Serum High-Density Lipoprotein-Cholesterol Levels Modify the Association Between Plasma Levels of Oxidatively Modified Low-Density Lipoprotein and Coronary Artery Disease in Men

Bo Zhang, Huai Bai, Rui Liu, Koichiro Kumagai, Hiroyuki Itabe, Tatsuya Takano, and Keijiro Saku

We investigated the association among plasma levels of oxidatively modified low-density lipoprotein (Ox-LDL), high-density lipoprotein-cholesterol (HDL-C), and the prevalence of coronary artery disease (CAD) in a case-control study. Cases (n = 183, male [M]/female [F]:138/45, age: 64.9 ± 10.6 years) were defined as patients with angiographically proven coronary atherosclerosis (≥50% stenosis) and controls were subjects with normal coronary arteries (n = 74, M/F:36/38, age: 57.6 ± 14.4 years). Plasma Ox-LDL levels were measured by a sensitive detection method using the monoclonal antibody DLH3. In women, both Ox-LDL and lipid variables were similar between cases and controls. In men, cases had significantly lower ($P < .05$) levels of HDL-C (39.1 ± 10.3 *v* 42.8 ± 10.9 mg/dL) and apolipoprotein (apo) A-I than controls, while the difference in Ox-LDL between cases and controls was not significant (1.05 ± 0.79 and 0.83 ± 0.65 ng/10 μg LDL protein, respectively). However, HDL-C levels interacted with the association between Ox-LDL levels and CAD in males: increased Ox-LDL levels were significantly associated with CAD after controlling for age when HDL-C levels were high, but were not associated with CAD when HDL-C levels were low, as assessed by a multiple logistic regression analysis. In addition, the combination of HDL-C and Ox-LDL levels was a better indicator for CAD in males than HDL-C levels alone (−2 log likelihood, 24.1 *v* 19.4) after controlling for age and conventional risk factors of CAD, while Ox-LDL levels were not significantly associated with CAD. HDL-C levels interact with the association between plasma Ox-LDL levels and CAD in men, and increased Ox-LDL levels are an indicator of CAD in male subjects with high HDL-C levels.

LOW-DENSITY LIPOPROTEIN (LDL) is oxidatively modified by endothelial cells and macrophages in the arterial wall. Oxidatively modified LDL (Ox-LDL) is known to be involved in the initiation and development of atherosclerotic lesions.[1] The uptake of Ox-LDL by macrophage and smooth muscle cells leads to the formation of foam cells, which accumulate lipid droplets.[2] Although it is not yet clear whether or not oxidative modification of LDL also occurs in plasma, there is evidence that Ox-LDL and anti-Ox-LDL antibodies exist in plasma.[3-6]

Ox-LDL exhibits several cell biologic activities, ie, it enhances the interaction between leukocytes and endothelial cells, inhibits endothelial cell migration, induces endothelin secretion from endothelial cells and macrophages, and induces apoptosis in vascular smooth muscle cells. Thus, it would be interesting to determine whether or not increased circulating levels of Ox-LDL could be a marker for coronary artery disease (CAD). Methods for detecting circulating Ox-LDL using monoclonal antibody against Ox-LDL and malondialdehyde (MDA)-modified LDL have been developed by the research groups of Holvoet et al[3,7-9] and Itabe et al.[2,4,10] Increased circulating levels of Ox-LDL have been shown to be associated with coronary heart disease,[4] coronary spastic angina,[11] transplant-associated CAD,[8,9] and acute coronary syndrome.[7] The findings in these studies strongly suggest that circulating Ox-LDL is clinically significant as a biomedical marker for CAD.

An inverse relationship has been established between plasma levels of high-density lipoprotein-cholesterol (HDL-C) and CAD.[12,13] Direct evidence for the antiatherogenic effects of HDL has recently been obtained in studies of the over- or underexpression of apolipoprotein (apo) A-I using genetic animal models of reverse cholesterol transport.[14-18] The antiatherogenic effects of HDL are partly attributable to its protective effects against LDL oxidation. HDL subclasses have been shown to inhibit the oxidation of LDL,[19] and reconstituted HDL reduces the capacity of Ox-LDL to accumulate cholesterol esters in mouse peritoneal macrophages.[20] However, HDL-C levels are not correlated with plasma levels of Ox-LDL in normal healthy subjects,[4] because Ox-LDL levels are also regulated by other factors, including oxidative stress, the lipid content of LDL, and LDL levels, etc.

Although LDL-C levels are known to predict the risk for CAD, data from 35 years of the Framingham Heart Study[21] have shown that the increases in the risk of CAD associated with higher LDL-C levels are greater when HDL-C levels are low than when HDL-C levels are high, indicating that HDL-C levels modify the relationship between LDL-C levels and CAD. Therefore, it is of interest and could be clinically important to determine whether and how HDL-C levels may affect the relationship between Ox-LDL and CAD, which, however, has not yet been examined. In this case-control study, we examined the association among plasma levels of Ox-LDL, HDL-C, and the prevalence of CAD.

From the Department of Cardiology, Fukuoka University School of Medicine, Fukuoka; and the Faculty of Pharmaceutical Sciences, Teikyo University, Kanagawa, Japan.

Submitted March 31, 2003; accepted October 10, 2003.

Supported by grants-in-aid from the Ministry of Education, Science and Culture of Japan (no. 10670221, 10670693, 11670724, 12670712, and 15790403), by a research grant from the Clinical Research, by research grants from the Ministry of Health and Welfare, by a grant from the Uehara Memorial Foundation (2002), and by a research grant (no. 026001) from the Central Research Institute of Fukuoka University.

Presented in part at the 71st Scientific Session of the American Heart Association, Dallas, TX, November 10, 1998.

Address reprint requests to Keijiro Saku, MD, PhD, FACP, FACC, Department of Cardiology, Fukuoka University School of Medicine, 7-45-1 Nanakuma Jonan-ku, Fukuoka 814-0180, Japan.

0026-0495/04/5304-0003$30.00/0

doi:10.1016/j.metabol.2003.10.028

MATERIALS AND METHODS

Subjects

The subjects consisted of 257 consecutive patients who underwent diagnostic coronary angiography (CAG) for suspected or known coronary atherosclerosis or for other reasons (mostly atypical chest pain) at the Fukuoka University Hospital from 1994 to 1996.[22] This study was approved by the ethics committee of Fukuoka University Hospital, and informed consent was obtained from each patient. Controls (CAD^- patients) were defined as those with less than 25% luminal narrowing, and cases (CAD^+ patients) were those who had 1, 2, or 3 stenosed ($>$ 50% luminal narrowing) epicardial coronary arteries . Patients with luminal narrowing of between 25% and 50% were excluded. Patients with spastic angina pectoris, ie, acetylcholine-positive, were excluded from the controls, and none of the controls had a history of myocardial infarction (MI). Patients with acute MI (within 3 weeks after onset), heart failure (Killip class $\geq$ 2 after MI), vascular disease (aortitis treated by prednisolone), hepatic dysfunction (viral and nonviral, transaminases more than 3 times the normal value), or uncontrollable diabetes mellitus (DM) were excluded from the study. Patients with systolic or diastolic blood pressure $>$ 140 mm Hg or 90 mm Hg, or who were under antihypertensive treatment were considered to have hypertension (HT). Patients under treatment for DM and/or with symptoms of DM and a fasting glucose concentration $\geq$ 126 mg/dL were considered to have DM. Otherwise, the results of a 75-g glucose tolerance test were used to give a diagnosis of DM.

CAG

Coronary arteries were cannulated by the Judkins technique[23] with 5F catheters, as described previously.[22,24,25]

Determination of Serum Lipids, Lipoproteins, and Apolipoproteins

Blood was drawn in the morning after an overnight fast. Serum total cholesterol (TC) and triglyceride (TG) concentrations were determined enzymatically. HDL-C was determined by the heparin Ca^{2+} precipitation method.[26] LDL-C was calculated as TC $-$ HDL-C $-$ TG/5, since the highest level of TG in all of the subjects was 367 mg/dL. HDL subfractions [HDL_2 (d 1.063 to 1.125 g/mL) and HDL_3 (d 1.125 to 1.21 g/mL)] were separated by standard sequential preparative ultracentrifugation techniques.[27] Apo A-I, apo A-II, apo B, apo C-II, apo C-III, and apo E were determined by the turbidity immunoassay method.[28] Serum lipoprotein (a) [Lp(a)] levels were measured by an enzyme-linked immunosorbent assay (ELISA) using Tint Eliza Lp(a) (Biopool, Umea, Sweden).[29] For all measurements in our laboratory, the coefficients of interassay and intra-assay variation were less than 5.0%, and blinded quality-control specimens were included in each assay.

Measurement of Plasma Ox-LDL Levels

Blood samples were obtained intravenously from all of the subjects using EDTA as an anticoagulant. To measure the oxidation of human plasma LDL, the LDL fraction (1.019 to 1.063 g/mL) was obtained by sequential centrifugation.[10,30] Plasma levels of Ox-LDL were determined by a sensitive detection method established by Itabe et al.[10] With this method, it is possible to detect 0.5 ng protein of Ox-LDL by precoating microliter wells with a murine monoclonal antibody against Ox-LDL (FOH1a/DLH3)[2] before sandwich ELISA using an antihuman apo B antibody. Briefly, 100 μL of the monoclonal antibody FOH1a/DLH3 (3 μg/mL in phosphate-buffered saline [PBS]) was incubated in each microtiter well (Flexible assay plate, Falcon 3912, Becton Dickinson, Winooski, CA) at room temperature for 2 hours. After the antibody solution was discarded, the wells were filled with Tris-buffered saline (TBS) containing 1% bovine serum albumin (BSA) (fraction V) at room temperature for at least 2 hours. After the blocking solution was discarded, sample (human LDL fractions, 5 μg protein/well) or diluted standard Ox-LDL (0.5 to 20 ng/well) was applied to the well and incubated at 4°C overnight. Standard Ox-LDL was prepared by incubating LDL with $CuSO_4$ at 37°C for 3 hours. The wells were then washed 3 times with TBS containing 0.05% Tween 20. The wells were incubated for 2 hours at room temperature with 100 μL sheep antihuman apo B antibody solution (diluted 1:5,000 in PBS). After the wells were washed 3 times with TBS-Tween, 100 μL alkaline phosphatase (ALP)-conjugated goat antisheep IgG antibody (diluted 1:10,000 in TBS containing 2% skim milk) was added, and the samples were incubated for another 2 hours. The reactivity of ALP was finally measured by incubating the samples with 100 μL p-nitrophenylphosphate (1 mg/mL, dissolved in 1 mol/L triethanolamine buffer, pH 9.8) at 37°C for appropriate intervals, and the absorbance was measured on an ELISA plate reader. Each sample was run with a control well that was precoated with nonimmune IgM (0.3 μg/well) instead of monoclonal antibody. In each ELISA plate, various concentrations of standard Ox-LDL were run simultaneously to determine a standard curve. The samples were measured in duplicate, and the coefficients of interassay and intra-assay variability were less than 10%.

Table 1. Characteristics of Patients With (CAD^+) and Without (CAD^-) CAD

	CAD^- Patients (n = 183)	CAD^+ Patients (n = 74)
Age (yr)	57.6 $\pm$ 14.4	64.9 $\pm$ 10.6*
BMI (kg/m^2)	22.9 $\pm$ 3.2	23.4 $\pm$ 3.1
Gender		
Female	38 (51.4%)	45 (24.6%)*
Male	36 (48.6%)	138 (75.4%)
Smoking	24 (32.4%)	78 (42.6%)
DM	11 (14.9%)*	70 (38.2%)
Hypertension	37 (50.0%)	102 (55.7%)

Abbreviations: BMI, body mass index; DM, diabetes mellitus.
*$P < .05$, by χ^2 test.

Statistical Analysis

Statistical analysis was performed using the SAS Software Package (version 6.12, SAS Institute, Cary, NC). Categorical variables (such as gender) were compared between CAD^+ and CAD^- patients by a χ^2 analysis. Distributions of continuous variables were examined by the Shapiro-Wilk test.[31] Differences in continuous variables (such as age and lipid parameters) were examined by an analysis of variance (ANOVA)[32] and the Wilcoxon rank-sum test.[32] Multiple comparisons were performed by the Scheffe's multiple comparison test.[32] Adjustment for age was performed by an analysis of covariance.[32] The association between Ox-LDL (2 levels) and CAD according to HDL-C (2 levels) after controlling for age were evaluated by a multiple logistic regression analysis.[32] In the logistic regression analysis, HDL-C and Ox-LDL were coded as dichotomous variables using 0 to represent the "low" group and 1 to represent "high" group. CAD^- and CAD^+ were coded as 0 and 1, and "descending" option was used in the procedure. For logistic regression coefficients, we show the standard error, and for odds ratios, we calculated 95% confidence intervals (CI). All P values are 2-tailed. The significance level was considered to be 5% unless indicated otherwise.

RESULTS

Table 1 shows the characteristics of patients with (CAD^+) and without CAD (CAD^-). CAD^+ patients were significantly ($P < .05$) older than CAD^- patients. There were also significantly ($P < .05$) more males and a higher prevalence of DM

Table 2. Serum Levels of Lipids, Lipoproteins, and Apolipoproteins in Patients With (CAD^+) and Without (CAD^-) CAD According to Gender

	Female		Male	
	CAD^- (n = 38)	CAD^+ (n = 45)	CAD^- (n = 36)	CAD^+ (n = 138)
Age (yr)	60.4 ± 13.8	68.9 ± 10.0*	54.5 ± 14.8	63.6 ± 10.5*
BMI (kg/m^2)	21.7 ± 3.1	23.1 ± 3.1	24.1 ± 2.9	23.7 ± 3.1
Smoking	6 (15.6%)	8 (17.8%)	18 (50.0%)	70 (50.7%)
DM	5 (13.2%)	16 (35.6%)*	6 (16.7%)	54 (39.1%)*
Hypertension	18 (47.4%)	27 (60.0%)	19 (52.8%)	75 (54.4%)
HDL-C (mg/dL)	48.8 ± 11.1	48.2 ± 10.3	42.8 ± 10.9	39.1 ± 10.3*†
HDL_2-C (mg/dL)	32.9 ± 10.4	31.4 ± 9.8	26.3 ± 10.6	24.8 ± 9.1
HDL_3-C (mg/dL)	15.9 ± 2.2	16.6 ± 2.6	17.2 ± 3.5	15.5 ± 3.3*
Apo A-I (mg/dL)	116.7 ± 22.8	118.3 ± 20.1	111.2 ± 23.5	101.4 ± 23.7*
Apo A-II (mg/dL)	29.2 ± 4.6	27.6 ± 5.3	29.9 ± 6.3	27.4 ± 7.1
Apo B	96.2 ± 26.1	108.9 ± 30.4	99.3 ± 22.0	102.7 ± 29.3
Apo C-II (mg/dL)	2.8 ± 1.2	3.7 ± 1.7*†	3.5 ± 1.5	3.9 ± 8.6
Apo C-III (mg/dL)	7.4 ± 2.2	9.8 ± 3.9*†	8.8 ± 2.9	8.5 ± 3.9
Apo E (mg/dL)	4.5 ± 1.1	5.2 ± 1.7*	4.4 ± 1.0	4.5 ± 1.5
TC (mg/dL)	196 ± 34	207 ± 34	185 ± 28	188 ± 41
TG (mg/dL)	96 ± 44	128 ± 79	124 ± 65	127 ± 80
Lp(a) (mg/dL)	27.5 ± 20.0	35.1 ± 35.3	24.3 ± 15.2	30.5 ± 30.8
LDL-C (mg/dL)	128 ± 31	133 ± 31	117 ± 25	124 ± 37
Ox-LDL (ng/10 μg LDL protein)	1.22 ± 0.91	1.34 ± 1.00	0.83 ± 0.65	1.05 ± 0.79

Abbreviations: HDL-C, high-density lipoprotein-cholesterol; Apo, apolipoprotein; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein-cholesterol; Lp(a), lipoprotein (a); Ox-LDL, oxidatively modified LDL; DM, diabetes mellitus.

*$P < .05$, CAD^+ *v* CAD^-, by an ANOVA and the Wilcoxon rank-sum test.

†$P < .05$, CAD^+ *v* CAD^-, adjusted for age by an analysis of covariance.

among CAD^+ patients than among CAD^- patients. On the other hand, the prevalence of smoking, hypertension, and body mass index (BMI) were not significantly different between the 2 groups.

Because CAD^+ and CAD^- patients were not matched with regard to gender, gender was used as a stratification variable in the data analysis. In Table 2, prevalence of smoking, DM, and HT, serum levels of lipids, lipoproteins, and apolipoproteins, and plasma Ox-LDL levels were compared between CAD^+ and CAD^- patients according to gender. For both females and males, CAD^+ patients were older and had higher prevalence of DM than CAD^- patients (Table 2). For females (Table 2), CAD^+ patients had significantly ($P < .05$) higher serum levels of apo C-II, apo C-III, and apo E than CAD^- patients. The differences in serum apo C-II and apo C-III levels between CAD^- and CAD^+ patients were also significant after adjusting for age, as assessed by an analysis of covariance. For males (Table 2), CAD^+ patients had significantly ($P < .05$) lower serum levels of HDL-C, HDL_3C, and apo A-I than CAD^- patients. However, after adjusting for age, only serum HDL-C levels significantly differed between CAD^- and CAD^+ patients (Table 2). Plasma Ox-LDL levels in CAD^+ patients were slightly, but not significantly, higher than those in CAD^- patients among both females (1.34 ± 1.00 *v* 1.22 ± 0.91 ng/10 μg LDL protein, not significant [NS]) and males (1.05 ± 0.79 *v* 0.83 ± 0.65 ng/10 μg LDL protein, NS).

As also shown in Table 2, for both CAD^- and CAD^+ patients, males were younger and had lower serum levels of HDL-C, HDL_2-C, and apo C-III than females, as assessed by an ANOVA and the Wilcoxon rank-sum test. For CAD^- patients, males had significantly ($P < .05$) higher BMI and serum apo C-II levels and lower plasma Ox-LDL levels (0.83 ± 0.65 *v* 1.22 ± 0.91 ng/10 μg LDL protein). For CAD^+ patients, males had significantly ($P < .05$) lower serum levels of HDL_3-C, apo A-I, apo E, TC, and Lp(a) than females.

Because HDL-C levels have been shown to affect the risk of CAD associated with increased LDL-C levels[21] and because the HDL-associated enzyme paraoxonase (PON1) has been shown to protect LDL from oxidation,[33] we were encouraged to examine whether or not HDL-C levels may interact with the relationship between Ox-LDL and CAD.

Table 3 shows the relationship among HDL-C (2 levels),

Table 3. Age and Prevalence of CAD for each Combination of OxLDL Level and HDL-C Level (High HDL-C-Low OxLDL, High HDL-C-High OxLDL, Low HDL-C-Low OxLDL, and Low HDL-C-High OxLDL) in Females and Males

Gender	HDL-C*	Ox-LDL†	Age (yr)	Prevalence of CAD (%)
Females				
	High	Low	65.0 ± 10.9	54.3
	High	High	65.3 ± 14.1	56.7
	Low	Low	69.7 ± 6.5	66.7
	Low	High	61.8 ± 15.2	41.7
Males				
	High	Low	58.4 ± 14.7	61.9
	High	High	65.6 ± 10.4	85.7‡
	Low	Low	62.3 ± 9.9	85.1‡
	Low	High	60.7 ± 13.0	83.3‡

*Median value of HDL-C (39 mg/dL) was used as a cut-off point.

†Median value of Ox-LDL (0.98 ng/10 μg LDL protein) was used as a cut-off point.

‡$P < .05$ *v* high HDL-C-low OxLDL, by an ANOVA and Scheffe's multiple comparison test.

Table 4. Age-Adjusted Associations Between Ox-LDL and CAD According to HDL-C Levels in Male Subjects as Assessed by a Multiple Logistic Regression Analysis

		Ox-LDL*	
		Low	High
HDL-C*	Low	3.59 (1.40-9.19)†	3.22 (1.00-10.4)
	High	1.00	3.27 (1.02-10.4)†

NOTE. Odds ratio and 95% confidence intervals were presented.

Abbreviations: HDL-C, high-density lipoprotein cholesterol; Ox-LDL, oxidatively modified low-density lipoprotein.

*HDL-C and Ox-LDL were coded as dichotomous variables using 0 to represent the "low" group and 1 to represent "high" group. Median values of HDL-C (39 mg/dL) and Ox-LDL (0.98 ng/10 μg LDL protein) were used as a cut-off point, respectively.

†$P < .05$, by multiple logistic regression analysis.

Ox-LDL (2 levels), and the prevalence of CAD (percentage of CAD^+ patients) in males and females. Age is also given for each combination of HDL-C and Ox-LDL. As shown in Table 3, for both males and females, age was not significantly different among patients with high HDL-C–low Ox-LDL, high HDL-C–high Ox-LDL, low HDL-C–low Ox-LDL, and low HDL-C–high Ox-LDL. For females, the prevalence of CAD was not significantly different among each combination of HDL-C and Ox-LDL (Table 3), which agrees with the results shown in Table 2 that the differences in HDL-C and Ox-LDL between female CAD^- and CAD^+ patients were not significant. For males, patients with high HDL-C–low Ox-LDL showed a significantly lower prevalence than patients with high HDL-C–high Ox-LDL, low HDL-C–low Ox-LDL, and low HDL-C–high Ox-LDL, as assessed by an ANOVA and Scheffe's multiple comparison test. This result suggests that high Ox-LDL was related to CAD when HDL-C was high, although among all of the males, the difference in Ox-LDL between CAD^- and CAD^+ patients was not statistically significant (Table 2).

Therefore, we examined the association between Ox-LDL and CAD in male subjects according to HDL-C strata and HDL-C–by–Ox-LDL interaction after adjusting for age by a multiple logistic regression analysis (Table 4). As shown, the association between high plasma Ox-LDL levels and CAD in males varied with the HDL-C levels: it was significant in males with high HDL-C levels, but was not significant in males with low HDL-C levels. HDL-C–by–Ox-LDL interaction showed a significance level of less than 0.1 (data not shown).

Therefore, we examined whether the combination of HDL-C and Ox-LDL may have a greater ability to predict CAD than HDL-C alone. Table 5 shows the results of a multiple logistic function analysis of the association of HDL-C (model 1), Ox-LDL (model 2), and the combination of HDL-C and Ox-LDL (model 3) with CAD in male subjects after controlling for age and additionally for DM, HT, and smoking. As shown, although Ox-LDL levels were not significantly associated with CAD after adjusting for age and additionally for DM, HT, and smoking, the combination of HDL-C and Ox-LDL fit the model for predicting the risk of CAD much better than did HDL-C alone, as judged by the criteria for assessing model fit, -2 log likelihood,[32] supporting the result in Table 4 that HDL-C levels interact with the association between Ox-LDL and CAD.

Table 6 shows odds ratios for each combination of HDL-C (2 levels) and Ox-LDL (2 levels) after controlling for age and for DM, HT, and smoking. As shown, patients with high HDL-C–low Ox-LDL had the lowest risk of CAD among each combination of HDL-C and Ox-LDL. High Ox-LDL levels were consistently associated with a higher risk of CAD in male subjects with high HDL-C levels, and the high HDL-C–high Ox-LDL group had a similar relative risk of CAD as the low HDL-C–low Ox-LDL group (Table 6). Therefore, these results suggest that the association between plasma Ox-LDL levels and CAD depends on the HDL-C level, and high Ox-LDL levels could be a useful marker for CAD in combination with the HDL-C level.

DISCUSSION

Circulating oxidized LDL originates from mild oxidation in the arterial wall by cell-associated lipoxygenase and/or myeloperoxidase and plays an important role in the initial step of

Table 5. Multiple Logistic Function Analysis of Coronary Risk Factors in Male Subjects After Adjusting for Age and Additionally for DM, HT, and Smoking

Model	Model Variable	Regression Coefficients ± SE	Wald χ^2	(*P* value)	−2 Log Likelihood
Adjusting for age					
Model 1	HDL-C*	−0.81 ± 0.39	4.26	.039	12.4
Model 2	Ox-LDL†	0.49 ± 0.42	1.37	NS	9.4
Model 3	Combined HDL-C and Ox-LDL‡	1.23 ± 0.41	8.92	.003	16.7
Adjusting for age, DM, HT, and smoking					
Model 1	HDL-C*	−0.61 ± 0.40	2.26	NS	19.4
Model 2	Ox-LDL†	0.64 ± 0.45	2.02	NS	19.2
Model 3	Combined HDL-C and Ox-LDL‡	1.15 ± 0.43	7.13	.008	24.1

Abbreviations: HDL-C, high-density lipoprotein cholesterol; Ox-LDL, oxidatively modified low-density lipoprotein; DM, diabetes mellitus; HT, hypertension; NS, not significant.

*In 2 levels, given a value of 1 for HDL-C > 39 mg/dL and 0 for HDL-C ≤ 39 mg/dL.

†In 2 levels, given a value of 1 for OxLDL > 0.98 ng/10 μg LDL protein and 0 for Ox-LDL ≤ 0.98 ng/10 μg LDL protein.

‡In 2 levels, given a value of 0 for HDL-C > 39 mg/dL and Ox-LDL ≤ 0.98 ng/10 μg LDL protein and 1 for all other cases (high HDL-C-high Ox-LDL or low HDL-C-Low Ox-LDL or low HDL-C-high Ox-LDL).

Table 6. Odds Ratios for Each Combination of Ox-LDL Levels and HDL-C Levels After Adjusting for Age and Additionally for DM, HT, and Smoking

High HDL-C–Low Ox-LDL	High HDL-C–High Ox-LDL*	Low HDL-C–Low Ox-LDL*	Low HDL-C–High Ox-LDL*
1.00	3.72 (1.09-12.6)†	2.98 (1.13-7.88)†	2.96 (0.86-10.2)

Abbreviations: HDL-C, high-density lipoprotein cholesterol; Ox-LDL, oxidatively modified low-density lipoprotein, DM, diabetes mellitus; HT, hypertension.

*Odds ratio and 95% confidence interval are given, calculated by using 3 dummy variables in a logistic regression analysis.

†$P < .05$ by multiple logistic regression analysis.

atherosclerosis, while HDL, which contains antioxidative and anti-inflammatory enzymes on its particle surface, such as lecithin-cholesterol acyltransferase (LCAT), PON1, and platelet-activating factor acetylhydrolase (PAF-AH), etc, protects LDL from oxidation and plays an important role in the reverse cholesterol transport. Therefore, HDL is an antagonist of Ox-LDL. We examined the association among plasma Ox-LDL levels, HDL-C levels, and CAD in this case-control study to test the hypothesis that HDL may affect the association between plasma Ox-LDL levels and CAD. To the best of our knowledge, this is the first study to investigate the interaction of HDL-C with the association between Ox-LDL and CAD.

Several studies that used monoclonal antibodies against both Ox-LDL and MDA-LDL[3,7-9] or specifically against oxidized phosphatidylcholine[4,10,11] to detect circulating Ox-LDL have shown that plasma Ox-LDL levels are an important indicator for CAD. In these studies, Ox-LDL levels were measured directly in plasma. In the present study, we did not observe a significant association between plasma Ox-LDL and CAD. However, this result does not contradict these previous results, because we measured Ox-LDL levels in LDL fraction separated by sequential ultracentrifugation and therefore expressed Ox-LDL levels as the amount of Ox-LDL relative to the amount of LDL protein. Therefore, our findings suggest that the relative proportion of Ox-LDL and native LDL may not be significantly altered in patients with CAD.

Our findings that DM and low HDL-C levels were associated with CAD agree with other reports.[35-37] Low HDL-C levels have been shown to be a marker for the presence of a small, dense LDL in the circulation, which is susceptible to oxidation.[38] HDL also decreases the accumulation of lipid peroxides on LDL.[39] However, we did not find a significant correlation between Ox-LDL levels and HDL-C levels in patients without CAD (data not shown), because the oxidation of LDL is known to be affected by many other factors. Our result agrees with that reported by Toshima et al[4] in normal volunteers. In male patients with CAD, Ox-LDL levels were positively, but not negatively, related to HDL-C levels ($r = .208$, $P < .05$, Table 3), suggesting that the ability of HDL to protect against LDL oxidation may be independent of HDL-C levels. The fact that knockout of PON1 in mice does not affect HDL-C levels[33] supports this notion. Therefore, although we did not measure serum activities of HDL-associated antioxidative enzymes, including PON1, PAF-AH, and LCAT, our present finding suggests that measurement of HDL-associated enzymes should provide valuable information on the relationship between HDL and Ox-LDL in vivo.

Our finding that high Ox-LDL levels were significantly associated with CAD in male subjects with high HDL-C levels (Tables 3 and 4) agrees with the notion that an increased plasma Ox-LDL level is a marker for CAD. This result may be attributable to the higher LDL-C levels that were associated with high Ox-LDL levels, because patients with high Ox-LDL had significantly ($P < .05$) higher LDL-C levels than those with low Ox-LDL among both males (131 ± 53 *v* 125 ± 34 mg/dL) and females (139 ± 30 *v* 122 ± 30 mg/dL) when HDL-C was high. When HDL-C was low, high Ox-LDL was not associated with CAD in male subjects with low HDL-C (Tables 3 and 4), possibly because serum LDL-C levels were relatively low and very similar in CAD^- (117 ± 27 mg/dL) and CAD^+ patients (120 ± 27 mg/dL).

Our finding that HDL-C interacts with the association between Ox-LDL and CAD in men is consistent with the finding of Castelli,[21] who reported that HDL-C affects the risk of CAD associated with high LDL levels. We did not observe an interaction between HDL-C and the association between Ox-LDL and CAD in female subjects, because in addition to a relatively small sample size, there was no significant difference in either HDL-C or Ox-LDL between CAD^- and CAD^+ (Table 2). We did not examine whether or not LDL-C levels may affect the association between Ox-LDL and CAD, because Ox-LDL levels were expressed as the amount of Ox-LDL relative to the amount of LDL protein, ie, Ox-LDL was adjusted by LDL protein, which should correlate with LDL-C levels.

The results of the Framingham study[21] indicated that HDL-C and LDL-C additively increased the risk of CAD. Our finding also shows that the combination of HDL-C and Ox-LDL had a greater ability to predict CAD than HDL-C alone. However, although we observed an increased risk of CAD in patients with high HDL-C–high Ox-LDL and in those with low HDL-C–low Ox-LDL relative to patients with high HDL-C–low Ox-LDL, the relative risk of CAD in patients with low HDL-C–high Ox-LDL was, unexpectedly, not significantly higher than that in patients with low HDL-C–low Ox-LDL. Although the reason for this phenomenon is not clear, our finding supports the notion that HDL-C is more important than LDL-C in patients with CAD, because while LDL-C levels can be controlled by cholesterol-lowering drugs, such as statin drugs, it is not easy to increase HDL-C levels. Recently, HDL-raising drugs, such as cholesteryl ester transfer protein inhibitor, have attracted much attention.[40] Because, in addition to protecting LDL from oxidation, HDL also has other antiatherogenic properties, including anti-inflammatory effects and its role in the reverse transport of cholesterol, it is possible that a high Ox-LDL level may have relatively minor effects on the risk of CAD when HDL-C levels are low. However, the fact that high Ox-LDL levels were

significantly associated with CAD in men with high HDL-C levels may indicate that not only low HDL-C levels, but also reduced antioxidative ability of HDL could be related to the risk of CAD. This should be confirmed by measuring HDL-associated enzymes, which, unfortunately, was not performed in the present study.

Limitations

In this case-control study, cases were not matched with controls with regard to age or gender (Table 1). Therefore, we performed a statistical analysis using gender stratification and by adjusting for age. Alcohol is known to affect HDL-C levels, but we did not obtain information on alcohol consumption in our study subjects. We selected angiographically defined normal subjects as controls and defined CAD as patients with significantly stenosed coronary arteries. Therefore, we excluded patients with 25% to 50% luminal narrowing, thus losing information on this group of patients. Angiographically defined normal subjects were subjected to diagnostic CAG because of suspected or known coronary atherosclerosis or other reasons (mostly atypical chest pain). However, a selection bias is known to exist: angiographically defined normal subjects generally have more risk factors for coronary disease than patients with clinical symptoms, but who have not been selected for angiography, because a person with both chest pain and a known risk factor, such as smoking, may be more likely to be referred for angiography than a person with just a clinical symptom.[41] In addition, the presence of any luminal disease on angiography is highly suggestive of the presence of substantial atherosclerosis. Thus, biases produced in selecting controls may have limited the power of this study.

In conclusion, HDL-C levels interact with the association between Ox-LDL and the risk of CAD, and increased Ox-LDL levels are a useful maker for CAD in men.

REFERENCES

1. Han J, Hajjar DP, Febbraio M, et al: Native and modified low density lipoproteins increase the functional expression of the macrophage class B scavenger receptor, CD36. J Biol Chem 272:21654-21659, 1997

2. Itabe H, Takeshima E, Iwasaki H, et al: A monoclonal antibody against oxidized lipoprotein recognizes foam cells in atherosclerotic lesions. Complex formation of oxidized phosphatidylcholines and polypeptides. J Biol Chem 269:15274-15279, 1994

3. Holvoet P, Mertens A, Verhamme P, et al: Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol 21:844-848, 2001

4. Toshima S, Hasegawa A, Kurabayashi M, et al: Circulating oxidized low density lipoprotein levels. A biochemical risk marker for coronary heart disease. Arterioscler Thromb Vasc Biol 20:2243-2247, 2000

5. Dotevall A, Hulthe J, Rosengren A, et al: Autoantibodies against oxidized low-density lipoprotein and C-reactive protein are associated with diabetes and myocardial infarction in women. Clin Sci (Lond) 101:523-531, 2001

6. Erkkila AT, Narvanen O, Lehto S, et al: Autoantibodies against oxidized low-density lipoprotein and cardiolipin in patients with coronary heart disease. Arterioscler Thromb Vasc Biol 20:204-209, 2000

7. Holvoet P, Vanhaecke J, Janssens S, et al: Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation 98:1487-1494, 1998

8. Holvoet P, Stassen JM, Van Cleemput J, et al: Oxidized low density lipoproteins in patients with transplant-associated coronary artery disease. Arterioscler Thromb Vasc Biol 18:100-107, 1998

9. Holvoet P, Van Cleemput J, Collen D, et al: Oxidized low density lipoprotein is a prognostic marker of transplant-associated coronary artery disease. Arterioscler Thromb Vasc Biol 20:698-702, 2000

10. Itabe H, Yamamoto H, Imanaka T, et al: Sensitive detection of oxidatively modified low density lipoprotein using a monoclonal antibody. J Lipid Res 37:45-53, 1996

11. Kugiyama K, Sugiyama S, Soejima H, et al: Increase in plasma levels of oxidized low-density lipoproteins in patients with coronary spastic angina. Atherosclerosis 154:463-467, 2001

12. Gordon DJ, Probstfield JL, Garrison RJ, et al: High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 79:8-15, 1989

13. Gordon DJ, Rifkind BM: High-density lipoprotein—the clinical implications of recent studies. N Engl J Med 321:1311-1316, 1989

14. Tall AR: An overview of reverse cholesterol transport. Eur Heart J 19:A31-35, 1998

15. Paszty C, Maeda N, Verstuyft J, et al: Apolipoprotein AI transgene corrects apolipoprotein E deficiency-induced atherosclerosis in mice. J Clin Invest 94:899-903, 1994

16. Rubin EM, Krauss RM, Spanler EA, et al: Inhibition of early atherosclerosis in transgenic mice by human apolipoprotein AI. Nature 353:265-266, 1991

17. Plump AS, Scott CJ, Breslow JL: Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E-deficient mouse. Proc Natl Acad Sci USA 91:9607-9611, 1994

18. Plump AS, Erickson SK, Weng W, et al: Apolipoprotein A-I is required for cholesteryl ester accumulation in steroidogenic cells and for normal adrenal steroid production [see comments]. J Clin Invest 97:2660-2671, 1996

19. Singh K, Chander R, Kapoor NK: High density lipoprotein subclasses inhibit low density lipoprotein oxidation. Indian J Biochem Biophys 34:313-318, 1997

20. Sakai M, Miyazaki A, Hakamata H, et al: Reconstituted high density lipoprotein reduces the capacity of oxidatively modified low density lipoprotein to accumulate cholesteryl esters in mouse peritoneal macrophages. Atherosclerosis 119:191-202, 1996

21. Castelli WP: Cholesterol and lipids in the risk of coronary artery disease—the Framingham Heart Study. Can J Cardiol 4:5a-10a, 1988

22. Saku K, Zhang B, Ohta T, et al: Quantity and function of high density lipoprotein as an indicator of coronary atherosclerosis. J Am Coll Cardiol 33:436-443, 1999

23. Judkins MP: A percutaneous transfemoral technique. Radiology 89:815-821, 1967

24. Saku K, Jimi S, Sasaki N, et al: Reduced coronary vasodilation in patients with familial hypercholesterolemia following intracoronary injection of isosorbide dinitrate. Jpn Circ J 59:808-814, 1995

25. Saku K, Zhang B, Shirai K, et al: Hyperinsulinemic hypoalphalipoproteinemia as a new indicator for coronary heart disease. J Am Coll Cardiol 34:1443-1451, 1999

26. Noma A, Nezu-Nakayama K, Kita M, et al: Simultaneous determination of serum cholesterol in high- and low-density lipoproteins with use of heparin Ca^{2+}, and an anion-exchange resin. Clin Chem 24:1504-1508, 1978

27. Havel RJ, Eder HA, Bragdon JH: The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 34:1345-1353, 1955

28. Ikeda T, Shibuya U, Sugiuchi H, et al: Automated immunoturbidimetric analysis of six plasma apolipoproteins: correlation with radioimmunodiffusion assays. J Clin Lab Anal 5:90-95, 1991

29. Abe A, Maeda S, Makino K, et al: Enzyme-linked immunosorbent assay of lipoprotein(a) in serum and cord blood. Clin Chim Acta 177:31-40, 1988

30. Bronzert T J, Brewer JB Jr: New micromethod for measuring cholesterol in plasma lipoprotein fractions. Clin Chem 23:2089-2098, 1977

31. SAS Institute Inc. SAS Procedures Guide, version 6 (ed 3). Cary, NC, SAS Institute, 1990

32. SAS Institute Inc. SAS/STAT User's Guide, version 6, vol 2 (ed 4). Cary, NC, SAS Institute, 1990

33. Shih DM, Gu L, Xia YR, et al: Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature 394:284-287, 1998

34. Clinical Studies by Multivariate Analysis: Introduction to Proportional Hazards Model and Logistic Model with Application Programs of SAS. Nagoya, Japan, Nagoya Univ. Publishing House, 1990

35. Washio M, Sasazuki S, Kodama H, et al: Role of hypertension, dyslipidemia and diabetes mellitus in the development of coronary atherosclerosis in Japan. Jpn Circ J 65:731-737, 2001

36. Miwa K, Nakagawa K: Risk factors that discriminate 'high-risk' from 'low-risk' Japanese patients with coronary artery disease. Jpn Circ J 64:825-830, 2000

37. Kambara H, Imoto A, Owada C, et al: Coronary risk factors used to predict coronary artery disease by logistic regression analysis. Jpn Circ J 56:1199-1205, 1992

38. Chait A, Brazg RL, Tribble DL, et al: Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. Am J Med 94:350-356, 1993

39. Mackness MI, Durrington PN, Mackness B: How high-density lipoprotein protects against the effects of lipid peroxidation. Curr Opin Lipidol 11:383-388, 2000

40. Okamoto H, Yonemori F, Wakitani K, et al: A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. Nature 406:203-207, 2000

41. Pearson TA, Derby AA: Invited commentary: Should arteriographic case-control studies be used to identify causes of atherosclerotic coronary artery disease? Am J Epidemiol 134:123-128, 1991