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Effects of weekly administration of pegylated recombinant human OB protein on appetite profile and energy metabolism in obese men[1–3]

Margriet S Westerterp-Plantenga, Wim HM Saris, Chris J Hukshorn, and L Arthur Campfield

ABSTRACT

Background: Results of leptin administration in mice, rats, and humans provide a rationale for therapeutic augmentation of circulating leptin (OB protein) concentrations in obese humans; this may reduce food intake, increase metabolic rate, and lower body mass.

Objective: We assessed the effects of weekly subcutaneous pegylated polyethylene glycol (PEG)-OB protein administration on appetite and energy metabolism in obese men.

Design: We performed a randomized, double-blind, placebo-controlled trial in 30 obese men [body mass index (in kg/m^2): 34.2 ± 3.6; age: 44.7 ± 7 y]. Subjects received 20 mg PEG-OB protein/wk for 12 wk while limiting their energy intake to 2.1 MJ/d.

Results: During treatment, appetite and hunger before breakfast decreased and remained lower in the PEG-OB-protein group, whereas they increased and remained higher in the placebo group ($P < 0.0001$). During treatment, hunger decreased in the PEG-OB-protein group ($P < 0.05$) and cognitive restraint increased in the placebo group ($P < 0.0001$). Neither appetite nor food intake changed significantly during the ad libitum evening meal. Under energy balance conditions in the respiration chamber, appetite at the end of treatment was not significantly different from baseline despite similar, significant reductions in 24-h energy intake, energy expenditure, sleeping metabolic rate, body mass, fat mass, and fat-free mass ($P < 0.01$ for all) in both groups.

Conclusion: Treatment with PEG-OB protein modified subjective appetite at a dosage that produced no changes in body composition, energy expenditure, or body mass loss relative to placebo treatment, suggesting that PEG-OB protein has central rather than peripheral biological activity in obese men. *Am J Clin Nutr* 2001;74:426–34.

KEY WORDS Obesity, leptin, OB protein, PEG-OB protein, appetite, respiration chamber, universal eating monitor, energy balance, hunger, satiety, food intake, underreporting, energy expenditure, body mass, body composition, weight reduction, weight loss

INTRODUCTION

Obesity is a medical condition associated with relatively high rates of morbidity and early mortality if it remains untreated (1–4). Commonly used weight-control methods, such as diet and exercise (5) and pharmacologic approaches (6), often produce short-term success, but sustained weight maintenance after weight reduction is generally difficult to achieve (5, 7). For this reason and also because the prevalence of obesity is increasing (8–10), other treatments that may be effective in the long term should be considered.

Leptin, or OB protein, is a circulating hormone that was identified as a possible energy balance regulator. Leptin was discovered after the positional cloning of the *OB* gene (11), which is expressed in tissues such as adipose tissue and the placenta (12). Serum leptin concentrations change in response to factors that are known to affect body weight (12, 13). Moreover, genetic evidence that leptin may be an important energy balance regulator in humans was obtained in research on subjects with congenital leptin deficiency; these individuals had severe obesity (14).

It was shown that blood leptin concentrations correlated with percentage body fat and were elevated in obese individuals (15). Despite the presence of elevated leptin concentrations, which should reduce food intake and body fat, obese persons appear to be insensitive or resistant to leptin and continue to maintain high body fat. It was also reported that leptin concentrations decreased with weight loss in obese individuals but leptin later increased with weight regain (16).

In lean and obese mice and rats, intraperitoneal or subcutaneous leptin administration was associated with dose-dependent reductions in food intake and body weight (17–19). Increased energy expenditure during leptin treatment was observed in food-restricted lean mice (20). The authors concluded that leptin controls thermoregulatory energy expenditure when food supplies are scarce but alters food intake, rather than energy expenditure, when food is abundant (20). These observations suggest that recombinant human leptin has potential in the treatment of human obesity. Moreover, it was suggested that exogenously administered leptin crosses the blood-brain barrier in humans, even

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though it is expected that the high serum leptin concentrations would saturate the transport system (21). The existence of a transport pathway that cannot be saturated was proposed, and more than one pathway may be involved in the transport of leptin to the brain (21). Thus, administration of exogenous leptin can be studied to determine whether increasing cerebrospinal fluid leptin concentrations results in weight loss in obese persons (21).

The only human studies that are available to support these suggestions are preliminary intervention trials. In trials sponsored by Amgen Pharmaceuticals (Thousand Oaks, CA), significant dose-related reductions in body fat and body weight were observed after daily subcutaneous injections of 0.01–0.30 mg recombinant human met-leptin/kg body weight for 24 wk in obese subjects (22). In addition, treatment of a young, severely obese girl with daily met-leptin caused dramatic reductions in appetite, food-seeking behavior, food intake, and body weight (23).

Polyethylene glycols (PEGs) are amphophilic polymers of ethylene glycol with various average molecular weights. PEGs can be activated and covalently attached to proteins. Modification of proteins with PEGs increased their serum half-lives and decreased immunogenicity to several proteins (24). The average molecular mass of the branched PEG that we used is 42 kD (25, 26).

In this study, we assessed the effects of weekly subcutaneous injections of human recombinant PEG-OB protein on the appetite profile and energy metabolism in obese men, under conditions of mild energy-intake deficit. We tested the null hypothesis that no differences in appetite, food intake, body weight, body composition, or energy expenditure would be observed. The term *PEG-OB protein* is used for the human recombinant pegylated PEG-OB protein that was administered and *leptin* for the endogenous substance.

SUBJECTS AND METHODS

Study design

The design was a prospective, randomized, double-blind, placebo-controlled group study of 30 obese men. The study was divided into 3 phases: *1*) screening and baseline characterization, *2*) PEG-OB protein or placebo treatment for 12 wk, and *3*) follow-up for 2 wk. Some of the results of this study were published elsewhere (27).

Subject selection and screening

Subjects were recruited by means of advertisements in local newspapers. Individuals who were willing to participate in the study underwent a medical screening after they gave their written, informed consent. This study was conducted according to the Guidelines for Good Clinical Practice and the Declaration of Helsinki (as amended in Tokyo, Venice, and Hong Kong) and was monitored by Hoffmann-La Roche Inc (Welwyn, United Kingdom). The screening included a full medical history and physical examination; clinical laboratory tests (eg, hematologic, biochemical, serologic, and urine analyses); a 12-lead electrocardiogram; and measurement of vital signs (supine and standing blood pressure and heart rate, body temperature, and respiratory rate). The screening visit was performed within the 6 wk before the baseline period.

To be eligible for inclusion, subjects had to be male; be 18–60 y old; have a body mass index (BMI; in kg/m^2) $\geq$27.0; be healthy on the basis of clinical laboratory assessments, physical examination, medical history, and vital signs; have no comorbid conditions; be nonsmokers or smoke $\leq$5 cigarettes or the equivalent/d; and be willing to give informed consent and comply with the study procedures. The exclusion criteria were obesity of a diagnosed endocrine origin; any significant physical or medical illness, including laboratory or electrocardiogram abnormalities, chronic infection, malignancy, or abnormal respiratory, cardiovascular, endocrine, gastrointestinal, renal, hepatic, hematologic, pancreatic, pulmonary, or neurologic function; and weight loss of >3 kg in the 3 mo previous to screening. Subjects selected for the study had to maintain their body weight from the time of screening until the study began. Additional exclusion criteria were current or past drug abuse or alcoholism, a history of psychotic illness at any time, attempted suicide or parasuicide at any time, current neurosis, use of any prescription medication within 2 wk or any over-the-counter medication within 72 h of the first administration of the test drug, or anticipation of the need for such medication during the course of the study. Potential subjects were also excluded if they had a known allergy or hypersensitivity to PEG-OB protein, PEG, or pegylated proteins; a relevant allergy to pharmaceutical agents; or a history of atopy.

PEG-OB protein

PEG-OB protein is a recombinant native human OB protein, expressed and purified from *Escherichia coli* and chemically conjugated to a species of branched PEG molecule with an average molecular weight of 42 kD in a 1-to-1 ratio. This resulted in a globular PEG–native human OB-protein polymer with increased molecular size (25, 26). PEG-OB protein at a concentration of 10 g/L was placed in sterile glass vials containing 1.3 mL; of the 1.3 mL available, 1.0 mL was taken out for use.

Procedures

Subjects were stratified and matched in pairs according to age, BMI, and fasting serum leptin and insulin concentrations to achieve balanced treatment with use of a frequency-based approach. Randomization numbers for the subjects were generated and incorporated into the double-blind labeling by a third party. The subjects then entered into the second part of phase 1, baseline characterization.

Baseline characterization

Baseline characterization was performed for each subject during the 2 wk before the 12-wk treatment phase. Baseline characterization included assessment of the appetite profile, daily energy intake, daily energy metabolism, 24-h energy metabolism, sleeping metabolic rate (SMR), and substrate utilization. We also measured body mass, body composition, and fasting serum leptin concentrations.

Appetite profile

The appetite profile was assessed at 3 different times: when subjects were fasting (before breakfast), in between meals during the day, and at an ad libitum evening meal. The appetite profile also included assessment of the degree of dietary restraint, which was determined by completion of a validated Dutch translation (28, 29) of the Three Factor Eating Questionnaire (TFEQ; 30) on day 1.

The appetite profile before breakfast and in between meals was determined from ratings of hunger, satiety, fullness, desire to eat, estimate of prospective consumption, thirst, and appetite

on 100-mm anchored visual-analogue scales (VAS) (29). The appetite profile before breakfast was completed on the day when subjects received the first injection (day 15 of the baseline characterization, which was day 1 of the treatment phase). It was completed 15 min before the first injection with PEG-OB protein or placebo. Breakfast was provided after the injection.

The appetite profile in between meals was completed during the 14-h daytime portion of a 36-h stay in the respiration chamber, which was the last day before the treatment phase started (day 14). The subjects were fed to maintain energy balance by matching their energy intake with their calculated energy expenditure, on the basis of their observed SMR and a physical activity level of 1.7. Subjects were fed meals of specified composition and size at fixed times during the day (29). Total energy intake was distributed as follows: 15% from breakfast, 25% from lunch, 40% from dinner, and 20% from snacks.

The macronutrient composition of the meals and snacks was as follows: 45% of energy from carbohydrate, 15% from protein, and 40% from fat. The energy density of breakfast was 6.7 kJ/g, of lunch was 8.4 kJ/g, of dinner was 4.4 kJ/g, and of snacks was 4.7 kJ/g. Calculations of energy density excluded water, coffee, and tea, which were available ad libitum. The appetite profile was completed at 10 fixed times in between meals: before and after breakfast, midmorning, before and after lunch, midafternoon, before and after dinner, midevening, and before going to sleep (29). Appetite during an ad libitum meal was assessed by measuring hunger, satiety, the amount eaten, meal duration, and the eating rate during a meal served from the Universal Eating Monitor (28, 31–34) on the evening before subjects entered the respiration chamber (day 13).

The Universal Eating Monitor consists of an electronic scale built into a table under the plate that the subject eats from; the scale is connected to a digital computer. Each time the subject takes a bite, the new weight of the plate is recorded by the computer. This technique provides a detailed record of food intake during a meal. In addition to recording the amount eaten, the meal duration, and the eating rate, it records bite frequency and bite size. As a result, it provides a cumulative food intake curve over time. The main course of the meal was pasta with sauce (energy density, 5.1 kJ/g; macronutrient composition: 44% of energy from carbohydrate, 16% from protein, and 40% from fat). The dessert was chocolate mousse (energy density: 10.5 kJ/g; macronutrient composition: 41.9% of energy from carbohydrate, 3.9% from protein, and 54.1% from fat).

Daily energy intake and daily energy metabolism

Daily energy intake under free-living conditions was assessed with food diaries that the subjects kept during the first week of baseline characterization. We checked these data by assessing energy expenditure with the doubly labeled water technique, according to the Maastricht protocol (35, 36). On the evening of day 0, subjects were given a weighed dose of a mixture of 99.84 atom% $^{2}H_{2}0$ in 10.05 atom% $H_{2}{}^{18}O$, such that baseline concentrations were increased to >300 ppm for ^{2}H and >2300 ppm for ^{18}O. A background urine sample was collected on the evening of day 0. Additional urine samples were collected on day 1 (the second void and again in the evening), in the morning and evening of day 8, and in the morning and evening of day 15 (35, 36). Samples were analyzed with isotope ratio mass spectrometry (Aqua Sira; VG Isogas Ltd, Middlewich, United Kingdom). In this type of spectrometer, ^{18}O is measured in water vapor. Water vapor is produced from the samples by online vacuum distillation. Deuterium is measured in hydrogen gas, which is produced from the samples online by the hot uranium technique (36).

24-h Energy metabolism, sleeping metabolic rate, and substrate utilization

SMR and 24-h energy metabolism were determined during each subject's stay in the respiration chamber. Measurements were made from the evening of day 13 until the morning of day 15, in the baseline-characterization period.

The respiration chamber is a 14-m^3 room furnished with a bed, a chair, a computer, a television, a radio-cassette player, a telephone, an intercom, a sink, and a toilet. The chamber is ventilated with fresh air at a rate of 70–80 L/min. The ventilation rate was measured with a dry gas meter (type G4; Schlumberger, Dordrecht, Netherlands). The concentrations of oxygen and carbon dioxide were measured with paramagnetic oxygen analyzers (Magnos 6G; Hartmann & Braun, Frankfurt, Germany and type OA 184A; Servomex, Crowborough, United Kingdom) and infrared carbon dioxide analyzers (type Uras 3G; Hartmann & Braun). During each 15-min period, 6 samples of outgoing air from each of the 2 chambers and 1 sample each of fresh air and calibration gas were measured. The gas samples to be measured were selected by a computer that also stored and processed the data (37). Subjects also had to follow a standardized physical activity program including controlled exercise on a bicycle ergometer (Lode, Groningen, Netherlands) at 45% of the predicted maximal capacity. Subjects cycled for 45 min during the morning (from 1000 to 1045). In the afternoon, 30 min of cycling was scheduled (from 1500 to 1530).

SMR was calculated from 0300 to 0600, with values controlled for physical activity by a Doppler radar system (37). Substrate utilization was calculated from oxygen consumption and carbon dioxide production over 24 h, corrected for protein utilization as indicated by nitrogen excretion in the collected 24-h urine sample.

Body mass and body composition

Body mass was measured with a digital scale accurate to 0.01 kg (model E1200; Sauter Inc, Ebingen, Germany) and height was measured to the nearest 0.001 m with use of wall-mounted calibrated meter scales. BMI was calculated from body weight and height (in kg/m^2). Body composition was determined by using hydrodensitometry and isotope dilution (36) with the combined equation of Siri (38; **Table 1**).

Serum leptin concentrations

Fasting blood samples for the measurement of serum leptin and PEG-OB protein concentrations were collected weekly before the next dose of PEG-OB protein or placebo. The samples were analyzed by Hoffmann-La Roche, Nutley, NJ. For the baseline characterization, the fasting blood sample was obtained on day 15 after the subjects left the respiration chamber. Serum leptin concentrations were measured with a double-antibody, sandwich-type enzyme-linked immunosorbent assay that used a monoclonal antibody specific for human leptin. The lower limit of detection is 0.5 μg/L and the upper limit is 50 μg/L. The intra- and interassay CVs were 9% and 12%, respectively. The leptin concentrations of normal-weight subjects range from 2 to 12 μg/L. To measure the

TABLE 1
Subject characteristics in the 2 matched groups at baseline (day 1) and after 12 wk of treatment (day 85) with 20 mg pegylated polyethylene glycol OB protein (PEG-OB protein) or placebo[1]

	PEG-OB protein group (n = 15)		Placebo group (n = 15)		P	
	Baseline	After treatment	Baseline	After treatment	Group × time interaction[2]	Time effect[3]
Body mass (kg)	107.3 ± 3.4	103.0 ± 3.0	108.6 ± 4.6	102.2 ± 4.1	NS	0.001
Fat mass (kg)	37.8 ± 3.3	33.9 ± 2.9	39.9 ± 4.4	33.9 ± 4.0	NS	0.01
Fat-free mass (kg)	69.5 ± 3.4	69.1 ± 3.0	68.7 ± 4.6	68.3 ± 4.1	NS	NS
Age (y)	45 ± 2		44 ± 2			
Leptin (μg/L)	21.9 ± 4.9	24.0 ± 4.0	20.4 ± 4.9	14.6 ± 4.1	0.05	0.05
Insulin (mmol/L)	19.6 ± 10.3	18.5 ± 7.8	20.0 ± 6.2	16.7 ± 4.2	NS	NS

[1] $\bar{x}$ ± SEM. There were no significant differences between the groups on day 1.
[2] Two-factor repeated-measures ANOVA.
[3] Repeated-measures ANOVA.

pharmacokinetics of leptin and PEG-OB protein, a frequent sampling schedule was applied in weeks 1 and 12 (27).

Treatment phase

Subjects received 20 mg PEG-OB protein (2 mL, 10 g/L) or placebo (2 mL of the buffer solution used for the PEG-OB protein) subcutaneously in the paraumbilical region once per wk for 12 wk while in a fasting state. Treatment started immediately after the screening and baseline characterization phase was completed (ie, after the subject left the respiration chamber). All subjects in both groups were prescribed a diet calculated to cause an energy deficit of 2.1 MJ/d throughout the study. Subjects discussed their diets every 2 wk with a dietitian. Vital signs and body mass were measured and standard laboratory tests were performed. The measurements made for baseline characterization were repeated in all subjects at the end of the treatment period by using the procedures described above.

In addition, subjects completed the appetite profile weekly before breakfast. Appetite between meals (over 14 h) was measured during a 36-h stay in the respiration chamber from day 83 to day 85 of the treatment period. During this same time period, SMR, 24-h energy metabolism, and substrate oxidation were also determined. Appetite and food intake during the ad libitum evening meal were assessed before entering the respiration chamber on day 83. Also, the TFEQ was completed on day 83 of the treatment period. During week 11, food intake was assessed again by using a food diary; the results were checked by using the doubly labeled water method. After 12 wk of treatment (on day 85), body mass and body composition were determined. The changes in subject characteristics from day 1 to day 85 are shown in Table 1.

Follow-up

The follow-up was conducted over the 2-wk period after the last dose of placebo or PEG-OB protein. We assessed the subjects' vital signs and body mass.

Safety

The safety of PEG-OB protein was monitored during each visit by documenting any adverse events and recording vital signs on case report forms. Routine clinical hematology and biochemical tests and urinanalyses were done weekly (27). The safety data were reviewed first for both groups combined and then for each group separately.

Data analysis

Changes from baseline to 12 wk were compared between the PEG-OB protein and placebo groups with a two-factor repeated-measures analysis of variance (ANOVA) with a group × time interaction. A post hoc Scheffe's procedure was used. To determine how representative the appetite profile ratings before breakfast were, these were compared with the average profile ratings from 10 time points over 14 h during the same day by using repeated-measures ANOVA. All statistics were executed with STATVIEW+GRAPHICS (Abacus Concepts Inc, Berkeley, CA). Significance was defined as $P < 0.05$.

RESULTS

During the 12-wk treatment period, sustained serum concentrations of PEG-OB protein measured just before the next dose ranged from 200 to 300 μg/L. Mean peak serum PEG-OB protein concentrations were reached 72 h after the dose was given; concentrations returned to the elevated predose concentrations after 1 wk (27). Baseline total leptin concentrations did not differ between the PEG-OB protein and placebo groups. Serum leptin concentrations increased to a new steady state amount in the PEG-OB protein group, whereas total leptin concentrations decreased with weight loss in the placebo group (Table 1). Serum leptin concentrations differed significantly between the groups from weeks 9 to 12 of the study (**Figure 1**; $P < 0.05$).

The weekly appetite profile ratings before breakfast showed a change from baseline; appetite and hunger decreased in the PEG-OB protein group but increased in the placebo group from days 1 to 8 (Figure 1). From days 8 to 78, these ratings were continuously significantly lower in the PEG-OB protein group than in the placebo group. The average values for desire to eat, estimate of prospective consumption, satiety, and fullness over the 12-wk period also differed significantly between the 2 groups (**Table 2**). Thirst scores remained stable during the 12-wk intervention period (Table 2). The differences between the groups in changes in appetite ratings coincided with the significant difference in change of serum leptin concentrations during weeks 9–11 (Figure 1). These ratings were assessed and blood samples were collected on the same day each week, before breakfast.

Appetite profile ratings recorded for 14 h during the day in the respiration chamber were not significantly different from baseline at the end of the 12-wk treatment; they also did not differ significantly between groups (**Table 3**). Appetite before breakfast in the

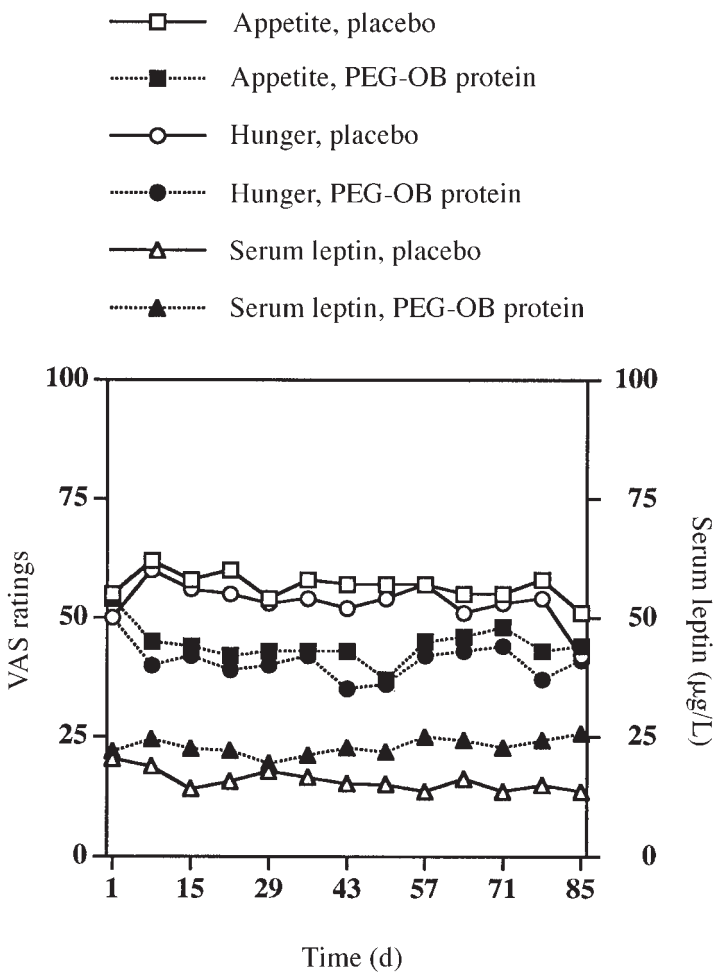


FIGURE 1. Visual analogue scale (VAS) ratings of appetite and hunger and serum leptin values in the subjects who received 20 mg pegylated polyethylene glycol OB protein (PEG-OB protein; $n = 15$) or placebo ($n = 15$). All data were collected before breakfast. There were significant differences in changes in appetite and hunger during treatment between the 2 groups (two-factor repeated-measures ANOVA with interaction; $P < 0.01$). There were significant differences in changes in serum leptin concentrations during treatment between the 2 groups (two-factor repeated-measures ANOVA with interaction; $P < 0.05$).

TABLE 2
Appetite profile ratings on the visual analogue scale before breakfast at baseline (day 1) and during 12 wk of treatment (average of weekly values); subjects were treated with 20 mg pegylated polyethylene glycol OB protein (PEG-OB protein) or placebo[1]

	PEG-OB protein group ($n = 15$)		Placebo group ($n = 15$)		*P*	
	Baseline	During treatment	Baseline	During treatment	Group × time interaction[2]	Within-group time effect[3]
	mm		*mm*			
Appetite	54 ± 1.1	44.4 ± 1.1[3]	55 ± 0.7	56.7 ± 0.8	0.0001	0.05
Hunger	50 ± 1.1	40.9 ± 1.1[3]	50 ± 1.2	53.2 ± 1.2	0.0001	0.05
Estimate of prospective consumption	54 ± 0.8	47.3 ± 0.8	57 ± 0.6	56.7 ± 0.6	0.0001	NS
Desire to eat	53 ± 1.0	45.9 ± 1.0	58 ± 1.0	56.4 ± 1.0	0.001	NS
Thirst	60 ± 1.1	60.2 ± 1.1	60 ± 0.7	61.0 ± 0.7	NS	NS
Satiety	35 ± 1.5	45.9 ± 1.5[3]	35 ± 0.7	36.8 ± 0.7	0.0001	0.05
Fullness	36 ± 1.2	44.0 ± 1.2[3]	32 ± 0.9	36.5 ± 0.9	0.0001	0.05

[1] $\bar{x}$ ± SEM.
[2] Two-factor repeated-measures ANOVA.
[3] Repeated-measures ANOVA with Scheffe's procedure.

TABLE 3
Appetite profile ratings on the visual analogue scale at baseline (day 1) and after 12 wk of treatment (day 85) with 20 mg pegylated polyethylene glycol OB protein (PEG-OB protein) or placebo[1]

	PEG-OB protein group (n = 15)				Placebo group (n = 15)				P[2]	
	Baseline		After treatment		Baseline		After treatment		Group × time interaction[4]	Within-group time effect[5]
	14 h	BB[3]	14 h	BB[3]	14 h	BB[3]	14 h	BB[3]		
	mm				*mm*					
Appetite	30 ± 2	54 ± 2	33 ± 5	44 ± 1[4,5]	37 ± 4	55 ± 2	33 ± 3	57 ± 2[4]	0.001	0.05
Hunger	27 ± 3	50 ± 1	30 ± 4	41 ± 1[4,5]	31 ± 5	50 ± 2	31 ± 3	53 ± 2[4]	0.001	0.05
Estimate of prospective consumption	36 ± 4	54 ± 2	36 ± 4	47 ± 1[4]	37 ± 4	57 ± 2	37 ± 4	57 ± 1[4]	0.001	NS
Desire to eat	31 ± 4	53 ± 1	34 ± 5	46 ± 1[4]	33 ± 3	58 ± 2	35 ± 4	58 ± 2[4]	0.001	NS
Thirst	44 ± 4	60 ± 1	44 ± 5	61 ± 2	54 ± 5	60 ± 2	35 ± 3	61 ± 2	NS	NS
Satiety	62 ± 5	35 ± 1	66 ± 5	46 ± 1[4,5]	63 ± 5	35 ± 1	66 ± 6	36 ± 1[4]	0.001	0.05
Fullness	62 ± 5	36 ± 1	64 ± 5	44 ± 2[4,5]	64 ± 6	32 ± 1	65 ± 5	35 ± 2[4]	0.001	0.05

[1] $\bar{x}$ ± SEM. Measurements were made over 14 h and before breakfast (BB) during the day in the respiration chamber (8–22 h).
[2] For BB values only.
[3] All values significantly different from 14-h values, $P < 0.01$ (repeated-measures ANOVA).
[4] Two-factor repeated-measures ANOVA.
[5] Repeated-measures ANOVA with Scheffe's procedure.

respiration chamber was not significantly different from appetite before breakfast measured separately during the same week (Tables 2 and 3) and differed significantly between the groups after treatment. However, the average appetite before breakfast in the respiration chamber differed from the average appetite throughout the same day (Table 3; $P < 0.0001$), thus, the appetite profile before breakfast was not representative of the appetite profile during the day.

Appetite and food intake did not differ significantly between the groups during the ad libitum meal at baseline or after the 12-wk intervention. There was no significant change in these appetite indexes from baseline to the end of treatment (**Table 4**).

With respect to the general attitude toward eating, scores on cognitive restraint (factor 1) in the placebo group increased significantly ($F_{1,14}$ = 26.4; $P < 0.0001$) and scores on hunger (factor 3) in the PEG-OB protein group decreased significantly ($F_{1,14}$ = 5.6; P = 0.03) from baseline to the end of the 12-wk treatment (**Table 5**). The other scores on the TFEQ did not change significantly from baseline to the end of treatment ($F_{1,14} \leq 4.3$; NS). The changes in TFEQ scores over the 12-wk treatment were significantly different between the groups with respect to cognitive restraint ($F_{1,28}$ = 4.94; P = 0.03); the other differences between groups in the changes [disinhibition (factor 2) and hunger] were not significant ($F_{1,28} \leq 0.85$; NS; Table 5).

Daily energy intake, calculated from self-reported food intake recorded in the food diaries, decreased by 2.6 MJ from baseline to the end of treatment in the PEG-OB protein group ($P < 0.001$) and by 2.2 MJ in the placebo group ($P < 0.001$). Self-reported energy intake did differ significantly between the groups. The diaries indicated that these decreases in energy intake resulted from reductions in meal size during breakfast and dinner and reductions in meal frequency from 5.3 to 4.4 eating episodes/d ($P < 0.03$). In both groups, the reported macronutrient composition shifted from 39% of energy from carbohydrate, 16% from protein, 39% from fat, and 6% from alcohol to 40%, 20%, 35%, and 5% of energy, respectively. The reported percentage of

TABLE 4
Food intake during the ad libitum meal from the Universal Eating Monitor (28) at baseline and after 12 wk of treatment[1]

	PEG-OB protein group (n = 15)		Placebo group (n = 15)	
	Baseline	After treatment	Baseline	After treatment
Amount eaten (MJ)				
Main course	2.3 ± 0.3	2.1 ± 0.2	2.0 ± 0.3	2.1 ± 0.3
Dessert	1.3 ± 0.2	1.3 ± 0.2	1.1 ± 0.2	1.2 ± 0.2
Eating rate (g/min)				
Main course	70 ± 8	63 ± 6	57 ± 6	63 ± 5
Dessert	62 ± 9	68 ± 7	57 ± 7	62 ± 9
Meal duration (min)				
Main course	6.5 ± 0.8	6.6 ± 0.8	6.8 ± 0.9	6.5 ± 0.7
Dessert	2.0 ± 0.3	1.8 ± 0.2	1.9 ± 0.2	1.8 ± 0.2
Change in hunger on VAS (mm/min)				
Main course	−5.9 ± 1.2	−5.9 ± 1.2	−2.1 ± 4.1	−2.1 ± 4.1
Dessert	−2.9 ± 0.8	−2.9 ± 0.8	−4.1 ± 1.3	−4.1 ± 1.3
Change in satiety on VAS (mm/min)				
Main course	6.6 ± 1.3	6.2 ± 1.6	7.8 ± 1	8.4 ± 1.9
Dessert	3.7 ± 3.1	3.7 ± 3.1	5.1 ± 1.6	5.1 ± 1.6

[1] $\bar{x}$ ± SD. There were no significant group × time interactions, time effects, or group differences. VAS, visual analogue scale.

TABLE 5
Scores on the Three-Factor Eating Questionnaire at baseline (day 1) and after 12 wk of treatment with 20 mg pegylated polyethylene glycol OB protein (PEG-OB protein) or placebo[1]

	PEG-OB protein group (n = 15)		Placebo group (n = 15)		P	
	Baseline	After treatment	Baseline	After treatment	Group × time interaction[2]	Within-group time effect[3]
Cognitive restraint	7.1 ± 1.4	9.3 ± 1.5	4.1 ± 0.6	9.5 ± 1.3[3]	0.05	0.0001
Disinhibition	4.8 ± 0.6	4.8 ± 0.6	4.9 ± 0.6	4.7 ± 0.6	NS	NS
Hunger	5.9 ± 1.2	4.3 ± 1.1[3]	3.8 ± 0.7	3.0 ± 0.7[3]	NS	0.05

[1] $\bar{x}$ ± SEM.
[2] Two-factor repeated-measures ANOVA.
[3] Repeated-measures ANOVA with Scheffe's procedure.

energy from protein increased ($P < 0.05$). However, when controlled for energy expenditure as measured by the doubly labeled water technique, reported energy intake showed an underreporting of 37% at baseline and of 48% after 12 wk of treatment (35).

Daily energy expenditures in the PEG-OB protein and placebo groups, respectively, were 16.5 ± 2 and 17.0 ± 2.8 MJ/d at baseline and 15.4 ± 2 and 14.7 ± 2.2 MJ/d at the end of the 12-wk treatment. This reflects energy expenditure reductions of 1.1 ± 1 MJ/d in the PEG-OB protein group and of 2.3 MJ/d in the placebo group; the difference between the groups was not significant.

In the respiration chamber, energy balance was achieved at a level of −0.02 to −0.20 MJ, which did not differ significantly from 0. After 12 wk of treatment with PEG-OB protein or placebo, there were no significant differences in energy expenditure, SMR, or substrate oxidation between the groups (**Table 6**). Energy expenditure was, on average, 93% of the energy expenditure at baseline. In both groups, 24-h energy expenditure and SMR during energy balance were significantly reduced after 12 wk. SMR expressed as a function of fat-free mass did not change significantly from baseline to the end of the 12-wk treatment, nor did it differ significantly between the groups after treatment. The respiratory quotient did not change significantly over the 12-wk treatment phase. The physical activity index in the respiration chamber (determined by using the standardized activity protocol) and the food quotient (determined by using the standardized macronutrient composition of the food provided to the subjects) did not change from baseline to the end of treatment. Because subjects were fed to maintain energy balance during their stays in the chamber, energy intake was significantly reduced after 12 wk of treatment in each group, corresponding to the significant reduction in energy expenditure.

There were no clinically relevant changes in mean laboratory values or vital signs during the study. No differences in standard chemistry or hematologic assessments were detected between the groups. Mean total serum protein decreased significantly (−2.3%) in the PEG-OB protein group but not in the placebo group (−0.5%). However, no significant differences in urinary protein were observed between the groups. In summary, at the dosage studied, PEG-OB protein appeared to be generally well tolerated and safe (27). At follow-up (2 wk after the last dose), vital signs were normal and body mass had not changed significantly from the last measurement made during the treatment phase.

DISCUSSION

Treatment of obese men with a weekly subcutaneous injection of 20 mg PEG-OB protein, under conditions of an energy deficit of 1–2 MJ/d, resulted in lower appetite and hunger ratings before breakfast than in the placebo group. This difference began during the first week after treatment started and it remained constant during the next 11 wk. The changes in the appetite profile before breakfast occurred while serum leptin concentrations changed in different directions in the 2 groups: serum leptin increased and remained higher in the PEG-OB protein group, whereas it decreased, as expected, and remained lower in the placebo group. As was shown before (12, 22, 23), increases in serum leptin con-

TABLE 6
Energy metabolism at baseline (day 1) and after 12 wk of treatment (day 85) with 20 mg pegylated polyethylene glycol OB protein (PEG-OB protein) or placebo[1]

	PEG-OB protein group (n = 15)		Placebo group (n = 15)		P for time effect (both groups)[2]
	Baseline	After treatment	Baseline	After treatment	
24-h EI (MJ/d)	14.1 ± 0.3[3]	13.1 ± 0.3	13.8 ± 0.4	12.6 ± 0.3	0.0001
24-h EE (MJ/d)	14.1 ± 0.3	13.4 ± 0.3	14.0 ± 0.4	12.8 ± 0.4	0.001
FQ[4]	0.8	0.8	0.8	0.8	
RQ[5]	0.84 ± 0.0003	0.84 ± 0.01	0.84 ± 0.0003	0.85 ± 0.01	NS
SMR (MJ/d)	8.5 ± 0.2	8.0 ± 0.2	8.3 ± 0.2	7.7 ± 0.2	0.001
PAL	1.7 ± 0.03	1.7 ± 0.03	1.7 ± 0.03	1.7 ± 0.02	NS

[1] There were no significant group × time interactions and no significant differences between groups. EI, energy intake; EE, energy expenditure; FQ, food quotient; RQ, respiratory quotient; SMR, sleeping metabolic rate; PAL, physical activity level (EE/SMR).
[2] Repeated-measures ANOVA.
[3] $\bar{x}$ ± SEM.
[4] FQ = carbon dioxide consumption ($\dot{V}CO_2$)/oxygen consumption ($\dot{V}O_2$) when the food is completely oxidized (30, 38).
[5] RQ = $\dot{V}CO_2/\dot{V}O_2$.

centrations are related to decreased hunger, which occurred in the PEG-OB protein group. Decreases in serum leptin are related to increased hunger (12), which occurred in the placebo group.

Furthermore, it is important to remember that the appetite profile VAS ratings were obtained in a fasted state, over 12 wk, while the subjects were in negative energy balance. The finding of a relatively reduced appetite under negative energy balance conditions is consistent with the reduced food intake in rats after PEG-OB protein treatment (17, 18) and with observations of appetite reduction by Farooqi et al (23). During the 12 wk of treatment, the subjects in both groups were in negative energy balance and appeared to be in compliance with the dietary instructions that created this deficit. Compliance with dietary instructions independent of treatment was indicated by the increases in cognitive restraint scores of the TFEQ (30, 39); these increases occurred in both groups but were significant only in the placebo group because of the relatively lower level of cognitive restraint (NS) at baseline in this group. At 12 wk, both groups had an average level of cognitive restraint of ≈9, which is the cutoff in our subject population for dietary restraint, as opposed to unrestraint (28, 32, 33). Compliance was also indicated by the average loss in body mass of 5.4 kg over 12 wk, which is consistent with a reduction in energy intake of 1–2 MJ/d and a loss of 1 kg body mass for every 30-MJ reduction in energy intake (5).

Another indicator of an effect of PEG-OB protein on the appetite profile was the significant decrease in the general feeling of hunger, as indicated by factor 3 (hunger) of the TFEQ. This change was consistent with the finding of reduced appetite and hunger as scored weekly in the fasting state. The hunger scores on the TFEQ and the appetite profile scores before breakfast appeared to be sensitive to the combination of PEG-OB protein treatment and negative energy balance in a fasted state. Also, self-reported daily energy intake was significantly reduced in the PEG-OB protein group at the end of treatment.

However, the relatively lower appetite before breakfast that we observed in the PEG-OB-protein group was not associated with reduced food intake during the ad libitum evening meal. Weekly administration of 20 mg PEG-OB protein may have failed to reduce observed food intake because the observed reduced appetite profile before breakfast was measured during negative energy balance conditions in the fasted state. It appeared that this finding was not representative of appetite during the rest of the day; therefore, the appetite profile data collected before breakfast was less meaningful. This also means that the results of the present study regarding food intake and body mass reduction did not agree with those of comparable animal studies (17, 18, 20) and initial intervention studies in humans (22, 23). The similar reductions in body mass that occurred in both groups probably resulted from compliance with the diet. Until now, only one study reported losses in body mass and fat mass that were significantly different in leptin-treated subjects than in placebo-treated subjects (22). In that study there was wide variability in the response of subjects to daily subcutaneous administration of 0.03 g recombinant methionyl human leptin/kg body wt (Amgen Pharmaceuticals), resulting in a dose of ≈30 mg/d.

There were no significant changes from baseline to the end of treatment in 24-h appetite profiles during energy balance. On the basis of the energy-balance measurements in the respiration chamber, energy expenditure decreased, on average, to 93% of the original average daily metabolic rate. Apparently, a decrease in energy expenditure was related to a lower body mass; together these factors did not result in any change in the 24-h appetite profile under energy-balance conditions. Moreover, the respiratory quotient did not change significantly from baseline to the end of treatment, nor did it differ significantly between the 2 treatment groups. The possible relations between substrate oxidation and both hunger and satiety, as reported previously (29), might explain, in part, why the appetite profiles did not change from baseline to the end of treatment.

The similar reduction in energy expenditure in both groups after 12 wk of treatment, under energy-balance conditions in the respiration chamber, must have resulted mainly from the similar losses of body mass, fat mass, and fat-free mass. In comparison, there was a diminished decrease of metabolic rate in OB protein–treated mice under conditions of negative energy balance (20). In the present study, subjects complied with dietary instructions designed to produce a negative energy balance, but the treatment did not affect this negative energy balance as in the OB protein–treated mice.

We conclude that a weekly subcutaneous injection of 20 mg PEG-OB protein in obese men results in lower appetite and hunger levels before breakfast than does placebo treatment. These lower appetite and hunger levels occurred in the fasted state during a period of negative energy balance. In contrast, appetite and hunger levels increased from baseline to the end of treatment in the placebo group. These effects occurred in parallel to increased serum leptin concentrations in the PEG-OB protein group and to decreased serum leptin in the placebo group. Moreover, a general reduction in hunger, as indicated by the TFEQ, in the PEG-OB protein group during a period of negative energy balance was observed compared with placebo treatment. Reduced hunger in the PEG-OB protein group did not result in reductions in daily food intake or body mass or changes in body composition. The treatment also did not have an independent effect on energy expenditure, ie, on the change in SMR as a function of fat-free mass.

When subjects were in short-term energy balance (at 93% of the original energy expenditure), the lack of change in the appetite profile from baseline to the end of treatment and the absence of differences between treatments implies that the relatively reduced hunger found in the fasted state before breakfast was not representative of the 14-h appetite profile measured in the respiration chamber.

The fact that PEG-OB protein treatment modified appetite at a dosage that did not result in significant changes in body composition, energy expenditure, or body mass loss compared with placebo treatment suggests that PEG-OB protein has central rather than peripheral biological activity in obese men.

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