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Table 4. Multivariable-adjusted HRs for CVD, CHD and cardiac death, stratified by hypertension, current smoking and body mass index, during the 24-year follow-up period

A. Hypertension

Hypertension (+)	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	4286	83753	690	1.08	0.99-1.17	127	1.35	1.12-1.62	263	1.19	1.05-1.36
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	676	12281	120	1.16	0.91-1.49	17	0.97	0.51-1.83	41	1.13	0.74-1.72
4.14-4.65	892	17334	136	1.00		23	1.00		49	1.00	
4.66-5.17	962	19079	133	1.02	0.80-1.30	20	0.90	0.49-1.65	50	1.09	0.73-1.62
5.18-5.68	813	16163	135	1.16	0.91-1.49	23	1.23	0.68-2.25	49	1.23	0.81-1.85
5.69-6.20	519	10550	88	1.15	0.87-1.52	22	1.63	0.89-2.99	36	1.30	0.83-2.03
6.21-6.71	265	5311	39	1.00	0.70-1.45	9	1.34	0.60-2.96	18	1.33	0.76-2.33
6.72-	159	3036	39	1.91	1.32-2.76	13	3.69	1.81-7.55	20	2.85	1.66-4.90
Hypertension (−)	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	4923	109269	194	1.06	0.91-1.24	45	1.27	0.94-1.71	85	1.24	0.99-1.55
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	1123	24819	32	0.77	0.49-1.21	6	0.75	0.27-2.11	11	0.70	0.33-1.51
4.14-4.65	1290	28927	53	1.00		11	1.00		20	1.00	
4.66-5.17	1116	24607	48	1.08	0.73-1.60	8	0.96	0.38-2.46	22	1.42	0.76-2.65
5.18-5.68	760	16863	34	1.21	0.77-1.88	13	2.34	0.99-5.50	19	1.99	1.03-3.86
5.69-6.20	362	8063	13	0.75	0.41-1.40	2	0.54	0.12-2.49	6	1.00	0.39-2.56
6.21-6.71	177	3889	9	1.06	0.52-2.19	2	1.04	0.22-4.93	4	1.40	0.47-4.23
6.72-	95	2104	5	1.16	0.46-2.94	3	3.44	0.90-13.16	3	1.98	0.57-6.88

B. Current Smoking

Current Smoking (+)	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	2998	61241	327	1.15	1.02-1.30	69	1.52	1.19-1.93	125	1.28	1.06-1.55
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	642	12648	69	1.00	0.72-1.40	9	0.75	0.32-1.74	24	1.05	0.60-1.84
4.14-4.65	756	15492	77	1.00		14	1.00		26	1.00	
4.66-5.17	713	14788	70	1.08	0.78-1.50	13	1.08	0.50-2.32	29	1.33	0.78-2.27
5.18-5.68	450	9262	51	1.29	0.89-1.86	11	1.47	0.65-3.35	17	1.30	0.69-2.44
5.69-6.20	268	5577	33	1.27	0.83-1.94	12	2.16	0.96-4.88	14	1.50	0.77-2.95
6.21-6.71	111	2286	17	1.48	0.87-2.55	6	2.59	0.95-7.01	10	2.54	1.19-5.43
6.72-	58	1189	10	1.81	0.92-3.56	4	3.29	1.02-10.57	5	2.64	0.98-7.13

between the TC level and each endpoint; however, the interaction term between the serum TC level and current smoking was significant for CHD death (p= 0.02). In addition, the multivariable HR for CHD based on the serum TC level was higher in the current smokers than in the non-smokers (**Table 4**).

We also calculated the population-attributable risk fractions for CVD, CHD and cardiac death, although the HRs for CVD were not significant (**Table 5**). The number of estimated excess deaths due

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(Cont Table 4)

B. Current Smoking

Current Smoking (−)	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	6211	131781	557	1.03	0.94-1.12	103	1.20	0.98-1.46	223	1.16	1.01-1.33
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	1157	24452	83	1.19	0.89-1.58	14	1.08	0.54-2.15	28	1.05	0.65-1.70
4.14-4.65	1426	30769	112	1.00		20	1.00		43	1.00	
4.66-5.17	1365	28898	111	0.99	0.76-1.29	15	0.73	0.37-1.44	43	1.05	0.68-1.61
5.18-5.68	1123	23764	118	1.10	0.84-1.43	25	1.29	0.70-2.35	51	1.32	0.87-2.01
5.69-6.20	613	13036	68	1.01	0.74-1.37	12	1.04	0.50-2.15	28	1.16	0.71-1.89
6.21-6.71	331	6913	31	0.85	0.56-1.27	5	0.72	0.27-1.96	12	0.93	0.48-1.79
6.72-	196	3950	34	1.76	1.19-2.62	12	3.49	1.66-7.33	18	2.68	1.52-4.74

C. Body Mass Index

Body Mass Index <25	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	7258	151366	700	1.11	1.03-1.21	135	1.33	1.12-1.59	278	1.22	1.08-1.39
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	1559	31987	127	1.00	0.79-1.27	20	0.80	0.45-1.42	45	1.00	0.67-1.50
4.14-4.65	1825	38630	156	1.00		30	1.00		55	1.00	
4.66-5.17	1640	34307	147	1.07	0.85-1.35	24	0.90	0.53-1.56	64	1.39	0.96-2.00
5.18-5.68	1160	24216	126	1.22	0.96-1.56	22	1.13	0.64-2.00	51	1.50	1.01-2.23
5.69-6.20	615	12899	77	1.19	0.90-1.57	19	1.49	0.82-2.70	32	1.47	0.94-2.30
6.21-6.71	293	6036	35	1.08	0.74-1.58	9	1.46	0.68-3.16	16	1.51	0.85-2.68
6.72-	166	3293	32	1.97	1.33-2.92	11	3.40	1.64-7.06	15	2.84	1.57-5.13
Body Mass Index >=25	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	1951	41656	184	0.95	0.81-1.11	37	1.32	0.95-1.84	70	1.17	0.92-1.51
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	240	5113	25	1.42	0.84-2.39	3	1.30	0.29-5.87	7	0.88	0.35-2.20
4.14-4.65	357	7631	33	1.00		4	1.00		14	1.00	
4.66-5.17	438	9379	34	0.88	0.54-1.43	4	0.86	0.21-3.49	8	0.47	0.20-1.14
5.18-5.68	413	8810	43	0.95	0.59-1.51	14	2.79	0.88-8.83	17	0.86	0.41-1.79
5.69-6.20	266	5714	24	0.78	0.46-1.34	5	1.17	0.30-4.57	10	0.70	0.30-1.63
6.21-6.71	149	3164	13	0.77	0.40-1.50	2	0.85	0.15-4.87	6	0.81	0.30-2.21
6.72-	88	1846	12	1.23	0.62-2.44	5	4.47	1.13-17.77	8	1.89	0.76-4.70

SD: standard deviation, HR: hazard ratio; 95% CI: 95% confidence interval, CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure

The HRs were adjusted based on the following factors:

A. age, sex, the serum albumin level, body mass index, diabetes, smoking status and drinking status

B. age, sex, the serum albumin level, body mass index, hypertension, diabetes and drinking status

C. age, sex, the serum albumin level, body mass index, hypertension, diabetes, smoking status and drinking status

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Table 5. PAF and excess deaths due to hypercholesterolemia for CVD, CHD and cardiac death

	CVD death	CHD death	Cardiac Death
JAS definition (TC >=5.69 mmol/L)			
HR (95%CI)	1.08 (0.92-1.28)	1.55 (1.10-2.19)	1.29 (1.00-1.66)
PAF	1.7%	10.6%	5.6%
Excess Death	14.6	18.2	19.5
ATPIII definition (TC >=6.21 mmol/L)			
HR (95%CI)	1.19 (0.95-1.48)	1.79 (1.16-2.74)	1.53 (1.11-2.12)
PAF	1.7%	6.9%	4.6%
Excess Death	14.6	11.9	15.6

HR: hazard ratio, 95%CI: 95% confidence interval, JAS: Japan Atherosclerosis Society, TC: total cholesterol, CVD: cardiovascular disease, CHD: coronary heart disease, PAF: population attributable fraction

to hypercholesterolemia was 14.6, 18.2 and 19.5 for CVD, CHD and cardiac death, respectively. The PAF of hypercholesterolemia determined based on the JAS definition (TC level ≥5.69 mmol/L) was 1.7%, 10.6% and 5.6% for CVD, CHD and cardiac death, respectively. Using the ATP Ⅲ definition (a TC level of ≥6.21 mmol/L), the PAFs for CVD, CHD and cardiac death were 1.7%, 6.9% and 4.6%, respectively. The number of excess deaths was also estimated to be 14.6 for CVD, 11.9 for CHD and 15.6 for cardiac death.

Discussion

In this 24-year Japanese cohort study, we found a 1-SD increment in the serum TC level to be positively associated with an increased risk of CVD, CHD and cardiac death. Similar results were also observed after classifying the participants by age (<65 or ≥65 years) and sex. Moreover, in the analyses of the seven TC level categories, the highest TC level (≥6.72 mmol/L) was found to be significantly associated with an increased risk of death due to CVD, especially CHD and cardiac death, and the PAFs of these diseases were moderately high. Furthermore, the relationship between the serum TC level and cardiovascular outcome was similar to that observed after classifying the subjects based on other risk factors, such as the prevalence of hypertension and current smoking. To the best of our knowledge, this is the first study to estimate the PAF for CVD death due to a high serum TC level in a Japanese population with a long follow-up period. Our results were based on data collected in 1980 and were not influenced by statins, as these drugs were not available in the market at the time of the survey.

In previous studies conducted in Western countries, the estimated PAF of CVD death due to hypercholesterolemia was found to be the highest among that for other risk factors[2-4]. However, the estimated PAFs in our study were lower than those for other risk factors, such as smoking (29%) and hypertension (8%), reported in previous studies assessing Japanese populations[6, 7]. Generally, the serum TC levels are lower in patients in Asian countries, including Japan, than in Western countries[20, 21]. This observation may reflect the different impact of PAF of CVD death due to hypercholesterolemia. Furthermore, the reason for this difference appears to be due to differences in the prevalence of CHD and stroke in the Japanese population. Compared to that observed in Western countries, the prevalence of CHD is lower than that of stroke in Japan[20]. Moreover, as demonstrated in this study, hypercholesterolemia is not related to stroke mortality, although hypertension, smoking and diabetes are positively associated with stroke as well as CHD. Consequently, these factors resulted in lower PAFs due to hypercholesterolemia than those due to other risk factors in the present study.

As described above, neither a 1-SD increment nor high serum TC level were found to be associated with death due to stroke. This observation is consistent with the findings of other studies conducted in Japan[22-24]. In Japan, hypertension is the strongest risk factor for stroke; therefore, the influence of hypercholesterolemia is thought to be relatively weak[25]. Moreover, one subtype of stroke, atherothrombotic infarction, is positively associated with hypercholesterolemia, although its incidence among stroke cases is relatively low in Japan[26, 27]. This may be why hypercholesterolemia is not positively associated with stroke in Japan. The absence of a positive association between the cholesterol levels and stroke mortality has also been noted in studies in Western countries, especially

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among elderly populations or patients with high blood pressure[28]. This is why the HRs and PAFs for total CVD death due to hypercholesterolemia were lower than those for CHD and/or cardiac death in the present study.

Moreover, we found a significant interaction between the serum TC level and smoking for CHD death, and the HR for smoking was higher than that for non-smoking in the subgroup analysis. These results are consistent with those reported by the Asia Pacific Cohort Studies Collaboration[29] and NIPPON DATA80[30] studies, although such findings have not been observed consistently[31]. Further research is therefore needed to confirm our findings.

As demonstrated in previous studies[8, 9], hypercholesterolemia is an important risk factor for CHD in Japan. However, the rate of fatalities from CHD among the Japanese population is lower than that observed in Western countries[32-34]. Heart failure is representative of end-stage CHD; therefore, HF may be registered as the cause of death in patients with CHD who survive a heart attack. Shiba *et al.* showed that the frequency of HF with an ischemic etiology is increasing in Japan[35]. Hence, we evaluated the endpoint "cardiac death," defined as death due to CHD or HF, and observed a similar result as that obtained for CHD only. Notably, the estimated PAF of CHD was higher than that of cardiac death, although this trend reversed when considering the estimated number of excess deaths.

As mentioned above, although the PAFs for smoking and hypertension estimated in other studies[6] are very high (29%), the rate of smoking is currently decreasing in Japan, and the prevalence of hypertension has not changed, even after adjusting for age[36, 37]. In contrast, the incidence of hypercholesterolemia is increasing[37]. As a result, the PAF of hypercholesterolemia may be higher in the future than that estimated in this study. Moreover, because the generation with hypercholesterolemia is reaching an age at which the risk for CVD increases, treating hypercholesterolemia is becoming increasingly important.

This study is associated with several limitations. First, we assessed risk factors, including the serum TC level, at baseline only; therefore, the relationship between the serum TC level and mortality may have been underestimated due to random errors in measuring the serum TC levels, known as the regression dilution effect[38]. Moreover, we did not have any information regarding changes in lifestyle or medications among the study subjects. For these reasons, it is necessary to apply the PAFs determined in this study to the present Japanese population with caution. Second, we were unable to obtain detailed information regarding the subtypes of stroke and heart failure because we could not view the subjects' death certificates. In Japan, the single underling cause of death is determined according to the ICD-9 code (until 1994) or ICD-10 code (from 1995 onwards) by a government officer based on a review of the death certificate. However, we believe this process may minimize the potential for information bias because the endpoints of NIPPON DATA were not defined by the researchers themselves. Third, we did not have access to data for other lipid parameters, such as high-density lipoprotein cholesterol, because the lipid profiles, with the exception of the TC level, were not generally evaluated in Japan during the baseline survey period.

Conclusion

In conclusion, in this long-term cohort study, hypercholesterolemia was shown to be significantly associated with an increased risk of death due to CVD, especially CHD and CHD plus HF, in both sexes among middle-aged and elderly community-dwelling Japanese individuals. The estimated PAFs of these diseases due to hypercholesterolemia were lower than those noted in Western countries and the values for other traditional risk factors. Nevertheless, we believe that managing hypercholesterolemia in Japanese patients is necessary in order to prevent the development of CVD in these subjects.

Declaration

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

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Original Article

The Relationship between Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 Ligands Containing Apolipoprotein B and the Cardio-Ankle Vascular Index in Healthy Community Inhabitants: The KOBE Study

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Aims: Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 ligands containing apolipoprotein B (LAB) and lectin-like oxidized LDL receptor-1 (LOX-1) are known as LOX-1-related modified LDL indicators. These indicators play an important role in the early phase atherosclerosis, but the relationship between these indicators and subclinical atherosclerosis, as represented by the cardio-ankle vascular index (CAVI), has not been assessed. We herein investigated the association of LOX-1-related modified LDL indicators and the CAVI in healthy, Japanese urban community inhabitants who were considered to be at low risk for cardiovascular disease (CVD).

Methods: The participants were 515 healthy Japanese (310 men and 205 women) without a history of CVD, cancer or the use of medication for hypertension, diabetes or dyslipidaemia. To estimate the association between LOX-1-related modified LDL indicators (LAB, soluble form of LOX-1 (sLOX-1)) and the CAVI, we performed multivariable linear regression analyses with possible confounders such as the serum LDL cholesterol level.

Results: The plasma LAB showed a positive association with the CAVI in men (standardized coefficient: 0.11, $p=0.04$). This relationship was not observed in women. On the other hand, no clear association was observed between the CAVI and the plasma sLOX-1 level in either sex.

Conclusions: The plasma LAB levels may represent a useful marker for detecting potential atherosclerosis in healthy individuals considered to be at low risk for atherosclerosis and CVD. Further studies are needed to confirm the present findings.

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Key words: Lectin-like oxidized low-density lipoprotein receptor-1, LOX-1 ligand containing ApoB, Cardio-ankle vascular index, Community-based study

Introduction

Endothelial dysfunction is currently considered to be an early phase in the development of atherosclerosis; the oxidative modification of low-density lipoprotein (LDL) is considered to play a key role in endothelial dysfunction[1]. Lectin-like oxidative LDL receptor-1 (LOX-1) is the receptor for oxidative and/

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or modified LDL in endothelial cells[2], and both LOX-1 and its ligands containing apolipoprotein B (LAB) are involved in endothelial dysfunction[3, 4]. Because a specific ELISA assay for LAB using a monoclonal anti-ApoB antibody can recognize ApoB-48 and ApoB-100, as well as recombinant LOX-1[5], this assay can measure the biological activity of ApoB-containing lipoprotein based on its binding to LOX-1. This parameter reflects the atherogenicity of whole modified LDL better than one specific antigenic determinant of oxidized LDL[6, 7]. Therefore, this ELISA assay is thought to be suitable for evaluating the biologic activity of atherogenic lipoproteins. In fact, recent studies have shown that the soluble form of LOX-1 (sLOX-1) and LAB both are possible biomarkers of not only the risk of cardiovascular disease (CVD), but also subclinical atherosclerotic disease[8-11].

On the other hand, since pathological studies have shown that atherosclerosis of the aorta preceded effects in other organs[12, 13], the cardio-ankle vascular index (CAVI) is considered to be a useful screening tool for subclinical atherosclerosis[14, 15]. The CAVI is also a novel arterial stiffness parameter[16, 17] that is associated with CVD[18-20]. In a recent clinical report, the serum LAB was associated with an increased intima-media thickness (IMT) in Caucasian men in the US, but not in Japanese men[11]. Since the absolute risk for CVD in Japanese men is lower than that in men in the US[21], a more sensitive measurement, such as the CAVI, may be more suitable for detecting potential atherosclerosis in Japanese people.

Accordingly, we conducted this cross-sectional study to investigate the association between LOX-1-related modified LDL indicators (LAB, sLOX-1) and the CAVI in Japanese urban community inhabitants considered to be healthy and at lower risk for CVD than the general population.

Methods

Study Participants

This study is based on data from the baseline survey of the Kobe Orthopedic and Biomedical Epidemiological study (the KOBE study). The KOBE study is a population-based cohort study; one of its endpoints is the incidence of lifestyle-related diseases such as hypertension, diabetes mellitus and dyslipidaemia. All study participants were volunteers, who resided in Kobe City (one of the major cities in the Kansai area, which is the second largest urban area in Japan). The subjects ranged in age from 40-74 years old. The KOBE participants had to meet the following criteria: 1) no current medication use for hypertension, diabetes mellitus or dyslipidaemia; and 2) no history of CVD or cancer. The details of the KOBE study were reported elsewhere[22]. Informed consent was obtained from each participant in writing. As part of the baseline survey of this cohort study, the CAVI was assessed in 549 individuals from July 2010 to December 2011. Of these, 17 participants were excluded; 16 had incomplete data and one had a high triglyceride level (≥400 mg/dL). Seventeen additional participants were excluded because they had a history of using medication for hypertension, diabetes mellitus or dyslipidaemia after the recruiting process. The remaining 515 individuals (310 men and 205 women) were included in this study. The present study was approved by the Ethics Committee at the Institute of Biomedical Research and Innovation.

Data Collection and Standardization

The study participants were asked to respond to questionnaires about lifestyle-related factors, such as the use of medications, smoking (current smoker or not) and alcohol consumption (current drinker or not). The body mass index (BMI) was calculated as the weight (kg) divided by the height squared (m^2). After a five minute rest period, the blood pressure was measured twice in each participant using an automatic sphygmomanometer (BP-103i II; Nihon Colin, Tokyo, Japan), and the mean value for each participant was recorded.

Blood samples were obtained from all participants after they had fasted for at least 10 h, and blood samples were tested in the commissioned clinical laboratory centre (SRL Inc., Tokyo, Japan). The haemoglobin A1c (HbA1c) level was measured by the latex coagulating method. Enzymatic methods were used to measure the serum total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride levels. The LDL cholesterol (LDL-C) level was calculated by Friedewald's formula. The serum high sensitivity C-reactive protein (hs-CRP, mg/L) level was measured by a BN II nephelometer (Dade Behring, Deerfield, IL, USA).

The plasma LAB levels were measured using an enzyme-linked immunosorbent assay (ELISA)[5]. The intra- and inter-assay coefficients of variance (CV) for LAB were 2.2% and 13.1%, respectively (n=26). The

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Table 1. The characteristics of the study participants

	Men	Women
Number of participants	310	205
Age (years)	61±9	63±8
BMI (kg/m^2)	23±3	21±2
SBP (mmHg)	123±17	117±18
DBP (mmHg)	78±10	71±10
HbA1c (%)	5.2±0.5	5.2±0.3
TC (mg/dL)	204±28	226±32
HDL-C (mg/dL)	61±14	72±15
TG (mg/dL)	88 (63, 120)	74 (56, 96)
LDL-C (mg/dL)	122±27	137±29
HR (beat/min)	58±9	61±8
Current smoker (%)	10	1.5
Current drinker (%)	76.8	33.2
hs-CRP (mg/L)	0.29 (0.14, 0.54)	0.24 (0.10, 0.55)
LAB (μg/L)	22700 (18090, 30470)	24660 (18090, 32240)
sLOX-1 (ng/L)	113 (93, 141)	116 (100, 140)
CAVI	8.0±0.9	7.9±0.9

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, TC: total cholesterol, HDL-C; high-density lipoprotein cholesterol, TG: triglyceride, LDL-C: low-density lipoprotein cholesterol, HR: heart rate, hs-CRP: high sensitivity, C-reactive protein, LAB: lectin-like oxidized LDL receptor-1 (LOX-1) ligand containing apolipoprotein B, sLOX-1: soluble form of LOX-1, CAVI: cardio-ankle vascular index
The values were expressed as the means ± standard deviation (SD) or medians (interquartile ranges) for continuous variables, and as percentages for categorical variables.

plasma sLOX-1 levels were measured by a sandwich ELISA using two types of monoclonal antibodies against the extracellular domain of LOX-1[10]. The blood collection tubes for LAB and sLOX-1 were identical to those used for blood glucose; the tubes contained sodium fluoride, heparin sodium and ethylenediaminetetraacetic acid (EDTA-2Na). The intra- and inter-assay CVs for sLOX-1 were 2.4% and 7.8%, respectively (n=26).

The CAVI was measured using a VaSera CAVI instrument (Fukuda Denshi Co. Ltd., Tokyo, Japan). After a five minute rest, the CAVI was assessed, and the heart rate was measured simultaneously. The CAVI was calculated using the formula below:

$$CAVI = a\ \{(2\rho/\Delta P) \times \ln (Ps/Pd)\ PWV^2\} + b$$

where Ps is the systolic blood pressure, Pd is the diastolic blood pressure, PWV is the pulse wave velocity, ΔP is Ps - Pd, ρ is the blood density and a and b are constants. The VaSera is equipped with both measurement and calculation systems, which allowed for automatic calculation of the CAVI.

Statistical Analysis

Sex-specific analyses were performed. The basic characteristics were expressed as the means ± standard deviation (SD) or medians (and interquartile ranges) for continuous variables, and as percentages for categorical variables. The correlation between the LAB and sLOX-1 (both log-transformed) was estimated by Pearson's product-moment correlation in each sex. The sex-specific CAVI was also compared by tertiles of the LAB or sLOX-1 using an analysis of covariance after adjusting for age, BMI, systolic blood pressure, heart rate, HbA1c, current smoking status and current alcohol consumption (drinker or not).

Univariable linear regression analyses were performed to determine the relationships between the CAVI and the variables used in multivariable models (LAB, sLOX-1, age, BMI, systolic blood pressure, heart rate, HbA1c, current smoker or not, current alcohol drinker or not, LDL-C, hs-CRP and HDL-C). The LAB, sLOX-1 and hs-CRP were log-transformed in all of the regression analyses. Multiple adjustments were also performed with linear regression models to estimate the association between LAB and sLOX-1 and the CAVI.

Model 1 included the age, BMI, systolic blood pressure, heart rate, HbA1c, current smoking status and current alcohol consumption. Model 2 included

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Table 2. The results of the univariable linear regression analyses for the relationship between the CAVI and the variables used in the multivariable models

	Men			Women		
	Coefficient	95%CI	*p* value	Coefficient	95%CI	*p* value
Ln LAB	0.525	0.260 to 0.790	<0.001	0.112	−0.159 to 0.383	0.415
Ln sLOX-1	0.212	−0.070 to 0.493	0.140	−0.133	−0.397 to 0.131	0.322
Age	0.061	0.052 to 0.071	<0.001	0.067	0.054 to 0.079	<0.001
BMI	−0.023	−0.062 to 0.017	0.258	−0.048	−0.098 to 0.001	0.056
SBP	0.015	0.009 to 0.021	<0.001	0.017	0.010 to 0.024	<0.001
HR	0.014	0.003 to 0.026	0.017	0.017	0.002 to 0.032	0.024
HbA1c	0.420	0.228 to 0.612	<0.001	0.556	0.182 to 0.930	0.004
Current smoker	0.235	−0.586 to 0.115	0.188	−0.845	−1.861 to 0.171	0.103
Current drinker	−0.170	−0.419 to 0.079	0.180	−0.114	−0.375 to 0.146	0.388
LDL-C	0.001	−0.003 to 0.005	0.616	0.003	−0.002 to 0.007	0.209
Ln hs-CRP	0.186	0.090 to 0.281	<0.001	0.168	0.072 to 0.263	0.001
HDL-C	−0.008	−0.015 to 0.000	0.037	−0.009	−0.017 to −0.001	0.026

CAVI: cardio-ankle vascular index, 95%CI; 95% confidence interval, LAB; lectin-like oxidized LDL receptor 1(LOX-1) ligand containing apolipoprotein B, sLOX-1; soluble form of LOX-1, BMI: body mass index, SBP: systolic blood pressure, HR: heart rate, LDL-C: low density lipoprotein cholesterol, hs-CRP: high sensitivity C-reactive protein, HDL-C; high density lipoprotein cholesterol

the variables in Model 1, plus the serum LDL-C levels. In Model 3, the hs-CRP was added to the variables in Model 2. Model 4 included all of the variables in Model 3, as well as the HDL-C. The adjusted coefficient of determination (adjusted R-squared) was also calculated for each model.

To evaluate the collinearity between variables, especially the LDL-C and LAB or sLOX-1 in regression models, we estimated the variance inflation factor (VIF) in each Model. If the estimated VIF for one variable is over 10, there is strong possibility of the existence of collinieariety[23]. All statistical analyses were performed using the R version 3.0.1 software program (R Foundation for Statistical Computing, Vienna, Austria). The significance level was set at $p<0.05$, and all statistical tests were two-tailed.

Results

Table 1 shows the characteristics of study participants. The mean age was 61 ± 9 years in men and 63 ± 8 years in women. The median LAB (μg/L) was 22,700 (18,090, 30,470) in men and 24,660 (18,090, 32,240) in women. The median sLOX-1 (ng/L) was 113 (93, 141) in men and 116 (100, 140) in women. Neither the LAB nor sLOX-1 was significantly different between the genders. The correlations between the LAB and sLOX-1 were not significant in either gender ($r=0.04$, $p=0.47$ in men and $r=0.05$, $p=0.51$ in women). The mean blood pressure and HbA1c did not meet the criteria for hypertension or diabetes. The mean LDL-C levels in women were higher than those in men, but did not reach the abnormal range. The current smoking rate was 10.0% in men and 1.5% in women, and the current drinking rate was 76.8% in men and 33.2% in women. The mean CAVI was 8.0 ± 0.9. The CAVI values did not differ significantly between men and women (8.0 ± 0.9 and 7.9 ± 0.9, respectively).

The results of the univariable linear regression analyses for the relationship between the CAVI and the variables used in the multivariable models were presented in **Table 2**. There was a significant positive association between the LAB and CAVI in men, but not in women. On the other hand, there were no associations between the sLOX-1 (log-transformed) and CAVI in either men or women.

The associations between the LAB (log-transformed) and CAVI in the multivariable linear regression models are presented in **Table 3**. After stratification by sex, statistically significant positive associations were shown in all three models for men. The coefficients or standardized coefficients in all models for men were not very different. In contrast, no associations were observed in any of the models for women. The plasma sLOX-1 (log-transformed) was also not associated with the CAVI in any model of the sex-specific analyses (**Table 4**). The serum LDL-C levels were not significantly associated with the CAVI in any of the results for model 2. Even if hs-CRP was added as an independent variable in the multivariable linear regression models, the results remained unchanged

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Table 3. The results of the multivariable linear regression analyses for the relationship between the LAB and the CAVI

		Coefficient	95%CI	Standardized Coefficient	*p* value	VIF
Men						
Model 1	Ln LAB	0.232	0.008 to 0.456	0.096	0.042	1.120
			Adjusted R-squared: 0.389			
Model 2	Ln LAB	0.255	0.005 to 0.506	0.106	0.046	1.398
	LDL-C	−0.001	−0.004 to 0.003	−0.021	0.685	1.325
			Adjusted R-squared: 0.387			
Model 3	Ln LAB	0.264	0.015 to 0.513	0.109	0.038	1.399
	LDL-C	0.000	−0.004 to 0.002	−0.029	0.572	1.333
	Ln hs-CRP	0.087	0.007 to 0.168	0.100	0.033	1.121
			Adjusted R-squared: 0.395			
Model 4	Ln LAB	0.247	−0.004 to 0.498	0.102	0.053	1.420
	LDL-C	−0.001	−0.005 to 0.002	−0.031	0.550	1.334
	Ln hs-CRP	0.081	0.000 to 0.162	0.093	0.051	1.146
	HDL-C	−0.004	−0.011 to 0.003	−0.055	0.290	1.378
			Adjusted R-squared: 0.395			
Women						
Model 1	Ln LAB	0.038	−0.177 to 0.253	0.019	0.727	1.092
			Adjusted R-squared: 0.424			
Model 2	Ln LAB	0.008	−0.235 to 0.251	0.004	0.949	1.392
	LDL-C	0.001	−0.003 to 0.005	0.034	0.598	1.487
			Adjusted R-squared: 0.422			
Model 3	Ln LAB	−0.001	−0.242 to 0.240	−0.001	0.993	1.393
	LDL-C	0.001	−0.003 to 0.005	0.042	0.512	1.491
	Ln hs-CRP	0.092	0.011 to 0.172	0.129	0.026	1.184
			Adjusted R-squared: 0.434			
Model 4	Ln LAB	−0.081	−0.326 to 0.164	−0.041	0.516	1.488
	LDL-C	0.002	−0.002 to 0.006	0.052	0.416	1.496
	Ln hs-CRP	0.091	0.012 to 0.170	0.129	0.024	1.184
	HDL-C	−0.008	−0.015 to −0.002	−0.142	0.011	1.153
			Adjusted R-squared: 0.449			

95%CI: 95% confidence interval, LAB: lectin-like oxidized LDL receptor-1 ligand containing apolipoprotein B, 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 LAB (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

(Model 3 in **Table 3** and **Table 4**). Moreover, these results were also similar when the serum HDL-C levels were added to both linear regressions (Model 4 in **Tables 3** and **4**).

The multivariable adjusted CAVI levels tended to be higher according to LAB tertile in men, but this trend was not significant ($p=0.15$, ANCOVA, **Fig. 1**). The estimated VIFs indicated that there was little evidence for the existence of collinearity. The estimated VIFs for LAB, sLOX-1, LDL-C, hs-CRP and HDL-C are shown in **Tables 3** and **4**. In addition, the VIFs for the other variables were not over 1.5 (data not shown).

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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

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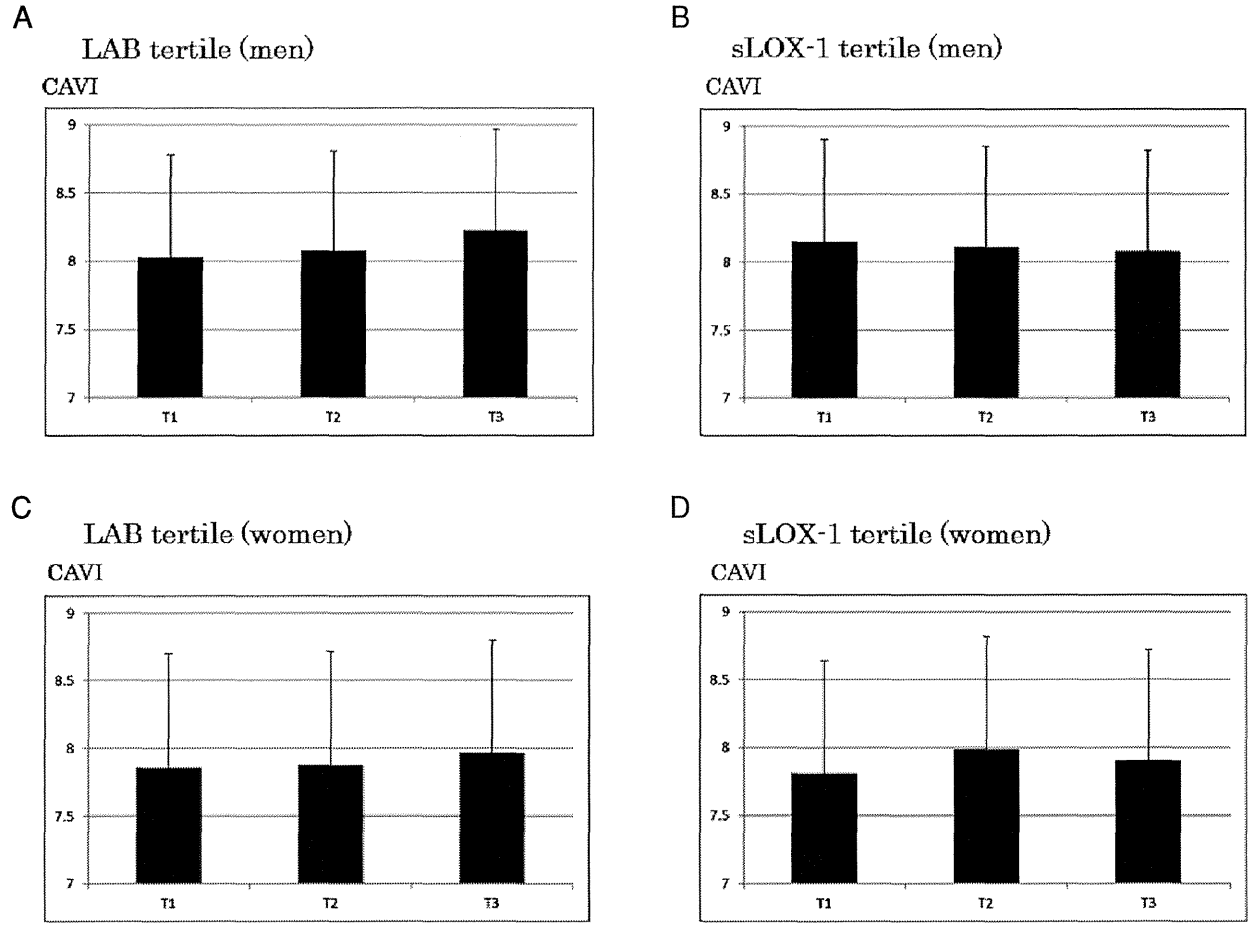

Fig. 1. The multivariable adjusted mean of the CAVI among tertiles of the LAB or sLOX-1.

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

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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.

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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.

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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.

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Lipoprotein particle profiles compared with standard lipids in association with coronary artery calcification in the general Japanese population

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ABSTRACT

Objective: The utility of lipoprotein particle profiles measured by nuclear magnetic resonance (NMR) spectroscopy beyond standard serum lipids remains inconclusive. Furthermore, few studies have compared NMR measurements with standard lipids in association with coronary artery calcification (CAC) in Japanese, where the coronary atherosclerotic burden is low. We examined whether NMR-based lipoprotein particle profiles are associated with CAC, and compared them with standard lipid and lipid ratios in the Japanese general population.

Methods and Results: We conducted a cross-sectional study in 851 men aged 40–79 years without cardiovascular diseases and lipid-lowering therapies. Adjusted odds ratios (ORs) (95% confidence intervals) for the top versus the bottom quartile of NMR-measured particle concentrations were 2.01 (1.24–3.23) for low-density lipoprotein (LDL-P), 1.04 (0.62–1.75) for high-density lipoprotein (HDL-P), 1.82 (1.13–2.95) for very-low-density lipoprotein (VLDL-P), and 1.92 (1.18–3.17) for LDL-P/HDL-P ratio. Similarly adjusted ORs of NMR-measured particle sizes were 0.59 (0.36–0.97) for LDL-P, 0.66 (0.40–1.10) for HDL-P, and 0.67 (0.40–1.12) for VLDL-P. The corresponding ORs were 1.82 (1.14–2.90) for total cholesterol (TC), 2.06 (1.28–3.30) for low-density lipoprotein cholesterol (LDL-C), 0.56 (0.34–0.91) for high-density lipoprotein cholesterol (HDL-C), 2.02 (1.24–3.29) for triglycerides, 2.08 (1.29–3.36) for non-high-density lipoprotein cholesterol (non-HDL-C), 2.27 (1.37–3.78) for TC/HDL-C ratio, and 1.73 (1.06–2.85) for LDL-C/HDL-C ratio. After mutual adjustment for total LDL-P concentration and TC/HDL-C ratio or non-HDL-C, LDL-P was no longer associated, whereas TC/HDL-C ratio remained significantly associated with CAC.

Conclusions: In community-based Japanese men, the overall association of CAC with NMR-measured lipoprotein indices is comparable, but not superior, to that with standard lipids.

1. Introduction

Lipoprotein particle profiles assessed by proton nuclear magnetic resonance (NMR) spectroscopy [1] are heterogeneous with respect to size and density, having a differential effect and strong

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connection with their atherogenic properties [2,3]. NMR-based lipoprotein profiles have thus been suggested as alternative lipoprotein measures for improved risk assessment of cardiovascular disease (CVD). These profiles have also been reported to be associated with an increased risk of CVD outcomes or subclinical atherosclerosis [4–8].

However, how well NMR-based lipoprotein indices predict CVD or subclinical atherosclerosis compared with standard serum lipids remains uncertain. Only a few prospective population-based studies have directly compared the predictability of clinical CVD risk between various NMR-based lipoprotein profiles and standard lipids including ratio measures [5,6]. In those studies, the association of CVD risk with NMR measures was statistically significant, yet of a lesser magnitude than that with standard lipids. Interestingly, the studies that compared these indices in association with subclinical atherosclerosis reported a stronger relation of NMR-based indices than standard lipids [7,8]. Additionally, these epidemiological studies were mainly conducted in Western countries.

The burden of coronary atherosclerosis in Japan has remained lower compared with that in Western populations. This has been confirmed with multiple levels of evidence, including comparative studies for clinical coronary artery disease (CAD) [9], an autopsy study [10], and studies on the subclinical stage of coronary atherosclerosis as measured by coronary artery calcification (CAC) [11–13]. More recently, however, the overall levels of total cholesterol (TC) in Japan have now become similar to or even higher than those in US [9,14,15]. The level of high-density lipoprotein (HDL) cholesterol (HDL-C) among Japanese adults is higher than that of the US [16], and has increased in recent years [17,18]. Literature is scarce on comparison of NMR-based lipid profiles among community-based samples from Japan and other countries. However, we have reported considerable differences in this profile among samples of Japanese men and Caucasians from the US [19]. To the best of our knowledge, no studies have examined the association of NMR-measured lipoproteins with subclinical atherosclerosis in the general Japanese population.

Therefore, we conducted a cross-sectional study of the general Japanese population to examine whether NMR-based lipoprotein particle profiles are associated with CAC, a marker of subclinical atherosclerosis, and compared them with standard lipid and lipid ratios. A community-based Japanese population tends to have a lower burden of CAD and subclinical atherosclerosis compared with Western populations. Therefore, this Japanese population would be useful for examining lipid profiles for CVD assessment in low-risk populations.

2. Methods

2.1. Participants and risk factor measurement

Participants eligible for the present study were 1094 Japanese men enrolled at baseline (May 2006 to March 2008) in the Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA). Detailed methods are described elsewhere [20,21]. In brief, SESSA participants were community-dwelling men aged 40–79 years, who were selected based on an age-stratified random sample from the Basic Residents' Register of the city, which includes information on the name, age, and sex of residents. The present study was approved by the Institutional Review Board of Shiga University of Medical Science (No. 17–19, 17–83).

A total of 243 men were excluded from this analysis for the following reasons: history of CVDs ($n = 103$), use of lipid-lowering medications ($n = 119$), missing information for lipid parameters ($n = 9$), and participants with a triglyceride (TG) level at or above 400 mg/dl ($n = 12$). The last criterion was used to adequately estimate low-density lipoprotein (LDL) cholesterol (LDL-C) levels following Friedewald's formula [22–24]. Therefore, 851 participants were finally included in the present analyses (mean [SD] age, 63.4 [10.0] years).

2.2. CAC

We assessed CAC either by electron-beam computed tomography (EBCT) ($n = 593$, 75.1%) using a C-150 scanner (Imatron, South San Francisco, CA, USA) or 16-channel multidetector-row computed tomography (MDCT, $n = 258$) scans using an Aquilion scanner (Toshiba, Tokyo, Japan). Images were obtained from the level of the root of the aorta through the heart at a slice thickness of 3 mm, with a scan time of 100 ms (EBCT) or 320 ms (MDCT). We acquired images at 70% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. Quantification of CAC was performed using a DICOM workstation and AccuImage software (AccuImage Diagnostics, South San Francisco, CA, USA). The presence of CAC was defined as a minimum of 3 contiguous pixels (area = 1 mm^2) with a density $\geq$ 130 Hounsfield units (HU). We placed a region of interest around each high-density lesion in the epicardial coronary arteries. Peak density (HU) and area (mm^2) of the individual coronary calcifications were measured, and then the CAC score was calculated according to the Agatston method [25]. All computed tomography (CT) images were read by one physician who was trained in CT reading at the Cardiovascular Institute of the University of Pittsburgh, and who was blinded to participants' demographics. The protocol described above was adopted from a separate cohort study performed by our research group [21], in which the reproducibility of the scans showed an intraclass correlation of 0.98 [11]. In stratified analysis by type of CT, we found that trends were similar between participants assessed by EBCT and those by 16-channel MDCT (data not shown). Additionally, CAC assessment by EBCT and MDCT has been reported to be comparable [26]. Therefore, we presented the combined results. To define the presence of CAC, a CAC score >0 was used [7].

2.3. Assays for lipids, lipoproteins, and other variables

Venipuncture was performed early in a clinical visit after a 12-h fast. We separated serum by centrifugation (3000 revolutions per min, for 15 min) at 4 °C within 90 min. Samples were sent for routine laboratory tests, including those for lipids and glucose. Plasma glucose levels were determined from NaF-treated plasma using a hexokinase glucose-6-phosphate-dehydrogenase enzymatic assay. Serum TC and TG were determined using enzymatic assays, and HDL-C was measured using a direct method (Determiner-C-TC, Determiner-C-TGL, Determiner-L HDL-C, respectively; Kyowa Medix, Tokyo, Japan). Serum lipids were determined at a single laboratory (Shiga Laboratory; MEDIC, Shiga, Japan) that has been certified for standardized lipid measurements according to the protocol of the Centers for Disease Control and Prevention/US Collaborating Center for Reference Method Laboratory Network Research in Blood Lipids (CDC/CRMLN) [27]. We used Friedewald's formula to estimate LDL-C levels [28]. Non-high-density lipoprotein cholesterol (non-HDL-C) was calculated by subtracting HDL-C from TC.

Serum samples were stored at −80C° and shipped on dry ice to LipoScience Inc, (Raleigh, NC) for lipoprotein particle analysis. NMR spectroscopy [1] was performed to quantify the particle concentrations of very-low-density lipoprotein (VLDL), LDL, and HDL [29]. Additionally, particle concentrations were further determined for 3 VLDL subclasses (large, >60 nm; medium, 35–60 nm; and small, 27–35 nm), 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) [30]. We calculated weighted average particle sizes of VLDL, LDL, and HDL.

2.4. Statistical analysis

Analyses were performed using the Statistical Package for the Social Sciences, version 18.0J (SPSS Inc., Chicago, IL, USA) and SAS version 9.3 (SAS Institute, Cary, NC, USA). Two-tailed P values <0.05 were considered significant. Participants' characteristics are shown using means and standard deviations (SDs) for continuous variables with approximately normal distributions or medians and interquartile ranges for continuous variables with skewed distributions, and percentages for categorical variables according to the absence or presence of CAC. Differences in characteristics were evaluated using the unpaired Student's t test, Mann–Whitney U-test, or χ^2 test, as appropriate. We calculated age-adjusted Spearman's rank correlation coefficients between the measured lipid levels. We divided each index of lipid into quartiles, and conducted logistic regression to estimate multivariable adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the presence of CAC (CAC score >0) according to the quartiles. In most analyses, the adjusted covariates were as follows: age, smoking status (former, current), ethanol consumption (g/day), BMI, blood glucose, systolic blood pressure, medication status (hypertension and diabetes), [above these covariates were defined as "non-lipid risk factors"], and type of CT. Further adjustment for exercise (defined as participants who regularly exercised ≥1 h/week) and a family history of coronary heart disease did not substantially affect the findings. Therefore, we did not include these variables in the model. The P value for linear trend across quartile was obtained with the median value for each quartile. We also calculated adjusted ORs and 95% CIs per 1-SD increase in lipid indices for the presence of CAC. The following lipid indices were log-transformed due to their skewed distributions: TG, small LDL particle (LDL-P), IDL, large and medium HDL particles (HDL-P), and total, large, medium, and small VLDL particles (VLDL-P). Furthermore, we analyzed non-HDL-C, total LDL-P concentration, and TC/HDL-C ratio in multivariable logistic regression models that were adjusted for other lipids according to previous studies. [7,8].

3. Results

The characteristics of the participants according to CAC are shown in Table 1. Participants with CAC tended to be older and to have less favorable risk factor distributions than those without CAC, including BMI, blood glucose, systolic and diastolic blood pressure, and medication status (hypertension and diabetes). Participants with CAC had higher levels of TG, non-HDL-C, NMR-measured lipoprotein particle concentrations of total LDL-P, small LDL-P, and all of the ratios than those without CAC. Participants with CAC also had lower levels of HDL-C, total and small HDL-P concentrations, and had a smaller size of LDL-P compared with those without CAC.

Supplementary table 1 shows the age-adjusted Spearman's correlation coefficients between NMR-based indices and standard lipid-based indices. Total LDL-P concentration was strongly correlated with LDL-C and non-HDL-C (r = 0.806 and 0.815, respectively). Large HDL-P concentration was positively correlated with HDL-C (r = 0.877), but inversely correlated with TC/HDL-C ratio (r = −0.813). Moreover, total and medium VLDL-P concentrations were strongly correlated with TG (r = 0.856 and 0.804, respectively). Additionally, LDL-P/HDL-P ratio was strongly correlated with TC/HDL-C and LDL-C/HDL-C ratios (r = 0.828 and 0.872, respectively).

Table 1
Characteristics of the participants according to the absence or presence of CAC in apparently healthy Japanese men aged 40–79 years: SESSA, Shiga, 2006–2008.

	CAC (−) (n = 328)	CAC (+) (n = 523)	P value
Age—years	58.4 (11.3)	65.9 (7.8)	<0.001
Smokers—%			
Current	32.9	33.8	0.783
Former	47.3	51.2	0.258
Ethanol consumption—g/day	26.8 (28.0)	33.5 (27.9)	<0.001
Body mass index—kg/m^2	22.9 (2.8)	23.6 (3.1)	<0.001
Blood glucose—mg/dl	98.5 (17.3)	103.9 (23.1)	<0.001
Blood pressure—mmHg			
Systolic	129.7 (17.4)	139.7 (19.6)	<0.001
Diastolic	77.7 (10.6)	80.9 (11.1)	<0.001
Exercise—%	37.2	46.3	0.009
Medication for hypertension—%	13.1	31.4	<0.001
Medication for diabetes—%	2.7	10.3	<0.001
Family history of coronary heart disease—%	11.3	11.1	0.932
Standard lipids—mg/dl			
TC	208.5 (32.6)	209.7 (35.0)	0.489
LDL-C	125.0 (31.2)	127.2 (31.9)	0.292
HDL-C	61.8 (16.2)	57.8 (17.6)	<0.001
TG[a]	94.0 (70.0, 133.5)	105.0 (77.0, 152.0)	0.001
Non-HDL-C	146.6 (34.4)	152.0 (35.3)	0.019
Lipoprotein particle concentrations			
LDL-P—nmol/l			
Total	1242.7 (371.2)	1320.8 (391.6)	0.003
Large	645.6 (282.8)	635.1 (290.9)	0.673
Small[a]	471.0 (94.0, 756.3)	559.0 (107.0, 888.0)	0.007
IDL[a]	100.0 (44.8, 169.0)	84.0 (40.0, 161.0)	0.276
HDL-P—μmol/l			
Total	35.1 (6.2)	33.5 (6.7)	<0.001
Large[a]	6.8 (4.8, 10.1)	6.4 (4.1, 9.6)	0.055
Medium[a]	7.4 (4.8, 10.6)	7.3 (4.9, 10.3)	0.566
Small	19.4 (5.3)	18.5 (5.1)	0.004
VLDL-P—nmol/l			
Total[a]	47.3 (27.3, 76.4)	51.2 (27.5, 87.8)	0.064
Large[a]	0.6 (0.3, 1.6)	0.5 (0.2, 1.6)	0.151
Medium[a]	16.7 (6.9, 33.6)	18.7 (9.1, 38.1)	0.184
Small[a]	24.1 (13.0, 42.3)	27.2 (12.2, 48.2)	0.289
Lipoprotein particle size—nm			
LDL-P	21.0 (0.5)	20.9 (0.6)	0.013
HDL-P	9.3 (0.5)	9.3 (0.5)	0.717
VLDL-P	44.7 (5.9)	44.4 (5.8)	0.590
Ratios			
TC/HDL-C	3.6 (1.0)	3.9 (1.1)	<0.001
LDL-C/HDL-C	2.2 (0.8)	2.4 (0.9)	0.001
LDL-P/HDL-P	36.9 (14.2)	41.4 (16.1)	<0.001

Values are expressed as mean (standard deviation) for continuous variables with approximately normal distributions and as % for categorical variables.
Abbreviations: CAC, coronary artery calcification; 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; non-HDL-C, non-high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; VLDL-P, very-low-density lipoprotein particle.
[a] Continuous variables with skewed distributions are expressed as median (interquartile range). The presence of CAC was defined as a CAC score >0. Participants who exercised were defined as those who regularly exercised ≥1 h/week.

Table 2 shows the ORs of CAC across quartiles for each lipid index adjusted for non-lipid risk factors and type of CT. Of the NMR measures, the association of total LDL-P concentration with CAC was strong, but not greater than associations of standard lipids or ratios. Small LDL-P, but not large LDL-P, concentration was associated with CAC. None of the NMR-measured HDL-P concentrations were significantly associated with CAC. Total, medium, and small VLDL-P concentrations were significantly associated with CAC. Among the three particle sizes that we studied (i.e., LDL-P, HDL-P, and VLDL-P), only LDL-P size was significantly and inversely