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Serum Leptin Levels Are Associated with Hyperinsulinemia Independent of Body Mass Index but Not with Visceral Obesity

Hoon Kim-Motoyama,* Takuhiro Yamaguchi,† Tomiyoshi Katakura,‡ Masakazu Miura,§ Yasuo Ohashi,† Yoshio Yazaki,* and Takashi Kadawaki*

**Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan; †Department of Biostatistics/Epidemiology and Preventive Health Sciences, School of Health Sciences and Nursing, University of Tokyo, Tokyo, Japan; ‡Nippon Express Health Insurance Society, Tokyo Hospital, Tokyo, Japan; and §Mitsubishi Kagaku Bio-Clinical Laboratories, INC, Tokyo, Japan*

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To examine the relationship between leptin levels and visceral obesity or plasma insulin levels, we studied serum leptin levels, fat distribution assessed by CT scan, and plasma insulin levels during 75 g oral glucose load in 100 Japanese men. Regression analysis adjusted by age and body mass index (BMI) showed leptin levels to be associated with visceral fat area(V)($p=0.003$), subcutaneous fat area(S)($p<0.0001$), and $V+S$($p<0.0001$), but not with V/S ratio($p=0.897$). By regression analysis adjusted by age, BMI, and $V+S$, serum leptin levels were still highly and positively correlated with plasma insulin levels during 75 g oral glucose load ($p<0.001$), insulin resistance index($p<0.001$), and β cell function index($p=0.009$) in homeostasis model assessment. These data suggest that hyperinsulinemia, but not visceral obesity, may be regulators of serum leptin levels independent of BMI.

Obesity is caused by an excess of adipogenesis. Leptin, the product of the obesity (ob) gene,[1] is one of the main regulators of adipogenesis affecting food intake and thermogenesis.[2] Leptin concentrations correlate with body mass index (BMI) and with percentage body fat.[3] Obesity is usually associated with insulin resistance causing hyperinsulinemia. This obesity-associated insulin resistance causing hyperinsulinemia may, at least in part, be due to hyperleptinemia itself.[4] Thus, although obesity, hyperleptinemia and hyperinsulinemia often coexist, their interrelationship may be quite complex, and whether the relationship between insulin and leptin concentrations is independent of obesity is unclear. In our previous report, subjects with visceral obesity with a higher visceral fat area(V)/subcutaneous fat area(S) ratio showed higher plasma insulin levels.[5] It is also unclear whether visceral obesity is associated with increased leptin concentrations in humans. To examine the relationship between serum leptin levels and visceral obesity or plasma insulin levels independent of BMI, we studied the association between serum leptin levels and visceral obesity assessed by CT scan, and plasma insulin levels during 75g oral glucose load(OGL) in 100 Japanese men.

METHODS

Study subjects. The subjects were 100 Japanese men (age: 21-65years mean 50.2years SD 9.4years, BMI: 18.9-41.6kg/m^2 mean 26.4kg/m2 SD 3.3kg/m^2) who underwent a medical check-up in a company-based clinic. They included 57 subjects with normal glucose tolerance, 25 subjects with impaired glucose torelance (IGT) and 18 subjects with non-insulin dependent diabetes mellitus (NIDDM). NIDDM and IGT were diagnosed according to the World Health Organization (WHO) criteria.[6] However, none required insulin injection or oral hypoglycemic agents.

Study protocol. Informed consent was obtained from all the subjects studied. Following 12 hr fast, venous blood samples were obtained. Body height, body weight, plasma glucose and insulin levels during a 75g OGL, HbA1c, V/S at umbilical level, waist to hip ratio (W/H), serum total cholesterol, triglyceride, γ-GTP levels and systolic and diastolic blood pressures were studied on the same day. Venous serum leptin levels were determined by a newly developed radioimmunoassay (Linco Research, St, Charles, MO).[7] In homeostasis model assessment(HOMA),[8,9] indexes of insulin resistance (fasting insulin $\times$ fasting glucose/ 405 mU$\times$g/10$\times$L^2) and β cell function (20$\times$fasting insulin/ fasting glucose-63 10mU/g) were performed.

Statistical analysis. Spearman's correlation coefficient was used to estimate linear relationships between variables. Regression analyses were carried out between leptin levels and each of the other parameters. The Statistical Analysis System package (SAS Institute, Cary, N.C.,USA) was used.

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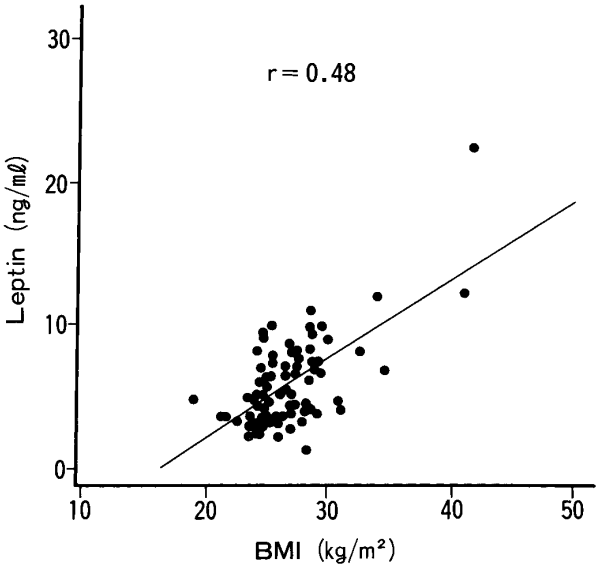

FIG. 1. Correlation of serum leptin concentrations with BMI for men.

RESULTS

Serum Leptin Levels, BMI, and Visceral Obesity

Serum leptin levels were positively correlated with BMI($r=0.48$) (Fig. 1). They were also positively correlated with subcutaneous fat area (S) ($r=0.63$) and total fat area (V+S) ($r=0.67$) (Fig. 2b and 2c). Spearman's correlation coefficient between serum leptin levels and visceral fat area (V) was 0.33,which was lower than that S or V+S (Fig2a). There was no positive correlation between leptin levels and V/S ratio($r=-0.20$) (Fig. 2d). By regression analysis adjusted by age and BMI, leptin levels were correlated positively with V ($p=0.003$), S ($p<0.0001$) or V+S ($p<0.0001$)(Table). V/S ratio, however, was not associated with leptin levels($p=0.897$).

Serum Leptin Levels, Plasma Insulin Levels, and Blood Pressure

Serum leptin levels were correlated with fasting plasma insulin (F-IRI) ($r=0.52$)(data not shown), 2hr insulin (2hr IRI) ($r=0.41$) (data not shown) and total insulin (Σ IRI)($r=0.53$)(Fig. 3) levels during 75g OGL. Since both leptin and insulin levels were influenced by BMI and age, we performed regression analysis adjusted by these two factors. Serum leptin levels were significantly associated with higher F-IRI ($p<0.0001$), 2hr IRI ($p<0.0001$), and ΣIRI ($p<0.0001$) levels, higher systolic blood pressure (SBP) ($p=0.005$), and higher V+S($p<0.0001$)(Table). Even after being adjusted by V+S and age and BMI, leptin levels, but not SBP, were still correlated positively with F-IRI ($p<0.0001$), 2hr IRI ($p<0.01$), and Σ IRI ($p<0.004$)(Table 1).

Serum Leptin Levels, Insulin Resistance Index, and β Cell Function Index in HOMA

Serum leptin levels were correlated with both insulin resistance index ($r=0.49$) and β cell function index($r=0.45$) in HOMA[8,9](data not shown). By regression analysis adjusted by BMI and age, serum leptin levels were associated with higher insulin resistance index($p<0.0001$) and higher β cell function index($p<0.0001$)(Table). Even after adjusted by V+S and age and BMI, leptin levels were still correlated positively with higher insulin resistance index ($p<0.001$) and higher β cell function index($p=0.009$)(Table 1).

DISCUSSION

This study is the first to show that visceral fat area (V), subcutaneous fat area(S) and total fat area (V+S) at the umbilical level, but not V/S ratio, are correlated positively with serum leptin levels in humans. It provides evidence that serum leptin levels are highly correlated with plasma insulin levels and systolic blood pressure independent of BMI and age. Furthermore, fasting, 2hr, and total plasma insulin levels during 75g oral glucose load, and both insulin resistance index and β cell function index assessed by HOMA were correlated with serum leptin levels independent of V+S, BMI, and age.

In this study, V/S ratio was not correlated positively with serum leptin levels. These data may be consistent with the previous report that leptin mRNA levels examined by quantitative reverse transcriptional polymerase chain reaction were higher in subcutaneous than in omental adipocytes in humans.[10] Our data is the first to show that higher V/S ratio, which is usually associated with insulin resistance, per se, are not associated with hyperleptinemia in humans.

It was previously reported that in vitro leptin downregulated insulin dependent tyrosine phosphorylation of insulin receptor substrate-1(IRS-1) in HepG2 cells[11] and that physical concentrations of leptin impaired several metabolic actions of insulin in rat adipocytes,[12] which suggested that leptin might cause insulin resistance in target tissues. However, another report showed that leptin enhanced insulin's ability to inhibit hepatic glucose production.[13] Thus leptin's influence on insulin action in vitro is highly controversial at present. A previous report in NIDDM patients, on the other hand, showed that there was no correlation between plasma leptin levels and insulin sensitivity by measuring the metabolic clearance rate of glucose during hyperinsulinemic euglycemic clamp, although plasma leptin levels were correlated with plasma insulin con-

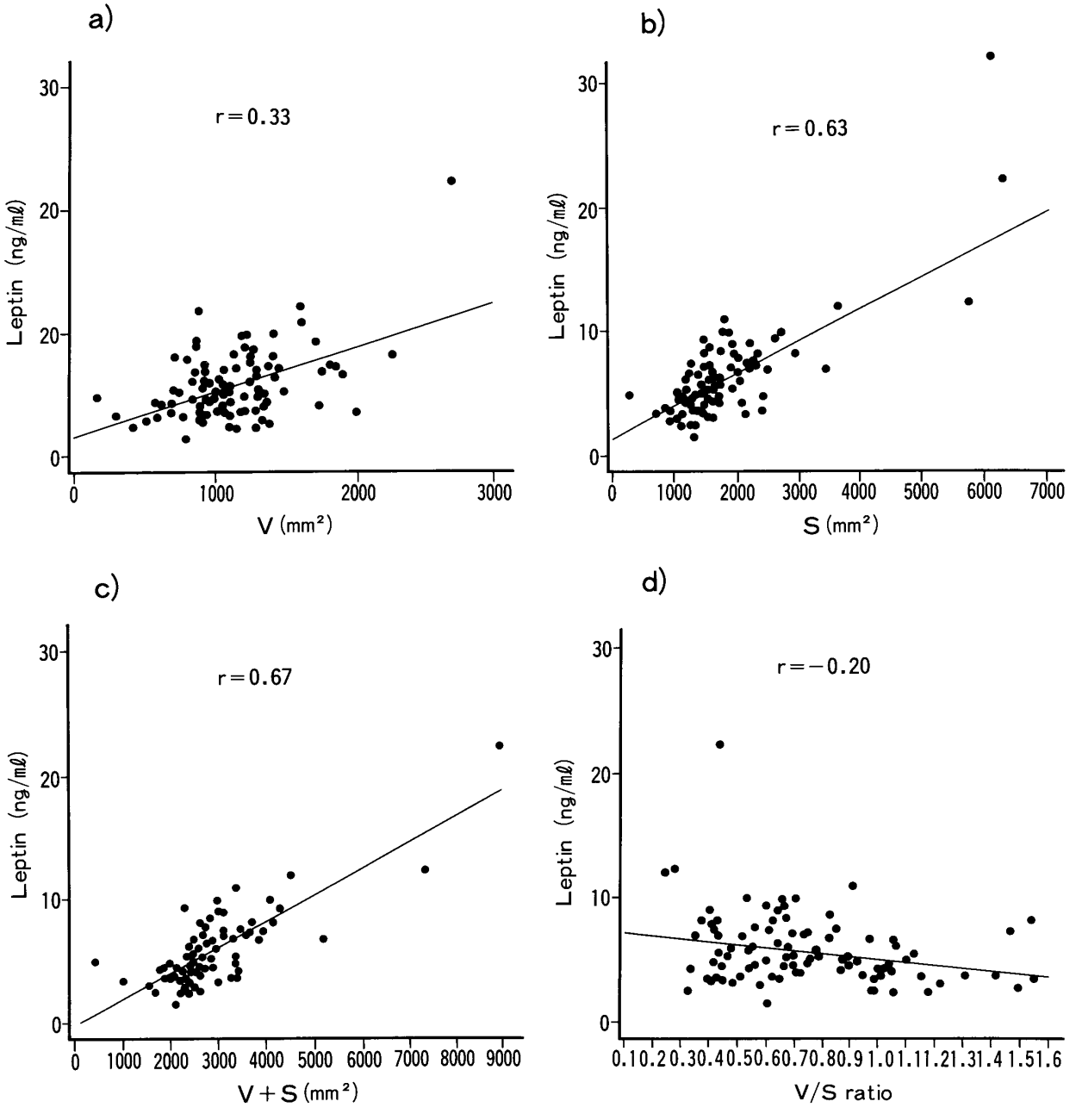

FIG. 2. Correlation coefficient of serum leptin concentrations with visceral fat area (V)(a). Correlation of serum leptin levels with subcutaneous fat area(S)(b), and total fat area(V+S)(c). V/S ratio(d) was not associated with serum leptin levels.

centrations.[14] In the present study, after adjusting by BMI and age, serum leptin levels were still highly correlated with plasma insulin levels during 75g OGL($p<0.001$) and systolic blood pressure($p=0.005$). This study shows that leptin levels strongly correlate with insulin levels and systolic blood pressure independent of BMI and age. Moreover, indexes of insulin resistance assessed by HOMA were correlated positively with higher serum leptin levels independent of V+S, BMI, and age. The reason for the discrepancy between this and the previous study[14] is not clear at present.

There are several possibilities to explain this positive relationship between leptin levels and insulin levels independent of BMI. Leptin may cause insulin resistance independent of obesity which may result in hyperinsulinemia. Alternatively, leptin itself may cause increased insulin secretion from pancreatic β cells, although there are reports that leptin may be responsible

TABLE 1

Regression Analysis between Serum Leptin Level and Each Parameter Adjusted by BMI and Age

Parameters	Mean ± SD	P value adjusted by BMI and age	P value adjusted by V+S, age and BMI
Fasting insulin (μ U/ml)	1.1 ± 1.1	$P < 0.0001$	$P < 0.0001$
2hr insulin (μ U/ml)	5.1 ± 3.2	$P < 0.0001$	$p = 0.013$
Σ insulin (μ U/ml)	20.3 ± 11.8	$P < 0.0001$	$p = 0.004$
I.R.I.	2.2 ± 2.2	$P < 0.0001$	$P < 0.0001$
FBS	106.3 ± 13.7	$p = 0.524$	$p = 0.573$
HbA1c	5.2 ± 0.6	$p = 0.038$	$p = 0.308$
β cell function	4.0 ± 3.3	$P < 0.0001$	$p = 0.009$
Systolic blood pressure (mmHg)	128.9 ± 18.3	$P = 0.005$	$p = 0.163$
Diastolic blood pressure (mmHg)	85.0 ± 11.4	$P = 0.151$	$p = 0.527$
Total cholesterol (mmol/l)	5.5 ± 0.9	$P = 0.294$	$p = 0.250$
Triglyceride (mmol/l)	1.7 ± 1.3	$P = 0.491$	$p = 0.789$
γ-GTP (μ Ukat/l)	0.99 ± 0.63	$P = 0.056$	$p = 0.020$
W/H	0.9 ± 0.04	$p = 0.145$	$p = 0.384$
V (mm^2)	1206.1 ± 3883.5	$P = 0.003$	—
S (mm^2)	16809.7 ± 8156.2	$P < 0.0001$	—
V + S (mm^2)	28015.8 ± 10459.0	$P < 0.0001$	—
V/S	0.73 ± 0.30	$P = 0.897$	—

Note. 2 hr insulin = insulin concentration of 2hr after 75g oral glucose load. Σ insulin = total insulin concentration during 75g oral glucose load. γ-GTP = gamma glutamyl transpeptidase.

for a decrease in insulin secretion.[15] It is also reported that insulin administration caused an increase in obese gene expression in rodent.[16] Thus, hyperinsulinemia itself may directly cause an increased release of leptin from adipose tissue. Finally, hyperleptinemia may be due to increased fat mass, which itself also causes insulin resistance independent of hyperleptinemia.

The results of this study revealed that leptin levels were highly correlated with plasma insulin levels, insulin resistance index, and β cell function index in HOMA independent of obesity. Therefore, leptin may not only modulate insulin sensitivity but also may itself be induced by insulin or induce insulin release.

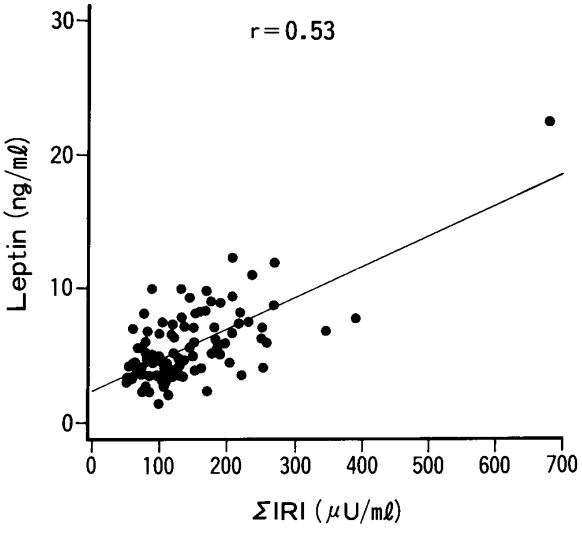

FIG. 3. Correlation of serum leptin concentrations with total insulin levels(Σ IRI) during 75g oral glucose load.

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