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Lipid Compositional Differences of Small, Dense Low-Density Lipoprotein Particle Influence its Oxidative Susceptibility: Possible Implication of Increased Risk of Coronary Artery Disease in Subjects With Phenotype B

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An increased susceptibility of low-density lipoprotein (LDL) to lipid peroxidative modification may be a key factor in the higher risk of coronary artery disease (CAD) among subjects with phenotype B. Compositional differences in the LDL particle may also be implicated in its atherogenicity and, in particular, may be associated with varying degrees of oxidative susceptibility of LDL, although this remains unclear. We hypothesized that the oxidative susceptibility of small, dense LDL was directly influenced by its lipid composition, which may lead to an increased risk of CAD in subjects with phenotype B. To test this hypothesis, we compared the differences in lipid compositions of LDL particles from subjects with phenotype A and those with phenotype B, and investigated the direct association of lipid composition with susceptibility to lipid peroxidative modification in 102 subjects who underwent a coronary angiographic examination. Subjects with phenotype B (n = 52) had a significantly higher incidence of CAD than subjects with phenotype A (77% *v* 44%; *P* < .005). In comparing the oxidative susceptibility of LDL, the lag time was significantly reduced in subjects with phenotype B compared to phenotype A (48.7 ± 8.6 *v* 41.5 ± 5.5 minutes; *P* < .0001). In addition, the lag time showed a positive correlation with LDL-peak particle diameter (PPD) (*r* = 0.324, *P* < .005). Lipid composition per LDL particle was expressed as the ratio of lipid content to apolipoprotein B (apoB) content (wt/wt). Subjects with phenotype B showed a significant depletion in the contents of free-cholesterol (FC), cholesterol ester (CE), and phospholipid (PL) per particle compared to subjects with phenotype A, although there was no significant difference in the triglyceride (TG) content per LDL particle. Except for TG, the lipid content per LDL particle showed a significant positive correlation with lag time in all subjects. Moreover, increased susceptibility of small, dense LDL to lipid peroxidative modification was most strongly associated with a depleted FC content per LDL particle. In conclusion, the greater risk of CAD in subjects with phenotype B may result, in part, from increased susceptibility to lipid peroxidative modification of LDL that is depleted in lipid contents, especially FC content per LDL particle.

INCREASED LEVELS of plasma low-density lipoprotein (LDL)-cholesterol are associated with a high incidence of coronary artery disease (CAD).[1-3] It is also well known that LDL particles are heterogeneous in size, density, and chemical composition on the basis of their physical properties.[4-6] Individuals with a predominance of large, buoyant LDL particles are identified as phenotype A, while individuals with a predominance of small, dense LDL particles are identified as phenotype B by their mobility on nondenaturing polyacrylamide gradient gels.[7,8] Approximately 15% of the population is reported to have a broad distribution of particles of intermediate size (phenotype I). A large and growing body of epidemiologic evidence shows a consistent association between subjects with phenotype B and the incidence of CAD.[8-11] This may be not independent of the characteristic lipid profile, in particular, high levels of triglycerides (TG) and low levels of high-density lipoprotein (HDL), although the mechanism(s) by which it underlies an increased risk of CAD in subjects with phenotype B remains to be established. Several possible hypotheses for the atherogenicity of small, dense LDL have been postulated, including an increased number of apolipoprotein B (apoB)-containing particles,[12] differences in glycosylation of LDL particles,[13] altered conformation and receptor affinity of

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the apoB molecule,[14] and increased susceptibility to lipid peroxidative modification.[15,16]

Growing evidence exists that lipid peroxidative modification of LDL plays an important role in the development of atherosclerosis.[17] Several clinical studies have demonstrated that LDL obtained from subjects with CAD showed an increased susceptibility to peroxidative modification compared to control subjects.[18-20] An increased risk of CAD in subjects with phenotype B may be explained, in part, by an increased susceptibility to peroxidative modification of small, dense LDL.

It has been considered that variability in the oxidative susceptibility of different LDL subclasses may be explained by compositional differences in the LDL particles, such as endogenous antioxidant concentrations,[21,22] fatty acid compositions, and lipid compositions.[15,23] Since clinical studies demonstrated that decreasing the lipid components of LDL induces an increase in oxidative susceptibility of LDL,[15,16] the lipid composition of LDL might be a key factor in the susceptibility to peroxidative modification. A recent study reported the results of analyzing differences in the lipid composition of LDL particles between phenotypes A and B; however, a direct association of lipid compositional differences and susceptibility to lipid peroxidative modification of LDL particles[24] could not be demonstrated.

Thus, we postulated that oxidative susceptibility of small, dense LDL was directly influenced by its lipid composition, which may lead to an increased risk of CAD in subjects with phenotype B. To resolve this hypothesis, we compared the differences in lipid compositions of LDL particles between subjects with phenotype A and subjects with phenotype B and investigated the direct association of lipid composition with susceptibility to lipid peroxidative modification in subjects who underwent a coronary angiographic examination.

MATERIALS AND METHODS

Subjects

Subjects in this study were selected from consecutive patients who underwent a coronary angiographic examination between January 1996 and April 1997 to evaluate CAD, including myocardial infarction suffered within the past 3 months, and cardiac function. Subjects who had liver dysfunction, renal dysfunction, or were taking lipid-lowering or antioxidant medication such as vitamin E or probucol were excluded. We did not adopt an exclusion criterion on age and we included subjects taking calcium-channel blockers, β-blockers, or an angiotensin-converting enzyme inhibitor.

Body mass index (BMI) was calculated as body weight in kilograms divided by height in square meters (kg/m^2) at admission. Hypertension was defined as systolic blood pressure greater than 140 mm Hg or diastolic pressure greater than 90 mm Hg or the use of antihypertensive medications. Diabetes mellitus was defined as generating the criteria of a 75-g oral glucose tolerance test or the use of insulin or hypoglycemic medications. Current smoker was defined as smoking more than 10 cigarettes per day without cessation in the previous 2-year period. The resulting sample size was 102 subjects (Table 1).

Table 1. Characteristics Based on LDL Phenotype A or B

	Phenotype A	Phenotype B	*P* Value
No. of subjects	50	52	NS
Age (yr)	57 ± 12	61 ± 9	NS
Sex (M/F)	35/15	44/8	NS
BMI (kg/m^2)	22.1 ± 3.5	24.6 ± 2.8	<.0001
HT (%)	42	60	NS
DM (%)	18	42	<.05
SM (%)	54	79	<.05
CAD (%)	44	77	<.005
TC (mg/dL)	188 ± 32	195 ± 25	NS
TG (mg/dL)	116 ± 52	163 ± 75	<.005
LDL-c (mg/dL)	113 ± 29	121 ± 21	NS
HDL-c (mg/dL)	52 ± 16	41 ± 11	<.0001
apoA-I (mg/dL)	131 ± 24	109 ± 17	<.0001
apoB (mg/dL)	98 ± 18	109 ± 17	<.005
Lag time (min)	48.7 ± 8.6	41.5 ± 5.5	<.0001
PPD (nm)	26.00 ± 0.37	25.02 ± 0.41	Designated

NOTE. Values are mean ± SD.

Abbreviations: BMI, body mass index; HT, hypertension; DM, diabetes mellitus; SM, smoker; CAD, coronary artery disease; TC, total cholesterol; TG, triglycerides; LDL-c, low-density lipoprotein-cholesterol; HDL-c, high-density lipoprotein-cholesterol; apo, apolipoprotein; PPD, peak particle diameter.

Coronary Angiographic Examination

Coronary angiographic examination was performed by the standard technique after an overnight fast. Blood pressure was monitored through the catheter, and standard 12-lead electrocardiograms were recorded continuously with a 6-channel recorder. The grade of organic coronary artery stenosis was judged by 2 independent observers, using a semiquantitative classification system, without any information about the subjects' clinical details. Patients with CAD were defined angiographically as having ≥ 50% stenosis in one major branch of the coronary artery trees.

Blood Sampling and Lipoprotein Preparation

Whole blood samples for measuring the plasma lipid profile and peak particle diameter (PPD) of LDL were obtained by venipuncture with vacutainer tubes after an overnight fast. LDL-cholesterol was calculated by Friedewald's formula.[25]

To prepare the LDL fraction, arterial blood samples were collected into test tubes containing 0.04*N* ethylenediaminetetraacetic acid (EDTA) disodium salt via a catheter sheath at the time of coronary angiographic examination. Plasma was isolated immediately by centrifugation at 3,000 rpm for 10 minutes. Then the LDL fraction (density = 1.019 to 1.063 g/mL) was isolated from plasma by sequential ultracentrifugation according to the procedures described by Havel et al.[26] The density of plasma was adjusted with adequate potassium bromide (KBr) solution followed by ultracentrifugation at 100,000 rpm for 4.5 hours at 15°C (Optima TL Ultracentrifuge, Beckman TL100.4 rotor). To remove KBr and EDTA, the LDL fraction was then dialyzed over 18 hours against 3 changes of a 2,000-mL volume of phosphate-buffered saline (PBS) with pH 7.4 at 4°C in a dark location. After the conclusion of dialysis, the concentration of LDL protein was quantified by the Lowry method[27] and stock samples were stored at 4°C.

Determination of LDL-Peak Particle Diameter

Nondenaturing 2.5% to 16% polyacrylamide gradient-gel electrophoresis was performed on whole plasma as previously described by Krauss.[7] Aliquots of 30-μL plasma samples were applied on ready-made gels (REALGEL PLATE; Biocraft, Tokyo, Japan) in a final concentration of 16% sucrose and 0.2% bromphenol blue. After a 20-minute prerun, electrophoresis was performed at 150 V for an 18-hour period with buffer at the final concentration of 0.09 mol/L tris

aminomethane/0.08 mol/L boric acid/0.0025 mol/L EDTA disodium salt at pH 8.3. Each sample and standard LDL sample (25.5 nm), which was obtained from a healthy young adult male and had already been evaluated for its average size by electric microscope, was stained with 0.04% oil-red O/60% ethanol over 24 hours; protein standards of known diameter such as thyroglobulin, apo-ferritin, and catalase were stained with 0.25% coomasie brilliant blue solution. Judging from the mobility of each sample and standards in each scan on a densitometric image analyzer, the estimated diameter for the major peak was calculated and identified as the LDL-PPD by negotiation between the 2 examiners. One LDL-PPD determination was performed for each subject. Analysis of the pooled plasma samples revealed that the identification of the major LDL peak was highly reproducible with an interassay coefficient of variation of less than 3%. Patients with phenotype B were defined as having a LDL-PPD of less than 25.5 nm, as previously described by Krauss et al.[7] For 8% of the subjects, an intermediate phenotype was observed, and these patients were excluded from the study.

Measurement of Oxidative Susceptibility

To compare the susceptibility to lipid peroxidative modification, we adopted the following method. The formation of conjugated dienes in the LDL fraction containing 100 μg of protein was monitored during incubation at 37°C with 5 μmol/L $CuSO_4$ in a final volume of 1 mL of PBS immediately after the conclusion of dialysis. The absorbance at 234 nm was measured at 5-minute intervals for a 240-minute period in a spectrophotometer (Beckman DU650). To assess oxidative susceptibility, the results were expressed as the lag time (minutes), propagation rate (absorbance units/minute), and maximum diene absorbance at 234 nm as previously described.[28]

Lipid Composition of the LDL Fraction

The concentrations of the following lipid composition parameters of the LDL fraction were analyzed enzymatically: total cholesterol (TC), free cholesterol (FC), TG, and phospholipid (PL). Esterfied cholesterol (CE) was calculated by the equation of (TC − FC)/0.59.[29] Since there is only 1 apoB molecule per LDL particle, the ratio of lipid content to apoB content of LDL (wt/wt) provides the lipid composition of individual particles in a sample of the LDL fraction. Accordingly, to compare the actual LDL particle composition, each value was expressed as the lipid content per protein of LDL fraction (wt/wt), in which protein was measured by the Lowry method and is nearly equal to apoB.

Statistical Analysis

Results are expressed as the mean ± SD. Statistical analyses for comparing phenotype A and B subjects were performed by Student's *t* tests. To compare the proportion of sex difference, current smokers, and the prevalence of hypertension and diabetes, the chi-square test was performed. For correlation analyses of individual parameters of oxidative susceptibility to variation in the constituents of LDL, Pearson's correlation coefficients were generated for all of the study population. Multivariate regression analysis was performed by a linear stepwise-regression method. An *F* value greater than 4 was accepted as indicating independent significance.

RESULTS

Subjects' Characteristics and Plasma Lipid Profile

Clinical characteristics and the plasma lipid profile of subjects with phenotype A and B are summarized in Table 1. There were no statistical differences in age, sex, or the prevalence of hypertension between subjects with phenotype A and subjects with phenotype B. Subjects with phenotype B had a higher mean BMI (24.6 ± 2.8 *v* 22.1 ± 3.5 kg/m^2; $P < .0001$), a greater prevalence of diabetes mellitus (42% *v* 18%; $P < .05$), and a higher habitual status of current smokers (79% *v* 54%; $P < .05$). In the association of small, dense LDL with CAD, our data demonstrated that subjects with phenotype B had a significantly higher prevalence of CAD than subjects with phenotype A (77% *v* 44%; $P < .005$). The prevalence of β-blocker medication use was not significantly different between subjects with phenotype A and those with phenotype B (26.0% *v* 28.8%; not significant [NS]).

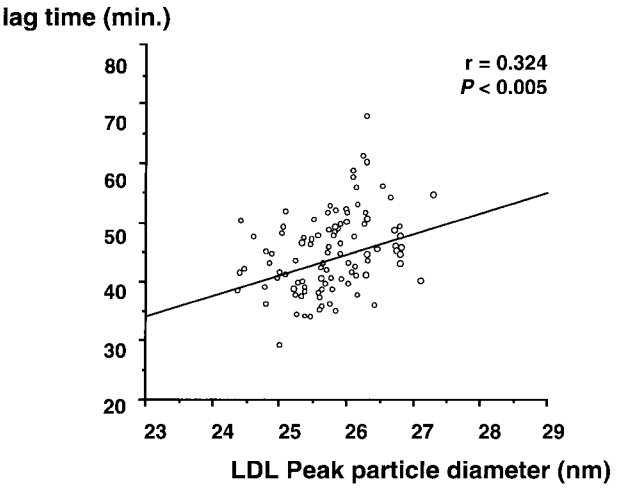


Fig 1. Correlation analysis between lag time and LDL-PPD. Lag time of LDL was estimated by monitoring of copper-induced conjugated diene formation at 234 nm. In the entire study population, lag time demonstrated a significantly positive correlation with LDL-PPD.

In addition, the analysis of plasma lipids demonstrated that subjects with phenotype B had significantly higher levels of TG (163 ± 75 *v* 116 ± 52 mg/dL; $P < .005$) and a significantly greater apoB concentration (109 ± 17 *v* 98 ± 18 mg/dL; $P < .005$), with significantly lower levels of HDL-cholesterol (41 ± 11 *v* 52 ± 16 mg/dL; $P < .0001$) and a significantly lower apoA-I concentration (109 ± 17 *v* 131 ± 24 mg/dL; $P < .0001$). The levels of TC and LDL-cholesterol concentration were not significantly different between subjects with phenotype A or B. LDL-PPD was significantly diminished in subjects with phenotype B compared with subjects with phenotype A (25.02 ± 0.41 *v* 26.00 ± 0.37 nm; $P < .0001$).

Susceptibility to Lipid Peroxidative Modification of LDL

Susceptibility to lipid peroxidative modification of LDL was assessed by copper-induced formation of conjugated dienes. The estimated lag time was significantly reduced in subjects with phenotype B compared to those with phenotype A (41.5 ± 5.5 *v* 48.7 ± 8.6 min; $P < .0001$) (Table 1). The LDL-PPD showed a significant positive correlation with lag time ($r = 0.324$, $P < .005$) (Fig 1). In contrast, there were no statistical differences in either the propagation rate (0.05 ± 0.02 *v* 0.04 ± 0.03 absorbance units/min) or the maximum diene absorbance (1.69 ± 0.50 *v* 1.59 ± 0.47) between the 2 phenotypes. We did

Table 2. Concentrations and Lipid Composition of LDL Fraction

	FC	CE	TG	PL	apoB
Concentration (mg/dL)					
Phenotype A	33 ± 8	143 ± 36	18 ± 6	77 ± 18	86 ± 19
Phenotype B	35 ± 7	155 ± 28	23 ± 8	83 ± 14	101 ± 21
P value	NS	NS	< .005	NS	< .005
Lipid composition (%)					
Phenotype A	12.4 ± 0.7	52.5 ± 4.7	6.8 ± 2.0	28.3 ± 0.7	
Phenotype B	11.7 ± 1.2	52.3 ± 2.6	8.0 ± 2.4	27.9 ± 1.1	
P value	< .005	NS	< .01	NS	

NOTE. Values are mean ± SD.

Abbreviations: LDL, low-density lipoprotein; FC, free cholesterol; CE, cholesterol ester; TG, triglyceride; PL, phospholipid; apoB, apolipoprotein B.

not observe any effect of β-blocker medication on particle size or susceptibility to peroxidative modification of LDL.

Lipid Composition of LDL

A comparison of the lipid composition of the LDL fraction (density = 1.019 to 1.063 g/mL) in both phenotypes (Table 2), uncontrolled for the number of LDL particles, demonstrated significantly greater concentrations of apoB (101 ± 21 *v* 86 ± 19 mg/dL; $P < .0005$) and TG (23 ± 8 *v* 18 ± 6 mg/dL; $P < .0005$) in subjects with phenotype B. The concentrations of FC (35 ± 7 *v* 33 ± 8 mg/dL; P = NS), CE (155 ± 28 *v* 143 ± 36 mg/dL; P = NS), and PL (83 ± 14 *v* 77 ± 18 mg/dL; P = NS) in the LDL fraction were not different between the 2 phenotypes.

Analysis of the fractional lipid composition of the LDL fraction revealed different trends. Percent TG (8.0% ± 2.4% *v* 6.8% ± 2.0%; $P < .01$) was significantly greater in the LDL fraction obtained from subjects with phenotype B. Although percent FC (11.7% ± 1.2% *v* 12.4% ± 0.7%; $P < .0001$) remained significantly lower, there were no differences in percent CE (52.3% ± 2.6% *v* 52.5% ± 4.7%; P = NS) and percent PL (27.9% ± 1.1% *v* 28.3% ± 0.7%; P = NS) in the LDL fraction between phenotype B and A.

Comparing the lipid composition per LDL particle, controlled for the number of particles by using the ratio of lipid components to the apoB content, revealed that subjects with phenotype B had a significantly depleted content of FC per particle (FC/apoB: 0.35 ± 0.06 *v* 0.43 ± 0.07; $P < .0001$), CE per particle (CE/apoB: 1.56 ± 0.23 *v* 1.77 ± 0.23; $P < .0001$), and PL per particle (PL/apoB: 0.83 ± 0.12 *v* 0.95 ± 0.12; $P < .0001$) compared with subjects with phenotype A (Fig 2). Although the subjects with phenotype B had significantly higher levels of TG concentration in the plasma and LDL fraction, there was no difference in the content of TG per LDL particle between phenotype B and A (TG/apoB: 0.23 ± 0.07 *v* 0.24 ± 0.07; P = NS).

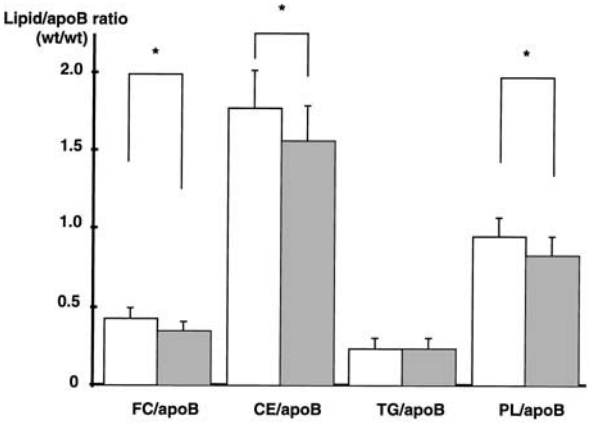
Furthermore, the estimated lag time was significantly positively correlated to FC ($r = 0.413$, $P < .0001$), CE ($r = 0.350$, $P < .005$) and PL ($r = 0.399$, $P < .0001$) content per particle in all of the subjects (Fig 3). However, there was no significant correlation between the lag time and TG content per LDL particle (Table 3).

Interrelation Between Lag Time and Other Parameters

Univariate correlation analysis was performed with lag time and concomitant factors such as age, BMI, plasma lipid components, and lipid composition per LDL particle (Table 3). Lag time showed a significantly positive correlation with HDL-cholesterol level ($r = 0.252$, $P < .005$) and a significantly negative correlation with BMI ($r = -0.202$, $P < .005$). In a stepwise-regression analysis adjusted for these confounding factors, only FC content per LDL particle demonstrated a significant correlation.

DISCUSSION

In this study, we demonstrated that subjects with phenotype B had a significantly higher incidence of CAD and higher susceptibility of LDL particles to lipid peroxidative modifica-



	FC/apoB	CE/apoB	TG/apoB	PL/apoB
typeA	0.46±0.07	1.77±0.23	0.24±0.07	0.95±0.12
typeB	0.35±0.06	1.56±0.23	0.23±0.07	0.83±0.12

* $p < 0.0001$

Fig 2. Lipid composition per LDL particle. Comparison of lipid composition per LDL particle, by using the ratio of lipid components to the apoB content per LDL particle. Subjects with phenotype B (■) had a significantly depleted content of FC, CE, and PL per particle compared with subjects with phenotype A (□). There was no significant difference in the TG content per LDL particle between phenotypes A and B.

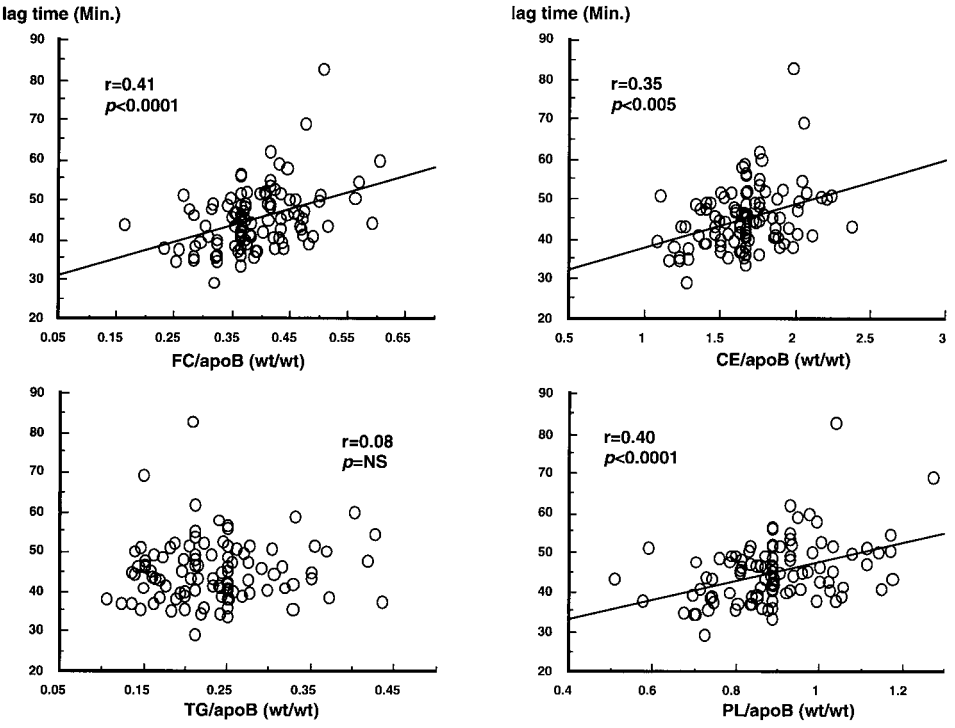


Fig 3. Correlation analysis between lipid composition per LDL particle and lag time. The lag time had a significantly positive correlation with FC content per particle, CE content per particle, and PL content per particle. However, there was no significant correlation between lag time and TG content per LDL particle.

tion compared to subjects with phenotype A. Further analysis of lipid compositional differences of LDL particles resulted in a significantly depleted content of FC, CE, and PL per LDL particle in phenotype B compared with phenotype A. Moreover, LDL-PPD was positively correlated to lag time and lipid contents, except for TG per particle and especially to the content of FC, in all subjects.

Many clinical studies have demonstrated that small, dense LDL is produced in the atherogenic lipoprotein metabolism and is associated with CAD.[5,8-11] This metabolism could also produce a characteristic plasma lipid profile such as higher levels of TG and lower levels of HDL-cholesterol without increasing the levels of LDL-cholesterol, as shown by our results.[10,11,30-32] It has also been considered to be associated with insulin resistance.[33-35] Although the atherogenicity of small, dense LDL itself has been widely accepted,[12-14] its precise mechanism has not yet been fully elucidated. One possible explanation has been the high susceptibility of small, dense LDL to lipid peroxidation.[17-20] Our results, which showed that subjects with phenotype B had a significantly higher incidence of CAD and a reduced lag time of LDL compared to those with phenotype A, supported this proposed atherogenic effect. In addition, concerning the size of the LDL particle, there was a positive correlation between LDL-PPD and the lag time of LDL in all of our subjects.

Table 3. Linear Regression Analysis for Lag Time

	Univariate	Multivariate	*F* Statistic
Age	−0.166	—	0.59
BMI	−0.202*	—	1.20
Plasma lipid			
TC	0.037	—	0.90
TG	−0.105	—	0.64
LDL	−0.057	—	0.03
HDL	0.252*	—	2.25
LDL-PPD	0.324‡	—	2.38
Lipid composition			
FC/apoB	0.413‡	0.413	20.51
CE/apoB	0.350†	—	0.41
TG/apoB	0.079	—	0.10
PL/apoB	0.399‡	—	1.29

*$P < .05$, †$P < .005$, ‡$P < .0001$ by Pearson's correlation coefficients.

Abbreviations: BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apo, apolipoprotein; FC, free cholesterol; CE, cholesterol ester; PL, phospholipid.

Subjects with phenotype B had a significantly higher plasma TG concentration, consistent with previous studies. This has been considered to result from very-low-density lipoprotein (VLDL) enriched in TG produced in the liver and hydrolysis of TG in lipoproteins reduced by low activity of lipoprotein lipase (LPL). Furthermore, our data demonstrated significantly greater concentrations of TG in the LDL fraction (density = 1.019 to 1.063 g/mL) in subjects with phenotype B, which may partially contribute to the higher plasma TG concentration. Although the concentrations of FC, CE, and PL in the LDL fraction were not different between the 2 phenotypes, subjects with phenotype B had higher concentrations of apoB in the LDL fraction, of which 1 molecule is contained in 1 LDL particle. This indicates an increased number of LDL particles in subjects with phenotype B, which is compatible with the hyperapoBnemia reported in previous studies. Therefore, an analysis comparing the lipid composition per LDL particle between the

2 phenotypes was performed using the ratio of lipid components to apoB content to control for particle number. As a result, LDL particles obtained from subjects with phenotype B demonstrated significantly depleted contents of FC, CE, and PL per LDL particle compared to subjects with phenotype A. In contrast, TG content per LDL particle did not differ between the 2 phenotypes. In addition, LDL-PPD was positively correlated to its lipid components of FC, CE, and PL, but not TG, per LDL particle (data not shown). Capell et al found similar results in LDL from healthy subjects.[24] Taken together, these results suggest that LDL particles of both phenotypes may be hydrolyzed to the same degree and support the hypothesis that TG enrichment of large VLDL depletes the FC, CE, and PL content per particle, and may lead to the formation of small, dense LDL.

Our findings indicate that the susceptibility of LDL to lipid peroxidative modification is most strongly associated with the FC content per particle. Thus, the increased risk of CAD in subjects with phenotype B may result in part from the higher susceptibility of LDL depleted in FC content per particle to lipid peroxidative modification. Mechanisms for such an effect have been suggested by studies in biomembranes and lipid bilayers showing that cholesterol incorporation affects metabolite permeability. Reduced accessibility of oxidants to the lipid core with increased amounts of FC may be a plausible explanation for a protective role in the oxidative susceptibility of the LDL particle.[36-38] In addition, decreased phospholipase A2 activity in LDL subfractions enriched in FC content could contribute to reduced oxidative susceptibility.[39] These reports indicate that an increased FC content in the LDL particle might directly regulate susceptibility to oxidative stress and contribute to preventing the LDL particle from undergoing subsequent oxidative modification. Thus, small, dense LDL with a depleted FC content per particle may have an increased susceptibility to lipid peroxidation. The other mechanism invovlved in this process is considered to be a reduction in the content of lipid-soluble antioxidants such as vitamin E in small LDL particles, as might be expected on the basis of their depleted lipid content,[16] although this was not measured in the current study.

Since the sample size in this study was small, we could not fully evaluate the advantages of subjects without CAD among the group with phenotype B. However, we found significant differences only in age (56 ± 8 *v* 62 ± 9 years, without CAD *v* with CAD; $P < .05$) and prevalence of hypertension (62% *v* 87%, without CAD *v* with CAD; $P < .05$). There was no significant difference in any index of susceptibility to lipid peroxidative modification. Further investigation of the advantages of the subjects without CAD over the subjects with CAD among those with phenotype B is required.

In conclusion, we found that an increased risk of CAD in subjects with phenotype B may result in part from an increased susceptibility to lipid peroxidative modification of LDL that has been depleted in lipid contents, especially FC content per particle. These results suggest that the differences in lipid composition of LDL particles between phenotype A and phenotype B may determine the physical properties of the LDL particles and may contribute to the higher incidence of CAD in subjects with phenotype B. Furthermore, the hypothesis that a high susceptibility of small, dense LDL to lipid peroxidative modification is directly influenced by the FC content of LDL particle remains to be investigated.

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