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Lipoprotein Particle Profiles Compared With Standard Lipids in the Association With Subclinical Aortic Valve Calcification in Apparently Healthy Japanese Men

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Background: Risk factors for atherosclerotic disease including dyslipidemia have been shown to be associated with aortic valve calcification (AVC). Nuclear magnetic resonance (NMR)-measured lipoprotein particles, low-density and high-density lipoprotein particles (LDL-p, HDL-p) in particular, have emerged as novel markers of atherosclerotic disease; however, whether NMR-measured particles are associated with AVC remains to be determined. This study aimed to examine the association between NMR-based lipoprotein particle measurements and standard lipids with AVC. The primary variables of interest were LDL-p (nmol/L), HDL-p (μmol/L), LDL-cholesterol, and HDL-cholesterol (both in mg/dL).

Methods and Results: A community-based random sample of Japanese men aged 40–79 years examined in 2006–2008, in Shiga, Japan was studied. Presence of AVC was defined as an Agatston score >0. Lipoprotein particles were measured using NMR spectroscopy. In the main analysis, multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for the prevalence of AVC across the higher quartiles of lipids in reference to the lowest ones were obtained. Of 874 participants analyzed, 153 men had AVC. Multivariable-adjusted ORs of prevalent AVC for the highest vs. the lowest quartile were significantly elevated for LDL-p (OR, 2.20; 95% CI: 1.23–3.93) and LDL-cholesterol (OR, 2.16; 95% CI: 1.23–3.78). In contrast, neither HDL-p nor HDL-cholesterol was associated with AVC.

Conclusions: The association of prevalent AVC with NMR-based LDL-p was comparable to that with LDL-cholesterol.

Key Words: Aortic valve calcification (AVC); High-density lipoprotein particles (HDL-p); Low-density lipoprotein particles (LDL-p)

Degenerative calcific aortic valve disease is one of the most common heart diseases worldwide, and its prevalence has increased in developed countries as populations have aged.[1–3] If mild valve obstruction exists, the disease seems to progress inevitably to hemodynamically more altered states. Once the disease becomes symptomatic and severe, the prognosis without surgery is dismal.[4] The subclinical early stage of degenerative calcific aortic valve disease is characterized by aortic valve calcification (AVC). The pathophysiology of AVC involves an active process, similar to that of atherosclerosis, characterized by 3 primary processes: lipid accumulation, inflammation, and calcification.[5–8]

Nuclear magnetic resonance (NMR)-based lipoprotein assessment that simultaneously measures the concentrations and sizes of various lipoprotein particles, presents novel markers of atherosclerosis and/or cardiovascular disease.[9–12] Although some of these measures are correlated

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with corresponding conventional lipid measures, some studies suggest that NMR-based measures have a role beyond the conventional lipids. For example, an observational study showed that low-density lipoprotein particle (LDL-p)-lowering therapy guided by change in LDL-p was more cost-effective than a low-density lipoprotein cholesterol (LDL-c)-guided approach in preventing cardiovascular disease.[13] With regard to atherosclerosis, we have previously reported that LDL-p and high-density lipoprotein particle (HDL-p) concentrations were more robust independent markers of subclinical atherosclerosis compared to the corresponding conventional cholesterols in a community-based sample.[10,11]

However, whether NMR-based lipoprotein particles are associated with AVC remains to be determined. In this cross-sectional study, we primarily examined an association of AVC with LDL-c, HDL-c and the corresponding NMR-based lipoproteins; that is, LDL-p and HDL-p. We selected those 4 lipid measures as the primary objectives of our investigation because of their clinical importance and the wealth of literature.

Methods

Study Participants

The Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA) is an ongoing prospective population-based study conducted in Japan. The study design and recruitment details have been reported previously.[14,15] In brief, we randomly selected and invited 2,379 Japanese men aged 40–79 years, residents of Kusatsu city, Shiga, Japan, from 2006 to 2008, based on the Basic Residents' Register of the city. A total of 1,094 men agreed to participate (participation rate, 46%), with their written informed consent obtained. For the present study, we excluded those participants who had any reported history of aortic valve surgery (n=4), or myocardial infarction or stroke (n=69). Because we used Friedewald's formula for estimating the serum concentration of LDL-c, we also excluded those who had serum triglycerides (TG) concentration ≥400 mg/dL (n=17) as the formula is not applicable for such individuals.[16] We further excluded those with an estimated glomerular filtration rate (eGFR) of <30 mL/min/1.73 m^2 using serum creatinine by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation modified for the Japanese (n=3),[17,18] those who used lipid-lowering medication (n=118), and those with missing pertinent information, which left 874 participants for analyses. The study conforms to the Declaration of Helsinki and was approved by the Institutional Review Board of Shiga University of Medical Science, Otsu, Japan.

Measurements

Data on medical history and lifestyle factors were collected from each participant using a self-administered questionnaire, and trained technicians confirmed the completed questionnaire with participants. Height, weight, blood pressure, and a variety of other measures were collected. Blood pressure was measured by using an automated sphygmomanometer (BP-8800; Omron Colin, Tokyo, Japan).

Blood samples were obtained early in the clinic visit after 12-h fasting.[14,15] Serum was separated by centrifugation (3000 revolutions per min, for 15 min) at 4°C within 90 min. A portion of the samples was sent for routine laboratory tests, including those for standard lipids and glucose. Concentration of blood glucose was determined from sodium fluoride-treated plasma using a hexokinase glucose-6-phosphate-dehydrogenase enzymatic assay. Concentrations of serum total cholesterol (TC) and triglycerides were determined using enzymatic assays, and that of HDL-c was measured using a direct method (Determiner- C-TC, Determiner-C-TGL, Determiner-L HDL-c, respectively; Kyowa Medix, Tokyo, Japan). Measurements were standardized according to guidelines from the Center for Disease Control and Prevention/Cholesterol Reference Method Laboratory Network (CDC/CRMLN). We used Friedewald's formula to estimate LDL-c levels[16] with TG <400 mg/dL. Non-HDL-c was calculated by subtracting HDL-c from TC.

The remaining serum samples were stored at −80°C. Then, a portion of them was shipped on dry ice to LipoScienceInc. (Raleigh, NC; now LabCorp, Burlington, NC, USA) for NMR-based lipoprotein particles measurement. NMR spectroscopy was performed to quantify the particle concentrations of LDL and HDL.[19,20] Additionally, particle concentrations were further determined for 3 LDL subclasses (intermediate-density lipoprotein [IDL], 23–27 nm; large, 21.3–23 nm; small, 18.3–21.2 nm), and 3 HDL subclasses (large, 8.8–13 nm; medium, 8.2–8.8 nm; and small, 7.3–8.2 nm). Weighted average particle sizes of LDL and HDL were also calculated.

Glycated hemoglobin (HbA1c) was measured using a latex agglutination assay according to the standardized method of the Japanese Diabetes Society (JDS). We then converted JDS values to those of the National Glycohemoglobin Standardization Program (NGSP) using the following formula recommended by the JDS: HbA1c (NGSP) = 1.02×HbA1c(JDS) + 0.25 (%).[21]

C-reactive protein (CRP) was measured by nephelometry using a BN II analyzer with an inter-assay coefficient of variation ranging from 4.5% to 4.6%.[2] eGFR was calculated according to the CKD-EPI equation modified for the Japanese as follows:[17,18]

eGFR (mL/min/1.73 m^2) = $141\times(\text{serum creatinine}/0.9)^{(-0.411)}\times0.993^{(\text{Age})}\times0.813$ for those with a serum creatinine ≤0.9 mg/dL (79.6 μmol/L).

eGFR (mL/min/1.73 m^2) = $141\times(\text{serum creatinine}/0.9)^{(-1.209)}\times0.993^{(\text{Age})}\times0.813$ for those with a serum creatinine >0.9 mg/dL (79.6 μmol/L).

Aortic Valve Calcification Assessment

The detailed method for cardiac computed tomography (CT) in SESSA was reported previously.[2,22] In brief, the aortic valve was assessed by either a GE-Imatron C150 Electron Beam Tomography system (EBCT; GE Medical Systems, South San Francisco, CA, USA) for participants examined from May 2006 through to August 2007, or a 16-row multi-detector row CT system (MDCT, Aquilion-16™; Toshiba Medical Systems, Tochigi, Japan) for participants examined thereafter.[23] A DICOM workstation and AccuImage software (AccuImage Diagnosis, San Francisco, CA, USA) were used to quantify calcium scores. The calcium score was calculated by multiplying the lesion area by a density factor derived from the maximal Hounsfield unit within this area, as described by Angaston et al.[23] AVC was identified according to the methods from the Multi-Ethnic Study of Atherosclerosis (MESA); this included any calcified lesion residing within the aortic valve leaflets.[24–26] Calcification of the aortic annulus, aortic sinuses, ascending

Table 1. Characteristics of Study Participants With or Without AVC (Shiga Epidemiological Study of Subclinical Atherosclerosis [SESSA] 2006–2008)

Characteristic	Overall (N=874)	No AVC (n=721)	Prevalent AVC (n=153)	P value
Age, years	63.3 (10.0)	61.8 (10.0)	70.3 (6.5)	<0.001
BMI, kg/m^2	23.4 (2.9)	23.4 (2.9)	23.3 (3.1)	0.904
Smoking status, %				0.984
Current	33.3	33.4	32.7	
Past	49.9	49.8	50.3	
Never	16.8	16.8	17	
Smoking,† pack-years	23.8 (5.0–43.0)	23.1 (5.0–42.0)	27.2 (8.4–47.0)	0.095
Drinking status, %				0.065
Current	77.4	78.6	71.2	
Past	5.3	4.6	8.5	
Never	17.3	16.8	20.3	
Systolic blood pressure, mmHg	135.8 (19.2)	134.3 (18.8)	142.5 (19.7)	<0.001
Diastolic blood pressure, mmHg	79.5 (11.0)	79.6 (11.1)	79.5 (10.5)	0.917
Anti-hypertensive medication, %	25.1	22.5	37.3	<0.001
Hypertension, %	50.9	47.4	67.3	<0.001
HbA1c, %	5.4 (5.2–5.8)	5.4 (5.1–5.7)	5.6 (5.3–6.0)	<0.001
Medication for diabetes, %	7.9	6.5	14.4	0.001
Diabetes, %	12.7	11.1	20.3	<0.001
eGFR, mL/min/1.73 m^2	74.9 (10.3)	75.9 (9.9)	70.0 (10.6)	<0.001
CRP, mg/dL	0.4 (0.2–0.9)	0.4 (0.2–0.8)	0.5 (0.3–1.0)	0.023
TC, mg/dL	209.1 (34.0)	208.7 (33.2)	211.0 (37.6)	0.490
LDL-c, mg/dL	126.4 (31.6)	125.3 (31.0)	131.2 (34.2)	0.036
HDL-c, mg/dL	59.3 (17.2)	59.8 (17.4)	57.0 (16.2)	0.065
TG, mg/dL	100.0 (74.0–145.0)	100.0 (73.0–145.0)	98.0 (77.0–143.0)	0.002
Non HDL-c, mg/dL	149.8 (34.9)	148.9 (34.4)	154.0 (37.0)	0.101
LDL-p (nmol/L)	1,292.5 (386.8)	1,280.7 (379.1)	1,348.3 (417.9)	0.049
Large LDL-p (nmol/L)	640.4 (286.7)	626.4 (280.8)	706.39 (305.3)	0.002
Small LDL-p (nmol/L)	535.1 (412.6)	534.8 (411.3)	536.6 (420.1)	0.960
IDL (nmol/L)	93.5 (42.0–165.0)	97.0 (42.0–169.0)	80.0 (40.0–148.0)	0.122
HDL-p (μmol/L)	34.1 (6.6)	34.6 (6.6)	31.6 (6.0)	<0.001
Large HDL-p (μmol/L)	6.6 (4.3–9.7)	6.6 (4.3–9.7)	6.7 (4.2–9.8)	0.861
Medium HDL-p (μmol/L)	7.3 (4.9–10.3)	7.5 (5.1–10.7)	6.4 (4.0–9.1)	<0.001
Small HDL-p (μmol/L)	18.9 (5.2)	19.1 (5.1)	17.7 (5.4)	0.002
LDL-p size (nm)	20.9 (0.6)	20.9 (0.6)	21.0 (0.6)	0.102
HDL-p size (nm)	9.3 (0.5)	9.3 (0.5)	9.4 (0.6)	0.204

The presence of AVC was defined as an Agatston score >0. Values are expressed as mean (standard deviation) for continuous variables with approximately normally distribution or by median (interquartile range) with skewed distribution and % for categorical variables. Differences in characteristics were evaluated by using the unpaired Student's t-test, Wilcoxon rank sums test, or Chi squared test. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or use of antihypertensive medications. Diabetes was defined as fasting plasma glucose ≥126 mg/dL, HbA1c (National Glycohemoglobin Standardization Program) ≥6.5%, or the use of diabetic medication. †The number of participants with and without aortic valve calcification were 149 and 717, respectively, owing to missing information. AVC, aortic valve calcification; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL-c, high-density lipoprotein cholesterol; HDL-p, high-density lipoprotein particle; IDL, intermediate-density lipoprotein; LDL-c, low-density lipoprotein cholesterol; LDL-p, low-density lipoprotein particle; TC, total cholesterol; TG, triglycerides.

aorta, or coronary arteries was excluded. All CT images were assessed by 1 trained medical technologist masked to the clinical information of the participants.

Statistical Analysis

Participants' characteristics are shown using means and standard deviations (SDs) for continuous variables with a bell-shaped distribution, medians and interquartile ranges for those with a skewed distribution, and percentages for categorical variables. Difference in characteristics by the presence or absence of AVC was evaluated using the unpaired Student's t-test, Mann-Whitney U-test, or Chi-squared test, as appropriate. We calculated age-adjusted Spearman's rank correlation coefficients between the measured lipid levels.

Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure (DBP) ≥90 mmHg, or use of antihypertensive medications. Diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dL, HbA1c (NGSP) ≥6.5%, or the use of diabetic medication. Hypertriglyceridemia was defined as triglycerides ≥150 mg/dL. Prevalent AVC was defined as a calcium score >0.[24,25]

Table 2. Multivariable-Adjusted OR for Prevalent AVC Across the Quartile of Primary Lipid Indices in Apparently Healthy Japanese Men (Aged 40–79 Years: 2006–2008, SESSA)

	1st quartile (ref)	2nd quartile	3rd quartile	4th quartile	P value for trend
	Range [Adjusted OR (95% CI)]				
LDL-c, mg/dL	34.6–105.6	106.0–123.6	123.8–146.8	147.4–275.8	0.006
	Reference	1.11 (0.62–1.95)	1.12 (0.64–1.96)	2.16 (1.23–3.78)	
HDL-c, mg/dL	25.0–46.0	47.0–56.0	57.0–68.0	69.0–150.0	0.323
	Reference	0.91 (0.53–1.55)	0.98 (0.56–1.70)	0.73 (0.39–1.33)	
LDL-p, nmol/L	297.0–1,009.0	1,010.0–1,273.0	1,275.0–1,543.0	1,544.0–3,156.0	0.011
	Reference	1.44 (0.81–2.56)	1.18 (0.65–2.11)	2.20 (1.23–3.93)	
HDL-p, μmol/L	13.9–29.7	29.8–33.3	33.4–37.5	37.6–68.9	0.297
	Reference	0.81 (0.50–1.34)	0.70 (0.40–1.24)	0.79 (0.43–1.43)	

ORs were adjusted for age, BMI, smoking (pack-years), drinking, systolic blood pressure, HbA1c, medications for blood pressure and diabetes, eGFR, CRP, and CT-type. The presence of AVC was defined as an Agatston score >0. P values for trend across the quartiles were calculated by inserting the median value for each quartile. CI, confidence interval; OR, odds ratio. Other abbreviations as in Table 1.

In the main analyses, we divided each lipid measure into quartiles, and computed multivariable adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of the prevalent AVC, using logistic regression, for the upper 3 quartiles (Q2–Q4) in reference to the lowest quartile (Q1). Quartiles of each lipid measure were used because there are no established cut-off points for those measures in relation to AVC. In the logistic regression model, we adjusted for the following risk factors: age, smoking (pack-years), drinking status (current, past, never), BMI, HbA1c, SBP, medication status (hypertension and diabetes), in addition to eGFR, CRP, and CT-type (EBCT/16-MDCT). A P value for trend across the quartiles was obtained by inserting a variable that takes median values for each quartile into a model.

In sensitivity analyses, we treated lipid measures as continuous, and calculated adjusted ORs and 95% CIs of prevalent AVC per 1-SD higher or lower lipids.

As post-hoc analyses, we conducted the following additional analyses: (1) we repeated analyses after stratification by presence/absence of metabolic syndrome (MetS), hypertension, diabetes mellitus and hypertriglyceridemia. The rationale for those stratified analyses was to explore potential interaction by those factors on the association between AVC and lipid measures.[27] For the definition of MetS, we used both revised National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria[28] and the Japanese criteria[29] (See the **Supplementary File** for detailed definitions). In conducting the stratified analyses, we excluded covariates that defined stratifying factor from regression models. Take stratification by MetS for example, we excluded BMI, systolic blood pressure, HbA1c, medications for blood pressure and diabetes from our multivariable adjusted model. The P value for interaction was calculated by inserting the interaction term and based on the Wald Chi-squared test (In quartile model testing interaction, we treated the quartiles as rank); (2) for completeness, we extended the exposure of interest to non-HDL-cholesterol and triglycerides, subclasses of LDL-p (large, small, and IDL), HDL-p (large, medium, and small), and size (nm) of LDL and HDL themselves; (3) we conducted sensitivity analysis repeating the main analysis including those with TG ≥400 mg/dL for the purpose of a comparison of HDL-c and HDL-p. We did not conduct the same sensitivity analysis for the comparison between LDL-c and LDL-p because the validity of Friedewald-based LDL-c concentration is uncertain in those with TG ≥400 mg/dL. Analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). A 2-tailed P value <0.05 was considered significant.

Table 3. Multivariable-Adjusted OR for the Presence of AVC per 1 Standard Deviation Higher (or *Lower) Value of the Primary Lipid Indices, in Apparently Healthy Japanese Men (Aged 40–79 Years: 2006–2008, SESSA)

	OR	95% CI	P value
LDL-c, mg/dL	1.40	1.15–1.72	0.001
HDL-c*, mg/dL	1.12	0.90–1.40	0.320
LDL-p, nmol/L	1.33	1.09–1.63	0.005
HDL-p*, μmol/L	1.28	1.02–1.61	0.033

ORs were adjusted for age, BMI, smoking (pack-years), drinking, systolic blood pressure, HbA1c, medications for blood pressure and diabetes, eGFR, CRP, and CT-type. The presence of AVC was defined as an Agatston score >0. Abbreviations as in Tables 1,2.

Results

Of the 874 men (aged 40–79 years) we analyzed, the mean (SD) age of our participants was 63.3 (10.0) years. One-hundred and fifty-three (17.5%) men had prevalent AVC (**Table 1**), and they tended to be older, more likely to have hypertension and diabetes and to take medication(s) for hypertension and/or diabetes. With regard to the standard lipids, those with prevalent AVC tended to have a higher concentration of LDL-c and a lower concentration of triglycerides. In NMR-measured lipid levels, the corresponding concentration of LDL-p tended to be higher and that of HDL-p tended to be lower than those without AVC (**Table 1**). By using an age-adjusted Spearman's correlation matrix, it was found that the LDL-p concentration was strongly correlated with LDL-c and non-HDL-c (r=0.81 and 0.82, respectively) (**Supplementary Table 1**). In a multivariable-adjusted logistic regression model, older age and higher SBP, in addition to greater LDL-c concentration, were independently associated with prevalent AVC (**Supplementary Table 2**).

In main analyses, the highest quartiles (Q4) of LDL-c and LDL-p were associated with prevalent AVC. ORs

(95% CIs) of prevalent AVC in Q4, in reference to Q1, were 2.16 (1.23–3.78) and 2.20 (1.23–3.93) respectively, after adjusting for age, BMI, smoking, drinking, SBP, HbA1c, medications for blood pressure and diabetes, eGFR, CRP, and CT-type (**Table 2**). Neither HDL-c nor HDL-p was associated with prevalent AVC.

In the sensitivity analyses treating lipid measures continuous, the strength of association with prevalent AVC per 1-SD higher value was the largest for LDL-c (adjusted OR=1.40, 95% CI: 1.15–1.72), followed by LDL-p (1.33, 1.09–1.63) (**Table 3**). HDL-p, but not HDL-c, was significantly inversely associated with prevalent AVC, such that 1 SD lower HDL-p was associated with 1.28 (1.02–1.61) higher OR.

The results of post-hoc analyses were as follows (**Supplementary Tables 3–10**): (1) in stratified analyses, LDL-c tended to be more strongly associated with AVC among participants with MetS than in those without it (**Supplementary Tables 3**,**4**). By contrast, in participants with hypertension, the association was lower compared to that with LDL-p (**Supplementary Table 5**). Similar to participants with MetS, the association of LDL-c tended to be stronger in those with diabetes (**Supplementary Table 6**), and those with hypertriglyceridemia (**Supplementary Table 7**) as compared to those without them. However, a statistically significant difference across the strata was observed only in the quartile model of hypertriglyceridemia stratification (**Supplementary Table 7**). Regarding HDL, the direction of association between HDL-p and AVC tended to be opposite by the presence/absence of MetS in the quartile models with a significant interaction by Japanese MetS criteria (P value for interaction=0.012, **Supplementary Table 4**). However, no such interaction was observed in a continuous model using either of the MetS definitions, and their directions of association were the same as the main results. Otherwise, there was no clear evidence of interaction by any of the stratifying factors. Likewise, according to continuous models, there was no clear evidence supporting stronger associations of LDL-p or HDL-p compared to those of corresponding conventional lipids in any of the strata (**Supplementary Tables 3**,**5**,**6**); (2) as for extended lipid indices, non-HDL-c showed a significant linear trend across the quartile (P value for trend=0.03) (**Supplementary Table 8**). In the continuous model, 1 SD higher non-HDL-c was associated with an adjusted OR of 1.39 (1.13–1.71). TG was not associated with prevalent AVC in either of the models (quartile, continuous). None of the NMR-based LDL, or HDL subclasses and their particle sizes were significantly associated with AVC in quartile models. In continuous models, only large LDL-p was significantly positively associated with AVC (OR=1.23, 1.02–1.49) (**Supplementary Table 9**); (3) including the participants with TG ≥400 mg/dL (n=9) resulted in no significant association of AVC with HDL-c or with HDL-p in either of the models (quartile, continuous), although the point estimates in the continuous model indicated a stronger association with HDL-p compared to HDL-c (**Supplementary Table 10**), which is similar to the main results.

Discussion

In this population-based, cross-sectional study of apparently healthy Japanese men, we found positive associations of LDL-p and LDL-c with prevalent AVC, independent of conventional cardiovascular disease risk factors. In contrast, an association of prevalent AVC was observed only with HDL-p in a continuous model and not with HDL-c. To the best of our knowledge, this is the first study that directly compared NMR-measured lipoproteins with standard lipids in relation to AVC in a Japanese population.

AVC is not uncommon in a general elderly population, and it may lead to more advanced aortic valvular disease.[1–3] Identifying risk factors for subclinical AVC is of the utmost importance to establish new therapeutic targets that might allow us to halt or at least slow the progression of disease. The positive association between LDL-c and AVC in our study is consistent with the results of previous studies.[26,30] Our study further suggests that LDL-p in addition to LDL-c may be a possible therapeutic target for preventing aortic valve calcification and consequent functional deterioration.

NMR-based lipoprotein parameters, LDL-p in particular, have been suggested as alternative lipid measures in assessing coronary artery diseases risk.[31] LDL-p is an alternate LDL measure, and although it is highly correlated with LDL-c, the cholesterol amount carried by each LDL particle varies. Higher levels of circulating LDL-p may increase the chances that these particles may contribute to the oxidative transformation of lipids within the aortic valve, promoting valvular inflammation and remodeling, and thus cause disease progression. A study showed that statin use, guided by changes in LDL-p, was more cost-effective than a LDL-c-guided approach in preventing cardiovascular disease.[13]

Some observational studies suggested that statin therapy may slow the progression of AVC.[32,33] A meta-analysis of 4 randomized controlled trials found no differences in the clinically relevant outcomes such as mean pressure gradient, valve area, freedom from valve replacement and death from cardiovascular causes between the statin group and the placebo group,[34] but the authors of the meta-analysis stated that "this issue is not over, mainly because the available evidence is based on studies which have limitations such as follow-up, randomization process, sample size and very elderly participants with many comorbidities". Indeed, the analyzed patients (n=269) in the meta-analysis had a mean age of 58 years, with asymptomatic mild aortic valve stenosis defined by maximum aortic valve velocity of 2.5–4.0 m/s. Agreeing with those authors, we think it is premature to conclude that there is no potential benefit for those in the subclinical stage of AVC to keep their lipids within the optimum range in hopes of preventing/delaying progression.

Comparison of Conventional Lipids and NMR-Based Lipoproteins

In comparing LDL-c and LDL-p in association with prevalent AVC, our results were sensitive to the choice of model. The point estimate (OR) in the 4th quartile for LDL-c in reference to 1st quartile was slightly smaller compared to the corresponding OR for LDL-p in the main model, whereas the opposite was the case in the continuous model. The post-hoc stratified analyses showed the association between LDL-c and AVC was stronger in individuals with hypertriglyceridemia in the quartile model, although the interaction by hypertriglyceridemia was not statistically supported in the continuous model (P value for interaction=0.262). Furthermore, no clear evidence supported a stronger association of LDL-p with AVC compared with that of LDL-c in any of the strata we examined. Taken together, LDL-p was similar but not clearly superior to LDL-c in the association with prevalent AVC.

Evidence of the potential role of HDL in AVC is scarce. Although serum HDL-c level has been shown to be inversely associated with hemodynamic progression of calcific aortic valve disease,[35] no studies have compared the strength of the association between HDL-c and NMR-based HDL-p in relation to AVC. Overall, the relationship of HDL-c or HDL-p with AVC was weaker compared to those of LDL counterparts in the main analyses. Among them, the association with HDL-p seemed be slightly stronger than HDL-c in the continuous model. Otherwise, post-hoc analyses did not show any different pattern of association from the main results. Some studies suggested that serum HDL-p concentration was more closely related to the performance rate of cholesterol efflux in the reverse cholesterol transport pathway than HDL-c;[12,36,37] however, whether this is translated into HDL-p being a better predictor for AVC remains uncertain, thus, warranting further study.

Other Conventional Lipids and Subclasses of LDL-p, HDL-p and Their Size

In post-hoc analyses, we observed a strong association between non-HDL-c and AVC, but not between TG and AVC. Non-HDL-c was shown to be similar to, or even better than, LDL-c in predicting cardiovascular disease incidence and mortality,[38,39] and our finding is consistent with those reports. In the literature, there is no single subclass of NMR-measured LDL/HDL and their size that is consistently associated with clinical atherosclerotic diseases. In our post-hoc analysis, only large LDL-p was significantly positively associated with AVC. Given that this was one statistically significant finding of all the post-hoc analyses we added, its interpretation should be made carefully. Further study is needed to examine whether our finding will be reproduced in a different setting, and to elucidate the potential role of the subclasses of NMR-based LDL-p, HDL-p and their sizes in the association with AVC.

Limitations and Strengths

Our study has several limitations. First, the study design was cross-sectional, so causality in any relationship could not be established; however, we excluded participants with any history of valvular surgery, myocardial infarction and stroke, and those on lipid medication, to minimize reverse causality. In addition, we believe it is unlikely that our participants changed their behavior as a result of their AVC status because we did not inform them of their status, due to its unclear clinical significance. Also, our results may not be applicable to women or non-Japanese individuals given our studied sample was limited to apparently healthy Japanese men. However, this may also offer an advantage in limiting the confounding factors associated with gender, race and genetic variation, as well as diet and lifestyle. The strengths of the study include community-based random sampling of apparently healthy men enhancing generalizability, and masked assessment of the key component, AVC, from the participants' characteristics to minimize information bias, as well as the standardized measurements of relevant parameters, including laboratory data, and a moderate sample size.

In conclusion, among LDL and HDL measures, both LDL-cholesterol and LDL-particle levels were positively associated with presence of AVC, and the strengths of association seemed similar in this community-based sample of asymptomatic Japanese men not using lipid-lowering medication.

Author Contributions

T.V., A.F., K.M. designed the study. T.V. performed analysis, interpreted the results, and drafted the manuscript. A.F. revised the manuscript. All authors contributed to the manuscript's critical review and approval of the final version.

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Disclosures

All authors declare no conflicts of interest.

IRB Information

The study was approved by the Institutional Review Board of Shiga University of Medical Science, Otsu, Japan.

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Supplementary Files

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