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Significance of Small Dense Low-Density Lipoprotein as a Risk Factor for Coronary Artery Disease and Acute Coronary Syndrome

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Small dense LDL (sd-LDL) has recently emerged as an important coronary artery disease (CAD) risk factor. This study was performed to investigate how LDL particle size is related to CAD and acute coronary syndrome (ACS). Blood samples were collected from 504 patients that underwent coronary angiography to evaluate chest pain. The LDL particle size of these samples was measured. The mean LDL particle size was smaller in patients with angiographically proven CAD than in the controls (26.41 ± 0.95 vs 26.73 ± 0.64 nm, $p < 0.001$), and was negatively correlated with the Framingham risk score ($r = -0.121$, $p = 0.007$). Patients with more extensive CAD had smaller LDL particles. LDL particle size was also smaller in patients with acute coronary syndrome as compared to non-ACS patients (26.09 ± 1.42 vs 26.54 ± 0.63 nm, $p = 0.011$). These results suggest that sd-LDL is independently associated with the incidence and extent of CAD, and can be a risk factor for the development of ACS in the Korean population.

Key Words: Small dense LDL, coronary artery disease

INTRODUCTION

The importance of low-density lipoprotein (LDL) cholesterol in the development of atherosclerosis has long been recognized. Thus it is logical that LDL cholesterol remains the primary therapeutic target for coronary artery disease (CAD) prevention. Nevertheless, an increasing amount of research over the past decade has been devoted to the heterogeneity of LDL particles and the atherogenicity of lipids and lipoproteins other than LDL. LDL heterogeneity, along with dietary and genetic influences, is now well recognized as an indicator of differences in lipoprotein composition, size, and metabolism.[1-3] For these reasons, small, dense low-density lipoprotein (sd-LDL) is viewed as an important CAD risk factor.[1,4,5]

There are several proposed biochemical and cellular mechanisms related to the sd-LDL atherogenicity. For example, sd-LDL may reside in the plasma longer,[6-10] not bind the LDL receptor as well, bind the scavenger receptor more avidly,[11-14] be more susceptible to oxidation,[15-17] have fewer antioxidants in its core,[18,19] enter the arterial wall more easily,[20,21] and bind to the glycosaminoglycans in the arterial wall more readily.[22,23] The cellular mechanisms include: an sd-LDL promotion of endothelial cell dysfunction,[24] induction of greater PAI-1 (plasminogen activator inhibitor-1) production in endothelial cells,[25] an increase in thromboxane secretion in endothelial cells,[26] and an increase in arterial smooth muscle intracellular calcium.[27]

Several large prospective studies have examined the relationship between sd-LDL and CAD using gradient gel electrophoresis to determine the peak particle size. These found the odds ratio of CAD to increase significantly when sd-LDL was the predominant LDL subclass present.[28-30] Evidence from several angiographic clinical trials indicated that successful treatment correlated to a

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decrease in the number of sd-LDL particles.[31-35]

Data regarding the relationship of LDL particle size to the coronary artery disease incidence are limited in the Korean population. Moreover, data on the relationship between LDL particle size and the extent of coronary artery disease or acute coronary syndrome (ACS) are limited worldwide. Therefore, this study was performed to investigate the relationship between LDL particle size and the extent of CAD or acute coronary syndrome. In addition, this study investigated the relationship between LDL particle size and the Global risk assessment score (GRAS) by using Framingham risk score to determine whether sd-LDL can be used as a cardiovascular event predictor.

MATERIALS AND METHODS

Subjects

This study enrolled 504 patients that underwent coronary angiography at Yongdong Severance Hospital, Yonsei University between October 2003 and June 2004. Any patients that had previously undergone coronary angiography, had a history of myocardial infarction, suffered chronic renal failure, were at endstage renal disease, suffered hepatic failure, liver cirrhosis, an infectious disease or had a malignancy were excluded from this study. In addition, data derived from repeated coronary angiography from the same patient were excluded.

Patients were considered hypertensive if they had a known history of hypertension, systolic blood pressure over 140 mmHg and/or diastolic blood pressure over 90 mmHg. Patients were considered diabetic if they had a fasting serum glucose over 126 mg/dL or if they were being treated with oral hypoglycemic agents or insulin. The height and weight of all subjects were recorded and a body mass index (BMI) was calculated with the formula: weight(kg) / height^2 (m^2).[9]

The control group included men and women showing normal or minimal CAD by coronary angiogram. The CAD group was divided into an ACS group and a non-ACS group. Diagnoses of myocardial infarction and angina pectoris were made based on: clinical symptoms, EKG changes and/or biochemical markers. CAD was defined as stenosis of one or more coronary artery branch with 50% of the diameter or more luminal narrowing as seen by coronary angiography.[9]

Estimation of the extent of CAD

The CAD extent was described by Gensini scores.[36] The Gensini score is a measure of the extent of myocardial ischemia. These were computed for each coronary artery stenosis, based on the degree of luminal narrowing and the geographic importance of the stenosis.

Global risk assessment scoring

A 10-year risk of major coronary events was calculated using the Framingham scoring system, based on Framingham Heart Study.[37,38]

Lipoprotein and metabolic parameter analysis

Fasting blood samples were obtained by venipuncture on the day of the coronary angiography prior to cardiac catheterization. Total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol were measured by the direct enzymatic method. The LDL subfraction was analyzed by polyacrylamide tube gel electrophoresis (Quantimetrix Lipoprint^{TM} LDL system, Redondo Beach, CA, USA).[39] It was then categorized as either pattern A or B based on the mean LDL particle size. The sd-LDL (subtypes 3-7) percentage of total LDL was measured.

$$\text{sd-LDL (\%)} = \frac{\text{LDL3} + \text{LDL4} + \text{LDL5} + \text{LDL6} + \text{LDL7}}{\text{LDL1} + \text{LDL2} + \text{LDL3} + \text{LDL4} + \text{LDL5} + \text{LDL6} + \text{LDL7}} \times 100 \ (\%)$$

LDL subtypes 1-2 were predominantly large, buoyant LDLs, whereas subtypes 3-7 were predominantly small, dense LDLs. The mean LDL particle size for 'Pattern A' was greater than 26.5 nm, hence named 'large, buoyant LDL dominant', while the mean value of particle size for 'Pattern B' was less than 26.5 nm, thus named 'small, dense LDL dominant'.

Statistical analysis

Statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc., IL, USA). Compari-

sons between the control and CAD groups were performed using a Student's t-test. All values are described as the mean ± standard deviation. Statistical significance was defined as $p < 0.05$.

The extent of CAD was evaluated by reviewing the coronary angiogram and was measured by the Gensini score. The extent of CAD, ACS and mean LDL particle size were investigated by multivariate analysis.

RESULTS

Comparison between the CAD patients and the controls

The demographic and metabolic characteristics of all patients are shown in Table 1. No difference was seen between CAD and control groups in BMI, total cholesterol, or triglyceride levels. A significant difference between the two groups was seen in age, hypertension, incidence of diabetes mellitus, LDL cholesterol, HDL cholesterol, mean LDL size, and sd-LDL fraction. The patients with angiographically proven CAD had a smaller mean LDL particle size than the control group (26.41 ± 0.95 vs 26.73 ± 0.64 nm, $p < 0.001$) (Table 2).

Multivariate analysis of risk factors for CAD

A multiple logistic regression analysis revealed small, dense LDL fraction to be an independent risk factor for CAD (odds ratio [OR] 2.312, 95% CI

Table 1. Comparison of the Baseline Demographic and Metabolic Characteristics between CAD and Control Groups

	Control (n = 242)	CAD (n = 262)	*p* value
Age (yr)	57.4 ± 11.6	63.1 ± 11.0	< 0.001
BMI (kg/m^2)	25.1 ± 3.5	24.4 ± 3.3	0.037
Hypertension (%)	40.9	57.3	< 0.001
Current smoker (%)	27.3	39.4	0.005
DM (%)	11.6	32.4	< 0.001
hs-CRP (mg/dL)	7.6 ± 22.7	10.5 ± 28.5	NS
T. chol (mg/dL)	172.9 ± 34.1	178.5 ± 38.3	NS
TG (mg/dL)	132.0 ± 73.6	144.5 ± 76.3	NS
HDL chol (mg/dL)	44.9 ± 11.9	41.1 ± 10.1	< 0.001
LDL chol (mg/dL)	102.1 ± 29.4	109.2 ± 35.7	0.015
Framingham score	11.0 ± 4.9	13.9 ± 3.1	< 0.001

Data are expressed as mean ± SD.
Control, normal control group; CAD, coronary artery disease group; DM, diabetes mellitus; BMI, body mass index; hs-CRP, high-sensitivity c-reactive protein; T. chol, total cholesterol; TG, triglyceride; HDL chol, high-density lipoprotein; LDL chol, low-density lipoprotein; NS, not significant.

Table 2. Comparison of the LDL Cholesterol Characteristics between the CAD and Control Groups

	Control (n = 242)	CAD (n = 262)	*p* value
Mean LDL size (nm)	26.73 ± 0.64	26.41 ± 0.95	< 0.001
LDL class (A/B) (%)	74.4 / 25.6	51.1 / 48.9	< 0.001
Fraction % of sd-LDL	12.2 ± 13.9	18.2 ± 18.0	< 0.001

Data are expressed as mean ± SD or percentage of total LDL.
Control, normal control group; CAD, coronary artery disease group; LDL, low-density lipoprotein; A, pattern A; B, pattern B; sd-LDL, small dense LDL.

Table 3. Multiple Logistic Regression Analysis for CAD

	OR	95% CI	*p* value
Age (yr)	3.763	2.085 - 6.791	< 0.001
Obesity	0.811	0.537 - 1.224	NS
Smoking	1.835	1.186 - 2.838	0.006
Hypertension	1.521	1.009 - 2.293	0.045
Diabetes Mellitus	3.291	1.957 - 5.537	< 0.001
Low HDL chol	1.208	0.714 - 2.044	NS
High LDL chol	2.220	0.754 - 6.538	NS
sd-LDL (Pattern B)	2.312	1.512 - 3.537	< 0.001

OR, odds ratio; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; chol, cholesterol; sd-LDL, small dense LDL; NS, not significant.

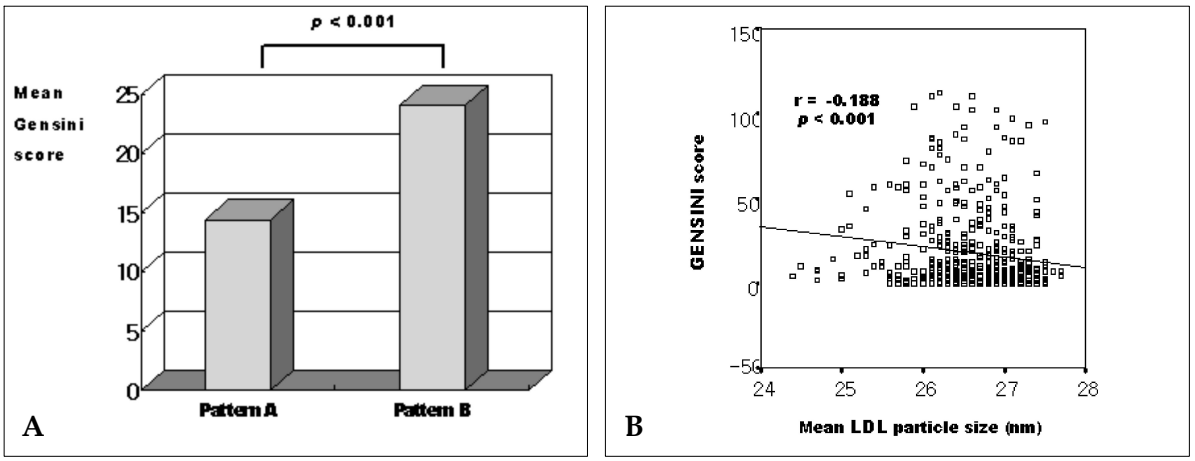

Fig. 1. (A) Comparison of pattern A and B mean Gensini scores. Mean Gensini scores for patterns A and B were significantly different (14.4 ± 22.9 vs 24.1 ± 28.9, $p < 0.001$). Pattern A represents a predominance of large, buoyant LDLs (mean particle size greater than 26.5 nm). Pattern B represents a predominance of small, dense LDLs (mean particle size smaller than 26.5 nm). (B) Correlation between mean LDL particle size and Gensini score. The mean LDL particle size had a significant negative correlation with the Gensini score (r = -0.188, $p < 0.001$). r, correlation coefficient.

1.512-3.537, $p < 0.001$) (Table 3).

Correlation between CAD severity and the mean LDL particle size

There was a significant difference in the mean Gensini scores between patients with pattern A and B LDL (14.4 ± 22.9 vs 24.1 ± 28.9, $p < 0.001$) (Fig. 1A). A univariate linear analysis between mean LDL particle size and the Gensini score showed a significant negative correlation (Correlation coefficient = -0.188, $p < 0.001$) (Fig. 1B).

Correlation between the mean LDL particle size and Framingham risk score

The mean LDL particle size showed a significant negative correlation with Framingham risk score (correlation coefficient = -0.121, $p = 0.007$) (Fig. 2).

Analysis between ACS and non-ACS CAD patients

The demographic and metabolic characteristics of the ACS and non-ACS groups are shown in

Table 4. There was no significant difference seen between ACS patients and non-ACS patients in age, diabetes mellitus, BMI, total cholesterol, LDL cholesterol, HDL cholesterol or triglyceride levels. A significant difference between the groups was noted in smoking, hypertension, mean LDL particle size, and sd-LDL fraction. The mean LDL particle size was smaller in the ACS group than the non-ACS group (26.09 ± 1.42 vs 26.54 ± 0.63 nm, p = 0.011) (Table 5).

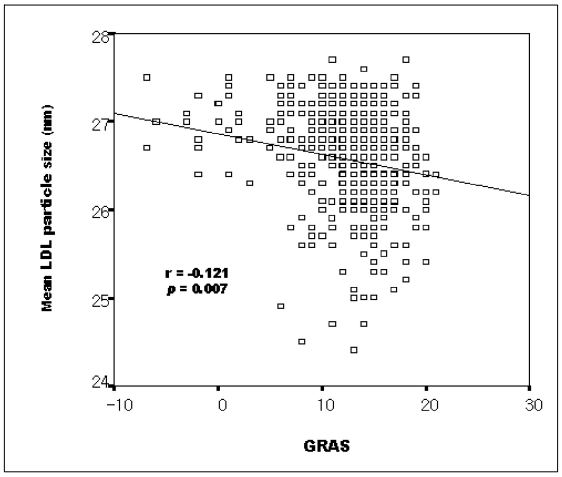

Fig. 2. Correlation between mean LDL particle size and Framingham risk score. The mean LDL particle size had a negative correlation with GRAS (r = -0.121, p = 0.007). GRAS (global risk assessment score) was obtained by using the Framingham risk scoring method; LDL, low-density lipoprotein; r, correlation coefficient.

Multivariate analysis of risk factors for ACS

A multiple logistic regression analysis showed that small dense LDL is not an independent risk factor for ACS (odds ratio [OR] 1.394, 95% CI, 0.765-2.540, p = NS) (Table 6).

DISCUSSION

Gradient gel electrophoresis under native conditions is commonly used to characterize LDL particle size distribution.[40] Densitometric LDL subfraction scans show a bimodal distribution. Pattern A LDL is characterized by a predominance of large, buoyant LDL particles with a major LDL diameter peak greater than 25.5 nm. Pattern B LDL is characterized by a predominance of sd-LDL particles with a major peak less than 25.5 nm.[41-43] sd-LDL is often accompanied by triglyceride, increased apo B and decreased high-density lipoprotein (HDL) levels. These dyslipoproteinemia are somewhat correlated to increased risk of CAD

Table 4. Comparison of the Demographic and Metabolic Characteristics between the ACS and Non-ACS Groups

	Non-ACS (n = 188)	ACS (n = 74)	p value
Age (yr)	63.6 ± 10.06	61.8 ± 13.2	NS
BMI (kg/m^2)	24.6 ± 3.4	23.9 ± 3.1	NS
Hypertension (%)	62.2	44.6	0.012
Current smoker (%)	35.6	49.3	0.048
DM (%)	33.0	31.1	NS
hs-CRP (mg/dL)	7.2 ± 23.7	18.4 ± 36.7	0.019
T. chol (mg/dL)	178.9 ± 36.5	177.4 ± 42.5	NS
TG (mg/dL)	145.1 ± 74.8	142.9 ± 80.3	NS
HDL chol (mg/dL)	41.1 ± 10.0	41.0 ± 10.4	NS
LDL chol (mg/dL)	109.2 ± 33.5	109.4 ± 40.9	NS
Framingham score	14.0 ± 2.8	13.6 ± 3.6	NS

Data are expressed as mean ± SD.

ACS, acute coronary syndrome; DM, diabetes mellitus; BMI, body mass index; hs-CRP, high-sensitivity c-reactive protein; T. chol, total cholesterol; TG, triglyceride; HDL chol, high-density lipoprotein; LDL chol, low-density lipoprotein;A, pattern A; B, pattern B; NS, not significant.

Table 5. Comparison of the Characteristics of LDL Cholesterol between the ACS and Non-ACS Groups

	Non-ACS (n = 188)	ACS (n = 74)	*p* value
Mean LDL size (nm)	26.54 ± 0.63	26.09 ± 1.42	0.011
LDL class (A/B) (%)	53.2 / 46.8	45.9 / 54.1	NS
Fraction % of sd-LDL	16.5 ± 15.0	22.9 ± 23.6	0.034

Data are expressed as mean ± SD or percentage of total LDL.
ACS, acute coronary syndrome; LDL, low-density lipoprotein; A, pattern A; B, pattern B; sd-LDL, small dense LDL; NS, not significant.

Table 6. Multiple Logistic Regression Analysis for ACS

	OR	95% CI	*p* value
Age	0.961	0.336 - 2.746	NS
Obesity	0.464	0.248 - 0.867	0.016
Smoking	2.135	1.163 - 3.922	0.014
Hypertension	0.600	0.334 - 1.077	NS
Diabetes Mellitus	0.891	0.477 - 1.665	NS
hs-CRP	1.010	1.000 - 1.021	NS
Low HDL chol	0.980	0.498 - 1.930	NS
High LDL chol	1.026	0.299 - 3.519	NS
sd-LDL (Pattern B)	1.394	0.765 - 2.540	NS

OR, odds ratio; CI, confidence interval; hs-CRP, high-sensitivity c-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; chol, cholesterol; sd-LDL, small dense LDL; NS, not significant.

development. It is not clear, however, whether sd-LDL's influence on CAD development is dependent on any other factors, including changes in lipoproteins and lipid parameters.

Our results indicate that the sd-LDL fraction of total LDL is significantly associated with CAD. This association is seen in both males and females. Even after adjustment for traditional risk factors, such as: age, obesity, smoking, diabetes mellitus, HDL cholesterol level, and LDL cholesterol level, our multiple logistic regression analysis still showed a significant correlation between sd-LDL and CAD. These findings suggest that sd-LDL can be viewed as an independent risk factor for CAD development apart from the traditional risk factors.

By using the Gensini score, the present study was able to investigate the correlation between mean LDL particle size and the extent of CAD. Several reports have suggested that sd-LDL may be an independent CAD risk factor and might contribute to CAD severity. These reports simply used the number of diseased coronary arteries as a measure of CAD severity.[5,44] One study reported the sd-LDL prevalence to be strongly associated with various CAD types. This study also found sd-LDL to be independent of traditional and nontraditional coronary risk factors. The study did not show, however, whether sd-LDL was related to the severity and extent of coronary artery lesions as indicated by Gensini scores.[43] The present study used the Gensini score as a measure of extent of CAD. Therefore, the present study is the first study to provide evidence of a significant correlation between mean LDL particle size and extent of CAD, as depicted by Gensini scores. Gensini scores should provide a more objective parameter than the number of diseased coronary arteries.

Many studies have analyzed the relationship

between triglyceride, LDL cholesterol, LDL particle size and CAD prevalence. Among these, acute myocardial infarction has been shown to have a strong negative correlation with high triglyceride concentrations and LDL particle size.[45-48] Several reports have suggested a negative correlation between LDL particle size and risk of acute myocardial infarction.[29,46] Furthermore, a negative association between LDL particle size and CAD development in general has also been reported.[49,50] When data were adjusted for triglyceride levels, these reports failed to prove that LDL particle size is an independent CAD risk factor. Meanwhile, other studies have demonstrated that the association between CAD and sd-LDL is independent of triglyceride level.[44,51] Our study revealed that LDL particle size is significantly correlated with total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol. After running the Student's t-test, however, no significant difference was seen in triglyceride level between the CAD and control groups. This is probably due to the relatively large variations in triglyceride level.

We also studied the correlation between mean LDL particle size and global risk assessment score (GRAS). There was a significant negative correlation between these two parameters. This result suggests that further studies are required to determine whether sd-LDL is a reliable 10-year predictor of coronary event risk.

sd-LDL is associated with increased triglyceride and decreased HDL levels.[52] The LDL size and density are partly affected by the exchange of triglycerides at the expense of cholesteryl esters from LDL, possibly mediated by cholesteryl ester transfer protein. This process causes LDL to become enriched in triglycerides at the expense of cholesteryl esters. Excess triglycerides in the LDL particle allows continued size reduction by hepatic triglyceride lipase, which may result in lipid-poor, thus, protein-rich LDL particles of relatively high density.[53] In addition to the addition of triglycerides, it has been suggested that genetic factors and increased hepatic lipase and lipid transfer activities also contribute to LDL heterogenicity. A carbohydrate-rich diet is also known to be associated with an increased sd-LDL level. Increased carbohydrate intake causes free fatty acid synthesis in the liver, which can, potentially, stimulate large triglyceride-rich VLDL production.[54]

LDL particle size seems to be more stable, and less influenced by meals than triglyceride levels, although fasting samples are needed for more definitive results. Therefore, LDL particle size is better than triglyceride levels at predicting the development of coronary artery disease. The problem is that the LDL subfraction is more difficult to measure than triglyceride levels. Conventional methods for measuring LDL subfractions, such as density gradient ultracentrifugation, native gradient gel electrophoresis, and nuclear magnetic resonance spectroscopy are not suitable for a clinical laboratory setting because they are labor intensive, require skillful and experienced technicians, are poorly reproducible and take a significant amount of time to analyze. The recently developed Quantimetrix Lipoprint™ LDL system uses polyacrylamide tube gel electrophoresis and provides more benefits than previous methods. Using this method, LDL subfractions can be easily analyzed in a short time.[55]

This study also investigated the correlation between sd-LDL and ACS. The CAD group was divided into an ACS and non-ACS group. The mean LDL particle size was smaller in the ACS group than the non-ACS group. The sd-LDL, as a percentage of total LDL, was higher in the ACS group than the non-ACS group. The multivariate analysis did not support the hypothesis that LDL particle size is an independent risk factor for ACS development. When we compared the lipid profiles of the ACS and non-ACS groups using a Student's t-test, we found no significant difference. The fact that the blood samples were collected on the day of coronary angiography might have affected LDL or HDL values. Several studies have reported a significant decrease in lipid profiles during the acute phase of acute coronary events.[56-59]

Lipoprotein (a) (Lp (a)) and oxidized LDL are modified forms of LDL that may also play important roles in the CAD development. Lp (a) accumulates in atherosclerotic lesions, accelerates smooth muscle proliferation and downregulates glucocorticoid receptors. Lp (a) levels are elevated in CAD and contributes to restenosis after angio-

plasty.[60] The relationship between LDL particle size and Lp (a) have not yet been fully explored. Malondialdehyde-modified LDL (MDA-LDL), an oxidized LDL candidate, could be a useful CAD indicator. MDA-LDL levels are, reportedly, significantly associated with LDL particle size. It has been suggested that circulating MDA-LDL plays an important role in atherosclerosis pathogenesis and might become a new therapeutic target for CAD prevention.[61] Further studies are needed to identify the relationship between MDA-LDL and CAD and ACS development.

Although a recent study reported that an LDL size increase was seen after intensive lipid-lowering therapy, and its decrease was strongly associated with CAD progression.[31] Further studies are needed to determine if there is a correlation between LDL particle size and the progression or regression of CAD and ACS. Further studies need to be done on patients after intensive lipid-lowering therapy to determine whether LDL particle size or cholesterol level is more important in CAD and ACS development. Furthermore, it would be interesting to examine CAD patients after percutaneous coronary intervention and explore the relationship between LDL particle size and restenosis.

In summary, LDL particle size was smaller among CAD patients, and correlated with the extent of CAD and ACS. The present study demonstrates that sd-LDL levels are strongly associated with CAD, are independent of both traditional and nontraditional coronary risk factors and are related to the extent of coronary lesions. Furthermore, sd-LDL plays an important role not only in the onset of CAD, but also in the progression of the disease.

In conclusion, sd-LDL is independently associated with the incidence and extent of CAD, and may be a risk factor for CAD and ACS development in the Korean population.

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