

SERUM IMMUNOREACTIVE-LEPTIN CONCENTRATIONS IN NORMAL-WEIGHT AND OBESE HUMANS

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Abstract Background. Leptin, the product of the *ob* gene, is a hormone secreted by adipocytes. Animals with mutations in the *ob* gene are obese and lose weight when given leptin, but little is known about the physiologic actions of leptin in humans.

Methods. Using a newly developed radioimmunoassay, we measured serum concentrations of leptin in 136 normal-weight subjects and 139 obese subjects (body-mass index, ≥ 27.3 for men and ≥ 27.8 for women; the body-mass index was defined as the weight in kilograms divided by the square of the height in meters). The measurements were repeated in seven obese subjects after weight loss and during maintenance of the lower weight. The *ob* messenger RNA (mRNA) content of adipocytes was determined in 27 normal-weight and 27 obese subjects.

Results. The mean (\pm SD) serum leptin concentrations were 31.3 ± 24.1 ng per milliliter in the obese sub-

jects and 7.5 ± 9.3 ng per milliliter in the normal-weight subjects ($P < 0.001$). There was a strong positive correlation between serum leptin concentrations and the percentage of body fat ($r = 0.85$, $P < 0.001$). The *ob* mRNA content of adipocytes was about twice as high in the obese subjects as in the normal-weight subjects ($P = 0.005$) and was correlated with the percentage of body fat ($r = 0.68$, $P < 0.001$) in the 54 subjects in whom it was measured. In the seven obese subjects studied after weight loss, both serum leptin concentrations and *ob* mRNA content of adipocytes declined, but these measures increased again during the maintenance of the lower weight.

Conclusions. Serum leptin concentrations are correlated with the percentage of body fat, suggesting that most obese persons are insensitive to endogenous leptin production. (N Engl J Med 1996;334:292-5.)

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THE *ob* gene is an adipocyte-specific gene that encodes leptin, a protein that regulates body weight.¹ In mice, mutations in the *ob* gene that result in a lack of circulating leptin cause obesity. The administration of recombinant leptin causes weight loss in these mice.²⁻⁴

We have reported the complete coding sequence of human *ob* complementary DNA (cDNA),⁵ a finding recently confirmed by others.⁶ We did not detect any mutations in the gene in five obese subjects. In eight normal-weight and eight obese subjects the amount of *ob* messenger RNA (mRNA) in adipocytes was correlated with body weight. An increase in expression of the *ob* gene in obese subjects has since been reported by other investigators.^{7,8} These results suggest that the *ob* gene encodes a protein that informs the brain of the amount of adipose tissue present in the body.

In this study we investigated whether leptin can be detected in serum at concentrations that correlate with body weight and whether serum leptin concentrations are reduced by weight loss.

METHODS

Subjects

We studied 136 lean subjects (84 women and 52 men; mean [\pm SD] age, 29 ± 7 years) and 139 obese subjects (99 women and 40 men;

mean age, 37 ± 11 years). The mean body-mass index (BMI), defined as the weight in kilograms divided by the square of the height in meters, was 23.0 ± 2.5 for the normal-weight subjects and 35.1 ± 7.2 for the obese subjects. Obesity was defined as a BMI ≥ 27.3 for men and ≥ 27.8 for women, which is approximately 120 percent of ideal body weight.⁹ None of the subjects were taking any medication or had any evidence of metabolic disease other than obesity, and all reported that their body weight had been stable for at least three months before the study. A blood sample was collected from each subject while fasting, and the serum was frozen at -80°C until analysis.

We performed biopsies of abdominal subcutaneous adipose tissue¹⁰ in 27 of the lean subjects (15 women and 12 men) and 27 of the obese subjects (17 women and 10 men). The tissue samples were transported in saline to the laboratory, where they were immediately digested with collagenase and the cells isolated.¹⁰

To study the effect of weight loss, seven of the obese subjects (six women and one man; BMI, 40.4 ± 5.2 ; age, 37 ± 13 years) were fed a liquid-protein diet providing 800 kcal per day (Optifast 800, Sandoz Nutrition, Minneapolis). In addition to the base-line studies described above, blood was drawn and biopsies performed when the subjects had reduced their body weight by 10 percent and again after they had maintained the lower body weight for four weeks.

The effect of food consumption on serum leptin concentrations was studied in a separate group of four normal-weight subjects (two women and two men; BMI, 24.3 ± 2.6 ; age, 40 ± 8 years) and three obese women (BMI, 32.2 ± 2.7 ; age, 43 ± 4 years). After an overnight fast, blood samples were drawn every 60 minutes for 8 hours. Breakfast (total energy, 848 kcal; protein, 14 percent; carbohydrate, 52 percent; and fat, 34 percent) was given after the fasting sample was collected, and lunch (total energy, 902 kcal; protein, 13 percent; carbohydrate, 52 percent; and fat, 35 percent) was given four hours later.

All protocols were approved by the institutional review board at Thomas Jefferson University, and all the subjects gave informed consent.

Radioimmunoassay for Serum Leptin

Antihuman leptin antiserum was raised in rabbits immunized with recombinant leptin.¹¹ This antiserum did not cross-react with insulin, insulin-like growth factor 1, or glucagon in doses of $10 \mu\text{g}$ per milliliter. The leptin was radiolabeled with iodine 125 by the Bolton-Hunter

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method¹² and purified by gel filtration using Sephadex G-25 (Pharmacia Biotech, Piscataway, N.J.). The specific activity was about 30 μCi per microgram. Unlabeled and ¹²⁵I-labeled leptin was stable for at least 30 days at 4°C.¹¹

In the leptin radioimmunoassay, recombinant leptin in charcoal-treated serum or 200 μl of test serum (in duplicate) was incubated in phosphate-buffered saline (pH 7.4) containing 0.1 percent Triton X-100 with antileptin serum (at a dilution of 1:2000) for 16 hours at 4°C in a total volume of 400 μl . ¹²⁵I-labeled leptin (about 30,000 counts per minute in 100 μl) was then added, and the incubation continued for an additional 24 hours. Antiserum-bound ¹²⁵I-labeled leptin was precipitated by the addition of 100 μl of sheep antirabbit IgG serum (Antibodies Inc., Davis, Calif.), 100 μl of normal rabbit serum (GIBCO BRL, Gaithersburg, Md.), and 100 μl of 10 percent polyethylene glycol. The tubes were centrifuged for 15 minutes at 2200 revolutions per minute, after which the supernatant was decanted and the pellet counted in a Packard 5000 gamma counter (Packard, Downers Grove, Ill.). In the absence of unlabeled leptin, the antiserum dilution used precipitated 12 \pm 1 percent of the ¹²⁵I-labeled leptin added; 1.4 \pm 0.1 percent was precipitated in the absence of antiserum. The limit of detection was 0.4 ng per milliliter, the intraassay standard coefficient of variation was 11.6 percent, and the interassay coefficient of variation was 20.8 percent. Serum values that were undetectable were assigned a value of 0.4 ng per milliliter for purposes of analysis.

Reverse-Transcriptase Polymerase Chain Reaction

Total adipocyte RNA was obtained by guanidinium thiocyanate-phenol-chloroform extraction.¹³ A reverse-transcriptase polymerase chain reaction¹⁴ was performed with a thermocycler (model 9600, Perkin-Elmer, Foster City, Calif.) with a final primer concentration of 10 pmol per 100- μl reaction, as described previously.⁵ The data are expressed as the ratio of *ob* cDNA to actin cDNA. There was no difference in the amount of actin cDNA among the subjects studied.

Other Analyses

Serum insulin was measured by radioimmunoassay (Linco Research, St. Charles, Mo.). Serum glucose was measured by the glucose oxidase method with a glucose analyzer 2 (Beckman, Brea, Calif.). The percentage of body fat was determined for 108 normal-weight and 71 obese subjects by bioelectric impedance analysis (RJL Systems, Mt. Clemens, Mich.).

Statistical Analysis

The results in the normal-weight and obese subjects were compared by means of t-tests. Because of extreme values in the distributions of serum leptin concentrations and percentages of body fat, the relations between continuous variables were evaluated by Spearman correlations. The Wilcoxon rank-sum test was used to evaluate differences in serum leptin concentrations and other measures according to sex and race. Three regression models were fitted to determine the relation between the serum leptin concentration and the percentage of body fat. The models included a simple linear relation of the percentage of body fat with the log of the serum leptin concentration, the log of the percentage of body fat with the log of the serum leptin concentration, and a quadratic model (percentage of body fat and the square of the percentage of body fat). A similar set of regression models was developed for the relation between expression of the *ob* gene and the percentage of body fat. Finally, multiple regression analysis with use of the quadratic model was performed to evaluate the relation of other variables to the serum leptin concentration, after control for the percentage of body fat. All analyses were two-tailed and conducted with SAS software (version 6.10 for Windows; SAS Institute, Cary, N.C.). No adjustments were made for multiple testing.

RESULTS

The mean serum leptin concentration in the 139 obese subjects was 31.3 \pm 24.1 ng per milliliter, as compared with 7.5 \pm 9.3 ng per milliliter in the normal-weight subjects ($P<0.001$). Seven percent of the latter group but none of the former had undetectable serum leptin con-

centrations. The lowest serum leptin concentration detected in an obese subject was 1.7 ng per milliliter. Body-composition analysis was performed in 108 of the normal-weight subjects (64 women and 44 men) and 71 of the obese subjects (47 women and 24 men). There was a strong positive correlation ($r=0.85$, $P<0.001$) between the serum leptin concentration and the percentage of body fat (Fig. 1). Serum leptin concentrations were also correlated with the BMI ($r=0.66$, $P<0.001$), fasting serum insulin concentration ($r=0.57$, $P<0.001$), and age ($r=0.26$, $P<0.001$). Serum leptin concentrations were significantly higher in normal-weight women than in normal-weight men and in obese women than in obese men when the groups were defined by BMI. However, when women and men with equivalent percentages of body fat were compared, there was no difference between the sexes.

A regression model to evaluate the relation between the serum leptin concentration and the percentage of body fat was developed. Of the models tested, the quadratic model ($r^2=0.72$) provided the best fit. There was no improvement in the fit of the model with the addition of the BMI, age, fasting serum insulin or glucose concentration, sex, or race. These factors therefore had no independent effect on the serum leptin concentration after we controlled for the percentage of body fat.

Ob mRNA in abdominal subcutaneous adipocytes was measured in 54 subjects. The *ob* mRNA content in the 27 obese subjects was about twice as high as in the 27 normal-weight subjects (29.0 \pm 8.7 vs. 18.8 \pm 10.9 relative units, $P=0.005$). Like the serum leptin concentration, the *ob* mRNA content of the adipocytes was correlated with the percentage of body fat ($r=0.68$,

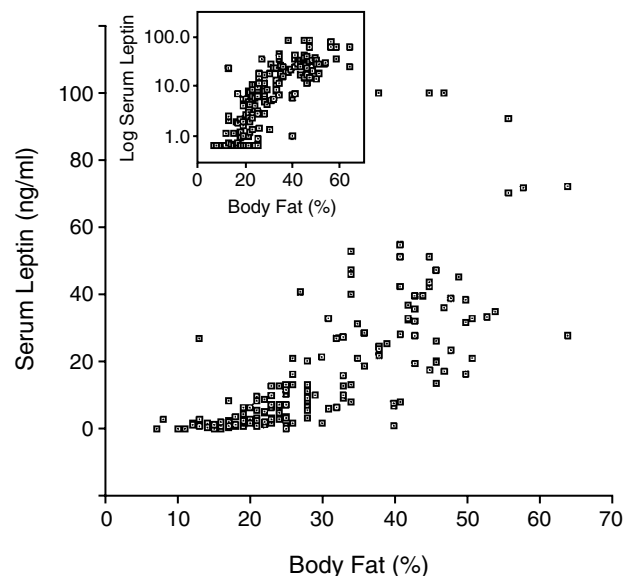


Figure 1. The Relation between the Percentage of Body Fat and the Serum Leptin Concentration in 136 Normal-Weight and 139 Obese Subjects.

The inset shows the natural log of the serum leptin concentration plotted against the percentage of body fat.

$P < 0.001$) (Fig. 2), BMI ($r = 0.70$, $P < 0.001$), and age ($r = 0.38$, $P = 0.01$). However, after we controlled for the percentage of body fat there was no independent effect of BMI, age, or sex in the analysis with the quadratic model.

Among the seven obese subjects who were fed 800 kcal daily, four lost 10 percent of their initial weight in 8 weeks and three in 12 weeks (Fig. 3). After weight reduction, these subjects' fasting serum insulin concentrations decreased significantly, but their fasting serum glucose concentrations did not change markedly (Table 1). The mean serum leptin concentration decreased by 53 percent and the *ob* mRNA content of adipocytes decreased by 38 percent during the same period (Fig. 3). During the subsequent four-week period of weight maintenance, the mean serum leptin concentration increased again slightly and the *ob* mRNA content rose, but not to values significantly different from those before weight loss.

Serum leptin concentrations did not change significantly in the seven subjects studied before and after two meals. Serum insulin and glucose concentrations increased transiently after each of the meals.

DISCUSSION

We found that leptin, the protein product of the *ob* gene, is detectable in serum and that obese subjects have higher serum leptin concentrations than normal-weight subjects. Although several factors may contribute to the elevation of serum leptin concentrations in obesity, the values were most closely correlated with the percentage of body fat. The accuracy of body-fat determination by bioelectric impedance analysis is limited,¹⁵ but the strength of the correlation ($r = 0.85$, $P < 0.001$) is much greater than that for any other variable tested. It therefore appears that, in humans, serum leptin

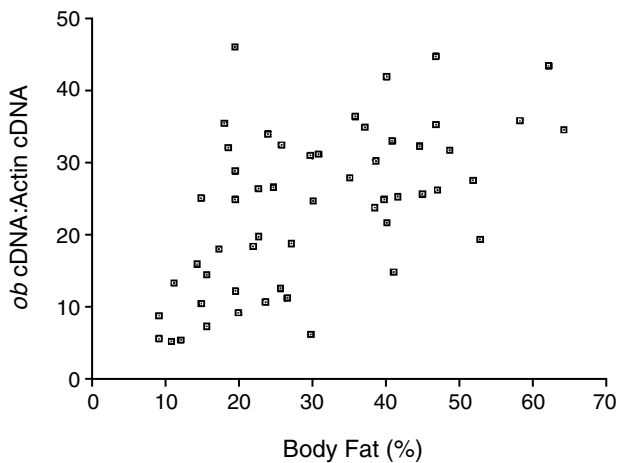


Figure 2. Correlation between Expression of the *ob* Gene in Adipocytes and the Percentage of Body Fat in 27 Normal-Weight and 27 Obese Subjects.

The data are expressed as the ratio of *ob* cDNA to actin cDNA. There was no difference in the amount of actin cDNA among the subjects studied.

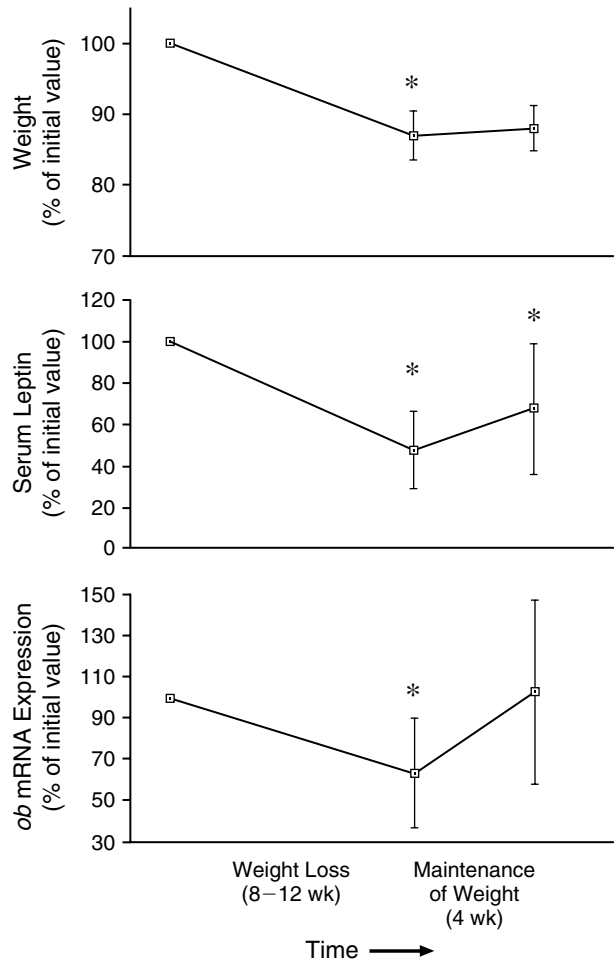


Figure 3. Effect of Weight Loss on Serum Leptin Concentrations and Expression of the *ob* Gene in Seven Obese Subjects, Expressed as a Percentage of the Initial Value.

A 10 percent reduction in body weight was achieved in seven obese subjects. The reduced body weight was then maintained for four weeks. The values shown are means \pm SD. The initial values were as follows: serum leptin, 50.2 ± 9.8 ng per milliliter; *ob* mRNA, 44.6 ± 9.4 relative units; and weight, 111.4 ± 13.9 kg. The asterisk denotes $P = 0.05$ for the comparison with the initial value.

concentrations reflect the amount of adipose tissue in the body.

The mechanism by which the increase in body fat is translated into an increase in serum leptin appears to involve induction of the *ob* gene. We found a significantly greater amount of *ob* mRNA in adipocytes from obese subjects than in those from normal-weight subjects. The fact that in obese subjects serum leptin increases significantly more (to four times the initial value) than does *ob* mRNA (to twice the initial value) suggests that hypertrophy of adipocytes leads to an increase in leptin production by individual cells, to approximately twice the initial value. Recent studies in humans^{7,8} and rodents¹⁶⁻¹⁸ support the concept that serum leptin concentrations are regulated by direct changes in the expression of the *ob* gene. It appears therefore that chang-

Table 1. Body-Mass Index and Fasting Serum Biochemical Values in Seven Obese Subjects before and after Weight Loss.

VARIABLE*	BEFORE WEIGHT LOSS	DURING WEIGHT LOSS	DURING MAINTENANCE OF WEIGHT
BMI	40.4±5.2	35.1±4.5†	34.7±4.5†
Fasting serum insulin (μU/ml)	16.1±4.5	11.9±5.0†	11.6±3.9†
Fasting serum glucose (mg/dl)	84±7	82±6	85±8
Serum triglyceride (mg/dl)	158±78	85±18†	98±32†
Serum cholesterol (mg/dl)	189±41	166±41†	175±23

*The body-mass index (BMI) was defined as the weight in kilograms divided by the square of the height in meters. Plus-minus values are means ±SD. To convert insulin values to picomoles per liter, multiply by 6.0; to convert glucose values to millimoles per liter, multiply by 0.056; to convert triglyceride values to millimoles per liter, multiply by 0.011; and to convert cholesterol values to millimoles per liter, multiply by 0.0256.

†P=0.05 for the comparison with the value before weight loss.

es in body fat are translated into changes in serum leptin at the level of *ob* gene expression.

We found that a reduction of 10 percent in body weight was associated with a reduction of 53 percent in serum leptin, but that serum leptin concentrations increased slightly during the maintenance period, during which body weight did not change. The large fluctuations in serum leptin concentrations in the presence of relatively small changes in body weight suggest that leptin secretion is regulated by other factors in addition to the size of the adipose-tissue depot. One of these factors may be caloric intake. While eating 800 kcal a day, the subjects were in negative caloric balance, which could be a signal to the body to reduce leptin production so that appetite would not be inhibited. During the maintenance period, food intake was increased to maintain the lower body weight. Energy expenditure probably also decreased during the maintenance period,¹⁹ aiding in the restoration of caloric balance and thus allowing serum leptin concentrations to increase again.

Several potential signals could mediate the reduction in serum leptin concentrations in response to caloric restriction. Fasting serum insulin concentrations decreased during weight loss, but the postprandial rise in serum insulin during the period of frequent sampling was not associated with any change in serum leptin concentrations. The feeding experiment does not rule out the possibility that long-term changes in insulin secretion alter serum leptin concentrations.

The significant correlation between the serum leptin concentration and the percentage of body fat suggests that adipocytes are signaling the brain about the size of the adipose-tissue depot. If the action of leptin in humans is similar to that in rodents,²⁻⁴ appetite should decrease and energy expenditure should increase, which together should result in weight loss. The finding of increased serum leptin concentrations in obese subjects suggests decreased sensitivity to leptin, although the detection of leptin by immunologic methods does not

prove that it is biologically active. No functional and structural abnormalities of the leptin-effector system in humans are currently known. However, diet-induced obesity in normal mice is an example of decreased sensitivity to leptin, because larger doses of leptin were required to induce weight loss in these mice than in leptin-deficient mice.⁴ The *db/db* mouse provides an example of unresponsiveness to leptin.^{3,4}

In summary, leptin, the protein product of the *ob* gene, is detectable in serum; its concentration is correlated with the percentage of body fat and is elevated in obese subjects. These results suggest that obesity in humans is more likely to be due to central mechanisms regulating food intake and energy expenditure than to defective signaling by adipocytes to these central mechanisms.

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