Comparison of Fasting Plasma Leptin Concentrations in Healthy Subjects With High and Low Plasma Insulin

I. Zavaroni, P. Gasparini, A.I. Barilli, P. Massironi, C. Campanini, M. Carantoni, R. Delsignore, and G.M. Reaven

This study was initiated to evaluate the role of hyperinsulinemia in the regulation of fasting plasma leptin. We measured plasma leptin and insulin concentrations in 404 healthy nondiabetic subjects. For analytical purposes, the population was divided into quartiles on the basis of the lowest (quartile 1) and highest (quartile 4) plasma insulin response to oral glucose, and fasting plasma leptin values in these 2 dichotomous groups were compared. The total plasma integrated insulin response was 4-fold greater in quartile 4, associated with significantly higher ($P < .001$) fasting plasma leptin (12.60 ± 0.85 *v* 8.53 ± 0.56 ng/mL). Fasting plasma leptin concentrations remained significantly higher in the hyperinsulinemic quartile when comparisons were made after subdividing the population on the basis of gender, body mass index (BMI), or waist to hip ratio (WHR). These results demonstrate that fasting plasma leptin concentrations are significantly higher in hyperinsulinemic individuals, and this difference is independent of either overall or central obesity.

LEPTIN, the product of the OB gene, is produced in adipose tissue, and initial measurements of plasma leptin in humans have emphasized the presence of elevated concentrations in obese individuals.[1-5] Plasma insulin levels are often increased in obese individuals,[6-8] raising the possibility that the relationship between obesity and the leptin concentration is mediated via the hyperinsulinemia associated with obesity. Indeed, evidence has recently been published demonstrating that the relationship between plasma insulin and leptin concentrations is independent of obesity.[9-14] However, the conclusion is based primarily on the results of multiple regression analyses performed in populations in whom the variables were distributed in a continuous fashion.

Although this statistical approach can provide useful insight as to the nature of the relationships between defined variables, it is not without its own problems. An alternative approach is to avoid the need for statistical adjustments by carefully creating groups that are dichotomous for the variable of interest. It is thereby possible to directly compare the two groups on this variable without relying on the statistical assumptions that underlie the use of multiple regression analysis. Although creating such dichotomous groups at the outset is more difficult than simply recruiting an undefined general population, the results are much simpler to evaluate if this hurdle can be overcome. Since this approach has not been heretofore applied to evaluate the relationship between obesity, leptin, and insulin, the present study was initiated.

SUBJECTS AND METHODS

Between 1993 and 1995, 421 presumably healthy factory workers were surveyed for a variety of risk factors for coronary heart disease. At that time, all subjects were instructed to consume 300 g carbohydrate for 3 days preceding the measurements. A complete medical history was obtained and a physical examination was performed. Venous blood was drawn after an overnight fast for determination of plasma glucose,[15] insulin,[16] and leptin[17] concentrations. In addition, plasma glucose and insulin levels were measured 1 hour and 2 hours after a 75-g oral glucose load.

On the basis of the medical history, physical examination, and plasma glucose response to the glucose load, 404 subjects were found to be without disease and without medication that would affect the variables measured. For the purpose of this study, we compared measurements in 100 subjects with the lowest total integrated plasma insulin response to the glucose challenge (quartile 1) and 101 subjects with the highest total insulin response (quartile 4). Results are expressed as the mean ± SEM, and values for the two groups have been compared, as appropriate, by Student's *t* test and analysis of variance, with subsequent tests for pairwise mean comparisons. The difference in gender distribution was evaluated by chi-square test.

RESULTS

Table 1 presents the baseline characteristics of the members of the two insulin quartiles. The integrated insulin response was increased by approximately 4-fold in the hyperinsulinemic group. The hyperinsulinemic group was also older, contained relatively more males, was more obese, and had higher fasting plasma glucose and insulin concentrations.

Figure 1 compares fasting plasma leptin concentrations of the subjects in quartile 1 and quartile 4. It is apparent from these data that plasma leptin concentrations were significantly ($P < .001$) higher in those with the greatest insulin response to oral glucose.

Although differences in the degree of adiposity between the two groups shown in Table 1 were relatively modest in magnitude, we thought it necessary to further evaluate the effect of obesity on the leptin concentration. For this purpose, a body mass index (BMI) of 27.0 kg/m^2 was used to subdivide the population into 4 groups. A comparison of fasting plasma leptin concentrations in the groups thus formed is illustrated in Fig 2. These results indicate that leptin concentrations were significantly higher ($P < .05$) in the hyperinsulinemic groups regardless of the BMI. Of particular interest was the comparison in the two groups with a BMI less than 27.0 kg/m^2. There were a substantial number of both low insulin responders (quartile 1, n = 81) and high insulin responders (quartile 4, n = 42) in this weight category, and it is obvious that the leptin concentration was significantly higher in quartile 4 in the absence of obesity. It

From the Department of Internal Medicine and Biomedical Science, Parma University, Parma, Italy; and Stanford University School of Medicine, Stanford, CA.

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Address reprint requests to I. Zavaroni, MD, Department of Internal Medicine and Biomedical Science, via Gramsci 14, 43100 Parma, Italy.

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Table 1. Clinical Characteristics of the Two Insulin Groups

Variable	Quartile 1 (n = 100)	Quartile 4 (n = 101)	P
Insulin response (μU/mL · min/10)	42 ± 1	161 ± 6	<.001
Gender (male/female)	45/55	67/34	<.001
Age (yr)	50 ± 1	53 ± 1	<.02
BMI (kg/m²)	24.5 ± 0.3	28.4 ± 0.4	<.001
WHR	0.88 ± 0.01	0.95 ± 0.01	<.001
Fasting plasma glucose (mg/dL)	97 ± 1	104 ± 2	<.001
Fasting plasma insulin (μU/mL)	7 ± 0.2	16 ± 0.6	<.001

should also be noted that leptin levels were higher ($P < .01$) in those with a BMI of at least 27.0 kg/m², irrespective of whether comparisons were made within quartile 1 or quartile 4. Since it appeared that both obesity and hyperinsulinemia increased fasting plasma leptin, it was not surprising that the leptin concentration in the group that was both obese (BMI ≥ 27.0 kg/m²) and hyperinsulinemic (quartile 4) was approximately twice as high as that in the non-obese (BMI < 27.0 kg/m²) group of low insulin responders (quartile 1).

Since the insulin quartiles also differed in terms of gender distribution, we further subdivided the population to evaluate the effects of both gender and obesity (Fig 3). These data again demonstrated that fasting plasma leptin concentrations are higher in the hyperinsulinemic (quartile 4) group in both males and females. Perhaps the most interesting comparison is between quartile 1 and quartile 4 males with a BMI less than 27.0 kg/m². There are substantial and relatively equal numbers of subjects in each subgroup (33 *v* 30), making the finding that fasting plasma leptin was almost twice as high in the hyperinsulinemic quartile a robust observation. It should also be pointed out that fasting plasma leptin concentrations were similar in hyperinsulinemic (quartile 4) males whether their BMI was less than 27.0 kg/m² (7.8 ± 0.4 ng/mL) or 27.0 kg/m² or higher (8.1 ± 0.3 ng/mL). Finally, fasting plasma leptin concentrations were much higher in females compared with males irrespective of the insulin response or degree of obesity. Specifically, plasma

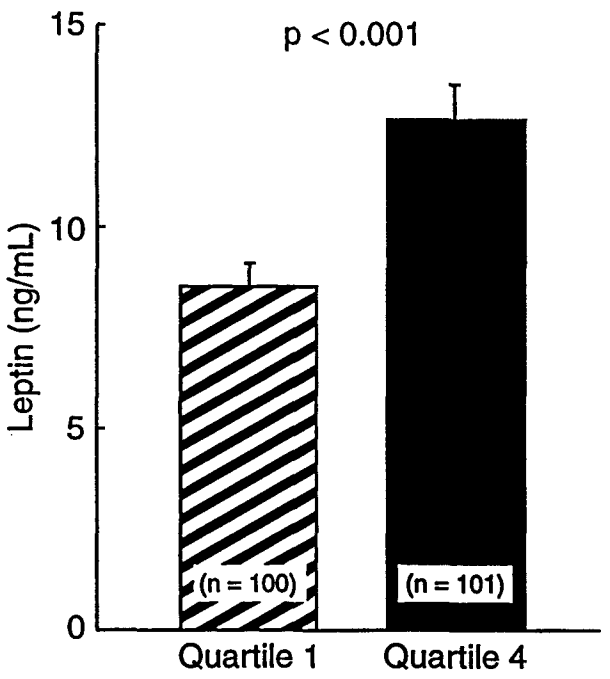

Fig 1. Plasma leptin concentration in quartiles with the lowest (quartile 1) and highest (quartile 4) plasma insulin response to oral glucose.

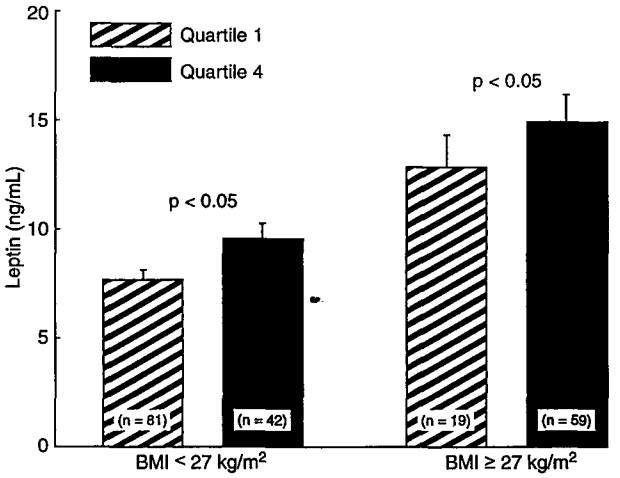

Fig 2. Plasma leptin concentration in quartiles with the lowest (quartile 1) and highest (quartile 4) plasma insulin response to oral glucose, subdivided into those with a BMI <27.0 kg/m² or ≥27.0 kg/m².

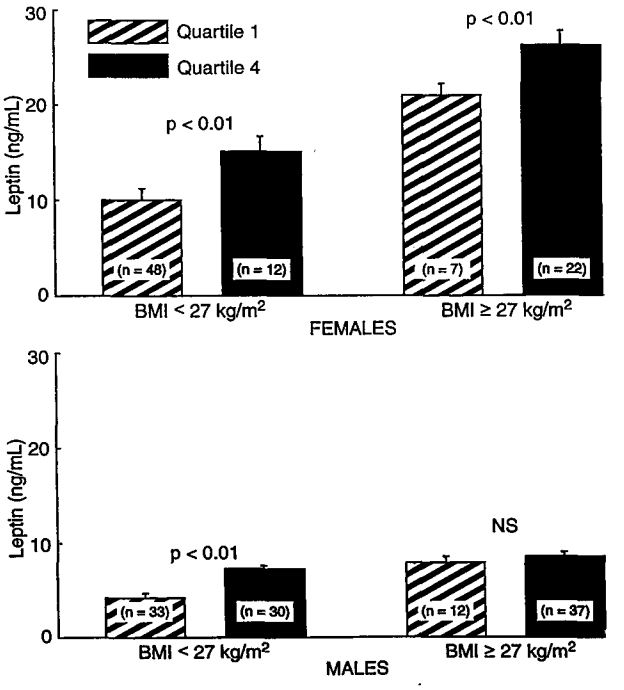

Fig 3. Plasma leptin concentration in quartiles with the lowest (quartile 1) and highest (quartile 4) plasma insulin response to oral glucose, subdivided on the basis of gender and BMI (<27.0 kg/m² or ≥27.0 kg/m²).

leptin concentrations were higher ($P < .001$) in females with a BMI less than 27.0 kg/m^2 (10.8 ± 0.6 *v* 5.7 ± 0.3 ng/mL) or at least 27.0 kg/m^2 (24.7 ± 1.4 *v* 8.5 ± 1.6 ng/mL).

To further evaluate the effect of obesity on the fasting plasma leptin concentration, the study population was divided on the basis of the waist to hip ratio (WHR). For this purpose, the median WHR was used as the cutoff point in both males (0.95) and females (0.87). The results of this comparison are shown in Fig 4, and indicate that hyperinsulinemic individuals have higher fasting plasma leptin, irrespective of their degree of central adiposity as estimated by the WHR.

DISCUSSION

To the best of our knowledge, this represents the first instance in which comparisons of fasting plasma leptin concentrations have been made in a large group of healthy subjects selected to create two dichotomous groups, with a very high or very low plasma insulin response to oral glucose. Table 1 indicates that the two groups varied approximately 4-fold in terms of plasma insulin concentrations. By establishing two discrete groups, we were able to directly compare their leptin concentrations without relying on statistical approaches to define the relationship between insulin and leptin concentrations in population-based studies, where both variables are continuously distributed. Although attempts to correct for the impact of covariates on the relationship of interest are widely used, it must be remembered that such approaches rely on assumptions as to the relevance of covariables, as well as the existence of linear relationships between all variables. By stratifying our groups, we avoid these potential confounders.

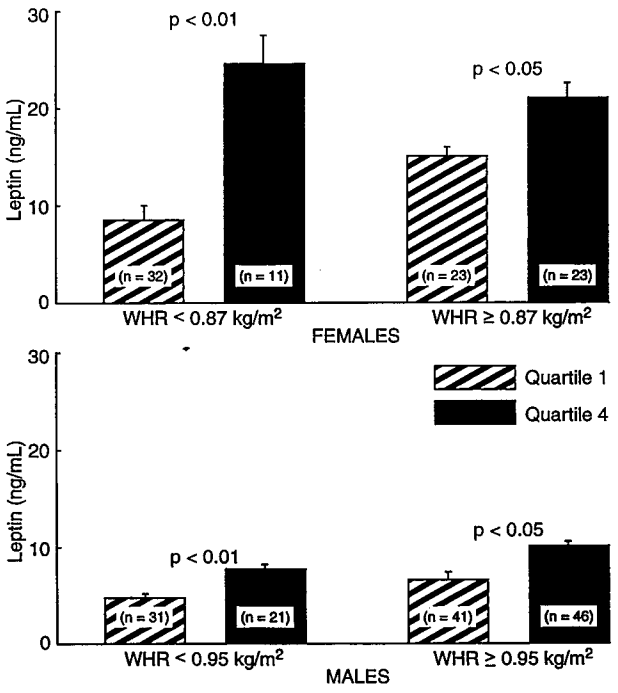

Fig 4. Plasma leptin concentration in quartiles with the lowest (quartile 1) and highest (quartile 4) plasma insulin response to oral glucose, subdivided on the basis of gender and WHR (0.95 in males and 0.87 in females).

The primary hypothesis tested in this study is that fasting plasma leptin is increased in hyperinsulinemic individuals and this relationship is independent of the presence of obesity. We believe the results provide strong support for this formulation. More specifically, fasting plasma leptin concentrations were significantly higher in the hyperinsulinemic group (quartile 4) as a whole, in the study population further divided into obese (BMI ≥ 27.0 kg/m^2) and non-obese (BMI < 27.0 kg/m^2) groups, and with gender and BMI used to stratify the population. Furthermore, evidence of higher fasting plasma leptin in hyperinsulinemic individuals was observed when the population was divided on the basis of the WHR. Indeed, if anything, the association between increases in the plasma insulin response and leptin concentration was more apparent in those with lower values for the BMI and WHR. The notion that the fasting plasma leptin concentration is tightly correlated with hyperinsulinemia is not surprising, given the evidence that sustained hyperinsulinemia can increase OB mRNA expression and leptin synthesis, as well as the plasma leptin concentration.[18-20]

In conclusion, we have selected two large groups of individuals divided on the basis of high and low plasma insulin responses to oral glucose. The results provide strong evidence that healthy hyperinsulinemic subjects have elevated fasting plasma leptin concentrations, independent of the degree of obesity and the gender. Plasma leptin also appears to increase as a function of female gender and/or obesity, with leptin values in hyperinsulinemic women with a BMI of 27.0 kg/m^2 or higher being 5-fold higher versus those with a BMI less than 27.0 kg/m^2 and in the lowest insulin group (quartile 4). On the other hand, two caveats must be made explicit. First, we have used the BMI and WHR as an index of obesity, rather than the fat mass. However, since highly significant relationships exist between leptin and both the percent body fat mass and the BMI,[3,4] we believe the appropriateness of our conclusion is not confounded by the use of the BMI and WHR as estimates of obesity. Similarly, we have relied on the fasting leptin concentration as our endpoint, and it is now clear that leptin levels vary throughout the day,[21-24] apparently associated with meals. However, although the absolute values are clearly different, there appears to be a very close relationship between the fasting plasma leptin concentration and the total integrated leptin response over 24 hours. For example, Saad et al[21] 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 versus 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 difference was the same whether fasting or daylong measures of leptin were used. Thus, it does not seem likely that our failure to measure total fat mass, or daylong leptin concentrations, would substantially affect our conclusion that hyperinsulinemic individuals have higher leptin concentrations independently of estimates of overall or central obesity.

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