Effect of prolonged moderate and severe energy restriction and refeeding on plasma leptin concentrations in obese women

Brent E Wisse, L Arthur Campfield, Errol B Marliss, José A Morais, Renata Tenenbaum, and Réjeanne Gougeon

ABSTRACT

Background: Plasma leptin in humans is subject to both long- and short-term regulation; it correlates with indexes of body fat that can only change slowly. However, short-term fasting causes large and rapid decreases.

Objective: We tested the interactions between energy intake and fat loss on plasma leptin during prolonged moderate and severe energy restriction, with a view to understanding mechanisms of control.

Design: Postabsorptive leptin was measured with an enzyme-linked immunosorbent assay specific for the human peptide in 21 obese women aged 41 ± 3 y (weight: 102 ± 4 kg; 48 ± 1% body fat) after 1 wk of a weight-maintaining diet and then weekly for 4 wk during a total fast (group 1); a 1.9-MJ/d all-protein, very-low-energy diet (VLED) (group 2); or a low-energy, balanced-deficit diet (BDD) providing 50% of maintenance energy (group 3). In groups 1 and 2, leptin was also measured after 1 wk of refeeding with a diet equivalent to the BDD.

Results: Mean leptin decreased markedly by up to 66% (P < 0.001) at week 1 of energy restriction and then gradually thereafter. The change in leptin per kilogram fat mass correlated with that in glucose concentrations [r = 0.538 (P = 0.012) at week 1 and r = 0.447 (P = 0.042) at week 4] but not with that in fat mass. During refeeding postfasting, leptin increased (P = 0.008), despite an ongoing loss of fat mass and correlated positively with changes in resting energy expenditure. At times with comparable cumulative energy restriction and fat loss between diets, the percentage change in leptin paralleled expenditure. At times with comparable cumulative energy restriction of fat mass and correlated positively with changes in resting energy expenditure in animal models. Systemic administration of exogenous leptin in the leptin-deficient ob/ob mouse results in normalization of food intake and an increase in energy expenditure (1). Lower amounts are effective if leptin is introduced into the central nervous system via the lateral ventricles (2, 3), where the leptin receptor is expressed in high amounts, especially in the hypothalamus (4). Its actions include inhibiting neuropeptide Y (5) and stimulating the production of melanocortin (6–8). In addition, leptin influences energy homeostasis via central regulation of thermogenesis (9).

With exceedingly rare exceptions, obese humans appear to have a normal OB gene (10) and normal leptin and leptin receptors. Plasma leptin concentrations in humans correlate closely and positively with indexes of body fat mass (10–13). Consumption of diets with moderate energy deficits is associated with decreases in plasma leptin (14–17), which appear to parallel changes in weight and fat mass (14), especially in men (17). Subjects who subsequently regain weight show corresponding increases in their plasma leptin concentrations (18).

Plasma leptin decreases markedly during short-term total fasting (18, 19), not in proportion to the loss in fat mass, and returns to baseline concentrations with refeeding (18). The cellular mechanism whereby fasting decreases leptin concentrations is as yet poorly defined, but correlates with markers of lipolysis (19). Plasma leptin concentrations correlate with serum insulin concentrations in weight-stable individuals, and both concentrations decrease in parallel after weight loss (20). The same findings were found in fed and fasted mice (21). If glucose and insulin are both “clamped” at basal concentrations, the decline in leptin with fasting is prevented (19). Four-day fasts are associated with a rapid decline in leptin concentrations that correlates best with that of insulin concentrations (22). Insulin does not increase leptin release by cultured rat adipocytes if glucose uptake is blocked.
In the present study, we determined leptin concentrations in 3 groups of obese women undergoing different degrees of dietary energy restriction for 4 wk as well as after 1 wk of refeeding. We hypothesized first that the initial change in energy intake would cause a decline in leptin concentrations over a period of days in response to the changes in hormone-fuel milieu and concurrent fat loss. The second hypothesis was that for comparable fat masses lost, independent of duration of energy restriction, decreases in plasma glucose, insulin, or both would parallel those in plasma leptin. Third, we postulated that leptin would increase during hypoenergetic refeeding in which energy intake is increased relative to that in the preceding period, but that net fat mobilization would continue.

SUBJECTS AND METHODS

Subjects and diets

Twenty-one obese women were studied as inpatients in the Clinical Investigation Unit of the Royal Victoria Hospital. Their characteristics are given in Table 1. Each subject was informed of the nature, purpose, and possible risks of the study and consent was obtained; the protocols were approved by the hospital’s Human Ethics Committee. Clinical and laboratory evaluations showed no evidence of gout or of hepatic, thyroid, cardiovascular, renal, or pulmonary dysfunction. The subjects did not smoke during the study, nor did they exercise, except for walking in the hospital ward. Results of studies of protein turnover in these subjects were published previously (25).

Postabsorptive (at 0800) plasma leptin concentrations were first measured after a 7-d period of isoenergetic feeding, which consisted of a weight-maintaining, liquid-formula diet (Ensure) and a glucose polymer powder (Polycose) (both from Ross Laboratories, Montréal) supplemented with protein that is derived from casein and soy (Bariatrix International Inc, Lachine, Canada) to meet individual energy requirements (10.8 ± 0.3 MJ/d) and provide a generous amount of protein (90 ± 2 g/d). Macronutrient energy distribution was as follows: 56% as carbohydrate (mostly from corn syrup solids), 15% as protein (from casein and soy), and 29% as fat (from corn oil). Water intake was ≥1.5 L/d.

Subsequently, 3 different levels of energy restriction were imposed for 4 wk. Eight subjects underwent total fasting. Six subjects consumed a mainly protein, very-low-energy diet (VLED) (Bariatrix International Inc). The protein formula provided 95 g protein, 10–15 g carbohydrate, 2 g fat, and 1.9 MJ/d. Seven other subjects received a low-energy, balanced-deficit diet (BDD) that provided 50% of individual weight-maintenance energy requirements and provided 5.5 ± 0.3 MJ/d with 30 ± 1% of energy as protein, 53 ± 1% as carbohydrate, and 17 ± 1% as fat. The diet was composed of a commercial meal-replacement product (Boost; Mead Johnson Canada, Belleville, Canada), a bran cereal (AllBran Cereal; Kellogg Canada Inc, Etobicoke, Canada), milk, and a casein-soy protein supplement (Bariatrix International Inc) to maintain a protein intake the same as that of the isoenergetic diet. All subjects were given one tablet of a multivitamin-multimineral supplement (Centrum Forte; Cyanamid Canada Inc, Montréal) daily and, with the total fast or the VLED, 20 mmol K as KCl (K-Dur; Key, Schering Canada Inc, Pointe-Claire, Canada). After 4 wk of fasting or of the VLED, subjects were refed for 1 wk a diet providing 3.35 MJ/d initially. The diet was composed of regular foods providing 3.35 MJ/d initially. The diet was composed of regular foods.

Weight was measured on a balance calibrated to 0.1 kg (Scaletronix digital scale; Ingram and Bell-Meditron, Le Groupe Inc, Don Mills, Canada). Initial body composition was assessed with a 4-terminal bioimpedance analyzer (BIA-103; RJL Systems, Detroit) by using the procedures, anatomic sites, and equation described by Lukaski et al (26). Body circumference measurements were taken at the site giving the minimal value between the xiphoid process and the iliac crest for the waist and at the level of maximal protuberance in the trochanteric region for the hips.

Resting energy expenditure (REE) was measured with a ventilated-hood indirect calorimeter (Deltatrac Metabolic Monitor; Sensormedics Corporation, Anaheim, CA) weekly in the morning, on awakening, in a quiet environment with a room temperature averaging 22°C. Data were collected while the subjects breathed

<table>
<thead>
<tr>
<th>Subject characteristics during isoenergetic feeding before consumption of the hypoenergetic diets</th>
<th>Fasting (n = 8)</th>
<th>VLED (n = 6)</th>
<th>BDD (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>32.1 ± 4.2</td>
<td>45.2 ± 4.1</td>
<td>47.7 ± 3.9</td>
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<tr>
<td>Weight (kg)</td>
<td>102.7 ± 6.8</td>
<td>105.2 ± 9.9</td>
<td>98.9 ± 7.1</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>37.8 ± 2.0</td>
<td>40.2 ± 3.4</td>
<td>38.3 ± 2.8</td>
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<tr>
<td>Body fat (%)</td>
<td>49.0 ± 2.7</td>
<td>48.7 ± 2.6</td>
<td>47.1 ± 1.5</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>51.0 ± 5.6</td>
<td>51.8 ± 7.2</td>
<td>46.8 ± 4.3</td>
</tr>
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<td>Waist circumference (cm)</td>
<td>NA</td>
<td>108.5 ± 6.5</td>
<td>106.5 ± 3.8</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>NA</td>
<td>0.83 ± 0.04</td>
<td>0.81 ± 0.02</td>
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<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.1 ± 0.2</td>
<td>5.5 ± 0.2</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/L)</td>
<td>194.8 ± 38.0</td>
<td>220.0 ± 26.3</td>
<td>162.6 ± 22.6</td>
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<tr>
<td>Fasting plasma leptin (μg/L)</td>
<td>74.4 ± 12.3</td>
<td>86.2 ± 13.8</td>
<td>71.1 ± 10.3</td>
</tr>
<tr>
<td>Resting NPRQ</td>
<td>0.82 ± 0.03</td>
<td>0.81 ± 0.02</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td>REE (MJ/d)</td>
<td>7.2 ± 0.4</td>
<td>7.2 ± 0.6</td>
<td>6.9 ± 0.4</td>
</tr>
</tbody>
</table>

1 ± SEM. NPRQ, nonprotein respiratory quotient; VLED, very-low-energy diet; BDD, balanced-deficit diet; REE, resting energy expenditure; NA, not available.

2 Significantly different from BDD, P < 0.05.
under the plastic canopy for 20 min. The average result from the last 15 min of calorimetry was used to calculate 24-h REE according to the de Weir equation (27). Overnight fasted blood samples were taken for determinations of serum glucose, electrolytes, calcium, phosphate, uric acid, and lipid concentrations; for liver and kidney function tests; and for complete blood counts. An electrocardiograph was performed weekly in all subjects.

Venous blood samples were drawn with minimal stasis after an overnight fast at the end of the isocaloric diet, then weekly at 0800 in all subjects. Heparin-containing plasma was collected with aprotinin (Trasylol, 10 000 000 Kallikrein inhibiting U/L; Bayer, Etobicoke, Canada). These samples were cooled, centrifuged at 2000 × g at 4°C for 15 min, and stored at −20°C until assayed for leptin (see below) and for insulin by using an anti-beef insulin antiserum, purified human insulin standards, [125I]porcine insulin (Linco Research Inc, St Charles, MO), and dextran-coated charcoal separation. This immunoadsorbent gives immunoreactive insulin results slightly lower than that of an assay (Linco Research Inc) specific for insulin that excludes proinsulin (R Gougeon, M Giroux, and EB Marliss, unpublished observations, 1991). Plasma values were corrected for dilution by aprotinin, based on concurrently determined hematocrit values. Perchloric acid supernates of deproteinized whole blood were assayed for 3-hydroxybutyrate with an enzymic microfluorometric method (28). Circulating total leptin concentrations were measured by using the human OB protein-specific enzyme-linked immunosorbent assay: samples were added to monoclonal anti-human OB–coated plates, then polyclonal rabbit anti-human OB was added to the plates and the complexes were detected by using horseradish peroxidase–labeled goat anti-rabbit immunoglobulin G. Optical density of the labeled reaction was measured at a wavelength of 450 nm in a plate reader (Titertek Multiscan MC; Eflab OY, Helsinki) (29, 30).

Daily urinary urea nitrogen and creatinine were determined with an autoanalyzer and daily ammonium nitrogen was measured by using a specific ion electrode (Orion Research Inc, Cambridge, MA). Total urinary nitrogen was measured with an automated micro-Kjeldahl technique by using a single-channel autoanalyzer (Technicon, Tarrytown, NY) (31) or by chemiluminescense (Antek Pyro-chemiluminescent Nitrogen System, Houston). All determinations were made in duplicate or triplicate. Daily nitrogen balance was calculated on the basis of measured intake minus the sum of losses (32).

Weekly fat (triacylglycerol) losses were calculated by using the following equation:

\[
\text{Fat loss (g)} = \left[ \frac{\text{energy expenditure}}{9} \right] - \left[ \frac{\text{energy from protein oxidation}}{\text{g N/d}} \times 6.25 \right] - \left[ \frac{\text{energy from dietary intake}}{\text{g N/d}} \times 4 \right]
\]

where energy expenditure is REE (measured) + energy expended in daily activity (estimated as 50% of baseline REE) and energy from protein oxidation is nitrogen excretion (g N) × 6.25 (g protein/g N) × 4 (kcal/g protein). (To convert kcal to kJ, multiply by 4.184.) The thermic effect of food was estimated to be 7% and was considered part of the energy expended in daily activity. Because the fasting group had no dietary intake, 7% was subtracted from total energy expenditure.

### Statistical analyses

Statistical analyses were carried out by using the PRIMER BIOSTATISTICS program (McGraw-Hill Inc, New York) and the SPSS for WINDOWS software package (release 6.0; SPSS Inc, Chicago). Data were analyzed by using repeated-measures analysis of variance (ANOVA): one factor (group) between and one factor (time) within subjects. When an interaction among groups was found with repeated measures, a one-way ANOVA was done to detect differences among groups at each point and the significant differences \((P < 0.05)\) were identified by Bonferroni adjustment. Linear correlations were calculated by using Pearson correlation coefficients. Data are presented as means ± SEMs.

### RESULTS

There were no significant untoward effects experienced by the subjects during any of the dietary, fasting, or refeeding periods, nor any abnormalities indicated by the hemograms, clinical biochemistry measurements, or electrocardiograms. Serum triacylglycerol decreased significantly from baseline to weeks 1 and 4: from 1.5 ± 0.1 to 1.0 ± 0.1 and 0.8 ± 0.1 mmol/L with fasting, from 1.8 ± 0.5 to 0.9 ± 0.2 and 0.8 ± 0.1 mmol/L with the VLED, and from 1.4 ± 0.2 to 1.1 ± 0.1 and 1.2 ± 0.1 mmol/L with the BDD, respectively. Blood 3-hydroxybutyrate increased significantly from baseline to weeks 1 and 4: from 31 ± 12 to 3949 ± 284 and 6180 ± 332 μmol/L with fasting, from 23 ± 10 to 811 ± 133 and 1954 ± 360 μmol/L with the VLED, and from 35 ± 6 to 102 ± 25 and 157 ± 43 μmol/L with the BDD, respectively. Serum uric acid increased transiently during the VLED and fasting (data not shown), in association with ketosis, but there were no related symptoms or clinical events.

Plasma leptin concentrations in the 3 groups of subjects are shown in Figure 1A. Baseline values on day 7 of isocaloric feeding were not significantly different between groups and correlated positively with fat mass \((r = 0.724, P = 0.0002)\), REE \((r = 0.501, P = 0.025)\), fasting plasma insulin \((r = 0.503, P = 0.020)\), and triacylglycerol \((r = 0.517, P = 0.03)\) and negatively with the nonprotein respiratory quotient (NPRQ) \((r = -0.633, P = 0.005)\). However, in stepwise linear multiple regression analyses, only fat mass correlated significantly with initial plasma leptin concentrations \((r = 0.786, P = 0.0003; n = 16)\). Leptin decreased significantly in all 3 groups in the first week \((P < 0.001)\), with values correlating with baseline values \((r = -0.625, P = 0.002)\), and continued to decrease but less dramatically so through week 4. At week 4, concentrations also correlated with baseline values \((r = -0.843, P < 0.001)\). The percentage decline in baseline values is shown in Figure 1B. Differences were greatest between the fasting and BDD groups and were significant at all time points. Thus, there was a trend for greater declines in leptin with greater energy deficits. Leptin concentrations adjusted per kilogram fat mass are shown in Figure 1C. The decrease in the BDD group was significantly lower than that in the fasting group from weeks 1 to 4 \((P = 0.026)\). After 1 wk of refeeding (week 5), leptin increased significantly \((P = 0.002)\) in the fasting group.

Cumulative losses in fat mass in absolute values and as percentage changes from baseline in the 3 groups of subjects are shown in Figure 2, A and B, respectively. Fat mass decreased significantly \((P < 0.001)\) from baseline in all 3 groups at week 1: 1.63 ± 0.06 kg in the fasting group, 1.48 ± 0.13 kg in the VLED group, and 0.87 ± 0.07 kg in the BDD group. The rates of loss remained constant in each subsequent week, up to week 4. The
The amount of fat lost during refeeding (1.1 ± 0.1 kg) was significantly different from the amount of fat lost during the preceding weeks of the study (P < 0.001). The amount of fat lost reflected the energy deficits, with the fasting and VLED groups losing more fat than the BDD group (both P < 0.001); differences in the amount of fat lost were not significant between the fasting and VLED groups.

Fasting plasma glucose in absolute values and as percentage changes from baseline are shown in Figure 3A and B, respectively. Plasma glucose concentrations decreased significantly in both the fasting and VLED groups (P < 0.05), significantly more so in the fasting group, whereas concentrations did not decline in the BDD group (Figure 3A). Concentrations in the fasting group were significantly different from those in the VLED and BDD groups from weeks 1 to 4. The percentage change in plasma glucose from initial concentrations was significant (P < 0.001) in the VLED and fasting groups, greatest in the fasting group and intermediate in the VLED group (Figure 3B). With refeeding, glucose increased significantly from weeks 4 to 5 in the fasting and VLED groups.

There were no significant differences between groups in plasma insulin concentrations at any time point, with repeated-measures ANOVA, in part because of interindividual variability, especially at baseline (Figure 4A). However, the percentage change from baseline during the BDD was significantly less than that during fasting and the VLED (Figure 4B). Insulin increased significantly with refeeding (from weeks 4 to 5) in the fasting group, in absolute terms (Figure 4A) and as a percentage change from baseline (Figure 4B).

Changes and percentage changes from baseline in REE are shown in Figure 5A and B, respectively. REE decreased significantly (P < 0.002, repeated-measures ANOVA) with time; by
FIGURE 2. Mean (±SEM) cumulative body fat mass loss (A) and percentage changes (B) from baseline (week 0) at the end of each week of energy restriction (weeks 1–4) and after 1 wk of refeeding (day 7 of week 5) in obese women receiving 1 of 3 treatments: all-protein, very-low-energy diet (VLED; n = 6); fasting (n = 8); and low-energy, balanced-deficit diet (BDD; n = 7). No fat was lost during the 7-d isoenergetic diet. A: From weeks 0 to 4 there were significant main effects of time (P < 0.002) and of group (P < 0.001) and a significant interaction (P = 0.021); from weeks 4 to 5 there was a significant main effect of time (P < 0.001), but not of group, and no significant interaction; significantly different from VLED and fasting, P < 0.001. B: From weeks 0 to 4 there were significant main effects of time (P < 0.001) and of group (P = 0.001) and a significant interaction (P < 0.001); from weeks 4 to 5 there was a significant main effect of time (P < 0.001), but not of group, and no significant interaction; significantly different from VLED and fasting, P < 0.002.

FIGURE 3. Mean (±SEM) fasting plasma glucose concentrations (A) and percentage changes (B) from baseline (week 0) at the end of each week of energy restriction (weeks 1–4) and after 1 wk of refeeding (day 7 of week 5) in obese women receiving 1 of 3 treatments: all-protein, very-low-energy diet (VLED; n = 6); fasting (n = 8); and low-energy, balanced-deficit diet (BDD; n = 7). A: From weeks 0 to 4 there were significant main effects of time (P < 0.001) and of group (P < 0.001) and a significant interaction (P < 0.001); from weeks 1 to 5 there were significant main effects of time (P < 0.001) and of group (P = 0.001) but no significant interaction; significantly different from VLED and BDD, P < 0.001. B: From weeks 0 to 4 there was a significant main effect of group (P < 0.001), but not of time, and no significant interaction; from weeks 4 to 5 there were significant main effects of time (P < 0.001) and of group (P = 0.050) and no significant interaction.
week 1 in the VLED group, by week 2 in the fasting group, and by week 3 in the BDD group. With refeeding, REE increased (NS) by 3.3% from weeks 4 to 5. The positive correlation between percentage changes in leptin (per kg fat lost) and percentage changes in glucose at week 4 in the BDD group and at week 2 in the VLED group are shown in Figure 6.

Pearson correlation coefficients between percentage changes in leptin concentrations and changes in anthropometric and metabolic variables from baseline to weeks 1 and 4 of energy restriction (pooled data) and from week 4 of energy restriction to day 7 of refeeding (week 5) are shown in Table 2. The percentage change in leptin correlated positively with the change in glucose, NPRQ, and fat mass at week 1; with the change in glucose and fat mass at week 4; and with the change in REE from weeks 4 to 5. We performed stepwise multiple linear regression with the percentage change in leptin concentrations from baseline as a dependent variable and the change in glucose, NPRQ, and fat mass at week 1 and the change in glucose and fat mass at week 4 as independent variables. Only glucose entered as a significant independent variable. At week 1, it explained 49% and at week 4, 63% of the variation in the percentage change in leptin. At week 5, 50% of the variation in the percentage change in leptin was explained by the change in REE.

When adjusted per kilogram of fat mass, the change in leptin correlated only with that in glucose at weeks 1 and 4 (Table 3). With refeeding, the change in leptin from weeks 4 to 5 correlated with that in REE and that in triacylglycerol. With stepwise linear multiple regression analysis, only REE was a significant independent variable, explaining 45% of the change in leptin.

Changes in leptin and in metabolic variables in the 3 groups at a time when all subjects had comparable fat losses and comparable energy deficits but of different durations, ie, week 4 for the BDD group and week 2 for the VLED and fasting groups, are shown in Table 4. There were significant differences between the BDD and fasting groups in the percentage change in leptin, the percentage change in leptin per kilogram fat mass lost, and the percentage change and change in absolute values of glucose. There was also a significant difference between the VLED and fasting groups in the percentage change and change in absolute values of glucose. There was no significant effect of age nor of group on baseline leptin concentrations or on leptin concentrations adjusted per kilogram fat mass (by simple-factorial ANOVA). Within the fasting group, of which half the subjects were aged < 30 y, age had no significant effect on leptin responses ($P = 0.650$, repeated-measures ANOVA).

DISCUSSION

We examined the effect of 3 approaches to prolonged inpatient energy restriction on plasma leptin concentrations in obese women with no other concurrent metabolic disorders. Fasting causes decreases in leptin that start at 12 h and reach a nadir of 30–40% of prefasting concentrations at 36 h (18). We likewise found the greatest decreases in leptin concentrations at the onset of the treatments, although our earliest observation occurred later than 12 h. The magnitudes of the changes in leptin clearly did not correspond to those in body fat. Our results confirm that changes in energy intake over the first week have a much more profound influence on leptin concentrations than do corresponding changes in body fat mass (16, 19, 21, 24, 33). Leptin concentrations observed with energy restriction do not reflect the size of body adipose mass (34). However, maintenance of the same low energy intake was associated with a smaller further decrease in leptin concentrations over the subsequent 3 wk, con-
consistent with an effect of the decreasing fat mass. In addition, the results indicated that the remaining large fat mass was unable to return leptin toward baseline values during energy restriction.

We found that the magnitude of the decrease in leptin related to that of its baseline value. However, the decrease in leptin at weeks 1 and 4 did not correlate with baseline values when expressed as a percentage change or when corrected per kilogram fat mass. Thus, we analyzed the response in change from baseline by adjusting leptin per kilogram fat mass to correct for the variability at baseline and found a significant correlation with the change in glucose at weeks 1 and 4. The change in glucose at weeks 1 and 4 did not correlate with baseline values. Furthermore, we pooled the data of subjects after comparable cumulative energy deficits and fat losses and found that per kilogram of fat lost, the percentage decline in leptin correlated with the change in glucose concentrations (Figure 6). These results support those of others (24) that a close relation exists between changes in leptin and glucose during energy restriction and suggest an effect of

**FIGURE 5.** Mean (±SEM) resting energy expenditure (A) and percentage changes (B) from baseline (week 0) at the end of each week of energy restriction (weeks 1–4) and after 1 wk of refeeding (day 7 of week 5) in obese women receiving 1 of 3 treatments: all-protein, very-low-energy diet (VLED; n = 6); fasting (n = 8); and low-energy, balanced-deficit diet (BDD; n = 7). A: From weeks 0 to 4 there was a significant main effect of time (P < 0.001), but not of group, and no significant interaction; from weeks 4 to 5 there were no significant main effects and no significant interaction. B: From weeks 0 to 4 there was a significant main effect of time (P < 0.001), but not of group, and no significant interaction; from weeks 4 to 5 there were no significant main effects and no significant interaction.

**FIGURE 6.** Relation between the percentage change in leptin concentrations adjusted per kilogram fat mass lost at week 4 of a low-energy, balanced-deficit diet (BDD; n = 7) and at week 2 of an all-protein, very-low-energy diet (VLED; n = 6) or of a fast (n = 8) and the percentage change in glucose in obese women.
TABLE 2
Pearson correlation coefficients between percentage changes in leptin concentrations and changes in anthropometric and metabolic variables from baseline to weeks 1 and 4 of 3 energy-restriction protocols and from week 4 of energy restriction to day 7 of refeeding (week 5).\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Week 1 (n = 21)</th>
<th></th>
<th>Week 4 (n = 21)</th>
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<th>Week 5 (n = 14)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
<td>(P)</td>
<td>(r)</td>
<td>(P)</td>
<td>(r)</td>
<td>(P)</td>
</tr>
<tr>
<td>(\Delta\text{Glucose})</td>
<td>0.713</td>
<td>0.0003</td>
<td>0.792</td>
<td>&lt;0.0001</td>
<td>0.345</td>
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<td>(\Delta\text{Insulin})</td>
<td>0.119</td>
<td>0.608</td>
<td>0.287</td>
<td>0.207</td>
<td>0.194</td>
<td>0.526</td>
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<tr>
<td>(\Delta\text{REE})</td>
<td>0.149</td>
<td>0.531</td>
<td>0.114</td>
<td>0.632</td>
<td>0.709</td>
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<td>(\Delta\text{NPRQ})</td>
<td>0.577</td>
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<td>0.288</td>
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<td>(\Delta 3\text{-OHB})</td>
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<td>0.0002</td>
<td>−0.765</td>
<td>0.0001</td>
<td>−0.063</td>
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<tr>
<td>(\Delta \text{Fat mass})</td>
<td>0.461</td>
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<td>0.533</td>
<td>0.013</td>
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<td>(\Delta \text{Triacylglycerol})</td>
<td>0.207</td>
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<td>0.087</td>
<td>0.730</td>
<td>0.434</td>
<td>0.182</td>
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\(^1\)3-OHB, 3 hydroxybutyrate; REE, resting energy expenditure; NPRQ, nonprotein respiratory quotient.

TABLE 3
Pearson correlation coefficients between changes in leptin and changes in metabolic variables from baseline to weeks 1 and 4 of 3 energy-restriction protocols and from week 4 of energy restriction to day 7 of refeeding (week 5).\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Week 1 (n = 21)</th>
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<th>Week 4 (n = 21)</th>
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<th>Week 5 (n = 14)</th>
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<td>(P)</td>
<td>(r)</td>
<td>(P)</td>
<td>(r)</td>
<td>(P)</td>
</tr>
<tr>
<td>(\Delta \text{Leptin (µg·L}^{-1}·kg fat mass}^{-1})</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(\Delta\text{Glucose})</td>
<td>0.538</td>
<td>0.012</td>
<td>0.447</td>
<td>0.042</td>
<td>0.235</td>
<td>0.418</td>
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<tr>
<td>(\Delta\text{REE})</td>
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<td>0.511</td>
<td>−0.031</td>
<td>0.878</td>
<td>0.675</td>
<td>0.024</td>
</tr>
<tr>
<td>(\Delta \text{Triacylglycerol})</td>
<td>−0.154</td>
<td>0.543</td>
<td>0.115</td>
<td>0.651</td>
<td>0.625</td>
<td>0.040</td>
</tr>
</tbody>
</table>

\(^1\)REE, resting energy expenditure.
concentrations differed significantly between the BDD and fasting groups, but changes in insulin did not (Table 4). The greatest decrease in glucose concentrations was associated with the greatest decrease in leptin per kilogram fat lost. This finding (all data pooled) supports a role for glucose and its availability to adipose tissue in determining circulating leptin concentrations. We present results from women only because their responses to energy deficits differ from those of men (24, 44), 2 result in smaller percentage decreases in insulin and glucose than in men (24), and 3 result in higher leptin concentrations than in men. Thus, our results cannot be extrapolated to men.

Our refeeding diet had greater effects in subjects after fasting than after the VLED: leptin, insulin, and glucose concentrations increased significantly after refeeding in the fasting group, whereas only glucose increased in the VLED group. Ketone bodies decreased considerably in both groups after refeeding (data not shown); the corresponding increase in insulin and glucose than in men (24), and 3 result in higher leptin concentrations than in men. Thus, our results cannot be extrapolated to men.

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In conclusion, we showed that prolonged changes in energy intake override the regulation of leptin concentrations by the fat mass. Our results support the premise that insulin-mediated glucose uptake by adipocytes primarily modulates leptin concentrations. Furthermore, after a fast, refeeding a diet providing energy intakes considerably below requirements increases leptin concentrations and energy expenditure even with ongoing fat mobilization.

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REFERENCES


