Decreases in fasting leptin and insulin concentrations after acute energy restriction and subsequent compensation in food intake

Monica Mars, Cees de Graaf, Lisette CPGM de Groot, and Frans J Kok

ABSTRACT
Background: The decrease in leptin after energy restriction is a starvation signal to the brain. Several studies have found an association between this decrease and subjective appetite; however, no solid data are available on the acute decrease in fasting leptin concentration and subsequent caloric compensation.

Objective: The objective was to assess the effect of acute decreases in fasting leptin concentrations, induced by energy restriction, on subsequent energy intake compensation. We hypothesized that men with a large decrease in fasting leptin concentrations would have larger ad libitum energy intakes than would men with a small decrease in leptin.

Design: Thirty-four male unrestrained eaters [age: 23 ± 3 y; body mass index (in kg/m²): 22.3 ± 1.6] participated in a semicontrolled intervention study. Fasting serum leptin and insulin concentrations were measured before and 2 d after 62% energy restriction. Energy intake was measured on the 2 following days on which food was provided ad libitum.

Results: During energy restriction, fasting leptin and insulin concentrations decreased by 27.2% (95% CI: −34.4%, −19.9%) and 30.7% (95% CI: −41.0%, −20.4%), respectively. Subjects consumed 143 ± 27% of their estimated energy requirements (18.3 ± 2.9 MJ) on the first day and 124 ± 20% (16.0 ± 2.6 MJ) on the second day of ad libitum intake. No significant correlations were observed between decreases in fasting leptin or insulin concentrations and subsequent ad libitum energy intake; however, decreases in insulin were correlated with an increase in carbohydrate intake (r = −0.49, P < 0.01).

Conclusion: Although fasting leptin concentrations decreased significantly during energy restriction and subjects showed compensatory behavior during subsequent ad libitum food intake, no association was observed between the decrease in fasting leptin concentrations and caloric compensation.

KEY WORDS Ob protein, insulin, appetite regulation, energy intake, caloric restriction, leptin

INTRODUCTION

Leptin is a hormone that is produced by adipose tissue and is secreted in the blood (1). Because leptin is produced by fat cells, serum leptin concentrations are positively correlated with the amount of fat mass in the body (2). However, short-term severe energy restriction causes circulating leptin concentrations to decrease to a larger extent than would be expected from the loss of fat mass alone (3–5). In a recent review, Flier (6) advocates that this decrease in leptin concentration serves as an important signal from fat to the brain that the body is starving. Moreover, he suggests that this function is likely to be as important as or more important than is leptin’s function to inform the brain about the amount of fat mass. Because of the low availability of glucose, insulin concentrations decrease during energy restriction. It has been suggested that this decrease in insulin mediates the acute decrease in leptin (5, 7). Moreover, growing evidence suggests that insulin itself is also involved in the central regulation of energy balance via a pathway similar to that for leptin (8, 9).

Although several human intervention studies have shown that decreases in leptin concentrations are associated with increased appetite feelings (10–13), only one study has shown that acute manipulations in energy balance are inversely associated with energy intake compensation (14). However, this study included only 6 obese subjects and the manipulations included both energy restriction and overfeeding. Therefore, the results of that study have to be interpreted carefully. Thus, no solid data are available on the effect of an acute decrease in leptin on energy intake compensation in humans.

For studies measuring energy intake compensation, it is important to select subjects that are lean and do not exhibit restraint eating, ie, a high cognitive awareness of food intake (15) or any other inhibition. Being overweight and a restrained eater may suppress voluntary energy intake after weight loss. Therefore, we performed a study in 34 lean, male, unrestrained, free-living subjects. An acute decrease in fasting leptin and insulin concentrations was induced by 2 d of 62% energy restriction and, subsequently, the ad libitum compensatory energy intake was measured during the 2 d after. We hypothesized that men with a large decrease in leptin would have higher ad libitum energy intakes than would men with a small decrease in leptin. Second, we investigated whether this decrease in leptin was associated with macronutrient composition of the ad libitum food intake. Additionally, the changes in insulin concentrations during energy restriction were investigated in relation to compensatory energy and macronutrient intakes.

1 From the Division of Human Nutrition, Wageningen University, Wageningen, Netherlands.
2 Supported by the Netherlands Association for Scientific Research (NWO-MW project no. 980-10-007).
3 Reprints not available. Address correspondence to M Mars, PO Box 8129, NL-6700 EV Wageningen, Netherlands. E-mail: monica.mars@wur.nl.
Received March 2, 2004.
Accepted for publication November 10, 2004.

the equation of Schofield (19):

\[\text{BMR (MJ)} = 0.0485 \times \text{weight (kg)} + 3.67 \quad (1)\]

Then, the physical activity level was estimated from a short retrospective physical activity questionnaire containing 6 activities (20). Basal metabolic rate and physical activity level were used to estimate individual energy needs:

\[E = \text{BMR} \times \text{PAL} \quad (2)\]

Taking one-third of the individual energy needs and rounding these values up in energy groups of 0.8 MJ resulted in 15 subjects receiving 4.2 MJ/d, 14 subjects receiving 5.0 MJ/d, and 5 subjects receiving 5.8 MJ/d. The energy-restricted diet consisted of nutrient-dense meal and snack replacements (Profiel; Nutricia, Zoetermeer, Netherlands), each of which contained 0.8 MJ, that were provided and taken home, also contained nutrient-dense meal and snack replacements (Profiel; Nutricia, Zoetermeer, Netherlands), each of which contained 0.8 MJ, that were supplied at the beginning of the study. Fifty-five percent of energy was derived from carbohydrate, 20% from fat, and 25% from protein (Table 2). Compliance was measured from preprinted daily food records, on which subjects recorded the time of consumption. Noncaloric beverages (e.g., diet cola, black coffee, and black tea) were allowed during the energy-restriction period; a list of these products was provided to the subjects at the start of the intervention. The products consumed were also recorded in the daily food record.

Ad libitum food intake

Food intake on days 3 and 4 of the protocol was not controlled, ie, the subjects’ intakes were ad libitum. During these days, the subjects ate a buffet-style breakfast and warm lunch at the laboratory. At least 200% of the estimated energy need was available to each subject, and empty packages and leftovers were used to record food intake. The remaining meals and snacks, which were provided and taken home, also contained ≥200% of estimated energy needs. The subjects recorded the foods consumed at home in a diary and were asked to bring all empty packages and leftovers back to the laboratory. The diary, leftovers, and empty packages were cross-checked, and portion sizes were verified with dummies of household measures by a trained dietitian. Only foods that are generally consumed in the Netherlands were provided during the ad libitum period (21) (Appendix A). In addition to these foods, the subjects were free to use other products, but they were instructed to write these products down in detail in their diaries.

To prevent the subjects from consuming amounts similar to those consumed habitually, foods were provided in unusual portions sizes. For example, bread rolls were smaller than the regular size (20 g instead of 30 g) and plates larger than regular dinner plates were used. Additionally, breakfast on day 3 consisted of a milk shake (400 g), which was offered ad libitum in a blinded beaker. The macronutrient composition of the milkshake was determined according to the Dutch national guidelines (21);
58%, 29%, and 13% of calories were derived from carbohydrates, fat, and protein, respectively. One beaker contained 2.6 MJ, which reflects the average energy intake in young adult men during breakfast (21). The subjects were instructed to drink until satiation. If necessary, a second and third serving were available. The subjects were not aware of the energy content of the breakfast meal.

Energy intake

During all 4 d, food intake was calculated by cross-checking the leftovers and diaries. Energy and macronutrient intakes were calculated by using the Dutch food-composition table (22) and product information from the manufacturers. To correct for individual differences in energy needs, energy intake proportional to estimated energy needs was calculated per day. Additionally, the percentages of energy derived from protein, fat, and carbohydrate were calculated to investigate the effect of leptin decreases on the macronutrient composition of the diet.

Blood sampling and biochemical analyses

Fasting blood samples were taken between 0730 and 0930 in the morning for each individual subject at the same time. Subjects were not allowed to drink or eat calorie-containing products ≤12 h before blood sampling. Blood samples were placed directly on ice after sampling and were centrifuged at 1187 g for 10 min after the serum samples were coagulated. Serum and plasma samples were then divided among aliquots and stored at −70 °C until analyzed.

Serum leptin concentrations were assessed in duplicate by radioimmunoassay (Linco Research Inc, St Charles, MO), with the lowest detection limit at 0.5 ng/mL. The intraassay CV was 3–8%; the interassay CV was 4–8%. Serum insulin was measured in duplicate by immunoassay (Immulite 2000 Analyzer; Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden), with the lowest detection at 2.0 μU/mL. Plasma glucose was measured quantitatively with a bichromatic endpoint assay (Glu FlexTM reagent; Dade Behring BV, Leusden, Netherlands). All samples for each subject were analyzed in one run. Means of the duplicates were used for data analyses.

Anthropometric measures

Height was measured with a wall-mounted stadiometer that was accurate to 0.5 cm, while subjects were shoeless, with the Frankfurt plane horizontal. The subjects were weighed wearing indoor clothing and no shoes and with empty pockets on a digital balance accurate to 0.1 kg in a fasting state after voiding.

Statistical analyses

Because of nonnormality, fasting hormonal concentrations were transformed with the natural logarithm (ln) before analyses. For these variables, geometric means and 95% CIs are provided. Other variables are provided as arithmetic means ± SDs or 95% CIs. The range represents the minimum and maximum value of the variable. Changes in weight and biochemical variables during the energy-restriction period and the ad libitum period were tested with paired Students’ t tests. Associations between baseline measures were calculated by means of Pearson’s correlation coefficients (r).

Changes in biochemical variables were presented as proportional changes in the descriptive tables [(day 3 − day 1)/day 1 × 100%]. Changes in biochemical variables within subjects between days were tested by repeated-measures analysis of variance; overall P values for a day effect are presented. Associations were tested by using changes in absolute values, ie, day 3 − day 1, or the ratios, ie, hormone concentrations on day 3/hormone concentrations on day 1. The latter resulted in a measure corrected for baseline concentrations. Associations between changes in biochemical variables and ad libitum food intake were calculated by regression analyses; slopes (β1) and 95% CIs are provided in the tables. Because of a nonnormal distribution, the natural logarithm (ln) of the ratios were used in the regression analyses, ie, ln(day 3/day 1). P values < 0.05 were considered statistically significant. For all data analyses we used the statistical package SAS (release 8.0; SAS Institute Inc, Cary, NC).

RESULTS

Energy restriction

At baseline, the serum leptin concentration was associated with body weight (r = 0.39, P < 0.05) and body mass index (r = 0.55, P < 0.001) but not with insulin concentration (r = 0.18, P = 0.30). The average change in body weight after energy restriction was −1.1 ± 0.7 kg (range: −2.6 to −0.3 kg), and serum leptin, serum insulin, and plasma glucose concentrations changed by −1.0 (95% CI: −1.4, −0.5) μg/mL, −2.4 (95% CI: −3.3, −1.5) μU/mL, and −0.3 (95% CI: −0.4, −0.2) mmol/L, respectively (Table 3). Decreases in fasting leptin concentrations were neither associated with changes in body weight (r =
These increases in fasting leptin and insulin concentrations were associated with their decreases during energy restriction: \( r = 0.88 \) (\( P < 0.0001 \)) and \( r = 0.47 \) (\( P < 0.01 \)), respectively.

**Leptin and insulin decreases in relation to energy intake**

Fasting serum leptin concentrations on day 3 were not associated with energy intake on the same day: energy intake in kJ \( (r = -0.11, P = 0.55) \) and energy intake as a percentage of energy \( (r = -0.13, P = 0.45) \). A small positive trend \( (r = 0.31, P = 0.08) \) was observed between the ratio of fasting leptin concentrations on day 3 and day 1 and energy intake (expressed as a percentage of estimated energy requirements) on the first ad libitum day (Table 4). In other words, a large decrease in leptin tended to be associated with a low energy intake directly after energy restriction (Figure 1). On the second ad libitum day, no such trend was observed \( (r = 0.15, P = 0.41) \). No statistically significant associations were observed between the ratio of fasting insulin concentrations on day 3 and day 1 and the ad libitum energy intake on day 3 \( (r = 0.27, P = 0.13) \) or day 4 \( (r = 0.22, P = 0.21) \).

**Leptin and insulin decreases in relation to macronutrient composition**

No statistically significant associations were observed between changes in leptin during energy restriction and macronutrient specific energy intake (Table 4). However, on the first ad libitum day, the ratio for fasting insulin concentrations was inversely associated with the percentage of energy derived from carbohydrates \( (r = -0.49, P = 0.006) \). On day 4, this association became weaker \( (r = -0.30, P = 0.08) \). In other words, a larger decrease in insulin was associated with a diet containing more energy from carbohydrate (Figure 2).
DISCUSSION

Although it is generally accepted that acute decreases in leptin, induced by severe energy restriction, are signals to the brain to stimulate energy intake, the present study is the first to investigate this in 34 young adult men. In the present study, we observed that leptin concentrations decreased significantly and that subjects showed compensatory energy intake. However, we did not observe a statistically significant association between the magnitude of the decrease in leptin and energy intake compensation.

As expected, serum leptin concentrations decreased after energy restriction and increased after refeeding. Additionally, other variables—such as body weight, insulin, and glucose—also showed the expected pattern: a decrease during energy restriction and an increase during ad libitum intake. Moreover, subjects showed compensatory behavior during the ad libitum period, ie, they consumed considerably more energy than their estimated energy needs. Compensatory energy intake was especially apparent on day 3; the subjects consumed an average of 43% more than their estimated energy needs. Nevertheless, we did not observe that men with a larger decrease in leptin concentration had a larger ad libitum energy intake than did men with a small decrease in leptin concentration.

It might be that we did not find an association between the magnitude of the decrease in fasting leptin concentration and energy intake compensation because our group of subjects was too homogeneous. As stated in the introduction, restraint eating, ie, a high cognitive awareness of food intake (15), and being overweight may suppress voluntary energy intake after weight loss and therefore affect energy intake compensation. For this reason, we selected a group of lean unrestrained-eating men for our intervention. However, by selecting this homogenous group, we presumably narrowed the variation in the leptin response. This may have resulted in a small contrast in energy intake compensation between the so-called hyporesponders and hyper-responders, thereby underestimating the existing associations.

During the ad libitum period, we used 2 methods to make the subjects aware of their internal cues for appetite and to prevent them from consuming their habitual meals. First, we provided the foods in unusual portion sizes, ie, greater or smaller portions (appendix A). Second, the breakfast on day 3 consisted of a milk...
shake, for which the energy content was not known to the subjects. It is possible that these 2 methods might have introduced extra variation in energy intake. However, we believe that these methods were necessary to make the subjects alert to their internal cues for appetite.

In our study, the energy intake compensation was defined as the proportional energy intake compared with the estimated energy needs. It might have been that the estimation of the energy needs affected this outcome measure. We used the equation of Schofield in combination with a physical activity questionnaire to estimate individual energy needs. The Schofield equation is a simple equation based on the sex, age, and weight of the subject (19), and it has been shown that over- or underestimation with these type of equations is more likely to occur in obese subjects than in lean subjects, as in our study (23). The physical activity questionnaire that we used included questions on 6 daily activities (20), of which the number of hours of sleep and hours of working or studying were the most important questions. These 2 activities are rather constant within a subject. Moreover, the physical activity levels we observed were similar to those found in other studies (: 1.8; range: 1.5–2.2) (24). Therefore, we do not think that this estimation affected our results.

It has been shown that leptin has a diurnal pattern, which peaks at night (25–27). Several studies have shown that, during energy restriction, not only fasting leptin concentrations, but also the diurnal variation of these concentrations, is decreased (26, 27). Additionally, several studies have shown that carbohydrate composition affects the diurnal pattern of leptin. Weigle et al (28) have observed that a high-carbohydrate, low-fat diet increased the amplitude of leptin. Moreover, they observed that this increase in amplitude was predictive for the degree of body weight and fat lost during this study. This is in contrast with the finding of Teff et al (29), who observed that fructose-containing beverages decreased the amplitude of leptin compared with glucose-containing drinks, resulting in an increase in energy derived from an ad libitum test meal in restrained-eating women. In our study design, the measurement of spontaneous energy intake was critical. Continuous or multiple blood sampling would require a medical setting, which may have affected spontaneous energy intake. We therefore chose to measure ad libitum energy intake as accurately as possible in a free-living situation, but had to make the concession to only measure fasting leptin concentrations. However, further studies should also consider the change in diurnal variation as an important starvation signal.

We observed that large decreases in insulin lead to a high carbohydrate intake, ie, energy derived from carbohydrates and the amount of mono- and disaccharides that was consumed. This is in contrast with a classic study by Rodin et al (30), which observed that not low, but high, insulin concentrations increase hunger, palatability of sucrose, sweetness, and food intake. However, this study investigated hyperinsulenic clamps under short-term (150 min) experimental conditions, which are not comparable with our study setting.

There is evidence that the decrease in insulin during energy restriction might induce the decrease in circulating leptin (5, 7). During energy restriction, glycogen stores are being depleted, insulin concentrations decrease, and the body switches over to fatty acid oxidation. Human and in vitro studies have shown that a decreased glucose metabolism results in a reduction in leptin production (31, 32). It has therefore been suggested that foods with a high glycemic index during energy restriction may blunt the decreases in leptin (33, 34) and thus lower the starvation signal to the brain, which eventually prevents weight regain after weight loss. However, others speculate that carbohydrates with a low glycemic index, ie, fructose, have the opposite effect; they do not stimulate the diurnal variation in leptin and basal leptin concentrations, which results in an increase in energy intake (29).

In our study, few data on the specific mono- and disaccharide contents of the carbohydrates were available; only 3–16% of the data were available in the Dutch nutrient database (22, 35). Because no reliable and accurate data are available, we can only speculate that the carbohydrate composition of the energy-restricted diet might have affected the leptin response and, therefore, the intake of carbohydrates during the ad libitum period.

Several human studies have observed that a greater decrease in leptin is associated with a greater appetite (10–13). However, no data are yet available on actual energy intake. Although it has been shown that the level of appetite is a good predictor of energy intake (36–38), it is possible that the relation between the motivation to eat and actual food intake is disturbed by a cognitive awareness in a human situation. This may affect food intake even after the exclusion of restrained-eating subjects from participation. The awareness of the subjects being on an energy-restricted diet might have disturbed the association between internal cues, ie, the decrease in fasting leptin, and subsequent energy intake compensation.

Animal models still provide most of the direct evidence for the role of the acute leptin response in energy intake compensation after energy restriction (6). It might be that the role of leptin in starvation and energy intake regulation is different in rodents and cannot be extrapolated to humans. First, leptin-deficient rodents and starving rodents show several neuroendocrine changes (1), which are not observed in leptin-deficient humans (39–41) or fasting humans (42). These observations stress that there might be important differences in the physiology of starvation between species. Second, the cognitive awareness of humans during energy restriction, which is always present, also makes it very difficult to extrapolate findings from rodents to humans.

Overall, we conclude that the decreases in fasting leptin concentrations induced by a 62% energy-restricted diet of 2 d was not associated with the energy intake compensation during the following 2 d. Although it is generally accepted that the acute response in fasting leptin after energy restriction is a starvation response, more studies have to be conducted to confirm this assumption. Controlled interventions in obese and nonobese subjects are especially needed to unravel the function of this starvation response in humans.

We thank the volunteers who participated in this study: Els Siebelink, Karin Borgonjen, and Lieneke Kolmus for their help during the preparation and implementation of the intervention study; and Lucy Ockma and coworkers for their assistance in the sample collection.

MM helped design the study, collected data, analyzed the statistical data, and wrote the manuscript. CdG, LCPMdG, and FJK helped design the study and provided significant advice and consultation. No conflicts of interest were declared by any of the authors.

REFERENCES


## Appendix A. Foods provided during the ad libitum period (days 3 and 4)

<table>
<thead>
<tr>
<th>Product (portion size)</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast and cold dinner</td>
<td>kJ/100 g</td>
</tr>
<tr>
<td>Test meal (400 g)(^1)</td>
<td>641</td>
</tr>
<tr>
<td>Bread rolls, natural, with raisins or with muesli (20 g)</td>
<td>1013 or 1073</td>
</tr>
<tr>
<td>Margarine (75 g)</td>
<td>2972</td>
</tr>
<tr>
<td>Cheese (±60 g, 3 slices)</td>
<td>1571</td>
</tr>
<tr>
<td>Ham (±60 g, 3 slices)</td>
<td>540</td>
</tr>
<tr>
<td>Bologna (±48 g, 6 slices)</td>
<td>1292</td>
</tr>
<tr>
<td>Strawberry-cherry jam (50 g)</td>
<td>1023</td>
</tr>
<tr>
<td>Chocolate spread (50 g)</td>
<td>2248</td>
</tr>
<tr>
<td>Apple syrup (75 g)</td>
<td>961</td>
</tr>
<tr>
<td>Sprinkles, chocolate-flavored (75 g)</td>
<td>1909</td>
</tr>
<tr>
<td>Sprinkles, fruit-flavored (75 g)</td>
<td>1671</td>
</tr>
<tr>
<td>Orange juice (300 g)</td>
<td>167</td>
</tr>
<tr>
<td>Milk (300 g)</td>
<td>202</td>
</tr>
<tr>
<td>Buttermilk (300 g)</td>
<td>137</td>
</tr>
</tbody>
</table>

### Warm lunch

#### Day 3
- Rice with spices, *mase* (on request) | 405 |
- *Ketiao* sauce (on request) | 718 |
- Scrambled egg (on request) | 707 |
- Dessert (100, 150, or 300 g) | 734 |

#### Day 4
- Pasta | 281 |
- Chili sauce (on request) | 613 |
- Minced meat (on request) | 972 |
- Or Quom, vegetarians (on request) | 469 |
- Cheese (30 g) | 1571 |
- Dessert (100, 150, or 300 g) | 337 |

### Snacks
- Cookie, *stroopwafel* (8 g) | 1787 |
- Cookie, *café noir* (10 g) | 1882 |
- Gingerbread (17 g) | 1128 |
- Kiwi fruit (per piece) | 168 |
- Apple (per piece) | 207 |
- Orange (per piece) | 198 |
- Banana (per piece) | 375 |
- Twix chocolate bar (60 g)\(^2\) | 2040 |
- Snickers chocolate bar (60 g)\(^2\) | 2128 |
- Crisps, cheese-flavored (45 g) | 2102 |
- Crisps, paprika-flavored (45 g) | 2288 |
- Coffee creamer (2.5 g) | 2306 |
- Sugar (5 g) | 1700 |

\(^1\) Only provided during the breakfast on day 3.
\(^2\) M&M/Mars Inc, Hackettstown, NJ.