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Effect of Fasting, Refeeding, and Dietary Fat Restriction on Plasma Leptin Levels*

DAVID S. WEIGLE, P. BARTON DUELL, WILLIAM E. CONNOR, ROBERT A. STEINER, MICHAEL R. SOULES, AND JOSEPH L. KUIJPER

Departments of Medicine (D.S.W.) and Obstetrics and Gynecology (R.A.S., M.R.S.), University of Washington, Seattle, Washington 98195; Department of Medicine, Oregon Health Sciences University (P.B.D., W.E.C.), Portland, Oregon 97201; and ZymoGenetics Corporation (J.L.K.), Seattle, Washington 98102

ABSTRACT

The factors responsible for the variability in plasma leptin levels observed among individuals with similar body compositions remain unclear. To examine the impact of dietary variables, we compared the changes in leptin levels induced by fasting and dietary fat restriction with the expected decrease following a significant loss in adipose mass. A 21.4 ± 3.7% weight loss led to a 76.3 ± 8.1% decrease in mean plasma leptin level (25.2 ± 9.3 to 6.1 ± 3.4 ng/mL, $P = 0.0001$) in a group of 9 obese males. Despite a weight loss of only 2.6 ± 0.8%, mean plasma leptin levels fell by 61.9 ± 25.2% (8.5 ± 4.5 to 2.4 ± 0.5 ng/mL, $P < 0.01$) in 7 nonobese females subjected to 3 days of fasting. Leptin levels in fasted subjects returned to baseline within 12 h of refeeding. Individual high- and low-fat meals given to 19 subjects after an overnight fast had no effect on plasma leptin levels. Reduction in dietary fat content from 37–10% of total calories for 7 weeks was also without effect on plasma leptin levels in these subjects. We conclude that plasma leptin levels primarily reflect total adipose mass, rather than meal consumption or dietary energy source, but that the reduction in leptin levels with ongoing fasting is disproportionate to the reduction in adipose mass. The ability of fasting to deactivate this presumed physiological satiety system may have been advantageous in environments characterized by rapid changes in food availability. (*J Clin Endocrinol Metab* **82:** 561–565, 1997)

LEPTIN has been postulated to be the primary afferent signal by which adipose tissue communicates with the central nervous system (CNS) for the purpose of regulating body composition (1–4). Although this hypothesis is supported by the positive relationship between plasma leptin levels and total adipose mass, the large variation in leptin levels among individuals with similar body compositions suggests that other factors modulate leptin secretion (5–9). One plausible modifier of leptin secretion is the individual's recent dietary history. Dietary variables of potential importance include duration of antecedent fasting, interval between a meal and leptin determination, fat content of a meal, and habitual dietary fat content. The latter variable is of particular interest, because reduction of dietary fat without enforced caloric restriction has been reported to lead to weight loss in human subjects (10–12). It is conceivable that this effect of dietary fat reduction is caused by enhancement either of leptin secretion or leptin sensitivity at any given total adipose mass. To investigate the impact of dietary manipulation on plasma leptin levels, we studied the effect of a 3-day fast and refeeding, low- and high-fat test meals, and sustained reduction in dietary fat intake in weight stable adult human subjects. These results were compared with the effect of a significant loss in body fat induced by caloric restriction in a group of obese men.

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Address all correspondence and requests for reprints to: David S. Weigle, Division of Endocrinology, Box 359757, Harborview Medical Center, 325 Ninth Avenue, Seattle, Washington 98104. E-mail: weigles@zgi.com.

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Subjects and Methods

Subjects

Nine healthy obese male volunteers between the ages of 37 and 62 yr (mean age 51 ± 9 yr) participated in the weight loss portion of these studies. No subject used tobacco or was actively attempting to reduce his body weight through caloric restriction, exercise, or medication before enrollment. Subjects were admitted to a metabolic ward for weight stabilization on a liquid formula diet that was adjusted over a 10- to 12-day observation period to precisely meet daily caloric needs (13). This diet supplied 15% of total daily calories as protein, 40% as fat, and 45% as carbohydrate. Body fat content was measured by hydrodensitometry as described previously (13), and plasma was obtained for leptin measurement in this baseline weight-stable state at 0800 h after an overnight fast. All subjects were then placed on an outpatient 700-kcal liquid diet supplying 30% of total daily calories as protein and 70% of calories as carbohydrate (13). Subjects consumed this diet for a mean of 95 days (range 74–110 days) resulting in a 19–25% weight loss. Following weight loss, caloric intake was increased to match estimated daily energy expenditure, and subjects were readmitted to the metabolic ward for 10–12 days for repeat weight stabilization, body fat determination, and plasma collection at the end of the stabilization period.

The effect of short-term fasting on plasma leptin levels was evaluated in a group of seven healthy weight-stable women between the ages of 27 and 32 yr (mean age 29 ± 2 yr) who were within 10% of ideal body weight as determined by the 1980 Metropolitan Life Insurance Company Tables (14). The mean weight of the subjects was 65.9 ± 7.7 kg, and their mean body mass index (BMI) was 22.9 ± 1.9 kg/m^2. Subjects were admitted to a metabolic ward for 5 consecutive days during the follicular phase of their menstrual cycle. During the admission (control) day, each subject ate three meals and an evening snack for a total of 2200 kcal (18.5% protein, 30% fat, and 51.5% carbohydrate). The same day at 2000 h, each subject began a 72-h fast ending at 2000 h of day 4. During the fasting days, subjects were allowed to drink only water and herb tea without restriction. Adherence to the fast was verified by daily deter-

minations of plasma glucose and insulin levels as well as urinary ketone measurements. Subjects were given a meal at 2000 h on day 4, and on the 5th (refeeding) day, they were fed as on the control day. Blood was sampled every 10 min from 0800–0900 h, 1330–1430 h, and 1900–2000 h during the control and refeeding days, as well as on day 3 of the fast. A pool of five plasma samples (equal aliquots) from each of these 1-h intervals was assayed for leptin as described below.

The effect of dietary fat manipulation on serum leptin levels was evaluated in a group of 19 weight stable men and women between the ages of 23 and 73 yr (mean age 47 ± 13 yr, male/female ratio = 11:8). Subjects received a high-fat diet (37% of calories from fat, 48% of calories from carbohydrate) for 5 weeks and a graded very low-fat diet for 7 weeks. The very low-fat diet consisted of a 30% fat diet for 10 days, a 20% fat diet for 10 days, and a 10% fat 75% carbohydrate diet for the subsequent 4 weeks. Protein comprised 15% of calories in both diets. The two dietary phases were separated by a 4- to 8-week washout period on an unsupervised diet. Diets were prepared in a metabolic kitchen and administered in random order. Subjects were weighed 6 days/week, and energy intakes were adjusted as necessary to achieve weight stability. At the end of week 5 of the high-fat diet after a 12 h overnight fast, plasma was drawn for leptin and insulin determinations, and a test meal containing 37% fat and 50% of total daily caloric intake was given. Plasma was drawn 4, 5, and 6 h postprandially and pooled for leptin and insulin measurements. At the end of week 7 of the low-fat diet after a 12-h overnight fast, plasma was drawn for leptin and insulin determinations, and a test meal containing 10% fat and 50% of total daily caloric intake was given. Plasma was drawn 4, 5, and 6 h postprandially and pooled for leptin and insulin measurements. Plasma insulin determinations were performed with a commercially available RIA (Diagnostic Products Corp., Los Angeles, CA). All protocols for human subjects were approved by either the University of Washington or Oregon Health Sciences University Human Subjects Review Committees.

Leptin measurements were made with a commercially available RIA kit based on a polyclonal antiserum raised against full-length recombinant human leptin (Linco, St. Charles, MO). The interassay coefficient of variation was 11.9%, and the intraassay coefficient of variation was 4.8%. Recovery of recombinant leptin added to human serum was 91.7 ± 5.1% at 2 ng/mL, 97.6 ± 4.2% at 4 ng/mL, and 105.5 ± 4.9% at 10 ng/mL.

Statistical analyses

Each subject served as his or her own control for the effects of weight loss, fasting, or dietary manipulation on plasma leptin levels. ANOVA, paired *t* tests, and Pearson correlation coefficients were used to compare mean leptin levels and other continuous measures by intervention. All data are expressed as mean ± SD, unless noted otherwise, with a significance level of 0.05.

Results

Significant reductions in total body mass and adipose mass were achieved in the nine male subjects who participated in this portion of the study (Table 1). This change in body composition was accompanied by a 76.3 ± 8.1% reduction in the mean plasma leptin level ($P = 0.0001$). The mean daily weight change of each subject was computed during the in-patient weight stabilization periods at baseline and reduced body weights to verify that energy balance was actually achieved at the time of leptin determinations. As shown in Table 1, group mean daily weight changes were not significantly different from zero or from each other in the baseline and reduced states.

The effect of a 3-day fast followed by refeeding on plasma leptin levels is shown in Fig. 1. Compliance with the fast was established by decreases in the 0800 h plasma insulin level from 9.9 ± 4.8 to 5.1 ± 2.2 μU/mL ($P < 0.0005$), the plasma glucose level from 71.1 ± 11.0 to 44.8 ± 15.6 mg/dL ($P < 0.0005$), and a qualitative increase in first void urinary ketones from the 1st to the 3rd day of the fast. Leptin levels measured on day 3 of the fast were lower than those measured during the control ($P < 0.01$) and refeeding ($P < 0.02$) days at all time points studied, whereas the levels on the control and refeeding days were indistinguishable from each other. The mean 0800 h plasma leptin level on day 3 of the fast was also lower than that observed in the reduced obese men (2.4 ± 0.5 *vs.* 6.1 ± 3.4 ng/mL, $P = 0.014$) despite the fact that the fasted nonobese subjects experienced only a 1.7 ± 0.6 kg (2.6 ± 0.8%) weight loss over the course of the fast. Figure 1 also clearly demonstrates a diurnal variation in plasma leptin levels with the 1900–2000 h values significantly exceeding the 0800–0900 h values on the control (13.2 ± 5.3 *vs.* 8.5 ± 4.5 ng/mL, $P = 0.0037$), fasting (3.1 ± 1.0 *vs.* 2.4 ± 0.5 ng/mL, $P = 0.047$), and refeeding (12.5 ± 6.5 *vs.* 8.5 ± 5.6 ng/mL, $P = 0.0057$) days.

All subjects tolerated the reduction in dietary fat from 37–10% of total calories for the full duration of this portion of the study. Body weight and BMI at the end of the low fat dietary phase were only slightly lower than at the end of the high fat phase (87.5 ± 13.7 *vs.* 88.5 ± 13.9 kg, $P = 0.04$; 29.4 ± 3.6 *vs.* 29.7 ± 3.5 kg/m^2, $P = 0.04$). The mean fasting plasma leptin level at the end of the low-fat phase (14.5 ± 14.9 ng/mL) was insignificantly lower than that observed at the end of the high-fat phase (15.6 ± 15.2 ng/mL, $P = 0.15$). The test meals given at the end of the low- and high-fat dietary phases produced significant increases in plasma insulin levels over fasting values (low fat: 10.6 ± 5.9 *vs.* 180 ± 84 μU/mL, $P < 0.001$; high fat: 12.2 ± 9.9 *vs.* 132 ± 89 μU/mL, $P < 0.001$). Mean postprandial leptin levels did not differ from fasting leptin levels following either the low-fat (14.5 ± 15.6 *vs.* 14.5 ± 14.9 ng/mL, $P = 1.0$) or the high-fat (16.0 ± 15.6 *vs.* 15.6 ± 15.2 ng/mL, $P = 0.29$) test meals. A plot of fasting plasma leptin levels of individual subjects at the end of the low-fat dietary phase against the levels observed at the end of the high-fat phase (Fig. 2A) demonstrates the lack of effect of chronic dietary fat reduction across a wide range of baseline leptin levels. Similar plots of fasting *vs.* postprandial leptin levels for both low- (Fig. 2B) and high-fat (Fig. 2C) test

TABLE 1. Comparison of subjects before and after weight loss (n = 9)

	Baseline	Reduced	Reduction (%)	*P*-value
Weight (kg)	117.0 ± 15.3	91.9 ± 12.5	21.4 ± 3.7	0.0001
BMI (kg/m^2)	35.7 ± 2.9	28.0 ± 2.3	21.4 ± 3.7	0.0001
Percent fat	33.0 ± 4.8	23.9 ± 6.5	28.5 ± 11.5	0.0001
Stability (g/day)[a]	−14.4 ± 33.8	−17.2 ± 19.1		NS
Leptin (ng/mL)	25.2 ± 9.3	6.1 ± 3.4	76.3 ± 8.1	0.0001

Values shown are mean ± SD.

[a] Mean of daily weight variations of subjects determined by regression analysis during admissions for weight stabilization.

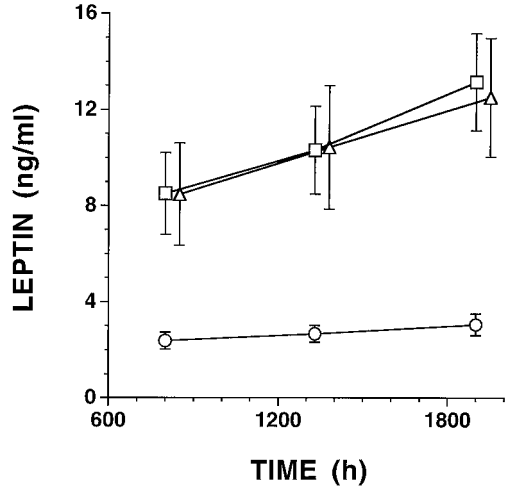

FIG. 1. Plasma leptin levels *vs*. clock time in seven female subjects studied during control (□), 3rd day of fasting (○), and refeeding (△) days. *Bars* represent SE.

meals reveals the complete absence of an effect of meal consumption on circulating leptin.

The changes in plasma leptin levels induced by weight loss, fasting, and dietary fat reduction are plotted against percent weight loss in Fig. 3. The results of the only comparable previously published weight loss study are also included in Fig. 3. It can be seen that the reduction in overnight fasted plasma leptin level following an intervention was proportional to the induced weight change. In contrast, the reduction in plasma leptin level following 3 days of fasting exceeded the decrease expected on the basis of the observed weight loss.

Discussion

If circulating leptin acts as a primary indicator to the CNS of total body energy content, the plasma leptin level should decrease after a major reduction in adipose mass, as our data and those of others indicate (5, 6). We observed a 76.3 ± 8.1% decrease in the mean plasma leptin level with a 21.4 ± 3.7% weight loss in nine obese men as compared with the 53% decrease observed by Considine and coworkers (6), with a 10% weight loss in a comparably obese group composed of six women and one man (Fig. 3). These results establish that the reduction in leptin level is proportional to the reduction in adipose mass and suggest that the system behaves in a similar fashion in men and women. The greater reduction in mean plasma leptin level that we observed was not caused by ongoing negative energy balance, because our subjects were refed to accurately stabilize body weight for 10–12 days before measuring their leptin levels.

The most extreme example of ongoing negative energy balance, a total fast, resulted in a decrease in plasma leptin levels that greatly exceeded the reduction expected on the basis of the weight loss observed in subjects over the course of 3 days (Fig. 3). The first plasma sample obtained 12 h after the meal that ended the fast revealed a leptin level indistinguishable from that observed on the control day, supporting the impression that the decrease in leptin with fasting was

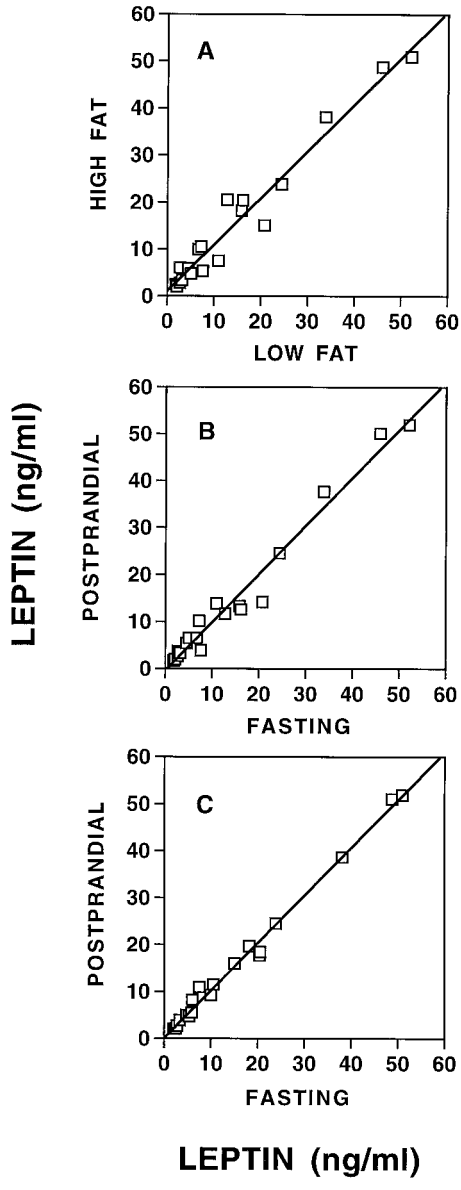

FIG. 2. A, Fasting plasma leptin levels of subjects studied at end of 37% (high-fat) and 10% (low-fat) dietary fat interventional periods. Slope of regression = 1.000 (r = 0.979, $P = <0.001$). B, Pooled 4-, 5-, and 6-h postprandial plasma leptin levels *vs*. fasting plasma leptin levels in subjects receiving a 10% fat test meal. Slope of regression = 1.029 (r = 0.985, $P = <0.001$). C, Pooled 4-, 5-, and 6-h postprandial plasma leptin levels *vs*. fasting plasma leptin levels in subjects receiving a 37% fat test meal. Slope of regression = 1.016 (r = 0.996, $P = <0.001$).

rapidly reversible and related to factors other than the small change in body composition sustained by the subjects. Although no human studies to date have examined the effect of more than 12 h of fasting, rodent studies have demonstrated similarly dramatic decreases in adipose tissue leptin messenger RNA levels after 48 or 72 h of fasting (15–17). Our results could be explained by the sustained decrease in mean circulating insulin level induced by several days of fasting, as suggested by the observation that withdrawal of insulin from 3T3-F442A adipose cells for 24 h results in a dramatic decrease in leptin messenger RNA content (18). In support of

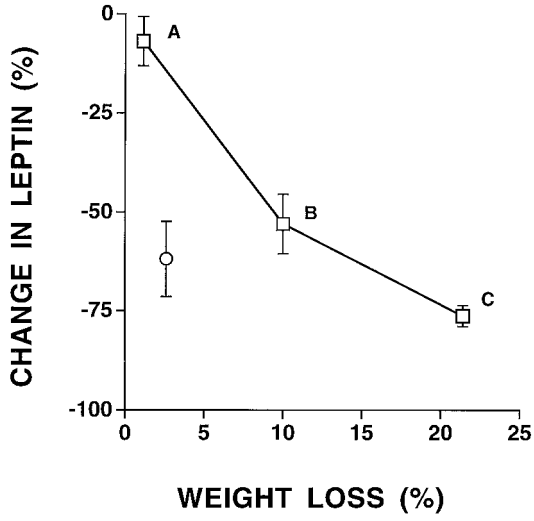

FIG. 3. Change in plasma leptin levels *vs.* percent weight loss in subjects fasted overnight (□) or 7 subjects fasted for 3 days (○). Point *A* represents mean difference in leptin level measured after a reduction in dietary fat content from 37% to 10% of total calories in 19 subjects. Point *B* represents mean difference in leptin level following a 10% weight loss in 7 subjects (6). Point *C* represents mean difference in leptin level following a 21.4% weight loss in 9 subjects. *Bars* represent SE.

an effect of sustained changes in insulin levels on leptin secretion, Kolaczynski and co-workers (19) recently demonstrated an increase in circulating leptin during the final 24 h of a 72-h hyperglycemic clamp in human subjects.

The ability of progressive fasting to suppress plasma leptin levels despite a minimal change in adipose mass suggests that ongoing caloric deficiency supersedes total energy reserve in setting chronic levels of satiety and thermogenesis. This regulatory mechanism might have conferred a survival advantage in environments characterized by rapid changes in food availability. By extrapolation from animal studies, the fasting-induced decrease in leptin should increase hunger, possibly by allowing an increase in arcuate nucleus neuropeptide Y production (20, 21), and decrease thermogenesis (2). The well-known ability of fasting to depress thermogenesis has been attributed to decreased peripheral conversion of T_4 to T_3. It has been demonstrated, however, that replacement of levothyroxine in subjects consuming hypocaloric diets fails to completely correct their lower resting metabolic rates (22). Perhaps full correction of impaired thermogenesis with fasting is impossible in the presence of subnormal leptin levels.

Our data confirm the diurnal variation in plasma leptin levels reported by Sinha and co-workers (23). An evening increase in leptin was observed in all subjects and was present, although greatly attenuated, on the 3rd day of fasting. Our data do not allow us to address the possibility that leptin levels rise to their maximum value after the onset of sleep (23).

Considine and co-workers (6) reported that food consumption did not increase plasma leptin levels. Our data confirm this observation for both low- and high-fat test meals given to overnight fasted subjects despite the fact that postprandial plasma insulin levels were 11- to 18-fold greater than fasting values. This lack of effect contrasts with the ability of a single meal to return leptin levels to normal following the suppression induced by 3 days of fasting, and suggests a fundamental difference in the mechanisms regulating leptin secretion in the fed and chronically fasted states.

It has been reported that human subjects placed on a low-fat diet without overt caloric restriction choose to consume fewer calories and lose weight (10–12). Indeed, when subjects were placed on a 10% fat diet in the present study, they reported feeling uncomfortably full both before and after meals and had to be encouraged to consume a sufficient number of calories to prevent excessive weight loss. Because leptin has been shown to act as a satiety signal (1–4), it would be reasonable to postulate that chronic dietary fat reduction either increases leptin secretion for a given adipose mass or increases CNS sensitivity (24) to circulating leptin. Plasma leptin levels might, therefore, have been expected either to rise (increased secretion) or fall (increased sensitivity) following a major sustained change in dietary fat content from 37% to 10% of total calories. Our finding that isocaloric dietary fat reduction was not associated with a significant change in plasma leptin levels suggests that other variables, such as increased dietary fiber, account for the satiating effect of low-fat feeding.

In conclusion, plasma leptin levels appear to primarily track total body adipose mass and are unaffected by isocaloric dietary fat manipulation or meal consumption. The lack of an effect of meals suggests that leptin serves a very different function from rapidly acting gastrointestinal meal termination signals such as cholecystokinin (25). A role for leptin as a major afferent signal to the CNS for modulating long-term energy balance is not inconsistent with our observation that fasting for 3 days causes plasma leptin to drop to levels well below those expected for the total adipose mass. This ability of sustained fasting to dissociate circulating leptin from adipose stores could reflect a permissive effect of insulin on leptin secretion and may have conferred a survival advantage during evolution. The practical implication of these observations is that plasma leptin determinations for research purposes should be performed after no more than a 12-h fast.

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