mini-osmotic pumps). In addition, these investigators determined that there was a downregulation of both the leptin receptor mRNA and protein in the hypothalamus. Thus, administration of leptin via mini-osmotic pump may not be an ideal method for delivering leptin because it may accelerate the onset of leptin resistance by subjecting the animal to constant high levels of leptin. Also, it may mask the circadian rhythm of the endogenous leptin release from adipose tissue.

The purpose of this study was to determine whether leptin

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Table 1. Diet Composition of All Diets (g/kg)

	Low-Fat Control (LF)	High Fat (HF)	Modified HF (MHF)
Cornstarch	465.392	141.52	46.395
Casein	140	200	360
Maltose dextrin	100	80	40
Cellulose	50	65	65
Soybean oil	40	350	325
Tert-butyl-hydroquinone	0.008	0.07	0.065
Salt mixture (minerals)	35	45.5	90
Vitamin mix	10	13	26
Vitamin E	0.3	0.39	0.39
L-Cystine	1.8	3.9	3.9
Choline bitartrate	2.5	3.25	3.25
Caloric content (kcal/g)	3.81	5.24	4.89
% as protein	14.89	15.56	29.79
% as carbohydrate	75.66	24.34	10.35
% as fat	9.45	60.10	59.86

NOTE. All diets were commercially prepared by Dyets, Inc (Bethlehem, PA).

treatment during refeeding in diet-induced obese rats may reduce both the enhanced BW gain and food intake seen in weight-reduced animals upon refeeding. We also assessed short-term and long-term effects of exogenous leptin treatment.

MATERIALS AND METHODS

Animals

One hundred female Wistar rats (Harlan Sprague Dawley, Indianapolis, IN) were used in this study. Rats were 70 days old at the start of the study, and their average BW was 257.0 ± 1.9 g (mean ± SEM). Animals were housed in stainless-steel hanging cages with a modified 12-hour light/dark cycle (lights on at midnight and off at noon). Rats had free access to water at all times.

Diets

Rats were fed commercially prepared diets that were formulated according to that of the AIN-93M formula for the maintenance of adult animals.[12] The low-fat (LF) diet was 4% fat (wt/wt), while the high-fat (HF) diet was 35% fat (wt/wt), in order to produce obesity. During food restriction periods, rats were fed a modified HF diet, which was fortified with increased protein, vitamin, and mineral contents to assure proper nutrition during the weight loss period. The composition of the described diets is shown in Table 1.

Experimental Design

The paradigm of this study is depicted in Fig 1. One hundred female Wistar rats were divided into 5 groups: (1) LEP rats (n = 20) were fed a HF diet for 8 weeks to induce an obese state and were then food-restricted to 50% of ad libitum amount with a modified HF diet for 3 weeks to induce a 20% BW loss. They were then given leptin injections (see below) for 19 days concomitant with being refed the HF diet ad libitum to the end of the study (11 weeks). (2) SAL rats (n = 20) were treated in the same manner as LEP rats except they were given saline injections during refeeding; (3) PF (n = 20) rats were treated similarly as SAL rats except they were pair-fed with the LEP rats during refeeding; (4) HFC rats (n = 20) were fed the HF diet ad libitum for the entire 21-week study duration; and (5) LFC (n = 20) were fed a LF diet for the entire study. Ten rats from each group were killed after leptin treatment was discontinued. The remaining 10 rats from each group were killed at the end of the study. The rats were killed by brief exposure to carbon dioxide and then decapitated. Trunk blood was collected, and the plasma was separated and stored for hormone and substrate determinations. Retroperitoneal, omental fat pads, and all other visible fat were dissected out, weighed, and their sum was considered as internal fat. The carcass was eviscerated and was stored for chemical body composition analysis.

Leptin Administration

Recombinant rat leptin supplied in phosphate-buffered saline (PBS) was purchased from R & D Systems (Minneapolis, MN). Leptin was administered intraperitoneally in doses of 200 μg/kg BW, twice daily for 19 days. The injection times were kept constant, with the first injection timed at 1 hour before the onset of the dark cycle and the second injection at 4 hours before the onset of the light cycle. These times were selected to increase both the nadir and the peak in leptin circadian rhythm,[13] and hence, to suppress the increase in food intake seen in rats with the onset and end of the dark cycle. PBS injections were given to SAL and PF rats in accordance with leptin injections in the LEP rats.

General Measurements and Evaluation

BW was measured at least once per week. During the food restriction period, BW was monitored every other day. Food intake was also determined at least once per week for all animals. During leptin treatment, food was measured twice daily, once during the light cycle and once during the dark cycle. This was done in order to monitor whether the rats consumed more food during periods without exogenous leptin to compensate for the reduction in food intake seen with leptin administration. At baseline, at obese BW (peak) and at reduced BW, fasting blood samples were taken from the retro-orbital sinus (1.5 mL), and the acquired plasma was used for the measurement of the following: glucose (kit, Sigma Chemical Co, St Louis, MO), insulin (radioimmunoassay [RIA] kit, ICN Pharmaceuticals, Costa Mesa, CA), and leptin (rat RIA kit, Linco Research, St Louis MO). Blood samples obtained at death were also assayed for glucose, insulin, and leptin. Carcass composition was determined by the method as described by Jen et al.[14] Body fat was determined according to the method of Folch et al.[15] Carcass protein content was assayed using the method of Lowry et al.[16] Because all visible internal fat had been previously removed, the fat mass derived from carcass composition analysis was considered subcutaneous fat. Total fat mass was defined as the summation of internal and subcutaneous fat.

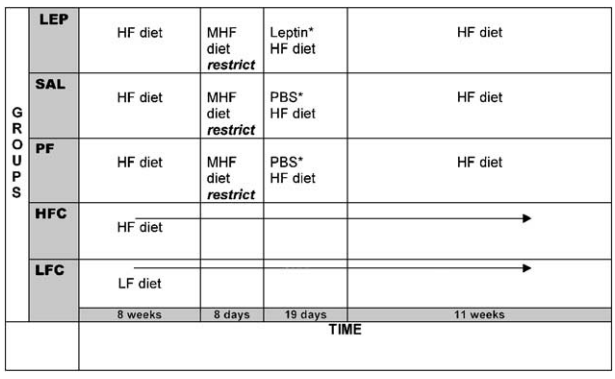

Fig 1. Experimental paradigm. *Leptin or PBS (phosphate-buffered saline) was administered to the respective groups by injection during the first 19 days of refeeding. Animals were fed ad libitum unless otherwise noted in bold italics. The entire study lasted approximately 22 weeks.

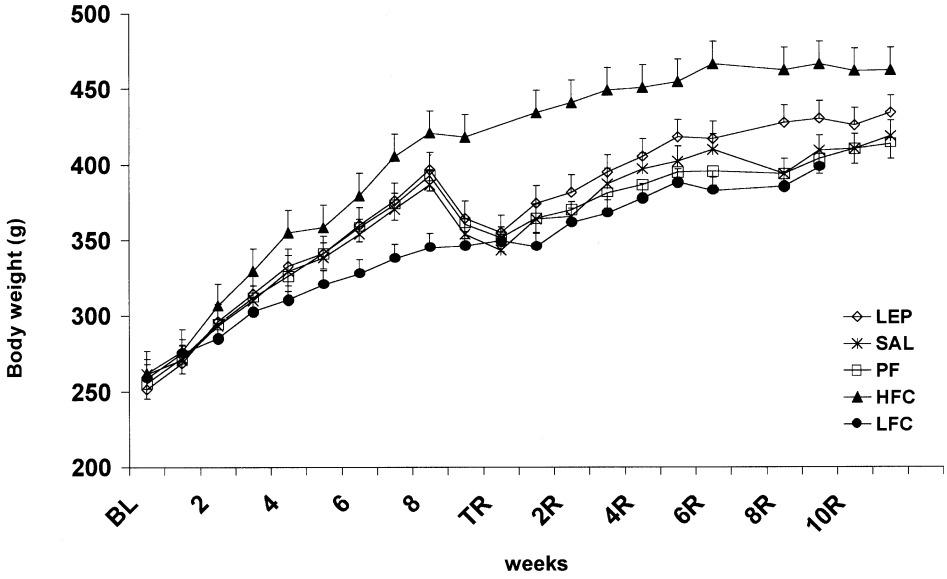

Fig 2. BWs of all rats throughout the entire study. BL, baseline; TR, BW trough; R, week of refeeding.

Bioelectrical Impedance Analysis

Body composition was estimated using bioelectrical impedance analysis (BIA; model 101, RJL Systems, Detroit, MI) at baseline, when rats reached obese BW, and when rats reached trough, according to the method of Hall et al.[17] Total body resistance, reactance, and the length between electrodes were obtained. The readings obtained at death and the final carcass composition analysis data were analyzed by regression analysis to establish an equation to estimate the body composition at each stage in the study. Based on regression analysis of total fat mass from chemical body composition analysis and BIA, the final equation was as follows: Fat Mass (g) = [0.539 · BW (g)] − [15.68 · (length)^2/resistance] − 92.638 (r = 0.851, $P < .0001$).

Statistics

Analysis of variance (ANOVA) or ANOVA with repeated measures were used to analyze data using SPSS (version 10.0, Chicago, IL). Significance level was set at $P < .05$. When significance was observed, post-hoc t tests (least significant difference) were performed to detect the groups that contributed to the overall difference.

RESULTS

Baseline

The mean BW of all rats was 254 ± 2 g (mean ± SE). HF and LF animals had similar fat contents and percents (g, HF: 28.9 ± 1.5; LF: 24.6 ± 3.5; %, HF: 10.7 ± 0.7; LF: 7.7 ± 0.7). HF and LF rats also had similar glucose (mmol/L, HF: 6.8 ± 0.6; LF: 6.2 ± 0.4), insulin (pmol/L, HF: 424.0 ± 68.2; LF: 377.4 ± 86.1), and leptin concentrations (μmol/L, HF: 14.2 ± 1.3; LF: 13.2 ± 1.1).

At Obese BW

After 8 weeks of HF feeding, all HF-fed rats weighed significantly more than the LF-fed rats (Fig 2; BW, g: HF: 395 ± 4; LF: 345 ± 7; $P < .01$). HF-fed rats had significantly more fat mass and percent than LF-fed rats (g, HF: 75 ± 6.0; LF: 55 ± 4, $P < .05$; %, HF: 20 ± 1; LF: 15 ± 0.8, $P < .0001$). HF-fed and LF-fed rats had similar glucose and insulin concentrations (data not shown). HF-fed rats had significantly higher leptin levels compared to LF-fed rats (μmol/L, HF: 26.8 ± 2.1; LF: 17.6 ± 2.9, $P < .05$).

At BW Trough

It took 11 days for the HF-fed animals (except those in the HFC group, which did not undergo weight reduction) to lose 20% of their obese BW. HF-fed rats had significantly less body fat mass compared to when they were at their obese weight (g, obese weight: 19.6 ± 1.0; trough: 12.2 ± 1.9, $P < .01$). HF-fed rats had leptin concentrations that were significantly reduced from their obese BW (15.0 ± 1.2 μmol/L, $P < .01$)

After Week 1 of Refeeding

During the first week of refeeding, LEP rats were given leptin injections, and SAL and PF rats were injected with PBS. HFC and LFC animals were not weight-reduced throughout the study. LEP, SAL, and HFC animals gained significantly more weight (Table 2, $P < .05$) than LFC rats while HFC and PF rats gained a similar amount of weight. More importantly, LEP and SAL rats gained the same amount of weight during this time, despite LEP rats receiving leptin treatment.

Immediately upon refeeding, weight-reduced rats were hyperphagic and consumed more food during the light cycle in day one of refeeding compared to the dark cycle of the same day. Despite this hyperphagia, the total food intake on day one of refeeding was no different from subsequent days of that week. LEP animals consumed significantly less than SAL animals during most days of this week (days 1, 3, 5, and 7, $P < .05$). LEP food consumption was affected only during the dark cycle for these days, when the exogenous leptin was still effective.

LEP rats consumed significantly less food than SAL and HFC rats (data not shown, $P < .05$). LEP and PF rats consumed significantly more energy compared to LFC rats ($P < .001$). LEP and SAL rats had significantly higher feeding efficiency (g

Table 2. Body Weight Gain, Energy Intake, and Feeding Efficiencies of All Rats During the Time Coinciding With Leptin Treatment

Group	Time		
	Week 1	Week 2	5 Days*
BW gain (g)			
LEP	19.3 ± 2.2[a]	13.6 ± 1.9	10.2 ± 3.3
SAL	22.0 ± 2.9[a]	7.8 ± 2.7	5.9 ± 2.1
PF	8.6 ± 2.2[bc]	8.5 ± 2.1	6.3 ± 1.4
HFC	16.6 ± 7.0[ab]	8.8 ± 2.2	4.0 ± 1.4
LFC	3.2 ± 2.3[c]	12.2 ± 2.1	4.6 ± 1.5
P value	.001	.229	.326
Energy intake (kJ)			
LEP	2.63 ± 1.10[b]	2.83 ± 0.67[a]	2.03 ± 0.64[ab]
SAL	3.01 ± 1.32[a]	2.68 ± 0.94[a]	1.98 ± 0.80[b]
PF	2.65 ± 0.81[b]	2.74 ± 0*[a]	2.06 ± 0*[ab]
HFC	3.21 ± 0.79[a]	2.93 ± 1.03[a]	2.18 ± 0.95[a]
LFC	2.03 ± 0.73[c]	2.29 ± 0.70[b]	1.55 ± 0.52[c]
P value	<.0001	<.0001	<.001
Feeding efficiency (g BW gained per kJ of intake)			
LEP	0.71 ± 0.09[a]	0.51 ± 0.06	0.36 ± 0.11
SAL	0.71 ± 0.09[a]	0.31 ± 0.11	0.25 ± 0.10
PF	0.31 ± 0.08[bc]	0.31 ± 0.07	0.28 ± 0.06
HFC	0.49 ± 0.22[ac]	0.28 ± 0.08	0.19 ± 0.07
LFC	0.13 ± 0.12[bc]	0.55 ± 0.09	0.27 ± 0.09
P value	.002	.061	.823

NOTE. All values are means ± SEM. Values within each column with different superscripts are significantly different from each other ($P < .05$).
*Last 5 days of leptin treatment.

BW gained per kJ of energy intake) than PF and LFC rats ($P < .05$).

After Week 2 of Refeeding

During week 2 of refeeding, LEP rats gained nonsignificantly more weight than rats from all other groups, and there were no differences in BW gain among all groups. LEP rats weighed significantly more than LFC rats (Fig 2, $P < .05$) but weighed similarly to SAL and PF rats. HFC rats weighed the most of all groups (Fig 2, $P < .05$). Daily food consumption was no longer affected by leptin treatment (Table 2). LEP, SAL, PF, and HFC groups had similar energy intake, which were all higher than the LFC group.

During Week 3 of Refeeding

At the end of the last 5 days of leptin treatment, the 5 groups were similar in weight gain and feeding efficiency. The LEP, SAL, PF, and HFC groups had similar energy intake and all were significantly higher than that of the LFC group.

Other Observations During Leptin Treatment

The total food intake during leptin treatment was no different between LEP and SAL rats. Over the first 3 weeks of refeeding, LEP rats consumed similar amounts of food each week (weekly food intake in g, week 1: 119.8 ± 5.0; week 2: 129.1 ± 3.0; week 3: 121.1 ± 5.3; difference not significant [NS]). On the contrary, SAL rats consumed significantly more food during the first week of refeeding compared to weeks 2 and 3 (weekly food intake in g, week 1:137.5 ± 6.0; week 2: 122.2 ± 4.3; week 3: 116.3 ± 4.4, $P < .05$).

After Leptin Treatment

After leptin treatment was discontinued, weekly food intake did not differ between LEP and SAL rats (data not shown) from the first week post-treamtent until the end of the study period.

After 19 days of leptin treatment (~3 weeks of refeeding), LEP rats weighed signficantly more than the PF rats (Table 3, $P < .0001$). LEP, SAL, and LFC animals were similar in BW, while SAL rats weighed similarly to both PF and LFC rats. HFC rats weighed significantly more than rats from all other groups ($P < .05$).

LEP, SAL, PF, and LFC rats were similar in internal, subcutaneous, and total fat contents, and in total fat percentage. HFC rats had the most fat in each fat pad measured and had the highest total fat percentage compared to all other groups ($P < .0001$ for all). LEP, HFC, and LFC rats had similar percentages of carcass protein, which was signficantly less than that of SAL and PF rats ($P < .05$). The ratio of carcass protein/fat was calculated to compare protein and fat composition in the rats, with low ratios meaning a high percentage of fat compared to percent of carcass protein (Table 3). HFC rats had the lowest ratios compared to all other groups ($P < .01$), but the value was similar to LFC rats.

Rats from all groups except HFC had similar glucose concentrations. LEP and PF rats had significantly lower plasma glucose than HFC rats (Fig 3, $P < .05$). LEP animals had plasma insulin levels that were significantly higher than SAL, PF, and LFC rats ($P < .05$) but were similar to HFC rats. SAL rats had significantly lower insulin levels compared to all groups ($P < .05$) except for the PF rats. In addition, LEP rats

Table 3. Body Composition of All Rats After 19 Days of Leptin Treatment and at Death

Group	BW (g)	Internal Fat (g)	Subcutaneous Fat (g)	Total Fat (g)	Total Fat %	% Carcass Protein	Protein-to-Fat Ratio
After 19 days of leptin treatment							
LEP	393.1 ± 11.2^{b}	34.6 ± 3.6^{a}	42.5 ± 4.5^{b}	77.1 ± 7.6^{b}	19.3 ± 1.5^{b}	13.80 ± 1.26^{a}	0.82 ± 0.12^{bc}
SAL	371.0 ± 14.1^{bc}	32.2 ± 3.9^{a}	34.6 ± 5.1^{b}	67.2 ± 8.5^{b}	17.6 ± 1.5^{b}	17.76 ± 1.30^{b}	1.03 ± 0.11^{b}
PF	363.0 ± 8.6^{c}	29.4 ± 1.7^{a}	36.9 ± 2.9^{b}	65.6 ± 3.8^{b}	18.1 ± 0.9^{b}	18.81 ± 1.60^{b}	1.06 ± 0.11^{b}
HFC	448.3 ± 10.7^{a}	58.6 ± 3.4^{a}	62.8 ± 4.8^{a}	124.8 ± 6.5^{a}	27.3 ± 1.2^{a}	14.4 ± 2.9^{a}	0.35 ± 0.09^{a}
LFC	377.0 ± 12.4^{bc}	35.4 ± 3.7^{b}	34.8 ± 5.5^{b}	71.6 ± 8.8^{b}	18.8 ± 2.0^{b}	11.94 ± 0.38^{a}	0.58 ± 0.06^{ac}
P value	<.0001	<.0001	<.0001	<.0001	<.0001	.001	.001
At death (after 11 weeks of refeeding)							
LEP	425.5 ± 14.1^{ac}	51.9 ± 2.7^{ac}	53.4 ± 4.2	107.6 ± 6.2	24.5 ± 1.1	10.40 ± 1.82^{a}	0.40 ± 0.07^{b}
SAL	413.7 ± 14.0^{ab}	46.2 ± 4.1^{ab}	51.3 ± 5.5	101.9 ± 9.6	23.8 ± 1.8	18.33 ± 2.73^{b}	0.82 ± 0.18^{a}
PF	409.7 ± 7.9^{bc}	45.1 ± 2.7^{bc}	46.4 ± 2.0	91.7 ± 4.8	22.3 ± 0.7	16.26 ± 1.41^{b}	0.75 ± 0.05^{a}
HFC	455.2 ± 12.9^{a}	54.8 ± 3.3^{a}	56.7 ± 3.7	109.6 ± 6.6	24.6 ± 0.08	12.69 ± 0.14^{ab}	0.51 ± 0.06^{ab}
LFC	382.0 ± 14.8^{b}	38.4 ± 2.9^{b}	48.1 ± 5.3	86.5 ± 7.9	22.4 ± 1.4	16.47 ± 1.15^{b}	0.68 ± 0.08^{a}
P value	.032	.019	.498	.113	.505	.001	.019

NOTE. All values are means ± SEM. Values within each row with different superscripts are significantly different from each other.

had significantly higher insulin/glucose (IG) ratios compared to SAL and PF rats ($P < .05$) but were similar to HFC and LFC rats.

LEP rats had signficantly higher plasma leptin levels than that of SAL and PF rats (Fig 4, $P < .05$). HFC animals had the highest plasma leptin levels. The leptin treatment elevated the LEP plasma leptin levels so that the leptin/fat mass ratio was similar to HFC rats (Fig 4). LEP rats had significantly higher leptin/fat mass ratios compared to SAL, PF, and LFC rats ($P < .05$). HFC and LFC rats had similar leptin/fat mass ratios.

Eight Weeks Post-leptin Treatment

At the end of the study (8 weeks post-leptin treatment, following 11 weeks of refeeding), HFC rats weighed significantly more than PF and LFC rats (Table 3, $P < .05$). LEP rats weighed significantly more ($P < .05$) than LFC rats but weighed similarly to rats from all other groups.

LEP, SAL, and PF rats were no different in BW and internal fat content. Also, LEP and SAL animals weighed similarly to HFC rats. SAL and PF rats weighed similarly to LFC rats. There were no differences among all groups for subcutaneous fat (g), total fat (g), and total fat percentage. LEP rats had significantly more internal fat than LFC rats. LEP rats had a significantly smaller carcass protein percentage compared to SAL, PF, and LFC rats ($P < .05$) but was similar to HFC rats. LEP rats had a further reduction in protein/fat ratios, but these were similar to that of HFC rats ($P < .05$).

Many of the metabolic differences that were observed im-

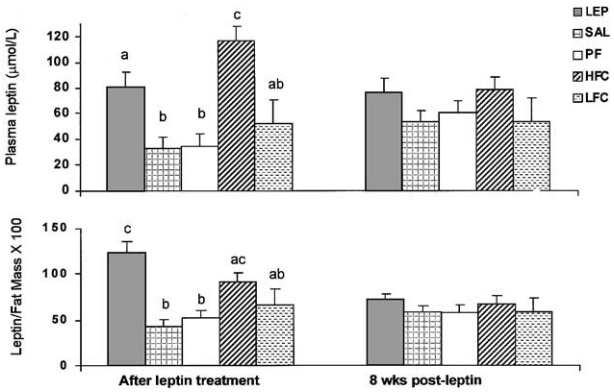

Fig 3. Plasma leptin levels (top) and leptin/fat mass% (bottom) after day 19 of leptin treatment and at the end of the study period. Columns with different letters within each time period are significantly different from each other ($P < .05$).

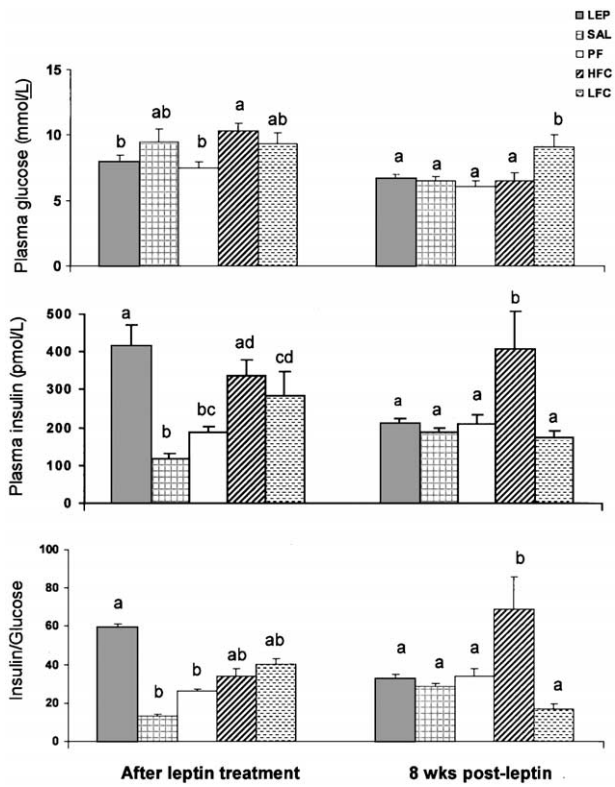

Fig 4. Plasma glucose levels (top), insulin (middle), and insulin/glucose ratios (bottom) after day 19 of leptin treatment and at the end of the study period. Columns with different letters within each time period are significantly different from each other ($P < .05$).

mediately after the leptin treatment period disappeared. LFC rats had significantly higher glucose levels compared to all other groups (Fig 3, $P < .05$), while all HF fed animals had similar glucose levels. HFC rats had both significantly higher insulin levels and IG ratios compared to all other groups (both $P < .05$). LEP, SAL, PF, and LFC rats all had similar insulin levels and IG ratios. There were no differences among all groups in plasma leptin levels and leptin/fat mass ratios. Leptin levels at sacrifice were positively correlated with all fat pad weights (retroperitoneal: $r = 0.622$, $P < .0001$; omental: $r = 0.657$, $P < .001$; subcutaneous: $r = 0.785$, $P < .0001$), total body fat ($r = 0.761$, $P < .0001$), and insulin ($r = 0.280$, $P < .05$).

DISCUSSION

Leptin injections given to weight-reduced, diet-induced obese rats during refeeding does not prevent weight regain and alter BW and composition compared to animals not receiving leptin. The anorectic effect of leptin was transient during refeeding, and by the end of the study, leptin-treated animals were similar in BW and fat percentage to their HF-fed counterparts. The current study also indicates that weight reduction does not make diet-induced obese rats more leptin-responsive than weight-steady animals. In addition, exogenous leptin administration caused rats to have significantly higher insulin levels and insulin/glucose ratios compared to rats of all other groups. It also elevated leptin to fat mass ratios to that of the high-fat ad libitum control (HFC) rats. Thus, exogenous leptin injections induced leptin resistance, in addition to insulin resistance, in obese weight-reduced rats fed a HF diet.

Insulin resistance has been reported to cause weight loss or prevent weight gain. Eckel[18] hypothesized that an insulin-resistant state changes the metabolism in a way that would prevent additional weight gain, and others also support this notion.[19-21] In contrast, leptin resistance acts to promote future BW gain. Chu et al[22] reported that the elevated leptin levels observed among overweight adult American men indicated leptin resistance, although it is difficult to determine whether the leptin resistance preceded or followed the obese condition.[23] The current finding suggests that the leptin treatment caused both a temporary insulin resistance and leptin resistance, acting to "cancel" each other out and resulting in very little change in BW compared to other weight-reduced animals.

In a previous study, we observed that diet-induced obese rats that have been weight-reduced responded similarly physiologically to that observed prior to obesity.[24] With this in mind, it was expected that weight reduction in obese rats would make the animals more leptin-sensitive than without a weight reduction. In the current study, it is apparent that despite this weight loss, the sensitivity to the exogenous leptin was only transient. This insensitivity may be due to 2 factors: the obese state of the rats and the diet composition. Numerous investigators have reported that obese animals and humans[25,26] are hyperleptinemic. While administering exogenous leptin can remedy the obese state of the genetically obese *ob/ob* mice, this is not the case in diet-induced obese animals. Because of this obese and hyperleptinemic state seen at peak body weight in the current study, exogenous leptin treatment may increase the self-limiting of the leptin influx to the brain and may therefore make it more difficult to bind to receptors within the hypothalamus.

The HF diet may have also been a factor in the observed leptin resistance. Normally, exogenous leptin administration to animals causes a decrease in food intake and an increase in energy expenditure to result in BW loss.[27,28] In contrast, Widdowson et al[29] and Lin et al[30] clearly demonstrated that a HF diet diminishes the response to central and peripheral leptin treatment, respectively. The current study further supports this notion. The peripheral leptin treatment administered to HF-fed animals caused only a brief and transient effect on food intake but affected plasma insulin levels and body composition.

The high blood leptin levels seen in obesity are unable to affect food intake. Thus, the ratio of cerebrospinal fluid levels to blood leptin levels is decreased in obese rats and humans.[31,32] Increased adiposity appears to affect leptin transport across the blood-brain barrier by self-inhibition of the transport system.[33] Banks et al[34] confirmed that adipose tissue mass plays a major factor in decreasing the entry of leptin into the brain, regardless of aging or of increased lean body mass. While it is apparent that the animals in the current study were leptin-resistant peripherally, we did not determine whether the animals were centrally resistant. Even so, it is possible that the adiposity coupled with exogenous leptin treatment, which adds to the self-inhibition of the central system, could have played a role in further exacerbating central leptin resistance. Further study is warranted to investigate this phenomenon.

Cha et al[13] found that a HF diet abolished the normal, diurnal variation of plasma leptin concentrations. Obese subjects have lower circadian amplitute of plasma leptin compared to their lean counterparts.[35,36] Without the distinct diurnal excursions in leptin levels, animals may feed continuously throughout their entire day. This impairment in leptin action may eventually lead to obesity. In addition, the consumption of HF diets alone can significantly increase leptin levels independent of body composition.[13,37] HF-fed, diet-induced obese rats were used for the current study, and when the rats reached their obese BW, they had elevated leptin levels exhibiting impaired leptin action. Thus, augmenting leptin levels according to circadian rhythm during this study may have been ineffective to create the conditions conducive to suppressing food intake and reduce BW and fat content.

We hypothesized that exogenous leptin administration given via intraperitoneal injection to augment the circadian rhythm may be more effective than using a mini-osmotic pump in reducing BW gain because it may not cause leptin resistance as quickly and may also be able to elicit the benefits of leptin (ie, reduction in BW and fat) before the onset of resistance. Unfortunately, this was not so. While the leptin treatment in this study caused changes in food intake, it did not cause changes in body weight and eventually had no effect by the second week of treatment. Intraperitoneal injections of leptin for 48 hours has been shown to cause a several-fold increase in mRNA encoding the suppressors of leptin/cytokine signaling in both the hypothalamus and peripheral tissues.[38] Both intracerebroventricular and intraperitoneal leptin treatment, coupled with HF diet feeding, caused reductions in STAT3 hypothalamic signaling in C57Bl/6J mice.[39] Under normal conditions, exogenous leptin treatment induces signaling through the STAT3

pathway in order to elicit the overall effect of a reduction in food intake. Hence, it appears that exogenous leptin administration efficacy diminishes over time when coupled with HF feeding.

An unusual finding in this study was the fact that LFC and HFC rats had similar body fat percents after almost 21 weeks of consumption of their respective diets ad libitum, even though HCA rats consumed significantly more calories than LFC rats. The LF diet was 4% soybean oil (wt/wt) and was 9.45% fat by calorie. Soybean oil comprises 24% oleic acid and 54% linoleic acid.[40] Ikemoto et al[40] observed that mice fed diets made with soybean oil as a fat source weighed significantly more and had significantly more body fat than mice fed isocaloric diets of palm oil, lard, rapeseed oil, safflower oil, perilla oil, or fish oil. Soybean oil may promote fat gain compared to other fat sources. Examing the BW data in detail, it was observed that 3 of 9 of the LF-fed rats were significantly heavier and fatter than the rest of their group. We speculate that some rats may be more sensitive to the weight- and fat-promoting effect of soybean oil and may therefore gain more weight than the other rats. This may mask the difference in BW between HF- and LF-fed rats.

In summary, this study demonstrates that exogenous leptin administration efficacy diminishes over time when coupled with HF feeding. Bolus leptin injections to manipulate leptin circadian rhythm were ineffective in influencing food intake, BW, and body fat content. Leptin treatment may be most effective in a low-dose infusion,[10] rather than in bolus injections due to an acute effect on energy intake and BW regain. However, as shown in both this study and in others, exogenous leptin treatment may, in the long run, increase leptin resistance in diet-induced obese animals. Hence, this may imply that long-term leptin treatment may not be beneficial to obese individuals consuming a HF diet.

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