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Weekly Subcutaneous Pegylated Recombinant Native Human Leptin (PEG-OB) Administration in Obese Men

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ABSTRACT

To assess the biological activity and tolerability of pegylated recombinant native human leptin (PEG-OB), 30 obese men (mean body mass index, 33.9 kg/m^2) were randomized to a double-blind treatment with weekly sc injections of 20 mg PEG-OB or placebo for 12 weeks, in addition to a hypocaloric diet (deficit, 2 MJ/day). Body composition, energy expenditure, and metabolic parameters were measured before and after treatment. PEG-OB was generally well tolerated based on adverse event reports, lab values, and vital signs. Weekly sc PEG-OB led to sustained serum concentrations of PEG-OB and leptin throughout treatment. No significant differences in the delta or percent weight loss, percent body fat, sleeping metabolic rate, or respiratory quotient were observed between the PEG-OB and placebo groups. Percent change in serum triglycerides from baseline was significantly correlated with body weight loss in the PEG-OB group, but not in the placebo group. Although larger reductions in serum triglycerides were observed in the PEG-OB group compared with the placebo group, these differences were not statistically significant. We concluded that weekly injection of PEG-OB leads to sustained serum concentration of PEG-OB and leptin throughout the 12-week treatment period and is generally well tolerated. The trends observed in serum triglycerides suggest that a weekly 20-mg sc treatment with PEG-OB may have biological effects in obese men. (*J Clin Endocrinol Metab* **85:** 4003–4009, 2000)

OBESITY IS A complex, increasingly prevalent and important health problem throughout the world (1). Human obesity is characterized by increased adipose tissue mass resulting from a complex interaction of genetic predisposition to metabolic efficiency and environmental/lifestyle factors (2–5). Obese individuals are at increased risk for conditions such as noninsulin-dependent diabetes mellitus, hypertension, hyperlipidemia, coronary heart disease, stroke, and certain cancers (1). Although treatment [*e.g.* healthy eating (including behavior modification), physical activity, drugs] is available and most people can achieve medically significant weight loss (5–10% initial body weight), the long-term maintenance of that weight loss and its associated improvement in health is, unfortunately, very rare (2–4). These factors and the psychological, social, and economic costs of obesity are matters of growing concern in the scientific, medical, and public health communities (1).

New scientific information concerning the regulation of energy balance and fat mass has emerged since the discovery of leptin (also known as OB protein). Evidence from many animal studies and observational studies in humans suggests that this hormone, which is secreted primarily from adipocytes in proportion to cell size, seems to play a role in the control of body fat stores by acting within the central nervous system to coordinate the regulation of feeding behavior, metabolism, autonomic nervous system, and body energy balance in rodents, primates, and humans (4–6). Although obese individuals have increased serum concentrations of leptin (7, 8) and concomitant decreased sensitivity to leptin, therapeutic augmentation of the circulating leptin levels, by administration of leptin, may result in increased leptin signaling and action in a manner similar to use of exogenous insulin to increase insulin signaling in noninsulin-dependent diabetes mellitus (4, 5). Thereby, reductions of food intake, body fat mass, and body weight in obese patients may result from treatment with recombinant leptin.

Since its identification, interest within the medical community in the effects (if any) of treatment of obese individuals with leptin has been significant (4). However, only initial interventional studies in humans are available to support these suggestions. In trials sponsored by Amgen Inc., significant dose-related reductions in body fat and body weight (up to −7.1 kg in the highest dose group) were observed following daily sc treatment with from 0.01–0.30 mg/kg recombinant human met-leptin for 24 weeks in obese subjects (9). In the treatment of a young very obese girl with a mutated *ob* gene, daily sc injection of low doses of met-leptin (dose of 0.028 mg/kg of lean mass calculated to cause a circulating concentration ~10% of what would be predicted based on her body fat) has been reported. Daily met-leptin treatment caused a dramatic reduction in appetite, food-seeking behavior, food intake, and body weight (10). The results of treatment of this girl with congenital leptin deficiency (10), taken together with the results of the clinical trials in obese adults (9), indicate that leptin has bio-

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logical activity in at least some obese humans and that among its activities are reductions in appetite and food intake at low doses and reductions in body fat and body weight at the maximal dose studied.

Leptin has been reported to have a very short apparent half-life in animals (11, 12) and humans (13). Polyethylene glycols (PEG) are amphiphilic polymers of ethylene glycol with varying average molecular weights that can be activated and covalently attached to proteins. Modification of proteins through pegylation, covalent linkage of PEG polymers to the protein, has resulted in increased serum half-life and reduced immunogenicity for a number of proteins (14–16). This technology was applied to leptin to produce pegylated recombinant native human leptin or PEG-OB protein (PEG-OB), which was used in this study. Preclinical studies with PEG-OB indicate an extended half-life in rodents (>48 h) compared with that reported for recombinant native human leptin (data not shown) and efficacy for reduction of food intake and body weight of rats treated daily with sc PEG-OB for 8 days (17, 18).

We undertook a double blind, randomized study to determine the biological activity and safety of elevating serum levels of leptin using PEG-OB in comparison with placebo in obese men on a mild hypocaloric diet.

Subjects and Methods

Experimental subjects

This single center trial had a prospective, randomized double blind placebo-controlled group design. This study was conducted according to the guidelines for Good Clinical Practice and was monitored by Hoffmann-La Roche, Inc. (Welwyn, UK).

Obese [body mass index (BMI), $\geq$27.0 kg/m^2] men 18–60 yr of age were eligible for inclusion. Recruitment was from the Institute waiting list or by local advertising. All aspects of the study conformed to the declaration of Helsinki. The ethics committee of the University of Maastricht approved the study, and all participants gave written informed consent. Patients with obesity-related diseases requiring pharmacological treatment (*e.g.* diabetes, hypertension, dyslipidemia) were excluded. Other exclusion criteria were: weight loss more than 3 kg in the previous 3 months; presence of any significant illness, including laboratory or electrocardiogram abnormalities; history or presence of drug abuse or alcoholism; and smoking more than five cigarettes or equivalent per day. Also, known allergy, history of atopy or hypersensitivity to pegylated proteins, and use of any drug that might have influenced body weight or serum lipids led to exclusion.

Study design

After screening, 30 patients were selected and enrolled. Baseline energy expenditure, body composition, and metabolic profile (including lipid profile and insulin sensitivity) were measured. Patients were stratified and matched into pairs according to age, BMI, and serum leptin and insulin concentrations to achieve balanced treatment groups. Randomization numbers for patients were generated and incorporated into the double blind labeling by a third party. During the treatment period, either an injection of 20 mg PEG-OB (2 mL, 10 mg/mL^{-1}) or placebo (2 mL) were given sc, in the para-umbilical region, weekly for 12 weeks. In addition, all subjects were prescribed a hypocaloric diet (500 kcal or 2 MJ/day deficit). The energy content of the diet was based on the measured energy expenditure of each subject (see below). The dietary prescription was discussed every 2 weeks with a dietitian. Subjects came to the laboratory in the morning, in a fasting state, each week to receive treatment. Vital signs and body weight were recorded, and blood samples were taken for standard laboratory tests. At the end of the 12-week treatment period, energy expenditure, body composition, and metabolic profile were measured again.

Measurements of energy expenditure and body composition

Energy expenditure and substrate utilization were measured during a 36-h stay in a respiration chamber while the subjects were maintained in energy balance by adjusting the food provided (19). Macronutrient composition of the diet was fixed at 45/15/40% of energy for carbohydrate, protein, and fat, respectively. The respiration chamber is a 14 m^2 room furnished with a bed, chair, wash bowl, toilet, and radio/TV set and telephone. While in the chamber, subjects had to follow a standardized physical activity program, including controlled exercise for 35 min on a bicycle ergometer (starting at 40 W for 5 min, followed by 80 W for 30 min). Two exercise sessions took place; one in the morning at 1000 h and the other one in the afternoon at 1500 h. Gas sampling and analysis from the chamber is described in detail elsewhere (20).

Total energy expenditure, sleeping metabolic rate (SMR), and respiratory quotient (RQ) were calculated during the last 24 h in the chamber. Energy expenditure was calculated from the O_2 consumption and CO_2 production according to the method of Weir (21). SMR was calculated from the sleep period between 0300 and 0600 h, controlled for physical activity by a Doppler radar system. Body weight was measured (with a calibrated digital scale accurate to 0.01 kg), and height was measured to the nearest 0.001 m. Body composition was determined after leaving the respiration chamber in the morning by using hydrodensitometry and deuterium dilution (22). Body composition was calculated using the combined equation of Siri (23).

Blood chemistry and pharmacokinetics

Fasting serum concentrations of glucose, insulin, free fatty acids, glycerol, triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol were measured at baseline (day 1) and after 12 weeks of treatment (day 85). Samples were stored at −80 C and analyzed by a certified laboratory. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation (24).

Insulin sensitivity was assessed by the short insulin tolerance test (25). After an overnight fast, sampling and injection catheters were placed. Arterialized venous blood samples were collected after insulin was iv injected (Human Actrapid; Novo Nordisk A/S, Bagsvaerd, Denmark; 0.1 U kg^{-1} body weight). The test was terminated after 16 min by an iv glucose injection. The rate constant for plasma glucose disappearance (K_{itt}) was calculated using a linear regression line fitted through the blood glucose values from 4–16 min because no changes in blood glucose were noted within 4 min after insulin injection.

Fasting blood samples for the measurement of serum concentrations of leptin and PEG-OB were collected weekly before the next dosing and analyzed at Hoffman-La Roche Inc. (Nutley, NJ). For the pharmacokinetics of leptin and PEG-OB, a frequent sampling schedule was applied in weeks 1 and 12. Serum leptin concentrations were measured using a double-antibody "sandwich" enzyme-linked immunosorbent assay using a monoclonal antibody specific for human leptin. The lower level of detection is 0.5 ng/mL, and the upper limit is 50 ng/mL. The intra- and interassay variations were 9% and 12%, respectively. The leptin levels of normal weight subjects ranged from 2–12 ng/mL. PEG-OB concentrations were measured using a similar enzyme-linked immunosorbent assay after separating PEG-OB from leptin by size exclusion. Quality control samples covering a range of leptin and PEG-OB concentrations were included in each assay. Both assays were verified by appropriate recovery and cross-reaction experiments.

Safety

Safety of PEG-OB was monitored during each visit by documentation of adverse events and the recording of vital signs on Case Report Forms. Routine clinical hematology and biochemical tests and urine analysis were done weekly.

PEG-OB

Recombinant native human leptin, expressed and purified from *Escherichia coli,* was chemically conjugated to a species of branched PEG molecule with an average molecular weight of 42 kD in a 1:1 ratio. The result was a globular PEG—native human leptin polymer with increased molecular size (26, 27). PEG-OB at a concentration of 10 mg/mL was placed in sterile glass vials containing 1.3 mL.

Statistical analysis

The number of subjects needed was calculated as follows. Results of clinical trials using a hypocaloric diet and orlistat treatment (120 mg t.i.d.) for 12 weeks reported weight loss in 1022 placebo-treated subjects as 2.23 kg with a SD of 3.04 kg (Roche orlistat protocols 14119B, M14119, M14149, M14161, and M14185). With this information, power calculation indicated that 25 subjects per treatment group were needed to detect an additional weight reduction in the PEG-OB group of 4.0 kg over that seen in the placebo group with a power of 90%, assuming a SD of 3 kg. Assuming a 20% dropout rate, 30 subjects per treatment group would be required. This number of subjects was also sufficient to detect a difference in change from baseline SMR between the groups of 0.21 kJ per min with a power of 90%. This calculation was based on a SD of SMR in a single subject of 0.16 kJ per min with an estimated SMR of 5.3 kJ per min, accordingly to the WHO equation (28). However, after the first 30 subjects completed the study, a planned interim analysis was conducted and the study of weekly 20-mg sc PEG-OB was prematurely stopped by the sponsor due to lack of efficacy for weight loss at the dose tested.

Changes from baseline after 12 weeks of treatment were compared between the PEG-OB-treated and placebo group using factorial ANOVA. *Post hoc,* for each comparison separately, ANOVA with repeated measures was used. Additional statistical tests were used when appropriate. All statistical tests were two-sided, and significance was defined as $P < 0.05$. All data are presented as mean ± SEM, unless otherwise indicated.

Results

Of the 38 subjects screened, 8 did not meet the inclusion criteria. The initial demographic characteristics of the subjects are shown in Table 1. The characteristics of the 15 subjects randomized to each treatment group were similar. All 30 subjects who were randomized into the two treatment groups completed the trial.

Serum concentrations of PEG-OB and leptin

Serum concentrations of PEG-OB during the study are shown in Fig. 1. Following weekly sc dosing, sustained serum levels of PEG-OB, measured just before the next dose, ranging from 200–300 ng/mL, were observed. The PEG-OB serum profile after sc injection was similar after the first (day 1) and 12th weekly (day 78) dose (Fig. 1). Following a sc injection, mean peak serum PEG-OB concentrations were achieved 72 h after dosing, followed by a return to the elevated pre-dose levels after 1 week.

Baseline total leptin concentrations during the study are shown in Fig. 2. Baseline total leptin concentrations were 20.4 ± 4.9 and 21.9 ± 4.9 ng/mL (mean ± SEM) in the placebo

TABLE 1. Subject characteristics

	Placebo (n = 15)	PEG-OB (n = 15)
Body weight (kg)	108.6 ± 4.6 (89.5–139.6)	107.3 ± 3.4 (85.6–137.4)
BMI (kg/m^2)	33.8 ± 1.2 (30.3–43.7)	34.0 ± 1.0 (29.3–39.7)
Age (yr)	44 ± 2 (33–54)	45 ± 2 (33–58)
Leptin concentration (ng/mL)	20.4 ± 4.9 (2.2–47.3)	21.9 ± 4.9 (5.5–41.0)
Insulin concentration (ng/mL)	21.3 ± 1.5 (6.4–28.0)	19.6 ± 2.6 (6.8–29.2)

Data are mean ± SEM.

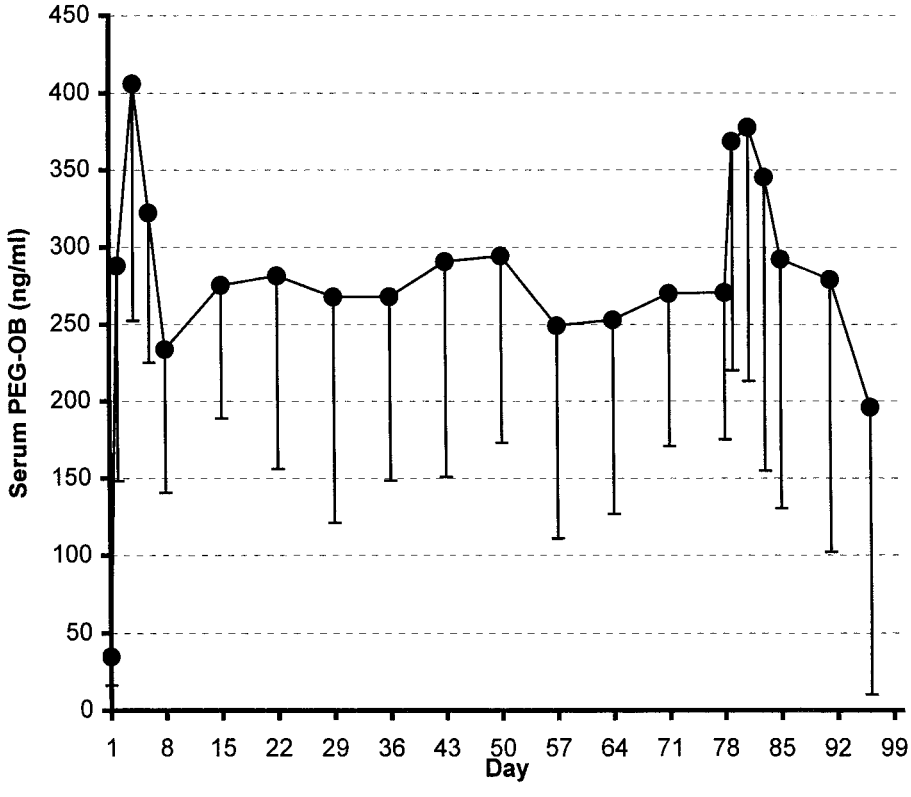

FIG. 1. Serum levels of PEG-OB following weekly sc dosing during the study. PEG-OB was administered sc on days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, and 78. PEG-OB concentrations are mean ± SD. More frequent blood sampling occurred after the first (day 1) and 12th weekly (day 78) dose. Note the sustained blood levels of PEG-OB in samples taken just before the next weekly dose of PEG-OB throughout the study; these sustained PEG-OB levels ranged from 200–300 ng/mL. Note that the PEG-OB serum profile following the first and 12th weekly sc injection was similar. Following a sc PEG-OB injection, mean peak serum PEG-OB concentrations were achieved 72 h after dosing, followed by a return to the elevated pre-dose levels after 1 week.

and PEG-OB treatment groups, respectively. Total leptin serum concentrations increased slightly to a new steady-state level (24.0 ng/mL during weeks 9–12 of the study) in subjects treated with PEG-OB, whereas total leptin concentrations fell with weight loss in placebo-treated subjects (14.6 ng/mL during weeks 9–12 of the study) (Fig. 2).

Body weight, body composition, and energy metabolism

The effect of PEG-OB treatment or placebo treatment on body weight, body composition, and energy expenditure are given in Table 2. The mean body weight change was −5.4 ± 0.8 kg in all 30 subjects studied. Subjects in both the placebo (n = 15) and PEG-OB (n = 15) treatment groups lost weight, and the amount of weight loss was similar in both groups (day 85 body weight: placebo, 102.2 ± 4.1 kg, delta, −6.4 kg; PEG-OB, 103.0 ± 3.0; delta, −4.3 kg). There was no significant difference in the delta or percent weight loss between the PEG-OB and placebo groups (Table 2). The mean changes in percent body fat and sleeping metabolic rate of all 30 subjects were −1.4 ± 0.4% and −0.4 ± 0.07 kJ/min, respectively, and there were no significant differences between PEG-OB and placebo. No differences were observed in RQ between the treatment groups. Using a technique pioneered in the clinical trials with the lipase inhibitor orlistat, subjects were classified as "good" losers (delta body weight at 4 weeks ≥2.5 kg) and "poor" losers (delta body weight at 4 weeks <2.5 kg) in each treatment groups (29–31). An imbalance of the distribution was observed with more good losers (8 of 15 or 53%) in the placebo group than the PEG-OB group (5 of 15 or 33%).

Metabolic profile

The effect of 20 mg PEG-OB or placebo treatment on the metabolic profile is shown in Table 3. No significant differences in serum glucose or insulin concentrations from baseline were observed at the end of the 12-week treatment period, and there were no differences between the treatment groups. Insulin sensitivity (measured by the short insulin tolerance test) also showed no significant differences between both groups before and after treatment.

Mean baseline (day 1) serum concentrations of free fatty

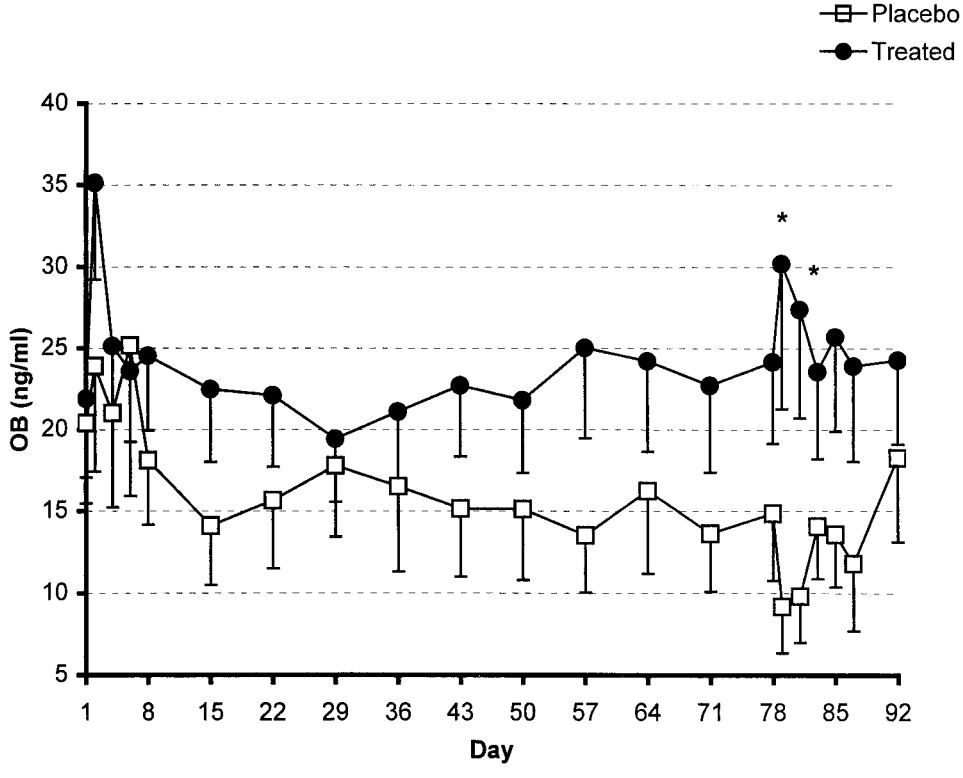

FIG. 2. Total serum leptin concentrations following weekly sc PEG-OB dosing during the study. PEG-OB was administered sc on days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, and 78. Total leptin concentrations are mean ± SEM. Baseline total leptin concentrations were 20.4 ± 4.9 and 21.9 ± 4.9 ng/mL in the placebo (□) and PEG-OB treatment (●) groups, respectively. Total leptin serum concentrations in blood samples taken just before the next weekly dose of PEG-OB increased to a new steady-state level during weeks 9–12 of the study in subjects treated with PEG-OB, whereas total leptin concentrations fell with weight loss in placebo-treated subjects during weeks 9–12 of the study. More frequent blood sampling occurred after the first (day 1) and 12th weekly (day 78) dose. Note that total leptin serum profile following the first and 12th weekly sc PEG-OB injection were similar. Following a sc PEG-OB injection, mean peak serum total leptin concentrations were achieved 72 h after dosing, followed by a return to the elevated pre-dose levels after 1 week. *, Significant differences between the PEG-OB and placebo groups.

TABLE 2. Effect of 12 weeks treatment with PEG-OB or placebo on body weight, body composition, and energy metabolism

	Placebo (n = 15)			PEG-OB (n = 15)		
	Day 1	Day 85	Delta (day 85-day 1)	Day 1	Day 85	Delta (day 85-day 1)
Body weight (kg)	108.6 ± 4.6	102.2 ± 4.1	−6.4	107.3 ± 3.4	103.0 ± 3.0	−4.3
Body fat (%)	36.7 ± 1.5	33.2 ± 1.7	−1.8[a]	35.2 ± 1.5	32.9 ± 1.5	−0.9[a]
24-h EE (MJ/day)	14.0 ± 0.4	12.8 ± 0.4	−1.2[b]	14.1 ± 0.3	13.4 ± 0.3	−0.7[c]
SMR (MJ/day)	8.3 ± 0.2	7.7 ± 0.2	−0.6[b]	8.5 ± 0.2	8.0 ± 0.2	−0.5[c]
RQ	0.84 ± 0.002	0.85 ± 0.01	−0.01	0.84 ± 0.001	0.84 ± 0.01	0.0

Data are mean ± SEM. For differences compared with baseline ([a] $P < 0.05$; [b] $P < 0.001$; [c] $P < 0.01$). There were no significant differences between treatments. EE, Energy expenditure.

TABLE 3. Effect of 12 weeks treatment with PEG-OB or placebo on metabolic and lipid profiles

Parameter	Placebo (n = 15)			PEG-OB protein (n = 15)		
	Day 1	Day 85	Delta (day 85-day 1)	Day 1	Day 85	Delta (day 85-day 1)
Glucose (mmol/L)	5.86 ± 0.17	5.58 ± 0.11	−0.29	6.18 ± 0.17	5.99 ± 0.18	−0.19
Insulin (μmol/L)	21.3 ± 1.5	17.0 ± 1.1	−4.3	19.6 ± 2.6	18.5 ± 2.0	−1.1
K_{itt} (%/min)	2.87 ± 0.18	3.11 ± 0.14	0.24	2.85 ± 0.16	3.04 ± 0.18	0.19
Leptin (ng/mL)	20.4 ± 4.9	13.6 ± 3.2	−6.8	21.9 ± 4.9	25.7 ± 5.8	3.8
Free fatty acids (μmol/L)	395 ± 47 (157–831)	365 ± 43 (154–707)	−29	474 ± 33 (170–761)	531 ± 45 (88–886)	58
Glycerol (μmol/L)	84.7 ± 9.4	84.5 ± 8.2	−0.2	82.7 ± 6.2	85.7 ± 5.1	3.1
Triglycerides (mmol/L)	1.54 ± 0.15 (0.88–2.78)	1.38 ± 0.14 (0.59–2.61)	−0.17	2.09 ± 0.23 (1.3–4.63)	1.73 ± 0.21 (0.65–3.26)	−0.35
Total cholesterol (mmol/L)	4.88 ± 0.19 (3.79–6.08)	4.71 ± 0.19 (3.36–5.72)	−0.18	5.06 ± 0.26 (3.78–6.31)	4.85 ± 0.25 (3.88–7.68)	−0.21
LDL-cholesterol (mmol/L)	3.31 ± 0.16	3.18 ± 0.18	−0.13	3.27 ± 0.27	3.18 ± 0.23	−0.09
HDL-cholesterol (mmol/L)	0.87 ± 0.05	0.90 ± 0.05	0.03	0.85 ± 0.03	0.88 ± 0.04	0.04

No significant differences were observed on day 1 between the treatment groups. Data are mean ± SEM. The range is given in *parentheses*.

acids, triglycerides, and total cholesterol were larger in the PEG-OB group than in the placebo group. However, when the range of the serum concentrations is considered (Table 3), no significant differences were observed. Although larger reductions in serum concentrations of triglycerides within subjects were observed at the end of the 12-week treatment period in the PEG-OB treatment compared with the placebo treatment group (PEG-OB, delta = −0.35 ± 0.15 mmol/L *vs.* placebo, delta = −0.17 ± 0.12 mmol/L), these differences were not statistically significant. The change in serum tri-glycerides from baseline as a function of body weight loss is shown in Fig. 3. Percent change in serum triglycerides from baseline was significantly correlated with the amount of body weight loss in the PEG-OB treatment group ($P < 0.01$) but not in the placebo treatment group. No significant differences in serum glycerol, LDL-cholesterol, and HDL-cholesterol concentrations from baseline were observed at the end of the 12-week treatment period, and there were no differences between the treatment groups.

Safety

The most common adverse events related to PEG-OB treatment are shown in Table 4. The most significant findings were pain at the injection site and pruritis. These occurred with similar frequency in both the placebo and the PEG-OB groups and generally occurred only with the first or second series of injections.

One subject in the placebo group developed a kidney stone on study day 78. The subject was hospitalized for 2 days, and the kidney stone was passed without intervention. This serious adverse event was classified as unrelated to treatment. The subject completed the study.

Safety data were reviewed as one composite group first and then by treatment group. There were no clinically relevant changes in the mean values of laboratory measurements or vital signs during the study. No difference was detected between the groups with regard to standard chemistry or hematology assessments. The mean total serum protein was reduced (−2.3%) in the PEG-OB group, but not in the placebo group (−0.5%). However, no difference in urinary protein was observed between the groups. In summary, at the dose studied, PEG-OB seemed to be generally well tolerated and safe.

Discussion

These results demonstrate that weekly 20-mg sc PEG-OB administration to obese men on a hypocaloric diet led to sustained serum concentration of both PEG-OB and leptin, measured just before the next dose, throughout the 12-week treatment period. Mean peak serum PEG-OB concentrations were achieved 72 h after dosing, followed by a return to the elevated pre-dose levels after 1 week. Total leptin serum concentrations increased to a new steady-state level in subjects treated with PEG-OB, whereas total leptin concentrations fell with weight loss in placebo-treated subjects. In addition, weekly 20-mg sc PEG-OB was generally well tolerated and safe in these subjects.

No significant differences in the delta or percent weight loss, percent body fat, or sleeping metabolic rate were observed between the PEG-OB and placebo groups. No differences were observed in RQ between the treatment groups. Due to the premature termination of the study based on the interim anal-

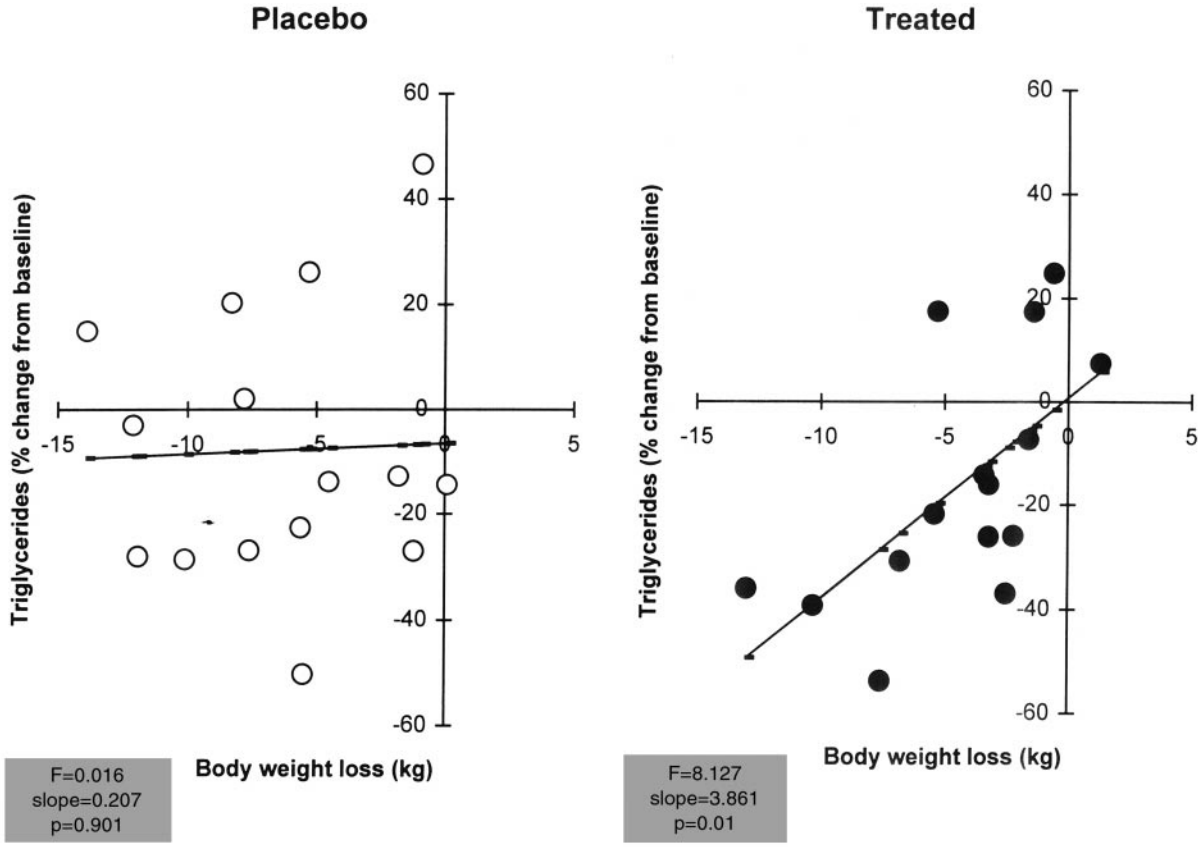

FIG. 3. The percent change in serum triglycerides from baseline following weekly sc PEG-OB dosing as a function of body weight loss at the end of the study. The percent change in serum triglycerides from baseline (day 1) at the end of the 12-week treatment period (day 85) in each subject in the placebo (○) and PEG-OB treatment (●) groups is plotted as a function of the body weight loss. Regression lines were determined in both groups. In the placebo group, the slope was 0.2 with a $P = 0.9$, whereas in the PEG-OB group, the slope was 3.9 with $P = 0.01$. Thus, percent change in serum triglycerides from baseline was significantly correlated with the amount of body weight loss in the PEG-OB treatment group but not in the placebo treatment group. When the data points for all 30 subjects were combined, the slope of the regression line between decrease in triglycerides (expressed as percent change from baseline) in the PEG-OB group was significantly different from the regression line in the placebo group (analysis of covariance; $P < 0.05$).

TABLE 4. Most common adverse events related to PEG-OB treatment

Event	Frequency (no. of subjects reporting)	
	Placebo	PEG-OB
Hematoma at injection site	5/15	3/15
Local irritation at the injection site	1/15	4/15
Pain at injection site	1/15	1/15
Itching or rash	1/15	2/15

ysis, the power of the study was suboptimal and it remains unknown whether PEG-OB has no effect on weight loss.

Percent change in serum triglycerides from baseline was significantly correlated with the amount of body weight loss in the PEG-OB treatment group, but not in the placebo treatment group. Although larger reductions in serum concentrations of triglycerides were observed at the end of the 12-week treatment period in the PEG-OB group compared with the placebo treatment group, these differences were not statistically significant. The trends in serum triglycerides are consistent with similar changes repeatedly observed in studies in which obese rats or mice were treated with leptin at doses that did not reduce body weight (4–6, 32). It has been proposed by Unger *et al.* (33) that an important function of leptin is to confine the storage of triglycerides to the adipocytes, while limiting triglyceride storage in nonadipocytes and to selectively mobilize fat. Together with a small but consistent reduction in hunger ratings after overnight fasts throughout the treatment period and a reduction in generalized hunger as measured by the three-factor eating inventory in the PEG-OB group (34), these trends in serum triglycerides suggest that weekly 20-mg sc treatment with PEG-OB may have biological effects in obese men.

In trials sponsored by Amgen Inc., lean and obese subjects received multiple daily sc injections of met-leptin ranging from 0.01–0.3 mg/kg. Significant dose-related reductions in body fat and body weight were observed following daily sc treatment with up to 0.30 mg/kg met-leptin for 24 weeks (9). Mean weight loss from baseline increased with increasing met-leptin dose in obese subjects at 24 weeks of treatment. The most effective dose for weight and fat loss was 0.3 mg/kg and would be equivalent to an average dose of 30 mg/week in our 100-kg subjects. Mild and moderate injection site reactions were the most common adverse events reported (9).

The treatment of a young very obese girl with a mutated *ob* gene, with daily sc injection of low doses of met-leptin (dose of

0.028 mg/kg of lean mass calculated to cause a circulating concentration ~10% of what would be predicted based on her body fat), has been reported. Daily met-leptin treatment for 12 months caused a dramatic reduction in appetite, food-seeking behavior, food intake, and body weight (10). After 12 months of treatment, her body weight was reduced by 16.4 kg, 95% of which was body fat. As in the studies reported here with PEG-OB, met-leptin treatment had no effect on metabolic rate or energy expenditure in this girl. However, her lipid profile, which was normal prior to treatment, was not effected by met-leptin administration. The results of treatment of this girl with congenital leptin deficiency (10), taken together with the results of the Amgen trials in obese adults (9), indicate that leptin has biological activity in at least some obese humans and that among its activities are reductions in appetite and food intake at low doses and reductions in body fat and body weight at the maximal dose studied.

Other biological effects of leptin and PEG-OB observed in animal studies were not observed in this study. Most prominent among these were serum glucose and insulin concentrations (5, 6). We failed to observe any treatment effects on these variables possibly due to the small sample size or 20-mg PEG-OB dose or weekly dosing schedule. However, neither parameter was elevated in the group of obese men enrolled in this trial. We also failed to observe a reduction in body fat, as well as total energy expenditure and sleeping metabolic rate (5, 6).

The steady-state concentrations of PEG-OB (200–300 ng/mL) following weekly 20-mg sc PEG-OB were lower than the blood levels of met-leptin associated with efficacy (13). Given the similarities in the biological activity to reduce appetite between weekly sc 20 mg PEG-OB (34) and daily low-dose met-leptin in congenital leptin deficiency (10) and the difference in blood levels, it is possible that increasing the dose of PEG-OB or altering the dosing schedule may result in significant reductions in body fat and body weight in addition to larger decreases in appetite, hunger, food intake, and serum triglycerides concentrations. The testing of this hypothesis will be the subject of future research.

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