

Leptin Responses to Weight Loss in Postmenopausal Women: Relationship to Sex-Hormone Binding Globulin and Visceral Obesity

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Abstract

VAN ROSSUM, ELISABETH F.C., BARBARA J. NICKLAS, KAREN E. DENNIS, DORA M. BERMAN, AND ANDREW P. GOLDBERG. Leptin responses to weight loss in postmenopausal women: relationship to sex-hormone binding globulin and visceral obesity. *Obes Res.* 2000;8:29–35.

Objective: Leptin concentrations increase with obesity and tend to decrease with weight loss. However, there is large variation in the response of serum leptin levels to decreases in body weight. This study examines which endocrine and body composition factors are related to changes in leptin concentrations following weight loss in obese, postmenopausal women.

Research Methods and Procedures: Body composition (DXA), visceral obesity (computed tomography), leptin, cortisol, insulin, and sex hormone-binding globulin (SHBG) concentrations were measured in 54 obese (body mass index [BMI] = 32.0 ± 4.5 kg/m²; mean \pm SD), women (60 \pm 6 years) before and after a 6-month hypocaloric diet (250 to 350 kcal/day deficit).

Results: Body weight decreased by 5.8 ± 3.4 kg (7.1%) and leptin levels decreased by 6.6 ± 11.9 ng/mL (14.5%) after the 6-month treatment. Insulin levels decreased 10% ($p < 0.05$), but mean SHBG and cortisol levels did not change significantly. Relative changes in leptin with weight loss correlated positively with relative changes in body weight

($r = 0.50$, $p < 0.0001$), fat mass ($r = 0.38$, $p < 0.01$), subcutaneous fat area ($r = 0.52$, $p < 0.0001$), and with baseline values of SHBG ($r = 0.38$, $p < 0.01$) and baseline intra-abdominal fat area ($r = -0.27$, $p < 0.06$). Stepwise multiple regression analysis showed that baseline SHBG levels ($r^2 = 0.24$, $p < 0.01$), relative changes in body weight (cumulative $r^2 = 0.40$, $p < 0.05$), and baseline intra-abdominal fat area (cumulative $r^2 = 0.48$, $p < 0.05$) were the only independent predictors of the relative change in leptin, accounting for 48% of the variance.

Discussion: These results suggest that obese, postmenopausal women with a lower initial SHBG and more visceral obesity have a greater decrease in leptin with weight loss, independent of the amount of weight lost.

Key words: weight loss, leptin, sex hormone-binding globulin, insulin, visceral obesity

Introduction

Leptin, an adipocyte-derived hormone encoded by the *ob* gene (1), plays an important role in controlling body weight by regulating both energy intake and energy expenditure (2,3). In humans, leptin concentration is directly proportional to the amount of adipose tissue in the body (4,5), and leptin resistance is thought to play a role in developing obesity (6,7). When obese humans lose fat mass, leptin levels decline (5); however, the decline in circulating leptin does not always correlate with the amount of weight lost in women (8,9). In fact, in some subjects leptin levels decrease more than would be expected from the amount of lost fat (8,9). This suggests that, in addition to the loss of body fat, other factors may affect changes in leptin levels with weight reduction.

It is our hypothesis that weight loss-induced changes in hormones that affect leptin production will influence leptin responses to weight loss. In particular, insulin and

Submitted for publication March 10, 1999.

Accepted for publication in final form July 1, 1999.

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cortisol stimulate the production and secretion of leptin in human fat cells (10–13), and their decrease with weight loss may reduce circulating leptin levels. Sex steroid hormones also affect leptin as demonstrated by gender differences in plasma leptin levels and in leptin gene expression in adipocytes (8,14). Specifically, estrogen administration stimulates leptin production and increases serum leptin levels in vivo (15–17), whereas testosterone suppresses leptin secretion (16,18). Sex hormone-binding globulin (SHBG), a serum glycoprotein that binds sex steroids with high specificity, is an index of relative androgenicity. Obese women have lower SHBG levels, and circulating SHBG increases with weight loss (19,20), suggesting that SHBG levels could contribute to the variability in leptin responses to weight loss.

Findings of previous studies show there is a positive correlation between leptin and insulin concentrations and a negative correlation between leptin and SHBG concentrations in certain subjects (21–26). However, the effects of these endocrine factors on leptin changes with weight loss are unknown. The purpose of this study was to determine whether changes in leptin concentrations following a 6-month weight loss program of caloric restriction are predicted by baseline concentrations of, or changes in, insulin, cortisol, and SHBG, independent of changes in body composition.

Methods

Subject Selection

All subjects were healthy, postmenopausal (no menstruation for at least 2 years, follicle-stimulating hormone > 30 IU/L), obese (body mass index [BMI] > 27 kg/m²) women. None of the women were on estrogen replacement therapy or medications affecting lipids, glucose metabolism, or blood pressure. The women were sedentary (< 20 min of exercise, twice weekly), weight stable (< 2.0 kg weight change in past year), and had not smoked for at least 5 years. All women provided informed consent to participate in the study according to the guidelines of the University of Maryland Institutional Review Board for Human Research.

Initial screening evaluations included a medical history, physical examination, fasting blood profile, and 12-lead resting electrocardiogram to exclude subjects with evidence of diabetes (fasting plasma glucose level > 6.4 mM), hypertension (blood pressure > 140/90 mmHg), hyperlipidemia, cancer, liver, renal or hematological disease, other medical disorders, or orthopedic limitations that would affect physical activity. The second screening visit included a graded exercise test to exclude women with an abnormal cardiovascular response to exercise. A total of 82 women met the study criteria and were enrolled.

Study Design

Dietary Control. Before beginning the diet intervention, the women completed an initial 7-day food record to provide information about their dietary habits. To establish dietary control before metabolic testing, all women met weekly with a registered dietitian for 6 to 8 weeks and were instructed in the principles of the American Heart Association (AHA) Step 1 diet (27). Subjects were weight-stable on this diet for at least 2 weeks before research testing. The dietitian monitored compliance by weekly review of 7-day food records and 24-hour dietary recalls.

Research Testing. Measurements of body composition, body fat distribution, and serum leptin concentration were performed in the morning after a 12-hour fast before and after a 6-month hypocaloric diet. All measures of body composition were performed by the same investigator before and after the 6-month intervention. Waist-to-hip ratio (WHR) was measured in duplicate and calculated as the ratio of the minimal waist circumference to the circumference at the maximal gluteal protuberance. Percent body fat, fat free (bone and muscle) mass, and adipose tissue mass were measured using DXA (Model DPX-L; Lunar Radiation Corporation, Madison, WI). A single-slice computed tomography (CT) scan taken midway between L4 and L5 was performed using a GE Hi-Light CT scanner to measure intra-abdominal and subcutaneous fat area as previously described (28).

Hormonal Assays. Venous blood samples were taken before and after weight loss in the morning after an overnight fast for the measurement of serum leptin, insulin, cortisol, and SHBG. All samples were stored at –70 °C until analysis, so the pre- and post-weight loss samples from each woman were run in the same assay. Concentrations of all hormones were measured in duplicate. Leptin and insulin were measured using commercially available radioimmunoassay kits (Linco, St. Louis, MO). The intra-assay and interassay coefficients of variation were 5.2% and 3.5%, respectively, for leptin and 5% and 9%, respectively, for insulin. Cortisol was measured using a commercial radioimmunoassay kit from Diagnostic Products Corporation (Los Angeles, CA) with intra-assay and interassay coefficients of variation of 4.3% and 5.2%, respectively. Serum SHBG was measured using an immunoradiometric assay (Diagnostic Systems Laboratories, Inc., Webster, TX) with intra-assay and interassay coefficients of variation of 2.0% and 8.3%, respectively.

Weight Loss Intervention. During the 6-month weight loss intervention, all subjects met weekly with a registered dietitian for instruction in the principles of a hypocaloric diet (250 to 350 kcal/day deficit) that followed the AHA guidelines. The program focused on eating behavior, stress management, control of portion sizes, modification

of binge eating and other adverse habits, and also encouraged low intensity walking 3 days/week for 30 to 45 min. The women walked 1 day a week on a treadmill at our exercise facility at 50% to 60% heart rate reserve under the supervision of an exercise physiologist and were instructed to walk the other 2 days on their own. After the 6-month intervention, the women were weight-stabilized (< 0.5 kg change) on a eucaloric diet for a period of 2 weeks before retesting.

Statistics

Statistical analyses were performed with a Macintosh Statview program (Abacus Concepts Inc., Berkeley, CA). Data were first tested for normal distribution using the Shapiro-Wilk test for normality. Leptin and insulin data were not normally distributed so the logarithm of each was used for parametric statistical analyses. Differences between variables before and after weight loss were determined using a paired *t*-test. Analysis of covariance (ANCOVA) was used to adjust leptin levels for fat mass when needed. Statistically significant relationships between changes in hormones and changes in body composition were determined with Spearman Rank correlational analysis. Stepwise multiple-regression analysis was used to determine the strongest predictors of the change in leptin, defined as the difference between the post-intervention values and the basal values. All data are presented as mean \pm SD, and the level of significance was set at $p < 0.05$ for all analyses.

Results

We report data on 54 of the 82 women who completed the weight loss intervention and all post-weight loss measurements. The remaining 28 women dropped out due to illness, relocation, or personal reasons. The baseline physical characteristics or hormone concentrations of the women who dropped out were not different from the 54 women who completed the study.

Body Composition and Hormone Levels

The 54 women lost an average of 7.1% of body weight during the 6-month weight loss intervention (Table 1). There was an average 14.3% decrease in fat mass, but no change in lean tissue mass. Because both waist and hip circumference decreased with weight loss, there was no change in WHR. There were comparable 16% and 12% decreases in intra-abdominal fat (IAF) and subcutaneous fat (SCF) areas, respectively; hence, there was no preferential loss of visceral fat with weight loss. On average, leptin decreased $14.5\% \pm 35.9\%$ with weight loss ($p < 0.0001$; Table 2). However, there was large variation in the leptin responses to weight loss (-59% to $+120\%$). Insulin levels decreased by 10% with weight loss ($p < 0.05$), but on average SHBG and cortisol levels did not change significantly (Table 2).

Relationship of Leptin to Body Composition and Hormones at Baseline

The logarithm (log) of plasma leptin correlated positively with body weight, fat mass, waist circumference, IAF area, and SCF area at baseline (Table 3). In addition, the log of leptin correlated with the log of insulin (Table 3), but not with SHBG ($r = 0.13$) or cortisol ($r = 0.11$) at baseline. After using ANCOVA to adjust baseline leptin levels for fat mass, the relationship of leptin to insulin remained significant ($r = 0.40$, $p < 0.01$), but there was still no relationship with the other hormones at baseline. There was an inverse relationship between baseline SHBG levels and WHR ($r = -0.43$, $p < 0.01$), but not BMI ($r = 0.04$) or IAF ($r = 0.05$).

Linear regression analysis was used to determine whether changes in body composition or hormones with weight loss correlated with baseline leptin levels. Baseline leptin levels did not predict changes in any of the hormones. However, relative changes in body weight ($r = 0.30$, $p < 0.05$) and fat mass ($r = 0.52$, $p < 0.0001$) were directly related to the log of baseline leptin levels. These relationships remained significant even after adjusting leptin levels for fat mass (change in body weight: $r = 0.30$; change in fat mass: $r = 0.32$; $p < 0.05$).

Predictors of the Relative Changes in Leptin with Weight loss

Linear regression analysis was used to determine the predictors of changes in leptin levels with weight loss. Because changes in leptin concentrations with weight loss were strongly related to leptin levels at baseline ($r = -0.49$, $p < 0.01$), we examined the factors associated with the relative changes in leptin with weight loss. Percent changes in leptin with weight loss correlated positively with relative changes in body weight ($r = 0.50$, $p < 0.0001$, Figure 1), fat mass ($r = 0.38$, $p < 0.01$), and SCF area ($r = 0.52$, $p < 0.0001$), but not with relative changes in IAF. Furthermore, changes in leptin did not correlate with changes in insulin, cortisol, or SHBG levels with weight loss.

We also examined whether changes in leptin with weight loss correlated with baseline measures of body composition and hormone levels. Relative changes in leptin with weight loss correlated with baseline values of SHBG ($r = 0.38$, $p < 0.01$; Figure 2), and tended to correlate with baseline IAF ($r = -0.27$, $p < 0.06$), but not with other measures of body composition or hormones. Thus, women with low SHBG levels and high IAF area showed a greater decline in leptin levels with weight loss.

To determine the independent predictors of the relative change in leptin with weight loss, stepwise multiple-regression analyses were performed using a regression model that included relative changes in body weight and SCF area, and baseline IAF area and SHBG levels. This analysis showed that baseline SHBG levels ($r^2 = 0.24$, $p < 0.01$), relative

Table 1. Body composition before and after the 6-month weight loss intervention

	Before weight loss	After weight loss	Change
Weight (kg)	83.5 ± 12.3	77.7 ± 12.5	-5.8 ± 3.4*
BMI (kg/m ²)	32.0 ± 4.5	29.8 ± 4.7	-2.2 ± 1.3*
Body fat (%)	47.1 ± 5.4	43.3 ± 6.6	-3.9 ± 3.0*
Fat mass (kg)	39.0 ± 9.1	33.8 ± 10.1	-5.2 ± 3.5*
Lean tissue mass (kg)	40.4 ± 4.3	40.4 ± 4.0	0.0 ± 1.5
Waist-to-hip ratio	0.83 ± 0.06	0.82 ± 0.06	0.01 ± 0.04
IAF area (cm ²)	154.7 ± 51.2	129.8 ± 47.2	-25.1 ± 37.4*
SCF area (cm ²)	449.2 ± 124.0	398.9 ± 121.2	-53.3 ± 55.7*

All data are mean ± SD; *n* = 54.

* Significant change (*p* < 0.0001) from before weight loss.

changes in body weight (cumulative $r^2 = 0.40$, $p < 0.05$) and baseline IAF area (cumulative $r^2 = 0.48$, $p < 0.05$) were the only independent predictors of the relative change in leptin with weight loss, and together accounted for 48% of the variance in the relative change in leptin with weight loss (Table 4).

Discussion

The main findings of this study are that, in postmenopausal women, relative changes in serum leptin levels with weight loss are predicted not only by changes in body weight, but also by baseline SHBG levels and IAF area. The decrease in serum leptin with weight loss was greater in women who initially had a lower SHBG and larger amount of visceral fat. This finding suggests that postmenopausal women with a more android distribution of body fat, accompanied by a lower SHBG level, experience greater declines in leptin with a comparable amount of weight lost after a hypocaloric diet. We are in the process of studying

the clinical significance of these findings in terms of maintenance of lost weight in these women.

Because leptin is produced and secreted by adipose tissue, a decrease in leptin levels with loss of body fat is expected. However, in this study, as well as others (8,9), changes in leptin levels with weight loss were not entirely accounted for by changes in body weight. In fact, not all subjects who lost weight decreased their leptin level and in some cases, leptin levels even increased with weight loss. This suggests that there are other factors that influence changes in leptin with weight loss. Our results show that pre-weight loss visceral fat mass and SHBG (a measure reflecting the androgen/estrogen ratio) also affect the change in leptin with weight loss.

We can only speculate on the physiological mechanism for the greater decline in leptin with weight loss in women with a lower SHBG. A lower SHBG level presumably reflects a higher testosterone-to-estrogen ratio. Because testosterone is thought to suppress leptin production and re-

Table 2. Leptin, insulin, SHBG, and cortisol levels before and after the 6-month weight loss intervention

	Before weight loss	After weight loss	Change
Leptin (ng/mL)	30.9 ± 20.2	24.3 ± 14.8	-6.6 ± 11.9*
Insulin (pmol/L)	79.1 ± 36.4	71.3 ± 30.4	-7.2 ± 23.3†
SHBG (nmol/L)	122.0 ± 75.3	136.1 ± 99.6	12.5 ± 92.5
Cortisol (nmol/L)	542.5 ± 302.9	509.8 ± 266.2	-45.4 ± 269.0

All data are mean ± SD; *n* = 54; except SHBG, *n* = 42.

* Significant change (*p* < 0.0001) from before weight loss.

† Significant change (*p* < 0.05) from before weight loss.

Table 3. Statistically significant correlates of leptin with body composition and hormones at baseline

	Leptin
Weight	0.59*
Fat mass	0.64*
Waist	0.52*
IAF area	0.30†
SCF area	0.62*
Insulin	0.32†

n = 54; * *p* < 0.0001, † *p* < 0.05; leptin and insulin were log-transformed.

lease (16,18) while estrogen stimulates leptin (15–17), SHBG levels would reflect the effects of these sex steroid hormones on leptin. Our findings suggest that a woman’s relative balance of androgen to estrogen, as approximated by the SHBG level, may affect the change in leptin with weight loss. In addition, leptin levels decreased more than would be expected for the amount of fat lost by some of these women, which could be explained by an improvement in leptin sensitivity with weight loss. This supports the finding of a smaller decline in leptin levels after the same proportional weight loss in normal weight women than in obese women (21).

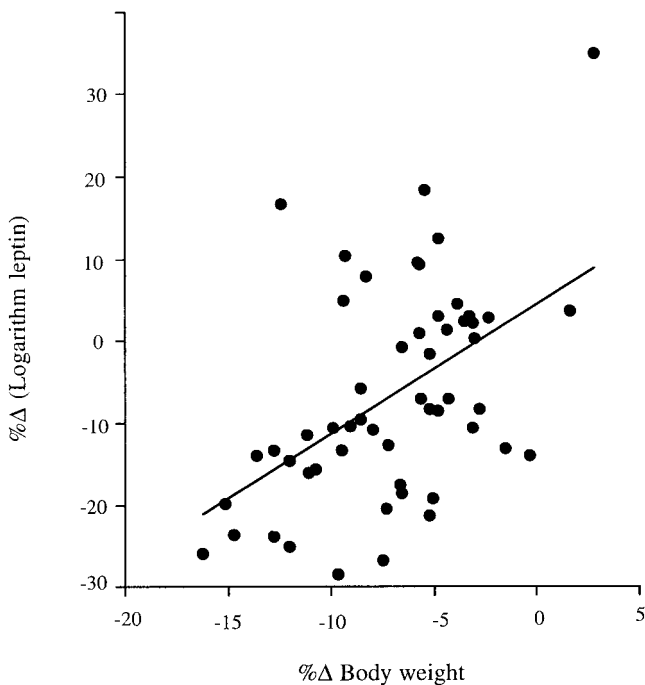


Figure 1: Relationship between relative changes in leptin concentrations with weight loss and relative changes in body weight (*r* = 0.50, *p* < 0.0001).

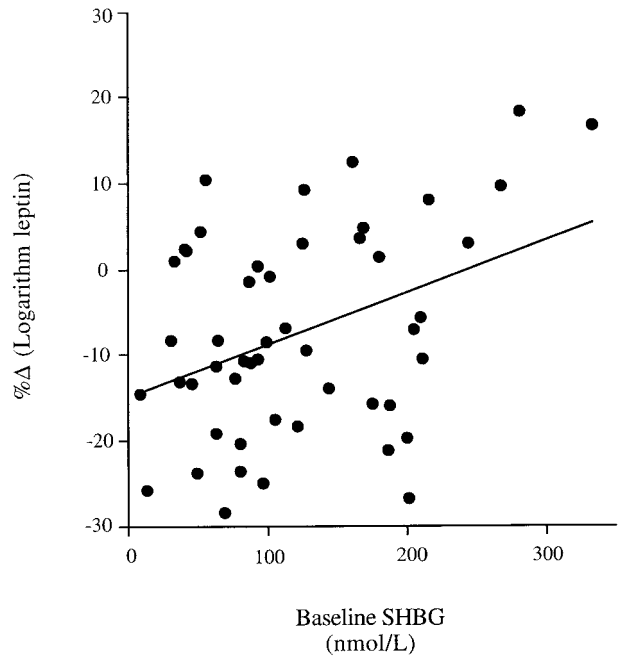


Figure 2: Relationship between relative changes in leptin concentrations with weight loss and baseline SHBG concentration (*r* = 0.38, *p* < 0.01).

The association between a low SHBG level and a high WHR suggests that an android fat distribution in postmenopausal women affects the magnitude of the decline in leptin levels following weight loss. This is supported by the contribution of baseline IAF area in accounting for a significant portion of the variance in relative changes in leptin with weight loss. The physiological mechanisms underlying our observance that women with an elevated IAF area experience greater declines in leptin with weight loss are unclear, but could be mediated by the lower leptin secretion from visceral relative to subcutaneous adipose tissue (29). A lowered leptin secretion from visceral fat could also explain

Table 4. Independent predictors of relative changes in leptin with weight loss

Variable	Coefficient	SEM	Cumulative <i>r</i> ²
Baseline SHBG	0.205	0.047	0.24*
% Δ body weight	2.953	0.906	0.40†
Baseline IAF area	−0.167	0.068	0.48†

n = 54; * *p* < 0.01; † *p* < 0.05.

why we found that relative changes in leptin with weight loss correlated with relative changes in subcutaneous, but not visceral, fat area. Furthermore, the metabolic consequences and clinical significance of the greater decrease in leptin levels in postmenopausal women with central obesity, an important risk factor for cardiovascular disease (30), remains to be determined.

While baseline levels of leptin and SHBG were not related in the healthy, postmenopausal women of our study, previous studies show that leptin concentrations are indirectly related to SHBG levels in men (23) and in women with hirsutism (25) and polycystic ovary syndrome (24,26). However, none of these relationships persisted after adjustment for differences in obesity, indicating that the influence of SHBG on leptin levels may be mediated by the effects of SHBG on obesity. In our study, there was no relationship between baseline SHBG levels and obesity at baseline, nor did baseline SHBG predict the amount of weight lost in these women (data not shown). Thus, the effects of SHBG on the leptin responses to weight loss are independent of obesity in these women.

The relationship between baseline leptin and insulin levels seen in our data is in accordance with the findings from other studies (21,22). This relationship persisted after adjustment for fat mass, suggesting that insulin's effects on circulating leptin levels in older obese women are independent of degree of obesity. Despite the fact that both leptin and insulin levels decreased with weight loss, there was no relationship between changes in leptin and changes in insulin with weight loss in these women. This is consistent with some previous reports (31,32), but contrasts Havel et al. who reported a significant relationship between change in leptin and change in insulin with weight loss in normal-weight and obese postmenopausal women (21). This discrepancy among these may be explained by the different obesity status of the subjects, or to the timing of the insulin measurements. A study in normal-weight and obese mice showed that leptin and insulin concentrations correlated only when the normal-weight mice were excluded from the analysis (33), suggesting that the physiological mechanism behind insulin's influence on serum leptin may differ between lean and obese subjects. On the other hand, Carantoni et al. recently demonstrated an independent relationship between change in leptin concentration with weight loss and change in 24-hour insulin response after weight loss, but found no correlation between changes in leptin and changes in fasting insulin levels (34).

Because cortisol promotes leptin production and increases plasma leptin concentrations in humans (11–13), we hypothesized that cortisol might also influence the leptin response to weight loss. Cortisol concentrations did not change significantly with weight loss in our subjects. However, our findings may be limited by our measurement of only one fasting serum cortisol concentration. Because cor-

tisol levels are subject to both pulsatile and diurnal variation, it may be that measurement of 24-hour integrated serum cortisol concentrations or cortisol response to some stimulus would show a relationship to leptin concentrations before and in response to weight loss.

In summary, our findings suggest that, in obese, postmenopausal women, changes in leptin with weight loss can be predicted by baseline SHBG levels and IAF area, as well as changes in body weight. Thus, postmenopausal women with a low initial SHBG level and a greater IAF mass tend to decrease their leptin levels more with weight loss. The clinical significance and the physiological mechanism(s) underlying the interaction of SHBG and body fat distribution with the leptin response to weight loss during hypocaloric feeding remains to be established.

Acknowledgments

We are grateful to Linda Bunyard, RD, MS and Naomi Tomoyasu, PhD, for their assistance with data collection and dietary counseling and to Agnes Kohler, MA, for the hormone assays. We would also like to thank the nursing and technical staff of the Division of Gerontology and GRECC for their assistance in the conduct of this project. Finally, we especially thank all of the women who volunteered to participate in this study. Supported by National Institutes of Health Grant RO1 NR03514, NIH Grant R29 AG14066–02, NIH Geriatric Leadership Academic Award K07 AG-00608, University of Maryland Intramural funding and Department of Veterans Affairs Baltimore Geriatric Research, Education and Clinical Center.

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