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Citation for published version (APA):

Hukshorn, C. J., Menheere, P. P. C. A., Westerterp-Plantenga, M. S., & Saris, W. H. M. (2003). The effect of pegylated human recombinant leptin (PEG-OB) on neuroendocrine adaptations to semi-starvation in overweight men. *European Journal of Endocrinology*, 148(6), 649-655.
<https://doi.org/10.1530/eje.0.1480649>

Document status and date:

Published: 01/01/2003

DOI:

[10.1530/eje.0.1480649](https://doi.org/10.1530/eje.0.1480649)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

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CLINICAL STUDY

The effect of pegylated human recombinant leptin (PEG-OB) on neuroendocrine adaptations to semi-starvation in overweight men

Chris J Hukshorn, Paul P C A Menheere¹, Margriet S Westerterp-Plantenga and Wim H M Saris

Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Department of Human Biology, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands and ¹Department of Clinical Chemistry, University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands

(Correspondence should be addressed to C J Hukshorn; Email: c.hukshorn@hb.unimaas.nl)

Abstract

Objective: Starvation induces a complex neuroendocrine response in humans thought to have evolved to defend against reduced energy intake. The drop in leptin levels observed during fasting has been implicated as a factor that triggers this adaptive response. To explore this hypothesis, we executed a randomized, double-blind, placebo-controlled study to investigate whether elevated leptin levels using long-acting pegylated human recombinant leptin (PEG-OB) influenced the neuroendocrine responses to semi-starvation in human subjects.

Design: Twenty-four overweight male subjects (mean \pm s.e.m.: 34.8 \pm 1.3 yrs; 28.8 \pm 0.5 kg/m²) were prescribed a very low energy diet (2.1 MJ/day) to induce a state of semi-starvation for the next 46 days. In addition, all subjects received a weekly treatment of 80 mg PEG-OB or matching placebo. Hormone measurements were performed throughout the study period and included 5-h frequent hormone samplings and 24-h urine collections.

Results: Weekly subcutaneous administration of PEG-OB led to significant additional weight loss (2.8 kg) but it did not reverse the fasting-induced changes in the thyroid, corticotropic, somatotrophic axes and sympathetic nervous system activity. However, after adjustment for weight loss, the drop in mean luteinizing hormone levels was attenuated in the PEG-OB group compared with the placebo group.

Conclusions: These results suggest that a reduced level of leptin accompanying food restriction might be a component of the fasting-induced neuroendocrine inhibition of the human reproductive axis.

European Journal of Endocrinology 148 649–655

Introduction

Starvation or severe energy restriction rapidly evokes several neuroendocrine adaptive responses in humans. These responses are thought to have survival value in periods of inadequate energy intake. Among these neuroendocrine adaptations is a suppression of both the reproductive and thyroid axes while selected components of the corticotropic and somatotrophic axes are stimulated (1, 2). The mechanisms that trigger these adaptive responses to energy restriction are still not clear.

Leptin, the protein hormone encoded by the *ob* gene, is primarily secreted from adipose tissue and plays an important role in body weight homeostasis by regulating food intake and energy expenditure (3–5). Serum leptin concentrations are directly proportional to adipocyte mass and are reduced in humans and animals that have lost body fat, or are energy-restricted (6).

Some investigators have suggested that leptin has a broader physiological role and evolved as a mediator of the adaptations to fasting, and thus promotes the chances of survival during periods of starvation (2, 7). In favor of this hypothesis is the fact that during fasting leptin levels drop rapidly and out of proportion to fat mass changes (8). In addition, leptin administration to rodents attenuates many of these fasting-induced neuroendocrine changes in gonadal, adrenal, and thyroid axes (7, 9). In male non-human primates conflicting reports exist regarding the effect of peripheral infusion of leptin during a short-term fast. Reversal of the inhibitory effects of fasting on gonadotropin release occurred in one study while no effect was found in two others (10–12). In one study, intravenous replacement with homologous leptin did not reverse the acute changes in growth hormone (GH) and cortisol secretion observed with fasting in the adult male macaque (12).

One approach to study whether the neuroendocrine effects of leptin administration to animals during fasting can be extended to humans is to administer exogenous leptin in conditions associated with a (relative) deficiency or absence of leptin, such as during severe energy restriction.

Therefore, we undertook a randomized, double-blind, placebo-controlled study to investigate whether elevated leptin levels using long-acting pegylated recombinant leptin (PEG-OB) were able to attenuate the neuroendocrine adaptations to semi-starvation induced by a very low energy diet (VLED) in healthy overweight male subjects.

Subjects and methods

Subjects

Twenty-four healthy overweight male volunteers were recruited by local advertizing, screened and enrolled in the study after execution of written informed consent. The study was approved by the Medical Ethical Committee of the Maastricht University. Overweight and mildly obese men aged 18–45 yrs and with a body mass index (BMI) between 25 and 32 kg/m² were eligible for inclusion. Additional inclusion criteria were: detailed medical and psychiatric histories and a physical examination with negative findings, including a normal adult sexual maturation, a normal 12-lead resting electrocardiogram, no medical condition present, no use of prescription medication, no smoking and no transmeridian travel in the previous 4 weeks. Each participant had normal biochemical tests of renal, hepatic, metabolic and hematological function, and morning fasting serum concentrations of insulin, cortisol, insulin-like growth factor-1 (IGF-1), thyroid stimulating hormone (TSH), prolactin (PRL), and total testosterone within the normal ranges. Subjects with a history or presence of drug abuse or alcoholism, atopy or hypersensitivity to pegylated proteins and with a weight loss of more than 3 kg in the previous 3 months were excluded from the study.

Study design

This single center trial had a prospective, randomized, double-blind, placebo-controlled group design. After screening, 24 subjects were selected and enrolled in the trial. The study was divided into three phases: a) baseline characterization (day –14, –8 and 1); b) PEG-OB or placebo treatment and a VLED for 46 days (day 1 to 46); and c) follow-up for 2 weeks (day 50 and 57). Baseline characterization consisted of a 5-h frequent blood sampling protocol (day –14) and a 24-h urine collection (day –8) to measure the 24-h excretion of nor-epinephrine (NE) performed in the 2 weeks before the start of the diet and treatment period. All subjects were asked to maintain a constant body weight until

the beginning of the diet and treatment phase and their normal levels of physical activity throughout the study period. Subjects were stratified and matched into pairs according to age, BMI, fasting serum leptin and insulin concentration 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 semi-starvation for the next 46 days. The VLED (Modifast, Novartis, Breda, The Netherlands) provides 2.1 MJ daily and is a protein enriched formula diet (containing 44, 14, and 42% of energy as protein, fat and carbohydrate, respectively). The dietary prescription was discussed every week with a dietician. Adherence to the diet was confirmed by measurements of body weight loss. Treatment consisted of either weekly 80 mg PEG-OB (8 ml, 10 mg ml⁻¹; produced and provided by Hoffmann-La Roche Inc., Nutley, New Jersey) or matching placebo (8 ml) given s.c. in the para-umbilical region during the VLED period. Subjects returned in a fasting state on day 8, 15, 22, 29, 36, and 43 to the laboratory to receive treatment after weight and vital signs (pulse, respiratory rate, and blood pressure) were recorded. Blood samples were drawn for safety laboratory tests and hormone measurements on day 1, 8, 15, 25, and 46. On day 24 the 24-h urine collection and on day 25 the 5-h frequent blood sampling protocol were repeated. After the treatment period (day 46) all subjects received less formula diet and were instructed to supplement this with a free choice of habitual food items in order to gradually increase their energy intake over the next 2 weeks. Vital signs and body weight during this follow-up period were measured on day 50 and 57. Safety was monitored by documentation of adverse events and recording of vital signs at each visit. In addition, urine analysis and routine measurements of serum chemistry and blood cell counts were conducted regularly throughout the study. Standard clinical chemistry and blood cell counts were conducted at the certified central laboratory of the University Hospital Maastricht, The Netherlands.

PEG-OB

Recombinant methionyl human leptin has a reported average terminal half-life of approximately 4 h in humans, which requires daily administration to obtain sustained blood levels (13). Modification of proteins through covalent linkage of polyethylene glycol polymers to the protein has resulted in reduced immunogenicity and increased serum half-life for a number of proteins (14). Recombinant native human leptin, expressed and purified from *Escherichia coli*, was chemically conjugated to a species of branched polyethylene glycols (PEGs) with an average molecular weight 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 (15). Our previous study in obese male subjects clearly showed sustained elevated blood levels following weekly s.c. 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 pre-dose levels after 1 week (16).

Hormone measurements

Venous blood was sampled on day 1, 8, 15, 25, and 46 after an overnight fast and allowed to clot for 20 min at room temperature. Immediately after clotting the samples were put on ice and serum was extracted by centrifugation. The sera obtained were frozen in liquid nitrogen and stored at -80°C for later measurements of serum cortisol, insulin, IGF-1, free 3,5,3'-triiodothyronine (FT₃), and free thyroxine (FT₄). Mean 5-h hormone levels were determined using a 5-h frequent blood sampling protocol. In both the fed (day -14) and the energy restricted (day 25) states, blood sampling was carried out at 10-min intervals for 5 h beginning at 0800 h, at least 1 h after venapuncture. Blood was withdrawn through an indwelling i.v. catheter placed into an antecubital vein and collected with aprotinin (Trasylol, 10 000 Kallikrein inhibiting U/l; Bayer, Etobicoke, Canada). Subjects remained in bed during sampling and daytime naps were not allowed. Serum concentrations of cortisol, GH, luteinizing hormone (LH), follicle stimulating hormone (FSH), TSH, PRL, total testosterone, sex hormone binding protein (SHBG) and albumin were assayed in a single 5-h pool of serum.

Assays

The serum concentrations of cortisol, insulin, IGF-1, FT₃, FT₄, GH, LH, FSH, TSH, PRL, total testosterone, SHBG and albumin were all measured at the certified central laboratory of the University Hospital Maastricht. Serum cortisol was measured with a luminescence enzyme-immunoassay (LEIA), Immulite 200, DPC, Los Angeles, USA), which has a sensitivity of 25 nmol/l and a reproducibility better than 10%. Serum insulin was measured by an immunofluorimetric sandwich assay (IFMA, Autodelphia, Perkin Elmer, Turku, Finland), which has a sensitivity of 1.4 mU/l and a reproducibility better than 6%. Serum IGF-1 was measured by IRMA (Nichols Institute Diagnostics, San Juan Capistrano, USA), which has a sensitivity of 17 ng/l and a reproducibility better than 15%. FT₃ and FT₄ were measured by two-step fluoroimmunoassay (FIA, Autodelphia), which has a sensitivity of 0.04 pmol/l and a reproducibility better than 4% for FT₃ and a sensitivity of 1.3 pmol/l and a

reproducibility better than 4% for FT₄. Serum GH was measured by IFMA, which has a sensitivity of 0.1 mU/l and a reproducibility better than 7%. Serum LH was measured by IFMA, which has a sensitivity of 0.01 U/l and a reproducibility better than 3%. Serum FSH was measured by IFMA, which has a sensitivity of 0.2 U/l and a reproducibility better than 10%. Serum TSH was measured by IFMA, which has a sensitivity of 0.012 mU/l and a reproducibility better than 2%. Serum PRL was measured by IFMA, which has a sensitivity of 0.0005 U/l and a reproducibility better than 6%. Total serum testosterone was measured by radioimmunoassay (DPC), which has a sensitivity of 0.7 nmol/l and a reproducibility better than 10%. Serum SHBG was measured by LEIA, which has a sensitivity of 1 nmol/l and a reproducibility better than 4.5%. Free testosterone was calculated from serum total testosterone, SHBG, and albumin according to Swinkels *et al.* (17, 18) using the equilibrium constants of testosterone to SHBG and albumin to testosterone derived from Dunn *et al.* (19). Urinary NE was determined by HPLC using the ClinRep kit from Recipe (Munich, Germany). Total leptin concentrations (endogenous leptin plus PEG-OB) were measured according to the method described previously (16).

Statistics

Data were analyzed using two-factor repeated-measures ANOVA to compare differences in parameters across the two groups over time. When significant differences were found, a *post hoc* Scheffe's procedure was used to determine the location of the difference. A multiple-linear regression model with the percentage change in 5-h mean hormone concentrations from baseline as the dependent variable and the percentage change in weight and treatment status as independent variables was used to analyze the difference in percentage change in 5-h mean hormone concentrations from baseline between both treatment groups, adjusted for the independent variables. All statistical tests were two-sided and significance was defined as $P < 0.05$. All data are presented as means \pm S.E.M., unless otherwise indicated.

Results

The characteristics of the subjects before the intervention are presented in Table 1. The parameters used to match subjects into pairs were not different and balanced treatment groups were achieved. The baseline hormone levels of the 12 subjects randomized to each treatment group were similar. All subjects in the trial were of Caucasian origin. Two subjects of the placebo group dropped out voluntarily after 1 week because they were not able to maintain the strict VLED

Table 1 Baseline characteristics of the 24 subjects at the screening.

Characteristic	Placebo (n = 12)	PEG-OB (n = 12)
Age (years)	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)
Cortisol (nmol/l)	333±26 (181–478)	349±34 (208–551)
IGF-1 (ng/ml)	166±11 (120–260)	177±11 (122–244)
TSH (mU/l)	1.6±0.1 (0.9–2.5)	1.5±0.2 (0.7–2.5)
PRL (U/l)	0.18±0.02 (0.12–0.30)	0.17±0.02 (0.09–0.31)
Total testosterone (nmol/l)	15.4±1.0 (9.0–21.4)	15.3±0.8 (10.0–21.7)

No statistical significant differences were found between both groups.
Data are presented as means±S.E.M.

regime. However, baseline characteristics of the two groups remained comparable (data not shown). As this study was a part of a greater study, results on body weight, body composition, energy expenditure and appetite have been published elsewhere (20). In summary, at the end of the treatment period (day 46), weight loss was 14.6±0.8 kg in the PEG-OB group, compared with 11.8±0.9 kg in the placebo group ($P = 0.027$). Significant reductions in appetite were observed in the PEG-OB group however.

Table 2 shows the effect of energy restriction and treatment on cortisol, FT₃, FT₄, IGF-1, insulin and total leptin measured on day 1, 8, 15, 25, and 46. In the first 3 weeks of the study cortisol levels increased significantly from initial values in the PEG-OB group. However, the rise of cortisol levels in the placebo failed to reach significance. At the end of the treatment period (day 46) cortisol fell below baseline levels in both groups. FT₃ declined significantly in the first 3 weeks of study in both groups but the fall remained significant in the PEG-OB group on day 46 only. FT₄ levels were increased in the placebo and PEG-OB groups during the whole study, but only on days 8 and 15 was this rise significantly different from the baseline. IGF-1 declined similarly in both groups to levels well below the normal range during the study and reached a minimum on day 25. Insulin levels declined from baseline in both groups and remained significantly reduced throughout the study. Urinary excretion of NE fell from 54.5±3.4 µg/24-h to 42.3±3.5 µg/24-h in the placebo group and from 51.5±4.3 µg/24-h to 44.6±5.4 µg/24-h in the PEG-OB group. No significant differences were observed in levels of cortisol, FT₃, FT₄, IGF-1, insulin and 24-h urinary NE excretion between treatment groups over time.

Food restriction for 6 weeks by means of a VLED reduced circulating leptin levels by 72% to 2.0±0.2 ng/ml in the placebo group ($P < 0.001$). Following weekly s.c. dosing, sustained serum levels of total leptin (endogenous leptin plus PEG-OB), measured just before the next dose on day 8 and 15, ranging from 950 to 3700 ng/ml were observed. Peak

total leptin concentrations on day 25 and 46, measured 72 h after s.c. injection, ranged from 2300 to 6050 ng/ml.

The 5-h mean concentrations of serum cortisol, GH, LH, FSH, TSH, PRL, total testosterone, SHBG, albumin and estimated free testosterone on both study days (days -14 and 25) for the two treatment groups are shown in Table 3. Serum 5-h mean cortisol, TSH, FSH and albumin levels were unchanged by energy restriction and treatment in both groups. The 3 weeks of semi-starvation resulted in a more than 4-fold increase in the mean (5-h) serum GH concentrations and an approximately 2-fold increase in mean SHBG levels in all subjects. In contrast to the group receiving PEG-OB, mean LH and PRL levels decreased significantly in the placebo group. Mean total testosterone levels increased significantly in the PEG-OB treatment group but remained unaltered in the placebo group. Calculated free testosterone concentrations declined significantly in both the placebo and PEG-OB groups. There was no significant difference in the delta or percent change from baseline of 5-h mean concentrations of serum cortisol, GH, LH, FSH, TSH, PRL, total testosterone, SHBG, albumin, and estimated free testosterone between the groups.

A multiple-linear regression model with the percentage change in 5-h mean hormone concentrations from baseline as a dependent variable and the percentage change in weight and treatment status as independent variables was used to analyze the differences between both treatment groups, defined by the binary variable group. The percentage change in 5-h mean LH concentrations of the PEG-OB group, plotted as a function of percentage change in weight, resulted in a positive relationship, i.e. a regression line that was superimposed on the regression line of the placebo group (Fig. 1). The regression line of the PEG-OB group was significantly different from the regression line of the placebo group ($P = 0.03$). The percentage change in 5-h mean LH concentrations adjusted for percentage weight loss resulted in a reduced decrease in percentage change in 5-h mean LH concentrations for the PEG-OB

Table 2 Changes throughout the intervention in endocrine measures.

Day	Cortisol (nmol/l)		Free T ₃ (pmol/l)		Free T ₄ (pmol/l)		IGF-I (ng/ml)		Insulin (mU/l)		Total leptin† (ng/ml)	
	Placebo (n = 10)	PEG-OB (n = 12)	Placebo (n = 10)	PEG-OB (n = 12)	Placebo (n = 10)	PEG-OB (n = 12)	Placebo (n = 10)	PEG-OB (n = 12)	Placebo (n = 10)	PEG-OB (n = 12)	Placebo (n = 10)	PEG-OB (n = 12)
Day 1	303±51	265±27	6.1±0.3	6.0±0.1	12.7±0.5	14.1±0.5	153±11	165±10	9.4±1.0	7.2±0.8	7.1±0.8	7.6±1.2
Day 8	383±29	422±34 ^c	5.0±0.2 ^b	4.8±0.1 ^c	13.9±0.4 ^b	15.4±0.4 ^c	149±9	140±10 ^b	4.5±0.7 ^c	3.4±0.6 ^c	3.4±0.6 ^c	1828±183 ^c
Day 15	354±26	413±27 ^b	5.0±0.2 ^b	5.2±0.3 ^b	14.1±0.4 ^b	15.8±0.7 ^b	129±13	129±9 ^c	5.0±1.1 ^a	3.2±0.5 ^c	3.1±0.5 ^c	2777±194 ^c
Day 25	395±34	393±23 ^c	5.2±0.2 ^b	5.0±0.2 ^b	13.4±0.5	15.0±0.5	121±11 ^b	116±11 ^c	3.4±0.6 ^c	2.2±0.4 ^c	2.1±0.3 ^c	4194±285 ^c
Day 46	245±22	227±18	5.5±0.3	5.4±0.2 ^c	13.0±0.5	14.8±0.7	133±13	128±10 ^c	3.6±0.7 ^c	2.0±0.3 ^c	2.0±0.2 ^c	3980±203 ^c

Data are presented as means±S.E.M.

† Note that the assay did not distinguish between endogenous leptin and PEG-OB. For differences compared to baseline (day 1): ^aP < 0.05, ^bP < 0.01, ^cP < 0.001.

group compared with the placebo group. For the complete study sample, both independent variables accounted for 31% of the variance in mean 5-h serum LH concentrations.

Discussion

We previously reported that exogenous leptin induces additional weight loss in humans under severe energy-restricted conditions by reducing appetite. In the present report, we studied the effect of elevated leptin levels using long-acting PEG-OB on the neuroendocrine responses to severe energy-restriction in human subjects.

Introducing a state of semi-starvation in this study triggered an array of neuroendocrine responses similar to those previously reported (21). Changes in the thyroid (FT₃ and FT₄), corticotropic (cortisol) and somatotrophic axes (GH and IGF-1) paralleled the severity of the energy deficit and were therefore more pronounced (but not significantly different) in the PEG-OB group, which lost significantly more weight. In addition, PEG-OB treatment did not prevent the inhibition of the sympathetic nervous system activity, which normally occurs during energy restriction. The suppression in several reproductive hormones (LH, PRL, and estimated free testosterone with the exception of FSH) tended to be reduced in the PEG-OB treated group but failed to reach significance. However, after adjustment for weight loss using multiple linear regression, the drop in mean LH levels was attenuated in the PEG-OB group compared with the placebo group.

A recent study reported reversal of the effects of sustained weight-reduction on circulating concentrations of thyroid hormones (T₃ and T₄ but not TSH) in four subjects by a low dose of exogenous leptin (22). In contrast, our study failed to demonstrate an effect of leptin administration on the weight-loss decline in FT₃. The findings of this study are in line with the characteristics of two human syndromes associated with absent or very low leptin levels.

Patients with total leptin deficiency or non-functional leptin receptors have been shown to be markedly hyperphagic and the majority of these rare very obese patients identified until now fail to attain normal adult reproductive capabilities (23–25). Daily s.c. recombinant human leptin treatment of a young very obese girl with a mutated *ob* gene caused rapid and progressive reductions in body weight as a result of decreased appetite and food-seeking behavior. Furthermore, after 1 year of treatment with a low dose of leptin, the appearance of pulsatile nocturnal changes in LH was noted, which suggested the onset of early puberty (26).

Four months of leptin replacement in seven female patients with severe lipodystrophy and leptin deficiency improved menstrual abnormalities and low

Table 3 Changes in mean (5-h) serum concentrations (pools) for all subjects.

	Placebo (n = 10)		PEG-OB (n = 12)	
	Day - 14	Day 25	Day - 14	Day 25
Cortisol (nmol/l)	204.2±24.0	240.8±19.4	214.5±17.6	241.8±18.4
GH (mU/l)	0.40±0.12	1.75±0.41 ^b	0.55±0.21	2.17±0.44 ^b
TSH (mU/l)	1.24±0.07	1.09±0.14	1.18±0.14	1.09±0.15
PRL (U/l)	0.121±0.010	0.101±0.013 ^a	0.135±0.013	0.126±0.015
LH (U/l)	3.72±0.75	3.07±0.70 ^a	3.63±0.47	3.44±0.39
FSH (U/l)	4.28±1.45	4.01±1.46	3.71±0.72	3.35±0.48
Total testosterone (nmol/l)	19.45±2.03	19.08±1.26	19.33±1.06	22.17±1.02 ^a
SHBG (nmol/l)	20.2±2.4	38.4±6.3 ^b	20.8±1.8	38.5±2.9 ^c
Albumin (g/l)	40.8±0.6	40.3±0.9	41.3±0.5	40.5±0.7
Free testosterone† (pmol/l)	438.4±32.1	295.7±20.1 ^b	432.2±25.3	345.5±20.5 ^b

Data are presented as means±s.e.m.

† Free testosterone was calculated from plasma total testosterone, SHBG and albumin (see Subjects and methods section).

For differences compared to baseline (day - 14): ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$.

estradiol levels and corrected the blunted LH response to luteinizing hormone-releasing hormone. No clinical or key regulatory effects of treatment were observed on thyroid or adrenocortical function (27).

Taken together, our results seem to support the hypothesis that the reduction in leptin is one of the factors that may be responsible for the fasting-induced inhibition of the reproductive axis and subsequent impaired fertility in humans (28, 29).

Several limitations of our study are of note that could account for the limited effect of leptin observed in this study. The lowest leptin levels reached in the placebo group in this study are still higher than the leptin levels found in the syndromes mentioned above. Furthermore, reproductive function is known to continue in males under limited energy availability in which it is completely blocked in females, suggesting that female reproduction is more sensitive to

nutritional deprivation (30, 31). Both observations could account for the limited effect of leptin therapy on reproductive hormones found in this study with severe energy-restricted overweight males. Also the serum leptin levels achieved in this pharmacological study are supraphysiological. Recent studies suggest that the threshold for the various responses to leptin is set at different levels. Some evidence suggests that especially the effects on neuroendocrine function elicited by hyperleptinemia might differ from those caused by leptin in the physiological range (32, 33). Finally, more subtle effects on these dynamic neuroendocrine axes might have been revealed by direct measurements of diurnal variations or the pulsatility of secretory patterns.

In conclusion, we demonstrated that although weekly 80 mg s.c. PEG-OB administration to severe energy-restricted healthy overweight men led to significant additional weight loss after 6 weeks, it did not reverse the fasting-induced changes in the thyroid, corticotropic, somatotrophic axes, and sympathetic nervous system activity. However, the drop in mean LH levels when adjusted for weight loss was attenuated in the PEG-OB group compared with the placebo group. This result suggests that a reduced level of leptin accompanying food restriction might be a component of the fasting-induced neuroendocrine inhibition of the human reproductive axis.

Acknowledgements

We thank Hoffmann-La Roche Inc. for kindly providing PEG-OB. We also wish to express our sincere appreciation to Inge van Ierssel, Marja van der Hulst and Fiona Ong for their commitment and assistance during this study. Finally, we greatly acknowledge the co-operation, patience, and contributions of all our subjects.

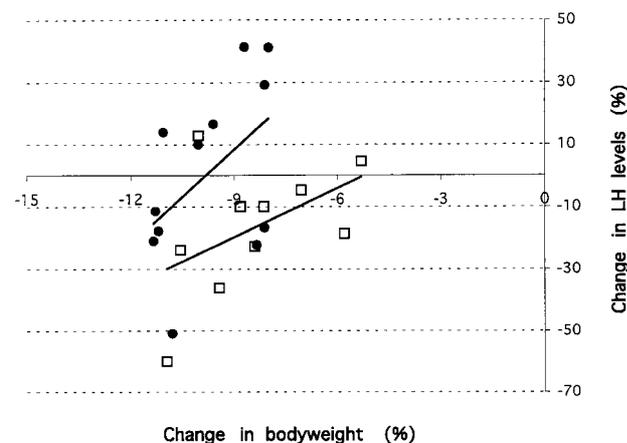


Figure 1 Percentage change in 5-h mean LH concentrations as a function of percentage change in weight plotted for the PEG-OB group (●) and placebo group (□). The regression line of the PEG-OB group is significantly different from the regression line of the placebo group (Multiple-linear-regression analysis: $P = 0.03$).

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Received 23 October 2002

Accepted 10 March 2003