Relationship of Plasma Leptin to Plasma Insulin and Adiposity in Normal Weight and Overweight Women: Effects of Dietary Fat Content and Sustained Weight Loss*

PETER J. HAVEL†, SIDIKA KASIM-KARAKAS, WENDY MUELLER, PATRICIA R. JOHNSON, RONALD L. GINGERICH‡, AND JUDITH S. STERN

Department of Nutrition (P.J.H., W.M., P.R.J., J.S.S.); Department of Internal Medicine, Divisions of Endocrinology and Metabolism (S.K.K., W.M.); and Clinical Nutrition (J.S.S.), School of Medicine; and Department of Anatomy, Physiology, and Cell Biology (P.J.H.), School of Veterinary Medicine, University of California, Davis, California 95616; and Linco Research Incorporated (R.L.G.), St. Charles, Missouri 63304

ABSTRACT

Leptin, the product of the human homologue of the ob gene, which is defective in the obese (ob/ob) mouse, may be a humoral regulator of human adiposity. Plasma leptin concentrations were measured by RIA in 19 normal weight (body mass index (BMI) = 24.5 ± 0.6 kg/m²) and 19 overweight to obese (BMI = 34.7 ± 1.2 kg/m²) nondiabetic postmenopausal women on sequential controlled weight-maintaining diets containing 31%, 23%, and 14% of energy as fat, each for 4–6 weeks. Thereafter, the subjects ate a very low fat diet (<15%) ad libitum; plasma leptin and insulin concentrations, BMI, percent body fat (%BF), and resting energy expenditure were determined after 6 and 8 months. Absolute and adiposity-corrected plasma leptin levels were higher in overweight/obese women (37.7 ± 3.5 ng/mL; 1.01 ± 0.07 ng/mL %BF⁻¹) than in normal weight women (16.9 ± 2.2 ng/mL; 0.57 ± 0.06 ng/mL %BF⁻¹, both P < 0.005 vs. obese), but were not different between the 31%, 23%, and 14% fat diets when body weight was stable. Plasma leptin was highly correlated with BMI (r = 0.81, P < 0.0001), %BF (r = 0.80, P < 0.0001), and fasting plasma insulin (r = 0.01, P < 0.0001). After 8 months on the ad libitum low fat diet, the women had lost an average of 6.9 ± 1.0% of body mass (-9.0 ± 0.3 kg/m², P < 0.0001). In 15 subjects who lost more than 7% of body mass (-12.3 ± 1.0%), plasma leptin concentrations decreased (-9.6 ± 1.9 ng/mL, P < 0.0005), and the decrease of plasma leptin per change of adiposity (Aleptin/A%BF) was greater in overweight/obese women (3.6 ± 0.5) than in normal weight women (0.9 ± 0.4, P < 0.01 vs. obese). In 18 other subjects who lost less than 7% of body mass (-2.7 ± 0.6%), plasma leptin was unchanged (+1.4 ± 1.4 ng/mL). Overall, the change of plasma leptin was significantly correlated with change of BMI (r = 0.43, P < 0.02), the change of %BF (r = 0.49, P < 0.005), the change of resting energy expenditure (r = 0.40, P < 0.02), and with the change of plasma insulin independently of changes of body adiposity (r = 0.45, P < 0.01). We conclude that plasma leptin concentrations are: 1) not affected by dietary fat content per se; 2) highly correlated with BMI, %BF, and plasma insulin in both overweight/obese and normal weight women; 3) decreased in parallel with plasma insulin after sustained weight loss; and 4) decreased more in overweight/obese than in normal weight women. (J Clin Endocrinol Metab 81: 4406-4413, 1996)
whereas ob gene expression (16–19, 24) and plasma leptin (26) were decreased in fasted vs. fed animals. Although no short-term effects of meal ingestion on plasma leptin levels in humans have been noted (22, 23), diet composition could be a significant factor in the regulation of plasma leptin, especially when low fat diets are used to achieve weight loss. Plasma leptin levels and adipose ob gene expression decrease with short-term weight loss on low fat diets in obese individuals (22, 23), although normal weight subjects were not examined, and in one study the decreases of plasma leptin after weight loss appeared to be transient (23). In the present study, with the use of a new sensitive and specific RIA for human leptin, we investigated the relationships between plasma leptin concentrations and body mass index (BMI), percent body fat, and plasma insulin in both normal weight and overweight to obese women when body weight was held constant on weight-maintaining diets containing 31%, 23%, and 14% of energy as fat, and again after 6 and 8 months on an ad libitum very low fat diet (<15% fat) during which a subset of the subjects achieved and maintained substantial reductions of body weight.

Subjects and Methods

Subjects

Nineteen normal weight (BMI < 27.8 kg/m²) and 19 overweight to obese (BMI > 27.8 kg/m²) women participated in the study. All participants were screened and shown to be postmenopausal defined as without menstruation for >12 months, removal of both ovaries, or FSH > 50 mIU/mL. Before being admitted to the study, subjects were screened and found to be free of diabetes mellitus, fasting hyperlipidemia (total serum cholesterol < 300 mg/dL and triglycerides < 300 mg/dL), renal, or hepatic disease by medical history, physical examination, and serum chemistry. Seventeen out of the 38 women (10 normal weight and 7 overweight/obese) were on estrogen or estrogen/progesterone replacement therapy. These women were all either on a stable continuous menstrual therapy. All subjects gave informed consent and the experimental protocols were approved by the Institutional Human Subjects Review Committee of University of California, Davis. Characteristics of the participants are shown in Table 1.

Weight-maintaining diets with variable fat content

To determine the effects of dietary fat content independent of changes of body mass on plasma leptin concentrations, all subjects were fed weight-maintaining diets at the University of California, Davis Medical Center during the first 4 months of the study. Menus were prepared by calculating the nutrient compositions of the diets with a food database system (Nutrition Data System, NDS93, University of Minnesota). The diets consisted of whole foods weighed to ±1 g. For the first 4 weeks, the diets consisted of 31% of energy from fat, 17% from protein, and 52% from carbohydrate. The following 6 weeks, the diets consisted of 23% of energy from fat, 17% from protein, and 60% from carbohydrate. During the last 6 weeks, the experimental diets consisted of 14% of energy from fat, 19% from protein, and 67% from carbohydrate. To ensure accuracy of the diets, the energy, fat, protein, and carbohydrate contents of the diets were verified by Hazleton Laboratories (Madison, WI).

All subjects ate their weekday evening meals at the facility. Breakfast, lunch, and weekend meals were prepared and taken home by the participants. Participants were given detailed instructions on the importance of consuming all the food provided and kept daily records of any discrepancies. Compliance was closely monitored and noncompliant subjects (>1% of calories altered from the experimental diet on more than one occasion) were excluded from the study. Subjects were instructed not to change their activity level during the experimental diet portion of the study.

Initial energy intake was individualized and based on the subjects resting energy expenditure (REE) measured by indirect calorimetry (27, 28) with a factor included for activity level (estimated by an activity questionnaire), and in accord with each subject’s BMI and body composition. Subjects were weighed five times each week, and the energy content of the diets was adjusted by increasing or decreasing daily caloric intake by 150 kcal when body weight varied by >1 kg to maintain body weight. The average daily caloric intake on the 31% fat diet was 2050 ± 60 kcal/day and was increased by approximately 6% to 2170 ± 70 kcal/day by the end of the 14% fat diet (Δ = 120 ± 30 kcal/day, P < 0.0005 vs. 31% fat diet).

Self-regulated low fat diet

To examine the effects of sustained weight loss on plasma leptin concentrations, after 4 months on the weight-maintaining experimental diets, the participants switched to a self-selected (ad libitum) very low fat diet for the remaining 8 months of the study. Subjects were given detailed instructions and support to adhere to a diet with <15% of energy as fat through a series of group classes and individual counseling sessions with a registered dietitian. Of the initial 38 women, 33 completed the entire study (16 normal weight and 17 overweight/obese). The mean dietary fat content on the low fat diet (assessed by 7-day food records analyzed by NDS93) after 6 and 8 months on the ad libitum low fat diet was 12 ± 1% and 11 ± 1%, respectively. The average weight loss after 8 months was 6.9 ± 1.0%. For analysis, subjects were divided into two groups; 18 women who lost less than 7% of body mass on the low fat diet, and 15 women who lost more than 7% of body mass. Dietary fat content was not significantly different between those who lost <7% (10 ± 1%) and those who lost >7% (12 ± 1%).

Data collection

Body weights were measured, and fasting blood samples for analysis of plasma leptin, insulin, and glucose concentrations were obtained 2 weeks after the start of the 31% fat diet, at 4 weeks after the start of the 23% fat and 14% fat diets, and after 6 and 8 months on the self-selected low fat diets (10 and 12 months after the start of the study). Percent body fat was measured by bioelectrical impedance (Bio-Analogics, Beaverton, OR) during the 31%, 23%, and 14% fat experimental diets, and again at 12 months. Measurements of percent body fat made with this instrument were very consistent within subjects (r = 0.9662, P < 0.0001). The coefficient of variation of the percent body fat measurements within subjects averaged 4.1 ± 0.5%. REE was measured at stable body weight and again at 12 months (27, 28).

Assays and data analysis

Plasma glucose concentration was measured by the glucose oxidase technique with a kit (Glucose Trinder, Sigma, St. Louis, MO). Immunoreactive plasma human leptin concentrations were determined by RIA (29) with reagents supplied by Linco Research, St. Charles, MO. The range of the standard curve in this assay is 0.5–100 ng/mL. The intra- and interassay coefficients of variation are <8%. The antibody used in the assay does not cross-react with human insulin, proinsulin, glucagon, pancreatic polypeptide, or somatostatin. Plasma insulin concentration was measured with a specific RIA for human insulin in plasma using an antibody that does not cross-react with proinsulin (Linco Research).
Data are expressed as means ± SEM. Statistical comparison of means within a group were made with a paired t test. Comparison of means of two different groups were made with a two-sample t test. Correlation coefficients and significance values between absolute values or changes of BMI, percent body fat, plasma leptin, insulin, and glucose were calculated by linear regression analysis. Partial regression analysis was performed with PC SAS (SAS Institute, Inc., Cary, NC) to examine the relationship between absolute or changes of plasma insulin or REE and leptin concentrations, independent of BMI or percent body fat.

Results

**BMI, percent body fat, plasma leptin, insulin, and glucose concentrations in normal weight and overweight/obese women on weight-maintaining diets**

BMI was slightly (~1%) but significantly decreased on the 14% fat diet, despite the caloric adjustments. Percent body fat was not different across the three weight-maintaining diets. Plasma insulin concentrations were doubled in obese (108 ± 12 pmol/L) vs. lean (48 ± 6 mmol/L) and were unaffected by dietary fat content. Plasma glucose was not significantly different between overweight/obese and normal weight women and decreased slightly during the 14% fat diet overall (from 5.6 ± 0.1 to 5.5 ± 0.1 mmol/L) and in the overweight/obese group (from 5.7 ± 0.2 to 5.5 ± 0.1 mmol/L). Plasma leptin concentrations were more than two times higher in overweight/obese than in normal weight subjects and were not affected by varying the dietary fat content (Fig. 3). Plasma leptin concentrations within subjects were remarkably stable across the three diets (r = 0.9079-0.9587, all P < 0.0001).

**Correlations between BMI, percent body fat, plasma leptin, insulin and glucose concentrations at stable body weight**

As expected, BMI was highly correlated with percent body fat overall (r = 0.85, P < 0.0001, Fig. 1A), and within both the normal weight (r = 0.88, P < 0.0001) and the overweight/obese groups (r = 0.65, P < 0.005). Plasma leptin was highly correlated with BMI overall (r = 0.81, P < 0.0001, Fig. 1B) and within both the normal weight (r = 0.58, P < 0.001) and overweight/obese groups (r = 0.59, P < 0.001). Percent body fat was similarly well correlated with plasma leptin overall (r = 0.80, P < 0.0001, Fig. 1C) in normal weight (r = 0.64, P < 0.005) and in overweight/obese subjects (r = 0.69, P < 0.002).

Fasting plasma insulin concentrations were well correlated with BMI (r = 0.70, P < 0.0001, Fig. 2A) and with percent body fat (r = 0.58, P < 0.0002, Fig. 2B). Plasma leptin concentrations were significantly correlated with fasting plasma insulin (r = 0.61, P < 0.0001, Fig. 2C). However, by partial regression analysis, plasma insulin was not significantly correlated with plasma leptin independently of BMI or percent body fat. BMI, percent body fat, plasma leptin, and plasma insulin were not correlated with fasting plasma glucose levels (data not shown).

**Effects of sustained weight loss on body fat, plasma leptin, and plasma insulin concentrations in normal weight and overweight/obese women**

Thirty-three of the original 38 subjects completed the entire study. After 6 months on the self-selected low fat diet (month 10 of the study), the participants lost an average 4.0 ± 0.5 kg (ΔBMI = -1.6 ± 0.2 kg/m², P < 0.0001, Table 3). After 8 months on the self-selected low fat diet (month 12 of the study), the average weight loss was sustained (Δ = -5.0 ± 0.7 kg, %ΔBMI = -6.9 ± 1.0, P < 0.0001). Subjects were divided into two groups for analysis; 18 women (8 normal weight and 10 overweight/obese) who lost less than 7% of body mass (~2.3 ± 0.6 kg; % weight loss = -2.7 ± 0.6%, range = +3.0 to -6.3%) on the low fat diet, and 15 women (8 normal weight and 7 overweight/obese) who lost more...
than 7% of body mass (−8.4 ± 0.5 kg, % weight loss = −12.3 ± 1.0%, range = −8.2 to −23.0%). The women with less than 7% weight loss had modest, but significant reductions of percent body fat (−0.9 ± 0.4%, P < 0.02), but both plasma leptin (Fig. 4A) and plasma insulin concentrations were unchanged (Table 2). Women with greater than 7% weight loss had predictably larger reductions of percent body fat (−5.6 ± 0.6%, P < 0.0005), and in these subjects both plasma leptin and plasma insulin concentrations both fell by approximately 35% (Table 2).

Weight loss in women losing >7% body mass was not different between normal weight women (Δ = −3.7 ± 0.4 kg/m²) and in overweight/obese women (Δ = −3.7 ± 0.4 kg/m²). Plasma leptin concentrations were decreased in both normal weight women and overweight/obese women who lost weight after 6 months on the ad libitum low fat diet, and the reductions were sustained after 8 months (Fig. 4B). The decrement of plasma leptin after weight loss was greater in overweight/obese women (Δ = −13.9 ± 2.2 ng/mL, P < 0.0005) than in normal weight women (Δ = −5.7 ± 2.4 ng/mL, P < 0.02 vs. normal weight), however plasma leptin levels remained significantly higher in the overweight/obese women than in normal weight women (P < 0.01, Fig. 4B).

Baseline characteristics of the subjects (age, BMI, percent body fat, total body fat, hormone replacement therapy, and family history of diabetes) and hormone/substrate measurements (plasma leptin, insulin, and glucose concentrations) were evaluated to determine whether any of these parameters was predictive of weight loss on the ad libitum low fat diet. Of these, only family history of diabetes was predictive of weight loss, with subjects with first degree diabetic relatives having significantly greater decreases of BMI (−2.9 ± 0.6 vs. 1.6 ± 0.3 kg/m², P = 0.033) and percent body fat (−4.9 ± 1.0 vs. −2.1 ± 0.5%, P = 0.012) than in women without family history of diabetes.

Correlation of changes of BMI, percent body fat, plasma leptin, insulin, and REE on a long-term low fat diet in normal weight and overweight/obese women

As expected, the changes of BMI were highly correlated with the change of percent body fat after 8 months on the
self-selected low fat diet ($r = 0.78$, $P < 0.0001$, Fig. 4A). The change of plasma leptin on the low fat diet across all subjects was significantly correlated with both the change of BMI ($r = 0.42$, $P < 0.02$, Fig. 4B) and with the change of percent body fat ($r = 0.49$, $P < 0.005$, Fig. 4C). In contrast to leptin, the changes of plasma insulin were not significantly correlated with the change of BMI ($r = 0.26$, $P = 0.15$, data not shown) or percent body fat ($r = 0.26$, $P = 0.14$, data not shown). However, the change of plasma leptin on the self-selected low fat diet correlated well with the change of fasting plasma insulin ($r = 0.52$, $P < 0.002$, Fig. 4D). In addition, by partial regression analysis, the change of plasma leptin was significantly correlated with both the change of plasma insulin (Table 3). After 8 months on the low fat ad libitum diet, only overweight/obese women had a significant change in their leptin/adiposity ratio, which decreased by approximately 30% (Table 3). There were no significant alterations of the absolute change of leptin per unit adiposity ($\Delta$leptin/Δ% body fat) or the proportional changes of leptin per proportional change in adiposity (%Δleptin/%Δ% body fat) in normal weight or overweight/obese women, overall, or in the women who lost less than 7% of body weight (data not shown). However, plasma leptin decreased with adiposity in normal weight and overweight/obese women who lost more than 7% body weight, and both the absolute changes of leptin per unit adiposity and the proportional changes of leptin per proportional change of adiposity were 2.5–3.5 times greater in overweight/obese women than in normal weight women (Table 3).

**Discussion**

The aim of this study was to determine the effects of dietary fat content, in the absence of weight loss, on plasma leptin levels in women with a wide range of adipose tissue mass and within normal weight and overweight/obese subjects. In addition, the relationship between plasma leptin and plasma insulin levels after sustained weight loss on a very low fat diet was examined in both normal weight and overweight/obese women. Plasma leptin levels were higher in overweight to obese women than in lean to normal weight women and were highly correlated with both BMI and adiposity as assessed by percent body fat measurements overall and within both the normal weight and the overweight/obese groups. Elevations of plasma leptin levels in obesity and correlation with adiposity across human subjects with a wide range of adiposity have been reported in two recent studies (22, 23) but not within groups of lean or obese subjects. When the relationship of plasma leptin to adiposity was examined by calculating the leptin/percent body fat ratio, we found that after correcting for adiposity, overweight/obese women have higher plasma leptin levels per unit of adiposity (Table 3).
adiposity than normal weight women. These data are supported by a recent study showing increased leptin production per unit body fat in obese humans (30).

At stable body weight, plasma leptin levels were correlated with fasting plasma insulin. However, it is unclear whether this relationship between plasma insulin and leptin is caused by the coexisting adiposity or reflects regulation of plasma leptin by insulin, particularly because fasting insulin did not correlate with plasma leptin independently of adiposity.

We found no effect of reducing the fat content of the diet on plasma leptin or insulin concentrations when body weight was maintained, even after several weeks on very low fat diets. Thus, plasma leptin concentrations are not altered by dietary fat content in the absence of weight loss or changes of plasma insulin levels. Furthermore, because subjects were fed more than they would consume ad libitum during low fat intake, changing the fat content of the diet appears to alter the regulated level of adiposity via a mechanism unrelated to changes of plasma leptin or plasma insulin levels. In fact, the amount of body weight and fat loss on the ad libitum low fat diet was best related to having a first degree relative with diabetes, whereas other parameters measured in the study (adiposity, plasma leptin, plasma insulin, or REE) were not predictive of weight loss. This suggests that very low fat diets may be an effective method for inducing and maintaining weight loss in subjects with a family history of diabetes.

After sustained weight loss of greater than 7% of body mass, there was a clear reduction of both plasma leptin and plasma insulin concentrations in normal weight and overweight/obese women. However, the plasma leptin/percent body fat ratio, which is elevated in overweight/obese women, decreased after weight loss in overweight/obese but not in normal weight women. In addition, the absolute and proportional decreases of plasma leptin per unit body fat after weight loss was greater in overweight/obese women than in normal weight women. For example, a 10% decrease of adiposity in obese women leads to an average 34% decrease of plasma leptin, whereas the same proportional change of adiposity only decreases plasma leptin by an average of 13% in normal weight women. Plasma leptin and insulin levels were unchanged in women who lost less than 7% of body mass, despite a modest, but significant, average weight loss (-3% of body mass) and more than 10 months on a very low fat diet.

The overall change of plasma leptin at the completion of the study was well correlated with changes of BMI on percent body fat. In addition, the change of plasma leptin was correlated with the change of fasting plasma insulin independently of changes of BMI or percent body fat. Insulin administration increases (16, 17) and insulin deficiency decreases (18, 19) leptin gene expression in animals. However, because absolute plasma leptin and insulin levels were not independently correlated, it would appear to be the dynamics of insulinemia during weight loss that is associated with changes of plasma leptin, rather than the absolute plasma insulin level at stable body weight. Furthermore, if insulin is involved in the regulation of plasma leptin, it is unlikely to be an acute effect because short-term infusions of insulin did not alter plasma leptin in two studies in humans (31, 32). Lastly, it should also be noted that the findings of this study conducted in women should not be extrapolated to men, because men have lower levels of adiposity, ob gene expression (21), and plasma and cerebrospinal fluid leptin concentrations (33).

In summary, we have found that plasma leptin is not altered by dietary fat content per se and is highly correlated with BMI, percent body fat, and plasma insulin when body weight is stable. Plasma leptin per unit of adiposity is nearly doubled in overweight/obese women compared with normal weight women. Furthermore, there are sustained decrements of plasma leptin in both normal weight and over-
TABLE 3. Leptin/adiposity relationships in normal weight and overweight/obese women at stable body weight and after 8 months on an ad libitum low fat diet

<table>
<thead>
<tr>
<th></th>
<th>Normal weight</th>
<th>Overweight/obese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>&gt;7% wt loss</td>
</tr>
<tr>
<td>Leptin</td>
<td>16.7 ± 1.9</td>
<td>17.2 ± 3.8</td>
</tr>
<tr>
<td>∆Leptin</td>
<td>-2.0 ± 1.8</td>
<td>-5.7 ± 2.4</td>
</tr>
<tr>
<td>%∆Leptin</td>
<td>-12.5 ± 9.1</td>
<td>-29.4 ± 11.1</td>
</tr>
<tr>
<td>%Body fat</td>
<td>28.3 ± 1.0</td>
<td>29.8 ± 1.3</td>
</tr>
<tr>
<td>∆%Body fat</td>
<td>-3.0 ± 1.0</td>
<td>-6.9 ± 0.8</td>
</tr>
<tr>
<td>%∆%Body fat</td>
<td>13.4 ± 1.8</td>
<td>-23.5 ± 2.7</td>
</tr>
<tr>
<td>(Leptin/%body fat)</td>
<td>0.57 ± 0.06</td>
<td>0.56 ± 0.12</td>
</tr>
<tr>
<td>∆(Leptin/%body fat)</td>
<td>-0.01 ± 0.05</td>
<td>-0.09 ± 0.07</td>
</tr>
<tr>
<td>%∆(Leptin/%body fat)</td>
<td>2.1 ± 9.4</td>
<td>-6.4 ± 13.5</td>
</tr>
<tr>
<td>(∆Leptin)/(%∆body fat)</td>
<td>0.9 ± 0.4</td>
<td>13.0 ± 0.7</td>
</tr>
<tr>
<td>(%∆Leptin)/(∆%body fat)</td>
<td>1.3 ± 0.5</td>
<td>3.4 ± 0.4</td>
</tr>
</tbody>
</table>

Values are mean ± se; a P < 0.01 vs. baseline; b P < 0.01 vs. normal weight women.

Fig. 5. Correlation of change of BMI (ΔBMI) with change of percent body fat (A), change of BMI with change of plasma leptin (B), change of percent body fat with change of plasma leptin (C), and change of plasma insulin with change of plasma leptin (D) in 33 normal weight and overweight/obese women after 12 months on study in which 15 subjects lost >7% and 18 subjects lost <7% body mass on a very low fat diet.

weight/obese women after substantial reductions of body weight, but the reductions of plasma leptin are greater in obese than in normal weight women. The changes of plasma leptin concentration after weight loss are correlated with changes of BMI, percent body fat, and REE, and parallel the changes of plasma insulin independently of the change of adiposity. Thus, if leptin is in fact a hormonal regulator of feeding behavior and adiposity in humans, decreases of plasma leptin after weight loss could contribute to the strong tendency for weight regain after successful dieting.

Acknowledgments

We would like to acknowledge the expert technical assistance of Liz Rinehart and Rogelio Almario.