Plasma Insulin Concentration Is More Tightly Linked to Plasma Leptin Concentration Than Is the Body Mass Index

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This study tested the hypothesis that the integrated plasma insulin response to oral glucose is a more sensitive predictor of the fasting plasma leptin concentration than the body mass index (BMI) or waist to hip ratio (WHR). For this purpose, we determined the fasting plasma leptin concentration and plasma insulin response to a 75-g oral glucose challenge in 76 healthy female subjects, with a BMI of 19.1 to 36.6 kg/m^2 and a WHR of 0.57 to 1.1. The results demonstrated that fasting plasma leptin concentrations were significantly correlated with both the BMI ($r = .64$, $P < .001$) and the plasma insulin response to glucose ($r = .61$, $P < .001$), but not with the WHR ($r = .27$). Since the BMI and the insulin response were also significantly related ($r = .34$, $P = .003$), multivariate analysis was performed to determine if the BMI and insulin response were independent determinants of the fasting leptin concentration. This analysis indicated that both the BMI and insulin response were significantly related to plasma leptin ($P < .001$). To pursue this issue further, the population was divided into tertiles on the basis of the (1) plasma leptin concentration, (2) BMI, and (3) integrated insulin response. The two variables that were most closely linked to each other were the leptin concentration and insulin response. In contrast, the BMI was relatively easily disassociated from the other two variables. These results indicate that while both the plasma insulin response to glucose and the BMI are significantly associated with the fasting plasma leptin concentration, the plasma insulin response appears more closely associated with the plasma leptin concentration.

LEPTIN, the product of the OB gene, is produced in adipose tissue, and there is abundant evidence that plasma leptin concentrations are increased in obese individuals.[1-13] As a consequence, it is generally assumed that the primary regulator of plasma leptin is the degree of obesity. However, plasma insulin is also increased in association with obesity,[14-16] and there is considerable evidence of an association between plasma insulin and leptin concentrations.[6-16] Indeed, it could be argued that the circulating insulin concentration rather than obesity per se is primarily responsible for hyperleptinemia. For example, there is substantial evidence that the relationship between plasma leptin and insulin is independent of differences in the degree of obesity.[6-13] In addition, it has been shown that experimental hyperinsulinemia in the absence of any change in weight can lead to higher adipose tissue OB mRNA levels, an enhanced adipose tissue leptin synthesis, and an increase in plasma leptin.[17-23] Of particular interest in the context of this study is the finding that plasma leptin, which was significantly elevated in non-obese patients with hyperinsulinemia secondary to insulinoma, decreased significantly following surgical intervention and normalization of insulin levels.[24] Finally, two recent publications have shown that the association between obesity and leptin is lost following a weight loss, whereas the association between leptin and insulin concentrations is maintained.[25,26] The first study,[25] performed by our research group, demonstrated that the decrease in the leptin concentration following dietary-induced weight loss was unrelated to changes in weight, body mass index (BMI), fat mass, or percent body fat, but was significantly related to the decrease in the ambient insulin concentration. Similarly, leptin decreased rapidly in association with weight loss after biliopancreatic diversion, and the decrease correlated with the reduction in the insulin concentration, not with changes in measures of obesity.[26]

Although the results of the two latter studies are consistent in their demonstration of a central role for circulating insulin in the regulation of plasma leptin, the conclusion is based on the study of relatively few individuals, ie, 12 and 10, respectively. The current study evaluates the hypothesis that plasma leptin is more closely related to plasma insulin than to obesity in a much larger group of healthy individuals over a much broader range of obesity.

SUBJECTS AND METHODS

The study participants were 76 women recruited from the San Francisco Bay Area through advertisement in local newspapers. Their age (mean ± SD) was 52 ± 10 years (range, 31 to 75), with a mean BMI of 28.5 ± 4.6 kg/m^2 (range, 19.1 to 35.6) and mean waist to hip ratio (WHR) of 0.80 ± 0.9 (range, 0.57 to 1.1). The Stanford University Human Subjects Committee approved the study protocol, and all subjects provided written informed consent before admission to the General Clinical Research Center. Participants were healthy as determined by history, physical examination, complete blood cell count, and chemistry panel. All participants were defined as nondiabetic on the basis of at least 2 fasting plasma glucose values less than 126 mg/dL.[30]

After an overnight fast, blood was drawn for measurement of the plasma insulin concentration before and 30, 60, 120, and 180 minutes after a 75-g oral glucose challenge. Plasma insulin concentrations were determined by radioimmunoassay[28] using a human-specific antibody (Linco Research, St. Charles, MO). This antibody selectively measures human insulin with practically no cross-reactivity (<0.2%) to proinsulin or the primary circulating split form, (des^{31,32})-proinsulin. The total integrated area of the plasma insulin concentration during this 180-minute period was used to quantify the insulin response. Fasting blood samples for leptin assay were collected in EDTA tubes and immediately centrifuged. The plasma was stored at −70°C. The leptin level was measured by a commercial radioimmunoassay (Linco Research). The degree of obesity was estimated by calculating the BMI and WHR.

The data are expressed as the mean ± SE. Pearson's product-moment correlations were calculated to determine relations between variables. Multiple regression analysis was performed to assess the independent

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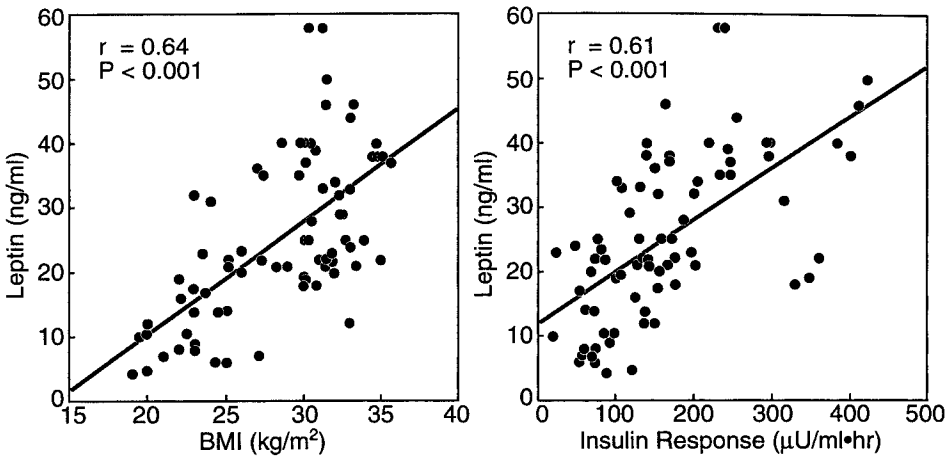

Fig 1. Relationship between plasma leptin and variations in the degree of obesity as estimated by the BMI and the plasma insulin response to glucose.

effect of related variables. To further define the relationship between the BMI and insulin response, participants were divided into 3 groups based on tertiles of leptin, insulin, and BMI. One-way ANOVA was performed to evaluate differences among variables within each group. Post hoc pairwise comparison was performed by Bonferroni test.

RESULTS

There were statistically significant correlations between the fasting plasma leptin concentration and both the BMI ($r = .64$, $P < .001$) and the plasma insulin response to oral glucose ($r = .61$, $P < .001$). However, the relationship between fasting plasma leptin and fasting insulin was not as strong ($r = .38$, $P < .01$), and the association between leptin and the WHR was not statistically significant ($r = .23$, $P > .05$). Thus, values for the integrated plasma insulin response and BMI were used for the remainder of the analyses. The relationships between fasting plasma leptin and the BMI and the total integrated insulin response to glucose are shown in Fig 1. In addition to depicting the significant correlation between leptin and both the BMI and the insulin response, these data emphasize the wide variation in both the plasma insulin response to glucose and plasma leptin in the study population.

Figure 1 shows that both the BMI and the plasma insulin response were significantly correlated with plasma leptin. Furthermore, the BMI and insulin response were also significantly correlated ($r = .34$, $P = .003$). In an effort to assess the relative contribution of these two measures to the variability in leptin concentration, multiple regression analysis was performed. The standardized regression coefficients in Table 1 indicate that both the BMI and the insulin response to glucose were independently related to the fasting plasma leptin concentration ($P < .001$). Parenthetically, adding the WHR to the regression analysis did not affect the outcome, nor was the WHR an independent predictor.

Table 1. Multiple Regression Analysis of the Relationships Between Leptin, Age, BMI, and Insulin Response

Independent Variable	Regression Coefficient	Standard Error	Standardized Regression Coefficient	*P*
Age	0.11	0.1	.08	.28
BMI	1.39	0.23	.53	<.001
Insulin response	0.06	0.01	.45	<.001

NOTE. The dependent variable is the fasting plasma leptin. R^2 for the entire model = .59.

To provide further quantitative information concerning the relationship between leptin, the BMI, and the insulin response to oral glucose, the following analysis was performed. To begin, the entire population was divided into tertiles on the basis of the leptin concentration, and comparisons were made for the BMI and insulin response of these 3 groups. These results are shown in Table 2, and demonstrate that there was no difference in the BMI (31.7 ± 0.5 *v* 29.5 ± 0.7 kg/m^2) of individuals in the highest (40 ± 1 ng/mL) and intermediate (24 ± 1 ng/mL) tertiles for plasma leptin. In contrast, the significant difference in the leptin concentration in these two tertiles was mirrored by comparable changes in the insulin response (234 ± 18 *v* 151 ± 15 μU/mL · h).

Table 2 shows the results for the population divided into tertiles on the basis of the BMI. Despite a significant difference between those with the highest (33.1 ± 0.3 kg/m^2) and intermediate (29.5 ± 0.3 kg/m^2) BMIs, both the leptin concentration (31 ± 2 *v* 30 ± 2 ng/mL) and the insulin response (191 ± 20 *v* 185 ± 17 μU/mL · h) of these 2 groups with different BMIs were essentially identical. Table 2 displays the results for the population divided into tertiles on the basis of the integrated plasma insulin response to oral glucose. These data demonstrate that although plasma leptin concentrations were significantly higher in individuals in the highest tertile compared with the intermediate tertile for the insulin response (35 ± 2 *v* 25 ± 2 ng/mL), there was no difference in the BMI of these two groups (30.1 ± 0.7 *v* 29.5 ± 0.9 kg/m^2).

DISCUSSION

The results shown in Fig 1 demonstrate that both the BMI and the plasma insulin response were correlated to a similar degree with the leptin concentration in a volunteer population of healthy women. In addition, multiple regression analysis (Table 1) indicated that both the plasma insulin response to oral glucose and the BMI were independently related to the leptin concentration. To compare the relative degree of association between leptin and the plasma insulin response to glucose as compared with that between leptin and the BMI, we divided the entire population into tertiles based on each of the 3 variables,

Table 2. Effect of Dividing the Population Into Tertiles on the Basis of the Leptin Concentration, BMI, and Insulin Response (mean ± SE)

Variable	Tertile	No. of Subjects	Age (yr)	Leptin (mg/mL)	BMI (kg/m²)	Insulin Response (μU/mL · h)
Leptin	Highest	26	51 ± 2	40 ± 1*†	31.7 ± 0.5†	234 ± 18*†
	Middle	25	52 ± 2	24 ± 1‡	29.5 ± 0.7‡	151 ± 15‡
	Lowest	25	52 ± 2	12 ± 1	24.1 ± 0.8	115 ± 16
BMI	Highest	26	48 ± 2	31 ± 2†	33.1 ± 0.3*†	191 ± 20†
	Middle	25	53 ± 2	30 ± 2‡	29.5 ± 0.3‡	185 ± 17
	Lowest	25	54 ± 2	14 ± 2	22.8 ± 0.4	125 ± 19
Insulin response	Highest	26	51 ± 2	35 ± 2*†	30.1 ± 0.7†	276 ± 15*†
	Middle	25	52 ± 2	25 ± 2‡	29.4 ± 0.9‡	148 ± 3‡
	Lowest	25	52 ± 1	16 ± 2	25.9 ± 0.9	74 ± 5

*$P < .05$, highest *v* middle tertile.
†$P < .05$, highest *v* lowest tertile.
‡$P < .05$, middle *v* lowest tertile.

leptin, insulin response, and BMI (Table 2). The results showed that the upper and middle tertiles for plasma leptin had essentially identical values for the BMI, but the upper leptin tertile had a significantly higher insulin response. Table 2 also shows that although the BMIs of the upper and middle tertiles were significantly different, their plasma leptin and insulin concentrations were comparable. When divided on the basis of the insulin response, the difference between the highest and middle insulin tertiles was associated with a significant difference in the leptin concentration, despite similar values for the BMI. Again, the insulin response and leptin concentration remained linked, whereas the BMI was easily dissociated. Thus, irrespective of the variable used to divide the population, insulin was more closely related to leptin than was the BMI.

Although the present results are consistent with the view that the linkage between insulin and leptin concentrations is tighter than that between leptin and the BMI, questions can be raised as to the implication of this finding. In the first place, we have used the fasting leptin concentration as our experimental variable, and it is now clear that leptin levels vary throughout the day,[29-32] apparently associated with meals. However, although the absolute values are clearly different, there appears to be a very close relationship between fasting plasma leptin and the total integrated leptin response over 24 hours. For example, Saad et al[29] have shown that the 24-hour leptin response area was increased by approximately 4-fold in obese women compared with obese men (35.6 *v* 9.1 ng/mL · min). The relationship between the fasting leptin concentrations in the two genders was essentially identical, 32.4 *v* 8.6 ng/mL. The gender difference in lean individuals was even greater, with women having an 8-fold increase in both fasting and 24-hour integrated leptin concentrations, but the relative difference was the same whether fasting or day-long measures of leptin were used. Thus, it does not seem likely that our failure to measure day-long leptin concentrations would substantially affect our conclusion.

Perhaps a greater problem involves our use of the BMI and WHR as measures of adiposity, rather than total fat mass or percent body fat. However, given the fact that the relationship between plasma leptin and the BMI is similar to that between leptin and either the fat mass or percent body fat,[3,7,9,10,12,29,30,32] it is not self-evident that the use of the BMI seriously confounds the interpretation of our results. The results from Saad et al[29] are again most revealing in this context. Thus, the correlation coefficients between the mean 24-hour leptin concentration and estimates of obesity in female subjects were .71, .64, .68, and −.09 for the BMI, percent body fat, fat mass, and WHR. If the total integrated leptin response during the 24-hour period was used as the measure, the correlation coefficients were .70, .64, .65, and −.12, for the BMI, percent body fat, fat mass, and WHR. It should be noted that, similar to our findings, Saad et al found that the BMI correlated closely with leptin concentrations, whereas the WHR was unrelated. Based on these data and the other publications cited herein, it does not seem reasonable to discard the present experimental findings on the basis of our use of the BMI as the estimate of obesity.

Finally, although the suggestion that the post–glucose load insulin concentration may be more closely related to plasma leptin than is obesity appears to be in conflict with the generally accepted view, this notion is not without substantial experimental support. Firstly, there is a relatively enormous amount of evidence from cross-sectional studies of a significant relationship between plasma insulin and leptin concentrations that is independent of any measure of obesity.[6-13] Secondly, there is also abundant evidence from interventional studies that prolonged infusion of insulin leads to increased plasma leptin concentrations in both humans and rodents.[20-23] In support of these findings, there is evidence of a direct stimulatory effect of insulin on leptin synthesis and/or OB mRNA in various cell types in culture.[17-19] In contrast are the results of weight-loss studies in humans, showing that the decrease in leptin following weight loss is dissociated from the concomitant loss of adipose tissue[2,4,25,26,33] but remains closely correlated with the ensuing decline in the insulin concentration.[25] In a similar manner, the presence of increased plasma leptin concentrations in non-obese patients with insulin-secreting tumors, and their prompt decline when the hyperinsulinemia was surgically corrected,[24] emphasizes the continued close linkage between insulin and leptin concentrations and the absence of such a connection between leptin and obesity.

In conclusion, the results of this study in healthy nondiabetic women provide further evidence for the view that the post–glucose load plasma insulin concentration is a significant predictor of the plasma leptin concentration, independently of obesity. In addition, based on the evidence presented herein and

in the relevant experimental literature, the hypothesis has been proposed that the post–glucose load insulin concentration is related more closely to plasma leptin than is obesity. A consideration of this alternative point of view does not involve disputing the fact that adipose tissue is the source of circulating leptin, nor that there is a significant relationship between obesity and hyperinsulinemia. However, it does mean that questions should be raised and further experimental studies performed to evaluate the relative importance of insulin and obesity as regulators of the plasma leptin concentration.

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