Table 4. The results of the multivariable linear regression analyses for the relationship between the sLOX-1 and the CAVI

		Coeffcient	95%CI	Standardized coefficient	*p* value	VIF
Men						
Model 1	Ln sLOX-1	−0.071	−0.299 to 0.158	−0.028	0.542	1.061
			Adjusted R-squared: 0.381			
Model 2	Ln sLOX-1	−0.066	−0.296 to 0.163	−0.026	0.571	1.068
	LDL-C	0.001	−0.002 to 0.004	0.023	0.623	1.069
			Adjusted R-squared: 0.380			
Model 3	Ln sLOX-1	−0.067	−0.295 to 0.161	−0.027	0.563	1.068
	LDL-C	0.001	−0.003 to 0.004	0.016	0.723	1.074
	Ln hs-CRP	0.085	0.004 to 0.166	0.097	0.040	1.120
			Adjusted R-squared: 0.387			
Model 4	Ln sLOX-1	−0.068	−0.300 to 0.160	−0.027	0.559	1.068
	LDL-C	0.000	−0.003 to 0.004	0.011	0.817	1.084
	Ln hs-CRP	0.077	−0.004 to 0.159	0.089	0.064	1.143
	HDL-C	−0.005	−0.011 to 0.002	−0.067	0.195	1.358
			Adjusted R-squared: 0.388			
Women						
Model 1	Ln sLOX-1	−0.091	−0.294 to 0.112	−0.048	0.376	1.026
			Adjusted R-squared: 0.426			
Model 2	Ln sLOX-1	−0.089	−0.292 to 0.114	−0.046	0.389	1.028
	LDL-C	0.001	−0.002 to 0.005	0.034	0.551	1.169
			Adjusted R-squared: 0.424			
Model 3	Ln sLOX-1	−0.092	−0.293 to 0.109	−0.048	0.367	1.028
	LDL-C	0.001	−0.002 to 0.005	0.040	0.482	1.171
	Ln hs-CRP	0.092	0.012 to 0.172	0.128	0.024	1.183
			Adjusted R-squared: 0.436			
Model 4	Ln sLOX-1	−0.057	−0.258 to 0.144	−0.030	0.575	1.050
	LDL-C	0.001	−0.002 to 0.004	0.032	0.574	1.175
	Ln hs-CRP	0.091	0.012 to 0.170	0.128	0.025	1.184
	HDL-C	−0.008	−0.014 to −0.001	−0.129	0.019	1.103
			Adjusted R-squared: 0.449			

95%CI: 95% confidence interval, sLOX-1: soluble form of lectin-like oxidized LDL receptor-1, CAVI: cardio-ankle vascular index, LDL-C: low-density lipoprotein cholesterol, hs-CRP: high sensitivity C-reactive protein, HDL-C; high density lipoprotein cholesterol
Variables for Model 1: age, body mass index, systolic blood pressure, heart rate, HbA1c, current smoker or not, current alcohol drinker or not and sLOX-1 (log-transformed).
Variables for Model 2: Model 1 + LDL-C
Variables for Model 3: Model 2 + hs-CRP
Variables for Model 4: Model 3 + HDL-C

Discussion

In the present cross-sectional study of healthy Japanese community-dwellers who were considered to be at low risk for atherosclerosis, there were positive associations between the CAVI and LAB, especially in men. Moreover, the positive associations between the LAB and the CAVI were not changed after adjustment for the LDL-C. These tendencies were not changed with further adjustment for the levels of hs-CRP and HDL-C. On the other hand, no clear association between sLOX-1 and the CAVI was observed in either

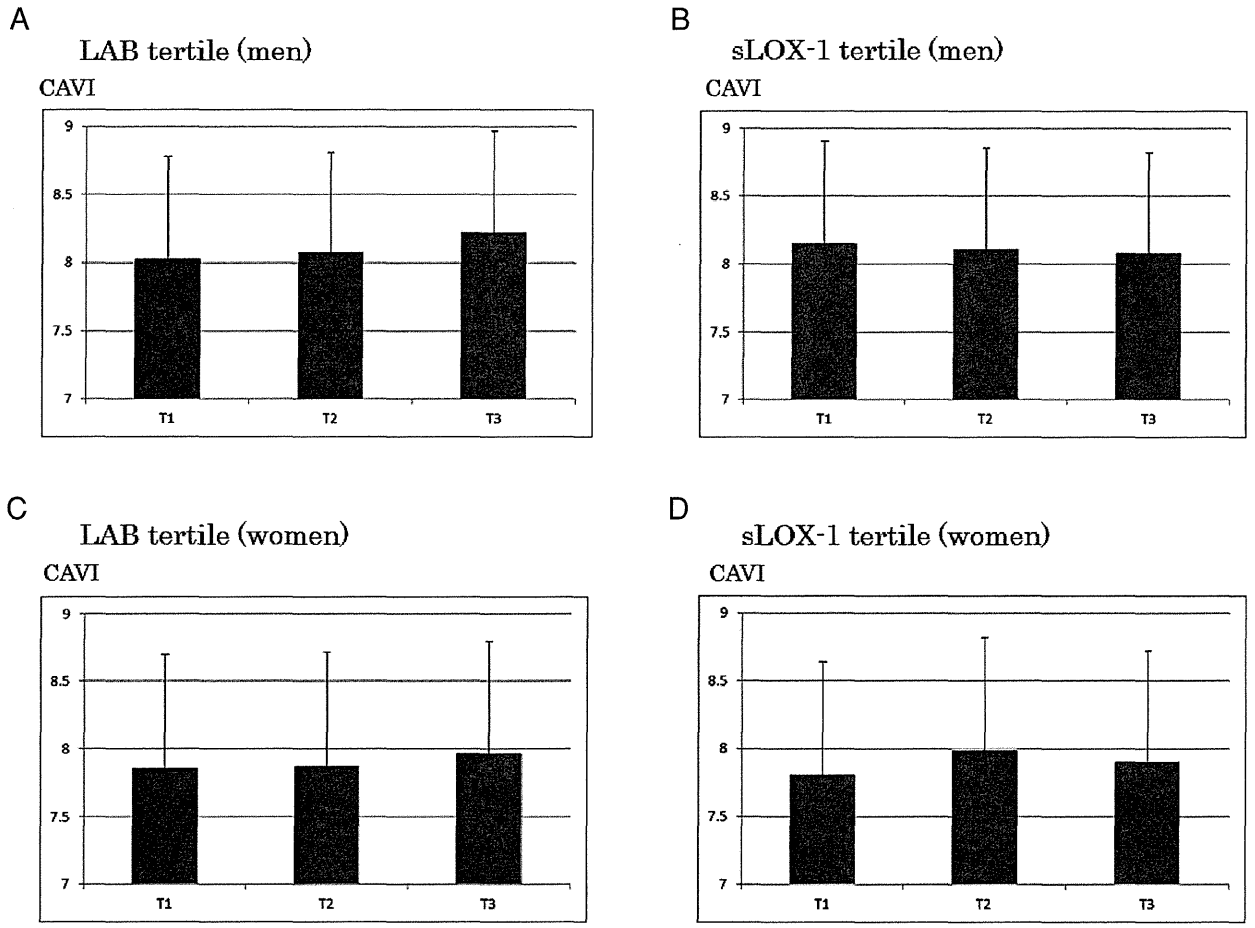


Fig. 1. The multivariable adjusted mean of the CAVI among tertiles of the LAB or sLOX-1.

A) The multivariable adjusted mean CAVI among tertiles of the LAB (men)
B) The multivariable adjusted mean of CAVI among tertiles of the sLOX-1 (men)
C) The multivariable adjusted mean of CAVI among tertiles of the LAB (women)
D) The multivariable adjusted mean of CAVI among tertiles of the sLOX-1 (women)
*The tertile of each category [minimum value to max value]
A) T1 [4126 to 19340], T2 [19390 to 27150], T3 [27180 to 61360] (μg/L)
B) T1 [57 to 101], T2 [101 to 125], T3 [126 to 1264] (ng/L)
C) T1 [6324 to 20430], T2 [20630 to 28500], T3 [28670 to 76860] (μg/L)
D) T1 [59 to 103], T2 [104 to 127], T3 [129 to 16460] (ng/L)
CAVI: cardio-ankle vascular index, LAB: LOX-1 ligand containing apolipoprotein B, sLOX-1: soluble form of LOX-1
Adjusted by age, body mass index, systolic blood pressure, heart rate, HbA1c, current smoker or not, current alcohol drinker or not

sex.

The major strength of the present study was that the participants were representative of the healthy population in Japan, without histories of CVD or medication use for risk factors, such as hypertension. Because healthy community dwellers rarely visit hospitals to receive health examinations for atherosclerosis, it is difficult to collect data about the parameters related to early-stage atherosclerosis in healthy individuals compared with unhealthy individuals. While health data obtained from work sites could contribute to investigations of the present study question, it may be impossible to accurately assess the atherosclerotic condition of individuals, because the mean age of patients included in occupational databases is usually lower than that in the general population. There is also a risk of bias due to the healthy worker effect[24].

Both *in vitro* and animal experiments have shown that the LAB is related to endothelial dysfunction, which leads to lipid sedimentation[3, 4], inflammation[25], migration and the proliferation of smooth muscle[26], and to the formation of foam cells[27]. All of

these phenomena are thought to enhance the progression of atherosclerosis. In addition, elevation of the serum LAB levels also increased the risk of CVD, including ischemic stroke, in a long-term Japanese cohort study[10]. These experimental and clinical findings were compatible with the results of the present study. LAB may reflect a subclinical state of atherosclerotic findings, so it could predict the risk of future CVD events[10]. In contrast, sLOX1 may be a useful marker for the diagnosis of CVD in the acute phase, because elevated sLOX-1 levels were observed in acute coronary syndrome in previous clinical studies[8, 9].

In a recent report, the serum LAB level was associated with an increased intima-media thickness in Caucasian men in the US after adjusting for other risk factors, but this relationship was not found in Japanese men[11]. The CAVI measures the heart-ankle pulse wave velocity, and is believed to reflect atherosclerosis of the aortic wall. Since previous studies indicated that the progression of atherosclerosis was observed earlier in the aorta than at other sites[12, 13], the CAVI is a useful tool for evaluating early-phase atherosclerosis. In contrast, the result of the intima-media thickness may indicate the progression of advanced atherosclerosis. One of the reasons why the results were different between this study and the previous one[11] may be the difference in the indicators, CAVI vs. intima-media thickness. In addition, there was a difference in the ages of the populations in these studies. The previous study had a younger study population (40-49 years) compared to this study (mean age of men: 61 ± 9 years).

In this study, a positive association between the LAB and the CAVI was observed in men, but not in women. The sex-specific positive association may reflect a potential sex difference in the risk for CVD among Japanese participants. The median levels of LAB and the mean CAVI were not significantly different between men and women, but the proportion of high CAVI levels (CAVI ≥ 9.0) was larger in men than in women (19% and 11%, respectively; $p=0.02$). The mean age in the high CAVI population did not differ by sex. This finding indicates that the early atherosclerotic changes detected by CAVI may tend to progress more in men compared to women of the same age, and this difference may be associated with the relationship between the CAVI and LAB observed in our study. Japanese women tend to have a much lower risk for coronary artery disease (CAD) than Japanese men[28], whose risk for CAD is also lower than that in US populations[21]. Therefore, the determination of risk factors for CAD is difficult to evaluate in Japanese women.

Our previous study[22] based upon the KOBE study showed that the hs-CRP level was positively associated with the CAVI, and the association between LDL-C and the CAVI was weak. These results indicated that the hs-CRP could be a more useful marker for atherosclerosis than LDL-C to detect early atherosclerosis in a healthy population without traditional risk factors for CVD, such as diabetes. In the present study, the serum hs-CRP level showed a positive association with the CAVI in all models of this study, and the results were concordant with those of the previous study. CRP is a marker for inflammation that is clinically useful in the evaluation of potential atherosclerosis, because inflammation enhances endothelial dysfunction. LAB is also involved in promoting inflammation[25], but it influences various other reactions leading to the progression of atherosclerosis[3, 4, 26, 27]. The positive relationship between the LAB and the CAVI in men in the present study was not influenced by adding the hs-CRP level to regression models; therefore, this result may indicate that the LAB has an influence on the development of atherosclerosis caused not only by inflammation, but also by other pathways. In addition, because the hs-CRP level is affected by infections or inflammatory diseases, the serum LAB levels may be represent another helpful marker that can be used to screen for subclinical atherosclerosis in individuals who have no or few risk factors for CVD.

The present study is associated with several possible limitations. First, this study was a cross-sectional study; thus, causality cannot be determined. The results of this study need to be confirmed in future prospective studies. Second, LOX-1-related modified LDL indicators are new markers for CVD and subclinical atherosclerotic diseases. Much is unknown about their relationship with environmental or lifestyle related factors. Third, the CAVI is also a relatively new method for assessing atherosclerosis; its relationship with future CVD events has not been sufficiently investigated. Fourth, collinearity may exist between the LDL-C and LAB or sLOX-1 in the linear regression models. However, the estimated VIFs for the LDL-C, LAB and sLOX-1 in Models 2, 3 and 4 were small, so there was little evidence for the existence of such collinearity. Finally, the generalizability of our study results is limited, because the KOBE study participants (volunteers) are believed to be more health conscious than the general population. Thus, the results of the present study should be applied to the general population with caution.

Conclusions

In this cross-sectional study of healthy Japanese city dwellers who were considered to be at low risk for atherosclerosis, the LAB was positively associated with the CAVI in men, but not in women, after adjustment for possible confounders. In contrast, no clear association between the sLOX-1 and the CAVI was observed. The LAB may have the potential to be a useful marker for detecting subclinical atherosclerosis, particularly in men, who seem to be at low risk based on the established CVD risk factors. However, the change in the mean CAVI affected by the level of LAB was very small, and there are limited evidences available to evaluate the association between the LAB and the incidence of CVD in epidemiologic studies, so further research will be needed to continue elucidating the relationship and to confirm the present findings.

Acknowledgements

The authors would like to express their sincere appreciation to the volunteers involved in the administration of the KOBE study, and to all of the research staff.

Sources of Funding

This study was supported by: 1) grants from the Regional Innovation Cluster Program, Global Type, Ministry of Education, Culture, Sports, Science and Technology; 2) a Grant-in-Aid for Researchers, Hyogo College Medicine, 2010; 3) a Grant-in-Aid for Young Scientists B 23790711 from the Japan Society for the Promotion of Science; 4) the Intramural Research Fund of the National Cerebral and Cardiovascular Center (22-4-5) and 5) a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (B 21390211, B 23390178, C 23590835, C25460778). These funding sources had no involvement in the present study, such as in the study design, interpretation of data, writing of the paper, and so on.

Conflicts of Interest

There are no conflicts of interest in the present study.

Declaration

Dr. Sugiyama had full access to all of the data generated in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1) Stocker R, Keaney JF Jr: Role of oxidative modifications in atherosclerosis. Physiol Rev, 2004; 84: 1381-478
2) Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Aiba Y, Tanaka T, Miwa S, Katsura Y, Kita T, Masaki T: An endothelial receptor for oxidized low-density lipoprotein. Nature, 1997; 386: 73-77
3) Sawamura T, Kakino A, Fujita Y: LOX-1: a multiligand receptor at the crossroads of response to danger signals. Curr Opin Lipidol, 2012; 23: 439-459
4) Ishigaki Y, Katagiri H, Gao J, Yamada T, Imai J, Uno K, Hasegawa Y, Kaneko K, Ogihara T, Ishihara H, Sato Y, Takikawa K, Nishimichi N, Matsuda H, Sawamura T, Oka Y: Impact of plasma oxidized low-density lipoprotein removal on atherosclerosis. Circulation, 2008; 118: 75-83
5) Sato Y, Nishimichi N, Nakano A, Takikawa K, Inoue N, Matsuda H, Sawamura T: Determination of LOX-1 ligand activity in mouse plasma with a chicken monoclonal antibody for ApoB. Atherosclerosis, 2008; 200: 303-309
6) Itabe H, Ueda M: Measurement of plasma oxidized low-density lipoprotein and its clinical implications. J Atheroscler Thromb, 2007; 14: 1-11
7) Yamazaki K, Bujo H, Taira K, Itou N, Shibasaki M, Takahashi K, Saito Y: Increased circulating malondialdehyde-modified LDL in the patients with familial combined hyperlipidemia and its relation with the hepatic lipase activity. Atherosclerosis, 2004; 172: 181-187
8) Hayashida K, Kume N, Murase T, Minami M, Nakagawa D, Inada T, Tanaka M, Ueda A, Kominami G, Kambara H, Kimura T, Kita T: Serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are elevated in acute coronary syndrome: a novel marker for early diagnosis. Circulation, 2005; 112: 812-818
9) Kobayashi N, Hata N, Kume N, Seino Y, Inami T, Yokoyama S, Shinada T, Tomita K, Kaneshige T, Mizuno K: Soluble lectin-like oxidized low-density lipoprotein receptor-1 as an early biomarker for ST elevation myocardial infarction. Circ J, 2011; 75: 1433-1439
10) Inoue N, Okamura T, Kokubo Y, Fujita Y, Sato Y, Nakanishi M, Yanagida K, Kakino A, Iwamoto S, Watanabe M, Ogura S, Otsui K, Matsuda H, Uchida K, Yoshimoto R, Sawamura T: LOX index, a novel predictive biochemical marker for coronary heart disease and stroke. Clin Chem, 2010; 56: 550-558
11) Okamura T, Sekikawa A, Sawamura T, Kadowaki T, Barinas-Mitchell E, Mackey RH, Kadota A, Evans RW, Edmundowicz D, Higashiyama A, Nakamura Y, Abbott RD, Miura K, Fujiyoshi A, Fujita Y, Murakami Y, Miyamatsu N, Kakino A, Maegawa H, Murata K, Horie M, Mitsunami K, Kashiwagi A, Kuller LH, Ueshima H; ERA JUMP Study Group: LOX-1 ligands containing apolipoprotein B and carotid intima-media thickness in middle-aged community-dwelling US Caucasian and Japanese men. Atherosclerosis, 2013; 229: 240-245
12) Bjurulf P: Atherosclerosis in different parts of the arterial

system. Am Heart J, 1964; 68: 41-50
13) Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Natural history of aortic and coronary atherosclerotic lesions in youth. Findings from the PDAY Study. Arterioscler Thromb, 1993; 13: 1291-1298
14) Miyoshi T, Doi M, Hirohata S, Sakane K, Kamikawa S, Kitawaki T, Kaji Y, Kusano KF, Ninomiya Y, Kusachi S: Cardio-ankle vascular index is independently associated with the severity of coronary atherosclerosis and left ventricular function in patients with ischemic heart disease. J Atheroscler Thromb, 2010; 17: 249-258
15) Kadota K, Takamura N, Aoyagi K, Yamasaki H, Usa T, Nakazato M, Maeda T, Wada M, Nakashima K, Abe K, Takeshima F, Ozono Y: Availability of cardio-ankle vascular index (CAVI) as a screening tool for atherosclerosis. Circ J, 2008; 72: 304-308
16) Noike H, Nakamura K, Sugiyama Y, Iizuka T, Shimizu K, Takahashi M, Hirano K, Suzuki M, Mikamo H, Nakagami T, Shirai K: Changes in cardio-ankle vascular index in smoking cessation. J Atheroscler Thromb, 2010; 17: 517-525
17) Shirai K, Hiruta N, Song M, Kurosu T, Suzuki J, Tomaru T, Miyashita Y, Saiki A, Takahashi M, Suzuki K, Takata M: Cardio-ankle vascular index (CAVI) as a novel indicator of arterial stiffness: theory, evidence and perspectives. J Atheroscler Thromb, 2011; 18: 924-938
18) Nakamura K, Tomaru T, Yamamura S, Miyashita Y, Shirai K, Noike H: Cardio-ankle vascular index is a candidate predictor of coronary atherosclerosis. Circ J, 2008; 72: 598-604
19) Izuhara M, Shioji K, Kadota S, Baba O, Takeuchi Y, Uegaito T, Mutsuo S, Matsuda M: Relationship of cardio-ankle vascular index (CAVI) to carotid and coronary arteriosclerosis. Circ J, 2008; 72: 1762-1767
20) Park JB, Park HE, Choi SY, Kim MK, Oh BH: Relation between Cardio-Ankle Vascular Index and Coronary Artery Calcification or Stenosis in Asymptomatic Subjects. J Atheroscler Thromb, 2013; 20: 557-567
21) NIPPON DATA80 Research Group: Risk assessment chart for death from cardiovascular disease based on a 19-year follow-up study of a Japanese representative population. Circ J, 2006; 70: 1249-1255
22) Higashiyama A, Wakabayashi I, Kubota Y, Adachi Y, Hayashibe A, Nishimura K, Sugiyama D, Kadota A, Imano H, Miyamatsu N, Miyamoto Y, Okamura T: Does high-sensitivity C-reactive protein or low-density lipoprotein cholesterol show a stronger relationship with the cardio-ankle vascular index in healthy community dwellers?: the KOBE study. J Atheroscler Thromb, 2012; 19: 1027-1034
23) Armitage P, Berry G, Matthews JNS: Statistical Methods in Medical Research, 4th ed. Blackwell Publishing. 2002
24) CY Li, FC Sung. A review of the healthy worker effect in occupational epidemiology. Occup Med, 1999; 49: 225-229
25) Honjo M, Nakamura K, Yamashiro K, Kiryu J, Tanihara H, McEvoy LM, Honda Y, Butcher EC, Masaki T, Sawamura T: Lectin-like oxidized LDL receptor-1 is a cell-adhesion molecule involved in endotoxin-induced inflammation. Proc Natl Acad Sci U S A, 2003; 100: 1274-1279
26) Hinagata J, Kakutani M, Fujii T, Naruko T, Inoue N, Fujita Y, Mehta JL, Ueda M, Sawamura T: Oxidized LDL receptor LOX-1 is involved in neointimal hyperplasia after balloon arterial injury in a rat model. Cardiovasc Res, 2006; 69: 263-271
27) Li L, Sawamura T, Renier G: Glucose enhances human macrophage LOX-1 expression: role for LOX-1 in glucose-induced macrophage foam cell formation. Circ Res, 2004; 94: 892-901
28) Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Dohi S, Egusa G, Hiro T, Hirobe K, Iida M, Kihara S, Kinoshita M, Maruyama C, Ohta T, Okamura T, Yamashita S, Yokode M, Yokote K; Japan Atherosclerosis Society: Executive Summary of Japan Atherosclerosis Society (JAS) Guideline for Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases for Japanese-2012 version. J Atheroscler Thromb, 2013 ; 20: 517-523

Atherosclerosis 239 (2015) 444–450

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

High-density lipoprotein particle concentration and subclinical atherosclerosis of the carotid arteries in Japanese men☆

Maryam Zaid [a], Akira Fujiyoshi [a, *], Katsuyuki Miura [a, b], Robert D. Abbott [b], Tomonori Okamura [c], Naoyuki Takashima [a], Sayuki Torii [a], Yoshino Saito [a], Takashi Hisamatsu [a, b], Naoko Miyagawa [a], Takayoshi Ohkubo [a, d], Aya Kadota [b], Akira Sekikawa [e], Hiroshi Maegawa [f], Yasuyuki Nakamura [a, g], Kenichi Mitsunami [h], Hirotsugu Ueshima [a, b], For the SESSA Research group

[a] Department of Public Health, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga, 520-2192, Japan
[b] Center for Epidemiologic Research in Asia, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga, 520-2192, Japan
[c] Department of Preventive Medicine and Public Health, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku, Tokyo, 160-8582, Japan
[d] Department of Hygiene and Public Health, Teikyo University School of Medicine, 2-11-1 Kaga Itabashi-ku, Tokyo, 173-8605, Japan
[e] Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, 130 De Soto Street, Pittsburgh, PA, 15261, USA
[f] Division of Endocrinology and Metabolism, Department of Internal Medicine, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga, Japan
[g] Department of Cardiovascular Epidemiology, Kyoto Women's University, 35 Kitahiyoshi-cho, Imakumano, Higashiyama-ku, Kyoto, 605-8501, Japan
[h] Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga, 520-2192, Japan

ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form
28 January 2015
Accepted 28 January 2015
Available online 31 January 2015

Keywords:
High-density lipoprotein (HDL) particle
High-density lipoprotein (HDL) cholesterol
Subclinical atherosclerosis
Carotid intima-media thickness (cIMT)
Plaque count

ABSTRACT

Objective: The association of high-density lipoprotein particle (HDL-P) with atherosclerosis may be stronger than that of HDL-cholesterol (HDL-C) and independent of conventional cardiovascular risk factors. Whether associations persist in populations at low risk of coronary heart disease (CHD) remains unclear. This study examines the associations of HDL-P and HDL-C with carotid intima-media thickness (cIMT) and plaque counts among Japanese men, who characteristically have higher HDL-C levels and a lower CHD burden than those in men of Western populations.

Methods: We cross-sectionally examined a community-based sample of 870 Japanese men aged 40–79 years, free of known clinical cardiovascular disease (CVD) and not on lipid-lowering medication. Participants were randomly selected among Japanese living in Kusatsu City in Shiga, Japan.

Results: Both HDL-P and HDL-C were inversely and independently associated with cIMT in models adjusted for conventional CHD risk factors, including low-density lipoprotein cholesterol (LDL-C) and diabetes. HDL-P maintained an association with cIMT after further adjustment for HDL-C ($P < 0.01$), whereas the association of HDL-C with cIMT was noticeably absent after inclusion of HDL-P in the model. In plaque counts of the carotid arteries, HDL-P was significantly associated with a reduction in plaque count, whereas HDL-C was not.

Conclusion: HDL-P, in comparison to HDL-C, is more strongly associated with measures of carotid atherosclerosis in a cross-sectional study of Japanese men. Findings demonstrate that, HDL-P is a strong correlate of subclinical atherosclerosis even in a population at low risk for CHD.

1. Introduction

Many studies have reported an inverse association between high-density lipoprotein cholesterol (HDL-C) and coronary heart disease (CHD) [1–3]. This has led to the notion that cardiovascular risk may drop significantly once HDL-C levels are increased [4]. However, recent trials involving pharmacological increases in HDL-C levels have reported no significant effects on the reduction of

☆ Institution where work was performed: Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga, 520-2192, Japan.

* Corresponding author.
E-mail address: afujiy@belle.shiga-med.ac.jp (A. Fujiyoshi).

http://dx.doi.org/10.1016/j.atherosclerosis.2015.01.031
0021-9150/

carotid intima-media thickness (cIMT) [5], the progression of coronary atherosclerosis [6], or any other cardiovascular measurement [7,8]. Also, a large mendelian randomization study has shown that some polymorphisms associated with genetically higher HDL-C levels do not lower risk of myocardial infarction [9]. Lack of improved cardiovascular outcomes with increased HDL-C has stressed the view that increasing HDL-C levels may not directly translate to decreases in cardiovascular risk [10] and, thus has led to a surge of interest in identifying other features of HDL that can be targeted for assessing cardiovascular risk.

Recently, total HDL particle (HDL-P) concentration has been shown to be a marker of reduced cardiovascular risk [11–13] and some evidence suggests that this is independent of HDL-C [12]. However, studies on HDL-P were largely limited to Western populations, which are known to have a higher risk of CHD and lower levels of HDL-C than less vulnerable regions of Asia, particularly Japan [14–16]. Whether associations persist in these regions at lower risk for CHD and with higher HDL-C levels remains unclear. Our objective is to evaluate the association of HDL-C and HDL-P with subclinical atherosclerosis in a population-based sample of Japanese men.

2. Methods

2.1. Study participants

The Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA) aims to examine various factors associated with subclinical atherosclerosis. The design of this study is described elsewhere [17]. In brief, from 2006 to 2008, 1094 Japanese men aged 40–79 years were randomly selected from the general population in Kusatsu City, Shiga, Japan. After excluding those on lipid-lowering medications (n = 168) and missing information on HDL-P, HDL-C or lipid-lowering medications (n = 56), 870 remained for analysis in the current report. All participants provided written informed consent. The study complies with *the Declaration of Helsinki* and was approved by the Institutional Review Board of Shiga University of Medical Science, Otsu, Japan.

Factors collected through physical examinations include height, weight, blood pressure, and a variety of other measures. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Blood pressure was measured twice in a seated position after a 5 min rest, using an automated sphygmomanometer (BP-8800; Omron Colin, Tokyo, Japan). The average of two measurements was used.

Hypertension was defined as systolic blood pressure (SBP) ≥140 mm Hg, diastolic blood pressure (DBP) ≥90 mm Hg, or as the use of antihypertensive medications. Diabetes mellitus (DM) was defined as a hemoglobin A1c (HbA1c) ≥6.1% (Japan Diabetes Society criteria; equivalent to HbA1c ≥ 6.5% in National Glycohemoglobin Standardization Program) [18] a fasting glucose ≥6.99 mmol/l (126 mg/dL), or the use of antidiabetic medications.

A self-administered questionnaire was used to collect data on medical history, medication use, smoking, alcohol intake, and other lifestyle behaviors with confirmation by trained technicians.

2.2. Laboratory measurements

Blood samples were drawn from participants after a 12-h fast and centrifuged soon after coagulation. Standard lipids, including total cholesterol and triglycerides (TG), were measured using enzymatic techniques. HDL-C was measured after heparin-calcium precipitation. Measurements were standardized according to guidelines from the Center for Disease Control and Prevention/Cholesterol Reference Method Laboratory Network (CDC/CRMLN) [19]. Friedewald's formula was used to estimate low-density lipoprotein cholesterol (LDL-C) levels in men with TG < 4.52 mmol/l (400 mg/dl). For higher TG levels, LDL-C was treated as missing.

HDL-P concentration was determined by nuclear magnetic resonance (NMR) spectroscopy using serum samples stored at −80 °C [20], and shipped on dry ice to LipoScience Inc (Raleigh, North Carolina, US). Concentrations were obtained from amplitudes of distinct spectroscopic NMR signals of the lipid methyl group, characteristic of each subclass. Reproducibility of NMR-measured HDL-P has been examined and measurements have a coefficient of variation <2% [21].

2.3. Intima-media thickness and plaque counts of carotid arteries

Ultrasound measurements of the carotid arteries were performed by sonographers following an established protocol of the Ultrasound Research Laboratory at the University of Pittsburgh [17,22]. A Toshiba XarioSSA-660A scanner (Toshiba Medical Systems, Japan), equipped with a 7.5 MHz linear-array imaging probe, was used for high-resolution B-mode ultrasound of the carotid arteries. Sonographers scanned both right and left carotid arteries.

In both arteries, the IMT of the common carotid artery (CCA), carotid bulb, and internal carotid artery were measured. For the CCA segment, both near and far walls were examined 1 cm proximal to the bulb. For the bulb and internal carotid artery segments, only far walls were examined. cIMT was defined as the mean of the eight IMT values measured in both arteries.

Plaque was defined as focal thickening lesion (>10% protrusion compared to adjacent areas) with an IMT of ≥1 mm. The total number of plaques in CCA, bulb, and internal carotid artery of both left and right carotid arteries were counted.

2.4. Statistical analyses

Participant demographics were described according to quartile of HDL-P and HDL-C. P-values for trend across the quartiles were determined either using linear regression when a response variable is continuous (such as age), or using logistic regression when it is categorical (such as current smoker or not).

A dose–response relationship between HDL measures and subclinical atherosclerosis was investigated by obtaining adjusted means of cIMT and plaque counts across quartiles of HDL-P and HDL-C using linear regression. We then calculated a difference in cIMT per 1 standard deviation (SD) increase in HDL-P or HDL-C, treating them as continuous variables.

For carotid plaque, we modeled plaque count as an overdispersed integer response following a negative binomial distribution. Regression coefficients have been transformed to percentages, indicating the percent reduction (or excess) in plaque counts per 1 SD increase in HDL-P or HDL-C.

In regression models, we chose the following adjusting covariates as they are established cardiovascular risk factors: age (years), SBP (mmHg), hypertension medication (yes/no), current smoker (yes/no), current alcohol intake (g/day), DM (yes/no), LDL-C (mmol/l) [this set was defined as "base covariates"] and HDL-P or HDL-C (mmol/l).

Analyses were performed using SAS version 9.3 (SAS Institute, Cary, North Carolina) and two-tailed *P*-values of <0.05 were considered significant.

3. Results

3.1. Study participants and characteristic trends with HDL-P and HDL-C

Characteristics of study participants according to quartiles of HDL-P and HDL-C are displayed in Table 1A and 1B. Mean (SD) characteristics of all participants included 63.3 (10) years for age, 834 (184)μm for cIMT and 2.4 (2.4) for plaque count (75.4% of all participants had presence of plaque ≥1). Men with higher HDL-P tended to be younger, leaner, have less prevalence of DM, and consumed more alcohol. The same was also true for HDL-C with the exception of age. Additionally, men with higher HDL-C tended to have less prevalence of hypertension and were less likely to be current smokers. Among lipids, HDL-P and HDL-C were positively related to each other. LDL-C was negatively associated with both HDL-P and HDL-C.

3.2. HDL-P and HDL-C associations with cIMT and carotid plaque

Results of quartile analyses are depicted in Fig. 1. With adjustment for base covariates, higher quartiles of HDL-P and HDL-C were both associated with smaller cIMT (panels A & B, dashed blue lines). The overall inverse relationship of HDL-P was maintained with further adjustments for HDL-C (panel A, solid red line). In contrast, the observed inverse association of HDL-C was noticeably absent after adjustments for HDL-P (panel B, solid red line). Higher quartiles of HDL-P were associated with lower mean plaque count in both models (panel C), with and without adjustments for HDL-C. Across quartiles of HDL-C, an association with plaque counts was absent (panel D).

In Table 2, a 1 SD increase in HDL-P and HDL-C was associated with 47.2 μm and 22.1 μm lower cIMT, respectively, (unadjusted models). In models adjusted for base covariates, 22.1 μm and 11.1 μm lower cIMT was estimated per 1 SD increase in HDL-P and HDL-C, respectively. After adjustment for HDL-C, the estimated cIMT differences in relation to HDL-P remained significant. In contrast, differences in cIMT with HDL-C were absent when adjusted for HDL-P.

Table 3 depicts the estimated reduction or excess in total number of carotid artery plaque counts per 1 SD increase in HDL-P and HDL-C. In unadjusted models, a 1 SD increase in HDL-P and HDL-C was associated with 20.4% and 8.8% reduction in total plaques, respectively. HDL-P was associated with significant reductions in plaque counts even after adjustment for base covariates and HDL-C. Here, a 1 SD increase in HDL-P was associated with 10.4% reduction in number of plaques in the final model adjusted for HDL-C. In contrast, HDL-C had no significant associations with carotid artery plaque in any of the adjusted models.

Table 1A
Characteristics of participants (n = 870), aged 40–79 years, across quartiles of HDL particle concentration, 2006–2008, Kusatsu, Shiga, Japan.

Characteristic	Quartile of HDL-P				*P* trend
	1	2	3	4	
Age, years	68.2 ± 8.3	64.4 ± 9.7	60.7 ± 10.2	60.0 ± 9.6	<0.001
Body mass index, kg/m^2	23.9 ± 3.2	23.1 ± 2.8	23.3 ± 3.0	23.1 ± 2.8	0.016
SBP, mmHg	138 ± 16	133 ± 19	133 ± 19	140 ± 22	0.089
Hypertension, %[a]	57.7	47.0	46.1	53.2	0.803
Diabetes, % (Type 2)	21.8	17.7	16.6	17.0	0.033
Current smoker, %	34.6	36.3	27.2	35.8	0.512
Alcohol intake (g/day)	12.3 ± 18.4	17.3 ± 20.8	23.4 ± 25.2	43.4 ± 34.1	<0.001
Triglycerides, mmol/l	1.36 ± 0.70	1.31 ± 0.74	1.38 ± 1.00	1.53 ± 1.11	0.010
LDL-C, mmol/l[b]	3.31 ± 0.87	3.33 ± 0.80	3.33 ± 0.71	3.05 ± 0.83	<0.001
HDL-C, mmol/l	1.20 ± 0.31	1.44 ± 0.34	1.62 ± 0.36	1.88 ± 0.47	<0.001

Values are mean ± SD, or % (as indicated).
SBP, systolic blood pressure; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HDL-P, HDL particle; LDL, low-density lipoprotein cholesterol.
Quartiles of HDL-P are as follows (1): 13.9–29.8 μmol/l; n = 220, (2): 29.9–33.4 μmol/l; n = 215, (3): 33.5–37.8 μmol/l; n = 217, and (4): 37.9–68.9 μmol/l; n = 218. P-values for trend were obtained using linear regression (for continuous variables) or logisitc regression (for categorical variables) as per 1 unit increase in HDL-P.
[a] Hypertension is defined as SBP ≥140 mmHg or DBP ≥90 mmHg or use of antihypertensive medication. Diabetes is defined as glycated hemoglobin ≥6.5% (NGSP) or fasting glucose ≥ 6.99 mmol/l or use of anti-diabetic medication.
[b] LDL-C was calculated by Friedewald equation. [LDL-C (mg/dl) = total cholesterol (mg/dl) - HDL cholesterol (mg/dl) -triglyceride (mg/dl)/5].

Table 1B
Characteristics of participants (n = 870), aged 40–79 years, across quartiles of HDL cholesterol concentration, 2006–2008, Kusatsu, Shiga, Japan.

Characteristic	Quartile of HDL-C				*P* trend
	1	2	3	4	
Age, years	64.1 ± 9.5	63.7 ± 9.5	62.2 ± 10.6	63.4 ± 10.3	0.129
Body mass index, kg/m^2	24.6 ± 3.0	23.9 ± 2.8	23.0 ± 2.9	21.9 ± 2.6	<0.001
SBP, mmHg	137 ± 17	136 ± 19	136 ± 18	135 ± 23	0.168
Hypertension, %[a]	55.2	50.5	56.4	41.4	0.008
Diabetes, % (Type 2)	20.8	22.0	20.5	9.5	0.001
Current smoker, %	42.5	32.7	35.0	23.3	<0.001
Alcohol intake (g/day)	18.4 ± 23.8	19.0 ± 24.2	27.9 ± 30.0	30.9 ± 30.9	<0.001
Triglycerides, mmol/l	1.91 ± 1.01	1.49 ± 1.11	1.23 ± 0.63	0.96 ± 0.43	<0.001
LDL-C, mmol/l[b]	3.36 ± 0.85	3.47 ± 0.82	3.18 ± 0.72	3.02 ± 0.79	<0.001
HDL-P, μmol/l	28.9 ± 4.6	32.5 ± 4.2	36.4 ± 5.7	39.0 ± 7.1	<0.001

Values are mean ± SD, or % (as indicated).
Quartiles of HDL-C are as follows (1): 0.67–1.19 mmol/l; n = 212, (2): 1.22–1.45 mmol/l; n = 214, (3): 1.46–1.78 mmol/l; n = 234, and (4): 1.81–3.88 mmol/l; n = 210. P-values for trend were obtained using linear regression (for continuous variables) or logisitc regression (for categorical variables) as per 1 unit increase in HDL-C.
[a] Hypertension is defined as SBP ≥140 mmHg or DBP ≥90 mmHg or use of antihypertensive medication. Diabetes is defined as glycated hemoglobin ≥6.5% (NGSP) or fasting glucose ≥ 6.99 mmol/l or use of anti-diabetic medication.
[b] LDL-C was calculated by Friedewald equation. [LDL-C (mg/dl) = total cholesterol (mg/dl) - HDL cholesterol (mg/dl) -triglyceride (mg/dl)/5].

4. Discussion

4.1. HDL and carotid atherosclerosis

In this cross-sectional study of Japanese men, free of clinical CVD and not on lipid-lowering medication, the inverse association of HDL-P with cIMT was independent of conventional cardiovascular risk factors, including HDL-C. In contrast, the association of HDL-C with cIMT was attenuated with adjustments for these factors and was absent after adjustment for HDL-P. Furthermore, higher HDL-P, but not higher HDL-C, was inversely and independently associated with lower number of carotid artery plaque after adjustment for cardiovascular risk factors. We demonstrated stronger associations of HDL-P, compared to HDL-C, with two different measures of carotid atherosclerosis (i.e. cIMT and carotid plaque) among a community-based sample of Japanese men. Whether effects of HDL-P are more noticeable in the higher ranges of HDL-C, normally thought to be atheroprotective, warrants consideration.

Our findings are consistent with those of other studies [11–13]. The Multi-Ethnic Study of Atherosclerosis (MESA) [12], in the United States, for example, reported a significant inverse association of cIMT with HDL-P, but not with HDL-C after adjustments for each other and known risk factors. The Woman's Health Study (WHS), however, did not find a significant inverse association of HDL-P with CHD [23] and instead, only confirmed the inverse association between HDL-C and cardiovascular risk. Possible explanations for the difference in findings may not only be due to the

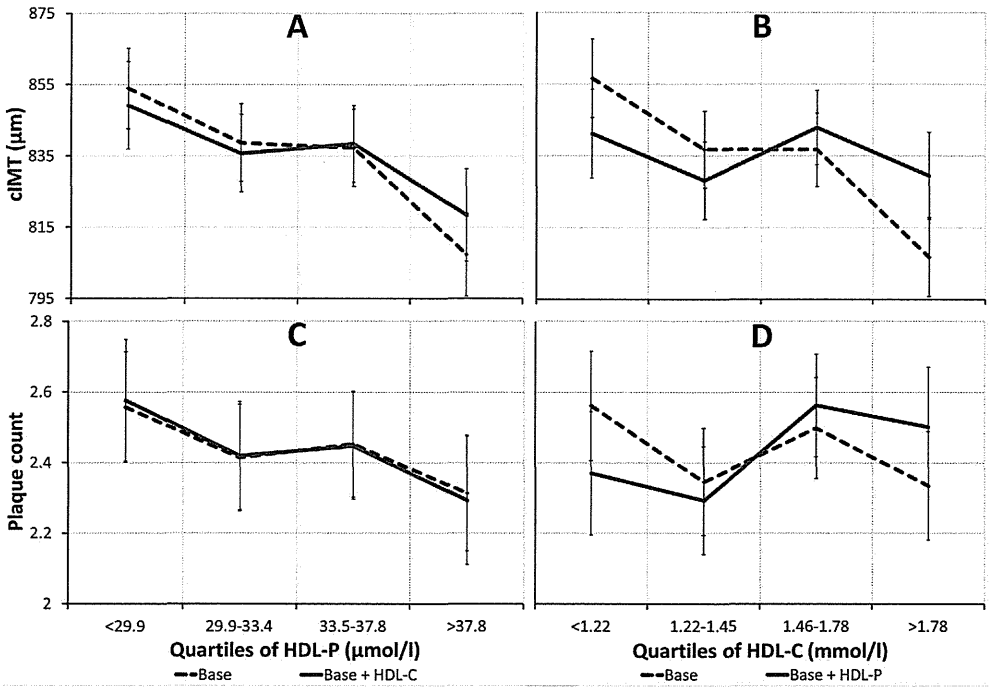


Fig. 1. Adjusted mean cIMT (n = 870) and plaque count across quartiles of HDL-P and HDL-C. Means were adjusted for base covariates [Base]: age (years), systolic blood pressure (mmHg), hypertension medication (yes/no), smoking status (yes/no), alcohol intake (g/day), diabetes (yes/no) and LDL-C (mg/dl), or adjusted for base covariates and HDL-C (mmol/l) or HDL-P (μmol/L) [Base + HDL-C or HDL-P]. P-values are for linear trend. All linear trends are significant $p < 0.05$, except for HDL-C models: Base + HDL-P ($p = 0.886$) for cIMT and Base ($p = 0.763$) and Base + HDL-P ($p = 0.187$) for plaque counts. Error bars represent standard error of mean.

Table 2
Estimated reduction (−) or excess (+) in cIMT per 1 standard deviation increase in HDL-P or HDL-C (n = 870), 2006–2008.

Parameter	Model	cIMT (μm)	95% CI	*P* value
HDL-P	Unadjusted	−47.2	−59.0, −35.3	<0.001
	Base covariates	−22.1	−34.4, −9.9	<0.001
	Base covariates + HDL-C	−22.8	−37.9, −7.7	0.003
HDL-C	Unadjusted	−22.1	−34.3, −10.0	<0.001
	Base covariates	−11.1	−22.1, 0.0	0.050
	Base covariates + HDL-P	+1.0	−12.6, +14.6	0.886

Base covariates include: age, SBP, hypertension medication (yes/no), and smoking status (yes/no), alcohol intake, LDL-C, diabetes (yes/no) and HDL-P or HDL-C. 1 standard deviation of HDL-P = 6.7 μmol/l and of HDL-C = 0.45 mmol/l.
cIMT, carotid intima-media thickness; CI, confidence interval.

sample population, but also to the randomized clinical trial study design of WHS, involving low-dose aspirin and vitamin E in primary prevention of CVD and cancer.

It is noteworthy that Japanese populations have higher HDL-C levels [16] and lower risk of CHD compared to populations of Western countries [24]. In addition, we previously reported significantly lower measurements of cIMT and higher levels of HDL-P among Japanese men compared to Caucasian men in the US [15]. Despite having a different cardiovascular risk profile, we found that in Japanese men, HDL-P, but not HDL-C, was significantly inversely associated with two measures of carotid atherosclerosis. Hence, our finding, together with results of other studies, suggests that HDL-P may be a novel marker for, and may possibly play a biological role against, the pathogenesis of atherosclerosis.

We have also analyzed HDL size subclass: small (7.3–8.2 nm), medium (8.2–8.8 nm), and large (8.8–13 nm) and their associations with cIMT and carotid plaque counts. However, we found no significant associations of any size with either measure of subclinical atherosclerosis in models adjusted for HDL-C. An atheroprotective effect of subclass size is also controversial [25] and is in need of focused attention.

Table 3
Estimated percent reduction (−) or excess (+) in total number of carotid artery plaque count per 1 standard deviation increase in HDL-P or HDL-C (n = 870), 2006–2008.

Parameter	Model	Estimate (%)	95% CI	*P* value
HDL-P	Unadjusted	−20.4	−27.4, −13.5	<0.001
	Base covariates	−7.8	−15.1, −0.5	0.037
	Base covariates + HDL-C	−10.4	−19.7, −1.1	0.029
HDL-C	Unadjusted	−8.8	−15.7, −1.9	0.012
	Base covariates	−2.0	−8.5, +4.5	0.552
	Base covariates + HDL-P	+3.7	−4.5, +11.9	0.380

Base covariates include: age, SBP, hypertension medication (yes/no), and smoking status (yes/no), alcohol intake, LDL-C, diabetes (yes/no) and HDL-P or HDL-C. 1 standard deviation of HDL-P = 6.7 μmol/l and of HDL-C = 0.45 mmol/l.

4.2. Potential mechanisms

The failure of recent randomized controlled trials on HDL-C-increasing drugs for CVD prevention resulted in questioning a causal protective role of HDL-C, which may only be an indicator of cardioprotective mechanisms at work. Nevertheless, the cardioprotective association of HDL is far from being ruled out. It has been suggested that increased particle concentrations of HDL may be indicative of higher reverse cholesterol transport activity [12]. The reverse cholesterol transport pathway mediates the efflux of cholesterol from peripheral cells to the liver [26] which is believed to be a key process in preventing plaque formation and progression [27] and, thus, many CVDs. Indeed, macrophage-specific cholesterol efflux was found to have a strong inverse association with cIMT and CHD [28]. Furthermore, recent studies have found that HDL-P, and not HDL-C, concentrations are positively associated with cholesterol efflux in patients with type 2 diabetes [29] and

patients undergoing coronary angiography [30]. These findings parallel our results, with total HDL-P having inverse associations with cIMT. It may be that serum HDL-P concentration is more closely related to the performance rate of cholesterol efflux in the reverse cholesterol transport pathway than HDL-C, with more particles being analogous to increased pathway activity. How HDL affects the cholesterol transport and protects against CVD may depend on its structure and composition, leading to a variety of biological activities, such as anti-inflammation, antioxidation, and vasodilation [25], all of which cannot be assessed by HDL-C alone [26, 31].

4.3. Limitations and strengths

As our study is cross-sectional and observational, causality cannot be proven in the associations of HDL-P with cIMT and carotid plaque counts. Other limits of our study include the study population being restricted to men of a single ethnic group. However, this is not without its advantage, as homogeneity in a population minimizes confounding from genetic variation. The size of carotid plaques was also not taken into account. Total plaque count may not define the grade or vulnerability of plaques, nevertheless it has been reported that the presence of plaque, alone, in the carotid arteries is positively associated with increased risk of cardiovascular events [36]. Thus, carotid plaque count can be used as an alternate indicator of subclinical atherosclerosis [37]. The main protein component of HDL particle, apolipoprotein A-1, is a strong predictor of CHD and a key player in reverse cholesterol transport [32,33]. Unfortunately, apolipoprotein A-1 levels were not measured in our serum samples and thus we were unable to look at possible confirmatory associations of HDL-P with cIMT and plaque.

This is the first study, of which we are aware of, to report a significant and inverse association of HDL-P, and not HDL-C, with plaque count in the carotid arteries, even after adjustments for conventional risk factors. This finding, as well as the confirmatory finding of cIMT associations, is in agreement with most literature published on HDL-P and both clinical CVD and subclinical atherosclerosis [11,12,38]. The fact that we could identify a relationship of HDL-P with cIMT and plaque in a population at low risk of CHD, indicates that HDL-P may be an important predictor of subclinical atherosclerosis and perhaps even more so in populations at high risk. Presently, there are few population-based studies on HDL-P, let alone any on a Japanese population, that characteristically has higher serum HDL-C levels compared to those of Western populations. Thus, our findings provide additional information to the current modest body of knowledge in this area.

5. Conclusion

In a community-based sample of Japanese men, free of clinical CVD, HDL-P was associated with measures of carotid atherosclerosis (cIMT and plaque count) independent of lipids or lipoproteins and other traditional CVD risk factors. In contrast, associations with HDL-C were absent after accounting for HDL-P. There is need for more scrutiny towards the properties of HDL in general, in order to better understand its involvement in CVD risk processes.

Funding

This work was supported by Grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology Japan [(A) 13307016, (A) 17209023, (A) 21249043, (A) 23249036, and (A) 25253046]; Glaxo-Smith Klein; and by National Institutes of Health (NIH), USA [R01HL068200].

Research was supported in part by Ichiro Kanehara Foundation Scholarship 12RY006 for Foreign Nationals in Japan [to MZ], for the 2013 fiscal year.

The SESSA research group

Chairperson: Hirotsugu Ueshima (Center for Epidemiologic Research in Asia, Department of Public Health, Shiga University of Medical Science, Otsu, Shiga).

Co-chairperson: Katsuyuki Miura (Department of Public Health, Shiga University of Medical Science, Otsu, Shiga).

Research members: Minoru Horie, Yasutaka Nakano, Takashi Yamamoto (Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu, Shiga), Emiko Ogawa (Health Administration Center, Shiga University of Medical Science, Otsu, Shiga), Hiroshi Maegawa, Itsuko Miyazawa (Division of Endocrinology and Metabolism, Department of Medicine, Shiga University of Medical Science, Otsu, Shiga), Kiyoshi Murata (Department of Radiology, Shiga University of Medical Science, Otsu, Shiga), Kenichi Mitsunami (Shiga University of Medical Science, Otsu, Shiga), Kazuhiko Nozaki (Department of Neurosurgery, Shiga University of Medical Science, Otsu, Shiga), Akihiko Shiino (Biomedical MR Science Center, Shiga University of Medical Science, Otsu, Shiga), Isao Araki (Kusatsu Public Health Center, Kusatsu, Shiga) Teruhiko Tsuru (Department of Urology, Shiga University of Medical Science, Otsu, Shiga), Ikuo Toyama (Unit for Neuropathology and Diagnostics, Molecular Neuroscience Research Center, Shiga University of Medical Science, Otsu, Shiga), Hisakazu Ogita, Souichi Kurita (Division of Medical Biochemistry, Department of Biochemistry and Molecular Biology, Shiga University of Medical Science, Otsu, Shiga), Toshinaga Maeda (Central Research Laboratory, Shiga University of Medical Science, Otsu, Shiga), Naomi Miyamatsu (Department of Clinical Nursing Science Lecture, Shiga University of Medical Science, Otsu, Shiga), Toru Kita (Kobe City Medical Center General Hospital, Kobe, Hyogo), Takeshi Kimura (Department of Cardiovascular Medicine, Kyoto University, Kyoto), Yoshihiko Nishio (Department of Diabetes, Metabolism, and Endocrinology, Kagoshima University, Kagoshima), Yasuyuki Nakamura (Department of Living and Welfare, and Cardiovascular Epidemiology, Kyoto Women's University, Kyoto), Tomonori Okamura (Department of Preventive Medicine and Public Health, School of Medicine, Keio University, Tokyo), Akira Sekikawa, Emma JM Barinas-Mitchell (Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA), Daniel Edmundowicz (Department of Medicine, Section of Cardiology, School of Medicine, Temple University, Philadelphia, PA, USA), Takayoshi Ohkubo (Department of Hygiene and Public Health, Teikyo University School of Medicine, Tokyo), Atsushi Hozawa (Preventive Medicine, Epidemiology Section, Tohoku University, Tohoku Medical Megabank Organization, Sendai, Miyagi), Nagako Okuda (Department of Health and Nutrition, University of Human Arts and Sciences, Saitama, Japan), Aya Kadota (Department of Public Health, Shiga University of Medical Science, Otsu, Shiga), Aya Higashiyama (Research and Development Initiative Center, National Cerebral and Cardiovascular Center, Suita, Japan), Shinya Nagasawa (Department of Epidemiology and Public Health, Kanazawa Medical University, Kanazawa, Ishikawa), Yoshikuni Kita(Tsuruga Nursing University), Akira Fujiyoshi, Naoyuki Takashima, Takashi Kadowaki, Sayaka Kadowaki (Department of Public Health, Shiga University of Medical Science, Otsu, Shiga), Yoshitaka Murakami (Department of Medical Statistics, Faculty of Medicine, Toho University, Tokyo, Japan), Robert D. Abbott, Seiko Ohno (Center for Epidemiologic Research in Asia, Shiga University of Medical Science, Otsu, Shiga), Takashi Hisamatsu (Center for Epidemiologic Research in Asia, Department of Public Health, Shiga University of Medical Science, Otsu, Shiga), Naoko Miyagawa, Sayuki Torii, and

Yoshino Saito (Department of Public Health, Shiga University of Medical Science, Otsu, Shiga).

Conflict of interest

None declared.

Acknowledgments

None.

References

[1] Emerging Risk Factors C, E. Di Angelantonio, N. Sarwar, P. Perry, S. Kaptoge, K.K. Ray, A. Thompson, A.M. Wood, S. Lewington, N. Sattar, C.J. Packard, R. Collins, S.G. Thompson, J. Danesh, Major lipids, apolipoproteins, and risk of vascular disease, JAMA 302 (18) (2009) 1993–2000.

[2] D.J. Gordon, J.L. Probstfield, R.J. Garrison, J.D. Neaton, W.P. Castelli, J.D. Knoke, D.R. Jacobs Jr., S. Bangdiwala, H.A. Tyroler, High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies, Circulation 79 (1) (1989) 8–15.

[3] A.R. Sharrett, C.M. Ballantyne, S.A. Coady, G. Heiss, P.D. Sorlie, D. Catellier, W. Patsch, Atherosclerosis Risk in Communities Study G, Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: the Atherosclerosis Risk in Communities (ARIC) Study, Circulation 104 (10) (2001) 1108–1113.

[4] P. Libby, The forgotten majority: unfinished business in cardiovascular risk reduction, J. Am. Coll. Cardiol. 46 (7) (2005) 1225–1228.

[5] M.L. Bots, F.L. Visseren, G.W. Evans, W.A. Riley, J.H. Revkin, C.H. Tegeler, C.L. Shear, W.T. Duggan, R.M. Vicari, D.E. Grobbee, J.J. Kastelein, Torcetrapib and carotid intima-media thickness in mixed dyslipidaemia (RADIANCE 2 study): a randomised, double-blind trial, Lancet 370 (9582) (2007) 153–160.

[6] S.E. Nissen, J.C. Tardif, S.J. Nicholls, J.H. Revkin, C.L. Shear, W.T. Duggan, W. Ruzyllo, W.B. Bachinsky, G.P. Lasala, E.M. Tuzcu, Effect of torcetrapib on the progression of coronary atherosclerosis, N. Engl. J. Med. 356 (13) (2007) 1304–1316.

[7] W.E. Boden, J.L. Probstfield, T. Anderson, B.R. Chaitman, P. Desvignes-Nickens, K. Koprowicz, R. McBride, K. Teo, W. Weintraub, Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy, N. Engl. J. Med. 365 (24) (2011) 2255–2267.

[8] G.G. Schwartz, A.G. Olsson, M. Abt, C.M. Ballantyne, P.J. Barter, J. Brumm, B.R. Chaitman, I.M. Holme, D. Kallend, L.A. Leiter, E. Leitersdorf, J.J. McMurray, H. Mundl, S.J. Nicholls, P.K. Shah, J.C. Tardif, R.S. Wright, Effects of dalcetrapib in patients with a recent acute coronary syndrome, N. Engl. J. Med. 367 (22) (2012) 2089–2099.

[9] B.F. Voight, G.M. Peloso, M. Orho-Melander, R. Frikke-Schmidt, M. Barbalic, M.K. Jensen, G. Hindy, H. Holm, E.L. Ding, T. Johnson, H. Schunkert, N.J. Samani, R. Clarke, J.C. Hopewell, J.F. Thompson, M. Li, G. Thorleifsson, C. Newton-Cheh, K. Musunuru, J.P. Pirruccello, D. Saleheen, L. Chen, A. Stewart, A. Schillert, U. Thorsteinsdottir, G. Thorgeirsson, S. Anand, J.C. Engert, T. Morgan, J. Spertus, M. Stoll, K. Berger, N. Martinelli, D. Girelli, P.P. McKeown, C.C. Patterson, S.E. Epstein, J. Devaney, M.S. Burnett, V. Mooser, S. Ripatti, I. Surakka, M.S. Nieminen, J. Sinisalo, M.L. Lokki, M. Perola, A. Havulinna, U. de Faire, B. Gigante, E. Ingelsson, T. Zeller, P. Wild, P.I. de Bakker, O.H. Klungel, A.H. Maitland-van der Zee, B.J. Peters, A. de Boer, D.E. Grobbee, P.W. Kamphuisen, V.H. Deneer, C.C. Elbers, N.C. Onland-Moret, M.H. Hofker, C. Wijmenga, W.M. Verschuren, J.M. Boer, Y.T. van der Schouw, A. Rasheed, P. Frossard, S. Demissie, C. Willer, R. Do, J.M. Ordovas, G.R. Abecasis, M. Boehnke, K.L. Mohlke, M.J. Daly, C. Guiducci, N.P. Burtt, A. Surti, E. Gonzalez, S. Purcell, S. Gabriel, J. Marrugat, J. Peden, J. Erdmann, P. Diemert, C. Willenborg, I.R. Konig, M. Fischer, C. Hengstenberg, A. Ziegler, I. Buysschaert, D. Lambrechts, F. Van de Werf, K.A. Fox, N.E. El Mokhtari, D. Rubin, J. Schrezenmeir, S. Schreiber, A. Schafer, J. Danesh, S. Blankenberg, R. Roberts, R. McPherson, H. Watkins, A.S. Hall, K. Overvad, E. Rimm, E. Boerwinkle, A. Tybjaerg-Hansen, L.A. Cupples, M.P. Reilly, O. Melander, P.M. Mannucci, D. Ardissino, D. Siscovick, R. Elosua, K. Stefansson, C.J. O'Donnell, V. Salomaa, D.J. Rader, L. Peltonen, S.M. Schwartz, D. Altshuler, S. Kathiresan, Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study, Lancet 380 (9841) (2012) 572–580.

[10] P.P. Toth, P.J. Barter, R.S. Rosenson, W.E. Boden, M.J. Chapman, M. Cuchel, R.B. D'Agostino Sr., M.H. Davidson, W.S. Davidson, J.W. Heinecke, R.H. Karas, A. Kontush, R.M. Krauss, M. Miller, D.J. Rader, High-density lipoproteins: a consensus statement from the National Lipid Association, J. Clin. Lipidol. 7 (5) (2013) 484–525.

[11] K. El Harchaoui, B.J. Arsenault, R. Franssen, J.P. Despres, G.K. Hovingh, E.S. Stroes, J.D. Otvos, N.J. Wareham, J.J. Kastelein, K.T. Khaw, S.M. Boekholdt, High-density lipoprotein particle size and concentration and coronary risk, Ann. Intern. Med. 150 (2) (2009) 84–93.

[12] R.H. Mackey, P. Greenland, D.C. Goff Jr., D. Lloyd-Jones, C.T. Sibley, S. Mora, High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (Multi-Ethnic study of atherosclerosis), J. Am. Coll. Cardiol. 60 (6) (2012) 508–516.

[13] S. Mora, R.J. Glynn, P.M. Ridker, High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy, Circulation 128 (11) (2013) 1189–1197.

[14] A. Sekikawa, K. Miura, S. Lee, A. Fujiyoshi, D. Edmundowicz, T. Kadowaki, R.W. Evans, S. Kadowaki, K. Sutton-Tyrrell, T. Okamura, M. Bertolet, K.H. Masaki, Y. Nakamura, E.J. Barinas-Mitchell, B.J. Willcox, A. Kadota, T.B. Seto, H. Maegawa, L.H. Kuller, H. Ueshima, Group EJS, Long chain n-3 polyunsaturated fatty acids and incidence rate of coronary artery calcification in Japanese men in Japan and white men in the USA: population based prospective cohort study, Heart 100 (7) (2014) 569–573.

[15] A. Sekikawa, H. Ueshima, K. Sutton-Tyrrell, T. Kadowaki, A. El-Saed, T. Okamura, T. Takamiya, Y. Ueno, R.W. Evans, Y. Nakamura, D. Edmundowicz, A. Kashiwagi, H. Maegawa, L.H. Kuller, Intima-media thickness of the carotid artery and the distribution of lipoprotein subclasses in men aged 40 to 49 years between whites in the United States and the Japanese in Japan for the ERA JUMP study, Metabolism 57 (2) (2008) 177–182.

[16] H. Ueshima, M. Iida, T. Shimamoto, M. Konishi, M. Tanigaki, N. Nakanishi, Y. Takayama, H. Ozawa, S. Kojima, Y. Komachi, High-density lipoprotein-cholesterol levels in Japan, JAMA 247 (14) (1982) 1985–1987.

[17] A. Kadota, K. Miura, T. Okamura, A. Fujiyoshi, T. Ohkubo, T. Kadowaki, N. Takashima, T. Hisamatsu, Y. Nakamura, F. Kasagi, H. Maegawa, A. Kashiwagi, H. Ueshima, Carotid Intima-Media thickness and plaque in apparently healthy Japanese individuals with an estimated 10-Year absolute risk of CAD death according to the Japan atherosclerosis society (JAS) guidelines 2012: the shiga epidemiological study of subclinical atherosclerosis (SESSA), J. Atheroscler. Thromb. 20 (10) (2013) 755–766.

[18] A.K.M. Kashiwagi, E. Araki, Y. Oka, T. Hanafusa, H. Ito, M. Tominaga, S. Oikawa, M. Noda, T. Kawamura, T. Sanke, M. Namba, M. Hashiramoto, T. Sasahara, Y. Nishio, K. Kuwa, K. Ueki, I. Takei, M. Umemoto, M. Murakami, M. Yamakado, Y. Yatomi, H. Ohashi, Committee on the Standardization of Diabetes Mellitus-Related Laboratory Testing of Japan Diabetes Society, International clinical harmonization of glycated hemoglobin in Japan: from Japan diabetes society to national glycohemoglobin standardization program values, J. Diabetes Investig. 3 (2012) 39–40.

[19] M. Nakamura, S. Sato, T. Shimamoto, Improvement in Japanese clinical laboratory measurements of total cholesterol and HDL-cholesterol by the US cholesterol reference method laboratory network, J. Atheroscler. Thromb. 10 (3) (2003) 145–153.

[20] J.D. Otvos, Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy, Clin. Lab. 48 (3–4) (2002) 171–180.

[21] E.J. Jeyarajah, W.C. Cromwell, J.D. Otvos, Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy, Clin. Lab. Med. 26 (4) (2006) 847–870.

[22] K. Sutton-Tyrrell, S.K. Wolfson Jr., T. Thompson, S.F. Kelsey, Measurement variability in duplex scan assessment of carotid atherosclerosis, Stroke 23 (2) (1992) 215–220.

[23] S. Mora, J.D. Otvos, N. Rifai, R.S. Rosenson, J.E. Buring, P.M. Ridker, Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women, Circulation 119 (7) (2009) 931–939.

[24] H. Ueshima, A. Sekikawa, K. Miura, T.C. Turin, N. Takashima, Y. Kita, M. Watanabe, A. Kadota, N. Okuda, T. Kadowaki, Y. Nakamura, T. Okamura, Cardiovascular disease and risk factors in Asia: a selected review, Circulation 118 (25) (2008) 2702–2709.

[25] L. Camont, M.J. Chapman, A. Kontush, Biological activities of HDL subpopulations and their relevance to cardiovascular disease, Trends Mol. Med. 17 (10) (2011) 594–603.

[26] K.C. Vickers, A.T. Remaley, HDL and cholesterol: life after the divorce? J Lipid Res. 55 (1) (2014 Jan) 4–12.

[27] G.H. Rothblat, M.C. Phillips, High-density lipoprotein heterogeneity and function in reverse cholesterol transport, Curr. Opin. Lipidol. 21 (3) (2010) 229–238.

[28] A.V. Khera, M. Cuchel, M. de la Llera-Moya, A. Rodrigues, M.F. Burke, K. Jafri, B.C. French, J.A. Phillips, M.L. Mucksavage, R.L. Wilensky, E.R. Mohler, G.H. Rothblat, D.J. Rader, Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis, N. Engl. J. Med. 364 (2) (2011) 127–135.

[29] H.C. Tan, E.S. Tai, D. Sviridov, P.J. Nestel, C. Ng, E. Chan, Y. Teo, D.C. Wai, Relationships between cholesterol efflux and high-density lipoprotein particles in patients with type 2 diabetes mellitus, J. Clin. Lipidol. 5 (6) (2011) 467–473.

[30] P. Linsel-Nitschke, H. Jansen, Z. Aherrarhou, S. Belz, B. Mayer, W. Lieb, F. Huber, W. Kremer, H.R. Kalbitzer, J. Erdmann, H. Schunkert, Macrophage cholesterol efflux correlates with lipoprotein subclass distribution and risk of obstructive coronary artery disease in patients undergoing coronary angiography, Lipids Health Dis. 8 (2009) 14.

[31] R.S. Rosenson, J.D. Otvos, D.S. Freedman, Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial, Am. J. Cardiol. 90 (2) (2002) 89–94.

[32] M. Andrikoula, I.F. McDowell, The contribution of ApoB and ApoA1 measurements to cardiovascular risk assessment, Diabetes Obes. Metab. 10 (4) (2008) 271–278.

[33] T. O'Brien, T.T. Nguyen, B.J. Hallaway, D. Hodge, K. Bailey, D. Holmes, B.A. Kottke, The role of lipoprotein A-I and lipoprotein A-I/A-II in predicting

coronary artery disease, Arterioscler. Thromb. Vasc. Biol. 15 (2) (1995) 228–231.
[36] J.F. Polak, M. Szklo, R.A. Kronmal, G.L. Burke, S. Shea, A.E. Zavodni, D.H. O'Leary, The value of carotid artery plaque and intima-media thickness for incident cardiovascular disease: the multi-ethnic study of atherosclerosis, J. Am. Heart Assoc. 2 (2) (2013) e000087.
[37] P.J. Touboul, M.G. Hennerici, S. Meairs, H. Adams, P. Amarenco, N. Bornstein, L. Csiba, M. Desvarieux, S. Ebrahim, R. Hernandez Hernandez, M. Jaff, S. Kownator, T. Naqvi, P. Prati, T. Rundek, M. Sitzer, U. Schminke, J.C. Tardif, A. Taylor, E. Vicaut, K.S. Woo, Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011, Cerebrovasc. Dis. 34 (4) (2012) 290–296.
[38] S. Parish, A. Offer, R. Clarke, J.C. Hopewell, M.R. Hill, J.D. Otvos, J. Armitage, R. Collins, Lipids and lipoproteins and risk of different vascular events in the MRC/BHF heart protection study, Circulation 125 (20) (2012) 2469–2478.

最新醫學 別冊　診断と治療のABC 101 （別刷）

高LDL-C血症・低HDL-C血症
第3章　診断

LDL-C測定法の現状と将来の課題

三井田　孝

最新医学社

第3章　診 断

LDL-C 測定法の現状と将来の課題

三井田 孝　順天堂大学大学院 臨床病態検査医学 教授

要旨

LDL-C は，動脈硬化性疾患の最も重要な危険因子である．LDL-C の測定は，脂質異常症をスクリーニングするだけでなく，動脈硬化性疾患を合併した患者のリスク管理の目的でも行われる．臨床では，1970 年代に Friedewald が提唱した計算法で LDL-C を求めてきた．計算式の変法も複数報告されている．1990 年代に登場した LDL-C 直接法は，食後検体でも測定できる利点があり，次第に普及した．計算法と直接法の正確性を中心に，LDL-C 測定法の現状と課題について概説する．

はじめに

低比重リポタンパク（LDL）は，コレステロールの含量が約 50 % を占め，全リポタンパク分画の中で，最もコレステロールに富むリポタンパクである．LDL は，前駆体である超低比重リポタンパク（VLDL）として，肝臓から血中に分泌される．血中の VLDL は，血管内皮に結合しているリポタンパクリパーゼによってトリグリセライド（triglyceride ： TG）が水解され，粒子サイズと TG 含量が減った中間比重リポタンパク（IDL）になる．IDL は，一部が肝臓に再び取り込まれ，一部が肝性リパーゼによってさらに TG が水解され，最終的に LDL（狭義）となる．LDL という名称は，リポタンパクを分離する超遠心法で用いられる名称である．一般に LDL と呼ぶ場合は，比重が 1.006 ～ 1.063 の分画に浮上する広義の LDL を意味し，IDL（比重 1.006 ～ 1.019）と狭義の LDL（比重 1.019 ～ 1.063）が含まれる．LDL 分画中のコレステロール含量が LDL の量と良好な相関を示すため，臨床的には LDL-コレステロール（LDL-C）が

● キーワード
LDL-コレステロール　ホモジニアス法　BQ 法　脂質標準化プログラム

LDLの量的指標として使われている．

多くの疫学研究と薬物介入研究により，LDL-Cが動脈硬化性疾患（特に冠動脈疾患）の重要な危険因子であることが示されている．正常のLDLは，コレステロールを必要とする末梢組織の細胞に，LDL受容体を介して取り込まれる．コレステロールが十分細胞内にあるときは，LDL受容体がネガティブフィードバックを受けて細胞表面から減少し，無制限にLDLが細胞内に取り込まれることはない．一方，LDLは一部が血管内皮下で酸化変性を受ける（変性LDL）．この変性LDLはLDL受容体に認識されず，スカベンジャー受容体を介してマクロファージに取り込まれる．スカベンジャー受容体にはLDL受容体のような負のフィードバック機構がないため，変性LDLが存在するとマクロファージはやがて泡沫細胞となる．このように動脈硬化の初期病変ができると，その後にさまざまな細胞の遊走や増殖が起きて，動脈硬化性病変が完成する．

LDL-Cは，動脈硬化性疾患の1次および2次予防のため，幅広い対象で測定される．一般には，Friedewaldらが報告した計算式[1]か，LDL-C直接法（ホモジニアス法とも呼ばれる）が最もよく使用される．2010年にMillerらは，LDL-Cのホモジニアス法は，患者検体において正確性に問題があると報告し[2]，臨床の現場に大きな混乱が生じた．その後に我が国でも同様の検討が行われた．そこで本稿では，LDL-C測定法の現状と，将来の課題について述べる．

LDL-Cの測定法と正確性

日常臨床では，LDL-Cは，計算法，直接法，アガロース電気泳動を利用した定量法（保険収載名「コレステロール分画」）[3]，陰イオン交換カラムを用いた高速液体クロマトグラフィー（high performance liquid chromatography：HPLC）法（2014年に保険収載「リポタンパク分画（HPLC法）」）[4]で測定できる．実験室レベルでは，超遠心法を用いてLDL-Cが定量される．超遠心法は古典的でスタンダードなリポタンパクの分離法だが，時間と手間がかかり，必要検体量も多いことから，特殊な検体でのみLDL-C測定に用いられる場合が多い．空腹時採血が可能な場合（入院患者など）には計算法が，食後採血が多い職場健診や特定健診では直接法が用いられる傾向がある．また，LDL-Cの標準化には，超遠心法と沈殿法を組み合わせた方法が採用されている．

1．計算法

1972年に，Friedewaldらによって最初に報告された方法[1]で，十分な絶食後の空腹時に採血し，総コレステロール（total cholesterol：TC），TG，HDL-コレステロール（HDL-C）を測定して，LDL-Cを計算式（F式）から求める［LDL-C＝TC－

表1 LDL-C の計算法

報告者	計算式
Friedewald W T, et al[1]	=TC－(TG / 5)－HDL-C
Hata Y, et al[5]	=TC－(TG / K*)－HDL-C
Vujovic A, et al[6]	=TC－(TG / 6.58)－HDL-C
Chen Y, et al[7]	= (0.9×non HDL-C)－(0.1×TG)
Anandaraja S, et al[8]	= (0.9×TC)－(0.9×TG / 5)－28
Martin S S, et al[9]	=TC－(TG / AF**)－HDL-C

* Kの値は，TG値により変更．
K=3（TG＜150 mg/dL），K=4（150≦TG＜300 mg/dL），
K=5（300≦TG≦400 mg/dL）
** AF（adjustable factor）は，TGとnon HDL-Cの値で細分化したグループのメディアンを使用．

（TG／5）－HDL-C］．F式は，（1）絶食時間が10～12時間以上，（2）TGが400 mg/dL未満，という条件を満たす場合にしか適用できない．これは，TG／5が，VLDL-コレステロール（VLDL-C）にほぼ等しいことを利用している．しかし，TGが400 mg/dL未満であっても，TGが200 mg/dLを越えるあたりから，超遠心法で定量したLDL-CよりF式で求めたLDL-Cの方が低くなる傾向がある．そこで，TGの係数を変えたさまざまな変法が報告されている（表1）[5~9]．Hataら[5]は，F式のTGを割る係数を，TGの値により変える計算式を提唱したが，一般的に使用されるには至っていない．

最近Martin[9]らは，TGとnon HDL-Cの値で規定される180のグループに分け，各グループのTG／VLDL-C比のメディアンを記載した表を示した．対象者が属するグループのメディアンでTG値を割り，これをVLDL-Cとみなしてを求める計算式を提唱した．別の研究グループにより検証が行われ，F式はLDL-Cが100 mg/dLを超える場合に，Martinの式はLDL-Cが100 mg/dL未満の場合に，それぞれ他法より基準法であるBQ法（β-quantification method）（後述）の値に近かった[10]．特にLDL-Cが70 mg/dL未満の場合にMartinの式が優っていた．しかし，LDL-Cの計算が煩雑であることと，これまでの多くの研究がF式を使っていることより，著者らは臨床でF式をこの方法に置き換えるメリットがあるか疑問を呈している．ほかの方法も，F式をしのぐ明らかなメリットはない[11]．

2．BQ法

BQ法は，Lipid Research Clinics Coronary Primary Prevention Trial（LRC-CPPT）

で用いられたLDLの定量法を基本としており，世界的なLDL-Cの標準化プログラムで基準測定法として採用されている[12]．簡潔に言えば，超遠心法と沈殿法を組み合わせてLDL-Cを測定する方法である．最初に，超遠心法で比重＜1.006のカイロミクロンとVLDLを除去して分画①を得る．次に，分画①にヘパリン-Mn^{2+}を添加し，LDLを沈殿させて分画②を回収する．分画①にはLDLとHDLが，分画②にはHDLが含まれるため，この2つの分画のコレステロールをAbell-Kendall法[13]で定量し，①－②からLDL-Cを求める．BQ法で超遠心法を沈殿法と組み合わせて用いる理由は，TGが高い検体では，ヘパリン-Mn^{2+}だけでカイロミクロン，VLDL，LDL，のすべてを沈殿させることが困難だからである．コレステロールの定量に使われているAbell-Kendall法は，化学的にコレステロールを定量する方法である．しかし，安定した値を得るには高度な技術を要するため，精度よく正確に実施できる施設は，世界的にも限定されている．

コレステロールのように，化学的に純品が入手できる項目と異なり，LDL-Cには絶対的な標準物質となる純品はない．そこで，米国の疾病対策予防センター（Centers for Disease Control and Prevention：CDC）を中心とする世界7ヵ国，9つの脂質基準分析室からなるCholesterol Reference Method Laboratory Network（CRMLN）が，LDL-Cの標準化プログラムを提供している．我が国では，1992年7月から2012年3月31日まで，大阪府立健康科学センターにCRMLNの脂質基準分析室が設置されていた．2012年4月1日以降は，脂質基準分析室は国立循環器病研究センターへ移り，脂質標準化事業を継続している．CRMLNによるLDL-Cの標準化には，BQ法が用いられている．CRMLNの分析室は，CDCから送られてくる管理血清を定期的に測定し，一定の基準を満たす精密度と正確度を保持していることを求められている．我が国の脂質基準分析室でBQ法を用いて測定されたLDL-CとHDL-Cが，CDCで同様に測定された値とどのような関係であったのかという，過去15年間のデータが発表された[14]．LDL-CとHDL-Cのいずれも，両施設の値の間に$R^2=0.997$の非常に良好な相関があった．BQ法は，後述する直接法の試薬の認証試験の基準法になっている．しかし，BQ法で測定したLDL-Cにも，潜在的な誤差がある点は心に留めておく必要がある．

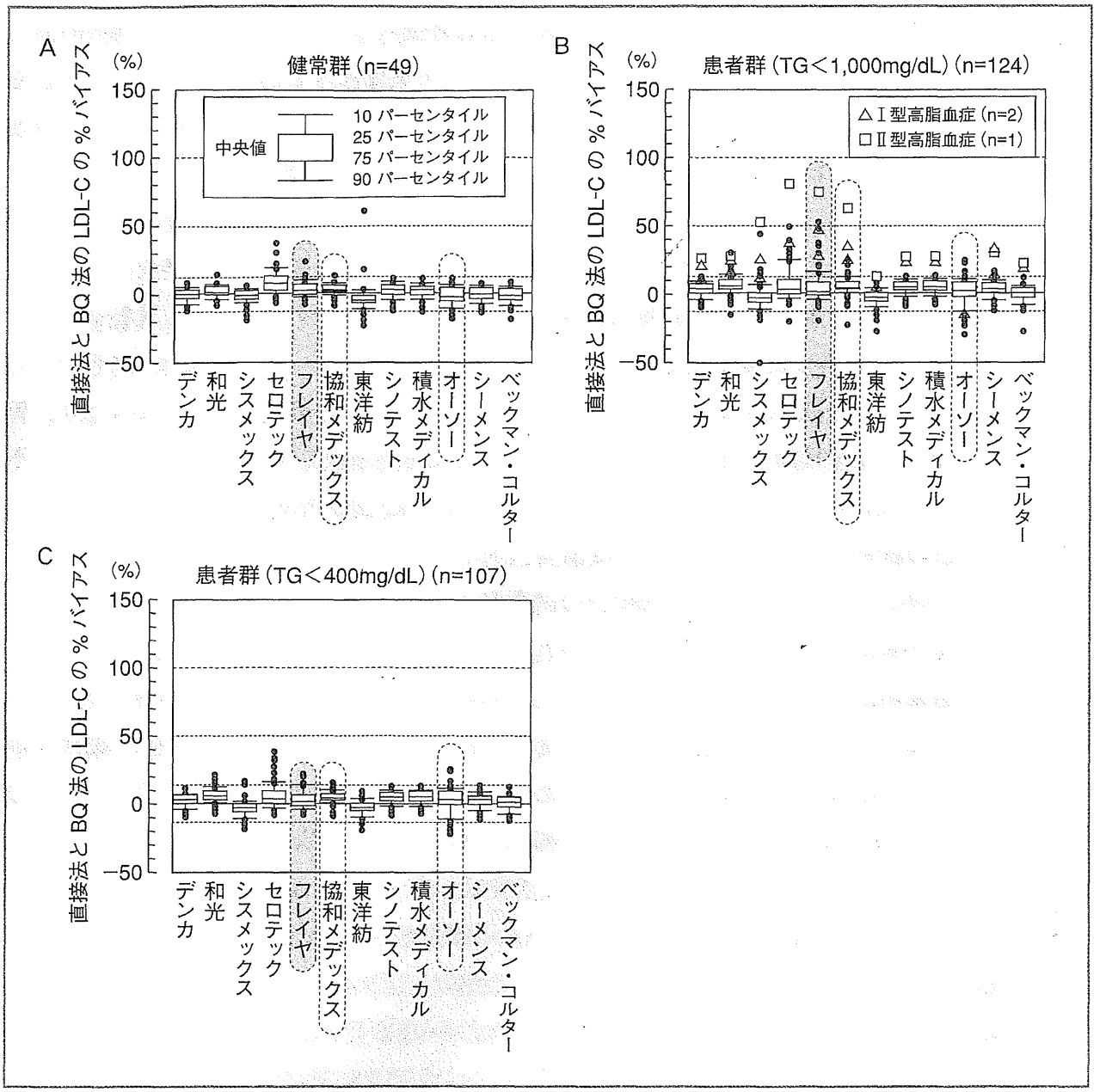
3．直接法（ホモジニアス法）

LDL-C直接法は，沈殿法のような前処理を必要とせず，血清を検体として直接大型自動分析器でLDL-Cを測定できる方法である．1990年代後半に，我が国の試薬メーカーが初めて直接法を発表した．一般臨床で広く用いられるようになったのは，2007年に動脈硬化性疾患予防ガイドラインが改訂されてLDL-C値で高コレステロール血症の診断や管理を行うよう勧告されたことと，2008年に特定健康診査・特定保健

指導（特定健診）が始まり，必須項目にLDL-Cが採用されて以降である．LDL-C直接法の測定原理は，選択的消去法と選択的阻害法の2つに大別できる．選択的消去法では，最初にLDL以外のリポタンパクのコレステロールを消去し，続いてLDLを界面活性剤で可溶化してLDLのコレステロールを定量する．選択的阻害法では，最初にLDL以外のリポタンパクが可溶化しないように保護し，続いてLDLを可溶化してコレステロールを定量する．現時点で直接法の試薬を販売している11社のうち，協和メデックス，積水メディカル，和光，デンカ生研の4社で，LDL-C直接法の市場シェアの約90％を占めている．直接法に共通するのは，新鮮血清とは異なる試料を用いると，LDLに対する試薬の反応性が変わることである（マトリックス効果）．そのため，超遠心などで分離したリポタンパクを使ってLDL-C直接法の反応性を調べたり，管理血清による全国的な精度管理調査を行って施設間差を検証することができない．そこで，CLMLNのLDL-C標準化プログラムでは，BQ法を基準測定法として，LDL-C直接法の試薬の認証試験を行っている．

2010年，米国からLDL-C直接法の試薬が正確でないと指摘を受け[2]，我が国でも研究班が組織され，同様の検討が行われた[15]．しかし，米国の検討では，リポタンパクの組成が正常と著しく異なる検体が，患者検体の17％程度含まれていた．我が国の検討では，TGが1,000 mg/dL以上の高TG血症や著明な低LDL-C血症，異常リポタンパクが出現する原発性胆汁性肝硬変などを除き，健常群49例と，脂質異常症，心血管系疾患，脂肪肝・アルコール性肝障害，糖尿病などを持つ患者群124例の計173例において，12社の，直接法とBQ法で測定したLDL-C値を比較した．その結果，LDL-C直接法は健常群ではほぼ満足のいく正確性を示したが，患者群では明らかに性能が不良な試薬があった（図1）．なお，Ⅰ型およびⅢ型高脂血症のように，LDL組成が正常と著しく異なる患者では，ほとんどの試薬でBQ法よりLDL-C直接法のLDL-C値が高く，LDL-Cを直接法で測定すべきでない患者であることも明らかとなった．この検討結果を受けて，フレイヤは試薬の製造・販売を中止した．協和メデックスは，高TG血症検体における試薬の反応性を改善させた改良品をすでに発売した．オーソーは，キャリブレーターの値付けが大きくずれていたため，新鮮な血清を用いてキャリブレーターの値付けを再検討し，それを反映させた試薬に変更した．性能不良と判定された試薬のうちの1つは，性能が良好な他社の試薬を導入することを検討中である．ほとんど使用されていないセロテックの試薬を除くと，TG＜400 mg/dLの検体ならば，市場に出ているLDL-C直接法でLDL-Cを正確に測定できることが分かる（図1-C）．現在，厚生労働科学研究費の助成を受けた研究班が，主要な国内4社（協和メデックを含む）の試薬の追加検討を行っている．食後検体におけるLDL-C直接法の正確性について，より明確な結果が出るものと期待される．

図1　直接法と BQ 法の LDL-C の % バイアス（文献[15]より引用改変）



$$\%\ バイアス = \frac{(直接法の\ LDL\text{-}C\ 値) - (BQ\ 法の\ LDL\text{-}C\ 値)}{BQ\ 法の\ LDL\text{-}C\ 値} \times 100$$

フレイヤは試薬の製造・販売を中止，協和メデックスは試薬を改良，オーソーはキャリブレーターの値付けを修正した．

おわりに

LDL-C 測定法の現状と問題点について述べた．LDL-C 直接法をめぐる混乱は，検査試薬の製造を承認する段階で，性能が不良な試薬をチェックできなかったことも一因である．LDL-C 直接法に限らず，臨床検査の正確性を検証する，公的システムの構築が必要と思われる．

文献

1) Friedewald W T, et al: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18 (6): 499-502, 1972.
2) Miller W G, et al: Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. Clin Chem 56 (6): 977-986, 2010.
3) Nauck M, et al: Quantitative determination of high-, low-, and very-low-density lipoproteins and lipoprotein (a) by agarose gel electrophoresis and enzymatic cholesterol staining. Clin Chem 41 (12): 1761-1767, 1995.
4) Hirowatari Y, et al: Measurement of cholesterol of major serum lipoprotein classes by anion-exchange HPLC with perchlorate ion-containing eluent. J Lipid Res 44 (7): 1404-1412, 2003.
5) Hata Y, et al: Application of Friedewald's LDL-cholesterol estimation formula to serum lipids in the Japanese population. Jpn Circ J 50 (12): 1191-1200, 1986.
6) Vujovic A, et al: Evaluation of different formulas for LDL-C calculation. Lipids Health Dis 9: 27, 2010.
7) Chen Y, et al: A modified formula for calculating low-density lipoprotein cholesterol values. Lipids Health Dis 9: 52, 2010.
8) Anandaraja S, et al: Low-density lipoprotein cholesterol estimation by a new formula in Indian population. Int J Cardiol 102 (1): 117-120, 2005.
9) Martin S S, et al: Comparison of a novel method vs the Friedewald equation for estimating low-densitylipoprotein cholesterol levels from the standard lipid profile. JAMA 310 (19): 2061-2068, 2013.
10) Meeusen J W, et al: Validation of a Proposed Novel Equation for Estimating LDL Cholesterol. Clin Chem (in press)
11) Oliveira M J, et al: Evaluation of four different equations for calculating LDL-C with eight different direct HDL-C assays. Clin Chim Acta 423: 135-140, 2013.
12) Lipid Research Clinics Program, Manual of Laboratory Operations. DHEW (NIH) Publication No 75-628, 1974 (revised 1982).
13) Abell L L, et al: Simplified method for the estimation of total cholesterol in serum, and demonstration of its specificity. J Biol Chem 195 (1): 357-366, 1952.
14) Nakamura M, et al: LDL cholesterol performance of beta quantification reference measurement procedures. Clin Chim Acta 431: 288-293, 2014.
15) Miida T, et al: A multicenter study on the precision and accuracy of homogeneous assays for LDL-cholesterol: comparison with a beta-quantification method using fresh serum obtained from non-diseased and diseased subjects. Atherosclerosis 225 (1): 208-215, 2012.