Plasma Leptin Concentrations Do Not Appear to Decrease Insulin-Mediated Glucose Disposal or Glucose-Stimulated Insulin Secretion in Women With Normal Glucose Tolerance

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The aim of this study was to test the hypothesis that plasma leptin concentrations contributed to the pathophysiology of NIDDM by decreasing both insulin-mediated glucose disposal and glucose-stimulated insulin secretion. The study was performed in 60 women with normal oral glucose tolerance. Differences in insulin-mediated glucose disposal were determined by comparing the steady-state plasma glucose (SSPG) concentrations attained at the end of a 180-min constant infusion of somatostatin, glucose, and insulin, while comparisons of glucose-stimulated insulin secretion were based on the incremental increase in insulin concentration 30 min after an oral glucose challenge (ΔIns) as compared with the fasting value. The results showed that the higher the fasting plasma leptin concentration, the greater the degree of insulin resistance ($r = 0.47$, $P < 0.01$). Furthermore, multiple regression analysis indicated that the relationship between leptin and SSPG was independent of age and degree of obesity as estimated by BMI. However, since the total integrated plasma insulin response was highly correlated with both SSPG ($r = 0.80$, $P < 0.001$) and leptin ($r = 0.55$, $P < 0.01$), multiple regression analysis was repeated, adding total insulin response to the model. When this was done, the significant relationship between leptin and SSPG disappeared, whereas both BMI ($P < 0.03$) and insulin response ($P < 0.001$) were correlated with SSPG. A significant relationship between leptin and ΔIns was seen, but it was a positive one ($r = 0.31$, $P < 0.02$), not a negative one as would be expected if circulating levels of leptin inhibited glucose-stimulated insulin secretion. Furthermore, multiple regression analysis could only confirm an independent relationship between ΔIns and SSPG, but not between ΔIns and leptin. The results of these studies do not support the view that circulating leptin has a primary effect on either insulin action or secretion in normal female volunteers. It seems more likely that chronic hyperinsulinemia in insulin-resistant individuals acts to increase adipose tissue leptin synthesis and secretion, leading to higher ambient leptin concentrations. *Diabetes* 47:244–247, 1998

Evidence from a prospective study on Pima Indians (1) has led to the view that both resistance to insulin-mediated glucose disposal and a decrease in the early insulin response to glucose were independent predictors of the progression from normal glucose tolerance to NIDDM. Based on these observations, it seems obvious that it would be important to understand the factors controlling these two crucial variables in the pathophysiology of NIDDM. In this context, it could be argued that leptin plays a central role in the development of NIDDM by postulating that leptin resistance contributes to obesity (2,3), a well-recognized risk factor for NIDDM (4,5). Furthermore, there is evidence that the increase in leptin levels characteristic of obese individuals (2,3) can lead to insulin resistance, as well as compromising the ability of the β-cell to compensate for the insulin resistance (6–10). The present study was initiated to examine this formulation by examining the relationship between leptin concentration, insulin resistance, and the early insulin response to oral glucose in 60 healthy women with normal oral glucose tolerance.

RESEARCH DESIGN AND METHODS

A total of 60 healthy women volunteered for this study; 51 were of European ancestry, 5 were Asian, 3 Hispanic, and 1 was African-American. Age range was 31–68 years (50 ± 10 [mean ± SD]). BMI, used as an estimate of degree of obesity, ranged from 19.1 to 35.6 kg/m^2 (29.3 ± 4.1). The volunteers were selected on the basis of a normal medical history, physical examination, routine laboratory tests, and electrocardiogram.

After an overnight fast, blood was drawn for measurement of plasma glucose (11) and insulin (12) concentrations before and 30, 60, 120, and 180 min after the ingestion of a 75-g oral glucose challenge. Only volunteer subjects with normal glucose tolerance by the criteria of National Diabetes Data Group (13) were included in the study. The incremental increase in plasma insulin concentration during the first 30 min (ΔIns) after the glucose challenge was selected as the measure of the insulin secretory response.

On a different day, the ability of insulin to promote glucose uptake was estimated by a modification (14) of the insulin suppression test as validated by our laboratory (15). After an overnight fast, an intravenous catheter was placed in each of the patient's arms. Blood was sampled from one arm for measurement of plasma glucose and insulin concentrations, and the contralateral arm was used for administration of test substances. Somatostatin was administered (250 μg/h in a solution containing 2.5% [wt:vol] human serum albumin) to suppress endogenous insulin secretion. Simultaneously, insulin and glucose were infused at rates of 25 mU · m^2 · min^{-1} and 240 mg · m^2 · min^{-1}. Blood was sampled every 30 min until

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ΔIns, oral glucose challenge; SSPG, steady-state plasma glucose; SSPI, steady-state plasma insulin.

150 min into the study, and then every 10 min until 180 min had elapsed. The four values obtained from 150 to 180 min were averaged and considered to represent the steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations achieved during the infusion. Because SSPI concentrations are similar in all individuals, SSPG concentrations provide a direct estimate of insulin-mediated glucose disposal in each individual: the lower the SSPG, the more insulin-sensitive the individual.

Fasting blood samples for leptin assay were collected in an EDTA tube and immediately centrifuged, and the plasma stored at 70°C. These samples were not thawed until leptin was measured (16) by a commercial radioimmunoassay (Linco Research, St. Louis, MO).

Data are expressed as mean ± SE or mean ± SD. Pearson's product moment correlations were calculated to determine relations between variables of interest. Multiple regression analysis was performed to assess the independent effect of related variables. Data were stored and analyzed using Systat 6.0 package for Windows, and the 0.05 level of probability was taken to be statistically significant.

RESULTS

The scatterplot in Fig. 1 demonstrates the presence of a significant relationship between fasting leptin levels and BMI ($r = 0.64$, $P < 0.001$) in our population of normal women with varying degrees of obesity.

To evaluate the possibility that elevated leptin levels lead to insulin resistance, we examined the degree of correlation between leptin and SSPG. Figure 2 displays the nature of this relationship in the 60 volunteers. It can be seen from these data that the two variables were significantly correlated ($r = 0.47$, $P < 0.01$). However, since both obesity and age (2,3,8,17) have been shown to correlate with leptin concentrations, multiple regression analysis was performed to evaluate the degree to which age, BMI, and leptin predicted SSPG.

The results of the multiple regression analysis are seen in Table 1 and indicate that leptin was an independent predictor of SSPG ($P < 0.002$). However, the plasma insulin response to oral glucose is known to correlate with SSPG (18), and leptin has been shown to be correlated with plasma insulin concentrations (19,20). Therefore, it could be argued that the relationship between SSPG and leptin identified in Table 1 was confounding, and actually due to the association between insulin and SSPG. Indeed, in the present study, the total integrated insulin response to glucose was highly correlated with both SSPG ($r = 0.80$, $P < 0.001$) and leptin ($r = 0.55$, $P < 0.01$). To pursue this issue further, the multiple regression analyses were repeated, adding insulin response to the model. These results are shown in Table 2 and demonstrate that under these conditions leptin was no longer independently related to SSPG, whereas insulin response ($P < 0.001$) and BMI ($P < 0.03$) were. Furthermore, the addition of insulin response to the model increased the r^2 of the model to a value of 0.69.

To address the issue of the link between leptin and β-cell secretion, we used as our measure the incremental increase in plasma insulin over baseline levels seen 30 min after the oral glucose challenge (ΔIns). The relationship between these two variables is displayed in Fig. 3, and shows that the higher the leptin concentration, the greater the insulin secretory response ($r = 0.31$, $P < 0.02$). To assess the role of other relevant variables in the prediction of ΔIns, multiple regression analyses were performed, with age, BMI, leptin, and SSPG as the independent variables. These results, given in Table 3, demonstrate that the only independent relationship was between ΔIns and SSPG ($P < 0.001$).

DISCUSSION

This study was initiated to evaluate the possibility that plasma leptin concentrations play a significant role in regulation of both insulin-mediated glucose disposal and pancreatic β-cell secretory function. Based on our results, we must conclude that both of these possibilities seem to be unlikely in normal, healthy women. On first glance, this conclusion seems to be at odds with the results of several published studies. On the other hand, after studying these publications, we believe the differences are more apparent than real.

The apparent disparity between our data and previous publications is greatest as regards the association between leptin

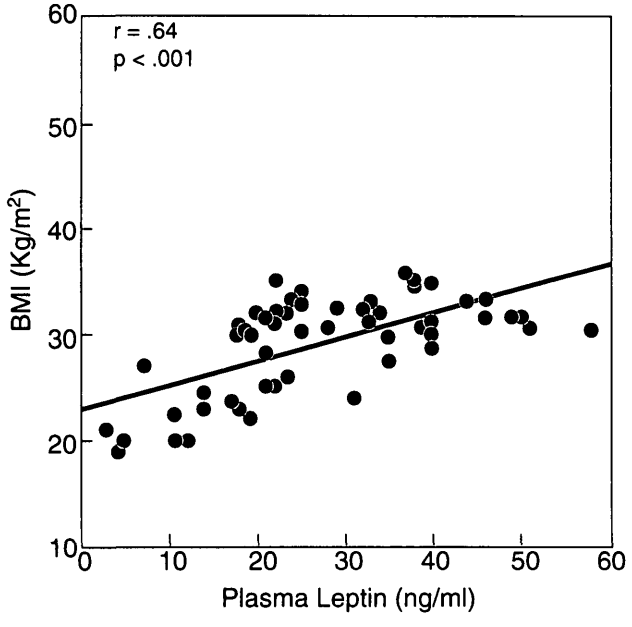

FIG. 1. Relationship between fasting plasma leptin concentration and BMI.

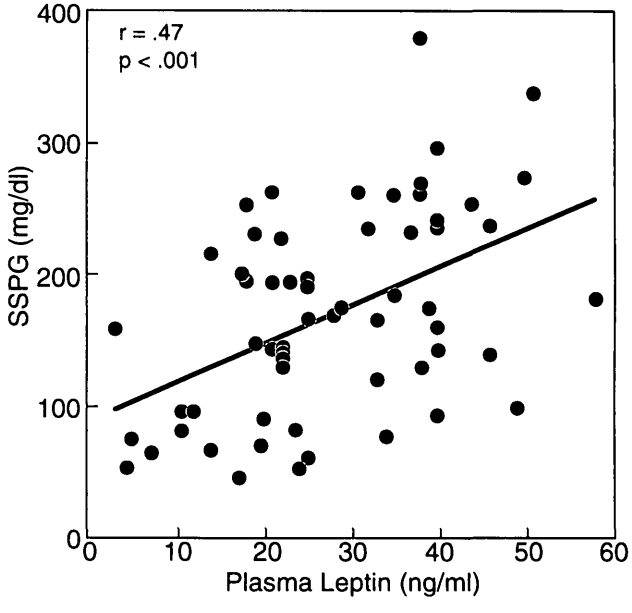

FIG. 2. Relationship between fasting plasma leptin concentration and resistance to insulin-mediated glucose disposal (SSPG).

TABLE 1
Multiple regression analysis of the relationships between insulin resistance and age, BMI, and plasma leptin

Variable	Regression coefficient	SE	Standardized regression coefficient	*P* value
Age	0.085	0.9	0.11	0.35
BMI	–0.23	2.7	–0.01	0.9
Leptin	2.99	0.9	0.49	0.002

Dependent variable, insulin resistance (SSPG); independent variables, age, BMI, and plasma leptin. R^2 for the entire model = 0.24.

levels and insulin resistance. Thus, evidence has been published that leptin concentrations are significantly related to direct measures of resistance to insulin-mediated glucose disposal (6,21). However, in neither of these studies was any effort made to consider the possibility that the observed relationship was secondary to the well-known relationship (18) between insulin resistance and plasma insulin concentrations. The results in Table 2 indicate that this was the case in our study, demonstrating that the integrated insulin response to oral glucose, but not insulin resistance, was independently correlated with leptin. Furthermore, the view that insulin and leptin are related is quite consistent with evidence that chronic hyperinsulinemia will increase leptin levels in humans, as well as inducing the expression of *OB* mRNA and leptin synthesis by cultured adipocytes (22). Given all of this information, we think the most likely sequence of events is that insulin resistance in nondiabetic individuals is associated with a state of compensatory hyperinsulinemia, which increases adipocyte leptin synthesis, resulting in higher circulating levels of plasma leptin. This conclusion does not rule out the possibility that leptin can also directly affect insulin action, as suggested by the in vitro demonstration that leptin attenuated several insulin-mediated activities when incubated with HepG2 cells (6). However, the significance of these findings to the in vivo situation remains to be clarified.

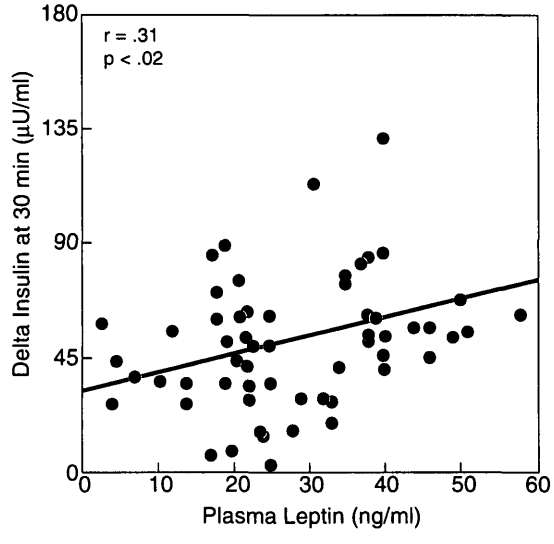

FIG. 3. Relationship between fasting plasma leptin concentration and the incremental insulin response over fasting 30 min after oral glucose (ΔIns).

TABLE 2
Multiple regression analysis of the relationships between insulin resistance and age, BMI, insulin response, and plasma leptin

Variable	Regression coefficient	SE	Standardized regression coefficient	*P* value
Age	0.17	0.59	0.02	0.7
BMI	4.1	1.8	0.23	0.028
Leptin	–0.89	0.73	–0.14	0.23
Insulin response	0.60	0.006	0.85	0.0001

Dependent variable, insulin resistance (SSPG); independent variables, age, BMI, plasma leptin, and insulin response. R^2 for the entire model = 0.69.

Evaluation of the possibility that leptin inhibits β-cell insulin secretion appears to be more straightforward. Thus, we are unaware of any evidence in humans that supports the evidence that leptin can inhibit in vitro insulin secretion in mice (9,10). Indeed, even in these experiments, the ability of leptin to directly inhibit insulin secretion was observed in islets isolated from *ob/ob* and wild-type mice, but had no effect on insulin secretion from the pancreas of Zucker *fa/fa* or islets from *db/db* mice. In contrast, the results of this and multiple other studies in humans demonstrate that the higher the leptin level, the higher the plasma insulin concentration.

Although the results of these studies do not support the view that elevated leptin levels play a significant role in regulation of either insulin-mediated glucose disposal or insulin secretion, they do offer a coherent view of the relationship between leptin concentrations and insulin metabolism. As discussed previously, there is evidence that chronic hyperinsulinemia will lead to increased adipose tissue leptin synthesis and higher leptin concentrations. Hyperinsulinemia is also known to be related to degree of insulin resistance (18). Irrespective of degree of obesity, it is suggested that the presence of chronic hyperinsulinemia in insulin-resistant subjects will increase adipose tissue leptin synthesis and secretion, resulting in higher leptin levels in insulin-resistant subjects. The addition of obesity will exaggerate the situation in any individual, both by increasing adipose tissue mass, the site of leptin synthesis and secretion, as well as by accentuating degree of insulin resistance. In this formulation, leptin does not play a regulatory role, and the increases in circulating leptin concentrations

TABLE 3
Multiple regression analysis of the relationships between insulin secretion and age, BMI, plasma leptin, and insulin resistance

Variable	Regression coefficient	SE	Standardized regression coefficient	*P* value
Age	–0.24	0.365	–0.084	0.51
BMI	–1.06	1.034	–0.161	0.31
Leptin	0.432	0.362	0.189	0.24
SSPG	0.216	0.058	0.575	0.0001

Dependent variable, insulin secretion (ΔIns 30); independent variables, age, BMI, plasma leptin, and insulin resistance (SSPG). R^2 for the entire model = 0.33.

in insulin-resistant individuals, obese or nonobese, are primarily a function of the ensuing hyperinsulinemia. We believe this to be the only formulation of the relationship between leptin, insulin action, and insulin secretion that is consistent with currently available data.

In conclusion, circulating leptin does not appear to have a primary effect on either insulin action or secretion in humans. Rather, we think it highly likely that chronic hyperinsulinemia in insulin-resistant individuals acts to increase adipose tissue leptin synthesis and secretion and elevate plasma leptin levels.

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