Relationship Between Insulin Sensitivity and Plasma Leptin Concentration in Lean and Obese Men

Karen R. Segal, Michael Landt, and Samuel Klein

Alterations in the production of or the sensitivity to leptin, the protein encoded by the *ob* gene, cause obesity and diabetes in rodents. We evaluated the isolated relationship between leptin and insulin sensitivity in lean and obese humans. Three groups of subjects who were carefully matched for either insulin sensitivity (determined by the modified intravenous glucose tolerance test and minimal model analysis) or adiposity (determined by hydrodensitometry) were studied: *1*) lean insulin-sensitive men (percentage body fat, 15 ± 1%); *2*) lean insulin-resistant men (percentage body fat, 16 ± 1%), matched on percentage body fat and fat mass with the lean insulin-sensitive group; and *3*) obese insulin-resistant men (percentage body fat, 31 ± 3), matched on insulin sensitivity with the lean insulin-resistant group. Basal plasma leptin concentrations were significantly lower in the lean insulin-sensitive than in the lean insulin-resistant men (1.90 ± 0.4 vs. 4.35 ± 1.21 ng/ml, $P < 0.05$) despite identical body composition. Plasma leptin in the obese men (9.27 ± 1.4 ng/ml) was significantly higher than values in the two lean groups ($P < 0.01$). Marked alterations in plasma glucose and insulin concentrations induced by glucose and tolbutamide injection did not cause any change in plasma leptin levels. These results demonstrate that insulin resistance is associated with elevated plasma leptin levels independent of body fat mass. However, plasma insulin itself does not acutely regulate leptin production. *Diabetes* 45:988–991, 1996

Identification of the obese gene and its protein product, leptin, has increased our understanding of the pathophysiology of obesity. In *ob/ob* mice, a mutation in the *ob* gene prevents normal leptin production by adipose tissue, which causes both obesity and diabetes (1). Treatment of *ob/ob* mice with leptin decreases body weight and normalizes blood glucose concentration (2). In fact, changes in glycemia precede changes in body weight (2), suggesting that leptin may have a direct effect on insulin action. Furthermore, insulin may also have a direct effect on leptin production. Studies performed in whole rats and in primary rat adipocytes demonstrate that insulin directly regulates *ob* gene expression (3). These data suggest that there are several important interactions between insulin and leptin, in which each hormone may be involved in regulating the function of the other.

However, the relationship between these two hormones is difficult to evaluate in humans because of the confounding influence of body fat mass on both plasma leptin levels and insulin sensitivity/concentration; plasma leptin and insulin concentrations increase with increasing body fat mass (4). Accordingly, the primary purpose of this study was to evaluate the relationship between insulin sensitivity and plasma leptin concentrations, independent of the confounding influence of body composition, and to evaluate whether acute alterations in plasma insulin and glucose concentrations affect plasma leptin levels. Three groups of subjects who were carefully matched for either insulin sensitivity or adiposity were studied: *1*) lean insulin-sensitive men; *2*) lean insulin-resistant men, matched by adiposity with the lean insulin-sensitive group; and *3*) obese insulin-resistant men, matched by insulin sensitivity with the lean insulin-resistant group. This approach served to avoid the usual colinearity and confounding between insulin sensitivity and body fat content. Plasma leptin concentrations were determined during basal conditions and during a modified intravenous glucose tolerance test, in which there are dramatic and reproducible changes in plasma glucose and insulin concentrations.

RESEARCH DESIGN AND METHODS

Subjects. Three groups of men, aged 25–40 years, who were carefully matched on the basis of body composition and insulin sensitivity participated in this study (Table 1). Two of the groups were lean men who were matched on percentage body fat (the modified intravenous glucose tolerance test) (5). The third group consisted of obese men matched to the lean insulin-resistant subjects on insulin sensitivity. All subjects had normal glucose tolerance despite differences in insulin sensitivity between some groups. The three groups had the same fat-free mass (FFM). All three groups were also matched for physical activity and aerobic fitness to eliminate these potential confounding influences on the interpretation of the study results. All subjects were sedentary, and subjects with a cycle ergometer maximum oxygen consumption (Vo_{2max}) measurement of >3.5 l/min were excluded. The study was approved by the Committee for Human Rights in Research of Cornell University Medical College and the Human Studies Committee of Washington University School of Medicine. Informed written consent was obtained from all subjects before their participation.

Study protocol. All subjects underwent body composition analysis and Vo_{2max} testing. An oral glucose tolerance test (OGTT) and an intravenous glucose tolerance test (IVGTT) were completed on separate occasions. The men consumed a weight-maintaining diet containing at least 300 g of carbohydrate per day and refrained from exertion for 3 days before each test. The subjects also abstained from caffeine and alcohol for 24 h before each test.

The OGTT was performed after the subjects fasted for 12 h overnight. Blood samples were drawn at baseline and every 30 min for 2 h after ingestion of 75 g of glucose to measure plasma glucose and insulin concentrations. The National Diabetes Data Group (6) criteria for normal glucose tolerance were used. The IVGTT was also performed

From the Department of Pediatrics (K.R.S.), Cornell University Medical College, New York, New York; and the Departments of Pediatrics (M.L.) and Internal Medicine (S.K.), Washington University School of Medicine, St. Louis, Missouri.

Address correspondence and reprint requests to Dr. Karen R. Segal, Cornell University Medical College, Division of Pediatric Cardiology, Room N-134, 1300 York Ave., New York, NY 10021. E-mail: krsega@med.cornell.edu.

Received for publication 1 April 1996 and accepted for publication 4 April 1996.

FFM, fat-free mass; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; S_I, insulin sensitivity index.

TABLE 1
Subject characteristics

	Lean insulin-sensitive	Lean insulin-resistant	Obese
n	10	8	7
Age	33 ± 2	34 ± 2	33 ± 3
Height (cm)	178 ± 1	178 ± 2	179 ± 3
Weight (kg)	79.0 ± 3.0*	81.7 ± 3.7	103.3 ± 5.5
Percentage body fat	15.2 ± 1.0*	16.4 ± 1.3	31.0 ± 2.5
Body fat mass (kg)	12.0 ± 0.9*	13.4 ± 1.3	32.3 ± 3.9
FM (kg)	67.0 ± 1.0	68.2 ± 3.2	71.0 ± 2.9
BMI (kg/m^2)	24.8 ± 1.0*	25.7 ± 1.1	32.5 ± 2.4
Vo_{2max} (ml/min)	3,326 ± 132	3,266 ± 187	3,392 ± 143

Data are means ± SE. *$P < 0.001$, lean insulin-sensitive and lean insulin-resistant versus obese group.

TABLE 2
Oral glucose tolerance

	Lean insulin-sensitive	Lean insulin-resistant	Obese
n	10	8	7
Fasting insulin (pmol/l)	37 ± 6*	87 ± 17	86 ± 15
Fasting glucose (mmol/l)	5.4 ± 0.1	5.4 ± 0.1	5.5 ± 0.2
Insulin area (pmol/l)	1,073 ± 103*	2,008 ± 324	2,120 ± 542
Glucose area (mmol/l)	31.1 ± 1.0	31.2 ± 1.4	30.6 ± 1.4

Data are means ± SE. Insulin and glucose areas are integrated over 2 h after 75 g oral glucose. *$P < 0.05$, lean insulin-sensitive versus lean insulin-resistant and obese groups.

after the subjects fasted for 12 h overnight as described by Bergman et al. (5). After an overnight fast, catheters were placed in a forearm vein and a hand vein of the contralateral arm. After 30 min, basal samples were collected at -20, -15, -5, and -1 min. Glucose (300 mg/kg) was injected as a bolus at time 0 over 1 min and flushed with saline to ensure complete delivery. At 20 min, tolbutamide (Orinase I.V., Upjohn, Kalamazoo, MI) was injected over 20 s (300 mg for the lean and 500 mg for the obese men) (5). Blood samples were drawn at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 min for determination of plasma glucose and insulin levels. Minimal model analysis was performed using the Minmod computer program to determine the insulin sensitivity index (S_I), which is a measure of the effect of changes in plasma insulin on the kinetics of plasma glucose clearance (5). The units for S_I are $\mu U \cdot ml^{-1} \cdot min \times 10^{-4}$. The range between 1.5 and 3.5 $\mu U \cdot ml^{-1} \cdot min \times 10^{-4}$ was used to match the insulin-resistant lean and obese groups, and the range from 5 to 8 $\mu U \cdot ml^{-1} min \times 10^{-4}$ was used for the insulin-sensitive lean group. Blood samples were also obtained at baseline and at 3, 5, 10, 16, 24, 25, 30, 60, 90, 120, and 180 min for determination of plasma leptin concentrations. These time points were selected to evaluate the effects of acute changes in plasma glucose and insulin levels on leptin concentration.

Body fat and FFM were determined by hydrodensitometry, after a 12-h fast, as described by Akers and Buskirk (7). Residual lung volume was estimated by the oxygen dilution method (8) while the subjects were in the water. Percentage body fat and FFM were derived from body density by use of the Siri equation (9).

Maximal aerobic fitness was determined by a continuous graded cycle ergometer test. The subjects began with unloaded cycling, and the work rate was increased by 25 W every 2 min until volitional exhaustion was reached. Standard criteria for achieving a true Vo_{2max} were applied (10). Ventilatory measurements were made continuously with use of a 2900 Metabolic Cart (Sensormedics, Yorba Linda, CA). The subjects breathed through a nonrebreathing valve. Oxygen uptake (Vo_2), carbon dioxide (Vco), and ventilation (VE) were measured according to standard procedures.

Sample analyses. Basal plasma leptin concentrations were determined by a newly developed radioimmunoassay (Linco Research, St. Louis, MO) using a polyclonal antibody raised in rabbits against highly purified recombinant human leptin (11). The coefficients of variation for within- and between-run analyses ranged from 3.4 to 8.3% and from 3.6 to 6.2%, respectively. Glucose was measured with a Beckman Glucose Analyzer II (12). Insulin was determined by radioimmunoassay (13).

Statistical analysis. One-way analyses of variance were applied to the leptin, insulin sensitivity data, and subject characteristics. Significant F ratios were followed by post-hoc analyses using the Newman-Keuls procedure (14). A two-way analysis of variance with repeated measures using group and time as the factors was applied to the repeated measurements of plasma leptin during the IVGTT (14). Significant F ratios were followed by post-hoc comparisons among cell means, as described above. The 0.05 level of probability was taken to be statistically significant.

RESULTS

The three groups of study subjects were similar with respect to age, height, aerobic fitness, and FFM (Table 1). By design, the two lean groups were matched for percentage body fat and body fat mass. Body fat mass and percentage body fat were more than two times greater in the obese men than in the two lean groups. Basal insulin and the integrated insulin response to oral glucose (Table 2) were significantly lower in the lean insulin-sensitive men than in the other two groups. Basal plasma glucose and the glucose response area were not different among the three groups. Insulin sensitivity was significantly lower in the lean insulin-sensitive group than the other two groups (Fig. 1).

Basal plasma leptin concentration was significantly greater in the insulin-resistant than in the insulin-sensitive lean men (Fig. 1) ($P < 0.05$). Plasma leptin was greater in the obese men than in the lean insulin-sensitive or insulin-resistant men ($P < 0.05$).

The time course of plasma leptin, glucose, and insulin changes during the intravenous glucose tolerance test is shown in Fig. 2. The S_I was calculated from the individual glucose and insulin levels; therefore, neither the changes in plasma glucose and insulin nor the differences between groups at individual time points were tested for statistical significance. Plasma leptin concentrations remained remarkably stable during the first 120 min of the IVGTT, when there were marked changes in plasma glucose and insulin concentrations (Fig. 2). However, there was a 12 ± 2 and 9 ± 2% decrease in plasma leptin concentrations at 180 min compared with basal values in the obese and lean insulin-resistant men, respectively ($P < 0.05$).

DISCUSSION

The present study determined the relationship between insulin sensitivity and plasma leptin concentration by use of an experimental design in which the usual correlations between adiposity, insulin resistance, and leptin production were uncoupled. The present investigation provides the first evidence for an independent relationship between insulin resistance and leptin levels. Compared with insulin-sensitivity in lean subjects, insulin-resistance in lean men is associated with an elevation in plasma leptin. Circulating leptin is further increased in obese men compared with lean men with a similar degree of insulin resistance, which demonstrates the distinct contribution of obesity and supports previous reports of increased expression of the human *OB* gene in obesity (15).

The interaction between insulin sensitivity and leptin concentration may be important in the regulation of body weight. It has been hypothesized that insulin resistance itself protects against weight gain. Longitudinal observations in Pima Indians suggest that future weight gain is associated with insulin sensitivity; subjects who were more insulin

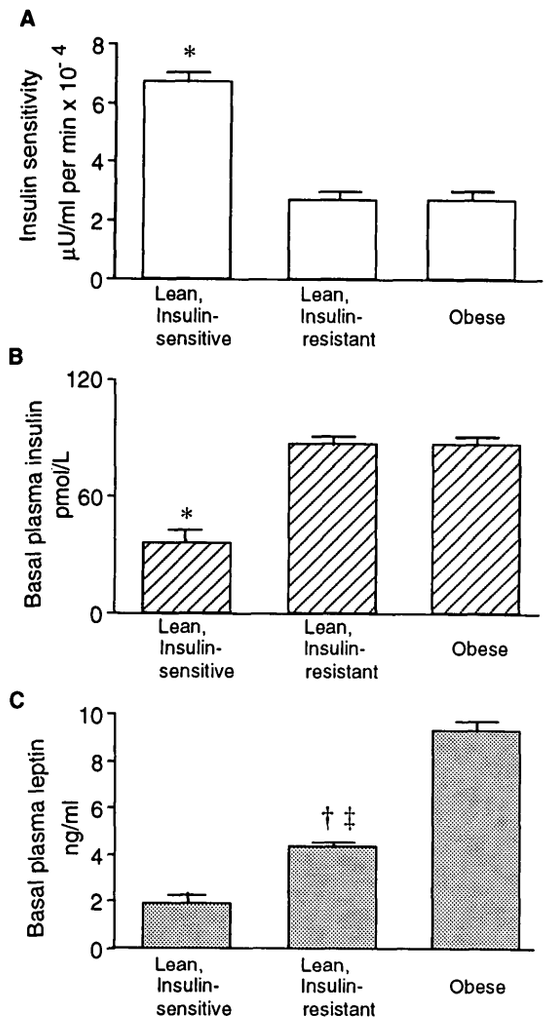

FIG. 1. *A:* S_I determined by minimal model analysis of the modified intravenous glucose tolerance test. *$P < 0.001$, lean insulin-sensitive versus lean insulin-resistant and obese groups. *B:* basal plasma insulin concentrations. *$P < 0.05$, lean insulin-sensitive versus lean insulin-resistant and obese groups. *C:* basal plasma leptin concentrations. †$P < 0.05$, lean insulin-sensitive versus lean insulin-resistant group. ‡$P < 0.05$, lean insulin-resistant versus obese groups.

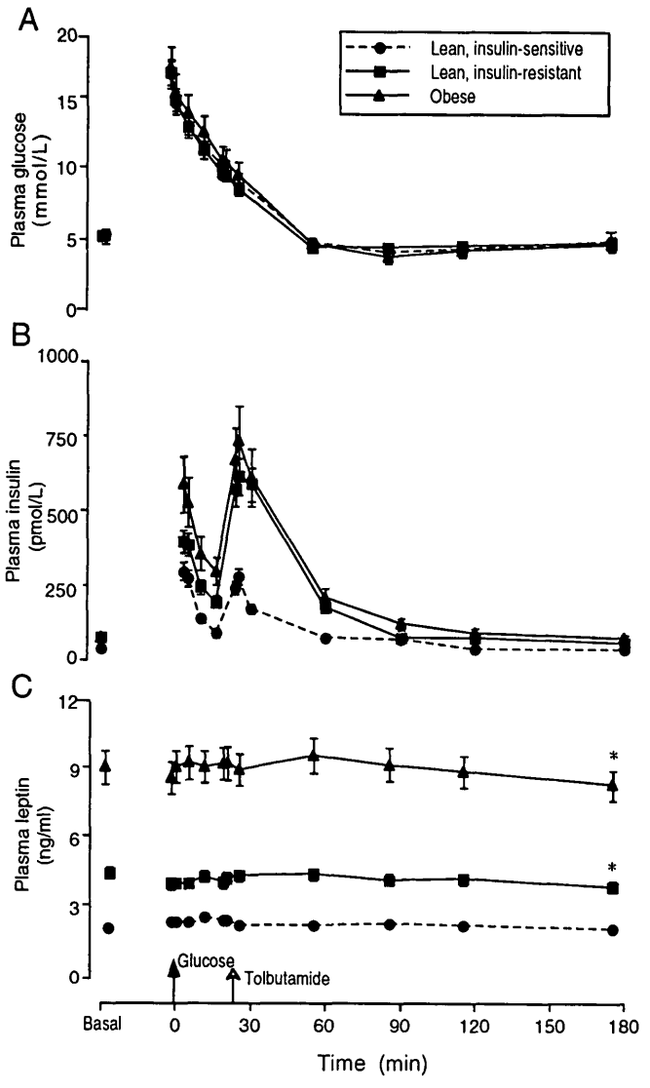

FIG. 2. Plasma glucose (*A*), insulin (*B*), and leptin (*C*) concentrations during the modified IVGTT. Glucose injection (16.7 mmol/kg body wt) was given at time 0, followed by tolbutamide injection (300 mg for lean subjects, 500 mg for obese subjects) at time 20 min. *$P < 0.05$ for 180 min versus basal plasma leptin.

resistant had a lower rate of weight gain than those who were more insulin sensitive (16). In addition, Yost et al. (17) found that the magnitude of improvement in insulin sensitivity after weight loss in obese subjects was directly related to recidivism; the greater the improvement in insulin sensitivity the greater and more rapid the regain in body weight. The results of the present study suggest that insulin resistance might help prevent obesity by increasing plasma leptin concentrations. However, the importance of leptin in regulating body weight in humans is not clear. The increase in plasma leptin concentration observed with increasing adiposity (18) suggests that obese humans may be resistant to the putative effects of leptin in modulating food intake and energy expenditure.

Plasma leptin levels remained remarkably stable during the first 2 h of the IVGTT protocol, despite two significant peaks in plasma insulin within the first 30 min. These data are consistent with recent studies that found circulating leptin did not change in response to meal ingestion (11,19). Therefore, in contrast to data observed in rats (3), acute changes in insulin concentration do not play a regulatory role in leptin production. However, we did find that plasma leptin concentration declined by ~10% at 180 min of the IVGTT, which coincides with the time of day that leptin is normally lowest, independent of feeding (19). Thus, it is likely that the decline in leptin observed at the end of the IVGTT in the present study may simply reflect a superimposed circadian rhythm in leptin secretion rather than a specific response to the intravenous glucose.

As body fat increases, the range of reported plasma leptin concentrations at any given level of body fat also increases (11,18). The variability in plasma leptin concentrations indicates that factors other than body fat mass contribute to the regulation of leptin production. The results of the present study raise the possibility that differences in the degree of insulin resistance may explain some of the variability in plasma leptin concentration in humans. However, our study is unable to elucidate the mechanism or mechanisms by which insulin sensitivity might regulate leptin production. The absence of a change in plasma leptin during the IVGTT excludes the possibility of an acute effect of insulin on leptin production or clearance.

In summary, the results of the present study demonstrate that insulin resistance, independent of adiposity, is associated with elevated plasma leptin concentrations. Although we found insulin did not regulate leptin production acutely, it is possible that chronically elevated plasma insulin concentrations stimulate *OB* gene expression.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health Grants DK-37948, DK-26678, and DK-49989 and General Clinical Research Center Grant RR-00036.

We appreciate the assistance of Yim Dam in performing the insulin assays.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–432, 1994
2. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F: Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269:540–543, 1995
3. Saladin R, DeVos P, Guerro-Millo M, Leturque A, Girard J, Staels B, Auwerx J: Transient increase in obese gene expression after food intake or insulin administration. *Nature* 377:527–529, 1995
4. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269:543–546, 1995
5. Bergman RN, Hope ID, Yang YJ, Watanabe RM, Meador MA, Young JH, Ader M: Assessment of insulin sensitivity in vivo: a critical review. *Diabetes Metab Rev* 5:411–429, 1989
6. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose tolerance. *Diabetes* 28:1039–1057, 1979
7. Akers R, Buskirk ER: An underwater weighing system utilizing force cube transducers. *J Appl Physiol* 26:649–652, 1969
8. Wilmore JH: A simplified method for determination of residual lung volume. *J Appl Physiol* 27:96–100, 1969
9. Siri WE: Body composition from fluid spaces and density: analysis of methods. In *Techniques for Measuring Body Composition.* Brozek J, Henschel A, Eds. Washington, D.C., National Academy of Science, 1961, p. 223–244
10. Astrand PO, Rodahl K: *Textbook of Work Physiology.* New York, McGraw-Hill, 1986
11. Ma Z, Gingerich RL, Santiago J, Klein S, Smith CH, Landt M: Analysis of human plasma leptin by radioimmunoassay. *Clin Chem.* In press
12. Hugget AS, Nixon DA: Use of glucose oxidase, peroxidase, and O-dianisidine in determination of blood and urinary glucose. *Lancet* ii:368–370, 1957
13. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–1384, 1965
14. Winer BJ: *Statistical Principles in Experimental Design.* 2nd ed. New York, McGraw-Hill, 1971
15. Lonnqvist F, Arner P, Nordfors L, Schalling M: Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. *Nature Med* 1:950–953, 1995
16. Swinburn BA, Nyomba BL, Saad MF, Zurlo F, Raz I, Knowler WC, Lillioja S, Bogardus C, Ravussin E: Insulin resistance associated with lower rates of weight gain in Pima Indians. *J Clin Invest* 88:168–173, 1991
17. Yost TI, Jensen DR, Eckel RH: Weight regain following sustained weight reduction is predicted by relative insulin sensitivity. *Obesity Res* 3:583–588, 1995
18. Considine RV, Sinha MK, Heiman ML, Kriaciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295, 1996
19. Sinha MK, Ohannesian JP, Heiman ML, Kriauciunas A, Stephens TW, Magosin S, Marco C, Caro JF: Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. *J Clin Invest* 97:1344–1347, 1996