Relationship Between Serum Leptin and the Insulin-Like Growth Factor-I System in Humans

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The growth hormone (GH)/insulin-like growth factor-I (IGF-I) system and leptin both play an important role in the regulation of body composition. Although the regulation of these two hormonal systems by insulin has been under intense investigation, the physiologic interactions between leptin and the GH/IGF-I system remain unknown. In this study, we examined the relationships among circulating leptin and key elements of the IGF-I system in 60 subjects (27 nondiabetic lean, 21 nondiabetic obese, and 12 type 1 diabetic subjects) with a wide range of insulin secretory capacity. Leptin, glucose, insulin, free IGF-I, total IGF-I, IGF-binding protein-1 (IGFBP-1), and IGFBP-3 levels were measured in the basal state after an overnight fast, and the acute insulin response to glucose (AIRG) was determined after intravenous glucose injection. AIRG was significantly higher ($P < .01$) in the obese (3,365 ± 562 pmol/L · min) versus lean subjects (1,624 ± 155 pmol/L · min). In simple regression analysis, the serum leptin concentration was positively correlated with the body mass index ([BMI] men, $r = .51$, $P = .005$; women, $r = .71$, $P < .001$), IGFBP-3 (men, $r = .20$, $P =$ nonsignificant; women, $r = .41$, $P < .025$), and AIRG (men, $r = .73$, $P < .001$; women, $r = .62$, $P < .01$). There was a nonlinear correlation between leptin and IGFBP-1, but there was no correlation between leptin and free or total IGF-I. In multiple regression analysis with leptin as the dependent variable, gender, BMI, and IGFBP-3 entered the equations at a statistically significant level. The correlation of leptin with IGFBP-3 was independent of obesity and persisted after correction for AIRG, suggesting a link between leptin and GH action.

THE GROWTH HORMONE (GH)/insulin-like growth factor-I (IGF-I) system plays a prominent role in muscle strength and development and has favorable effects on body composition.[1] GH has direct lipolytic effects and indirect anabolic and growth-promoting effects mediated by IGF-I. IGF-I also has been shown to promote adipose tissue differentiation.[1,2] The majority of IGF-I circulates in blood bound to a family of IGF-binding proteins (IGFBPs), which modulate its actions.[2,3] Presumably, the anabolic effects of GH are mediated by the unbound ("free") fraction of IGF-I, which is buffered by the IGFBPs, among which IGFBP-1 and IGFBP-3 play a predominant role. Serum IGFBP-1 is mainly dependent on circulating insulin, whereas IGFBP-3 is mainly under GH control.[3]

The *ob* gene product, leptin, is also involved in the regulation of body composition. When administered to ob/ob mice that lack this protein, leptin decreases food intake, increases energy expenditure, and decreases body weight.[4] Leptin-deficient mice also have stunted growth and an immature hypothalamic-pituitary axis,[5] and leptin treatment promotes growth in these animals,[4] suggesting that leptin stimulates GH or IGF-I secretion or action. Numerous studies have shown close correlations between circulating leptin concentrations and body fat mass, with higher leptin concentrations in women than in men.[6,7] The increase in body fat mass in obesity is accompanied by an increase in muscle mass, the mechanism of which has not been elucidated.[8] Because of a prominent role of IGF-I in muscle development, it is possible that muscle hypertrophy in obesity results from enhanced IGF-I secretion or action. In obese subjects, free IGF-I has been reported to be elevated[9] and GH secretion is blunted,[10] suggesting that obesity may be associated with an increased sensitivity to GH.

Taken together, these studies suggest the existence of an interaction between leptin and the IGF system in the regulation of body composition. The present study examines the relationships between leptin, IGF-I, and IGFBPs in humans with various insulin secretory capacity.

SUBJECTS AND METHODS

Subjects and Protocol

Study subjects (27 nondiabetic lean, 21 nondiabetic obese, and 12 type 1 diabetic subjects) were recruited from the Winnipeg metropolitan area. Based on a medical history and physical examination, these subjects were healthy and were not taking any medication, except for insulin in those with diabetes. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Manitoba. Informed written consent was obtained from all subjects before participation.

After an overnight fast, a bolus of 50% glucose (0.3 g/kg) was injected at time 0 and blood samples were drawn at −30, −15, 0, 1, 2, 3, 4, 5, 6, 7, 8, and 10 minutes for the determination of glucose and insulin. In addition, basal samples were used for measurement of leptin, total IGF-I, free IGF-I, IGFBP-1, and IGFBP-3. The samples were separated by centrifugation immediately after clotting, and serum was stored in aliquots at −20°C until analysis.

Serum Analysis

Serum leptin was assayed using a Linco radioimmunoassay (RIA) kit (Linco Research, St. Charles, MO). The coefficient of variation within and between assays was 3.0% to 5.0% and 3.5% to 6.0%, respectively. The detection limit was 0.5 ng/mL. Free IGF-I, total IGF-I, IGFBP-1, and IGFBP-3 were determined with two-site coated-tube immunoradiometric assays, using commercial kits (Active; Diagnostic Systems Laboratories, Webster, TX) as previously described.[11] Serum insulin was determined by a double-antibody RIA using a kit from Pharmacia Canada (Baie D'Urfe, Quebec). The serum glucose concentration was

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measured by the glucose oxidase method using a YSI 2300 STAT PLUS Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Data Analysis

The acute insulin response to glucose (AIRG) was calculated as the area under the insulin curve from 0 to 10 minutes after intravenous glucose injection, using the trapezoid rule. Statistical analyses were performed using SPSS for Windows after logarithmic transformation where data distribution was not normal (SPSS, Chicago, IL). One-way ANOVA with Duncan's multiple-range test was used to detect differences among the three study groups. Where a two-group comparison was applicable, the Student t test was used. Correlation coefficients were derived from regression analysis. To determine the independent effects of the variables on serum leptin levels, multiple linear regression analyses were performed. The results were considered statistically significant at a P level less than .05. Data are presented as the mean ± SE.

RESULTS

Physical and metabolic characteristics of the subjects are summarized in Table 1. Groups were matched for age and gender. By design, obese subjects had a higher body mass index (BMI) and diabetic patients had a higher serum glucose level than normal subjects. Among nondiabetic individuals, the AIRG was significantly higher in obese compared with lean subjects ($P < .025$), and fasting serum insulin tended to be elevated in the obese group. Obese subjects had higher leptin concentrations than lean subjects, and women had higher leptin levels than men at a similar BMI (Fig 1 and Table 1). Total and free IGF-I concentrations were lower in diabetic patients versus the normal and obese subjects. IGFBP-3 concentrations were significantly higher in the obese group compared with diabetic and normal subjects, whereas IGFBP-1 concentrations were about fourfold higher in diabetic patients compared with the nondiabetic groups.

Because of a major gender difference in leptin levels, simple correlation studies involving leptin are presented for men and women separately. In simple regression analysis, we found a positive correlation between leptin and IGFBP-3 in women, but not in men (Fig 2). However, there was a nonlinear inverse relationship between leptin and IGFBP-1 in both men and women (Fig 3). In nondiabetic subjects, there was a positive correlation between leptin and AIRG (Fig 4), but we found no significant correlation between IGFBP-3 and AIRG. There was no significant relationship between leptin and fasting insulin or between leptin and free or total IGF-I. There was a strong correlation between the leptin concentration and BMI (Fig 1), but neither IGFBP-1, IGFBP-3, free IGF-I, nor total IGF-I concentrations had a significant relationship with the BMI. IGFBP-3 correlated positively with free IGF-I ($r = .35, P < .01$) and total IGF-I ($r = .47, P < .0001$) and inversely with IGFBP-1 ($r = .26, P < .05$).

We performed a multiple regression analysis in all subjects with leptin as the dependent variable and gender, BMI, IGFBP-1, and IGFBP-3 as independent variables (Table 2). In nondiabetic subjects, fasting insulin and AIRG were also included. Gender, BMI, and IGFBP-3 significantly accounted for the variability in leptin levels, whereas the contribution of fasting insulin and IGFBP-1 was not significant. However, when AIRG and fasting insulin were both added to the regression equation, the AIRG contribution became significant ($P = .04$).

Table 1. Characteristics of the Subjects

Characteristic	Normal	Obese	Diabetic
No. of subjects	27	21	12
Sex ratio (women/men)	14/13	11/10	6/6
Age (yr)	36 ± 3	38 ± 2	37 ± 2
BMI (kg/m²)	23.3 ± 0.4	31.7 ± 0.9†	23.6 ± 0.7
Glucose (mmol/L)	4.4 ± 0.1	4.6 ± 0.2	10.5 ± 1.5†
Insulin (pmol/L)	67.2 ± 16.7	82.3 ± 18.7	
AIRG (pmol/L · min)	1,624 ± 155	3,365 ± 562†	
Leptin (ng/mL)	7.3 ± 1.0	19.3 ± 3.6†	7.8 ± 1.8
Total IGF-I (μg/L)	219 ± 21	214 ± 20	145 ± 21*
Free IGF-I (μg/L)	0.99 ± 0.13	0.80 ± 0.10	0.45 ± 0.12*
IGFBP-1 (μg/L)	25.8 ± 3.1	20.3 ± 5.6	110.3 ± 31.4†
IGFBP-3 (mg/L)	2.6 ± 0.1	2.9 ± 0.2*	2.3 ± 0.2

NOTE. Values are the mean ± SE. AIRG was determined in 22 normal and 15 obese subjects.

*$P < .05$, †$P < .01$ v the other group(s).

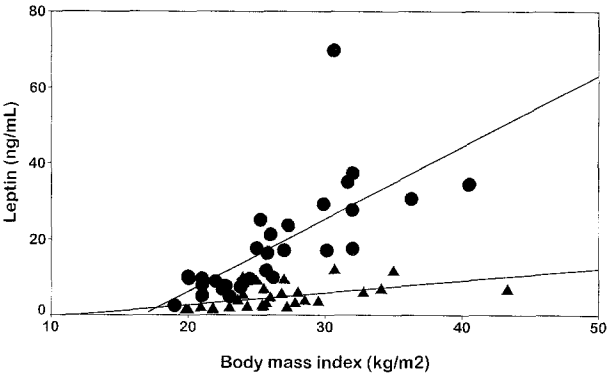

Fig 1. Relationship between serum leptin and BMI in men (▲, $r = .51, P < .005$) and women (●, $r = .71, P < .001$).

DISCUSSION

The main finding in this study is the association between leptin and IGFBP levels. Leptin levels were directly related to IGFBP-3 and inversely related to IGFBP-1 concentrations, but we found no significant relationship between leptin and IGF-I. Both IGF-I and IGFBP-3 are regulated by GH and are used as

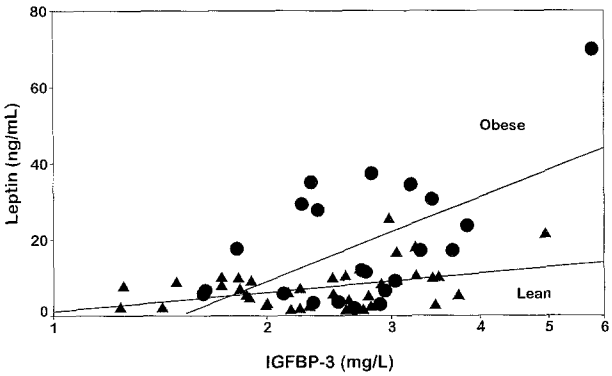

Fig 2. Relationship between serum leptin and IGFBP-3 in lean (▲, $r = .46, P < .005$) and obese (●, $r = .65, P < .001$) subjects. In men and women separately, $r = .20$ (P = nonsignificant) and $r = .41$ ($P < .025$), respectively.

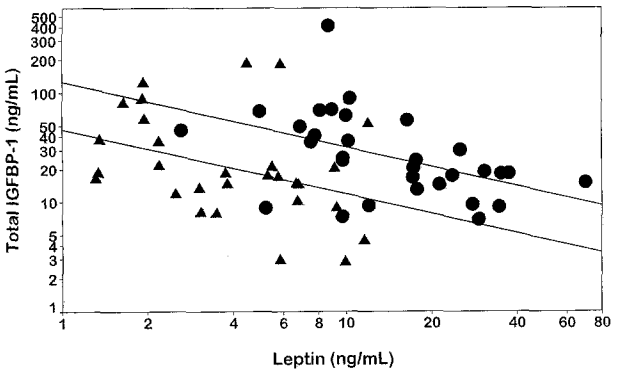

Fig 3. Relationship between serum leptin and IGFBP-1 in men (▲, $r = -.37$, $P < .05$) and women (●, $r = -.48$, $P < .01$). The nonlinear relationship is illustrated by the log-transformation.

Table 2. Multiple Regression Analysis of Leptin and Covariates

Covariate	B	SE(B)	*t*	*P*
BMI	1.015	0.204	4.965	<.0001
IGFBP-1	−0.00635	0.0173	−0.366	.7155
IGFBP-3	0.434	0.140	3.110	.003
Gender*	10.848	2.196	4.941	<.0001

NOTE. Analysis was performed for all 3 groups of subjects with leptin as the dependent variable and BMI, gender, IGFBP-1, and IGFBP-3 as independent variables. B is the regression coefficient; SE(B) is the standard error of the regression coefficient. When diabetic subjects were excluded from the analysis and fasting insulin was introduced into the model, the contribution of IGFBP-3 to the variability of leptin levels remained highly significant (B = 0.584, SE(B) = 0.155, $t = 3.772$, $P = .0005$), but insulin did not enter the equation at a significant level ($P = .464$).

*Men = 1 and women = 2.

indices of GH secretion and action,[12-16] whereas IGFBP-1 is mainly controlled by insulin.[3,17] Recent studies have reported elevated serum leptin in hypopituitary patients,[18-21] which was reduced by GH treatment.[20,21] Others have found a positive correlation between leptin and GH-binding protein in children and postulated that leptin might regulate adipocyte sensitivity to GH.[22] Leptin antiserum inhibited GH secretion in rats,[23] whereas leptin itself reversed fasting-induced inhibition of GH secretion in these animals. These studies suggest the presence of a feedback loop whereby GH inhibits leptin secretion and leptin stimulates GH secretion or action.

On the other hand, Bianda et al[24] found increased leptin levels following GH treatment of GH-deficient patients. Because leptin levels in this study decreased when insulin secretion was suppressed by IGF-I treatment, it is possible that leptin stimulation by GH may have resulted from increased insulin secretion.[4] Such inhibition of leptin secretion by IGF-I might also explain previous reports of an inverse relationship between leptin and IGF-I[21] or the IGF-I/IGFBP-3 molar ratio,[25] an index of the free IGF-I concentration. Because IGFBP-3 is also regulated by insulin,[26] it may be anticipated that an increase in IGFBP-3, by sequestrating the free IGF-I fraction, would further increase insulin secretion and, as a consequence, leptin levels, providing an additional explanation for a direct relationship between leptin and IGFBP-3.

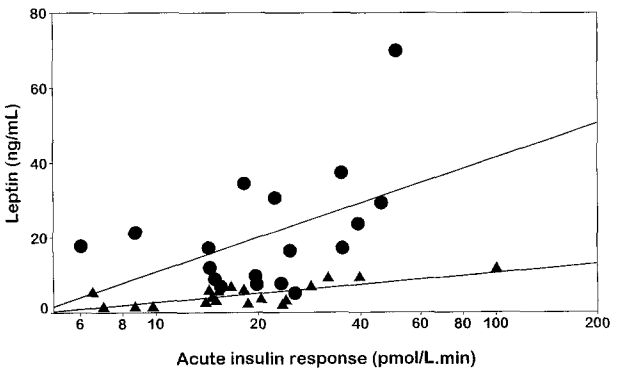

Fig 4. Relationship between serum leptin and AIRG in men (▲, $r = .73$, $P < .001$) and women (●, $r = .62$, $P < .01$). AIRG was determined in nondiabetic subjects only.

Thus, leptin and IGFBP-3 may be correlated because they are both regulated by insulin. To further support the role of insulin, subjects with a wide range of insulin levels were included in this study and the relationship between leptin and IGFBP-1 was investigated. IGFBP-1, whose synthesis by the liver is inhibited by insulin,[3,17,27] was associated with leptin by an inverse nonlinear relationship, whereas IGFBP-3, which is under positive control by both GH[12-16] and insulin,[26] showed a positive relationship with leptin. We also found an association between the AIRG and serum leptin, which indicates that leptin concentrations are higher in subjects with elevated insulin secretion.[28] However, since we found no significant relationship between IGFBP-3 and insulin and since the association between leptin and IGFBP-3 was independent of the AIRG, it cannot be solely explained by a common regulation of leptin and IGFBP-3 by insulin.

As in previous studies,[4,6,7] we found a direct association between serum leptin and body fatness as expressed by the BMI. The slope of this association was steeper in women than in men, and women had a higher leptin concentration at a similar BMI. Studies have shown that leptin correlates better with percent fat than with the BMI and that the relationship between leptin and the degree of obesity is not gender-specific when percent fat is used instead of the BMI.[6,7] The significance of the relationship between leptin and IGFBP-3 might therefore change if the true fat mass is used as a covariate. Leptin is mostly synthesized by subcutaneous fat,[29] which is more prominent in women than in men, and there is a negative association between leptin and testosterone concentrations,[30-32] explaining the gender difference in leptin levels. The association of leptin with adiposity is thought to reflect leptin resistance in obese subjects.[6,33] Both leptin[4,6,7] and IGFBP-3[34] increase with body weight, suggesting that hormonal factors associated with weight gain could explain our results. The activity of the hypothalamic-pituitary-adrenal axis may increase in obesity,[35] and glucocorticoids increase leptin secretion.[36,37] However, these steroids do not stimulate IGFBP-3 production and may actually decrease IGFBP-3 levels.[3] Obesity may be associated with increased sensitivity to GH,[10] and this could explain the

elevated IGFBP-3 levels with increasing body weight. The association between leptin and IGFBP-3 observed with simple correlation studies was significant in women but not in men, probably reflecting gender-related differences in the regulation of these proteins[30-32,38] and GH.[39]

There was a discrepancy in the relationship of leptin with IGF-I and IGFBP-3, two indices of GH secretion and action. The lack of correlation between leptin and IGF-I has been reported,[18] and the responses of IGF-I and IGFBP-3 to GH are not always parallel. Several studies have reported a smaller increase of IGFBP-3 compared with IGF-I during GH treatment,[13-15] while others have reported that IGFBP-3 increases more during GH treatment.[12,16] These studies have suggested that IGFBP-3 may be a better reflector of GH secretion compared with IGF-I, whereas IGF-I may be more informative about the growth response to GH. More recently, Lee et al[40] have reported that after GH injection in humans, IGF-I increases rapidly. The correlation between IGF-I and IGFBP-3 in the current study was only .50, in agreement with these reports. The metabolic correlates of these two peptides therefore may not be identical.

When this report was in preparation, Van Den Berghe et al[41] reported in chronically ill patients a stimulatory effect of GH secretagogues on leptin levels and a positive correlation between the concentrations of leptin and the acid-labile subunit, a GH-regulated protein that is a component of an approximately 150-kd complex also comprising IGFBP-3 and IGFs. These results and our observations suggest a role for GH or its secretagogues in the regulation of leptin secretion.

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