Impact of Weight Loss on Serum Leptin in Obese Postmenopausal Women

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ABSTRACT

A 6-month weight loss program consisting of energy restriction (1,200 kcal) in combination with phentermine hydrocholoride (Fastin) treatment evaluated changes in plasma leptin, insulin, and glucose concentrations in 39 obese postmenopausal women (body mass index 30-38 kg/m^2). Values for these parameters were correlated with plasma lipids concentrations and body composition measurements previously reported for this population. Over 6 months, participants had a 9% weight loss ($P < 0.001$) and a 9% reduction in body mass index ($P < 0.001$) compared to baseline with a 20% reduction in total fat mass and 25.8% in trunk fat ($P < 0.001$). Plasma leptin levels were reduced by 27 % ($P < 0.001$) at 3 months and by 32% ($P < 0.001$) at 6 months. In contrast, plasma glucose and insulin levels did not change over time. Significant positive correlations were found between changes in plasma leptin and changes in LDL cholesterol ($r = 0.366$), weight ($r = 0.570$) and trunk fat ($r = 0.362$) over 6 months indicating that reductions in leptin are associated with improved parameters in plasma lipids and body composition.

INTRODUCTION

Leptin, a circulating protein produced by the adipose tissue has been shown to be highly correlated with body fat suggesting that obese persons are insensitive to endogenous leptin production.[1] Montague et al[2] reported that 2 severely obese children, members of the same consanguineous pedigree, had very low serum leptin levels despite markedly elevated fat mass. These findings provide the first genetic evidence that leptin is a significant regulator of energy balance in humans. Increasing hunger has also been negatively correlated with leptin levels.[3]

Decreases in serum leptin, glucose, and insulin concentrations have been reported following energy restriction.[4] Interestingly gender differences have

been observed in leptin, with serum levels being higher in women compared to men both before and after energy restriction.[4] In another study, the short-term effect of energy restriction in centrally obese African American subjects with impaired glucose tolerance paralleled reductions of plasma leptin and basal insulin concentrations[5] suggesting that a negative energy balance may reduce serum leptin concentrations and improve glucose tolerance and insulin action in obese individuals.
Weigle et al[6] compared the effects of fasting, re-feeding, and dietary fat restriction on plasma leptin levels. When fasting and refeeding effects were compared, fasting resulted in a mean decrease of 62% in plasma leptin levels, which returned to baseline within 12 hours after subjects were re-fed.[6] Other studies have reported improvement in plasma lipids and decreases in plasma leptin concentrations following a 16-week weight reduction program[7] or after an exercise intervention associated with weight loss.[8,9]

In addition, chronic hyperinsulinemia has been shown to increase serum leptin levels, indicating a long-term effect of insulin on increased leptin production.[10] The authors suggested that this increased leptin production could be due to the trophic effects of insulin on adipocytes.

The aim of the present study was to evaluate the effects of a 6-month weight reduction program consisting of caloric restriction and Phenthermine hydrochloride therapy, as an appetite suppressant, on serum leptin, insulin, and glucose levels in postmenopausal women. In addition, we evaluated potential correlations between changes in serum leptin levels and our previously observed changes in body composition parameters and plasma lipid concentrations in this population.[11]

SUBJECTS AND METHODS

Post menopausal Caucasian women 40 to 70 years of age (n=39) on hormone replacement therapy (HRT) were recruited by direct mailings and by advertisement enclosed with Loma Linda University paychecks to participate in a 6-month long prospective clinical trial to examine the effects of weight loss on total and regional body composition, plasma lipids profile, and plasma leptin, insulin and serum glucose concentrations. A body mass index (BMI) of at least 30 kg/m^2 or a BMI of 27 kg/m^2 plus a cardiovascular disease risk factor such as hypertension, diabetes mellitus, hyperlipidemia or degenerative joint disease were required for participation in the study. Exclusion criteria were participation in any weight control program during the previous 3 months, use of serotonin re-uptake inhibitors, untreated hypertension, hyperparathyroidism, hypersensitivity to sympathomimetic amines, glaucoma, agitated states, history of drug abuse, or use of monoamine oxidase inhibitors, either ongoing or within 2 weeks prior to recruitment. All subjects underwent an initial physical examination, a 12 lead electrocardiogram, and blood tests that included complete blood count, lipid profile, leptin, insulin, glucose, and random chemistry profile. Monthly check-ups by the study physician or nurse educator were performed thereafter. Of the 49 women who enrolled, 39 completed the study. All subjects gave written informed consent to participate. The study protocol was approved by Loma Linda University Institutional Review Board.

Experimental Design

A phentermine hydrochloride (Fastin) dose of 15 mg/day was initially prescribed for study subjects. If a subject did not experience depressed appetite in response to this dose, the Fastin dose was increased to 15 mg twice per day

(8:00 AM and 5:00 PM). In addition to the drug therapy, a low calorie diet (1,200 Kcal/d) was prescribed, and attendance at monthly support sessions was required. The diet instructions included a recommendation to reduce saturated fat and cholesterol consumption, and to increase dietary fiber.

The support sessions provided skill-building techniques to encourage behavior changes in stress management, exercise, nutrition, and management of emotions without excess food consumption. Patients were encouraged to set a goal for themselves (ie, increased walking). The guidelines of the American College of Sports Medicine were followed. These include an increase of 10% effort/week, which equals approximately an increase of 1 to 2 minutes of exercise per week. In addition to the intake of 1,200 kcal/d, nutritional counseling included recommendations to eat at regular meal times and to keep food records for self-monitoring. For the management of emotions, recommendations were made on how to deal with stressful situations, for example, instead of eating, call a friend to vent feelings, exercise or do a pleasant task.

Food frequency dietary records were collected from all participants at baseline, 3, and 6 months to ascertain compliance to the low energy diet and to assess dietary intakes of total and saturated fat, cholesterol, and fiber. The Nutrient Profile Plus Program (Well Force Inc, Clackamas, Ore) was used to calculate nutrient intake and percentage of energy derived from macro-nutrients and saturated fat. The results from the dietary records are reported elsewhere.[11]

Serum Insulin, Leptin, and Glucose

Insulin, leptin, and glucose were measured at baseline, three, and six months during the program. Serum insulin was measured by radioimmunoassay (Quest Diagnostics, Teterboro, NJ; Nichols Institute Diagnostics, San Juan Capistrano, Calif) using guinea pig antibodies to the porcine ^{125}I-insulin (Linco Research Inc, St. Louis, Mo) radioactive tracer. The serum samples and a human insulin standard were incubated with antibody and tracer for 4 hours at room temperature. Antibody-bound insulin was then precipitated by a second antibody (goat anti-guinea pig gamma globulin), 10% guinea pig serum and polyethylene glycol. The precipitated complex was then counted in a gamma counter. Serum leptin analyses were carried out by the Obesity Research Center, Columbia University, New York, NY using radioimmunoassay (Cat.3 HL-81K, Linco Research, Inc, St. Louis, Mo).

Serum glucose was determined by reacting subjects' serum with glucose oxidase, which converts glucose to gluconolactone. This conversion relies on the consumption of oxygen and produces hydrogen peroxide as a byproduct. The hydrogen peroxide is reacted with a peroxidase to release the oxygen, which can then be measured and quantified by an oxygen specific electrode. In this reaction, the production of hydrogen peroxide from glucose oxidase is expected to be directly proportional to the amount of glucose in the plasma.[12]

Plasma Lipids

Two blood samples were obtained to determine plasma lipid (total, HDL, and LDL cholesterol and triacylglycerol) concentrations at baseline, 3, and 6 months for all subjects. Standardization and quality control for plasma total cholesterol and triacylglycerol assays have been maintained by participation in the Centers for Disease Control-National Lung and Blood Institute (CDC-NLBI) Lipid Standardization Program since 1989. An enzymatic method[13] was used to determine plasma total cholesterol against cholesterol standards (Boehringer Mannheim Corp,

Table 1. Baseline Characteristics of Study Subjects*

Measure	(N=39)
Anthropometrics	
Age (y)	58.39 ± 4.69
Weight (kg)	91.52 ± 17.6
Height (m)	1.62 ± 0.06
BMI (kg/m^2)	35.95 ± 5.32
Waist to Hip Ratio	0.85 ± 0.05
DXA	
Total Body Fat (kg)	42.19 ±11.01
Trunk Fat (kg)	20.31 ± 5.73
Leg Fat (kg)	14.86 ± 4.02
Lipids	
Total Cholesterol (mg/dL)	208.0 ± 24.2
LDL Cholesterol (mg/dL)	129.1 ± 35.5
HDL Cholesterol (mg/dL)	49.7 ± 10.3
Triglycerides (mg/dL)	144.8 ± 76.1

*Values are expressed Mean ± SD.

Indianapolis, Ind). Plasma HDL cholesterol was measured in the supernatant after precipitation of apolipoprotein B-containing lipoproteins,[14] and LDL cholesterol was calculated as described by Friedewald et al.[15] Plasma triacylglycerol was determined using the adjustment for free glycerol according to the method of Carr et al.[16]

Body Composition

Total and regional body composition was measured by DXA using the Hologic QDR-4500A instrument and body composition analysis software version 8.1A (Hologic Inc, Waltham, Mass). Scans were obtained with the subject in the supine position, wearing only a hospital gown and undergarment, and with metal and jewelry removed. Whole body scans were taken and regions of interest were isolated. Scan time was approximately 3 minutes for each assessment with a radiation exposure of 1.5 mrem. DXA scans were obtained at baseline, 3, and 6 months for all subjects.

Statistical Analysis

Statistical analyses were calculated using SPSS for Windows version 10.05 (SPSS Inc, Chicago, Ill) with significance defined as $P<0.05$. Data is presented as mean ± SD.

Repeated measures ANOVA was performed to detect differences over time within subjects in the measures of anthropometrics assessment, leptin, insulin, glucose, and plasma lipids. Contrasts were used to test for differences among the levels of a factor.

Means and standard deviation were used to summarize the outcomes at each time point. Pearson product-moment correlations were performed to relate trends in body-composition changes with changes in plasma lipid profile, leptin, glucose, and insulin concentrations.

RESULTS

The descriptive characteristics of the subjects at baseline (n=39) are summarized in

Table 1. All women were postmenopausal on HRT with weight ranging from 89 to 125 Kg and BMI ranging from 26 to 46 kg/m^2 at baseline.

Subjects complied with the diet as assessed by the dietary records. The major dietary changes were a decreased in kcal intake from 2233 ± 1233 kcal/d to an average1460 ±623 kcal/d, a decrease in the percent contribution of fat to total energy from 38.6 to 32% (from 13.2 to 10.3% saturated fat). The percent of energy derived from carbohydrate was maintained at 49% of total calories. Leptin levels significantly ($P< 0.01$) decreased (-27%) after 3 months of treatment compared to baseline levels. At 6 months treatment, there was a 34.7% decrease compared to baseline ($P < 0.01$).

Differences over time (MANOVA) in body compositions are summarized in

Table 2. Changes in Plasma Insulin, Leptin, and Glucose Over Six-Months Weight Loss Treatment*

Measures	Baseline	3 Months	%Difference	6 Months	%Difference
Insulin (μU/mL)	11.38 ± 6.48	11.55 ± 6.56	+1.5	11.42 ± 11.29	+ 0.35
Leptin (μg/L)	36.72 ± 12.95	26.51 ± 15.60†	-27.8	23.98 ± 12.96†	-34.7
Glucose (mg/dL)	96.33 ± 22.70	102.56 ± 25.90	+ 6.5	103.51 ± 20.57	+ 7.5

*Values represent mean ± SD for N = 39 subjects
†Values marked with † in the same row are significantly different at $P < 0.001$ from baseline as analyzed by repeated measures ANOVA.

Table 3. Changes in Fat Mass, Leg and Trunk Fat, Weight and Body Mass Index Over the Six-Month Weight Loss Treatment*

Measures	Baseline	3 Months	%Difference	6 Months	%Difference
Fat Mass (kg)	42.19 ±11.01	38.56 ±10.03†	-8.60†	33.68 ±9.08†	-20.20†
Leg Fat (kg)	14.86 ±4.02	13.93 ±3.73	-6.30†	11.03 ±3.08	-25.80†
Trunk Fat (kg)	20.30 ±5.73	18.24 ±5.15†	-10.00†	16.81 ±4.68†	-17.20†
Weight (kg)	95.2 ± 16.1	88.5 ± 16.0†	-7	85.4 ± 15.4†	-9
BMI (kg/m^2)	35.95 ±5.32	32.89 ±5.27	-8.50†	32.53 ±5.33	-9.40†

*Values represent mean ± SD for N = 39 subjects
†Values with † in the same row are significantly different at $P < 0.001$from baseline as analyzed by repeated measures ANOVA.
BMI indicates body mass index.

Table 3. Significant (P<0.001) reduction in body composition continued over 6 months as consequence of treatment. Compared to baseline there was a significant (P< 0.01) reduction in weight, BMI, fat mass, leg fat, and trunk fat at 3 and 6 months. From baseline the reductions were 7% (3 months) 9% (6 months) for weight, 8.5% (3 months) 9.4% (6 months) for BMI, 8.6% (3 months) 20.2% (6 months) for fat mass, 6% (3 months) 26% (6 months) for leg fat, and 10% (3 months) 17% (6 months) for trunk fat respectively.

Changes over time in total cholesterol (TC) were 10 and 11.4% respectively at 3 and 6 months compared to baseline (P<0.01) (Table 4). Likewise, LDL-C and TG were decrease 11.4 and 12% following 3 months and 18.1 and 15% after 6 months of treatment. HDL-C was significantly increased by 9.2% (P<0.001) after 6 months of treatment. The total cholesterol to HDL ratio was significantly decreased following 6 months by 20% (Table 4).

There were significant (P<0.05) correlations between plasma leptin, insulin, plasma lipids, and body composition changes as shown in Table 5. After 3 months treatment, subjects showed a significant positive correlation between changes in leptin and BMI (r = 0.400), total fat (r = 0.482), total cholesterol (r = 0.317) and LDL-C (r = 0.380) and insulin (r = 0.399). After 6 months of treatment, there were significant positive correlations between leptin changes and total cholesterol (r = 0.338), LDL-C (r = 0.366), weight (r = 0.507), BMI (r = 0.325), insulin (r = 0.738) and glucose (r = 0.333).

DISCUSSION

The metabolic changes seen in obese post-menopausal women in response to a comprehensive weight reduction intervention involve interactions between many different but related physiologic systems. Due to the many factors comprising the intervention including weight loss, dietary changes, Fastin treatment, and counseling, it is difficult to assess the effects of individual components on weight reduction. However, our hypothesis is that weight loss in this study had a

Table 4. Changes in Plasma Total Cholesterol, Triglycerides, LDL and HDL Cholesterol, and Total Cholesterol to HDL Ratio Over the Six-Month Weight Loss Treatment*

Parameters	Baseline	3 Months	%Difference from baseline	6 Months	%Difference from Baseline
TC (mg/dL)	208.0 ± 24.2	187.3 ± 36.9†	-10	184.2 ± 31.9†	-11.4
TG (mg/dL)	144.8 ± 76.1	127.7 ± 75†	-12	123.4 ± 55.4†	-15
HDL (mg/dL)	49.7 ± 10.3	47.5 ± 9.4	-5	54.3 ± 10.2†	+ 9.2
LDL (mg/dL)	129.1 ± 35.5	114.4 ± 31.1†	-11.4	105.7 ± 29.9†	-18.1
TC/HDL	4.19 ± 2.34	3.94 ± 1.92	+ 6	3.39 ± 1.22†	+ 20

*Values represent mean ± SD for N = 39 subjects
†"Values with † in the same row are significantly different at $P < 0.001$from baseline as analyzed by repeated measures ANOVA.
TC indicates plasma total cholesterol; TG, triglycerides

Table 5. Correlation Between Changes in Leptin, Total Cholesterol, LDL Cholesterol, Weight, Body Mass Index, Insulin, Glucose, Trunk Fat and Total Fat Over Six Months

Measures (N=39)	TC	LDL-C	WT	BMI	Insulin	Glucose	Trunk Fat	Total Fat
Leptin								
Δ Baseline to 3 Months	0.317*	0.380*		0.400*	0.399*			0.482†
Δ Baseline to 6 Months	0.338*	0.366*	0.507†	0.325*	0.738†	0.333*	0.362*	

*$P < 0.05$ (2 tailed), †$P < 0.01$ (2 tailed)
TC indicates total cholesterol; LDL-C, LDL cholesterol; WT, weight; BMI, body mass index.

major contribution to the observed changes in serum leptin concentrations. This study was conducted with free-living subjects over a six-month period. It was anticipated that Fastin would ensure a low energy dietary intake, which would result in a 10% body weight reduction. After 6 months of intervention, subjects lost an average of 9% of their initial body weight. We reported these results in a previous paper[11] and they are in agreement with reported data.[17-21]

An average of 4 kg of trunk fat and 4 kg of thigh fat were lost as assessed by DXA. Previously, we reported the positive effects of these losses on lipid profile profile.[11] These findings are in agreement with other studies.[21] Datillo et al[20] conducted a meta-analysis on the effects of weight reduction on serum lipid levels and reported favorable effects of weight loss on blood lipid profiles as seen by other investigators.[22,23] The positive effect of the weight loss program on plasma lipids in this study has been previously reported.[11] The correlations between changes in plasma lipids and changes in plasma leptin concentrations are the novel information in the current study. We also found a significant reduction in serum leptin concentrations with an average decrease of 13 µg/L over 6 months.

Total fat mass and trunk fat were significantly decreased with weight loss. These decreases may have significantly impacted the decrease in leptin levels. Van Harmelen[24] reported findings that suggest that subcutaneous adipose tissue has a higher rate of leptin secretion compared to omental adipose tissue due to enlarged fat cell size and increased expression of the leptin gene.

Plasma insulin and glucose levels did not change over 6 months. Clinically, "normal" plasma glucose and insulin levels range between 75 to 105 mg/dL

and less than 22 µU/mL, respectively. Yamashita[7] found similar results to ours in a group of women after weight reduction. After 16 weeks of weight reduction the mean plasma glucose and insulin concentration did not change, while the mean plasma leptin concentration decreased significantly from 35.4 to 26.8 ng/mL. Similar to our results, other studies involving weight loss failed to find differences in insulin levels after weight reduction.[3,25]

In conclusion, following 6 months of this weight reduction program, postmenopausal women experienced a decrease in body weight accompanied by a significant reduction in serum leptin levels and an improved lipoprotein profile. The positive correlations between leptin levels and plasma lipids and between body weight and body composition, and leptin levels suggest that changes in these parameters play a significant role in determining serum leptin concentrations.

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