### Title

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# Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women[1–3]

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**See corresponding editorial on page 761.**

**ABSTRACT**

**Background:** On the basis of observations in rodents, leptin is thought to play a key role in the regulation of energy expenditure and food intake, but less is known of its influence on ingestive behavior and energy balance in humans.

**Objective:** We examined the effect in women of a chronic energy deficit on plasma leptin concentrations and self-reported appetite and explored possible relations between leptin and appetite sensations.

**Design:** Twelve healthy women (body mass index, in kg/m$^2$: 23–37) participated in a metabolic ward study in which 3 wk of neutral energy balance was followed by 12 wk of energy deficit (energy intake reduced by 2 MJ/d and energy expenditure increased by 0.8 MJ/d). Body weight and composition were monitored, fasting leptin concentrations were measured 4 times, and feelings of hunger, fullness, desire to eat, and prospective consumption were monitored hourly throughout the day on 7 selected days.

**Results:** Adiposity-adjusted leptin decreased by 54% after 1 wk of a moderate energy deficit and remained low after 6 and 12 wk. Leptin was associated with self-reported hunger, desire to eat, and prospective consumption (range of $r$: $-0.6$ to $-0.7$, $P < 0.01$). The greatest hunger increase coincided with the largest percentage drop in circulating leptin and the lowest final leptin concentration. The relation between leptin and hunger was not influenced by amount of weight or body fat loss.

**Conclusions:** These findings support the idea that leptin is a physiologic regulator of hunger during energy deficits in humans; the role of leptin in the long-term regulation of food intake warrants further study. *Am J Clin Nutr* 1998;68:794–801.

**KEY WORDS** Energy intake, energy expenditure, appetite, hunger, satiety, women, weight loss, leptin

## INTRODUCTION

Leptin, a hormone secreted by the adipocyte as a product of the obese (*OB*) gene, is thought to play a role in the regulation of body weight via its central actions on energy expenditure and food intake (1). Genetic leptin deficiency in rodents (1) and humans (2) results in hyperphagia and marked obesity. Numerous studies have shown that leptin circulates in proportion to body fat mass in humans (3–6). However, leptin concentrations among persons with similar body compositions vary ≈10-fold at any given percentage of body fat (3). Also, leptin concentrations decrease after weight loss (4, 6, 7) and the decreases are disproportionate to changes in adiposity (7–9). These observations suggest that other factors in addition to adipose mass modulate leptin secretion [for review, *see* Havel (10)].

The state of energy balance may affect leptin concentrations. Leptin concentrations decrease in response to a state of negative energy balance produced by fasting for 2–3 d (7, 8) or low energy intakes (2.6–3.5 MJ/d) for 7 d (9) and increase substantially in response to a state of positive energy balance produced by short-term, massive overfeeding for 12 h (11). With long-term dietary interventions lasting 5 wk to 3 mo, leptin concentrations decrease with low energy intakes (2.2–4.2 MJ/d) (3, 4, 12) and rise when energy intakes are nearly doubled above requirements (11). However, such interventions are extreme. It is not clear whether leptin secretion is responsive to more moderate changes in energy balance. To our knowledge, the effect of a long-term, moderate energy deficit on circulating leptin concentrations has not been examined.

Little is known about the role of leptin in human appetite regulation. Administration of exogenous leptin inhibits food intake in rodents (13, 14) and in nonhuman primates (M Tang-Christensen, PJ Havel, R Jacobs, PJ Larsen, and JL Cameron, unpublished observations, 1997). Central administration of antibodies to leptin increases food intake in rodents, suggesting that a decrease in endogenous leptin may result in increased hunger (15). Whereas the results of these animal studies implicate leptin as a mediator of appetite, the role of leptin in determining hunger

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in humans is less clear. In one study, increased leptin concentrations coincided with decreased feelings of hunger immediately after hip replacement surgery (16). In another study, fasting leptin concentrations in obese women were linked with salivation during food exposure, but were not related to self-reports of hunger or desire to eat (17). One preliminary report found no relation between leptin and hunger over several hours after food ingestion (18). However, to our knowledge, there are currently no published studies linking circulating leptin with appetite during long-term energy deficits in humans.

In the present study we sought to determine whether plasma leptin concentrations were related to increased hunger and appetite-related variables in humans during a prolonged energy restriction rather than in response to feeding. We chose to examine these variables during a chronic energy-deficient state because leptin does not change in response to short-term fasting and refeeding and is therefore more likely to function as a long-term regulator of energy balance than as a short-term satiety signal. We examined the relation in women between changes in circulating leptin concentrations and changes in several self-reported appetite indexes during a moderate energy deficit.

## SUBJECTS AND METHODS

### Subjects

Healthy, overweight women aged 20–40 y with normal menstrual cycles were sought to participate in a study of the effects of a prolonged, moderate energy deficit on body composition, energy expenditure, mood, and appetite. Women were excluded if they were pregnant, anemic, or hyperlipidemic; reported tobacco use; or had positive results on urine tests for nicotine, narcotics, or mood-altering drugs. Before the study, candidates were evaluated by medical and dietary histories, physical and dental examinations, resting electrocardiogram, and a standard battery of blood tests, including a complete blood cell count and a serum chemistry panel. All subjects gave their informed consent and the experimental protocols were approved by the human subjects review committees of the University of California–Davis and the US Department of Agriculture. Participants lived in the metabolic suite at the Western Human Nutrition Research Center 24 h/d, 7 d/wk for the 105 d of the study. Twelve women completed the study.

The physical characteristics of the subjects are listed in **Table 1**. At the start of the study, 2 subjects were in the desirable weight range for height, with body mass indexes (BMIs; in kg/m$^2$) of 23 and body fat percentages of 27% and 28%, 7 subjects were overweight with BMIs between 25 and 29.9 and body fat percentages ranging from 35% to 42%, and 3 subjects were obese with BMIs ≥30 and body fat percentages ranging from 45% to 49%.

**TABLE 1**
Subject characteristics[1]

| | Value |
|---|---|
| Age (y) | 31 ± 1 (24–39) |
| Height (m) | 1.68 ± 0.02 (1.56–1.76) |
| Weight (kg) | 79.6 ± 3.2 (68.0–107.4) |
| BMI (kg/m$^2$) | 28.5 ± 1.3 (22.8–37.3) |
| Body fat (%) | 38.8 ± 1.9 (27.5–48.9) |
| Leptin (μg/L) | 28.0 ± 4.8 (15.4–71.5) |

[1] $\bar{x}$ ± SEM; range in parentheses. $n$ = 12. Values represent measurements taken at the beginning of the study.

### Experimental protocols

The study consisted of a 3-wk stabilization period for weight maintenance followed by a 12-wk intervention period for weight loss (**Figure 1**). During the stabilization period, the energy intake provided by the daily diet was sufficient for maintaining body weight and was individually prescribed by using Harris and Benedict (19) estimates of resting energy expenditure, adjusted for activity by a factor of 1.5. Energy intakes during the stabilization period ranged from 121 to 135 kJ · kg body wt$^{-1}$ · d$^{-1}$. To achieve an energy deficit during the intervention period, energy intakes were reduced by 2 MJ/d below the stabilization level. Energy intakes during the intervention period ranged from 99 to 118 kJ · kg body wt$^{-1}$ · d$^{-1}$. The diet used for this study consisted of 4-d rotating menus composed of conventional foods and was served in 4 meals: breakfast at 0800, lunch at 1130, dinner at 1630, and an evening snack at 2000. The macronutrient composition of the diet remained constant throughout the stabilization and intervention periods, averaging ($\bar{x}$ ± SD) 60 ± 1% carbohydrate, 18 ± 1% protein, and 22 ± 1% fat.

Throughout the study, activity levels were controlled carefully. The subjects took a 5-km walk every day beginning at 1330. In addition, during the intervention period, moderate-intensity [65% of maximal oxygen uptake ($\dot{V}O_2$max)] aerobic exercise workouts, consisting of treadmill walking and ergometer cycling, were scheduled 5 d/wk. Each aerobic exercise session lasted between 35 and 55 min so that the total energy expended during each workout was held constant at 1.25 MJ. Resistance exercise sessions, consisting of a circuit of weightlifting exercises performed on a Universal machine (Paramount Fitness Equipment Corp, Los Angeles), were scheduled 3 d/wk. The resistance exercise sessions lasted 20–25 min and the total energy expended per session was 0.3 MJ. All exercise sessions were scheduled to start at 0900. The additional exercise during the intervention period increased daily energy expenditure by ≈0.8 MJ/d above that expended during the stabilization period, thereby amplifying the energy deficit. Using the procedure described below for estimating daily energy expenditure, we determined that energy intake during the intervention period averaged ≈74% of daily energy expenditure.

### Body weight and composition measurements

Body weight was measured daily in the morning before breakfast with subjects wearing standard-issue surgical gowns. Cali-

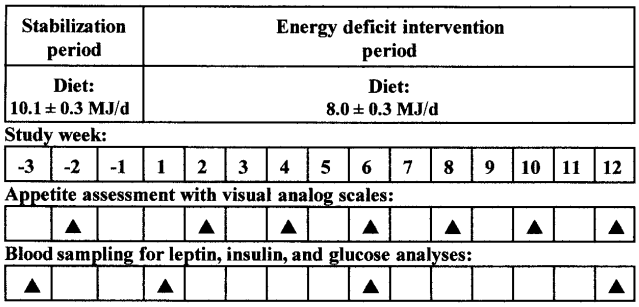


**FIGURE 1.** Diagram of study time line. Symbols (▲) indicate the time points for appetite evaluation or blood sampling.

brated platform scales with digital displays were used (model SX-501; Circuits & Systems Inc, East Rockaway, NY, and Acme Series 7000 and Ohaus PBI; Acme Scale Co, San Leandro, CA). Twice each week, body fat percentages were measured by total-body electrical conductivity (HA-2 body composition analyzer; EM-SCAN, Springfield, IL) immediately after the subjects were weighed (20).

### Resting metabolic rate and energy expenditure

Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured with an automated respiratory gas exchange system (2900; SensorMedics, Anaheim, CA). The system was calibrated with standard gas mixtures. Respiratory gases were collected for 30 min in the morning before breakfast while subjects rested comfortably in a semirecumbent position; subjects wore facemasks that were connected to the gas analyzers via a tubing assembly. Immediately before the gas collection began, subjects had rested for 20 min and had fasted for 11–12 h. Resting metabolic rate was calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ measurements by using the equation of de Weir (21).

Daily energy expenditure was calculated by multiplying resting metabolic rate by an activity factor of 1.5, appropriate for light activity (22). In addition, energy expenditure rates associated with aerobic and resistance exercise were measured during typical sessions by using the automated system and these values were incorporated into the estimate of daily energy expenditure.

### Blood analyses

Blood was drawn 4 times during the study for the analysis of leptin, insulin, and glucose (Figure 1). All samples were collected before breakfast and exercise and after an overnight fast into tubes containing EDTA. Plasma was separated by low-speed centrifugation (1100 $\times$ *g* for 10 min at 0–4°C) and stored at −70°C. Plasma leptin concentrations were measured by radioimmunoassay (23) with reagents supplied by Linco Research (St Charles, MO). The range of the standard curve in this assay was 0.5–100 μg/L and the intraassay and interassay CVs were <8%. The antibody used in the assay does not cross-react with human insulin, proinsulin, glucagon, pancreatic polypeptide, or somatostatin. A modified version of the method of Yalow and Berson (24) was used to measure plasma insulin concentrations by radioimmunoassay, with antisera from ICN Diagnostics (Costa Mesa, CA), [$^{125}$I]insulin (Amersham, Arlington Heights, VA), and polyethylene glycol precipitation (25). Plasma glucose concentrations were measured by the glucose oxidase method with a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH).

### Self-reported appetite

Appetite was assessed with use of visual analogue scales (VASs) administered every waking hour and immediately before and after subjects consumed a meal or snack. A separate VAS was included for 4 appetite-related variables: hunger, fullness, desire to eat, and prospective consumption (an assessment of the amount of food that could be eaten). The day-long appetite assessment was scheduled 7 times throughout the study (Figure 1), once during the stabilization period and 6 times during the intervention period. Aerobic and resistance exercise sessions were scheduled on all appetite assessment days during the intervention. Each VAS included a written question about a subjective feeling (eg, "How hungry do you feel right now?"), accompanied by a 100-mm long answer line anchored at either end with opposite descriptors (eg, "not at all hungry" and "extremely hungry") (26).

Note that VASs are limited in that intervals may be interpreted differently by individuals. We used 2 approaches to minimize any potential differences. First, for each subject on each assessment day, serial data were reduced to yield a cumulative, 1-d score for each of the 4 VASs by calculating the area under the curve (AUC) for a plot of the VAS response versus time elapsed from waking in the morning to sleeping at night (27). Then, the response to the energy deficit intervention was determined by adjusting the AUC value obtained during the intervention period by subtracting the respective AUC value obtained during the stabilization period. A second approach was used to overcome any between-subject inconsistencies with the use of the VAS answer line. From the serial VAS data collected on the stabilization collection day, means ± SDs were calculated for the 4 appetite variables for each subject. Then, for each subject on each assessment day, any single time point response higher than the stabilization mean + 1 SD was considered an incident of "hunger greater than usual," "fullness greater than usual," "desire to eat greater than usual," or "prospective consumption greater than usual" (28). These data are expressed as incidence (% of responses) of hunger, fullness, desire to eat, or prospective consumption for the stabilization or intervention assessment days.

### Statistical analyses

Data are expressed as means ± SEMs. A general linear model with time taken as a repeated measure was used to determine the effect of the duration of the energy deficit on body fat, appetite variables, and plasma concentrations of leptin, glucose, and insulin. The Student-Neuman-Keuls test was used to identify means that were significantly different ($P < 0.05$). To evaluate a possible relation between appetite and plasma leptin, insulin, or glucose concentrations, Pearson correlation coefficients were calculated. In addition, linear regression models were used with hunger, fullness, desire to eat, or prospective consumption as the dependent variable and plasma leptin or percentage change in leptin during the intervention period as the independent variable. Change in body weight or body fat mass was included as a potential covariate in the regression models. Percentage change in glucose during the intervention period was included as an independent variable in some models. Standardized regression coefficients were calculated in all regression analyses. All statistical analyses were performed with SAS for WINDOWS (version 6.12; SAS Institute Inc, Cary, NC).

## RESULTS

### Changes in plasma leptin with the energy deficit

There were significant decreases in weight, percentage body fat, and plasma leptin, insulin, and glucose concentrations during the intervention period (**Table 2**). The magnitude and time course of the changes differed for each variable. Leptin concentrations dropped ≈57% after 1 wk of the intervention; a similar decline of ≈49% occurred in circulating insulin concentrations at 1 wk. When leptin concentrations were corrected for adiposity by dividing by percentage body fat, the decline in adiposity-adjusted leptin concentrations averaged 54% after 1 wk of the energy deficit. After 6 and 12 wk of continued dietary restriction and exercise, adiposity-adjusted leptin concentrations remained

**TABLE 2**
Mean weight, body fat, and plasma leptin, insulin, and glucose concentrations and mean change during the 12-wk moderate energy deficit[1]

| | Stabilization period | Intervention week 1 | Intervention week 6 | Intervention week 12 |
|---|---|---|---|---|
| Weight (kg) | 79.6 ± 3.2[a] | 79.2 ± 3.2[a] | 75.6 ± 3.2[b] | 72.5 ± 3.2[c] |
| Change (kg) | — | –0.4 ± 0.2 | –4.0 ± 0.3 | –7.0 ± 0.5 |
| Percentage change (%) | — | –0.5 ± 0.2 | –5.1 ± 0.4 | –9.0 ± 0.7 |
| Fat mass (kg) | 31.3 ± 2.5[a] | 30.2 ± 2.5[b] | 27.3 ± 2.4[c] | 25.1 ± 2.5[d] |
| Change (kg) | — | –1.1 ± 0.1 | –4.0 ± 0.3 | –6.2 ± 0.4 |
| Percentage change (%) | — | –3.6 ± 0.5 | –13.3 ± 0.9 | –21.0 ± 1.9 |
| Leptin (μg/L) | 28.0 ± 4.8[a] | 13.0 ± 3.3[b] | 10.5 ± 2.6[b] | 9.2 ± 2.7[b] |
| Change (μg/L) | — | –15.0 ± 2.2 | –17.6 ± 2.4 | –18.9 ± 2.3 |
| Percentage change (%) | — | –56.9 ± 4.8 | –66.0 ± 3.1 | –71.9 ± 3.4 |
| Insulin (pmol/L) | 103 ± 13[a] | 47 ± 5[b] | 62 ± 10[b] | 54 ± 7[b] |
| Change (pmol/L) | — | –56 ± 12 | –41 ± 11 | –50 ± 12 |
| Percentage change (%) | — | –48.7 ± 5.9 | –35.8 ± 8.0 | –42.9 ± 6.4 |
| Glucose (mmol/L) | 4.8 ± 0.1[a] | 5.0 ± 0.1[a] | 4.6 ± 0.1[b] | 4.4 ± 0.1[b] |
| Change (mmol/L) | — | 0.2 ± 0.1 | –0.3 ± 0.1 | –0.4 ± 0.1 |
| Percentage change (%) | — | 3.6 ± 2.7 | –5.2 ± 1.7 | –8.4 ± 1.7 |

[1] $\bar{x}$ ± SEM; $n$ = 12. Intervention change (and percentage change) values represent change from the stabilization period. Means within a row with different superscript letters are significantly different, $P < 0.05$.

low (**Figure 2**). The loss of body fat during this study was more linear than and dissociated from both the changes in leptin and insulin (Table 2) and the adiposity-adjusted changes in leptin (Figure 2).

Plasma glucose concentrations were significantly lower at 6 and 12 wk and plasma insulin concentrations were significantly lower at 1, 6, and 12 wk of the intervention period than during the stabilization period (Table 2). The percentage change in plasma leptin or adiposity-adjusted plasma leptin concentrations was significantly correlated with the change in plasma glucose after 1 and 6 wk ($r > 0.65$, $P < 0.025$) and after 12 wk ($r = 0.68$, $P < 0.02$; **Figure 3**) of the energy deficit. Changes in plasma leptin were not significantly correlated with changes in plasma insulin.

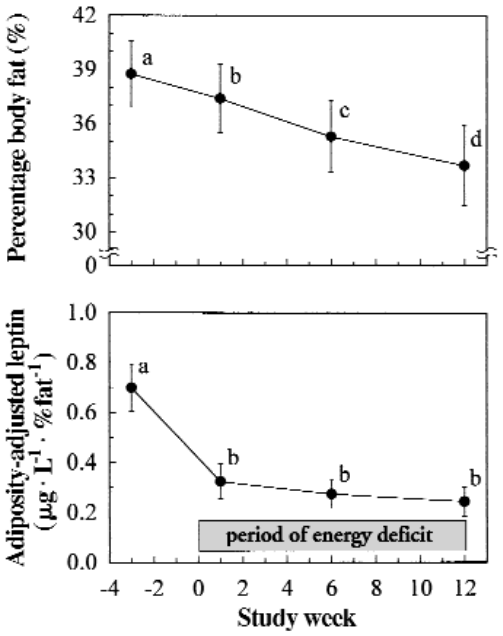


**FIGURE 2.** Mean (±SEM) percentages of body fat and concentrations of circulating leptin (adjusted for adiposity) in 12 women during a prolonged energy deficit. Means with different superscript letters are significantly different, $P < 0.05$.

### Changes in self-reported appetite with the energy deficit

Ratings of hunger and desire to eat (summarized as the AUC, representing the serial VAS responses throughout the day) increased and the incidence of hunger and desire to eat doubled in response to the energy deficit (**Table 3**). Feelings of fullness did not change significantly during the intervention. The mean AUC of prospective consumption did not change significantly during the intervention, but the incidence of "prospective consumption greater than usual" did. The magnitude and direction of the changes in appetite in response to the energy deficit varied considerably among subjects; the CVs for mean change in AUCs were >100% for all 4 appetite variables.

### Relation between leptin and appetite indexes

Circulating leptin concentrations and percentage change in leptin were significantly correlated with the changes in the AUCs for hunger, desire to eat, and prospective consumption (**Table 4**).

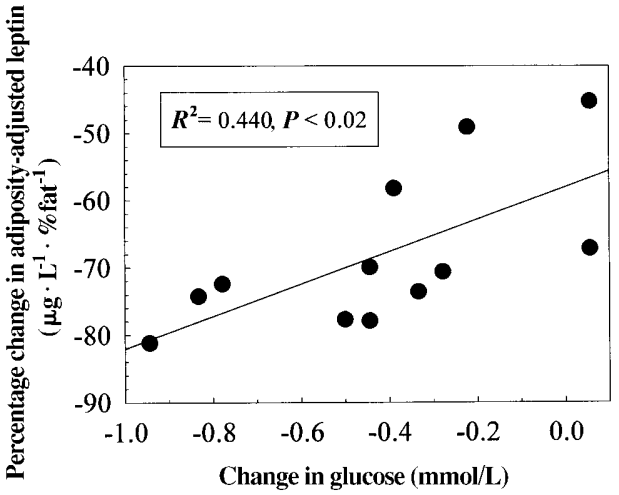


**FIGURE 3.** Relation between the change in plasma glucose concentrations and the percentage change in adiposity-adjusted plasma leptin concentrations in 12 women after 12 wk of an energy deficit. Similar correlations between changes in plasma glucose and plasma leptin were observed after 1 and 6 wk of the energy deficit.

**TABLE 3**
Mean ratings of hunger, fullness, desire to eat, and prospective consumption and change in these ratings during the 12-wk moderate energy deficit[1]

| | Stabilization period | Intervention weeks 2, 4, and 6 | Intervention weeks 8, 10, and 12 |
|---|---|---|---|
| Hunger | | | |
| AUC (mm · h) | 376 ± 69[a] | 529 ± 83[b] | 494 ± 95[a,b] |
| Change in AUC (mm · h) | — | 153 ± 65 | 118 ± 66 |
| Incidence (% of response)[2] | 16 ± 2[a] | 33 ± 5[b] | 29 ± 5[b] |
| Fullness | | | |
| AUC (mm · h) | 863 ± 70[a] | 771 ± 73[a] | 800 ± 98[a] |
| Change in AUC (mm · h) | — | –92 ± 51 | –63 ± 75 |
| Incidence (% of response) | 19 ± 2[a] | 16 ± 4[a] | 22 ± 6[a] |
| Desire to eat | | | |
| AUC (mm · h) | 387 ± 77[a] | 562 ± 91[b] | 539 ± 99[b] |
| Change in AUC (mm · h) | — | 175 ± 65 | 152 ± 66 |
| Incidence (% of response) | 17 ± 1[a] | 37 ± 4[b] | 35 ± 5[b] |
| Prospective consumption | | | |
| AUC (mm · h) | 512 ± 84[a] | 644 ± 97[a] | 616 ± 106[a] |
| Change in AUC (mm · h) | — | 132 ± 72 | 104 ± 77 |
| Incidence (% of response) | 18 ± 1[a] | 38 ± 5[b] | 38 ± 6[b] |

[1] $\bar{x}$ ± SEM; $n$ = 12. Intervention change values represent change from the stabilization period. AUC, area under the curve. Means within a row with different superscript letters are significantly different, $P < 0.05$.

[2] Percentage of response indicates the number of responses, as a percentage of total responses, when subjects reported greater than usual feelings of hunger, fullness, desire to eat, or prospective consumption.

**TABLE 4**
Pearson correlation coefficients for change in appetite variables versus plasma leptin, insulin, and glucose concentrations and percentage change in response to the energy deficit[1]

| | Hunger | Fullness | Desire to eat | Prospective consumption |
|---|---|---|---|---|
| Leptin | –0.701[2] | 0.504 | –0.690[2] | –0.743[2] |
| Leptin, percentage change | –0.726[2] | 0.385 | –0.697[2] | –0.647[3] |
| Adjusted leptin | –0.704[2] | 0.509 | –0.687[2] | –0.755[2] |
| Adjusted leptin, percentage change | –0.723[2] | 0.401 | –0.695[2] | –0.640[3] |
| Insulin | –0.521 | 0.401 | –0.532 | –0.425 |
| Insulin, percentage change | –0.196 | 0.227 | –0.212 | –0.119 |
| Glucose | –0.234 | –0.082 | –0.217 | –0.325 |
| Glucose, percentage change | –0.577[3] | 0.493 | –0.583[3] | –0.529 |

[1] The numerical values for the appetite variables used in the analysis were mean areas under the curve (AUCs) of intervention weeks 8, 10, and 12, adjusted by subtracting the respective AUC value reported during the stabilization period. Leptin, insulin, and glucose values were determined at intervention week 12, and the percentage change values represent change at intervention week 12 compared with the stabilization period. Adjusted leptin is leptin concentration adjusted for adiposity by dividing by percentage body fat.

[2] $P < 0.01$.

[3] $P < 0.05$.

Relations between leptin concentrations and hunger-related variables were similar at 6 and 12 wk of the intervention period; therefore, we report only the 12-wk data. The inverse relations between leptin (or adiposity-adjusted leptin) and these indexes of hunger indicate that lower leptin concentrations were associated with greater feelings of hunger, desire to eat, and prospective consumption measured throughout the day, whereas higher leptin concentrations were associated with attenuated feelings of hunger, desire to eat, and prospective consumption. The negative correlation between percentage change in leptin (or adiposity-adjusted leptin) and hunger sensations (Table 4 and **Figure 4**) indicates that those reporting the greatest increase in hunger, desire to eat, and prospective consumption experienced the largest percentage drop in circulating leptin concentrations. Percentage change in leptin was also negatively correlated with percentage incidence of hunger ($r = -0.567$, $P < 0.05$) and percentage incidence of desire to eat ($r = -0.604$, $P < 0.05$). Neither leptin nor percentage change in leptin was significantly related to sensations of fullness (Table 4). Plasma insulin and glucose concentrations were not related to hunger or fullness sensations, but the percentage change in glucose concentration was negatively correlated with hunger and desire to eat food. When percentage change in leptin and percentage change in glucose were both included as independent variables in a multiple linear regression model, the percentage change in leptin contributed to the variance in hunger response (standardized estimate: −0.627, $P = 0.077$), whereas percentage change in glucose did not (standardized estimate: −0.143, $P = 0.660$).

Changes in body weight or body fat did not influence the relation between appetite and leptin variables. When change in body weight (or body fat) was included in multiple linear regression analyses examining the association of hunger (or desire to eat) with circulating leptin (or percentage change in leptin), neither body weight change nor body fat change had a significant effect on the regression models (**Table 5**).

## DISCUSSION

We observed that leptin concentrations were reduced by 57% after 1 wk of negative energy balance imposed by a combination of moderately reduced energy intake and increased energy expenditure. The magnitude and time course of this response are similar to those reported by Dubuc et al (9), who observed a 61% reduction in leptin concentrations after 1 wk of energy restriction (2.6 MJ/d) in 13 normal-weight women. Scholz et al (12) reported a 50% reduction in leptin concentrations in 12 obese women consuming an energy-restricted diet (4.2 MJ/d) for 10 wk and Considine et al (4) reported a 53% decline in leptin concentrations in 7 obese persons consuming 3.3 MJ/d for 8–12 wk. The energy intakes in these studies were significantly less than the 8.0 ± 0.3 MJ/d consumed in the present study. However, our intervention also included regular aerobic and resistance exercise. The addition of these activities increased energy expenditure by ≈0.8 MJ/d. Reports on the effect of exercise on leptin concentrations are conflicting. In women, aerobic training had no effect on leptin in one study (29), whereas it led to an 18% decrease in circulating leptin concentrations in another study (30). Also, the leptin response to acute exercise is not consistent (29, 31). In the present study, exercise energy expenditure was increased as energy intake was decreased. Thus, it is not possible to know whether, in addition to its role in augmenting the energy deficit, the exercise component per se contributed to the reductions in leptin concentrations.

Along with the reduction in circulating leptin concentration, plasma glucose and plasma insulin concentrations declined during the intervention period. At all times tested during the energy deficit period, there were significant correlations between the change in plasma glucose and the percentage change in circulat-

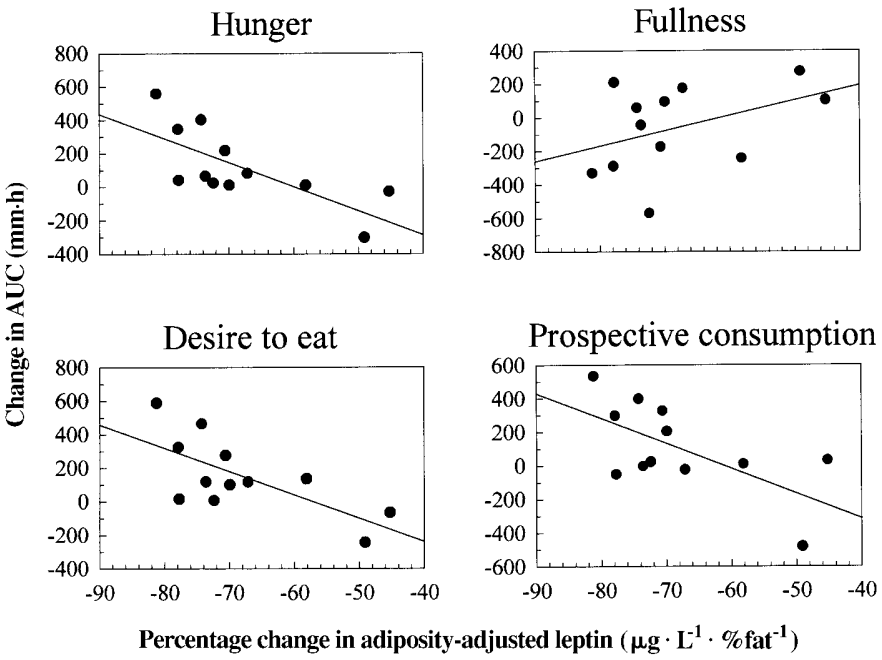


**FIGURE 4.** Relation between change in appetite indexes and percentage change in circulating adiposity-adjusted leptin concentrations in 12 women during a prolonged energy deficit, reported as mean areas under the curve (AUCs) for intervention weeks 8, 10, and 12 adjusted by subtracting the AUC for the stabilization period. The value for adiposity-adjusted leptin represents the percentage change at intervention week 12 compared with the first week of the stabilization period. For hunger: $R^2 = 0.522$, $P < 0.01$; for fullness: $R^2 = 0.161$, $P > 0.10$; for desire to eat: $R^2 = 0.483$, $P < 0.01$; for prospective consumption: $R^2 = 0.409$, $P < 0.05$.

ing plasma leptin or adiposity-adjusted leptin concentrations. Similar correlations between changes in plasma glucose and in plasma leptin were observed after 1 wk of more marked energy restriction in human subjects (9). In addition, plasma leptin does not decrease in humans when small amounts of glucose are infused to prevent the decline of glycemia during a 72-h fast (8), and glucose infusion increases plasma leptin concentrations in rhesus monkeys (32) and humans (33). These data are consistent with the observation that leptin secretion from isolated adipocytes in vitro is dependent on glucose uptake and metabolism (34) and provide support for the hypothesis that changes in leptin secretion in vivo, after energy restriction or refeeding, reflect decreases or increases in adipocyte glucose uptake and metabolism, respectively.

In this study we examined the relation between leptin and self-reported feelings of hunger and satiety under controlled diet and activity conditions that were held constant for 3 wk (energy-adequate, weight-maintaining diet) and 12 wk (energy-deficient diet plus exercise). For this purpose, we used composite estimates of hunger, fullness, desire to eat, and prospective consumption based on ratings made frequently throughout several days during the study. We hypothesized that if leptin acted as a factor regulating food intake, then as leptin concentrations decreased in response to the energy deficit, hunger would increase. In fact, using our composite values for hunger-related indexes, we found significant correlations between changes in plasma leptin concentrations and hunger sensations. Subjects with lower leptin concentrations and greater percentage decreases in circulating leptin reported greater feelings of hunger, desire to eat, and prospective consumption and those with higher leptin concentrations and smaller percentage decreases in leptin reported lesser

**TABLE 5**
Regression models examining the association between appetite, leptin, and change in body weight or fat during the energy deficit[1]

| Dependent and independent variables | Standardized coefficient | *P* |
|---|---|---|
| Change in hunger, AUC | | |
| Leptin concentration | –0.700 | 0.016 |
| Change in body weight | –0.007 | 0.976 |
| Change in hunger, AUC | | |
| Leptin concentration | –0.688 | 0.020 |
| Change in body fat | –0.052 | 0.835 |
| Change in hunger, AUC | | |
| Percentage change in leptin | –0.734 | 0.011 |
| Change in body weight | –0.084 | 0.720 |
| Change in hunger, AUC | | |
| Percentage change in leptin | –0.710 | 0.012 |
| Change in body fat | –0.117 | 0.620 |
| Change in desire to eat, AUC | | |
| Leptin concentration | –0.690 | 0.019 |
| Change in body weight | –0.011 | 0.966 |
| Change in desire to eat, AUC | | |
| Leptin concentration | –0.685 | 0.022 |
| Change in body fat | –0.022 | 0.932 |
| Change in desire to eat, AUC | | |
| Percentage change in leptin | –0.705 | 0.016 |
| Change in body weight | –0.085 | 0.728 |
| Change in desire to eat, AUC | | |
| Percentage change in leptin | –0.685 | 0.019 |
| Change in body fat | –0.089 | 0.718 |

[1] AUC, area under the curve. Numerical values used in the analysis for appetite and leptin variables are described in Table 4. Change in body weight or fat was measured from the beginning to the end of the 12-wk intervention period.

feelings of these hunger-related variables in response to the energy deficit. These relations were independent of decreases in body weight and body fat.

Frank hypoglycemia is known to trigger sensations of hunger. In the present study, plasma glucose concentrations averaged ≈4.5 mmol/L and did not decrease below 4.0 mmol/L in any of the subjects during the energy restriction period. Accordingly, absolute glucose concentrations were not related to hunger sensations, but we did find a correlation between hunger and the modest changes in fasting glucose concentrations that occurred at 12 wk of the intervention. However, in multivariate analyses, the leptin concentration (or percentage change in circulating leptin) was the dominant independent variable related to hunger sensations and the percentage change in glucose was not a significant contributor to hunger.

In another study of leptin and appetite, Karhunen et al (17) found no association between leptin and hunger or desire to eat measured by VASs in obese women. However, their study was designed to examine the relation between leptin and short-term appetite processes in a single eating situation. We also did not find a significant relation between leptin and fullness ratings, which can be considered to be an indicator of satiety and to be associated primarily with the presence of food in the stomach. The lack of a relation between leptin and fullness is consistent with the idea that circulating leptin is more a long-term regulator of energy balance than a short-term satiety signal. In fact, our study design was better suited to explore the possible effects of prolonged energy imbalance on the relation between circulating leptin and appetite.

The leptin-hunger relations observed in our study suggest that leptin may be involved in the process that determines appetite in response to a sustained energy deficit in humans. Leptin receptors have been identified in rodents in the hypothalamus, an area regulating appetite and energy balance, suggesting that the brain is an important site of leptin action (1). The association between leptin and hunger observed in our study is consistent with the effects of leptin on food intake in animals (13–15; M Tang-Christensen, PJ Havel, R Jacobs, PJ Larsen, and JL Cameron, unpublished observations, 1997) and with the hyperphagia resulting from congenital leptin deficiency in humans (2). However, we can only speculate on the possible effect that changes in circulating leptin concentrations may have on food intake behavior in humans. Although hunger evaluations after dietary manipulations have been used widely in studies of human ingestive behavior, the association between ratings of hunger and actual food intake is uncertain: some investigators have shown a relation between hunger and food intake (35–37), whereas others have not (28, 38, 39). Many factors, including psychologic and social factors, can obscure the relation between feelings of hunger and food intake. Our study provides evidence that circulating leptin concentrations are linked to hunger sensations during a prolonged energy deficit resulting from a moderate restriction of energy intake and a modest increase in energy expenditure with exercise. Further studies will be required to determine the effect of leptin on food intake in humans.

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## REFERENCES

1. Caro JR, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin: the tale of an obesity gene. Diabetes 1996;45:1455–61.
2. Montague CT, Farooqi IS, Whitehead JP, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 1997;387:903–8.
3. Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1995;1:1155–61.
4. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292–5.
5. Ostlund RE Jr, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J Clin Endocrinol Metab 1996;81:3909–13.
6. Havel PJ, Kasim-Karakas S, Mueller WM, Johnson PR, Gingerich RL, Stern JS. Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss. J Clin Endocrinol Metab 1996;81:4406–13.
7. Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. J Clin Endocrinol Metab 1997;82:561–5.
8. Boden G, Chen X, Mozzoli M, Ryan I. Effect of fasting on serum leptin in normal human subjects. J Clin Endocrinol Metab 1996; 81:3419–23.
9. Dubuc GR, Phinney SD, Stern JS, Havel PJ. Changes of serum leptin and endocrine and metabolic parameters after 7 days energy restriction in men and women. Metabolism 1998;47:429–34.
10. Havel PJ. Leptin production and action: relevance to energy balance in humans. Am J Clin Nutr 1998;67:355–6 (editorial).
11. Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF. Response of leptin to short-term and prolonged overfeeding in humans. J Clin Endocrinol Metab 1996;81:4162–5.
12. Scholz GH, Englaro P, Thiele I, et al. Dissociation of serum leptin concentration and body fat content during long term dietary intervention in obese individuals. Horm Metab Res 1996;28:718–23.
13. Pelleymounter MA, Cullen MJ, Baker MB, et al. Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science 1995;269:540–3.
14. Halaas JL, Gajiwala KS, Maffei M, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 1995; 269:543–6.
15. Brunner L, Nick HP, Cumin F, et al. Leptin is a physiologically important regulator of food intake. Int J Obes Relat Metab Disord 1997;21:1152–60.
16. Stratton RJ, Dewit O, Crowe E, Jennings G, Villar RN, Elia M. Plasma leptin, energy intake and hunger following total hip replacement surgery. Clin Sci 1997;93:113–7.
17. Karhunen L, Haffner S, Lappalainen R, Turpeinen A, Miettinen H, Uusitupa M. Serum leptin and short-term regulation of eating in obese women. Clin Sci 1997;92:573–8.
18. Joannic JL, Oppen JM, Lablou N, et al. Plasma leptin and hunger ratings in healthy humans. Obes Res 1997;5(suppl):20S (abstr).
19. Harris JA, Benedict FG. Biometric studies of basal metabolism in man. Washington, DC: Carnegie Institute of Washington, 1919. (Publication no. 297.)
20. Van Loan MD, Keim NL, Belko AZ. Body composition assessment of a general population using total body electrical conductivity (TOBEC). In: Hermans GPH, ed. Sports, medicine and health. New York: Elsevier Science Publishers, 1990:665–70.
21. de Weir JB V. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949;109:1–9.
22. National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
23. Ma ZA, Gingerich RL, Santiago JV, Klein S, Smith HC, Landt M.

Radioimmunoassay of leptin in human plasma. Clin Chem 1996;42:942–6.
24. Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. J Clin Invest 1960;39:1157–75.
25. Desbuquois B, Aurbach GD. Use of polyethylene glycol to separate free and antibody bound peptide hormones in radioimmunoassay. J Clin Endocrinol Metab 1971;33:732–8.
26. Hetherington M, Rolls BJ. Methods of investigating human eating behavior. In: Toates F, Rowland N, eds. Feeding and drinking. Amsterdam: Elsevier Science Publishers, 1987:77–109.
27. Matthews JNS, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. Br Med J 1990;300: 230–5.
28. Mattes R. Hunger ratings are not a valid proxy measure of reported food intake in humans. Appetite 1990;15:103–13.
29. Perusse L, Collier G, Gagnon J, et al. Acute and chronic effects of exercise on leptin levels in humans. J Appl Physiol 1997;83:5–10.
30. Hickey MS, Houmard JA, Considine RV, et al. Gender-dependent effects of exercise training on serum leptin levels in humans. Am J Physiol 1997;272:E562–6.
31. Alderson NL, Ferguson MA, Essig DA, Durstine JL. Effects of two different energy expenditures on plasma leptin concentrations. Med Sci Sports Exerc 1997;29(suppl):S156 (abstr).
32. Havel PJ. Glucose, but not fructose, infusion increases circulating leptin in proportion to adipose stores in rhesus monkeys. J Endocrinol Diabetes 1997;105(suppl):37–8.
33. Sonnenberg GE, Krakower GR, Hoffmann RG, Maas DL, Hennes MMI, Kissebah AH. Plasma leptin concentrations: effects of extended fasting and stepwise increases in glucose infusions. Obes Res 1996;4(suppl):13S (abstr).
34. Mueller WM, Gregoire F, Stanhope KL, et al. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology 1998;139:551–8.
35. Blundell JE, Rogers PJ, Hill AJ. Evaluating the satiating power of foods: implications for acceptance and consumption. In: Solms J, Booth DA, Pangborn RM, Raunhardt O, eds. Food acceptance and nutrition. New York: Academic Press, 1987:205–19.
36. Wolkowitz OM, Doran AR, Cohen MR, Cohen RM, Wise TN, Pickar D. Single-dose naloxone acutely reduces eating in obese humans: behavioral and biochemical effects. Biol Psychiatry 1988;24:483–7.
37. de Castro JM, Elmore DK. Subjective hunger relationships with meal patterns in the spontaneous feeding behavior of humans: evidence for a causal connection. Physiol Behav 1988;43:159–65.
38. Rogers PJ, Carlyle J, Hill AJ, Blundell JE. Uncoupling sweet taste and calories: comparison of the effects of glucose and three intense sweeteners on hunger and food intake. Physiol Behav 1988; 43:547–52.
39. Rolls BJ, Hetherington M, Laster LJ. Comparison of the effects of aspartame and sucrose on appetite and food intake, hunger and satiety in obese men. Appetite 1988;11:62–7.