Is Fasting Leptin Associated With Insulin Resistance Among Nondiabetic Individuals?

The Miami Community Health Study

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OBJECTIVE — Whether serum leptin levels are associated with insulin resistance independent of the effects of hyperinsulinemia and adiposity is an important unanswered question. We examined the relationship between the rate of insulin-mediated glucose uptake and serum leptin concentrations among nondiabetic men and women.

RESEARCH DESIGN AND METHODS — A cross-sectional analysis was performed among 49 young to middle-aged men and women who participated in the Miami Community Health Study. All participants had measures of insulin resistance (euglycemic-hyperinsulinemic clamp), postchallenge insulin levels, fasting serum leptin levels, and several measures of adiposity.

RESULTS — The rate of insulin-mediated glucose uptake (M in milligrams per kilogram per minute) was significantly associated with leptin concentrations in both men ($r = -0.83$; $P < 0.001$) and women ($r = -0.59$; $P < 0.001$). M was also inversely related to percent body fat and to the 2-h insulin area under the curve (AUC). After covariate adjustment for sex, percent body fat, and AUC, leptin remained a significant correlate of M ($P = 0.04$).

CONCLUSIONS — Cross-sectionally, leptin was significantly associated with insulin resistance in this nondiabetic sample of men and women. There may be a different physiological mechanism to explain the leptin/insulin resistance association apart from the insulin/adiposity link. Confirmatory evidence awaits the results of clinical trials.

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Regulation of weight and weight gain is a complex process that involves a number of behavioral, social, and hormonal factors. Among the latter, roles for insulin and leptin have been postulated, although the evidence is not consistent (1–4). A current hypothesis holds that leptin is a "lipostat," regulating appetite through a negative feedback mechanism, and may counteract the anabolic fat-storing effects of insulin (5,6).

Increased body fat is also accompanied by increased insulin resistance (7). Under normal conditions, insulin resistance is followed by peripheral hyperinsulinemia to maintain euglycemia. Whether high levels of leptin are associated with insulin resistance per se, independent of concomitant hyperinsulinemia and adiposity, is not completely understood (8–10). If an independent association were found, this would support the hypothesis that leptin may regulate insulin resistance, and thus play an important pathophysiologic role in type 2 diabetes separate from the effects of adiposity and hyperinsulinemia.

The purpose of this study was to examine the association between insulin resistance (determined directly with the hyperinsulinemic-euglycemic clamp), and plasma leptin concentrations among a random sample of nondiabetic men and women. The results suggest that even after accounting for adiposity and hyperinsulinemia (as indexed with the 2-h insulin area under the curve [AUC]), leptin was significantly related to the rate of insulin-mediated glucose disposal.

RESEARCH DESIGN AND METHODS

— The details of this study have been previously described (11). In brief, the Miami Community Health Study is an epidemiological study designed to comprehensively assess the physiological and behavioral correlates of blood pressure and other cardiovascular disease risk factors in African-Americans, Cuban-Americans, and non-Hispanic whites living in Dade County, FL. The study cohort was selected from census tracts (1990 U.S. Census) located within 10 miles of the University of Miami School of Medicine, with populations composed of at least 80% of the targeted ethnic groups. Participants were recruited and examined between 1991 and 1995. The participation rate of those known to be eligible was 53%.

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Abbreviations: AUC, area under the curve; M, insulin-mediated glucose uptake.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

All subjects underwent extensive examinations for cardiovascular risk factor testing, which included a standard 75-g oral glucose tolerance test. People testing positive for diabetes (World Health Organization criteria; fasting glucose ≥140 mg/dl or a 2-h glucose >200 mg/dl) were ineligible to participate in the clamp protocol. All people included in this report had a fasting glucose <126 mg/dl. Glucose was assayed by the glucose-oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Insulin was assayed with a double-antibody radioimmunoassay technique (Diagnostic Products, Los Angeles, CA). The 2-h insulin AUC was calculated using the trapezoidal rule and was used as a measure of circulating hyperinsulinemia. The interassay coefficient of variation for fasting glucose was 2.7%, and that for fasting insulin was 15%. Intra-assay coefficients were <2 and 11%, respectively.

Table 1—*Selected variables of study populations according to sex*

	Men	Women
n	23	26
Age (years)	34.5 ± 5.9	35.7 ± 7.0
BMI (kg/m^2)	27.3 ± 7.1	27.5 ± 7.7
% Non-Hispanic white	60.8 (14/23)	50.0 (13/26)
% African-American	30.4 (7/23)	42.3 (11/26)
% Cuban-American	8.7 (2/23)	7.8 (2/26)
% Body fat	27.9 ± 7.1	36.7 ± 7.0
Fasting insulin (pmol/l)	4.8 ± 0.4	4.7 ± 0.3
Fasting glucose (mmol/l)	4.8 ± 0.4	4.7 ± 0.3
ln (leptin) (ng/dl)	1.69 ± 0.77	2.69 ± 0.96
M (mg · kg^{-1} · min^{-1})	7.7 ± 3.6	8.19 ± 3.0
2-h insulin AUC (pmol · l^{-1} · 120 min^{-1})	714 ± 696	606 ± 354

Data are means ± SD or % (*n*/total).

Hyperinsulinemic-euglycemic clamp

Beginning in January 1993, 58 of 107 (54.2%) consecutive subjects participated in an investigation designed to directly measure insulin resistance using the euglycemic-hyperinsulinemic clamp technique (12). The details of this examination have been previously published (13).

Participants were required to fast for 10–12 h the night before their clinic visit. No formal instructions were given with respect to dietary intake. Sensitivity to insulin-mediated glucose disposal was determined with the hyperinsulinemic-euglycemic clamp procedure. At 8:30 A.M., compliance with fasting instructions was determined (noncompliance resulted in rescheduling). An angiocatheter (20 gauge) was placed in the right forearm for infusion, and a second angiocatheter (22 gauge) was placed in retrograde fashion in a vein in the left hand or wrist. Blood samples were obtained for fasting glucose and insulin concentrations. An insulin primer was infused over a 10-min period, followed by a constant infusion of 40.0 μU · m^{-2} · min^{-1} for 120 min. Euglycemia was maintained within 5% of the fasting value by a variable infusion of 20% dextrose solution. The serum glucose level was determined every 5 min, and the rate of glucose infusion was adjusted accordingly. Euglycemia was maintained for 2 h. During the second hour in steady-state hyperinsulinemia, the quantity of glucose metabolized was calculated as the mean of the glucose infusion rate. This infusion rate (M) reflects the total insulin-stimulated glucose metabolism and assumes complete suppression of hepatic glucose output. Radiolabeled glucose was not used to quantify hepatic glucose output because the insulin dose used in this study (1 μU · ml^{-1} · kg^{-1} body wt · min^{-1}) suppresses endogenous glucose production in healthy individuals (14). This rate of insulin infusion half-maximally stimulates whole body glucose uptake in normal people. Changes in both insulin sensitivity and responsiveness will alter glucose uptake when this insulin concentration is used. Upon termination of the clamp procedure, participants remained in the clinic until their glucose concentrations returned to the baseline level.

Leptin assay

Fasting serum samples were frozen at −70°C for a median time of 2 years. Adequate frozen samples were available for 49 of the 58 participants (84.5%). These samples were sent on dry ice to Melbourne, Australia, for analysis. Leptin concentrations were measured with a solid-phase double-antibody enzyme immunoassay with affinity-purified polyvalent antibodies. Concentrations were calculated from standard curves generated with recombinant human leptin. The limits of detection for the leptin assays were 20 pg/ml in serum or plasma. The interassay coefficient of variation was 8.45% and the intra-assay coefficient of variation was 7.7% for the high standard and 10.5% for the low standard.

Anthropometry

All anthropometric measures were made with the participant wearing light clothes and no shoes. Weight was recorded on a balance-beam scale and recorded to the nearest 0.25 lb. Height was measured to the nearest 0.5 cm. BMI was calculated as weight (kilogram) divided by height (meter^2). To estimate percent body fat, body density was calculated from subcutaneous skinfold-thickness measurements using age- and sex-specific prediction equations published by Durnin and Womersley (15). For non-Hispanic white and Cuban-American participants, percent body fat was calculated from body density using the Siri equation (16): percent body fat = (4.95/density − 4.50) × 100. For African-Americans (who have a greater density of lean body mass than whites), percent body fat was calculated using the equation published by Schutte et al. (17): percent body fat = (4.374/density − 3.928) × 100. The study was approved by the University of Miami School of Medicine Internal Review Board, and all participants gave written informed consent.

Statistical analysis

Preliminary analyses indicated that the distribution of leptin was skewed to the right. Log transformation (natural log) was used, which yielded more normally distributed data. Pearson product-moment correlations were used to assess the association among continuous variables. Multiple linear regression models were fit to estimate the association between the rate of insulin-mediated glucose uptake (M), and fasting leptin concentrations, adjusted for covariates. With this technique, the rate of insulin-mediated glucose uptake (M) served as the dependent variable. The major predictor variable was ln (leptin). The effects of several covariates, including sex, ethnicity, percent body fat, and the

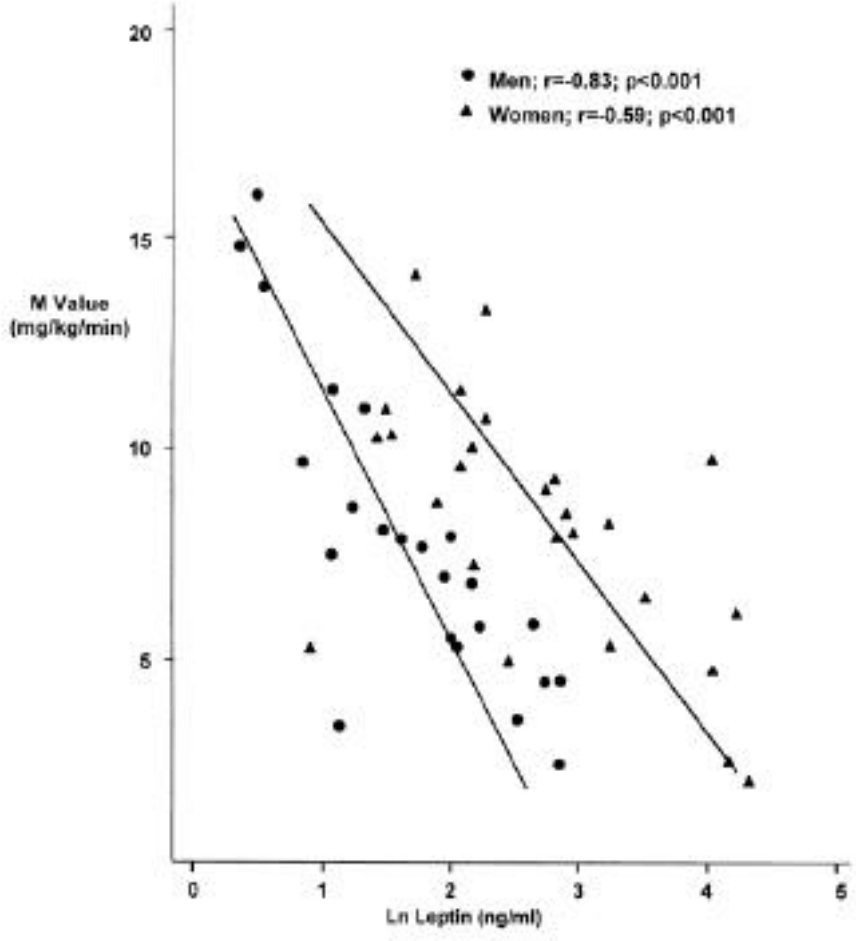

Figure 1—*Correlation of ln (leptin) versus the rate of insulin-mediated glucose uptake (M mg/kg/min) according to sex.*

2-h insulin AUC, were controlled in the final model. Before fitting the model, potential effects of collinearity were examined using correlation matrices. To examine whether the effect of leptin on M was modified by sex, for example, interaction terms were modeled by including the appropriate cross-product term. All were nonsignificant ($P = 0.20$). The β-coefficient associated with each independent variable may be interpreted as the predicted change in M, given a unit change of the independent variable. All analyses were performed using the Statistical Analysis System (18). All statistical tests were two-sided.

RESULTS — Table 1 presents selected descriptive characteristics of the study sample according to sex. The mean age was ~35 years in both men and women. The study population was composed mainly of non-Hispanic white and African-American participants. As expected, women had a higher mean percentage of body fat than men, even though their mean BMIs were nearly identical. Mean ln (leptin) concentrations were higher among women, and the rate of insulin-mediated glucose disposal (M milligrams per kilograms per minute) was similar between the sexes, as were mean levels of fasting insulin and fasting glucose. The 2-h insulin AUC was slightly higher among men. A scatterplot of ln (leptin) on M is provided in Fig. 1 separately for each sex. There was a clear inverse association among both men ($r = -0.83$) and women ($r = -0.59$; $P < 0.0001$ for both).

The association between M (milligrams per kilograms per minute) and selected variables is presented in Table 2. In both sexes, measures of adiposity were significantly correlated with M (inversely) and ln (leptin) (positively). The 2-h insulin AUC was correlated with both M and ln (leptin) among men ($r = -0.52$ and 0.53, respectively) and women ($r = -0.40$ and 0.34; $P < 0.10$ for each).

To evaluate the association between ln (leptin) and M independent of the covariates, multiple linear regression was used (Table 3). The data for men and women were combined because there was no interaction noted between sex and leptin. After adjustment for the effects of sex, percent body fat, and the insulin AUC, ln (leptin) was significantly and inversely related to the glucose disposal rate ($\beta = 1.14$; $P = 0.04$). Thus, for each unit increase in ln (leptin), M was predicted to change by 1.14 mg · kg^{-1} · min^{-1}. As shown, the insulin AUC was also negatively related to M after considering the effects of ln (leptin) and other covariates. These results were essentially unchanged when BMI was substituted for percent body fat, or when an indicator variable for ethnicity was included in the model.

CONCLUSIONS — This analysis was undertaken to test the hypothesis that fast-

Table 2—*Matrix of Pearson correlation coefficients among selected variables according to sex*

	% Body fat	AUC	M value	ln (leptin)
Men				
BMI	0.75*	0.46†	−0.71*	0.78*
% Body fat	—	0.40†	−0.70*	0.71*
AUC	—	—	−0.52‡	0.53‡
M value	—	—	—	−0.83*
Women				
BMI	0.80*	0.26	−0.75*	0.81*
% Body fat	—	0.09	−0.67*	0.72*
AUC	—	—	−0.40	0.34
M value	—	—	—	−0.59*

*$P < 0.001$; †$P < 0.05$; ‡$P < 0.01$.

Table 3—*Multiple linear regression of selected variables on M value (mg · kg^{-1} · min^{-1})*

Independent variable	β	SE(β)	*P* value
Sex	−3.2	0.84	<0.001
% Body fat	−0.19	0.06	0.01
Ln (leptin)	−1.14	0.56	0.04
Insulin AUC (pmol/l)	−0.54	−0.27	0.04

Independent variables included % body fat, ln (leptin) (ng/ml), insulin response (pmol · l^{-1} · 120 min^{-1}), and sex (coded as 0 - women, 1 - men).

ing leptin was associated with insulin resistance, independent of adiposity or hyperinsulinemia. Our results provide evidence in favor of this hypothesis and further suggest that leptin may effect the risk of type 2 diabetes separate from the risk due to obesity. These results are consistent with the observations of Haffner et al. (10), who showed that leptin was inversely related to the rate of glucose disposal among men after adjustment for BMI or the waist-to-hip ratio. The current study extends these findings to premenopausal women, and uses an estimate of percent body fat rather than BMI. We have previously shown that fasting plasma insulin was inversely related to fasting leptin levels independent of adiposity and other covariates in this multiethnic cohort (19). The current study furthers our previous work by using a direct assessment of insulin resistance (e.g., the hyperinsulinemic-euglycemic clamp), and demonstrates that the relationship between leptin level and insulin resistance per se was reduced, but not eliminated, after accounting for concomitant hyperinsulinemia.

An additional strength of the current study is the inclusion of both men and women from a defined population with a wide range of obesity. Studies that have examined women have generally included many who may be peri- or postmenopausal (8,9,20). The current study is one of the few to extend this research to premenopausal women, although the sample size was limited. Thus, the mechanism(s) that links leptin with insulin resistance may be common to both sexes. Kennedy et al. (9) failed to note an association between insulin resistance and leptin levels among 35 normoglycemic women (mean age 36 years), although it is unclear whether these women were randomly sampled from a defined population. Whether a sexual dimorphism exists in the leptin/insulin resistance linkage is an important topic for future research.

Our results fail to confirm the findings of others who concluded that the association between hyperleptinemia and insulin resistance was secondary to hyperinsulinemia (8). Instead, our data suggest that increased leptin levels were directly associated with insulin resistance independent of adiposity and hyperinsulinemia. This result is consistent with the work of Schwartz et al. (20) and Segal et al. (21), who reported that plasma leptin levels are associated with insulin and adiposity through different mechanisms. Plasma leptin levels clearly reflect the amount of body fat, consistent with the hypothesis that leptin works as a "lipostat" to regulate food intake (1,5,6). Our present results suggest that leptin may be involved in regulating insulin resistance independent of the concomitant body fat and circulating insulin levels. Others (22) have indicated that leptin may modify the β-cell response, perhaps allowing the islets to secrete more insulin when fat content is high. We had no measure of insulin secretion in the current study, and thus could not directly address this possibility. Unfortunately, we were unable to assess β-cell secretion in this study.

Overall, our data are consistent with the view that the inverse association between insulin resistance and leptin is partly, but not entirely, dependent upon the level of obesity and hyperinsulinemia. This hypothesis can be directly tested in clinical trials by administering leptin and measuring its effect on insulin sensitivity. Such trials are already underway.

In conclusion, we have found that plasma leptin levels are associated with insulin resistance in this nondiabetic sample of young to middle-aged men and women. Our data are consistent with the hypothesis that plasma leptin has an independent effect on insulin resistance that is not entirely explained by adiposity or hyperinsulinemia. Indeed, there may be a pathway through which leptin regulates insulin resistance that is separate from the insulin/obesity mechanism. Thus, risk of developing obesity-related diseases, including type 2 diabetes, may be, at least in part, a function of both insulin resistance and leptin resistance among susceptible individuals.

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