Original Article

Associations between Small Dense LDL, HDL Subfractions (HDL2, HDL3) and Risk of Atherosclerosis in Japanese-Americans

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***Aim*: Small dense low-density lipoprotein (sdLDL) has been suggested to be more atherogenic than large buoyant LDL. High-density lipoprotein (HDL) consists of two major subfractions (HDL2, HDL3), and just as controversy remains regarding which of the two is the more powerful negative risk factor for atherosclerosis, associations between sdLDL and these HDL subfractions are unclear.**
***Methods*: We measured sdLDL cholesterol (sdLDL-C), HDL2 cholesterol (HDL2-C) and HDL3 cholesterol (HDL3-C) by a newly developed method in 481 Japanese-Americans who were not using lipid-lowering medication, and examined the associations of these cholesterol concentrations with variables related to atherosclerosis.**
***Results*: In multivariate analysis, sdLDL-C was positively correlated with the body mass index (BMI), fasting glucose and insulin, 2-h glucose, HOMA-IR, high sensitivity C-reactive protein (hsCRP), and carotid artery intima-media thickness (IMT) after adjustment for age and sex. In particular, sdLDL-C was positively correlated with IMT, even after adjustment for sex, age, smoking status, hypertension, diabetes mellitus and hsCRP. HDL2-C was more closely inversely correlated than total HDL-C with BMI, fasting glucose and insulin, 2-h glucose, HOMA-IR, and hsCRP, whereas HDL3-C was not correlated with these factors. Additionally, HDL2-C was more closely correlated than total HDL-C or HDL3-C with sdLDL-C, LDL-C, triglycerides (TG), and apolipoprotein B (apoB).**
***Conclusions*: SdLDL-C was closely associated with insulin resistance and glucose tolerance, lending credence to its potential as a useful risk marker in assessing carotid artery IMT and the present degree of atherosclerosis in Japanese-Americans. The findings also suggest that subjects with higher HDL2-C levels were better protected from atherosclerosis.**

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Key words; Small dense low-density lipoprotein-cholesterol, High-density lipoprotein subfractions, Carotid artery intima-media thickness (IMT), Glucose tolerance

Introduction

Elevated serum levels of low-density lipoprotein-cholesterol (LDL-C) and low serum levels of high-density lipoprotein-cholesterol (HDL-C) have been established as important risk factors for atherosclerosis and coronary heart disease (CHD). Compared with large buoyant LDL, sdLDL has been suggested to be more atherogenic as a result of its higher degree of penetration of the arterial wall, lower binding affinity for LDL receptors, prolonged plasma half-life, and lower resistance to oxidative stress[1, 2]. Indeed, an increased risk of CHD in subjects with higher levels of sdLDL was previously documented[3]. Furthermore, measurement of sdLDL-C concentration is useful for assessment of not only the presence of CHD[4, 5] but also its severity[6] as well as carotid artery IMT[7].

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HDL consists of two major subfractions: large buoyant HDL2 and small dense HDL3. Controversy remains regarding which of the two is the more powerful negative risk factor in atherosclerosis. Several studies suggest that the level of the protective effects of HDL may be better represented by the concentration of HDL2 than that of HDL3[8, 9], whereas other studies offer no support for this[10, 11]. Additionally, the associations between sdLDL-C and these HDL subfractions are unclear.

Japanese-Americans, who are genetically identical to their Japanese progenitors, have typically lived a Westernized lifestyle for decades, consuming high-fat and high simple-carbohydrate diets[12, 13]. We reported that such Japanese-Americans are more hyperinsulinemic and insulin resistant than native Japanese, with a prevalence of diabetes two to three times higher than that of their Japanese counterparts[12]. We also reported that the mortality rate from CHD was higher among Japanese-Americans with diabetes than among native Japanese with diabetes[13]. In Japanese-Americans with such Westernized lifestyles, the effects of sdLDL and HDL subfractions on the development of atherosclerosis are expected to be more apparent than such effects in native Japanese.

In the present cross-sectional study, we investigated the associations between sdLDL-C, HDL2-C, HDL3-C and the risk of atherosclerosis, assessing which of the major HDL subfractions more accurately represents the protective effects of HDL among Japanese-Americans living in Los Angeles.

Methods

Study Subjects

This study is part of a long-term epidemiological survey (Hawaii-Los Angeles-Hiroshima study) initiated in 1970 that investigates risk factors for diabetes and cardiovascular disease among subjects limited to a population genetically identical to the native Japanese population. The epidemiological survey was previously described in detail elsewhere[13-15].

The subjects of our study were Japanese-Americans enrolled in medical examinations of the aforementioned epidemiological survey conducted in Los Angeles in 2004. The study population consisted of 183 men and 298 women who were not using medication for the treatment of diabetes mellitus and/or dyslipidemia, but included subjects diagnosed with diabetes mellitus, hypertension, and dyslipidemia. Glucose tolerance status was ascertained in individual subjects (NGT: normal glucose tolerance, IGT: impaired glucose tolerance, DM: diabetes mellitus) by the 75g oral glucose tolerance test (GTT). Diabetes was diagnosed in accordance with 1998 WHO criteria (fasting plasma glucose ≥126 mg/dL or 2-h glucose ≥200 mg/dL after 75gGTT)[16]. Hypertension was diagnosed if the subject used anti-hypertensive medication or had a blood pressure of 140/90 mmHg or higher[17]. Dyslipidemia was diagnosed in accordance with criteria developed by the Japan Atherosclerosis Society[18], if the subject had one or more of the following: LDL-C ≥140 mg/dL, TG ≥150 mg/dL or HDL-C <40 mg/dL. Smoking status (current or past, none) was also assessed using standard interview procedures. This study was approved by the ethics committee of Hiroshima University and by the Council of the Hiroshima Kenjin-Kai Association in Hawaii and Los Angeles.

Measurements

All subjects underwent physical measurements and provided blood and urine samples after an overnight fast. The collected blood was centrifuged, and the obtained serum, whole blood and urine samples were immediately frozen and subsequently brought back to Japan.

SdLDL-C was measured using a commercially available assay kit (sdLDL-EX; Denka Seiken Co., Tokyo, Japan) based on a homogenous method detailed elsewhere[19]. In short, surfactant A reacts with TG-rich lipoproteins and HDL, and the cholesterol in these lipoproteins is dissociated by the actions of cholesterol-oxidase/esterase and catalase. Sphingomyelinase mainly reacts with large buoyant-LDL, while surfactant B protects sdLDL from the actions of sphingomyelinase and the cholesterol-oxidase/esterase reaction. Thus, the sdLDL-C that escapes from the actions of these enzymes can be measured by a standard total-cholesterol assay. Measurement using this method was highly correlated ($y=0.99x-0.09$, $R^2=0.91$ Pearson correlation) with cholesterol concentration in the dense-LDL fraction (1.044-1.063 g/mL) isolated by ultracentrifugation[19]. The coefficients of variation (CV) for within-run precision were less than 1.1%. Plasma TG concentrations <11 mmol/L (<1,000mg/dL) did not affect sdLDL-C measurement.

HDL2-C and HDL3-C values were determined by a newly developed method for assaying HDL subspecies that combines single precipitation with a direct HDL-C assay[20]. In brief, a precipitation reagent (0.06 mL) containing 1,071 U/mL heparin, 500 mmol/L $MnCl_2$, and 12 mg/mL dextran sulfate was added to serum (0.3 mL). The sample was incubated and centrifuged at 10,000 rpm for 10 min. HDL3-C was measured by a homogenous HDL-C assay (HDL-EX;

Table 1. Clinical characteristics of study subjects

	Total	NGT	IGT	DM
N (men/women)	481 (183/298)	371 (133/238)	79 (37/42)	31 (13/18)
Age (y)	61.7 ± 13.7	60.4 ± 14.0	65.0 ± 10.8*	68.3 ± 13.6*
BMI (kg/m^2)	23.7 ± 3.6	23.4 ± 3.4	24.8 ± 3.5*	25.5 ± 5.6*
SBP (mmHg)	134.0 ± 17.9	132.4 ± 17.4	136.7 ± 18.6	145.4 ± 16.7*
DBP (mmHg)	77.3 ± 10.7	76.9 ± 10.0	77.7 ± 11.6	80.9 ± 13.9
Fasting glucose (mg/dL)	92.0 ± 20.1	87.6 ± 9.6	93.4 ± 9.6*	142.0 ± 47.7*†
2-h glucose (mg/dL)	119.6 ± 56.5	97.1 ± 20.3	160.6 ± 17.6*	284.6 ± 80.2*†
Fasting insulin (μU/mL)	6.9 ± 5.3	6.2 ± 4.3	7.8 ± 4.5*	11.9 ± 11.2*†
HOMA-IR	1.7 ± 1.7	1.3 ± 1.0	1.8 ± 1.0	4.4 ± 4.6*†
LDL-C (mg/dL)	140.6 ± 36.8	138.9 ± 36.7	147.3 ± 35.9	143.5 ± 39.9
sdLDL-C (mg/dL)	35.7 (25.3-49.3)	33.7 (24.4-46.2)	43.7 (28.5-58.2)*	47.5 (29.4-61.7)*
TG (mg/dL)	117 (82-177)	109 (77-169)	147 (93-206)	141 (106-198)*
HDL-C (mg/dL)	50.7 ± 13.8	51.3 ± 13.8	49.0 ± 15.0	47.7 ± 10.0
HDL2-C (mg/dL)	29.7 ± 12.0	30.4 ± 12.1	27.7 ± 12.7	26.2 ± 8.7
HDL3-C (mg/dL)	21.0 ± 4.8	20.9 ± 4.8	21.3 ± 4.8	21.4 ± 4.4
apoB (mg/dL)	116.3 ± 28.7	114.2 ± 27.5	124.7 ± 28.5*	120.0 ± 37.9
hsCRP (mg/L)	0.79 (0.39-1.71)	0.71 (0.36-1.55)	1.16 (0.55-2.57)	1.33 (0.61-1.83)
IMT (mm)	0.81 (0.71-0.92)	0.80 (0.71-0.92)	0.83 (0.73-0.94)	0.92 (0.81-1.09)*
Hypertension, n (%)	232 (48)	160 (43)	49 (62)	23 (74)
Dyslipidemia, n (%)	305 (63)	224 (60)	60 (76)	21 (67)
Smokers, n (%)	158 (33)	118 (32)	32 (40)	8 (26)

Data are expressed as the number, percentage, mean ± S.D. or median (25th-75th percentile). NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment for insulin resistance; LDL-C, low density lipoprotein cholesterol; sd, small dense; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; IMT, intima media thickness. *$p<0.05$ compared to NGT, †$p<0.05$ compared to IGT by Tukey-Kramer method.

Denka Seiken Co., Tokyo, Japan) in the supernatant, and HDL2-C was estimated by subtracting HDL3-C from the direct HDL-C. The HDL3-C and HDL2-C values determined by this method were identical to those determined by ultracentrifugation, with excellent correlation between the methods in terms of HDL3-C and HDL2-C measurements ($r=0.933$ and 0.978).

LDL-C and HDL-C were directly measured by homogenous assays. Insulin was measured by a double-antibody radioimmunoassay. Insulin resistance was evaluated by HOMA-IR[21]. CRP levels were measured using a highly sensitive, latex-enhanced immunonephelometric assay[22].

Carotid artery IMT was measured by B-mode ultrasonography (EUB-405X; Hitachi Ltd., Tokyo, Japan) with the 10-MHz probe using a technique developed by Pignoli *et al.*[23]. We examined the far walls of the left and right common carotid arteries. Examinations were made from three different longitudinal projections (i.e. anterior-oblique, lateral, and posterior-oblique). IMT was assessed as the greatest IMT at any location in the far walls of the carotid arteries, including atheromatous plaques on both sides. The greatest unilateral IMT value, representing a higher value when compared with that of the other side, was defined as max IMT. Images were analyzed off-line by specifically designed software (IntimaScope; MEDIA CROSS Co., Ltd., Tokyo, Japan). All measurements (scans and image analyses) were performed by one physician with the same equipment.

Statistical Analyses

Data are expressed as the mean ± S.D. or median (25th-75th percentile levels), depending on data distribution. Continuous variables were compared by analysis of covariance (ANCOVA), and if they were found to be significant, the Tukey-Kramer method was used to assess association between categories. Univariate correlation was evaluated by Spearman's rank correlation analysis. Multivariate correlation was tested by multiple regression analysis. For variables with skewed distribution, log-transformation was performed before multiple regression analysis. Such trans-

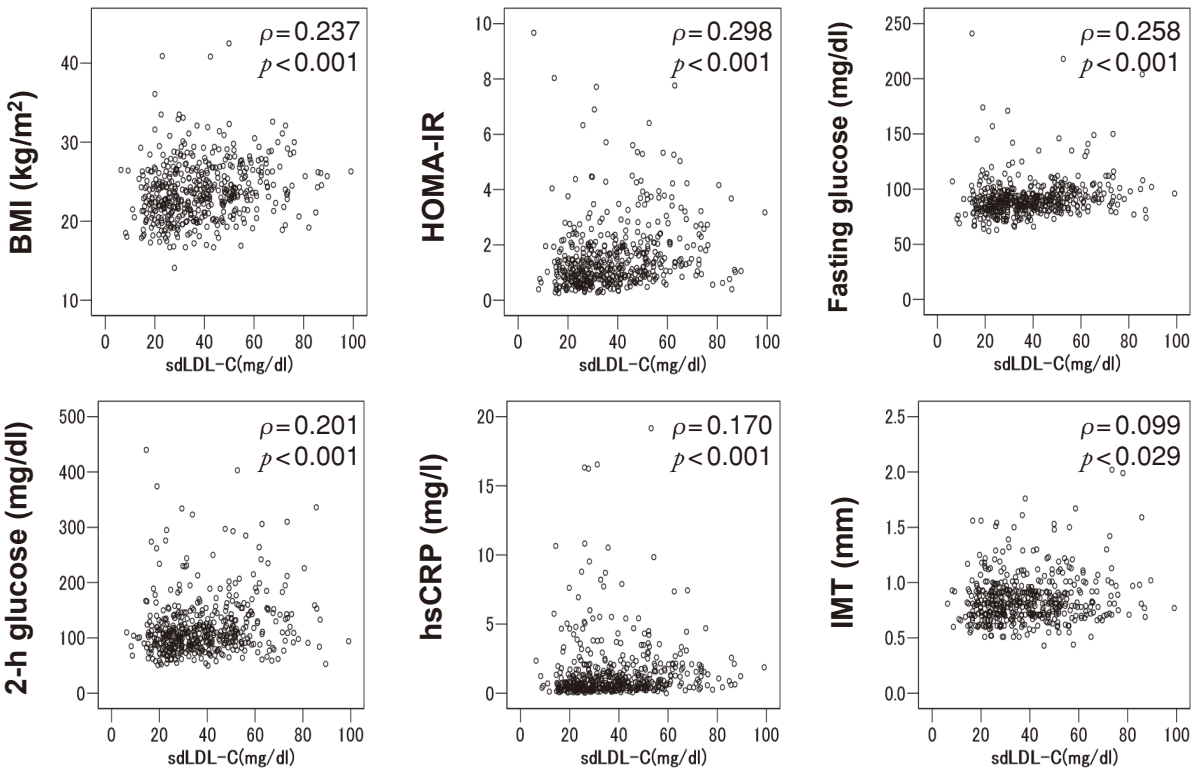

Fig. 1. Univariate correlation of sdLDL-C with variables in all subjects. Spearman's correlation coefficients (ρ) and p-values are given. SdLDL-C, small dense low-density lipoprotein cholesterol; BMI, body mass index; hsCRP, high sensitivity C-reactive protein; IMT, intima-media thickness.

formation was applied to sdLDL-C, hsCRP and IMT. For all analyses, SPSS for Windows (version 12·0; SPSS Inc., Chicago, IL) was used. $P<0.05$ was considered significant.

Results

Table 1 shows the general and metabolic characteristics of all subjects studied. Among the DM subjects, age, BMI, systolic blood pressure, fasting glucose and insulin levels, 2-h glucose levels, HOMA-IR, sdLDL-C levels, TG levels, and IMT were all significantly higher than these variables among NGT subjects. Additionally, among the IGT subjects, age, BMI, fasting glucose and insulin levels, 2-h glucose levels, and sdLDL-C levels were significantly higher than the variables among NGT subjects. No significant differences were observed in diastolic blood pressure, LDL-C, HDL-C, HDL2-C, HDL3-C, or hsCRP levels. We showed that the sdLDL-C level was significantly higher in DM subjects and IGT subjects than in NGT subjects, although no significant difference was observed from the LDL-C level in **Table 1**.

Fig. 1 indicates univariate correlation coefficients of sdLDL-C levels with non-lipid parameters, which are risk factors for atherosclerosis, by Spearman's rank correlation analysis. SdLDL-C levels showed a significant positive correlation with BMI ($\rho=0.237$, $p<0.001$), fasting glucose ($\rho=0.258$, $p<0.001$) and insulin ($\rho=0.270$, $p<0.001$, data not shown), 2-h glucose ($\rho=0.201$, $p<0.001$), HOMA-IR ($\rho=0.298$, $p<0.001$), hsCRP levels ($\rho=0.170$, $p<0.001$), and IMT ($\rho=0.099$, $p=0.029$).

Next, we investigated associations between sdLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C concentrations and non-lipid parameters by multiple regression analysis (**Table 2**). After adjustment for age and sex, the sdLDL-C level was positively associated with BMI ($\beta=0.178$, $p<0.001$), fasting glucose ($\beta=0.143$, $p=0.002$) and insulin ($\beta=0.150$, $p=0.001$), 2-h glucose ($\beta=0.136$, $p=0.002$), HOMA-IR ($\beta=0.111$, $p=0.016$), hsCRP levels ($\beta=0.137$, $p=0.003$), and IMT ($\beta=0.085$, $p=0.034$); however, LDL-C level was positively associated with only BMI ($\beta=0.094$, $p=0.034$) and fasting glucose levels ($\beta=0.090$, $p=0.046$). Thus, the sdLDL-C level was more closely associated than the LDL-C level with metabolic parameters. HDL-C and HDL2-C levels were

Table 2. Multivariate correlation of variables with LDL-C, SdLDL-C, HDL-C, HDL2-C and HDL3-C in all subjects

	LDL-C		SdLDL-C		HDL-C		HDL2-C		HDL3-C	
	β	p	β	p	β	p	β	p	β	p
BMI	0.094	0.034	0.178	<0.001	−0.267	<0.001	−0.282	<0.001	−0.055	0.218
Fasting glucose	0.090	0.046	0.143	0.002	−0.148	0.002	−0.169	<0.001	−0.002	0.973
2-h glucose	0.053	0.239	0.136	0.002	−0.099	0.034	−0.137	0.003	0.056	0.215
Fasting insulin	0.017	0.706	0.150	0.001	−0.281	<0.001	−0.284	<0.001	−0.088	0.057
HOMA-IR	0.005	0.921	0.111	0.016	−0.209	<0.001	−0.217	<0.001	−0.052	0.260
hsCRP †	0.033	0.463	0.137	0.003	−0.208	<0.001	−0.240	<0.001	0.004	0.928
IMT †	0.058	0.149	0.085	0.034	−0.078	0.061	−0.077	0.064	−0.029	0.468

Data are expressed after adjustment for age and sex.
† Parameters are transformed logarithmically before analysis.

Table 3. Univariate correlation between lipid variables in all subjects

	SdLDL-C		LDL-C		apoB		TG	
	ρ	p	ρ	p	ρ	p	ρ	p
HDL-C	−0.355	<0.001	−0.119	<0.001	−0.282	<0.001	−0.466	<0.001
HDL2-C	−0.431	<0.001	−0.208	<0.001	−0.356	<0.001	−0.483	<0.001
HDL3-C	0.076	0.098	0.210	<0.001	0.109	0.017	−0.103	0.023

Spearman's correlation coefficients (ρ) and p-values.

inversely associated with BMI ($\beta = -0.267, -0.282$), fasting glucose ($\beta = -0.148, -0.169$) and insulin ($\beta = -0.281, -0.284$), 2-h glucose ($\beta = -0.099, -0.137$), HOMA-IR ($\beta = -0.209, -0.217$), and hsCRP levels ($\beta = -0.208, -0.240$). The HDL2-C level, however, was more strongly associated than the HDL-C level with these parameters. On the other hand, the HDL3-C level was not associated with any of these metabolic parameters. Although HOMA-IR does not always reflect insulin resistance in diabetic subjects, we included DM subjects in the analysis of **Table 2**; therefore, we also investigated associations between lipid parameters and HOMA-IR among the subjects, excluding DM subjects. HOMA-IR was positively associated with the sdLDL-C level ($\beta = 0.190$, $p < 0.001$) and inversely associated with HDL-C ($\beta = -0.311$, $p < 0.001$) and HDL2-C levels ($\beta = -0.308$, $p < 0.001$). LDL-C and HDL3-C levels were not associated with HOMA-IR. Accordingly, the result that sdLDL-C, HDL-C and HDL2-C levels were significantly associated with HOMA-IR did not change.

Further analyses were performed to investigate correlations among lipid variables (**Table 3**). HDL-C and HDL2-C levels were inversely correlated with sdLDL-C ($\rho = -0.355, -0.431$), LDL-C ($\rho = -0.119, -0.208$), apoB ($\rho = -0.282, -0.356$), and TG levels ($\rho = -0.466, -0.483$). The HDL2-C level, however, was more closely correlated than the HDL-C level with these factors. The HDL3-C level was inversely correlated with TG levels ($\rho = -0.103$), whereas the HDL3-C level was positively correlated with LDL-C and apoB levels ($\rho = 0.210, 0.109$).

As shown in **Table 2**, after adjustment for age and sex, IMT was significantly associated with sdLDL-C levels, but not LDL-C, HDL-C, HDL2-C and HDL3-C levels. To clarify the association between IMT and sdLDL-C levels, we investigated using multiple regression models after adjustment for age, sex, smoking status, hypertension, diabetes mellitus, and hsCRP levels (**Table 4**). To fit the linear models, sdLDL-C and IMT were log-transformed. The sdLDL-C level was significantly positively associated with IMT even after multivariate adjustment ($\beta = 0.090$, $p = 0.029$).

Discussion

In the present study, we measured sdLDL-C, HDL2-C and HDL3-C levels, and examined the associations between these parameters and BMI, fasting glucose, 2-h glucose, fasting insulin, HOMA-IR, hsCRP, and IMT. Additionally, we investigated the

Table 4. Relationship of SdLDL-C by regression analysis with IMT as the dependent variable

Adjustment	β	p
SdLDL-C only	0.121	0.008
Adjusted for age and sex	0.085	0.034
Adjusted for age, sex, and smoking status	0.102	0.013
Adjusted for age, sex, smoking status and hypertension	0.093	0.023
Adjusted for age, sex, smoking status and diabetes mellitus	0.097	0.019
Adjusted for age, sex, smoking status and hsCRP	0.102	0.013
Adjusted for age, sex, smoking status, hypertension, diabetes mellitus and hsCRP	0.090	0.029

associations between sdLDL-C and HDL subfractions. As a result, sdLDL-C was closely associated with insulin resistance and glucose intolerance, and is therefore thought to be more important than LDL-C in atherogenesis. In terms of HDL-C, subjects with higher HDL2-C seem to be better protected from atherosclerosis.

In another study, Austin *et al*. measured the size of LDL particles and demonstrated an increased risk for myocardial infarction in subjects with LDL pattern B showing sdLDL, compared with LDL pattern A marked by large buoyant LDL[3]. Moreover, it was reported that not only the prevalence of sdLDL but also its concentration were increased in subjects with CHD or diabetes mellitus[4]. Our study showed that the sdLDL-C level was significantly higher in DM subjects and IGT subjects than in NGT subjects, although no significant difference was observed for the LDL-C level.

In univariate analysis, the sdLDL-C level was positively correlated with a higher risk of atherosclerosis, including higher levels of BMI, fasting and 2-h glucose, HOMA-IR, and hsCRP. HsCRP is the most reliable and accessible clinical measure among plasma markers of inflammation. Elevated hsCRP may be an indicator of systemic inflammation but also may be directly involved in atherosclerosis pathogenesis[24]. Many studies have described the epidemiologic relations between obesity and CRP. It is reported that elevated CRP levels are more likely to be found in obese and overweight subjects and measurements of abdominal obesity are associated with systemic inflammation as measured by hsCRP[25]. Therefore, we performed multivariate analyses after adjustment for age, sex and BMI to assess the association between sdLDL-C levels and hsCRP levels. As a result, the significance of the association was attenuated and disappeared after further adjustment was made for BMI ($\beta = 0.086$, $p = 0.054$). Accordingly, sdLDL-C may be positively associated with hsCRP through obesity.

The sdLDL-C level was positively correlated with a 1-h glucose level (data not shown). It was shown that 1-h and 2-h post-load glucose levels during 75g GTT were correlated with IMT, and represented independent predictors of atherosclerosis[26]. After adjustment for age and sex, the correlations between sdLDL-C levels and these variables remained and were closer than those of LDL-C (**Table 2**). These results revealed that sdLDL-C was more closely associated with insulin resistance and glucose intolerance than LDL-C. Although it is controversial how the presence of insulin resistance or impaired glucose tolerance increases the amount of small dense LDL, metabolism of VLDL1 is thought to be an important mechanism. Suppression of VLDL1 production by insulin was impaired in subjects with high liver fat, resulting in overproduction of VLDL1[27]. In addition, the presence of insulin resistance prolonged VLDL1 residence time for LDL. This condition permitted sufficient time for the processes of lipid exchange and lipolysis to generate small dense LDL particles[28].

Carotid artery IMT has long been utilized as one of the surrogate markers for atherosclerosis. In a 1998 study, we reported that no significant difference was observed in serum LDL-C concentrations between Japanese-Americans and native Japanese. In the same study, however, IMT in Japanese-Americans was reported to be greater than in native Japanese[15]. We suggested that the difference in IMT values between the two groups was due to exposure to coronary risk factors such as hypercholesterolemia for a longer duration and to a higher degree. We concluded that the LDL-C level was not correlated with IMT in Japanese-Americans in a cross-sectional study. Similarly, in this study, the LDL-C level was not correlated with IMT; however, the sdLDL-C level was positively correlated with IMT, even after adjustment for age, sex, smoking status, hypertension, diabetes mellitus and hsCRP levels. These results suggest that sdLDL-C indicates the present degree of atherosclerosis. Shoji *et al*. demonstrated that the sdLDL-C level was more closely associated with IMT than other lipid parame-

ters, such as LDL-C, apo B, total cholesterol, HDL-C, apo A-1 levels, and so on[7]. Although we investigated the association between IMT and HDL subfractions, no significant correlation was observed. Thus, sdLDL-C is thought to potentially be a useful risk marker in assessing carotid artery IMT.

The protective role of HDL against the development of CHD is well accepted. Several studies reported that HDL3-C was the strongest predictor of CHD[11, 29]. Inversely, another study suggested that the cardioprotective effect of elevated HDL-C levels may be attributed to the HDL2 subfraction[9]. In our study, the HDL2-C level was more closely inversely correlated than the total HDL-C level with metabolic parameters or hsCRP levels, whereas the HDL3-C level was not correlated with such factors (**Table 2**). Similar to sdLDL-C, we performed multivariate analyses after adjustment for age, sex and BMI to assess the association between HDL-C or HDL2-C levels and hsCRP levels. Even after adjustment for age, sex and BMI, HDL-C and HDL2-C levels were significantly inversely correlated with hsCRP levels ($\beta = -0.138, -0.169$). Additionally, the HDL2-C level was more closely correlated than the total HDL-C or HDL3-C level with sdLDL-C, LDL-C, TG, and apoB levels (**Table 3**). Thus, the HDL2 fraction is more variable and more closely associated with risk factors for atherosclerosis. These results indicate that subjects with higher HDL2-C seem to be better protected from atherosclerosis. Interestingly, the HDL3-C level was positively correlated with LDL-C and apoB levels. Elevated serum levels of LDL-C and apoB are recognized as risk factors for atherosclerosis. A shift toward smaller HDL particles, such as HDL3, was reported in atherosclerotic disorders such as diabetes[30] and metabolic syndrome[31]. One theory for this shift may result either from impaired maturation of HDL3 into HDL2 or from enhanced production of HDL3. The lecithin-cholesterol acyltransferase (LCAT) is a critical enzyme in HDL metabolism, and LCAT-mediated cholesterol esterification leads to the maturation of HDL3 into HDL2. Hepatic lipase (HL) and cholesteryl ester transfer protein (CETP) promote the conversion of HDL2 to HDL3 by modulating the content of these particles; therefore, impaired LCAT and enhanced HL or CETP activity could result in elevated HDL3-C levels.

This study has several limitations. Firstly, it is noted that all participants in this study were Japanese-Americans. In Japanese subjects, the HDL2-C level is higher than the HDL3-C level[20, 32]. Conversely, in Caucasians, several studies reported that the HDL3-C level was higher than the HDL2-C level[8, 10, 33]. In Japanese-Americans, as shown in **Table 1**, the HDL2-C level was higher than the HDL3-C level. Although the reason for this is unclear from our study, which of the two subfractions predominates appears to be affected more by genetic factors than a Westernized lifestyle. For example, HL activity and HL gene promoter polymorphism are important determinants of HDL2-C. One allelic variation in the HL gene associated with higher HDL2-C was more frequent in Japanese-Americans than in Caucasians[33]. In addition, CETP activity and CETP gene polymorphism were associated with the variance in HDL3-C[34]. Such factors may result in the observed predominance of HDL2-C in Japanese-Americans. Secondly, although sdLDL-C is thought to potentially be a useful risk marker in assessing carotid artery IMT, we did not examine the size of LDL; therefore, it is unknown which is more important, sdLDL-C concentration or LDL size for carotid artery IMT. Finally, because of the cross-sectional nature of this study, the associations do not necessarily indicate causality.

Conclusion

SdLDL-C was closely associated with insulin resistance and glucose intolerance, and is thus thought to potentially be a useful risk marker in assessing carotid artery IMT and the present degree of atherosclerosis in Japanese-Americans. Moreover, we hypothesized that subjects with higher HDL2-C levels were less prone to the development of atherosclerosis.

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