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Citation for published version (APA):

Hukshorn, C. J., Westerterp-Plantenga, M. S., & Saris, W. H. M. (2003). Pegylated human recombinant leptin (PEG-OB) causes additional weight loss in severely energy-restricted, overweight men. *American Journal of Clinical Nutrition*, 77(4), 771-776. <https://doi.org/10.1093/ajcn/77.4.771>

Document status and date:

Published: 01/01/2003

DOI:

[10.1093/ajcn/77.4.771](https://doi.org/10.1093/ajcn/77.4.771)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

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Pegylated human recombinant leptin (PEG-OB) causes additional weight loss in severely energy-restricted, overweight men¹⁻³

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ABSTRACT

Background: Increasing evidence suggests that falling leptin concentrations observed during fasting act as a peripheral signal of starvation, which serves to conserve energy in the face of limited reserves. An extension of this hypothesis is that exogenous leptin should affect energy regulation during severe energy restriction.

Objective: To explore this hypothesis, we assessed whether elevated leptin concentrations achieved with the use of long-acting pegylated human recombinant leptin [polyethylene glycol-OB protein (PEG-OB)] affected weight loss and changes in body composition, energy expenditure, appetite, and metabolic variables during semistarvation in healthy overweight men.

Design: A randomized, double-blind, placebo-controlled study was executed in overweight men with a mean (\pm SEM) age of 34.8 ± 1.3 y and body mass index (in kg/m^2) of 28.8 ± 0.5 . All subjects received weekly treatment with 80 mg PEG-OB ($n = 12$) or matching placebo ($n = 10$) for 46 d while their energy intake was reduced to 2.1 MJ/d by means of a very-low-energy diet. Body composition (hydrodensitometry and deuterium dilution), energy expenditure (ventilated hood), and appetite (visual analogue scales) were evaluated at the start and the end of the study. Metabolic variables were measured throughout the study period.

Results: Compared with placebo treatment, treatment with PEG-OB led to significant ($P < 0.03$) additional weight loss (14.6 ± 0.8 compared with 11.8 ± 0.9 kg) and a reduction in appetite ($P < 0.05$) after 46 d, but the 2 treatment groups did not differ significantly in changes in body composition, energy expenditure, and metabolic variables.

Conclusion: Our observations support the hypothesis that the decrease in leptin concentrations during starvation increases appetite in humans. *Am J Clin Nutr* 2003;77:771-6.

KEY WORDS Leptin, pegylated human recombinant leptin, polyethylene glycol-OB protein, PEG-OB, semistarvation, very-low-energy diet, body composition, energy expenditure, appetite, weight loss, men

INTRODUCTION

Leptin, the 16-kDa protein hormone encoded by the *ob* gene, is secreted primarily from adipose tissue and is involved in the regulation of food intake and metabolism via hypothalamic mechanisms (1-4). Other extrahypothalamic areas in which leptin has been implicated are glucose homeostasis, hematopoiesis, angiogenesis, and immune responses. Furthermore, leptin may act as an important regulator of neuroendocrine and reproductive functions (5).

Initially, leptin was considered to function as the predicted and long-sought adipostatic hormone. According to this hypothesis, rising concentrations of leptin with increasing adiposity would generate a signal to reduce food intake and increase energy expenditure to limit further weight gain. However, resistance to the proposed antiobesity action of leptin is observed in both animals (6, 7) and humans (8). In addition, even the supraphysiologic leptin concentrations obtained during several clinical trials to date failed to significantly affect weight loss during mild hypoenergetic conditions (9-11) or energy expenditure during weight maintenance (12).

The widespread occurrence of leptin resistance may reflect the fact that the inability to store energy efficiently at times of abundance is evolutionarily disadvantageous. According to this alternative view proposed on the basis of animal data, evolution would favor a leptin dose-response curve that functions briskly as a switch between the fed and fasted state but fails to limit further energy storage as concentrations increase with increasing energy stores (13, 14). An extension of this hypothesis is that exogenous leptin should affect energy regulation when administered during severe energy restriction. To test this hypothesis, we executed a randomized, double-blind, placebo-controlled study to investigate whether elevated leptin concentrations achieved with the use of long-acting pegylated human recombinant leptin [polyethylene glycol-OB protein (PEG-OB)] affected weight loss and changes in body composition, energy expenditure, appetite, and metabolic variables during semistarvation induced by a very-low-energy diet (VLED) in healthy, overweight men.

SUBJECTS AND METHODS

Subjects

Twenty-four healthy, overweight male volunteers were recruited by local advertising, screened, and enrolled in the study after they provided written informed consent. The study was

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²Hoffmann-La Roche Inc (Nutley, NJ) provided the pegylated human recombinant leptin (PEG-OB).

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Received July 17, 2002.

Accepted for publication August 23, 2002.

approved by the Medical Ethical Committee of Maastricht University. Overweight and mildly obese men who were 18–45 y of age and had a body mass index (BMI; in kg/m²) between 25 and 32 were eligible for inclusion. Additional inclusion criteria were detailed medical and psychiatric histories and a physical examination with negative findings, including no medical condition present, no use of prescription medication, and no smoking. Each participant had normal biochemical tests of renal, hepatic, metabolic, and hematologic function and no electrocardiogram abnormalities. Subjects who had a history of drug abuse or alcoholism or were currently using drugs or alcohol, had atopy or hypersensitivity to pegylated proteins, or had experienced a weight loss of > 3 kg in the previous 3 mo were excluded from the study.

Study design

This single-center trial had a prospective, randomized, double-blind, placebo-controlled group design. After the screening, 24 subjects were selected and enrolled in the trial. The study was divided into 3 phases: 1) baseline characterization (days -14, -8, and 1), 2) PEG-OB or placebo treatment and a VLED for 46 d (days 1–46), and 3) follow-up for 2 wk (days 50 and 57). Baseline measurements were performed before (days -14 and -8) and at the start (day 1) of the diet and treatment period. The measurements on day 1 consisted of measurements of energy expenditure, body composition, and metabolic profile (including oral glucose tolerance). Subjects were stratified and matched into pairs according to age, BMI, and fasting serum leptin and insulin concentrations to achieve balanced treatment groups. Randomization numbers for subjects were generated and incorporated into the double-blind labeling by an independent third party.

At the start of the treatment period (day 1) all subjects were prescribed a VLED to induce a state of semistarvation for the next 46 d. The VLED (Modifast; Novartis, Breda, Netherlands) was a protein-enriched formula diet (containing 44%, 14%, and 42% of energy as protein, fat, and carbohydrate, respectively) that provided 2.1 MJ/d. The dietary prescription was discussed every week with a dietitian. Adherence to the diet was confirmed by measurements of body weight loss. Treatment consisted of weekly administration of either 80 mg PEG-OB [8 mL of a 10-mg/mL solution; mean (\pm SEM) of 1.17 ± 0.02 mg/kg fat-free mass (FFM); Hoffmann-La Roche Inc, Nutley, NJ] or matching placebo (8 mL) that was given subcutaneously in the paraumbilical region during the VLED period. The subjects returned to the laboratory in a fasting state on days 8, 15, 22, 29, 36, and 43 to receive treatment after their weight and vital signs (pulse, respiratory rate, and blood pressure) were recorded. Blood samples were drawn for safety laboratory tests and measurements of metabolites on days 1, 8, 15, 25, and 46. At the end of the 46-d treatment period, measurements of energy expenditure, body composition, and metabolic profile were repeated. During the next 2 wk, the subjects received less formula diet and were instructed to supplement this with a free choice of habitual food items. Vital signs and body weight during this follow-up period were measured on days 50 and 57. Safety was monitored by documentation of adverse events and recording of vital signs at each visit. In addition, urine analyses, routine serum chemistry measurements, and blood cell counts were conducted regularly throughout the study. Standard clinical chemistry measurements and blood cell counts were conducted at the certified central laboratory of the University Hospital Maastricht, Netherlands.

Polyethylene glycol-OB protein

Recombinant methionyl human leptin has a reported average terminal half-life of ≈ 4 h in humans, and daily administration is required to obtain sustained blood concentrations (9). Modification of proteins through covalent linkage of polyethylene glycol polymers to the proteins results in reduced immunogenicity and increased serum half-life for many proteins (15). Recombinant native human leptin, expressed and purified from *Escherichia coli*, was chemically conjugated to a species of branched PEGs with an average molecular mass of 42 kDa in a 1:1 ratio. The result was a globular PEG-native human leptin polymer (PEG-OB) with increased molecular size. PEG-OB at a concentration of 10 mg/mL was placed in sterile glass vials containing 1.3 mL. Preclinical studies with PEG-OB indicate an extended half-life (>48 h) and efficacy for reduction of food intake and body weight in animals (16). Our previous study in obese male subjects clearly showed sustained elevated blood concentrations after weekly subcutaneous dosing of PEG-OB in humans. Mean peak serum PEG-OB concentrations were achieved 72 h after dosing, followed by a return to the elevated predose concentrations after 1 wk (10).

Body composition, energy expenditure, and appetite profile

Body weight was measured on a calibrated digital scale accurate to 0.1 kg, and height was measured to the nearest 0.01 m. Body composition was determined after the ventilated-hood measurements obtained in the morning by using the combination of hydrodensitometry and deuterium dilution according to the Maastricht protocol (17). Body composition was calculated according to the equations of Siri (18). Energy expenditure and substrate utilization were measured with the use of a ventilated hood after the subjects fasted overnight. After the subjects had been supine for 15 min, their oxygen consumption and carbon dioxide production were measured for 45 min by means of a computerized, open-circuit ventilated-hood system. The resting metabolic rate and the respiratory quotient were calculated according to the method of de Weir (19) over the 15-min interval with the lowest SD.

The subjects' appetite profile was measured while they were in a fasted state before breakfast and included determination of the degree of dietary restraint. The appetite profile was determined by averaging the ratings of actual appetite, hunger, and desire to eat on 100-mm anchored visual analogue scales (20). The appetite profile before breakfast was completed on days 1 and 46.

Measurements of metabolic profile and pharmacokinetics

Venous blood was sampled on days 1, 8, 15, 25, and 46 after the subjects had fasted overnight. Plasma or serum was extracted by centrifugation for 10 min at $1000 \times g$ and 4 °C, frozen in liquid nitrogen, and stored at -80 °C until further analysis. Plasma substrate concentrations were determined enzymatically in duplicate by using the hexokinase method (Roche, Basel, Switzerland) for glucose, the Wako NEFA C kit (Wako Chemicals, Neuss, Germany) for free fatty acids, the glycerol kinase method (Boehringer Mannheim GmbH, Mannheim, Germany) for free glycerol, the lipase method (Sigma Diagnostics, St Louis) for triacylglycerol, the CHOD-PAP method (Boehringer Mannheim GmbH) for cholesterol, and the β -hydroxybutyrate dehydrogenase method (Sigma Diagnostics, St Louis) for β -hydroxybutyrate. To avoid interassay variability, all specimens for a given substance were run in a single assay.

TABLE 1
Baseline characteristics of the 2 matched groups¹

Characteristic	Placebo (n = 12)	PEG-OB (n = 12)
Age (y)	34.9 ± 1.3 (24.0–40.0)	34.6 ± 1.2 (26.0–41.0)
Weight (kg)	95.0 ± 3.2 (77.2–113.9)	97.1 ± 1.8 (87.5–106.6)
BMI (kg/m ²)	28.6 ± 0.5 (25.2–31.2)	28.9 ± 0.4 (27.3–31.2)
Leptin (ng/mL)	7.3 ± 0.9 (4.3–16.3)	7.0 ± 0.8 (2.3–12.9)
Insulin (mU/L)	9.7 ± 1.8 (4.0–26.0)	7.8 ± 1.3 (1.9–17.0)

¹ $\bar{x} \pm \text{SEM}$; range in parentheses. PEG-OB, pegylated human recombinant leptin (polyethylene glycol-OB protein). There were no significant differences between the groups.

Serum insulin concentrations were measured at the certified central laboratory of the University Hospital Maastricht, Netherlands. Total leptin concentrations (endogenous leptin plus PEG-OB) were measured according to the method described previously (10).

Insulin resistance throughout the study was estimated by using the HOMA-R method with fasting plasma glucose and serum insulin concentrations (21). For determining oral glucose tolerance, a standard 2-h oral-glucose-tolerance test was performed on days 1 and 46 after the subjects had fasted overnight. In short, an intravenous cannula was placed in an antecubital vein. After a baseline blood sample was drawn, the subjects ingested 75 g glucose dissolved in 250 mL water. Next, blood samples were obtained 30, 60, and 120 min after the consumption of the solution for measurements of plasma glucose concentration.

Statistics

Changes from baseline to the end of the study were compared between the PEG-OB and placebo groups with two-factor repeated-measures analysis of variance with a group \times time interaction. When significant differences were found, a post hoc Scheffe's procedure was used to determine the location of the difference. All statistics were executed with STATVIEW (version 5.0; SAS Institute Inc, Cary, NC). All statistical tests were two-sided, and significance was defined as $P < 0.05$. All data are presented as means \pm SEMs.

RESULTS

The characteristics of the subjects before the intervention are presented in **Table 1**. The variables used to match subjects into pairs were not significantly different, and thus balanced treatment groups were achieved. All subjects in the trial were of white origin. Two subjects in the placebo group dropped out voluntarily after 1 wk because they were not able to maintain the strict VLED regime. However, after exclusion of the data for these 2 subjects, the 2 groups still did not differ significantly in baseline characteristics (data not shown). The most common adverse events related to treatment were injection-site ecchymosis, pruritis, and pain. These occurred with similar frequency in the PEG-OB and placebo groups and generally occurred only with the first or second series of injections. No significant differences between the groups in vital signs, standard chemistry or hematology measures, or urine analysis results were detected.

Weight loss, body composition, energy expenditure, and appetite profile

By day 46, treatment and energy restriction had resulted in an absolute weight loss of 14.6 ± 0.8 kg ($14.9 \pm 0.7\%$) in the PEG-OB group and of 11.8 ± 0.9 kg ($12.4 \pm 1.1\%$) in the placebo group ($P = 0.027$; **Figure 1**). There was a significant

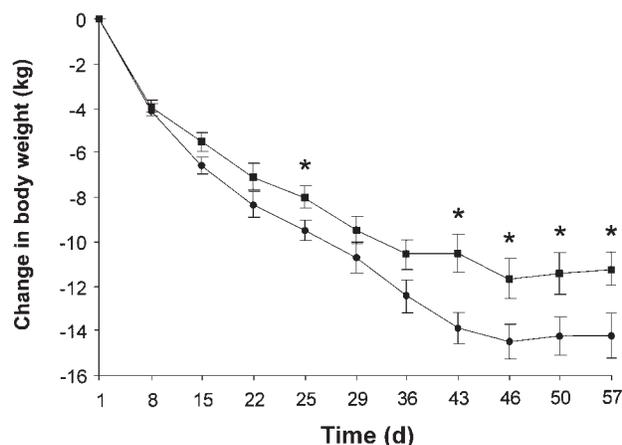


FIGURE 1. Effect of 80 mg pegylated human recombinant leptin [polyethylene glycol-OB protein (PEG-OB)] (●; n = 12) or matching placebo (■; n = 10) administered weekly and of severe energy restriction (2.1 MJ/d) on mean (\pm SEM) weight loss from the start of the treatment (day 1), through the diet period (day 46), to the end of the 2-wk follow-up period (day 57). *Significantly different from the PEG-OB group, $P < 0.05$ (interaction of time and treatment; two-factor repeated-measures ANOVA with Scheffe's F procedure).

difference in absolute weight loss between the PEG-OB and placebo groups on days 25, 43, and 46 (during the treatment period) and on days 50 and 57 (during the follow-up period) (Figure 1). The percentage of weight loss on days 43 and 50 was significantly different between the 2 groups ($P = 0.013$ and 0.045 , respectively). A tendency for a difference in percentage of weight loss was observed on days 46 and 57 of the study ($P = 0.062$ and 0.068 , respectively). Absolute and percentage changes in BMI were significantly different between the PEG-OB and placebo groups on days 43, 46, and 50 and day 43, respectively.

The loss of fat mass accounted for most of the loss of body mass in both the PEG-OB group and the placebo group (74% compared with 79%; NS). The resting metabolic rate significantly decreased in both groups after 6 wk of the VLED and treatment, as did the respiratory quotient. The mean absolute changes in resting metabolic rate and respiratory quotient of all 22 subjects were -0.817 ± 0.096 MJ/d and -0.05 ± 0.01 , respectively. None of the changes in body composition and energy expenditure were significantly different between the 2 groups (**Table 2**). In addition, comparison of the changes in resting metabolic rate adjusted for FFM over the treatment period did not show any significant differences between the PEG-OB and placebo groups. Appetite, which was measured before breakfast, significantly changed from baseline after the VLED and treatment: appetite decreased from 40 ± 17 to 34 ± 16 mm on the visual analogue scale in the PEG-OB group but increased from 38 ± 17 to 48 ± 16 mm in the placebo group ($P = 0.03$).

Metabolic profile and pharmacokinetics

The weight reduction observed during treatment was accompanied by a significant decrease in glucose, cholesterol, and triacylglycerol concentrations and by a significant increase in

TABLE 2

Effect of 46 d of weekly treatment with 80 mg pegylated human recombinant leptin [polyethylene glycol–OB protein (PEG-OB)] or matching placebo and of severe energy restriction (2.1 MJ/d) on body composition and energy expenditure¹

	Placebo (n = 10)		PEG-OB (n = 12)	
	Day 1	Day 46	Day 1	Day 46
FFM (kg) ²	67.3 ± 2.3	64.9 ± 2.7	69.3 ± 1.3	65.6 ± 1.2
FM (kg) ³	29.3 ± 1.9	19.9 ± 1.7	28.2 ± 1.8	17.3 ± 1.8
%FM (%) ³	30.1 ± 1.1	23.2 ± 1.2	28.8 ± 1.5	20.6 ± 1.9
RMR (MJ/d) ³	8.62 ± 0.25	7.27 ± 0.30	8.71 ± 0.19	7.67 ± 0.25
RQ ⁴	0.814 ± 0.012	0.769 ± 0.012	0.822 ± 0.013	0.770 ± 0.010

¹ $\bar{x} \pm \text{SEM}$. FFM, fat-free mass; FM, fat mass; %FM, percentage of fat mass; RMR, resting metabolic rate; RQ, respiratory quotient. There were no significant differences for time-by-group interaction (two-factor repeated-measures ANOVA).

^{2–4}Significant main effect of time (repeated-measures ANOVA): ² $P < 0.01$, ³ $P < 0.001$, ⁴ $P < 0.05$.

β -hydroxybutyrate, free fatty acid, and free glycerol concentrations in the PEG-OB and placebo groups (Table 3). The changes in metabolites were not significantly different between the 2 groups throughout the study. There were no effects of PEG-OB treatment on glycemic control, as evidenced by serum insulin concentrations (Table 3), glucose profiles obtained during the baseline and end-of-treatment oral-glucose-tolerance tests, and the estimated insulin resistance (HOMA-R method) at different time points (data not shown).

Food restriction for 6 wk by means of a VLED reduced circulating leptin concentrations to 2.0 ± 0.2 ng/mL (72%) in the placebo group (Table 3). After weekly subcutaneous dosing, sustained serum concentrations of total leptin (endogenous leptin plus PEG-OB), which were measured just before the next dose on days 8 and 15 and ranged from 950 to 3700 ng/mL, were observed. Peak total leptin concentrations on days 25 and 46, which were measured 72 h after subcutaneous injection, ranged from 2300 to 6050 ng/mL.

DISCUSSION

In the present study, we show for the first time that exogenous leptin induces additional weight loss in humans under severely energy-restricted conditions. Weight loss in both the PEG-OB- and placebo-treated subjects was primarily due to fat loss, which is in line with the results of previous animal studies (2–4). However, the fact that changes in fat mass contrary to changes in body weight did not differ significantly between the 2 groups in the present study may be explained by the small sample size. Conservation of FFM, which was observed in rodents treated with leptin (3, 22), was not observed in the present study, in which both treatment groups lost equal amounts of FFM after 6 wk of severe energy restriction. The loss of FFM in both groups was within the normal physiologic range of $\approx 25\%$ of total body weight loss.

Treatment of normal mice with leptin prevented the decrease in energy expenditure that would normally occur with a reduction in food intake (22). As reported before, we failed to reproduce similar results with humans under different energy-deficit conditions and PEG-OB doses (10). Also, a study of never-obese subjects on a weight-maintenance regimen failed to show an effect of leptin administration on energy metabolism (12). In contrast, a recent study reported reversal of the effects of sustained weight reduction on energy expenditure by a low dose of exogenous leptin (23). A reduction in appetite before breakfast under negative energy-balance conditions was present in the PEG-OB-treated group at the end of the present study. This result is consistent with the results of several

studies in rodents and primates that also showed a decline in food intake after leptin administration (2–4, 24). Furthermore, reductions in appetite were also observed in our first study with 20 mg PEG-OB/wk, but the effect was too small to result in additional weight loss (25). Because the decrease in energy expenditure was not significantly different in the 2 groups, it is conceivable that the additional weight loss caused by PEG-OB administration was largely attributable to reductions in appetite that resulted in better compliance with the dietary restriction. Thus, the results above suggest that exogenous leptin may influence human energy balance during starvation by reducing appetite.

No other metabolic effects of leptin and PEG-OB observed in animal studies were detected in the present study (5). Most prominent among these were effects on fasting plasma glucose and insulin, oral glucose tolerance, and triacylglycerol. We failed to observe an additional enhancement of the well-known effects of weight loss on these variables by weekly PEG-OB treatment in the presence of severe energy restriction. These findings suggest that leptin may not be directly involved in the regulation of these systems in humans under the conditions of this experiment. However, these variables were not elevated in the group of overweight and mildly obese men enrolled in this trial. Because the weight loss induced by the energy restriction and negative energy balance decreased fasting plasma glucose and insulin, oral glucose tolerance, and triacylglycerol, these improvements may have masked the effects of PEG-OB treatment on these variables.

The findings of the present study are consistent with the characteristics of homozygous human patients with total leptin deficiency or nonfunctional leptin receptors (26–28). These rare and very obese individuals have been shown to have a normal body temperature and normal resting energy expenditure but to be markedly hyperphagic. Also, the few such patients studied up to now exhibit normal glucose homeostasis, a normal cholesterol concentration, and only moderate impairments in metabolites such as free fatty acids and triacylglycerol despite their severe obesity. In addition, mice heterozygous for the *ob* gene (29, 30) and persons who are genetically partially deficient in leptin (31) appear to have an intermediate phenotype. When a young, very obese girl with a mutated *ob* gene received daily subcutaneous treatment with human recombinant leptin, she experienced a rapid and progressive reduction in body weight. This weight loss was mainly due to a reduction in food intake as a result of decreased appetite and food-seeking behavior but not to changes in energy expenditure (32).

The hypothesis first proposed by Flier and coworkers (13, 14) suggests that the falling concentration of leptin during starvation and its effects may constitute part of the thrifty genotype, a set of genes postulated to promote survival during periods

TABLE 3

Effect of 46 d of weekly treatment with 80 mg pegylated human recombinant leptin [polyethylene glycol-OB protein (PEG-OB)] or matching placebo and of severe energy restriction (2.1 MJ/d) on metabolites and endocrine measures¹

	Placebo (n = 10)	PEG-OB (n = 12)
Glucose (mmol/L) ²		
Day 1	5.2 ± 0.07	5.3 ± 0.08
Day 8	4.7 ± 0.11	4.5 ± 0.10
Day 15	4.7 ± 0.16	4.6 ± 0.09
Day 25	4.6 ± 0.13	4.5 ± 0.11
Day 46	4.7 ± 0.16	4.7 ± 0.09
Cholesterol (mmol/L) ²		
Day 1	4.8 ± 0.27	4.7 ± 0.24
Day 8	4.7 ± 0.31	4.1 ± 0.25
Day 15	4.0 ± 0.21	3.4 ± 0.22
Day 25	3.3 ± 0.19	2.9 ± 0.16
Day 46	3.7 ± 0.17	3.2 ± 0.13
β-Hydroxybutyrate (μmol/L) ²		
Day 1	67 ± 10	80 ± 7
Day 8	848 ± 212	1146 ± 138
Day 15	987 ± 282	1131 ± 177
Day 25	1303 ± 402	1401 ± 252
Day 46	794 ± 199	806 ± 186
Triacylglycerol (mmol/L) ³		
Day 1	1.15 ± 0.19	1.13 ± 0.26
Day 8	0.69 ± 0.09	0.59 ± 0.06
Day 15	0.77 ± 0.10	0.67 ± 0.10
Day 25	0.83 ± 0.17	0.58 ± 0.06
Day 46	0.58 ± 0.07	0.48 ± 0.06
FFA (μmol/L) ³		
Day 1	408 ± 48	382 ± 31
Day 8	704 ± 131	825 ± 67
Day 15	592 ± 73	664 ± 85
Day 25	553 ± 46	638 ± 34
Day 46	600 ± 50	597 ± 44
Free glycerol (μmol/L) ²		
Day 1	63.3 ± 3.2	67.3 ± 2.6
Day 8	134.8 ± 16.9	137.9 ± 14.0
Day 15	125.0 ± 11.5	132.7 ± 13.9
Day 25	85.0 ± 4.0	83.1 ± 4.9
Day 46	67.7 ± 4.4	77.8 ± 4.9
Insulin (mU/L) ²		
Day 1	9.4 ± 1.0	7.2 ± 0.8
Day 8	4.5 ± 0.7	3.4 ± 0.6
Day 15	5.0 ± 1.1	3.2 ± 0.5
Day 25	3.4 ± 0.6	2.2 ± 0.4
Day 46	3.6 ± 0.7	2.0 ± 0.3
Total leptin (ng/mL) ⁴		
Day 1	7.1 ± 0.8	7.6 ± 1.2
Day 8	3.4 ± 0.6	1828 ± 183
Day 15	3.1 ± 0.5	2777 ± 194
Day 25	2.1 ± 0.3	4194 ± 285
Day 46	2.0 ± 0.2	3980 ± 203

¹ $\bar{x} \pm \text{SEM}$. FFA, free fatty acids. There were no significant differences for time-by-group interaction (two-factor repeated-measures ANOVA), except for total leptin ($P < 0.001$).

^{2,3}Significant main effect of time (repeated-measures ANOVA): ² $P < 0.01$, ³ $P < 0.05$.

⁴The assay did not distinguish between endogenous leptin and PEG-OB.

of insufficient energy intake in human evolution by increasing the efficiency of energy storage. In this updated thrifty genotype concept, the decrease in leptin concentrations during periods of limited energy intake might signal the brain to initiate

several physiologic responses to increase the chances of survival. In addition, this concept suggests that an effective adipostatic role of leptin would subvert the thrifty genotype by limiting the capacity of energy storage during periods of abundance, which would result in reduced survival in subsequent periods of food shortages. Stated differently, evolution would favor resistance to leptin action when leptin concentrations are rising or high during periods of sufficient energy intake or storage. The fact that exogenous leptin resulted in additional weight loss only during severe energy restriction in the present study but failed to significantly affect weight loss during mild hypoenergetic conditions in the previous study (11) with comparable total leptin concentrations appears to support this concept.

Whether the results of the present study can be generalized to subjects with a BMI > 32 is uncertain. However, evidence showing that the marked decrease in leptin production and leptin concentrations after fasting is reduced in obesity suggests that fasting-induced adaptations to starvation and the effect of exogenous leptin may depend on the degree of obesity present (33).

In summary, our results support the hypothesis that the physiologic role of leptin is as a signal of starvation. We propose that falling concentrations of leptin after severe energy restriction influence human energy regulation by increasing appetite. 🌱

We express our sincere appreciation to Inge van Ierssel, Marja van der Hulst, and Fiona Ong for their commitment and assistance during this study. We also greatly acknowledge the cooperation, patience, and contributions of all of our subjects.

CJH participated in the study design, data collection, data analysis, and the writing of the manuscript. MSW-P and WHMS discussed the study design, data analysis, and versions of the manuscript. All authors declare that they had no conflicts of interest.

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