Angiology
64(3) 216-222

DOI: 10.1177/0003319712441855
ang.sagepub.com
SAGE

Changes in Small Dense Low-Density Lipoprotein Levels Following Acute Coronary Syndrome

Mahdi Reza Emadzadeh, MD[1,2], Maryam-Sadat Alavi, MD[1], Mohammad Soukhtanloo, PhD[1], Toktam Mohammadpour, MS[1], Amir Ali Rahsepar, MD[1,3], Shima Tavallaie, MSc[1,3], Roshanak Khojasteh, BSc[3], Roghayeh Paydar, BSc[1], Maral Amini, BSc[1], Seyyed Mohammad Reza Parizadeh, PhD[3], Saeed Akhlaghi, MSc[3], Majid Ghayour-Mobarhan, MD, PhD[3], and Gordon A. Ferns, MD, DSc, FRCP[4]

Abstract

Low-density lipoprotein (LDL), especially small dense LDL (sdLDL), plays a role in atherogenesis. We compared baseline sdLDL levels between healthy controls and patients with acute coronary syndrome (ACS). Blood samples were taken from patients diagnosed with myocardial infarction ([MI] n = 104) and unstable angina ([UA] n = 100). Both sdLDL and high-sensitivity C-reactive protein (hsCRP) levels were determined on admission and in the next 24 hours after the onset of symptoms. Baseline concentration of sdLDL was significantly higher in patients presenting with ACS than controls ($P < .05$). In the 24 hours following ACS, the sdLDL levels decreased insignificantly in both groups of patients with ACS ($P > .05$). The changes in sdLDL values were not significantly different between MI and UA participants ($P > .05$). Patients with ACS have higher concentration of sdLDL compared with the controls.

Keywords

small dense low-density lipoprotein, acute coronary syndrome, high-sensitive C-reactive protein

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide. The major independent risk factors for CVD are hypertension, smoking, diabetes mellitus, advanced age, as well as elevated serum total and low-density lipoprotein cholesterol (LDL-C) and low high-density lipoprotein cholesterol (HDL-C). However, these established risk factors account for no more than 25% to 30% of the excess cardiovascular risk factors in patients,[1] suggesting that other risk factors play an important role in the pathogenesis of atherosclerosis. Coronary heart disease (CHD) can manifest itself with stable or acute coronary syndrome (ACS) including unstable angina (UA) and also myocardial infarction (MI).

Low-density lipoprotein, especially small dense LDL (sdLDL), plays a role in atherogenesis. To date, many studies have investigated the possible role of sdLDL in CVD and most have reported a positive association between the sdLDL levels, LDL particle size, and presence and severity of CVD.[2-6] However, contrary evidence also exists.[7] Small dense LDLs are highly atherogenic due to lower affinity for the LDL receptor,[8] lower resistance to oxidative modification having lesser antioxidant concentrations,[9] prolonged half-life in the circulation,[10] and entering the arterial wall more easily.[11] Recently, the pathophysiology, atherogenicity, and measurement issues related to LDL subclasses have been reviewed.[12,13]

It has been reported that the mean LDL size was smaller in those patients with proven CHD based on angiography results than the healthy control participants; the authors have suggested after multiple regression analysis the presence of high

[1] Cardiovascular Research Center, Faculty of Medicine, Mashhad University of Medical Science Mashhad, Iran
[2] Department of Cardiology, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Iran
[3] Biochemistry of Nutrition Research Center, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Iran
[4] Institute for Science and Technology in Medicine, Faculty of Health, University of Keele, Staffordshire, United Kingdom

Corresponding Author:
Majid Ghayour-Mobarhan, Biochemistry of Nutrition Research Center, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Iran.
Email address: ghayourm@mums.ac.ir

levels of sdLDL would be an important and independent risk factor for coronary artery disease (CAD).[2] Moreover, the presence of sdLDL phenotype has been associated with an approximately 3-fold increased risk of CAD,[4] and it has been proposed that sdLDL particles and high concentrations of sdLDL are potent risk factors for CVD.[3] Moreover, previous studies have reported that there is a linear correlation between the sdLDL concentrations and the risk of development cardiovascular events.[5,6] It has also been demonstrated in healthy middle-aged individuals that predominance of sdLDL particles is associated with increased proinflammatory activation of peripheral mononuclear cells.[14] In addition to CVD, it has been also reported that carotid artery intima-media thickness was most closely associated with sdLDL-cholesterol among different lipid parameters, indicating that sdLDL-cholesterol is the best marker of carotid atherosclerosis among the different lipid parameters.[15]

Serum C-reactive protein (CRP) is a marker of systemic inflammation.[16] In addition, CRP may play an active role in CVD, from initial recruitment of circulating leukocytes to the arterial wall to eventual rupture of the unstable plaque.[17] Furthermore, CRP has been positively related to future cardiovascular events in high-risk individuals, as we[18] have previously shown that CRP is associated with CVD in Iranian patients with angiographically defined CAD. High-sensitivity (hs) CRP independently predicted the frequency of recurrent coronary events in patients with ACS.[19]

We evaluated the changes in sdLDL concentrations during the ACS (MI and UA) and the possible association between sdLDL concentrations and extent and severity of CAD.

Methods

Participants

A total of 204 patients (81 males, 123 females, mean age: 59.4 $\pm$ 9.7) with ACS were recruited. The patients with chest pain attended the Accident and Emergency Center within the preceding 12 hours and subsequently admitted to the coronary care unit (CCU) of Ghaem Hospital, Mashhad, Iran. Two blood samples were taken from each patient for analysis; the first sample on admission and the second about 24 hours later. The presence of MI or UA was determined by a cardiologist, according to the World Health Organization (WHO) criteria using electrocardiography, echocardiography, and serum troponin I positivity. Exclusion criteria were age <35 or >75 years; previous history of MI, pregnancy and lactation, cerebrovascular events, consumption of lipid-lowering agents, active liver disease or hepatic dysfunction; acute or chronic pancreatitis, and renal dysfunction (blood creatinine $\geq$2.0 mg/dL). Fifty-two healthy participants without a history of CAD or any cardiovascular risk factors who were referred to the Mashhad Central Laboratory for a routine checkup of serum biochemical parameters were recruited as the control group. In addition, 46 and 73 patients were candidates for coronary angiography in the MI and UA groups, respectively. Significant CAD based on coronary angiography results was defined as stenosis $\geq$50% of the luminal diameter in $\geq$1 branch of the coronary arteries. In addition, echocardiography was performed for all the patients. The stages of systolic function were determined based on the ejection fraction (EF) as follows: normal, EF $\geq$ 55%; stage I, 45% $\leq$ EF < 55%; stage II, 30% $\leq$ EF < 44%; and stage III, EF < 30%. Moreover, as previously has been described[20] the stages of diastolic function were determined to normal, stage I: impaired, stage II: pseudonormal, and stage III: restrictive.

Hypercholesterolemia was diagnosed by a serum total cholesterol concentration $\geq$200 mg/dL ($\geq$5.2 mmol/L) and/or LDL-cholesterol (LDL-C) $\geq$130 mg/dL (3.36 mmol/L), and/or treatment with lipid-lowering drugs, diabetes by a fasting blood glucose level $\geq$126 mg/dL ($\geq$7 mmol/L) on $\geq$2 occasions or treatment with antidiabetic drugs, and hypertension by blood pressures $\geq$140/90 mm Hg on $\geq$2 occasions or if patients were on treatment with antihypertensive medication. Patients were considered smokers if they smoked $\geq$1 cigarette/d at the time of admission or in the preceding 12 months. Each participant gave written consent to participate in the study, which had previously been approved by the Mashhad University of Medical Sciences' research ethics committee.

Blood Sampling

Venous blood samples were collected from each patient on hospital admission and approximately 24 hours later, and for the control group 1 blood sample was taken on the day of laboratory sampling after 12 hours of fasting. Following venipuncture of an antecubital vein, blood samples were collected into Vacutainer tube Pole Ideal Pars, Iran and centrifuged at 5000*g* for 15 minutes at 4°C. After separation, serum aliquots were frozen at −80°C until the day of analysis. A full lipid profile comprising total cholesterol, triglycerides, and LDL-C and HDL-C was determined for each patient. Serum lipid and fasting serum glucose concentrations were measured by routine enzymatic methods.

Determination of sdLDL

Small dense LDL was determined using a validated method.[21] Briefly, precipitation reagent (150 U/mL heparin–sodium salt and 90 mmol/L $MgCl_2$) was added to 0.5 mL of serum sample, mixed, and incubated for 10 minutes at 37°C. The samples were placed in an ice bath and allowed to stand for 15 minutes, and centrifuged at 15 000 rpm for 15 minutes at 4°C. The precipitate was packed tightly at the bottom of the tube, and the supernatant was clear. An aliquot of the supernatant was removed for measurement of LDL-C and apolipoprotein B (apo B). In the overhead solution, all lipoproteins were precipitated except HDL-C and sdLDL, and the concentration of the apo B_{100} or LDL-C in the supernatant equivalent with sdLDL the apo B_{100} was measured by immunoturbidometric assay (Apo-B, Biosystems, Barcelona, Spain). In this method, as

Table 1. Demographic, Clinical, and Baseline Biochemical Characteristics of 3 Groups of Participants.[a]

Groups	MI	UA	Control	*P*
Number	104	100	52	
Age, year	59.0 ± 0.9	59.9 ± 1.0	56.8 ± 0.8	>.05
Females (%)	73 (70.2%)	50 (50%)	29 (55.8%)	<.05
Diabetes mellitus (%)	25 (24%)	30 (30%)	0 (0%)	<.05
Hypertension (%)	35 (33.7%)	66 (66%)	0 (0%)	<.05
Hyperlipidemia (%)	9 (8.7%)	0	0 (0%)	<.05
Smoking (%)	17 (16.3%)	14 (14%)	0 (0%)	<.05
Family history (+) (%)	5 (4.8%)	2 (2%)	0 (0%)	>.05
BMI, kg/m^2 (%)				
<20	6 (5.8%)	2 (2%)	5 (9.6%)	
20-25	89 (85.6%)	87 (87%)	25 (48.1%)	<.05
25-30	7 (6.7%)	8 (8%)	17 (32.7%)	
>30	2 (1.9%)	3 (3%)	5 (9.6%)	
Diastolic function				
Normal	22 (21.2%)	31 (31%)	100 (100%)	
Stage I	61 (58.7%)	52 (52%)	0 (0%)	<.05
Stage II	13 (12.5%)	11 (11%)	0 (0%)	
Stage III	8 (7.7%)	6 (6%)	0 (0%)	
Systolic function				
Normal	2 (1.9)	15 (15%)	100 (100%)	
Stage I	94 (90.4%)	80 (80%)	0 (0%)	<.05
Stage II	6 (5.8%)	4 (4%)	0 (0%)	
Stage III	2 (1.9%)	1 (1%)	0 (0%)	
FBS (mg/dL)	113.55 ± 7.50	117.95 ± 5.19	79.83 ± 1.36	<.05
TG (mg/dL)	137.58 ± 6.97	158.74 ± 11.18	107.10 ± 4.34	<.05
TC (mg/dL)	173.67 ± 4.71	172.04 ± 3.74	164.62 ± 3.67	>.05
HDL-C (mg/dL)	50.51 ± 3.38	38.00 ± 1.25	43.5 ± 1.55	<.05
LDL-C (mg/dL)	89.46 ± 4.02	96.69 ± 3.16	102.81 ± 2.42	>.05
sdLDL (mg/dL)	51 (38-69)	46 (36-65)	13 (11-19)	<.05
hsCRP (mg/L)	8.7 (6.22-11.57)	6.8 (4.12-10.8)	2.1 (1.4-2.78)	<.05

Abbreviations: UA, unstable angina; MI, myocardial infarction; FBS, fasting blood sugar; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; sdLDL, small dense low-density lipoprotein; hsCRP, high sensitivity C-reactive protein.
[a] Values are presented as mean ± SEM or median (interquartile range).

previously described, the cholesterol and apo B values obtained by the precipitation method were similar to those obtained in the lipoprotein separated by ultracentrifugation, and the authors have found there was an excellent correlation between the two methods for sdLDL-C and apo B.[21]

Statistical Analysis

All data were analyzed by using SPSS for Windows, version 16 software package (SPSS Inc, Chicago, Illinois). Values were expressed as mean ± standard error of the mean for normally distributed data, and in the case of nonnormally distributed data as median and interquartile range. Data that were normally distributed were analyzed using Student *t* test and for nonnormally distributed data using nonparametric Mann-Whitney test (for 2 groups). For comparison between 2 related samples, the Wilcoxon signed rank test was used. Chi-square test was used to compare the qualitative factors such as hypertension between control and patients. A 2-sided $P < .05$ was considered significant. To analyze the relationship between sdLDL concentrations and individual ACS factors, Spearman correlation was used.

Results

We measured the serum levels of sdLDL and hsCRP in 104 patients with MI, 100 patients with UA and compared the results with 100 healthy participants. Demographic data and clinical and biochemical characteristics of the patients are summarized in Table 1. The MI group had a higher proportion of females when compared with the other 2 groups ($P < .05$). In addition, 46 and 73 patients underwent angiography in the MI and UA groups. Based on the results of angiography, in the MI group, 1, 20, 17, and 8 patients had normal, single-, double-, and triple-vessel disease, respectively. In the UA group, 4, 35, 18, and 16 patients had normal, single-, double-, and triple-vessel disease, respectively.

Small Dense LDL and hsCRP Levels

Baseline concentrations of sdLDL were significantly higher in patients presenting with MI and UA compared with the control group ($P < .001$). The same results were observed for hsCRP levels ($P < .001$). The comparison between the patients with MI and UA showed the significant difference in hsCRP levels ($P = .01$), while there was no significant difference in baseline

Table 2. The Levels of sdLDL and hsCRP in the First and Second Samples.[a]

	MI Group		UA group	
	First Sample	Second Sample	First Sample	Second Sample
sdLDL (mg/dL)	51 (38-69)	48 (33-66)	46 (36-65)	44 (37-63)
hsCRP (mg/L)	8.7 (6.22-11.57)	8.3 (6.6-11.35)	6.8 (4.12-10.8)	7.5 (5.5-11.2)

Abbreviations: sdLDL, small-dense low-density lipoprotein; hsCRP, high sensitive C-reactive protein.
[a]Data have been shown as median (interquartile range). There was insignificant difference between first and second sample in both groups.

sdLDL values between the 2 groups of patients ($P = .326$). The sdLDL levels in the second sample reduced from 51 (38-69) to 48 (33-66) mg/dL in MI group; and in patients with UA the same pattern was found, as the sdLDL levels decreased from 46 (36-65) mg/dL in the first sample to 44 (37-63) mg/dL in the second sample (Table 2). These changes were not significant in both groups ($P > .05$). Also, the changes in hsCRP levels were not significantly different between the first and second samples in both groups of the patients ($P > .05$). The changes in sdLDL in patients with MI and UA were 2 (−7-8) and 0.5 (−7-7) mg/dL, respectively. The changes in sdLDL were not significantly different between the 2 groups of patients ($P = .720$). Moreover, the changes in hsCRP levels were 0.25 (−1.67-1.5)(mg/L) and 0.0 (−2.1-1.1) (mg/L) in patients with MI and UA, respectively. The same as sdLDL concentration, the changes in hsCRP levels were not statistically different between the 2 groups ($P = .340$). The changes in hsCRP levels and sdLDL levels in each group were not correlated ($P > .05$).

In addition, because of the significantly different proportion of females in the MI group in comparison with other groups, the sex effect was adjusted. After the adjustments, sdLDL and hsCRP levels were significantly different among the 3 groups ($P < .05$). In addition, the comparison between the first and second serum samples was not significantly different in both groups of patients with MI and UA, after adjustment ($P > .05$). After adjustments, the changes in sdLDL concentrations were not significantly different between patients with MI and UA, while the changes in hsCRP values showed a significant difference between the 2 groups of patients ($P < .05$).

Small Dense LDL and hsCRP Levels and Their Association With Other Parameters

After the analysis, using the Mann-Whitney test, we found that sdLDL levels were not significantly associated with any cardiovascular risk factors, as diabetes, hypertension, smoking, and hyperlipidemia; patients had comparable levels of sdLDL when compared with patients without the above CVD risk factors. In addition, hsCRP levels were significantly different in patients with hypertension when compared with normotensive participants ($P = .011$), however, for other CVD risk factors there were no significant difference. Moreover, patients with different BMI levels had significantly different sdLDL and hsCRP concentrations ($P = .001$). The sdLDL levels did not differ significantly between males and females ($P > .05$).

Based on echocardiography, the patients in MI and UA groups were divided into different groups, with different stage of diastolic and systolic dysfunction. After analysis using Kruskal-Wallis test, there was a significant association between baseline sdLDL levels and systolic function; however, we did not find this association for diastolic function. Moreover, the baseline levels of hsCRP were not associated with echocardiographic results.

Based on the results of angiography, the baseline levels of sdLDL were compared between different groups of patients with different extent of coronary involvements. The analysis showed that the sdLDL levels were significantly higher in single- versus triple-vessel disease ($P = .023$). However, for baseline hsCRP levels, there was no significant difference between different groups of patients with different stenosed vessels ($P > .05$).

Multiple Linear Regressions

The variation in the baseline sdLDL levels could be explained by the best-fitting models derived from stepwise multiple linear regressions: in this model the variation in sdLDL levels were only explained by cholesterol levels ($\beta = .270$, $P = .008$).

Discussion

In the present study, we found that the baseline concentration of sdLDL was significantly higher in CAD patients presenting with ACS in comparison with healthy controls, whereas LDL-C and total-cholesterol levels were comparable between patients with CHD and controls. Consistent with our finding, there are several studies that have reported a positive association between the sdLDL levels, LDL particle size, and presence and severity of CVD.[2-6] The mean LDL size was smaller in the patients with proven CAD based on angiography results than the healthy control participants; moreover, the authors have suggested after multiple regression analysis the presence of sdLDL would be an important and independent risk factor for CAD development apart from the traditional cardiovascular risk factors.[2] Furthermore, the presence of sdLDL phenotype has been associated with an approximately 3-fold increased risk of CAD,[4] and it has been proposed that sdLDL particles and high concentrations of sdLDL are important risk factors for CVD.[3] In addition, previous studies have reported that there is a linear correlation between the sdLDL concentrations and the risk of development cardiovascular events.[5,6] It has been

reported that small LDL particles have been associated with circulating inflammation markers even before clinical evidence of atherosclerosis.[22] However, in our study, we have not observed any association between hsCRP and baseline sdLDL levels. It has been also demonstrated in healthy middle-aged individuals that predominance of sdLDL particles is associated with increased proinflammatory activation of peripheral mononuclear cells.[14]

We further observed that in the next 24 hours following ACS, the sdLDL levels decreased in patients with ACS, though this did not attain significance. Nor did the changes in sdLDL values differ significantly between MI and UA participants. The same pattern as sdLDL was observed for hsCRP levels, except that there were significantly higher hsCRP values in MI group when compared with UA group. The insignificant reduction in sdLDL concentration may be attributed to commencing statin and β-blocker therapies after the hospital admission of the patients. As previously reported, these medications are able to affect the LDL size and composition.[23,24] The particle size of LDL has been investigated in patients with ACS, and the authors found the LDL sizes were similar at admission and at discharge.[25] In contrast, in a case–control study among healthy middle-aged women, it was found that baseline LDL particle concentrations were higher and LDL particle size was lower among women who subsequently had cardiovascular events in comparison with those who did not.[26] In the latter study, the number of LDL particles but not LDL size remained significantly related to MI or angina. Moreover, patients with acute MI show an early reduction in LDL size, which persists during hospitalization[27] and also acute MI is accompanied by profound plasma lipid and lipoprotein modifications that have a great relevance and should be kept in mind for therapeutic decisions.[28] However, despite the changes in lipid and lipoprotein levels during ACS, it is less defined how sdLDL concentrations change during acute phase. In this study, we observed the insignificant decrease in sdLDL levels, however, unfortunately after 24 hours following the hospital admission, the sdLDL values were not measured to elucidate how sdLDL values change after ACS.

A variety of studies have evaluated the effect of lipid-lowering agent such as statins and fibrates on sdLDL size and concentrations. It has been reported that statin therapy may lead to increase in LDL particle size,[23] and treatment with fibrates also increases the LDL particle size and decreases CVD risk.[29] Some studies have reported that statin therapy was not associated with the increase in LDL size.[30]

Moreover, we did not find any difference in sdLDL levels in patients with different cardiovascular risk factors including diabetes mellitus, hyperlipidemia, hypertension, and smoking in comparison with those who do not. However, overall the baseline sdLDL levels were significantly different based on BMI and obesity categorizations. The multiple regressions showed that the baseline sdLDL levels were associated with total cholesterol levels and no other biochemical parameters. In addition, based on echocardiographic findings there was a significant association between baseline sdLDL levels and systolic but not diastolic function.

Different studies have reported various results; some of them have reported that sdLDL particles are closely associated with diabetes mellitus.[31,32] Feingold et al[32] reported a 2-fold increase in the incidence of sdLDL phenotype in normolipidemic noninsulin-dependent diabetic patients. In contrast, other studies failed to confirm these findings.[33] In addition, we did not find any difference in sdLDL concentration between males and females. A recent study[3] confirms our results, while a significant difference in LDL particle size has also been reported.[5]

In the present study, the sdLDL concentrations were only correlated with total cholesterol levels. In another study, in multivariate models for women that included triglycerides and HDL cholesterol, the number of LDL particles (but not LDL size) remained significantly related to MI and angina.[34] It has been reported that LDL particle size is associated with serum triglyceride level.[33] Furthermore, it has been reported that LDL particle diameter is associated with risk of MI but not after adjustment for triglyceride level.[35]

When defining CHD severity by various clinical features and angiographic findings, the baseline sdLDL but not hsCRP levels were significantly different between single- and triple-vessel diseases, being higher in patients with single-vessel disease. Patients with more severe lesions had lower levels of sdLDL concentrations, whereas LDL-C and total cholesterol levels were not different between different groups of patients. The reports about the association of sdLDL levels and LDL size are controversial. In another study, it has been reported that high sdLDL concentrations but not the LDL size were closely associated with CAD severity, determined by Gensini scores, independent of classical coronary risk factors[3]; the inefficacy of using LDL size in determining CAD severity has been confirmed by others.[36,37] However, in other studies, significant correlation between LDL particle size and extent of CAD has been reported.[2,29]

In conclusion, our results indicate that patients with ACS have a higher concentration of sdLDL compared with controls. The changes in sdLDL levels were not significantly different between the patients with MI and UA. Moreover, the baseline sdLDL levels differed between patients with different extent of CHD independent of the coronary risk factors. Based on our finding, sdLDL may act as an independent risk factor for CAD. In addition to assessing the impact of lipid-lowering agents on sdLDL size, further studies are needed to assess the effect of lipid-lowering drugs in reduction of sdLDL concentrations.

This study has limitations; first, we did not examine the size spectrum of sdLDL as would be possible by other techniques. We hope future studies will provide more information about the importance of size or concentrations of sdLDL. Therapeutic modulation for reduction of sdLDL concentration and LDL size would be an interesting field of investigation. However, before being used in clinical practice, the effect of this modulation on cardiovascular events should be studied further.

Acknowledgments

We are grateful to the patients and their family members who volunteered to participate in this study. The results presented in this work have been taken from Maryam-Sadat Alavi's MUMS thesis (ID: 88650).

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The MUMS (Mashhad University of Medical Science).

References

1. Bierman EL. George Lyman Duff Memorial Lecture. Atherogenesis in diabetes. *Arterioscler Thromb*. 1992;12(6):647-656.
2. Kwon SW, Yoon SJ, Kang TS, et al. Significance of small dense low-density lipoprotein as a risk factor for coronary artery disease and acute coronary syndrome. *Yonsei Med J*. 2006;47(3):405-414.
3. Koba S, Hirano T, Ito Y, et al. Significance of small dense low-density lipoprotein-cholesterol concentrations in relation to the severity of coronary heart diseases. *Atherosclerosis*. 2006;189(1): 206-214.
4. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA*. 1988;260(13):1917-1921.
5. Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. *JAMA*. 1996;276(11):875-881.
6. St-Pierre AC, Cantin B, Dagenais GR, et al. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec Cardiovascular Study. *Arterioscler Thromb Vasc Biol*. 2005;25(3):553-559.
7. Mykkanen L, Kuusisto J, Haffner SM, Laakso M, Austin MA. LDL size and risk of coronary heart disease in elderly men and women. *Arterioscler Thromb Vasc Biol*. 1999;19(11):2742-2748.
8. Chen GC, Liu W, Duchateau P, et al. Conformational differences in human apolipoprotein B-100 among subspecies of low density lipoproteins (LDL). Association of altered proteolytic accessibility with decreased receptor binding of LDL subspecies from hypertriglyceridemic subjects. *J Biol Chem.* 1994;269(46): 29121-29128.
9. Tribble DL, Rizzo M, Chait A, Lewis DM, Blanche PJ, Krauss RM. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. *Am J Med*. 2001;110(2):103-110.
10. Packard CJ, Demant T, Stewart JP, et al. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *J Lipid Res*. 2000;41(2):305-318.
11. Bjornheden T, Babyi A, Bondjers G, Wiklund O. Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. *Atherosclerosis*. 1996; 123(1-2):43-56.
12. Mikhailidis DP, Elisaf M, Rizzo M, et al. "European panel on low density lipoprotein (LDL) subclasses": a statement on the pathophysiology, atherogenicity and clinical significance of LDL subclasses: executive summary. *Curr Vasc Pharmacol*. 2011;9(5): 531-532.
13. Mikhailidis DP, Elisaf M, Rizzo M, et al. "European panel on low density lipoprotein (LDL) subclasses": a statement on the pathophysiology, atherogenicity and clinical significance of LDL subclasses. *Curr Vasc Pharmacol*. 2011;9(5):533-571.
14. Norata GD, Raselli S, Grigore L, et al. Small dense LDL and VLDL predict common carotid artery IMT and elicit an inflammatory response in peripheral blood mononuclear and endothelial cells. *Atherosclerosis*. 2009;206(2):556-562.
15. Shoji T, Hatsuda S, Tsuchikura S, et al. Small dense low-density lipoprotein cholesterol concentration and carotid atherosclerosis. *Atherosclerosis*. 2009;202(2):582-588.
16. Atar I, Gulmez O, Atar A, et al. The effects of prior beta-blocker therapy on serum C-reactive protein levels after percutaneous coronary intervention. *Clin Cardiol*. 2005;28(5):243-246.
17. Burke AP, Tracy RP, Kolodgie F, et al. Elevated C-reactive protein values and atherosclerosis in sudden coronary death: association with different pathologies. *Circulation*. 2002;105(17):2019-2023.
18. Kazemi-Bajestani SM, Ghayour-Mobarhan M, Ebrahimi M, Moohebati M, Esmaeili HA, Ferns GA. C-reactive protein associated with coronary artery disease in Iranian patients with angiographically defined coronary artery disease. *Clin Lab*. 2007;53(1-2): 49-56.
19. Ferreiros ER, Boissonnet CP, Pizarro R, et al. Independent prognostic value of elevated C-reactive protein in unstable angina. *Circulation*. 1999;100(19):1958-1963.
20. Garcia MJ, Thomas JD, Klein AL. New Doppler echocardiographic applications for the study of diastolic function. *J Am Coll Cardiol*. 1998;32(4):865-875.
21. Hirano T, Ito Y, Saegusa H, Yoshino G. A novel and simple method for quantification of small, dense LDL. *J Lipid Res*. 2003;44(11):2193-2201.
22. Vekic J, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V, et al. High serum uric acid and low-grade inflammation are associated with smaller LDL and HDL particles. *Atherosclerosis*. 2009; 203(1):236-242.
23. Melenovsky V, Malik J, Wichterle D, et al. Comparison of the effects of atorvastatin or fenofibrate on nonlipid biochemical risk factors and the LDL particle size in subjects with combined hyperlipidemia. *Am Heart J*. 2002;144(4):E6.
24. Croft KD, Dimmitt SB, Moulton C, Beilin LJ. Low density lipoprotein composition and oxidizability in coronary disease—apparent favourable effect of beta blockers. *Atherosclerosis*. 1992;97(2-3):123-130.
25. Ban Y, Koba S, Tsunoda F, et al. Predominance of small dense low-density lipoproteins and abnormal glucose regulation in patients with acute coronary syndrome. *Circ J*. 2006;70(4): 393-401.
26. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. *Circulation*. 2002;106(15):1930-1937.

27. Miwa K. Low density lipoprotein particles are small in patients with coronary vasospasm. *Int J Cardiol*. 2003;87(2-3):193-201.
28. Ryder RE, Hayes TM, Mulligan IP, Kingswood JC, Williams S, Owens DR. How soon after myocardial infarction should plasma lipid values be assessed? *Br Med J (Clin Res Ed)*. 1984; 289(6459):1651-1653.
29. Vakkilainen J, Steiner G, Ansquer JC, et al. Relationships between low-density lipoprotein particle size, plasma lipoproteins, and progression of coronary artery disease: the Diabetes Atherosclerosis Intervention Study (DAIS). *Circulation*. 2003;107(13):1733-1737.
30. Soedamah-Muthu SS, Colhoun HM, Thomason MJ, et al. The effect of atorvastatin on serum lipids, lipoproteins and NMR spectroscopy defined lipoprotein subclasses in type 2 diabetic patients with ischaemic heart disease. *Atherosclerosis*. 2003;167(2):243-255.
31. Grundy SM, Howard B, Smith S, Jr, Eckel R, Redberg R, Bonow RO. Prevention Conference VI: Diabetes and Cardiovascular Disease: executive summary: conference proceeding for healthcare professionals from a special writing group of the American Heart Association. *Circulation*. 2002;105(18):2231-2239.
32. Feingold KR, Grunfeld C, Pang M, et al. LDL subclass phenotypes and triglyceride metabolism in non-insulin-dependent diabetes. *Arterioscler Thromb*. 1992;12(12):1496-1502.
33. Tsunoda F, Koba S, Hirano T, et al. Association between small dense low-density lipoprotein and postprandial accumulation of triglyceride-rich remnant-like particles in normotriglyceridemic patients with myocardial infarction. *Circ J*. 2004; 68(12):1165-1172.
34. Kuller L, Arnold A, Tracy R, et al. Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the cardiovascular health study. *Arterioscler Thromb Vasc Biol*. 2002;22(7):1175-1180.
35. Stampfer MJ, Krauss RM, Ma J, et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *JAMA*. 1996;276(11): 882-888.
36. Campos H, Moye LA, Glasser SP, Stampfer MJ, Sacks FM. Low-density lipoprotein size, pravastatin treatment, and coronary events. *JAMA*. 2001;286(12):1468-1474.
37. Ruotolo G, Ericsson CG, Tettamanti C, et al. Treatment effects on serum lipoprotein lipids, apolipoproteins and low-density lipoprotein particle size and relationships of lipoprotein variables to progression of coronary artery disease in the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT). *J Am Coll Cardiol*. 1998;32:1648-1656.