Association Between Serum Soluble Low-Density Lipoprotein Receptor Levels and Metabolic Factors in Healthy Japanese Individuals

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Background: Soluble low-density lipoprotein receptor (sLDL-R) is formed by cleavage of the extracellular domain of low-density lipoprotein receptor (LDL-R). It is unclear whether serum sLDL-R is a marker of diseases associated with triglyceride (TG) metabolism. We investigated the association between serum sLDL-R concentrations and other biochemical parameters in healthy Japanese individuals. **Methods:** Study subjects consisted of 102 healthy adult Japanese volunteers (42 men, 60 women) with body mass index (BMI) < 30 kg/m² and serum TGs, LDL cholesterol (LDL-C), aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transpeptidase, and glucose concentrations within normal ranges. Serum sLDL-R concentrations were determined

by enzyme-linked immunosorbent assay and their correlations with biochemical parameters were analyzed. **Results:** Mean serum sLDL-R concentration was 120.9 ± 39.9 ng/ml. Serum sLDL-R levels were significantly and positively correlated with BMI ($r_s = 0.252$) and TG ($r_s = 0.408$) and LDL-C ($r_s = 0.325$) concentrations. Multiple regression analysis adjusted for age, gender, and smoking showed that BMI ($\beta = 0.274$), TG ($\beta = 0.328$), and LDL-C ($\beta = 0.224$) were factors independently correlated with sLDL-R levels. **Conclusion:** Serum sLDL-R concentration may be a marker of diseases associated with TG metabolism. This is the first report to date describing the clinical relevance of sLDL-R. J. Clin. Lab. Anal. 29:52–56, 2015. © 2014 Wiley Periodicals, Inc.

Key words: ADAM17; ectodomain shedding; ELISA; sLDL-R; TG metabolism

INTRODUCTION

Soluble low-density lipoprotein receptor (sLDL-R) is formed when the extracellular domain of low-density lipoprotein receptor (LDL-R) is cleaved from the cell surface (1). sLDL-R, which is involved in the metabolism of LDL cholesterol (LDL-C) (2, 3), has been observed in culture supernatants of dermal fibroblasts and HepG2 cells (1, 4). Mechanisms by which LDL-R becomes soluble include a genetic mutation and ectodomain shedding (4, 5). The mutation in question also causes familial hy-

percholesterolemia, with individuals homozygous for this mutation showing poor cellular uptake of LDL (6). In contrast, ectodomain shedding results from the cleavage

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of the extracellular domain of LDL-R in the area adjacent to the lipid bilayer and has been reported to involve a disintegrin and metalloproteinase (ADAM) (7), in particular ADAM17, a TNF- α converting enzyme (1, 5).

Although serum sLDL-R concentration is regarded as a marker of lipid metabolism, its relationships with known parameters of lipid metabolism and clinical features remain to be determined. Therefore, this study investigated associations between serum sLDL-R levels and biochemical parameters in a healthy population.

MATERIALS AND METHODS

Subjects and Samples

This cross-sectional study was designed to assess clinical characteristics possibly associated with serum sLDL-R concentrations in healthy adult Japanese individuals. Subjects were adult volunteers who provided signed consent agreements to participate in this study, performed at the Department of Pathobiological Science and Technology, School of Health Science, Tottori University, from September 2011 to July 2013. The subjects included 102 adult volunteers not taking periodic medications and without regular visits to hospital based on self-report. All subjects had a body mass index (BMI) < 30 kg/m² and triglyceride (TG; <150 mg/dl), LDL-C (<140 mg/dl), aspartate aminotransferase (AST; <31 U/l), alanine aminotransferase (ALT; <31 U/l), γ -glutamyl transpeptidase (γ -GTP; <51 U/l), and glucose (<110 mg/dl) concentrations within normal reference values, as determined by the criteria of the Japan Society of Ningen Dock (<http://www.ningen-dock.jp>).

Blood samples were collected in the morning after fasting for approximately 10 hr since 10 p.m. the previous night and mixed by inversion for 15 min at room temperature. Serum was separated by centrifugation at $1,700 \times g$ for 10 min and stored at -30°C until used. We avoided using serum samples that were repeatedly frozen and thawed. Serum concentrations of TG, high-density lipoprotein cholesterol (HDL-C), LDL-C, free fatty acids (FFAs), AST, ALT, γ -GTP, cholinesterase (ChE), and glucose were measured using the same assays as used in clinical laboratories. Height and weight were measured using methods based on international standards.

This study was conducted with the approval of the Ethics Committee, School of Health Science, Tottori University (approval number: 1744).

sLDL-R Measurements

Serum sLDL-R concentrations were measured using a sandwich enzyme-linked immunosorbent assay (ELISA), formulated using a Human LDL R DuoSet[®] (R&D Sys-

tems, Minneapolis, MN) and an IMMUNO-TEK ELISA Construction System[®] (ZeptoMetrix Corporation, Buffalo, NY), according to the manufacturers' instructions. For measurements, serum samples were diluted 100-fold; each was assayed in duplicate and the mean was used for analysis.

Statistical Analysis

Continuous variables are reported as means $\pm$ standard deviation, unless noted otherwise, and categorical variables as numbers. The distributions of variables were checked for normality using the Shapiro–Wilk method. Continuous variables were compared using Mann–Whitney *U*-tests, and categorical variables were compared using chi-square tests. Associations between two variables were assessed using Spearman rank correlation analysis. Linear univariable and multivariable regression analyses were used to investigate relationships between metabolic parameters and serum sLDL-R concentrations. All statistical analyses were performed using IBM SPSS version 19.0 software (IBM Japan, Tokyo) on a computer with a Windows operating system. A *P* value < 0.05 was considered statistically significant.

RESULTS

The demographic characteristics and blood biochemistry results for the study subjects are shown in Table 1. Serum sLDL-R concentrations ranged from 61.1 to 291.6 ng/ml. Median sLDL-R concentrations were 117.5 ng/ml (range, 70.7–225.1 ng/ml) in men and 108.4 ng/ml (range, 61.1–291.6 ng/ml) in women (*P* = 0.311).

ADAM17 has been reported to be involved in the cleavage of LDL-R to form sLDL-R (1). Univariate and multivariate analyses of correlations between serum sLDL-R levels and biochemical parameters associated with inflammatory cytokines, such as TNF- α , are shown in Table 2. Fasting serum sLDL-R concentrations were significantly correlated with BMI and serum TG and LDL-C concentrations. Serum sLDL-R levels, however, were not significantly correlated with subject age, gender, or smoking habits; or with HDL-C, FFA, AST, ALT, γ -GTP, ChE, or glucose concentration.

Multiple linear regression analysis was performed to investigate possible independent predictors of serum sLDL-R concentrations. Variables with *P* values < 0.2 on univariate analysis (i.e., TG, BMI, and LDL-C) were included in the multivariate analysis, along with subject age, gender, and smoking habits. The three biochemical factors associated with sLDL-R on univariate analysis were independently and positively correlated with serum sLDL-R levels on multivariate analysis (Table 2 and Fig. 1). We also

TABLE 1. Demographic and Biochemical Characteristics of the Study Participants

	All (n = 102)	Males (n = 42)	Females (n = 60)	P
sLDL-R (ng/ml)	120.9 ± 39.9 (111.4, 61.1–291.6)	123.7 ± 36.5(117.5, 70.7–225.1)	118.9 ± 42.3 (108.4, 61.1–291.6)	0.311
Age (years)	27.8 ± 8.4 (24, 21–55)	26.8 ± 7.5 (23, 21–51)	28.6 ± 9.1 (24, 21–55)	0.657
BMI (kg/m ²)	20.3 ± 2.4 (20.2, 15.6–28.5)	21.2 ± 2.3 (20.9, 16.5–27.0)	19.7 ± 2.2 (19.7, 15.6–28.5)	0.001
Smoking (n)	12	11	1	<0.001
TG (mg/dl)	67.6 ± 26.5 (62, 24–147)	74.1 ± 25.2 (75.5, 31–143)	63.1 ± 26.7 (55, 24–147)	0.011
HDL-C (mg/dl)	68.6 ± 17.1 (66.5, 40–158)	61.5 ± 13.9 (58, 40–102)	73.5 ± 17.4(73, 45–158)	<0.001
LDL-C (mg/dl)	98.3 ± 18.5 (95.5, 58–139)	98.8 ± 15.8 (96, 67–136)	98.0 ± 20.3 (94.5, 58–139)	0.676
FFA (mEq/l)	0.424 ± 0.187 (0.400, 0.140–1.360)	0.381 ± 0.148 (0.375, 0.140–0.730)	0.454 ± 0.206 (0.420, 0.170–1.360)	0.085
AST (U/l)	17.3 ± 3.9 (17, 11–30)	17.8 ± 4.2 (17, 11–30)	16.9 ± 3.7 (16, 11–30)	0.296
ALT (U/l)	12.1 ± 5.1 (11, 4–28)	13.4 ± 5.1 (12.5, 5–25)	11.2 ± 5.0 (10, 4–28)	0.009
γ-GTP (U/l)	16.7 ± 6.2 (15, 7–37)	19.7 ± 6.6 (18, 11–37)	14.6 ± 4.9 (13.5, 7–32)	<0.001
ChE (U/l)	300.7 ± 51.5 (295, 199–419)	327.8 ± 45.4 (323, 219–419)	281.8 ± 47.1 (269.5, 199–418)	<0.001
Glucose (mg/dl)	90.8 ± 7.1 (90, 75–109)	92.3 ± 7.5 (93.5, 75–109)	89.3 ± 6.4 (88.5, 78–109)	0.008

Results are expressed as mean ± standard deviation (median, minimum–maximum) or number. *P* values were calculated using Mann–Whitney *U*-tests for continuous variables or the chi-square test for categorical variables, with *P* < 0.05 considered statistically significant. sLDL-R, soluble low-density lipoprotein receptor; BMI, body mass index; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FFA, free fatty acid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; ChE, cholinesterase.

performed stepwise regression analysis for men only and for women only. For men, serum TG levels were significantly correlated with serum sLDL-R levels, whereas, for women, age, BMI, and serum TG levels were significantly associated with serum sLDL-R levels.

DISCUSSION

LDL-R is controlled by intracellular cholesterol levels and is involved in LDL-C metabolism (2). We found that,

in addition to its positive and independent association with serum LDL-C concentrations, circulating sLDL-R concentrations were positively and independently associated with BMI and serum TG concentrations in healthy Japanese adults. In particular, serum TG levels showed the strongest association with serum sLDL-R levels. TG metabolism has been associated with fatty liver and arteriosclerosis (8, 9).

To our knowledge, no study to date has investigated sLDL-R concentrations in human serum. Although the

TABLE 2. Associations Between Serum sLDL-R Concentrations and Clinical Parameters

	All (n = 102)				Males (n = 42)				Females (n = 60)			
	Spearman correlation coefficient		Multivariate analysis		Spearman correlation coefficient		Multivariate analysis		Spearman correlation coefficient		Multivariate analysis	
	<i>rs</i>	<i>P</i>	β	<i>P</i>	<i>rs</i>	<i>P</i>	β	<i>P</i>	<i>rs</i>	<i>P</i>	β	<i>P</i>
Age	0.088	0.379	0.144	0.101	−0.136	0.391	−0.137	0.359	0.224	0.085	0.294	0.012
Gender	−0.101	0.313	0.079	0.396	—	—	—	—	—	—	—	—
BMI	0.252	0.011	0.234	0.008	0.219	0.163	0.134	0.368	0.238	0.067	0.245	0.034
Smoking	−0.146	0.144	−0.012	0.895	−0.252	0.107	−0.151	0.326	0.132	0.316	0.197	0.070
TG	0.408	<0.001	0.326	<0.001	0.341	0.027	0.394	0.010	0.400	0.002	0.424	<0.001
HDL-C	−0.108	0.282	—	—	0.054	0.734	—	—	−0.086	0.511	—	—
LDL-C	0.325	0.001	0.222	0.014	0.287	0.066	0.157	0.321	0.333	0.008	0.211	0.057
FFA	0.045	0.656	—	—	0.186	0.237	—	—	0.009	0.948	—	—
AST	0.046	0.645	—	—	0.083	0.603	—	—	0.039	0.767	—	—
ALT	0.089	0.371	—	—	0.037	0.817	—	—	0.091	0.490	—	—
γ-GTP	0.127	0.204	—	—	0.141	0.374	—	—	0.049	0.712	—	—
ChE	0.110	0.271	—	—	0.100	0.527	—	—	0.050	0.702	—	—
Glucose	0.053	0.595	—	—	−0.076	0.632	—	—	0.126	0.339	—	—

BMI, body mass index; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FFA, free fatty acid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; ChE, cholinesterase. *rs*, simple correlation test (Spearman rank correlation analysis); β, multiple linear regression analysis for sLDL-R.

Fig. 1. Correlations between serum sLDL-R concentration and (A) age, (B) BMI, (C) TG concentration, and (D) LDL-C concentration in 102 healthy Japanese adults. Spearman correlation coefficients (r_s) and P are indicated. sLDL-R, soluble low-density lipoprotein receptor; BMI, body mass index; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol.

physiological role of sLDL-R is unclear, its concentrations were significantly and positively correlated with BMI and serum TG and LDL-C concentrations. sLDL-R is produced by cleavage of the extracellular domain of LDL-R, a receptor involved in LDL-C metabolism (1). The cleavage mechanism is complex, and it is difficult to separately consider LDL-R expression and cleavage levels.

The level of expression of LDL-R is controlled by intracellular cholesterol. About 70% of blood LDL-C is metabolized by endocytosis through LDL-R in the liver (3, 10). LDL-R is cleaved to produce sLDL-R by ADAM17, an α secretase involved in the cleavage of cell membrane receptors (1, 5). Addition of 4 β -phorbol 12-myristate 13-acetate

to HepG2 cells and fibroblasts cultured in lipoprotein-deficient serum has been reported to activate protein kinase C, with sLDL-R detected in the culture medium (1). Furthermore, addition of an ADAM17 inhibitor to the culture medium reduced the concentration of sLDL-R (1). Similarly, in humans, the incorporation of LDL into cells may be downregulated when cell-surface LDL-R is cleaved, increasing blood LDL-C and resulting in a positive correlation between sLDL-R and LDL-C concentrations. Furthermore, when blood LDL-C concentration is high, intracellular cholesterol is upregulated through LDL-R, leading to the downregulation of cell-surface LDL-R. The positive correlation between sLDL-R and LDL-C concentrations, despite cell-surface LDL-R

being decreased, requires activation of the cleavage mechanism. Since it was difficult to measure the ADAM17 concentration using our current experimental setup, we could not determine whether it had been activated. However, during the synthesis of endogenous TG from sugars and FFA in the liver, FFA was reported to activate ADAM17 through extracellular signal-regulation kinase and mitogen-activated protein kinases p38 (5, 11, 12).

It remains unclear why sLDL-R correlated with TG. Endogenous TG is mainly synthesized from liver-derived very low density lipoprotein; and if sLDL-R is also formed in the liver, it is reasonable to think that perhaps a certain hepatic factor can affect correlations between sLDL-R and TG. We speculate this hepatic factor to be hepatic fat accumulation, since several studies have reported that mild hepatic steatosis is diagnosed in healthy nonobese subjects (13–18). Although we did not investigate hepatic steatosis by abdominal ultrasonography in the current study, mild hepatic fat accumulation may be present in our subjects. Numerous studies have reported that hepatic steatosis induces increases in TNF- α production and activation of ADAM17 via various signaling pathways within hepatocytes (5, 12). Therefore, the significant correlation between sLDL-R and TG may be explained by the presence of hepatic fat accumulation as a background factor.

Age was found to be significantly associated with serum sLDL-R levels in women, but not in men. Sex hormones may be involved in the formation of sLDL-R, because estrogen has been reported to be involved in LDL-R expression (10, 19).

Our study had several limitations. First, the relatively small number of study subjects limited our ability to detect weak correlations on both univariable and multivariable analyses. Second, we were unable to measure serum ADAM17 concentrations, which would have provided further information regarding the cleavage mechanism. Finally, we cannot rule out the possibility of additional unknown confounding variables that correlate significantly with sLDL-R levels.

In summary, serum sLDL-R concentrations were significantly and positively correlated with BMI and serum TG and LDL-C concentrations, with serum TG levels showing the strongest association. To our knowledge, this is the first report describing the possible clinical association between sLDL-R and parameters of lipid metabolism in healthy individuals.

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