Relationships Between Serum Soluble Leptin Receptor Level and Serum Leptin and Adiponectin Levels, Insulin Resistance Index, Lipid Profile, and Leptin Receptor Gene Polymorphisms in the Japanese Population

Takeo Ogawa, Hiroshi Hirose, Yukihiro Yamamoto, Kanako Nishikai, Kiichi Miyashita, Haruo Nakamura, Ikuo Saito, and Takao Saruta

Leptin plays an important role in the regulation of body weight and is known to circulate in both free and bound forms. One of the leptin receptor isoforms exists in a circulating soluble form that can bind leptin. Clinical studies have shown that soluble leptin receptor (sOB-R) levels are lower in obese individuals. In the present study, we measured the serum sOB-R level in 419 healthy Japanese subjects (198 men and 221 women, aged 30 to 65 years, body mass index [BMI] 21.7 ± 2.6 [SD] kg/m^2) and in 150 type 2 diabetic patients (96 men and 54 women, BMI 24.3 ± 3.8 kg/m^2). We investigated the relationships between serum sOB-R level and BMI, blood pressure, homeostasis model assessment-insulin resistance index (HOMA-IR), serum leptin and adiponectin levels, lipid profile, and leptin receptor (LEPR) gene Lys109Arg and Gln223Arg polymorphisms. Serum leptin and sOB-R levels were measured by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), respectively. The serum sOB-R level in men was significantly higher than that in women. The serum sOB-R level was negatively correlated with BMI, fasting insulin, HOMA-IR, and serum leptin level and positively correlated with high-density lipoprotein (HDL)-cholesterol and serum adiponectin levels. The correlations between serum sOB-R level and fasting insulin, HOMA-IR, serum leptin, adiponectin, and HDL-cholesterol levels were significant even after adjustment for age, sex, and BMI in healthy subjects. There was no association between serum sOB-R level and the LEPR polymorphisms examined. These findings suggest that the serum sOB-R level is negatively correlated with HOMA-IR and serum leptin level and positively correlated with HDL-cholesterol level and serum adiponectin level, independent of age, sex, and BMI, in the Japanese population.

OBESITY, WHICH is characterized by the accumulation of excess adipose tissue, is a risk factor for diabetes mellitus, hypertension, hyperlipidemia, and atherosclerotic diseases. Epidemiologic studies demonstrate that the incidence and prevalence of obesity are also increasing in Japan. Leptin, the product of the *obese (ob)* gene,[1] is produced by adipose tissue and is secreted into the circulation. Leptin plays a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure.[2] In humans, the circulating leptin level is increased in obesity[3] and is positively correlated with the total body fat mass, suggesting that a hallmark of obesity is not leptin deficiency, but leptin resistance. Leptin acts mainly in the hypothalamus,[4] but also in other tissues by binding to specific leptin receptors, which belong to the cytokine receptor family. Besides membrane-bound isoforms of the leptin receptor with varying cytoplasmic length, a soluble form of the leptin receptor (sOB-R) has been demonstrated.[5] sOB-R consists entirely of the extracellular ligand-binding domain and lacks the transmembrane residues and intracellular domain responsible for signal transduction. Sinha et al[6] demonstrated the presence of leptin binding proteins and reported that in lean subjects the majority of leptin circulated in the bound form, whereas in obese subjects, the majority of leptin circulated as the free form. Landt et al[7] reported that only free leptin was detectable in cerebrospinal fluid (CSF), suggesting that it was the biologically active form. Lammert et al[8] observed that leptin binding activity was coeluted with levels of the sOB-R and that sOB-R was the major leptin binding protein in the circulating human blood.

Following the establishment of an enzyme-linked immunosorbent assay (ELISA) for human sOB-R, it has been reported that serum sOB-R level is low in obese individuals.[9-13] Contrary to leptin level, the sOB-R level increased after weight loss by a low-calorie diet[9] or gastric restrictive surgery.[14,15] Regarding the relationship between sOB-R and insulin levels, no association has been reported.[11,16] In the metabolic syndrome, Sandhofer et al[17] reported that sOB-R level was negatively correlated with homeostasis model assessment-insulin resistance (HOMA-IR), independent of age, body mass index (BMI), and fat mass, suggesting that a low sOB-R level is a marker of leptin resistance, and may constitute an additional component of the metabolic syndrome.

There are, however, few studies in healthy or diabetic subjects concerning the relationships between sOB-R level and HOMA-IR, lipid profile, and adiponectin level. In the present study, we measured serum sOB-R level in Japanese healthy subjects and type 2 diabetic patients and investigated its relationships with BMI, HOMA-IR, lipid profile, and serum leptin and adiponectin levels. Also, to our knowledge, there have been no studies concerning the relationship of leptin receptor (LEPR) gene polymorphisms with the sOB-R level or free leptin index, leptin/sOB-R ratio.[10] Therefore, we also investigated the relationship of the LEPR gene polymorphisms, Lys109Arg and Gln223Arg, with BMI, leptin and sOB-R levels, HOMA-IR, and clinical and metabolic parameters in healthy Japanese subjects.

From the Department of Internal Medicine; and Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo; and the Department of Internal Medicine, Mitsukoshi Health and Welfare Foundation, Tokyo, Japan.

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Address reprint requests to Hiroshi Hirose, MD, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.

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SUBJECTS AND METHODS

Subjects

This study included 198 healthy Japanese men and 221 women, aged 30 to 65 years, who received an annual health check-up, and their fasting plasma glucose (FPG) levels were less than 110 mg/dL. Subjects with endocrine disease, significant renal or hepatic disease, and those receiving medication for diabetes mellitus, hypertension, or hyperlipidemia were excluded. Also, 96 male and 54 female type 2 diabetic patients, who were diagnosed and followed up since an age of less than 65 years at Keio University Hospital or Mitsukoshi Clinic (Tokyo, Japan) were included as the diabetic group. Concerning LEPR gene polymorphisms, we analyzed 127 healthy men and 90 healthy women whose characteristics were the same as described above.

The present study was conducted according to the principles expressed in the Declaration of Helsinki. Informed consent was obtained from each subject after full explanation of the purpose, nature, and risk of all procedures used. The protocol was approved by the ethical review committee of the Keio University School of Medicine, Tokyo, Japan.

Measurements

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice with the subjects in the sitting position after resting for at least 5 minutes. Height, weight, FPG, serum insulin, leptin, sOB-R, adiponectin, total cholesterol, triglycerides (TG), high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)-cholesterol levels were measured in the morning after an overnight fast. Plasma glucose and lipid profiles were assayed by routine automated laboratory methods as described previously.[18-20] The serum insulin level was measured by an enzyme immunoassay (EIA) using a commercially available kit (Tosoh, Tokyo, Japan). The insulin resistance index was assessed by HOMA-IR.[21] Serum leptin level was measured with a commercially available kit (Linco Research, St Charles, MO) based on radioimmunoassay (RIA), as described previously.[18-20] Serum adiponectin was measured (in healthy subjects only) by ELISA without a denaturing step (Chugai Diagnostic Science and Fujirebio, Tokyo, Japan), as described previously.[22]

Measurement of Soluble Leptin Receptor by ELISA

Total sOB-R was measured by ELISA using a commercially available kit (Biovendor Laboratory Medicine, Brno, Czech Republic). Briefly, we diluted standards, quality controls, and samples 1:3 with dilution buffer prior to use. Then 100 μL diluted standards, quality controls, and samples were pipetted into 96-well microtiter plates coated with antileptin receptor monoclonal antibody. After incubation at room temperature for 1 hour, the wells were washed 3 times and incubated for 1 hour with the monoclonal antibody labeled with horseradish peroxidase. The wells were again washed 3 times and incubated for 5 minutes with tetramethylbenzidine reagent. Then 100 μL 0.2 mol/L H_2SO_4 was added to each well to stop the reaction, and the absorbance at 450/655 nm was measured. The limit of detection was 0.4 U recombinant leptin receptor/1 mL sample. The standard material used in this kit was recombinant human IgG-Fc-fragment-human leptin receptor dimeric chimera, which was different from the native soluble leptin receptor that was measured in serum. For this reason, we used 2 ng of the recombinant standards as 1 U equivalent of soluble native human leptin receptor. The dilution curve was parallel to the standard curve. Intra- and interassay coefficients of variance were 2.6% to 4.7% and 6.3% to 7.2%, respectively.

Determination of Polymorphisms

Lys109Arg and Gln223Arg polymorphisms were determined by TaqMan (Applied Biosystems, Tokyo, Japan) polymerase chain reaction (PCR) method. The following primers and probes for the Lys109Arg polymorphism were included in the reaction: forward primer, 5′-TTT CTA ACT TAT CCA AAA CAA CTT TCC A-3′; reverse primer, 5′-GCT AAT GCT TAC CTA TTT GTT GAA AAA C-3′; Lys-allele-specific probe, 5′-VIC-TTG AAG GAA AGA CAT TTG-MGB-3′; and Arg-allele-specific probe, 5′-FAM-TTG AAG GAA GGA CAT TT-MGB-3′. The following primers and probes for the Gln223Arg polymorphism were included in the reaction: forward primer, 5′-TTT GAA AAT CAC ATC TGG TGG AGT A-3′; reverse primer, 5′-ACC CAT ATT TAT GGG CTG AAC TG-3′; Gln-allele-specific probe, 5′-VIC-ATT TTC CAG TCA CCT CTA-MGB-3′; and Arg-allele-specific probe, 5′-FAM-TTT CCG GTC ACC TCT-MGB-3′. PCR was performed with an ABI Prism 7700 (Applied Biosystems) under the following conditions: initial denaturation at 95°C for 10 minutes followed by 35 cycles of 92°C for 15 seconds and 60°C for 60 seconds.

Statistical Analyses

All statistical analyses were performed using the StatView program for Windows (version 5.0J; SAS Institute, Cary, NC). Relationships between sOB-R level and other parameters were analyzed by simple correlation and by multiple linear regressions. Relationships between the LEPR Lys109Arg and Gln223Arg polymorphisms and clinical variables were examined by the Mann-Whitney *U* test in the 2 groups. Bonferroni's correction for multiple comparisons was performed where appropriate. Because serum insulin, TG, leptin, sOB-R and adiponectin levels and HOMA-IR were normally distributed after logarithmic transformation, the logarithms of these parameters were used for the analyses. All data were expressed as mean ± SD, and *P* values less than .05 were considered statistically significant.

RESULTS

Serum Leptin, Adiponectin and Soluble Leptin Receptor Levels in Healthy Subjects and Diabetic Patients

As shown in Table 1, serum leptin and adiponectin levels in healthy men were significantly lower ($P < .0001$) than those in healthy women. In contrast, the serum sOB-R level in men (24.9 ± 5.8 U/mL) was significantly higher ($P < .0001$) than that in women (22.9 ± 7.4 U/mL). Thus, the free leptin index, leptin/sOB-R ratio in men (0.09 ± 0.06) was significantly lower ($P < .0001$) than that in women (0.19 ± 0.15).

Similarly in the diabetic group, the serum leptin level in men was significantly lower ($P < .0001$) than that in women, and the serum sOB-R level in men (25.3 ± 6.6 U/mL) was significantly higher ($P = .0013$) than that in women (22.1 ± 6.3 U/mL). Thus, the leptin/sOB-R ratio in men was significantly lower ($P < .0001$) than that in women (Table 2).

Relationships Between Serum sOB-R Level and Clinical and Metabolic Parameters in Healthy Subjects

Because sex was an independent determinant of serum sOB-R level, we analyzed the relationships between serum sOB-R level and clinical and metabolic parameters in men and women separately.

As shown in Table 3, the serum sOB-R level was negatively correlated with BMI, fasting insulin, HOMA-IR, TG level, serum leptin level, and leptin/sOB-R ratio and positively correlated with age, HDL-cholesterol level, and serum adiponectin level in men. The correlations between serum sOB-R level and fasting insulin, HOMA-IR, HDL-cholesterol level, serum lep-

Table 1. Clinical and Laboratory Characteristics of 419 Healthy Subjects

Parameter	Male (n = 198)	Female (n = 221)	*P* Value*
Age (yr)	44.5 ± 9.3	41.7 ± 9.7	.0011
BMI (kg/m^2)	22.9 ± 2.2	20.6 ± 2.4	<.0001
SBP (mm Hg)	119 ± 16	112 ± 15	<.0001
DBP (mm Hg)	75 ± 12	68 ± 10	<.0001
Glucose (mg/dL)	91 ± 8	89 ± 7	0.0025
Insulin (μU/mL)	4.8 ± 2.6	4.9 ± 2.6	NS
HOMA-IR	1.1 ± 0.6	1.1 ± 0.6	NS
Total cholesterol (mg/dL)	197 ± 28	194 ± 33	NS
Triglycerides (mg/dL)	112 ± 70	68 ± 37	<.0001
HDL-cholesterol (mg/dL)	55 ± 14	69 ± 15	<.0001
LDL-cholesterol (mg/dL)	123 ± 27	112 ± 29	<.0001
Leptin (ng/mL)	3.9 ± 1.8	7.3 ± 4.1	<.0001
Adiponectin (μg/mL)	7.2 ± 4.4	13.4 ± 7.2	<.0001
sOB-R (U/mL)	24.9 ± 5.8	22.9 ± 7.4	<.0001
Leptin/sOB-R	0.09 ± 0.06	0.19 ± 0.15	<.0001

NOTE. Values are mean ± SD.
Abbreviations: NS, not significant ($P > .0033$); SBP, systolic blood pressure; DBP, diastolic blood pressure.
*Mann-Whitney *U* test.

tin level, and leptin/sOB-R ratio were significant even after adjustment for age and BMI.

As shown in Table 4, the serum sOB-R level was negatively correlated with BMI, fasting insulin, HOMA-IR, TG level, serum leptin level, and leptin/sOB-R ratio and positively correlated with HDL-cholesterol level and serum adiponectin level in women. The correlations between serum sOB-R level and fasting insulin, HOMA-IR, TG level, HDL-cholesterol level, serum adiponectin level, and leptin/sOB-R ratio were significant even after adjustment for age and BMI.

When we analyzed the whole healthy 419 subjects combined (Table 5), the serum sOB-R level was negatively correlated with BMI, fasting insulin, HOMA-IR, serum leptin level, and leptin/sOB-R ratio and positively correlated with HDL-cholesterol level and serum adiponectin level. The correlations between serum sOB-R level and fasting insulin, HOMA-IR, TG level, HDL-cholesterol level, serum leptin and adiponectin levels, and leptin/sOB-R ratio were significant even after adjustment for age, sex, and BMI.

Relationships Between Serum sOB-R Level and Clinical and Metabolic Parameters in Diabetic Patients

The serum sOB-R level in diabetic men (n = 96) was negatively correlated with BMI ($r = -0.453$, $P < .0001$), fasting insulin ($r = -0.512$, $P < .0001$), HOMA-IR ($r = -0.483$, $P < .0001$), serum leptin level ($r = -0.486$, $P < .0001$), and leptin/sOB-R ratio ($r = -0.731$, $P < .0001$) and positively correlated with HDL-cholesterol level ($r = 0.439$, $P < .0001$). The correlations between serum sOB-R level and fasting insulin ($r = -0.402$, $P = .0006$), HOMA-IR ($r = -0.357$, $P = .0029$), HDL-cholesterol level ($r = 0.384$, $P < .0001$), and leptin/sOB-R ratio ($r = -0.861$, $P < .0001$) were significant even after adjustment for age and BMI.

Table 2. Clinical and Laboratory Characteristics of 150 Diabetic Patients

Parameter	Male (n = 96)	Female (n = 54)	*P* Value*
Age (yr)	58.5 ± 7.8	59.2 ± 9.1	NS
BMI (kg/m^2)	24.4 ± 3.6	24.0 ± 4.1	NS
SBP (mm Hg)	130 ± 16	128 ± 19	NS
DBP (mm Hg)	80 ± 9	76 ± 10	NS
Glucose (mg/dL)	138 ± 21	135 ± 22	NS
HbA_{1c} (%)	6.9 ± 0.9	6.8 ± 1.1	NS
Insulin (μU/mL)	5.8 ± 3.5	6.5 ± 4.0	NS
HOMA-IR	2.0 ± 1.3	2.2 ± 1.5	NS
Total cholesterol (mg/dL)	203 ± 33	206 ± 32	NS
Triglycerides (mg/dL)	137 ± 87	109 ± 50	NS
HDL-cholesterol (mg/dL)	52 ± 10	59 ± 14	.0021
LDL-cholesterol (mg/dL)	126 ± 31	125 ± 31	NS
Leptin (ng/mL)	5.6 ± 3.3	10.4 ± 6.2	<.0001
sOB-R (U/mL)	25.3 ± 6.6	22.1 ± 6.3	.0013
Leptin/sOB-R	0.12 ± 0.10	0.28 ± 0.23	<.0001

NOTE. Values are mean ± SD.
Abbreviations: NS, not significant ($P > .0033$); SBP, systolic blood pressure; DBP, diastolic blood pressure.
*Mann-Whitney *U* test.

Table 3. Simple and Multiple Regression Analyses Between Log (serum sOB-R) and Several Parameters in 198 Healthy Men

Parameter	Simple Regression		Adjusted for Age and BMI	
	R	*P*	*R*	*P*
Age (yr)	0.211	.0032	—	—
BMI (kg/m^2)	−0.381	<.0001	—	—
SBP (mm Hg)	0.012	NS	0.059	NS
DBP (mm Hg)	−0.023	NS	−0.028	NS
Glucose (mg/dL)	0.058	NS	0.048	NS
Log [Insulin (μU/mL)]	−0.348	<.0001	−0.239	.0011
Log [HOMA-IR]	−0.326	<.0001	−0.219	.0027
Total cholesterol (mg/dL)	0.054	NS	−0.022	NS
Log [triglycerides (mg/dL)]	−0.226	.0016	−0.134	NS
HDL-cholesterol (mg/dL)	0.351	<.0001	0.222	.0018
LDL-cholesterol (mg/dL)	−0.059	NS	−0.086	NS
Log [leptin (ng/mL)]	−0.392	<.0001	−0.258	.0009
Log [adiponectin (μg/mL)]	0.324	<.0001	0.192	NS
Log [leptin/sOB-R]	−0.683	<.0001	−0.649	<.0001

NOTE. Values are mean ± SD.

Abbreviations: NS, not significant ($P > .0036$); SBP, systolic blood pressure; DBP, diastolic blood pressure.

The serum sOB-R level in diabetic women (n = 54) was negatively correlated with BMI ($r = -0.644$, $P < .0001$), fasting insulin ($r = -0.635$, $P < .0001$), HOMA-IR ($r = -0.592$, $P < .0001$), serum leptin level ($r = -0.763$, $P < .0001$), and leptin/sOB-R ratio ($r = -0.876$, $P < .0001$), but was not correlated with HDL-cholesterol level. The correlations between serum sOB-R level and fasting insulin ($r = -0.348$, $P = .0020$), serum leptin level ($r = -0.702$, $P < .0001$), and leptin/sOB-R ratio ($r = -0.960$, $P < .0001$) were significant even after adjustment for age and BMI.

LEPR Gene Lys109Arg and Gln223Arg Polymorphisms

The genotype frequencies were governed by Hardy-Weinberg law in all subject groups in this study. In male subjects, only 6 (4.7%) were Lys109/Lys109 homozygous, 44 (34.6%) were heterozygous, and 77 (60.6%) were Arg109/Arg109 homozygous for the LEPR gene. Also in female subjects, only 5 (5.6%) were Lys/Lys homozygous, 27 (30.0%) were heterozygous, and 58 (64.4%) were Arg/Arg homozygous for the LEPR gene. In this study, we performed analyses between Lys109-positive subjects and Arg/Arg homozygotes, because the number of Lys/Lys homozygotes was small. Both in male and female subjects, there were no significant differences in any parameter measured between the 2 groups.

In male subjects, only 4 (3.1%) were Gln223/Gln223 homozygous, 25 (19.7%) were heterozygous, and 98 (77.2%) were Arg223/Arg223 homozygous for the LEPR gene. Also in female subjects, only 3 (3.3%) were Gln/Gln homozygous, 22 (24.4%) were heterozygous, and 65 (72.2%) were Arg/Arg homozygous for the LEPR gene. In this study, we performed analyses between Gln223-positive subjects and Arg/Arg homozygotes, because the number of Gln/Gln homozygotes was small. Both in male and female subjects, there were no significant differences in any parameter measured between the 2 groups.

Table 4. Simple and Multiple Regression Analyses Between Log (serum sOB-R) and Several Parameters in 221 Healthy Women

Parameter	Simple Regression		Adjusted for Age and BMI	
	R	*P*	*R*	*P*
Age (yr)	0.150	NS	—	—
BMI (kg/m^2)	−0.401	<.0001	—	—
SBP (mm Hg)	−0.076	NS	−0.018	NS
DBP (mm Hg)	0.027	NS	0.045	NS
Glucose (mg/dL)	−0.168	NS	−0.118	NS
Log [insulin (μU/mL)]	−0.476	<.0001	−0.363	<.0001
Log [HOMA-IR]	−0.477	<.0001	−0.358	<.0001
Total cholesterol (mg/dL)	0.003	NS	−0.034	NS
Log [triglycerides (mg/dL)]	−0.262	<.0001	−0.201	.0025
HDL-cholesterol (mg/dL)	0.315	<.0001	0.224	.0003
LDL-cholesterol (mg/dL)	−0.134	NS	−0.164	NS
Log [leptin (ng/mL)]	−0.403	<.0001	−0.189	NS
Log [adiponectin (μg/mL)]	0.295	<.0001	0.204	.0010
Log [leptin/sOB-R]	−0.736	<.0001	−0.823	<.0001

NOTE. Values are mean ± SD.

Abbreviations: NS, not significant ($P > .0036$); SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 5. Simple and Multiple Regression Analyses Between Log (serum sOB-R) and Several Parameters in 419 Healthy Subjects

Parameter	Simple Regression		Adjusted for Age and BMI	
	R	*P*	*R*	*P*
Age (yr)	0.193	<.0001	—	—
BMI (kg/m^2)	−0.261	<.0001	—	—
SBP (mm Hg)	0.003	NS	0.016	NS
DBP (mm Hg)	0.058	NS	0.016	NS
Glucose (mg/dL)	−0.048	NS	−0.060	NS
Log [insulin (μU/mL)]	−0.412	<.0001	−0.305	<.0001
Log [HOMA-IR]	−0.402	<.0001	−0.298	<.0001
Total cholesterol (mg/dL)	0.028	NS	−0.029	NS
Log [triglycerides (mg/dL)]	−0.137	NS	−0.167	.0014
HDL-cholesterol (mg/dL)	0.204	<.0001	0.233	<.0001
LDL-cholesterol (mg/dL)	−0.064	NS	−0.123	NS
Log [leptin (ng/mL)]	−0.431	<.0001	−0.262	<.0001
Log [adiponectin (μg/mL)]	0.156	.0015	0.215	<.0001
Log [leptin/sOB-R]	−0.708	<.0001	−0.814	<.0001

NOTE. Values are mean ± SD.

Abbreviations: NS, not significant ($P > .0036$); SBP, systolic blood pressure; DBP, diastolic blood pressure.

DISCUSSION

In the present study, we demonstrated relationships between serum sOB-R level and BMI, serum leptin and adiponectin levels, HOMA-IR and lipid profile, which are important risk factors for the development of the metabolic syndrome and atherosclerosis. Contrary to leptin and adiponectin, the serum sOB-R level in men was significantly higher than that in women. A sex difference has already been reported in sOB-R level, which was consistently higher in men than in women.[9,11,23] Ogier et al[9] reported that the sex difference in sOB-R level could be explained by a difference in fat mass, but unfortunately, we were not able to measure the percentage of body fat in this study. Although we found that healthy women of reproductive age (<50-years-old) had significantly lower sOB-R level ($P = .007$) than those who were probably menopausal (>50-years-old), we did not find a significant difference in healthy men of different age groups (data not shown). It has been, however, reported that serum sOB-R level did not change significantly during the menstrual cycle,[12] and there was no significant difference in serum sOB-R level during in vitro fertilization (IVF) cycles.[13]

In this study, the serum sOB-R level was negatively correlated with fasting insulin, HOMA-IR, TG level, and serum leptin level and positively correlated with HDL-cholesterol and serum adiponectin level in healthy subjects, even after adjustment for age, sex, and BMI. An inverse correlation between sOB-R level and BMI or serum leptin level has already been reported.[9-13] Recently, Sandhofer et al[17] reported that serum sOB-R level was negatively correlated with fasting insulin and HOMA-IR, independent of age, BMI, and fat mass, in 76 middle-aged obese men. However, they demonstrated that HOMA-IR was not an independent determinant of the sOB-R level in stepwise linear regression.

To our knowledge, this is the first report demonstrating a relationship between serum sOB-R level and HDL-cholesterol or serum adiponectin level. Adiponectin is secreted specifically by adipose tissue, and its levels are reported to be lower in individuals with obesity, diabetes mellitus, or coronary artery disease.[24,25] We have previously reported that the serum adiponectin level was negatively correlated with HOMA-IR and positively correlated with HDL-cholesterol level, independent of age, sex, and BMI.[22] The present study has shown that when the sOB-R level is high, insulin resistance is low, and HDL-cholesterol and adiponectin levels are high, probably reflecting improvement of insulin resistance and the metabolic syndrome.

Concerning diabetic patients, the serum sOB-R level was also negatively correlated with BMI, HOMA-IR, and serum leptin level in both men and women. There are a few studies about the sOB-R level in diabetic subjects. Lewandowski et al[26] reported that insulin-dependent diabetic women had significantly higher sOB-R levels than normal pregnant women. Pecoits-Filho et al[27] reported that sOB-R level was significantly higher in diabetic end-stage renal disease (ESRD) patients than that in nondiabetic ESRD patients, and that the sOB-R level was positively correlated with glycosylated hemoglobin (HbA_{1c}) value. Although we had expected that the serum sOB-R level in healthy subjects might be higher than that in diabetic patients, who generally had a higher BMI, HOMA-IR, and serum leptin level, the serum sOB-R level was not significantly different between healthy subjects and diabetic patients in the present study (data not shown).

Although originally, free leptin levels could be measured only by a gel filtration chromatography method, Ogier et al[9] reported that the ratio of circulating leptin to sOB-R (leptin/sOB-R) was strongly related to the percentage of body fat, and this ratio was thought to be an index of free leptin.[28] In this study, the leptin/sOB-R ratio was significantly higher in women than in men. It was significantly higher in diabetic patients than in healthy subjects, but this significance disappeared after adjustment for BMI (data not shown). Moreover, the leptin/sOB-R ratio was positively correlated with BMI, HOMA-IR, and leptin level and negatively correlated with HDL-cholesterol, adiponectin, and sOB-R levels in healthy subjects. The leptin/sOB-R ratio did not show additional correlation in the relationship with HOMA-IR or BMI compared with leptin itself in the present study.

Since 1997, several single nucleotide polymorphisms (SNPs)

of the human LEPR gene have been reported in different ethnic groups.[29-33] Matsuoka et al[30] reported that 7 SNPs were identified in Japanese, but the allele frequency of each variant showed no significant difference between obese and non-obese subjects. Wauters et al[31] reported that in postmenopausal Caucasian women, total abdominal fat was higher in Gln223 homozygotes and leptin levels were higher in Lys109 homozygotes. Quinton et al[32] investigated the Gln223Arg polymorphism of the LEPR gene in postmenopausal Caucasian women and reported that the Gln223-positive group showed a higher BMI, fat mass, and serum leptin level and lower serum leptin-binding activity. Heo et al[33] reported that there was no association between common LEPR polymorphisms (Lys109Arg, Gln223Arg, Lys656Asn) and BMI or waist circumference in a meta-analysis. In the present study, there were no significant associations between the Lys109Arg or Gln223Arg LEPR polymorphism and clinical or metabolic parameters. We concluded in this study that the LEPR Lys109Arg and Gln223Arg polymorphisms were not associated with BMI, fasting insulin, HOMA-IR, serum leptin, or sOB-R level in the Japanese population.

In summary, the serum sOB-R level was negatively correlated with HOMA-IR and serum leptin level and positively correlated with HDL-cholesterol and serum adiponectin levels, independent of age, sex, and BMI, in healthy Japanese subjects. These findings suggest that sOB-R may be one of the factors influencing insulin resistance and leptin resistance.

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