

Plasma Leptin and Insulin Levels in Weight-Reduced Obese Women With Normal Body Mass Index

Relationships With Body Composition and Insulin

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Obesity is a complex disease with multiple features that has confounded efforts to unravel its pathophysiology. As a means of distinguishing primary from secondary characteristics, we compared levels of fasting plasma leptin and insulin in a cohort of weight-reduced obese women who have attained and maintained a normal BMI for more than 1 year with the levels in cohorts of never-obese and currently obese women. Weight-reduced obese women showed decreased plasma concentrations of leptin and insulin compared with obese women, but these levels remained significantly higher than those of never-obese women. Plasma leptin levels were highly correlated with plasma insulin levels ($r = 0.60$, $P < 0.001$). To further explore relationships with body composition, total body fat was determined by dual-energy X-ray absorptiometry and body fat distribution by computed tomography in subsets of these groups. Weight-reduced obese women had a significantly greater percent body fat and subcutaneous abdominal fat mass than did the never-obese women, and these were highly correlated with plasma leptin ($r = 0.90$, $P < 0.001$, and $r = 0.52$, $P < 0.001$, respectively). In these weight-reduced obese women, visceral fat mass was similar to that of the never-obese. The insulin sensitivity index and first-phase insulin response were also comparable. These results demonstrate that higher leptin levels in weight-reduced obese women are related to the higher total fat and particularly the subcutaneous fat masses. Normalization of visceral fat mass in the weight-reduced obese was accompanied by normalization of insulin sensitivity index and first-phase insulin response. This study suggests that increases in plasma leptin and insulin in obesity are secondary features of the obese state. *Diabetes* 48:347–352, 1999

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Received for publication 7 May 1998 and accepted in revised form 7 October 1998.

CT, computed tomography; DEXA, dual-energy X-ray absorptiometry; S_i , insulin sensitivity index.

Obesity is a highly prevalent disorder that is associated with decreased longevity and increased morbidity from a variety of disorders and diseases—including hyperglycemia, hyperlipidemia, hypertension, and cardiovascular disease (1)—and carries significant economic costs. Efforts to treat obesity and its associated health risks have been largely disappointing, however. One reason for this is that its primary features have not been distinguished from those secondary events resulting from increased adipose tissue mass. One way to distinguish between these is to study weight-reduced obese individuals who have achieved and maintained a normal BMI.

The process of weight gain in obesity is complex and involves interactions of genetic, environmental, and neuroendocrine factors. Recent research suggests that leptin, a peptide hormone secreted by adipocytes, signals feedback inhibition of food intake and stimulates energy expenditure (2,3). In this regard, aberrant secretion of leptin, deficient leptin receptor function, and/or postreceptor signaling have all been shown to cause obesity in animal models (4–6) and humans (7,8). That leptin deficiency and/or receptor defects cause the same obese phenotype as in the genetically obese rodent models suggests a role for leptin in human obesity. Resistance to leptin perception by specific brain centers (9) and reduced efficiency of leptin uptake into the central nervous system among obese individuals (10) have been proposed as possible mechanisms for unrestricted weight gain. Leptin levels are nearly always increased in obesity in humans. Leptin levels, on the other hand, are reduced or increased to reflect the size of adipose tissue mass (11,12), suggesting that the increased plasma levels might be secondary to increased BMI.

The comorbidities of obesity, including type 2 diabetes, coronary heart disease, and hypertension, are closely associated with a variety of disorders and diseases, including hyperglycemia, hyperlipidemia, hypertension, and cardiovascular disease. Insulin resistance and the accompanying hyperinsulinemia represent a central component of these adversities (13). This relationship is most apparent in the abdominally obese, and particularly in those with increased visceral fat mass. As with leptin, it is not clear whether insulin resistance and hyperinsulinemia are primary events or occur secondarily to increased body fat, particularly its visceral component. On the one hand, research has demonstrated that the insulin resistance of obesity is likely to

involve a rate-limiting step in skeletal muscle glucose metabolism (14,15), implying a primary defect. On the other hand, other studies suggest that insulin resistance is an adaptive secondary response to prevent further weight gain (16,17).

The present study was therefore undertaken to distinguish leptin and insulin resistance as primary versus secondary events in obesity. Normalization of leptin and insulin concentrations with BMI in weight-reduced obese individuals would suggest that increases of their levels in obesity could be a consequence of increased fat mass. Within the TOPS (Take Off Pounds Sensibly) Club, a nonprofit weight support group, there exists a subset of the membership known as KOPS (Keep Off Pounds Sensibly) who have verifiable chapter records of having been obese, reduced their weight to normal BMI, and maintained this weight loss for at least 1 year. In a first protocol, we determined plasma leptin and insulin levels in a cohort of these women. Results were compared with groups of never-obese and currently obese individuals. In a second protocol, we extended this study to obtain more precise measurements of body composition and insulin sensitivity in subsets of these subject groups.

RESEARCH DESIGN AND METHODS

Subjects. In the first protocol, we determined fasting plasma leptin and insulin levels in cohorts of 22 currently obese (age 46.3 ± 1.9 years), 20 weight-reduced obese with normal BMI (age 46.0 ± 2.3 years), and 29 never-obese (age 34.1 ± 1.3 years) women, all premenopausal. BMI was determined from the individual's weight (kg) and the square of the height (m^2). Caucasian women were recruited from the TOPS organization. Their weight has been recorded weekly by their local chapter. Obese individuals were defined as having BMI of $>30 \text{ kg}/m^2$ throughout their adult lives. Weight-reduced obese subjects—whose recorded BMIs were initially $>32 \text{ kg}/m^2$; who lost $>15 \text{ kg}$ via diet, exercise, and group support to BMI $<27 \text{ kg}/m^2$; and who successfully maintained this weight goal for 1–5 years—were recruited from the KOPS membership. The never-obese group was recruited from individuals with BMI $<27 \text{ kg}/m^2$ throughout their weight history. Subject characteristics are detailed in Table 1.

In the second protocol, subset cohorts of 14 currently obese women (age 28.6 ± 4.3 years), 9 weight-reduced obese women with normal BMI (age 43.9 ± 2.3 years), and 20 never-obese women (age 30.8 ± 1.4 years) participated in further evaluations of plasma leptin levels in relation to body composition and insulin dynamics. These included dual-energy X-ray absorptiometry (DEXA) to measure total body fat and computed tomography (CT) scanning to determine abdominal-visceral and -subcutaneous fat components. They also underwent minimal model procedures to determine insulin sensitivity and early-phase insulin response. Subject characteristics are listed in Table 2.

Investigations were conducted at the General Clinical Research Center. The study protocol was approved by the Human Research Review Committee of the Medical College of Wisconsin. Written consent was obtained before the studies after thorough explanation of the nature of the study and the details of all procedures involved. After giving informed consent, all subjects underwent physical examination and history. Fasting blood samples were drawn to ascertain normal kidney, liver, and thyroid function. Subjects were taking no medications at the time of the study.

DEXA. DEXA measurements (18) were performed using a total-body scanner (XR-26; Norland Medical Instruments, Fort Atkinson, WI). The size of the scanning area is $198 \times 65 \text{ cm}$. A series of transverse scans from head to toe are performed. Algorithms used for analysis were provided by the software program as part of the XR-26 Scanner, which allows delineation of different regions of interest. Percent body fat, total fat volume, and lean body mass are estimated. The coefficient of variation for percent body fat reported is $\sim 2\%$.

CT. Fat distribution within the abdomen was determined by CT scanning. The intra-abdominal and subcutaneous areas of fat were assessed from three contiguous slices, with the center slice located at the midplane of the fourth lumbar vertebra (19). Scans were performed on a Highspeed Advantage CT Scanner (General Electric Medical Systems, Waukesha, WI) using a scan circle diameter of 48 cm. Axial slices of 1 mm were obtained from the superior to inferior surfaces of the third lumbar vertebra. Images were generated at 120 kV, 1-s scanning and 150–240 mA, and images were displayed on a 512×512 matrix. Quality control procedures were followed throughout the study. In addition, a trabecular bone constancy phantom consisting of three sections with known densities was placed on the subjects' abdomen during the abdominal scans. CT data

were expressed as cross-sectional mass of tissue. The total adipose tissue area, total soft tissue area, and mean attenuation of soft tissue on each cross-sectional image were determined. The subcutaneous and intra-abdominal adipose tissue areas were differentiated by encircling the abdominal muscular wall. The number of volume elements in the scan containing fat was determined by thresholding techniques (20). Computer software delineates tissue areas from which quantitative estimates of the amounts of adipose tissue, muscle, or bone could be estimated. The coefficient of variation for our laboratory with a single observer over 3 consecutive days is 1.75%.

Minimal model analysis: insulin sensitivity and acute insulin response.

We used the minimal model procedure to obtain detailed quantitative data of insulin sensitivity (21,22). Subjects were studied after a 12-h overnight fast. Throughout the study, subjects remained in a supine position and did not ingest any food or fluids except for iced water. After drawing two baseline samples (-15 and -5 min), intravenous glucose ($0.3 \text{ g}/\text{kg}$) was administered via a constant infusion over 1 min using accurately calibrated Harvard Infusion pumps (Harvard Apparatus, South Natick, MA). Twenty minutes after glucose administration, an intravenous bolus of insulin ($0.03 \text{ U}/\text{kg}$ of Regular insulin) (Humulin; Eli Lilly, Indianapolis, IN) was administered. Blood samples ($2\text{--}3 \text{ ml}$) were taken at the following time points, with time 0 indicating the beginning of the glucose infusion: 2, 3, 4, 5, 6, 8, 10, 14, 16, 19, 22, 24, 28, 30, 35, 40, 50, 70, 100, 120, 140, and 180 min. Blood samples were immediately placed on ice. After centrifugation, the plasma was separated and stored at -20°C until assayed for glucose and insulin. Plasma glucose and insulin levels were analyzed by mathematical modeling (23) using MinMod Program 3.0 (provided by R.N. Bergman). To obtain the insulin sensitivity index (S_i), the time course of serum glucose was fitted by using nonlinear least-squares methods, with the serum insulin values as a known input into the system.

The minimal model procedure was also used to determine the acute insulin response to intravenous glucose (23). First-phase insulin response was calculated from the areas under the curve (when expressed above basal values) at 2, 3, 4, 5, 6, 8, and 10 min after glucose administration, again using MinMod Program 3.0.

Analytical methods. Glucose was measured by the glucose oxidase method with a Beckman Glucose Analyzer II (Beckman Instruments, Brea, CA). Fasting insulin and leptin were assayed using solid-phase ^{125}I -labeled radioimmunoassay. Reagents were purchased from Linco Research (St. Charles, MO). Five pooled sera of increasing peptide concentrations were used to evaluate intra-assay coefficients of variation. Quality controls were included with each assay. The intra-assay coefficient of variation for insulin ranged from 4.4 to 10.6% over a concentration range of 20–660 pmol/l, and the intra-assay coefficient of variation for leptin ranged from 3.4 to 6.8% over the concentration range of 2–76 ng/ml.

Statistical analysis. Results are expressed as means \pm SE. One-way analysis of variance was used to compare the groups. A log transform was used to normalize the data. The Sidak multiple comparisons procedure was used to compare individual means after analysis of variance. Pearson correlations were used together with a log transform to normalize data. Multiple regression analysis with dummy variables was used to compare the regression lines.

RESULTS

Protocol 1. Figure 1A shows scattergrams of fasting plasma leptin levels in weight-reduced obese individuals relative to currently obese and never-obese subjects. Even though the two nonobese (the never-obese and the weight-reduced obese) groups did not differ in BMI or glucose (Table 1), despite the wide variances in each group, mean leptin levels were nonetheless significantly greater in the weight-reduced obese group ($P < 0.002$). Each was far less than the mean concentration seen in the obese cohort. Plasma leptin levels were highly correlated with BMI over the entire range of BMI ($r = 0.80$, $P < 0.001$; Fig. 2). Regression analysis yielded the following slopes and years-intercepts for the three groups: currently obese, 1.20 and -4.9 ; weight-reduced obese, 1.14 and -15.0 ; and never-obese, 1.22 and -18.4 , respectively. As evidenced by the slopes, the three groups demonstrated similar relationships between leptin and BMI, and the regression lines for the two nonobese groups showed no statistical difference.

Fasting plasma insulin levels in the never-obese and the weight-reduced obese with similar BMIs were also lower than those in the currently obese (Table 1, Fig. 1B). Like leptin, insulin levels in the weight-reduced obese women were

TABLE 1
Subject characteristics: protocol 1

	Weight-reduced obese	Never obese	Currently obese
<i>n</i>	20	29	22
BMI (kg/m ²)	24.2 ± 0.4	22.2 ± 0.5	35.6 ± 0.9
Plasma leptin (ng/ml)	12.2 ± 1.9*	7.4 ± 0.7	38.3 ± 2.9
Plasma glucose (mmol/l)	4.69 ± 0.17	4.83 ± 0.07	5.13 ± 0.20
Plasma insulin (pmol/l)	68.9 ± 9.7*	44.7 ± 3.4	126 ± 16

Data are means ± SE. **P* < 0.001 vs. never-obese women of comparable BMI.

significantly higher compared with those in the never-obese women (*P* < 0.005). Fasting plasma leptin and insulin levels were highly correlated in all subjects (*r* = 0.60, *P* < 0.001) and among individuals within the normal range of BMI (*r* = 0.47, *P* < 0.001).

Neither leptin nor insulin was significantly correlated with age.

Protocol 2. As with the larger subject groups in protocol 1, subjects participating in the more detailed evaluations (protocol 2) demonstrated a similar trend in plasma leptin levels (Table 2). Leptin levels in weight-reduced obese women were significantly higher than in never-obese women with comparable BMI (*P* < 0.006), whereas mean plasma leptin levels in both nonobese groups were greatly reduced from those in the currently obese group.

Despite comparable BMI in the weight-reduced obese and the never-obese groups, DEXA-determined percent body fat was significantly greater in the former (*P* < 0.001, Table 2). Total body fat was highly correlated with leptin in both nonobese groups (*r* = 0.90, *P* < 0.001, Fig. 3A). The relationship between plasma leptin levels and total body fat was significant in both the lean (*r* = 0.62) and the weight-reduced obese (*r* = 0.58) groups, with a *P* value of 0.003 between groups. There was no difference in mean CT-determined visceral fat masses between the two groups of nonobese subjects. However, despite similar levels of BMI, mean subcutaneous abdominal fat mass was significantly greater in the weight-reduced obese than in the never-obese (*P* < 0.001). As with percent total body fat, subcutaneous fat mass was significantly correlated with plasma leptin levels (*r* = 0.52, *P* < 0.001, Fig. 3B) in the two nonobese groups. The relationship

between subcutaneous fat mass and leptin in the never-obese (*r* = 0.37) versus the weight-reduced obese (*r* = 0.52) groups was significant at *P* < 0.001, and adjusting plasma leptin levels for subcutaneous fat mass eliminated the significance between the two lean groups. Total body fat was the only variable found to be significantly correlated with age (*P* = 0.02).

*S*₁, determined from the minimal model procedure, was significantly higher in the never-obese and weight-reduced obese than in currently obese subjects (Table 2). Mean *S*₁ values in the weight-reduced obese were not significantly different from those of the never-obese (3.10 ± 0.90 vs. 2.58 ± 0.58, respectively). Over the entire range of subjects, leptin and *S*₁ are correlated (*r* = -0.49, *P* = 0.002). The correlation is much weaker among the individual groups and is only significant for the never-obese group (*r* = -0.47, *P* = 0.04). When adjusted for visceral fat, there are no longer any significant differences among the three groups. Similarly, the first-phase insulin response to intravenous glucose was significantly lower in the two nonobese groups compared with currently obese subjects, and this difference disappears after correcting for visceral fat. As shown in Table 2, there is also no significant difference in the 10-min areas under the curve between weight-reduced obese and never-obese subjects with similar BMI and mean visceral fat mass (298 ± 45 vs. 347 ± 30, respectively). Leptin is correlated with acute insulin response over the three groups (*r* = -0.47, *P* = 0.003), but this relationship is not significant within any of the groups.

DISCUSSION

A number of studies suggest that leptin may play a regulatory role in weight control (7,8,11,24,25). Overfeeding increases leptin gene expression (26), whereas fasting results in reduc-

TABLE 2
Subject characteristics: protocol 2

	Weight-reduced obese	Never obese	Currently obese
<i>n</i>	9	20	14
BMI (kg/m ²)	24.3 ± 0.4	21.2 ± 0.5	39.3 ± 1.3
Plasma leptin (ng/ml)	13.6 ± 2.2*	7.8 ± 1.0	31.0 ± 3.1
Total body fat (%)	40.0 ± 2.0*	33.0 ± 1.1	49.6 ± 1.1
Abdominal-subcutaneous fat (g)	157 ± 20*	81.2 ± 7.5	414 ± 36
Abdominal-visceral fat (g)	56.1 ± 7.7	42.8 ± 5.8	194 ± 17
Insulin sensitivity (× 10 ⁻⁵ pmol · l ⁻¹ · min ⁻¹)	3.10 ± 0.90	2.58 ± 0.58	0.49 ± 0.14
First-phase insulin response (area under the 10-min curve)	298 ± 45	347 ± 30	676 ± 74

Data are means ± SE. Total body fat was measured by DEXA, and subcutaneous- and visceral-abdominal fat areas were measured by CT. *S*₁ and first-phase insulin response were determined from minimal model analysis as described in METHODS. **P* < 0.001 vs. never-obese women of comparable BMI.

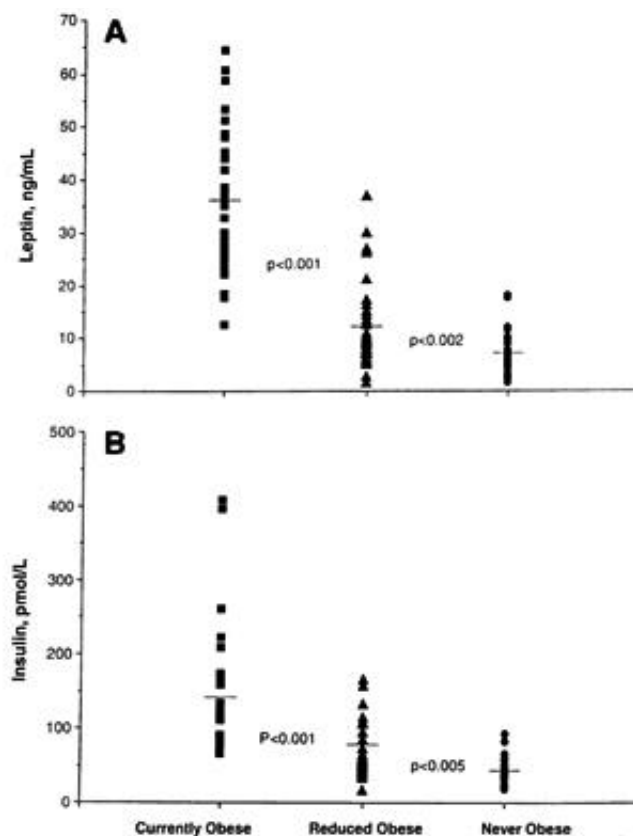


FIG. 1. Scattergrams of fasting plasma levels of leptin (A) and insulin (B) in currently obese, weight-reduced obese, and never-obese women.

tions in serum leptin (8,11). Serum leptin levels are also altered by changes in caloric intake, and changes in leptin concentrations are positively correlated with changes in fat mass in individuals undergoing weight loss (27). What is not known, however, is whether plasma leptin levels represent a primary cause of obesity. Were this so, then leptin levels would not be expected to decline to normal levels with normalization of BMI. Indeed, normalization of BMI in weight-reduced women did not reduce plasma leptin levels to those of the never-obese, even though values in weight-reduced women were significantly lower than those of obese women. Based on these initial results, one might thus conclude that the elevated plasma concentration of leptin in obesity might be a primary feature.

An alternative explanation comes from the detailed examination of body composition. These studies demonstrated strong associations between plasma leptin levels and DEXA-determined total body fat, as well as CT-determined abdominal subcutaneous fat. Although the never-obese range of BMI was achieved, the weight-reduced obese women retained greater levels of total body fat. Weight loss was associated with greater loss and, indeed, normalization of the visceral fat mass, but not of the subcutaneous abdominal fat component. Despite normalization of BMI, this fat depot remained nearly twice the size of that in never-obese individuals. The strong correlation between plasma leptin levels and subcutaneous fat mass represents one possible explanation for persistence of elevated plasma leptin levels in the weight-reduced obese. It is well known that leptin and leptin

gene expression vary among different regional fat depots (28,29). Cells from the subcutaneous fat depot produce more leptin mRNA than those from other sites and thus may be more consequential in increased leptin production. This regional specialization is also supported by a recent study (30) that demonstrated a stronger relation between leptin and hip circumference (predictive of subcutaneous fat) than between leptin and waist circumference (predictive of visceral fat) and by studies that measured subcutaneous adipose mass with magnetic resonance imaging (31,32).

As with plasma leptin, fasting insulin concentrations were not normalized with weight reduction to the normal range of BMI. The close correlation between plasma leptin and insulin levels suggests that the prevailing insulin concentration could contribute to the regulation of plasma leptin levels. Insulin directly regulates *ob* gene expression (33,34), and elevations of plasma insulin levels have resulted in increased leptin concentrations, as well (34–37). A recent study showing that increases of adipose tissue glucose metabolism appear to mediate insulin's actions on leptin production suggests that leptin production might be increased if more glucose is transported into adipocytes and subsequently metabolized (38). The fact that leptin undergoes a diurnal rhythm that is entrained to meal pattern is further support that insulin levels may alter leptin production (39). Leptin could thus represent a secondary response not only to high levels of subcutaneous fat but also to elevated plasma insulin levels.

Although plasma leptin levels were highly correlated with both total fat and subcutaneous fat mass in women, insulin resistance was more closely associated with abdominal visceral fat mass. Visceral fat mass, insulin sensitivity, and first-phase insulin response were all normalized upon weight reduction, whereas subcutaneous fat mass and leptin levels remained elevated. Intrigued by the possibility that insulin sensitivity might influence the tendency toward gaining weight, we examined relations in S_1 between the never-obese and the weight-reduced obese groups after adjusting for total body fat. There was no significant difference between groups, even after this adjustment. These results support our notion that the insulin resistance and accompanying hyperinsulinemia of obesity are closely linked to visceral fat mass (40). It is well

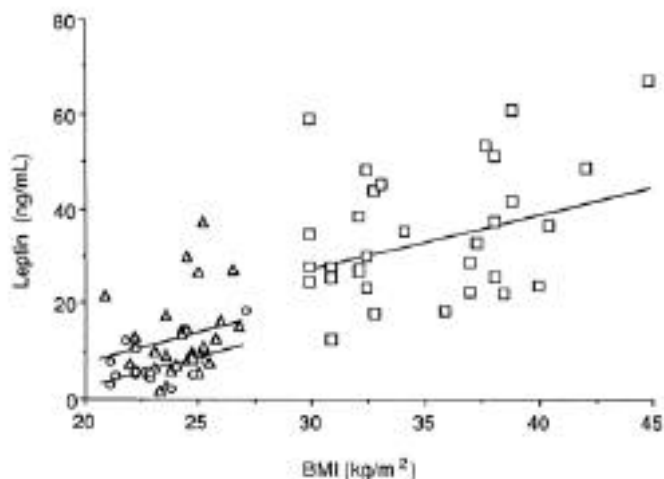


FIG. 2. Correlations between BMI and plasma values of leptin in never-obese (○), weight-reduced obese (△), and currently obese (□) women.

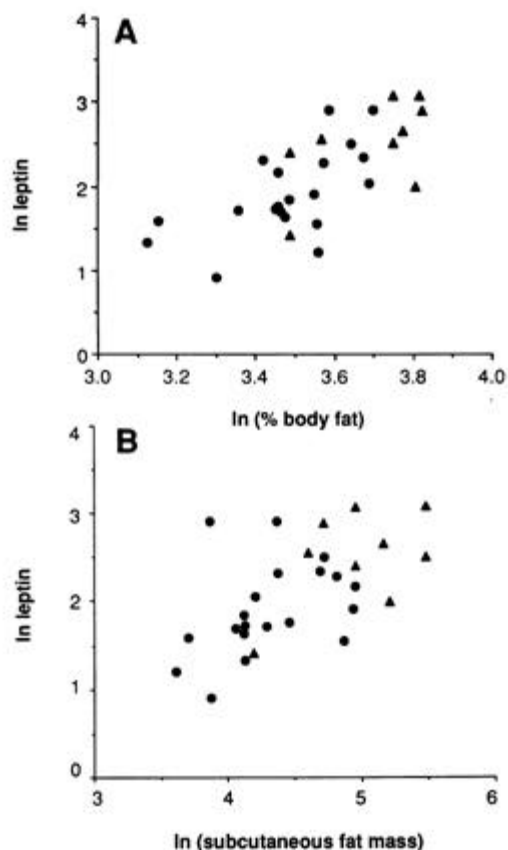


FIG. 3. Relationships of plasma leptin with DEXA-determined total body fat (A) and CT-determined subcutaneous abdominal fat mass (B) in never-obese (●) and weight-reduced obese (▲) women.

established that some of the hyperinsulinemia of obesity is due to increased β -cell secretory capacity, correlating with increased total body fat mass (41). Because increased insulin secretion is due to both hypertrophy and hyperplasia of pancreatic islets, the insulin secretory capacity may remain elevated, even after normalization of BMI by weight loss. This may explain why plasma insulin levels remain elevated in weight-reduced individuals. Weight reduction is, in fact, associated with diminished acute insulin response to intravenous glucose. Like insulin sensitivity, however, there is no difference in first-phase insulin secretion between the two nonobese groups; and the differences between the obese and nonobese groups in both S_I and acute insulin response are eliminated when adjusted for visceral fat, which again supports the close relationship between these parameters and abdominal visceral fat.

Our results are in discrepancy with those reported previously by Wing et al. (42), who demonstrated that plasma leptin levels are reduced and remained low in weight-reduced obese women. Their studies included individuals who were observed over a 6-month follow-up period with an average weight loss of 8.1 kg. Individuals in our studies, however, lost more than 15 kg and maintained their weight-reduced state for 1–5 years. On the other hand, a recent report in 14 postmenopausal women found that serum leptin concentrations after a mean weight loss of 12.0 kg declined from 31.8 ± 16 to 11.5 ± 5.4 ng/ml (43), which is comparable to levels seen in protocol 1 and higher than those seen in our never-obese group.

In the weight-reduced obese state in this study, leptin concentrations were not related to changes in fat mass or percent body fat. It is therefore possible that the amount and duration of weight loss, as well as the degree of residual subcutaneous obesity, could influence prevailing leptin levels in the weight-reduced obese, thus accounting for this discrepancy.

The natural history of obesity is characterized by repeated bouts of weight loss and regain. Although inclusion of behavioral and activity changes seems to improve the outcome, the majority of individuals experience relapses of weight regain (44). Biological adaptations may play a role in restoration of the obese state. Obese individuals who reduced their weight by 10% or more exhibit lower levels of energy expenditure than expected from their lean body mass (45). That biological adaptations may occur in response to weight loss has extremely important implications for both weight reduction and weight maintenance. A reduction in plasma levels of leptin with weight loss may interfere with the neural control of food intake. If reductions in plasma leptin contribute in this manner to the frequently common lack of success in weight maintenance, then giving leptin to women who have successfully lost weight may improve chances for successful maintenance of the weight-reduced state. Validity rests on prospective studies and clinical trials of leptin.

In summary, this pilot study indicates that in premenopausal women, elevation in plasma leptin levels is a feature of the weight-reduced state, even in individuals who achieved normal levels of BMI. The increase in plasma leptin could be secondary to the greater amount of fat in the subcutaneous compartment. This fat depot, which expresses increased capacity to produce leptin, may also be responsible for the elevated plasma insulin level, which, in turn, can support overproduction of leptin. Whether this increase in plasma leptin levels plays a role in successful maintenance of the weight-reduced state, as least in some individuals, remains speculative. Our results also support the notion that in premenopausal women, insulin resistance and impaired first-phase insulin response are secondary features of obesity that are completely ameliorated by reduction of visceral fat, but not total body fat, to the normal range. Prospective studies are needed to further explore the validity of these concepts.

ACKNOWLEDGMENTS

This study was supported in part by grants HL-34989 and RR-00058 from the National Institutes of Health.

We greatly appreciate the skillful assistance of the General Clinical Research Center nursing, nutrition, data management, and laboratory staff. We especially thank Keryl Jones, RN, for her recruitment and coordination of volunteers.

This work was presented in part at the annual meeting of the North American Association for the Study of Obesity (NAASO), Breckenridge, Colorado, 1996.

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Author Queries (please see Q in margin and underlined text)

Q1: AU: Please list the supplement number, if available, for ref. 40.