

Fasting leptin and appetite responses induced by a 4-day 65%-energy-restricted diet

M Mars¹, C de Graaf¹, C P G M de Groot¹, C T M van Rossum² and F J Kok¹

¹Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands

²Centre for Nutrition and Health, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

Correspondence: Dr C de Graaf, Division of Human Nutrition, Wageningen University, PO Box 8129, NL-6700 EV Wageningen, The Netherlands. E-mail: Kees.deGraaf@wur.nl

Received 30 November 2004; Revised 29 June 2005; Accepted 31 July 2005; Published online 13 September 2005.

Abstract

Objective:

Animal studies show that the leptin decline after acute severe caloric restriction is a peripheral signal to increase food intake. However, most human studies have failed to observe such a relationship. We studied the acute effects of severe caloric restriction on the association between serum leptin concentrations and subjective appetite.

Subjects:

A total of 44 healthy adult men (aged: 43 ± 5 years; BMI: 27.3 ± 3.2 kg/m²).

Measurements:

Fasting serum leptin concentrations and self-perceived appetite levels were measured during a 4-day diet containing 36% of the estimated energy requirements. Appetite levels were assessed with a 10-point Likert scale, reflecting hunger, fullness, desire to eat, prospective consumption and total appetite.

Results:

After the 4-day energy deficit, fasting leptin concentrations decreased by 39.4% (95% CI: -43.6; -34.9%). This decline was associated with an increase in fasting hunger ($r=-0.42$; $P<0.01$), desire to eat ($r=-0.39$; $P<0.05$) and total appetite ($r=-0.38$; $P<0.05$). Furthermore, the association between fasting leptin concentrations and fasting appetite levels became stronger during the energy restriction period (for total appetite: day 0 $r=-0.15$, $P=0.32$; day 2 $r=-0.31$, $P<0.05$; day 4 $r=-0.41$, $P<0.01$).

Conclusions:

The acute proportional reduction in fasting leptin after 4-day energy restriction is associated with an increase in self-perceived appetite. Additionally, the inverse association between proportional fasting leptin concentrations and self-perceived appetite response becomes stronger as energy restriction is prolonged. These findings suggest that leptin has an instrumental role in restoring energy balance in humans through the expression of appetite.

Keywords:

ob-protein, appetite regulation, hunger, caloric restriction, adult men

Introduction

Leptin is a hormone that is produced by fat cells and secreted into the blood stream. From animal studies it is known that leptin has an inhibiting effect on food intake. Ob/ob mice, transgenic mice that lack the gene to produce leptin, are leptin deficient and extremely obese.¹ Also in humans some cases of extreme obesity are known, which are caused by leptin deficiency, as a result of rare mutations in the leptin gene.² Injecting these humans or ob/ob mice with leptin reduces their food intake and brings them back to lower body weight.^{3, 4, 5}

Serum leptin concentrations are positively correlated with the amount of fat mass in the body.⁶ However, during short-term severe energy restriction, serum leptin concentrations rapidly fall to a larger extent than would be expected from loss of fat mass alone.^{7, 8, 9} Therefore, leptin is thought to be instrumental for the restoration of negative energy balances.^{10, 11, 12} This acute reduction in leptin might be the result of reductions in insulin concentrations that occur during energy restriction.¹³

Leptin may regulate energy balance by affecting energy intake, that is, appetite and food intake.¹¹ Up till now, several studies have investigated the association between leptin levels and appetite or food intake in humans, either during conditions of energy balance^{14, 15, 16, 17, 18, 19, 20} or energy imbalance.^{21, 22, 23} Especially, the latter studies found evidence for the hypothesis that leptin is involved in the restoration of negative energy balances via appetite. Westerterp-Plantenga *et al.*²² found a decrease in appetite ratings by administration of exogenous leptin during energy restriction.^{22, 23} Keim *et al.*²¹ found a strong inverse association between leptin concentrations and appetite ratings during energy restriction, independent of weight loss. However, both studies were based on several weeks of energy restriction, and did not study the effects of severe energy restriction that take place within days.

Thus, it is generally assumed that leptin is instrumental in restoring energy balance via appetite or food intake. However, if leptin has an important role in energy balance via appetite, leptin may affect appetite after short-term changes in energy balance. Therefore, the objective of our study was to assess the association between leptin and appetite responses induced by an acute, that is 4-day, energy restriction.

Methods

Subjects

A total of 44 adult men were recruited from the Doetinchem Cohort Study.^{24, 25, 26} Subjects were eligible if they did not use any drugs known to affect energy metabolism, and if they were not on a prescribed or weight loss diet during the previous 2 months. Individuals with a history of diabetes mellitus, diseases affecting the thyroid gland, liver or the gastrointestinal tract were excluded as well as those with glucosuria, anaemia or fasting plasma glucose concentrations >6.9 mmol/l.²⁷

All measurements were carried out at the Municipal Health Service Centre in Doetinchem (The Netherlands). All study participants gave their written informed consent. The study protocol was approved by the Ethics Committee of Wageningen University.

Experimental procedure

Before intervention, subjects came in a fasting state (minimum fast of 12 h) to the research centre between 0800 and 0900 hours. Fasting was defined as not consuming any food or calorie-containing drinks 12 h prior to the visit. An oral glucose tolerance test (OGTT) was performed as described earlier.²⁵ During collection of all seven blood samples, subjects rated their subjective appetite.

After 2 days of intervention, subjects came, again between 0800 and 0900 hours, in a fasting state (minimum fast of 12 h) to the research centre. One blood sample was taken and subjective appetite was rated. After 4 days of intervention, the procedure of the first study day was repeated.

Caloric restriction

The energy content of the diet was calculated by taking 33.3% of the individual energy needs and rounding this up in units of 0.8 MJ. Details on the calculated individual energy needs are described previously.²⁵ In total, 16 subjects received a 4.2 MJ, 23 received a 5.0 MJ, four received a 5.9 MJ and one subject received a 6.7 MJ diet. For each diet, macronutrients were set at protein 26 energy percent, fat 19 energy percent and carbohydrates 55 energy percent. Calculations beforehand showed that the micronutrient and mineral content of the supplied diet met the Dutch recommended dietary allowance.²⁸

The diet consisted of micronutrient-dense meal and snack replacements, for example, shake mixes, muesli bars and soups (Nutricia, Zoetermeer, The Netherlands). The products were supplied at the beginning of the study and subjects were instructed to consume these products according to a fixed schedule; subjects were allowed to shift products within a day, but not between days. Compliance was measured by preprinted daily food records in which subjects wrote down the time of consumption of each product. Subjects were advised to use only the provided food products, but if necessary, additional foods were written down in full detail. Subjects were allowed to use non-nutritive beverages *ad libitum*. At the end of the study, food intake during the 4 days of intervention was calculated by means of the daily food records and chemical analyses of the meal and snack replacements. Subjects consumed on average 36% (range 23–40%) of their estimated energy needs, that is, 4.9±0.7 MJ/day (21.7% protein, 21.5% fat and 56.8% carbohydrates as percent of total energy consumed).

Measurements

Before the experiment, restraint eating behaviour was assessed by means of the Dutch Eating Behaviour Questionnaire (DEBQ).²⁹ The score derived from this questionnaire is an indicator for cognitive awareness of food intake.

Height was measured to the nearest 0.1 cm, using a wall-mounted stadiometer with the Frankfurt plane horizontal. Weight was determined to the nearest 0.5 kg on an analogue scale (Seck Bascule MT, USA) with subjects wearing light clothes, without shoes and with empty pockets. Body mass index (BMI) was calculated by weight (kg) divided by height (m) squared.

Appetite was measured by means of 10-point Likert scales, including hunger, fullness, desire to eat and prospective consumption, as described by Hill *et al.*³⁰ Second, a score for total appetite was calculated by adding up the scores of hunger, desire to eat and prospective consumption, and subtracting the score of fullness. Additionally, a score for appetite after the glucose load was calculated. The area under the curve as percentage of the total area was calculated, as described previously.^{25, 31} A high score on hunger, desire to eat, prospective consumption and total appetite represents high appetite. For fullness, low scores represent high appetite.

Blood samples were centrifuged at 2500 rpm for 10 min, before being divided into aliquots. Aliquots were stored at -20°C before laboratory analyses. Serum leptin was assessed by a radioimmunoassay (LINCO Research Inc., MO, USA), with an analytical sensitivity of 0.5 ng/ml. Intra-assay coefficient of variation was 3–8% and the interassay coefficient of variation was 4–8%. Serum insulin was measured by immunoassay (Immulite[®] 2000 analyser, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden), with an analytical sensitivity of 2 μ U/ml. Intra-assay coefficient of variation was 3.3–5.5% and the interassay coefficient of variation was 4.1–7.3%. Plasma glucose was measured quantitatively by a dichromatic end point assay (Glu FlexTM reagent, Dade Behring BV, Leusden, The Netherlands). All samples of each subject were analysed in one run and in duplicate. Means of the duplicates were used for data analyses. Incremental areas under the curve (AUCs) were calculated for glucose and insulin during the OGTT, in order to assess the glucose tolerance and insulin sensitivity.

Statistical analyses

Owing to non-normality, geometric means and 95% confidence intervals (95% CI) are presented for the appetite parameters, leptin and insulin levels. The proportional changes in leptin and insulin after intervention are presented as geometric means. Absolute changes after intervention were normally distributed and therefore presented as arithmetic means (95% CI). Since only the acute leptin changes, which are independent of changes in body weight loss, were of interest, adjustments for changes in BMI were made with general linear models (GLM).

Pearson correlation coefficients (r_p) were calculated between changes in appetite and normally distributed variables, that is, absolute changes in leptin, insulin and glucose. The nonparametric Spearman correlation coefficient (r_s) was calculated for variables that were not normally distributed, that is, proportional changes in leptin, insulin and glucose. Since only the acute leptin changes, which are independent of body weight loss, were of interest, adjustments for changes in weight were calculated by means of partial correlation (R). Additionally Pearson coefficients (r_p) were calculated between appetite parameters and leptin concentrations for each intervention day.

The power, that is 1 minus the chance of type II error, of the present analyses was sufficient (80%) to detect a correlation coefficient of 0.41 (tested two sided, with a *P*-value of 0.05 considered as statistically significant). *P*-values smaller than 0.05 were considered statistically significant. All statistical tests were performed with the SAS package (v8.0).

Results

General characteristics of the 44 men who completed the study are shown in Table 1. Subjects were on average 43 ± 5 years old, and had an average BMI of 27.3 ± 3.2 kg/m². The frequencies of normal weight (BMI 18.5–24.9), overweight (BMI 25.0–29.9) and obese (BMI \geq 30.0) subjects were 9 (20%), 29 (66%) and 6 (14%), respectively. At baseline, BMI was highly correlated to fasting leptin levels ($r_p=0.72$; $P<0.0001$) and fasting insulin levels ($r_p=0.49$; $P<0.001$). There was no association between BMI and any of the appetite parameters (data not shown). During the intervention, subjects had lost on average 2.4 kg (range: 0.5–3.5 kg). Body weight loss was positively associated with baseline body weight ($r_s=0.34$; $P=0.02$).

Table 1 - Characteristics of the subjects participating in the intervention study, consisting of a 65% energy restriction of 4 days (n=44).

	<i>Means.d. Range</i>		
General			
Age (years)	43	5	31–52
Height (cm)	181	7	167–196
Baseline weight (kg)	89.5	10.3	71.5–118.4
Baseline BMI (kg/m ²)	27.3	3.2	22.7–39.8
Energy balance			
BMR (MJ/day) ^a	8.0	0.5	7.2–9.4
PAL ^b	1.7	0.2	1.4–2.2
Estimated energy needs (MJ/day) ^c	13.6	1.6	10.8–19.9
Energy intake during intervention (MJ/day) ^d	4.9	0.7	3.0–6.9
Energy deficit during intervention (%) ^e	63.9	2.6	60.0–75.7
Weight loss after intervention (kg)	2.4	0.7	0.8–4.4
Restrained eating score ^f	2.5	0.6	1.0–4.0

^a BMR=basal metabolic rate at baseline, estimated by the equation of Schofield.³⁸

^b PAL=physical activity level, estimated by a questionnaire.³⁸

^c Calculated by PAL \times BMR.

^d Calculated by dietary records.

^e Calculated by energy intake as percentage of estimated energy needs.

^f Assessed with Dutch Eating Behaviour Questionnaire.²⁹ 1=not restraint at all, 5=extremely restraint.

Serum leptin concentrations were steadily decreasing during the intervention (Table 2). As expected, obese subjects tended to show larger decreases than normal weight and overweight subjects; -4.1 (95% CI: -6.1; -2.1), -2.2 (-2.7; -1.8) and -1.5 (-2.1; -0.8) ng/ml, respectively. For the relative decrease in leptin,

no such tendency was observed. This was -35.2% (95% CI: -49.3; -21.1), -39.1% (-45.0; -33.2) and -34.6% (-46.9; -22.2) for normal weight, overweight and obese subjects, respectively. Insulin and glucose concentrations declined during the first 2 days, and did not decline further during the next 2 days. No clear differences were seen among normal weight, overweight and obese subjects. There were small increases in glucose tolerance and insulin sensitivity during the 4-day intervention.

Table 2 - Fasting levels on days 0, 2 and 4, and responses in physiological parameters during a 65% energy restriction of 4 days in adult men (n=44).

	<i>Before intervention (t₀)^a</i>	<i>2 days of intervention (t₂)^a</i>	<i>4 days of intervention (t₄)^a</i>	<i>Response</i>		
				<i>t₂-t₀^b</i>	<i>t₄-t₀^b</i>	<i>t₄-t₀ (%)^a</i>
Fasting						
Leptin (ng/ml)	5.5 (4.7; 6.4)	4.3 (3.6; 5.0)	3.3 (2.8; 3.9)	-1.3 (-1.7; -0.8) ^d	-2.3 (-1.7; -3.0) ^d	-39.4 (-43.6; -34.9) ^d
Insulin (μU/ml)	9.2 (7.8; 10.9)	7.0 (5.8; 8.9)	7.1 (5.8; 8.6)	-2.4 (-3.5; -1.3) ^d	-2.4 (-3.5; -1.3) ^d	-24.4 (-32.6; -14.9) ^d
Glucose (mmol/l)	5.8 (5.6; 6.0)	5.4 (5.2; 6.7)	5.4 (5.2; 5.5)	-0.4 (-0.5; -0.3) ^d	-0.5 (-0.6; -0.3) ^d	-7.8 (-10.1; -5.5) ^d
AUC ^c after 75 g glucose load						
Insulin (μU/ml x 120 min) (x10 ³)	3.9 (3.1; 5.0)	—	3.7 (3.0; 4.5)	—	0.3 (0.1; 0.7) ^d	-3.5 (-13.9; 8.2)
Glucose (mmol/l x120 min) (x10 ²)	1.5 (1.2; 1.8)	—	1.7 (1.5; 2.1)	—	-0.6 (-1.3; 0.1)	21.6 (-0.02; 48.1)

^a Fasting levels (t_0, t_2, t_4) and proportional changes (t_4-t_0 (%)) are expressed as geometric mean (95% CI).

^b Absolute changes (t_2-t_0, t_4-t_0) are expressed as arithmetic mean (95% CI).

^c Area under the curve.

^d Statistically significant different from 0, $P < 0.05$.

Fasting appetite levels increased between t_0 and t_2 , and did not change during the subsequent 2 days (Table 3). Furthermore, levels of appetite during the glucose tolerance test, in all dimensions except fullness, increased significantly after 4 days of intervention.

Table 3 - Fasting levels on days 0, 2 and 4, and responses in appetite parameters during a 65% energy restriction of 4 days in adult men (n=44).

	Before intervention (t_0) ^a	After 2 days of intervention (t_2) ^a	After 4 days of intervention (t_4) ^a	Response	
				$t_2 - t_0$ ^b	$t_4 - t_0$ ^b
<i>Fasting</i> ^c					
Hunger	2.8 (2.3; 3.4)	4.1 (3.3; 5.0)	4.1 (3.4; 4.9)	1.5 (0.9; 2.1) ^d	1.3 (0.8; 1.9) ^d
Fullness	3.7 (3.2; 4.2)	3.2 (2.8; 3.8)	3.4 (2.9; 3.9)	-0.5 (-0.9; 0.9)	-0.3 (-0.8; 0.2)
Desire to eat	3.7 (3.2; 4.4)	4.8 (4.0; 5.7)	5.0 (4.3; 5.8)	1.3 (0.6; 1.9) ^d	1.2 (0.5; 1.9) ^d
Prospective consumption	4.0 (3.4; 4.7)	4.9 (4.2; 5.6)	4.7 (4.0; 5.4)	0.8 (0.3; 1.4) ^d	0.7 (0.2; 1.2) ^d
Total appetite ^e	7.8 (6.5; 9.3)	11.0 (9.1; 13.3)	9.6 (7.8; 11.9)	4.0 (2.2; 5.7) ^d	3.6 (1.9; 5.3) ^d
<i>After 75 g glucose load (%)</i> ^f					
Hunger	43 (37; 49)	—	51 (46; 57)	—	8 (3; 13) ^d
Fullness	48 (43; 53)	—	46 (41; 51)	—	-2 (-6; 11)
Desire to eat	48 (43; 54)	—	57 (52; 63)	—	9 (4; 13) ^d
Prospective consumption	50 (44; 56)	—	55 (49; 61)	—	5 (1; 9) ^d
Total appetite	43 (39; 47)	—	49 (44; 53)	—	6 (2; 10) ^d

^a Fasting levels (t_0 , t_2 , t_4) expressed as geometric mean (95% CI).

^b Absolute changes ($t_2 - t_0$, $t_4 - t_0$) expressed as arithmetic mean (95% CI).

^c Fasting appetite was measured by a 10-point scale; for example, 1 is low hunger, 10 is high hunger.

^d Statistically significantly different from 0, $P < 0.05$.

^e Individual score calculated by: Total appetite = hunger - fullness + desire to eat + prospective consumption.

^f Calculated by the area under the curve as percentage of the total area over a period of 2 h after a 75 g glucose load, dissolved in 200 ml tea. This method has been fully detailed by Hulshof *et al.*³¹

The proportional changes in leptin concentrations after 4 days of intervention were associated with changes in hunger ($r_s = -0.42$; $P < 0.01$), desire to eat ($r_s = -0.33$; $P < 0.05$) and total appetite ($r_s = -0.33$; $P < 0.05$) (Table 4). Adjusting for changes in BMI during the intervention slightly increased the correlations of desire to eat ($R = -0.39$; $P < 0.05$) and total appetite ($R = -0.38$; $P < 0.05$). No associations were observed between the absolute changes in leptin concentrations and the appetite parameters after 2 days of intervention (data not shown). For absolute changes in insulin and glucose, no statistically significant

correlations were observed with the changes in appetite parameters, respectively, for total appetite: $r_s=0.01$ (ns) and $r_s=-0.14$ (ns) (Table 4).

Table 4 - Associations between absolute and proportional responses in leptin, and changes in appetite parameters, during a 65% energy restriction of 4 days in adult men (n=44).

	Pearson correlation coefficient (P-value) Absolute response ^a	Spearman correlation coefficient (P-value) Proportional response ^b
<i>Leptin response</i>		
▲ Hunger	0.18 (0.25)	-0.42 (0.005)
▲ Fullness	0.05 (0.76)	0.19 (0.21)
▲ Desire to eat	0.09 (0.57)	-0.33 (0.03)
▲ Prospective consumption	0.13 (0.42)	-0.17 (0.29)
▲ Total appetite	0.13 (0.41)	-0.33 (0.03)
<i>Leptin response adjusted for change in BMI ^c</i>		
▲ Hunger	0.09 (0.55)	-0.42 (0.006)
▲ Fullness	0.09 (0.56)	0.20 (0.19)
▲ Desire to eat	0.00 (0.98)	-0.39 (0.01)
▲ Prospective consumption	0.06 (0.69)	-0.22 (0.17)
▲ Total appetite	0.05 (0.75)	-0.38 (0.01)
<i>Insulin response</i>		
▲ Total appetite	0.07 (0.69)	0.01 (0.94)
<i>Glucose response</i>		
▲ Total appetite	-0.14 (0.39)	-0.14 (0.37)

^a Calculated by fasting leptin on day 4 minus fasting leptin level on day 0.

^b Calculated by the difference in leptin on day 4 and day 0 as a percentage of leptin level on day 0.

^c Adjusted for changes in BMI during the intervention by partial correlation.

Correlations between fasting appetite and fasting leptin became stronger during continuation of the energy restriction (Table 5). While no significant relation with leptin is observed at baseline of the study, that is, for total appetite $r_p=-0.15$ (ns), after 2 days this association becomes significant, that is, $r_p=-0.31$ ($P<0.05$), and after 4 days it increases to $r_p=-0.41$ ($P<0.01$).

Table 5 - Associations between leptin and appetite parameters during a 65% energy restriction in adult men, that is, at baseline, after 2 days and after 4 days of energy restriction (n=44).

	Spearman correlation coefficients (P-values)		
	Day 0	Day 2	Day 4
<i>Leptin</i>			
Hunger	-0.13 (0.42)	-0.25 (0.10)	-0.44 (<0.01)
Fullness	0.04 (0.79)	0.11 (0.49)	0.19 (0.22)
Desire to eat	0.05 (0.76)	-0.25 (0.10)	-0.27 (0.08)
Prospective consumption	-0.21 (0.16)	-0.31 (<0.05)	-0.26 (0.09)
Total appetite	-0.15 (0.32)	-0.31 (<0.05)	-0.41 (<0.01)
<i>Leptin adjusted for BMI^a</i>			
Hunger	-0.10 (0.54)	-	-0.22 (0.16)
Fullness	0.15 (0.35)	-	0.15 (0.36)
Desire to eat	0.02 (0.91)	-	-0.23 (0.15)
Prospective consumption	-0.10 (0.51)	-	-0.10 (0.52)
Total appetite	-0.13 (0.41)	-	-0.24 (0.13)
<i>Insulin</i>			
Total appetite	-0.29 (0.06)	-0.41 (<0.01)	-0.19 (0.24)
<i>Glucose</i>			
Total appetite	-0.29 (0.06)	-0.33 (<0.05)	-0.39 (<0.01)

^a Adjusted for BMI by partial correlation.

The correlation between insulin and leptin concentrations increased with prolonged caloric restriction: $r_s=0.35$ ($P<0.05$) at t_0 , $r_s=0.63$ ($P<0.0001$) at t_2 and $r_s=0.71$ ($P<0.0001$) at t_4 . At baseline, the correlation between insulin concentrations and total appetite was $r_p=-0.29$ ($P=0.06$), after 2 days this increased to $r_p=-0.41$ ($P<0.01$), while after 4 days this decreased to $r_p=-0.19$ (ns). For glucose, increasing associations were found; at baseline, the correlation between glucose and total appetite was $r_p=-0.29$ ($P=0.06$), after 2 days this increased to $r_p=-0.33$ ($P<0.05$) and after 4 days to $r_p=-0.39$ ($P<0.01$).

Discussion

The present study is the first study that investigated the association between leptin and appetite responses induced by an acute energy restriction. We observed that the proportional decrease in leptin during the energy restriction was associated with an increase in appetite. Additionally, we observed that the association between fasting leptin and appetite became more pronounced during the energy restriction.

The aim of the 4-day energy restriction was to obtain a decrease in leptin, which was not caused by a reduction in body weight. From the studies of Kolaczynski *et al.*⁷ and Dubuc *et al.*,⁸ we knew that the acute decline had to take place within 36 h when fasting and within 7 days when eating 35% of the estimated energy needs. From our data, we can conclude that a 4-day diet intervention containing 35% of the estimated energy needs is sufficient to induce a decrease in leptin of about 40%, which is independent of change in body weight.

After 2 days of energy restriction, our subjects came in a fasting state to the research centre. One may speculate that they were glycogen depleted and they had lowered insulin levels (Table 2), which induced the oxidation of fatty acids. This metabolic stress situation most likely caused the decrease in leptin levels. However, Kolaczynski *et al.*⁷ found an association between changes in leptin levels and beta-OH-butyrate levels after a 36-h fast, but could not confirm a causal relation. Also, no association was found with the decrease of leptin and insulin in that study, although they proved that glucose infusions prevented leptin levels from dropping.⁷ From *in vitro* studies, it has been observed that refraining fat cells from insulin treatment decreased their leptin secretion.¹³ In our study, we observed an increasing correlation of insulin with leptin concentrations, which is in line with the hypothesis that leptin might be excreted from the adipose tissue under control of insulin.

Before energy restriction, we observed a small correlation of insulin levels with appetite ($r_s = -0.29$, $P = 0.06$), which increased after 2 days ($r_s = -0.41$; $P < 0.01$) and disappeared after 4 days of energy restriction ($r_s = -0.19$; ns). Simultaneously, the association between leptin levels and appetite became stronger during continuation of the energy restriction, t_0 : $r_s = -0.15$ (ns), t_2 : $r_s = -0.31$ ($P < 0.05$), t_4 : $r_s = 0.41$ ($P < 0.01$). Based on these observations, one may speculate that during the first 2 days of energy restriction, appetite is mainly driven by changes in insulin (and possibly fatty acid oxidation) together with leptin, and that after 4 days of intervention leptin takes over. However, with our study design it is not possible to make any causal conclusions.

Our findings are in line with other studies that were performed with longer-term energy restrictions. Keim *et al.*²¹ observed a Pearson correlation coefficient of 0.7 between changes in leptin and appetite after a moderate energy restriction of 12 weeks in women (aged: 20–40 years, BMI: 22–37 kg/m²). This association is considerably higher than the correlation found in the present study (total appetite: $r = 0.38$). However, one may question whether our study is comparable to their study. First, they studied appetite parameters measured several times during the day, which may have increased the statistical power.²¹ However, appetite measured in the fasting state does not have to be representative for appetite during the day. Additionally, they measured baseline appetite after 1 week of energy restriction, while our study only lasted 4 days. Consequently, the appetite feelings as measured by Keim *et al.* might reflect other motivation reasons to eat than in our study, which makes it difficult to compare. Furthermore, their study took place in a laboratory setting; participants lived in a metabolic unit at the time of the study, while the subjects in the present study were free living.

Westerterp-Plantenga *et al.*²² and others found lower hunger and appetite levels in obese male subjects receiving pegylated leptin intravenously than in BMI- and age-matched subjects receiving a placebo.²³ Like in the present study, they only observed an effect on appetite while measured in a fasting state. Moreover, they observed the highest effect of the exogenous leptin after the first week of intervention. This confirms our finding that the acute decrease in leptin, which takes place within days, is important for the increase in fasting appetite during energy restriction. Although a parallel change in leptin levels and appetite is observed in the study of Westerterp-Plantenga, data on an association between leptin levels and appetite are not given. This makes it difficult to compare their study to the present study.

It has been suggested that obesity is associated with a blunted leptin response to severe energy restriction.³² In our analyses it appeared that subjects with a high

leptin level (the obese subjects) had a relatively high leptin response. We therefore presented proportional changes in leptin, which are indirectly corrected for baseline BMI. The obese subjects in our study did not show blunted responses to weight gain. Even more, the large range in BMI in our study might have enlarged the variation in leptin response and therefore enlarged the power of our study.

Only men were eligible to participate in our study. It is difficult to study changes in leptin levels and appetite in women as menstrual cycles are suggested to influence both leptin levels³³ and subjective appetite responses.³⁴ It is well known that women have higher leptin levels in energy balance;⁶ this might imply that they also have lower proportional changes in response to energy restriction. This suggests that lower associations would be found in women than in men. However, it has been suggested by Dubuc and co-workers that the changes in leptin may be the predominating peripheral signal for changes in energy balance in women. On the other hand, in men these predominating signals would be the changes in insulin and cortisol.⁸ Our results were not in line with this hypothesis.

With this study we wanted to assess the role of the acute leptin decline after energy restriction in the regulation of food intake. For this objective, appetite would be preferably measured by *ad libitum* intake. Since subjects were on an energy-restricted diet, this method was not feasible in our study setting. Therefore, we used appetite ratings,³⁰ which have been shown to be good indicators of the motivation to eat and predictors of actual food intake.^{35, 36, 37} We observed that the proportional decline in leptin was associated with an increase in appetite ratings. This suggests that subjects with a relatively high decrease in leptin would also sense a relatively high motivation to eat. In a setting with *ad libitum* food, these subjects may compensate the energy deficit faster than subjects with a smaller leptin response. However, further studies should be conducted before we can confirm these speculations.

Leptin is part of a negative feedback loop that helps to stabilise body weight via influences on appetite.¹² However, given the high variation in weight gain in the Western World, and weight management after weight loss, one may speculate that the leptin signal to energy imbalance is not adequate to control homeostasis for each individual. Many factors, including environmental influences, such as diet, physical activity and other lifestyle factors, and possible genetic factors may affect the magnitude of the leptin signal after energy restriction. However, no data are available on the interindividual variation in the leptin response to energy imbalances or on its dose-response relationship, which are needed to confirm such a speculation.

Overall, it may be concluded that the acute decline in leptin after 4-day caloric restriction is associated with an increase in appetite. Moreover, the inverse association between leptin levels and appetite response becomes stronger with continuation of energy deprivation. These findings indicate that leptin might have an instrumental role in negative energy balances in humans, presumably by affecting the expression of appetite. These results may contribute to the understanding of energy homeostasis and its regulation by leptin and appetite.

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Acknowledgements

We thank the volunteers who participated in this study; the project steering committee of the Peilstation and MORGEN-project; Caroline de Rover, Irma Thus, Ina Hengeveld of the Municipal Health Service Center in Doetinchem; Els Siebelink, Maartje Jansen and Carolien Bouwman for their assistance during the intervention study. The Netherlands Association for Scientific Research supported this research (ZonMW project no. 980-10-007).