Plasma Leptin and Insulin Relationships in Obese and Nonobese Humans

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Hyperinsulinemia is associated with an overexpression of mRNA for the *ob* protein leptin in rodent models of genetic obesity, and insulin has been reported to directly stimulate leptin mRNA in rat adipocytes. Human obesity is also associated with increased leptin mRNA as well as plasma levels, but there have been no reports of the effect of insulin on leptin secretion. We, therefore, tested the hypothesis that insulin stimulates leptin secretion in humans. Using a newly developed leptin assay, immunoreactive leptin was measured in fasting and postprandial plasma samples from 27 healthy adults and in samples before and during euglycemic-hyperinsulinemic then stepped hypoglycemic (hourly steps at 85, 75, 65, 55, and 45 mg/dl) clamps from 10 healthy subjects and 11 patients with IDDM. Plasma leptin was correlated ($r = 0.84$, $P = 0.0005$) with BMI in obese but not nonobese subjects and with fasting ($r = 0.75$, $P = 0.008$) but not postprandial plasma insulin levels. (Leptin levels did not change postprandially.) Euglycemic hyperinsulinemia did not alter leptin levels, nor did hyperinsulinemic hypoglycemia. Thus, because circulating leptin levels are not increased during postprandial hyperinsulinemia or during euglycemic (or hypoglycemic) hyperinsulinemia, we conclude that, at least in the short term, insulin does not increase leptin secretion in humans and that hyperleptinemia in obese individuals is not likely the result of hyperinsulinemia. *Diabetes* 45:695–698, 1996

The *ob* gene (1) encodes a protein, leptin, that reverses obesity in *ob/ob* mutant mice lacking the protein by suppressing appetite and stimulating calorigenesis (2–5). Subanorexogenic doses of leptin that had no effect on body weight corrected obesity-associated diabetes in the *ob/ob* mice, most likely through the amelioration of insulin resistance (2). Recently, *ob* mRNA levels in rat adipose tissue have been reported to be stimulated by two days of insulin infusion (6) as well as by postprandial hyperinsulinemia (7) and a single injection of insulin (7). Insulin also directly stimulated *ob* mRNA levels in primary rat adipocytes (7). Thus, insulin appears to be a potent regulator of *ob* gene expression in rat adipose tissue. However, since synthesis and secretion of polypeptide hormones may be regulated differently, we specifically tested the hypothesis that insulin is a secretagogue for leptin in humans.

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Received for publication 2 February 1996 and accepted in revised form 19 February 1996.

RESEARCH DESIGN AND METHODS

Subjects. We studied 27 (18 women and 9 men) subjects after overnight fast (12 were restudied 3 h after eating their usual lunch) and 10 healthy subjects and 11 IDDM patients during hyperinsulinemic clamps. The group comprised 7 African-Americans and 20 whites. Based on a definition of obesity as a BMI of ≥27.3 for men and ≥27.8 for women (8), 13 subjects were classified as obese (9 women and 4 men, age 36.4 ± 9 years, BMI 33.6 ± 8 [means ± SD]) and 14 as nonobese (9 women and 5 men, age 33 ± 8, BMI 23.6 ± 2). Blood specimens for measurement of leptin and insulin levels were collected in EDTA tubes and immediately separated and stored at −70°C. Blood samples from overnight-fasted subjects were collected between 0700 and 0800, and postprandial specimens were obtained between 1400 and 1500. Patients with IDDM (five women and six men, age 25.8 ± 4.6 years, BMI 25.3 ± 0.8) were admitted to the General Clinical Research Center in the evening before clamp studies after discontinuing intermediate- or long-acting insulin. Their diabetes was managed with intravenous regular insulin (Novolin; Novo Nordisk, Bagsvaerd, Denmark) throughout, with hourly monitoring of plasma glucose levels to prevent hypoglycemia. Nondiabetic control subjects (six women and four men, age 27.6 ± 4 years, BMI 24.6 ± 2.7) were studied as outpatients after overnight fast. Hyperinsulinemic (12 pmol · kg^{-1} · min^{-1}, 2.0 mU · kg^{-1} · min^{-1}) initially euglycemic then stepped hypoglycemic (hourly steps at 4.7, 4.2, 3.6, 3.1, and 2.5 mmol/l; 85, 75, 65, 55, and 45 mg/dl) clamps (9) were performed starting at ~0800. The plasma glucose concentration was held at ~4.7 mmol/l (~85 mg/dl) during the initial 60 min of insulin infusion.

Analytic methods. Plasma glucose was measured with a glucose oxidase method (Beckman, Fullerton, CA). Plasma free insulin (10) and immunoreactive human leptin levels (Linco, St. Charles, MO) were measured by radioimmunoassays. The leptin radioimmunoassay detects immunoreactive human leptin with a sensitivity (lowest detectable limit) of 0.5 ng/ml in plasma. The intra- and interassay coefficients of variation were <8%; and the assay did not detect human insulin, proinsulin, glucagon, pancreatic polypeptide, or somatostatin.

Statistical analysis. The data are expressed as means ± SE except when the SD is indicated. The mean plasma leptin values in different groups (men vs. women, obese vs. nonobese, African-American vs. white, and IDDM vs. control) were compared by means of *t* tests. Fasting and postprandial plasma leptin levels were compared by means of paired *t* tests. Owing to the scatter in BMI and plasma leptin and insulin values, the relationship among these continuous variables under basal conditions was assessed by Spearman's correlations. The significance of the differences in serial plasma leptin levels during hyperinsulinemic clamps was analyzed using a general linear models procedure repeated measures analysis of variance.

RESULTS

Plasma leptin levels. Plasma immunoreactive human leptin was readily measurable in all samples. The range of values for fasting plasma leptin in this population was 5 to ~50 ng/ml, and the BMI range was from 20 to 57. The mean plasma leptin concentration in the obese subjects was 37.2 ± 3.6 ng/ml, as compared with 14.2 ± 2.2 ng/ml in the nonobese subjects ($P = 0.0009$). The plasma leptin concentration in the 9 men (BMI 26 ± 0.7) was 9.5 ± 1.1 ng/ml, as compared with

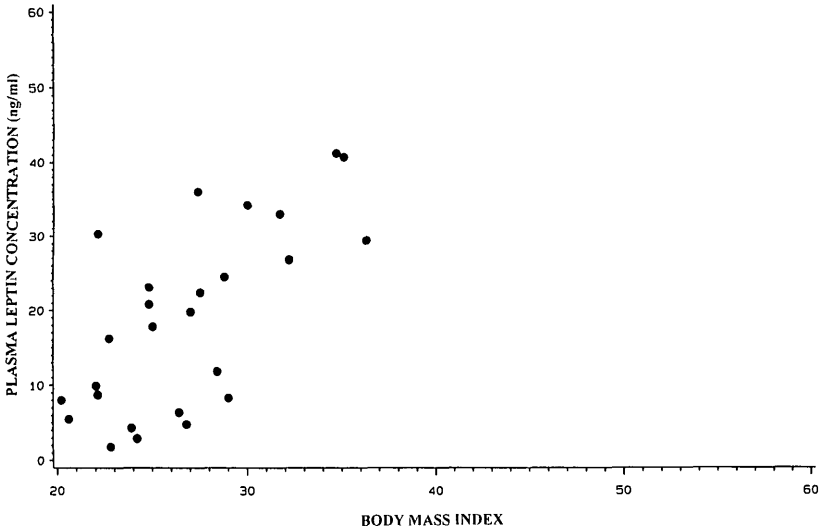

FIG. 1. Correlation of plasma leptin concentrations with BMIs in 27 subjects. The correlation coefficients were 0.76 (P = 0.0009) for the entire group, 0.84 (P = 0.0005) for obese subjects, and 0.02 (P > 0.5) for nonobese subjects.

19.8 ± 2.5 ng/ml in 14 age-matched women (BMI 25.2 ± 0.8) (P = 0.009). Plasma leptin levels (Fig. 1) were correlated with BMI (r = 0.76, P =0.0009) for the entire group. Because the distribution of plasma leptin appeared to be nonhomogeneous among obese and leaner subjects, we reanalyzed the correlations according to body size: The correlation between BMI and leptin was no longer significant in the nonobese group (r = 0.02), but that in the obese group became stronger (r = 0.84, P = 0.0005). The seven African-American subjects (BMI 32 ± 4.5) had a mean plasma leptin concentration similar to that in age- and BMI-matched whites, but showed the strongest correlation between leptin and BMI (r = 0.95, P = 0.013). Plasma leptin levels were also correlated with fasting plasma insulin (r = 0.75, P = 0.008) in all subjects. Among the 12 subjects studied in the fasting and postprandial states, the plasma insulin concentrations were 11.3 ± 0.7 μU/ml (67.8 ± 4 pmol/l) during fasting and 24.6 ± 3 μU/ml (148 ± 18 pmol/l) 3 h after food (P = 0.001). The corresponding plasma leptin concentrations were 27.2 ± 4.5 ng/ml (fasting) and 23.2 ± 4 ng/ml (postprandial).

Hyperinsulinemic clamps. The mean plasma leptin concentration before insulin infusion was 13.5 ± 3 ng/ml in patients with IDDM, as compared with 12.8 ± 3.3 ng/ml in matched nondiabetic subjects. Euglycemic hyperinsulinemia (Fig. 2) did not alter plasma leptin levels in IDDM patients or control subjects. Plasma leptin levels remained unchanged throughout the 85, 75, and 65 mg/dl hourly glycemic steps during the clamp (Figs. 3 and 4). Plasma leptin levels apparently increased by ~30% in two control subjects and decreased by 22% in one IDDM patient during the 55 to 45 mg/dl glycemic steps, but these changes were not significant.

DISCUSSION

We found that immunoreactive human leptin was readily measurable in unextracted plasma samples and that plasma leptin levels were correlated with BMI in humans, as previously reported (11). However, unlike the previous report, our data indicate that such correlation might be limited to obese subjects. Admittedly, our sample size was small; nonetheless, it showed sufficient power in discriminating leptin values by sex, body mass, and other correlates. Thus, inferences from this albeit small population may well have some biological validity. One such inference from the lack of correlation between BMI and leptin in nonobese subjects is the possibility of an inflection point downstream of the BMI scale below which signaling for leptin secretion may be quiescent.

Assuming that the metabolic effects of leptin in mice (2–5) extend also to humans and that plasma leptin levels among nonobese subjects represent the norm, then obese subjects have hyperleptinemia and may thus be relatively leptin resistant (operationally defined as obesity in states of plasma leptin abundance), similar to the findings in laboratory

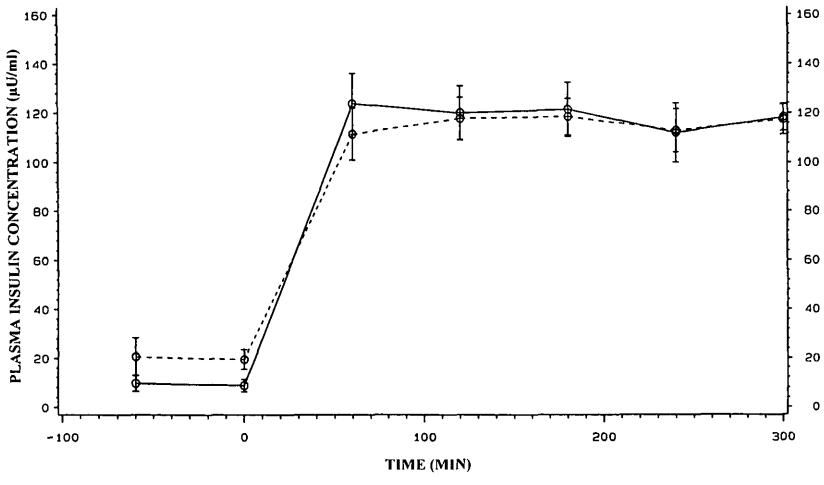

FIG. 2. Plasma free insulin concentrations in normal subjects (———) and IDDM patients (– – – –) undergoing hyperinsulinemic clamps. Steady-state plasma insulin was ~120 μU/ml (~720 pmol/l).

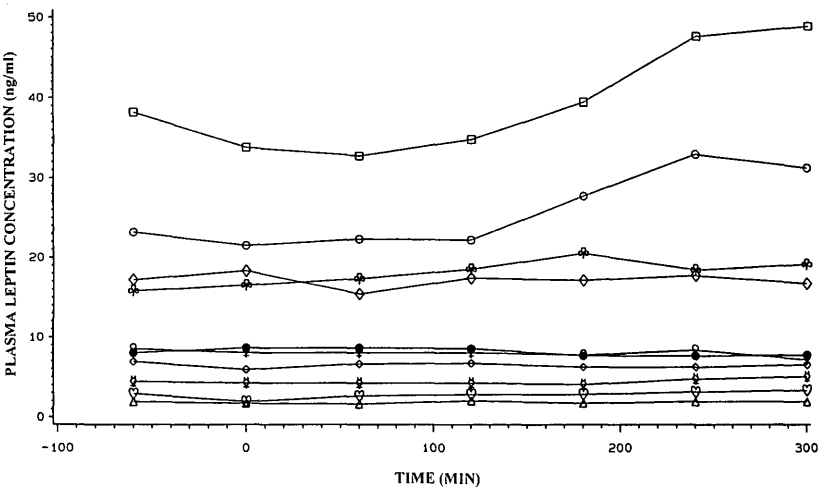

FIG. 3. Plasma leptin concentrations during hyperinsulinemic clamp in 10 normal subjects. Insulin was infused to a steady-state plasma level of ~120 μU/ml (~720 pmol/l) for 300 min. Blood glucose was maintained at ~85 mg/dl during the 1st hour of insulin infusion and then lowered in steps of 10 mg/dl each hour to a nadir of 45 mg/dl at study time 240 min and maintained at that level until time 300 min. Each data point represents the mean of two measurements; error bars have been omitted for clarity.

models of obesity (12). We also found that women had significantly higher plasma leptin levels than men matched for age and BMI. Correction for percentage body fat abolished this sex difference in a previous report (11). Thus, the sexual dimorphism in plasma leptin secretion is not likely due to relative leptin resistance in females.

In this study, we specifically tested the hypothesis that insulin is a physiological secretagogue for leptin in humans, based on earlier reports of insulin regulation of *ob* mRNA in rats (6,7) and the upregulation of *ob* mRNA in hyperinsulinemic animal models of obesity (13,14). We tested this hypothesis in three ways: *1*) by analyzing the relationship between plasma leptin and insulin levels in fasting subjects, *2*) by assessing the effect of physiological (meal-stimulated) hyperinsulinemia on plasma leptin levels, and *3*) by evaluating plasma leptin response to insulin infusion that results in high-physiological to pharmacological plasma insulin levels. We found that plasma leptin and insulin levels were correlated in fasting subjects. Hypothetically, this relationship could result from insulin stimulation of leptin secretion, leptin stimulation of insulin secretion, or stimulation of both leptin and insulin secretion by a third factor. Indeed, there are additional fundamental grounds for exploring a possible relationship between insulin and leptin. Insulin has long been implicated in the regulation of body weight (15) and has recently been confirmed to cause weight gain (16) through the attenuation of glycosuria and suppression of calorigenesis (17) in IDDM patients.

Notably, plasma insulin levels decreased in *ob/ob* mice and remained unchanged in wild-type mice treated with intraperitoneal injection of recombinant leptin (3), so leptin is not an insulin secretagogue, at least in mice. Our finding that postprandial plasma leptin levels were similar to fasting values in 12 subjects, despite a significant meal-stimulated rise in plasma insulin, indicates that physiological levels of insulin do not stimulate endogenous leptin secretion. Exposure to higher insulin levels maintained over a longer (5-h) period during the hyperinsulinemic clamp procedures also did not alter plasma leptin levels. (Furthermore, patients with IDDM treated with imperfect insulin replacement regimens nonetheless had normal plasma leptin levels for their age and body mass, suggesting relative independence of plasma leptin secretion from chronic iatrogenic under- or overinsulinization.) It is possible that a third factor that affects both leptin and insulin secretion accounts for the correlation that was observed in fasting subjects. Obesity might be one such factor, but we found a similar correlation between leptin and insulin among obese and nonobese subjects. Another possibility is insulin resistance, which may occur in obese as well as nonobese subjects. However, insulin resistance has not so far been specifically associated with alterations in leptin secretion.

Finally, because circulating leptin levels are not increased during postprandial hyperinsulinemia or during euglycemic (or hypoglycemic) hyperinsulinemia, we conclude that, at least in the short term, insulin does not stimulate leptin

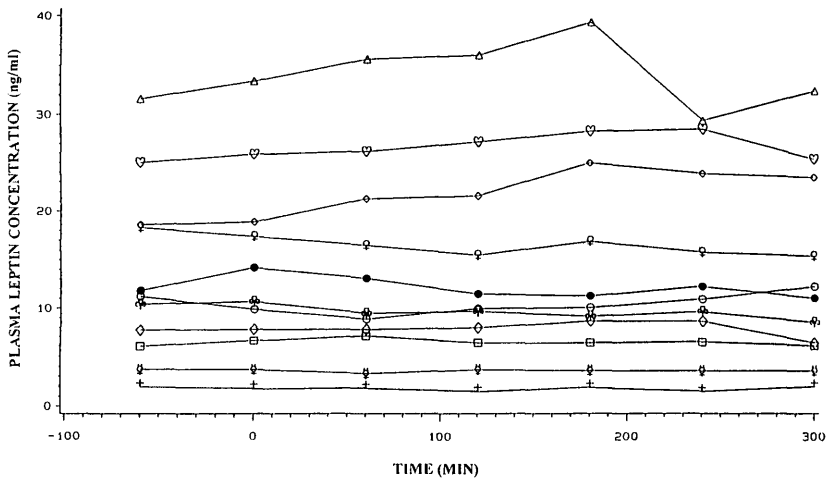

FIG. 4. Plasma leptin concentrations during hyperinsulinemic clamp in 11 patients with IDDM (see Fig. 3 for experimental details).

secretion in humans and that hyperleptinemia in obese individuals is not likely the result of hyperinsulinemia.

ACKNOWLEDGMENTS

This work was supported in part by U.S. Public Health Service Grants M01-RR00036 and P60-DK20579.

We thank Zina Lubovich, Michael Morris, and Carolyn Fritschle of the Washington University Diabetes Research and Training Center laboratory staff for technical assistance and Daniel Flasar for assistance with data management and analysis.

NOTE ADDED IN PROOF

Since the completion of our work and submission for publication, we have become aware of another study that found a correlation between fasting plasma insulin and leptin levels in humans (see 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).

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