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ceptibility to oxidation[9]. Thus, sd-LDL particles possess elevated atherogenic potential. Furthermore, elevated concentrations of sd-LDL can be found in patients with type 2 diabetes, metabolic syndrome, chronic kidney disease, and familial combined hyperlipidemia[10-14], all of which have been found as highly atherogenic conditions. Although Hirano *et al.* showed that sd-LDL-C is significantly higher in patients with coronary artery disease (CAD) in a cross-sectional study[14], no prospective study has addressed whether sd-LDL-C can predict a risk for CVD in non-Western populations. Recently, the Québec Cardiovascular Study has shown prospectively that men with an elevated proportion of LDL with a diameter less than 25.5 nm had a 3.6-fold increased risk of CAD compared with men with relatively normal LDL[15], indicating the strong link of sd-LDL to CVD as a biomarker of cardiovascular disease. Due to its atherogenic properties it is useful to measure sd-LDL for risk assessment; however, a reliable routine method is lacking.

sd-LDL has been measured by ultracentrifugation[16] or gradient gel electrophoresis[17]; however, these methods are both unsuitable for routine analysis, because each requires expensive equipment, complicated techniques, and long assay times. Hirano *et al.* have recently developed a simple precipitation method for sd-LDL-C quantification consisting of 2 steps: removal of apolipoprotein B-containing sd-LDL-free lipoproteins by precipitation with heparin and magnesium, followed by LDL-C measurement by the homogeneous method[18, 19]. This assay allowed us to screen sd-LDL-C in a large cohort. Using this assay, Ai *et al.* recently performed a case control study using samples from the Framingham Offspring Study and found significantly higher sd-LDL-C in women with CAD[20]. Koba *et al.* also showed that sd-LDL-C is more powerful than LDL-C for the determination of CAD[21]; however, these are cross-sectional studies and a prospective study is required to determine whether sd-LDL is an independent predictor of CVD. Therefore, the aim of this study was to address the role of sd-LDL-C for incident CVD in a large cohort study in Japan, the Suita study.

Methods

Population

The Suita study, a cohort study on CVD of urban residents, was established in 1989. The details of this study have been described elsewhere[22]. Briefly, 6485 men and women aged 30-79 years underwent a baseline survey at the National Cerebral and Cardiovascular Center between September 1989 and March 1994, and received medical examinations every 2 years. For these participants, we set the baseline of the present study at medical examinations held between April 1994 and February 1995, since at that time serum samples were collected and stored at −80℃. During this period, 2,437 participants attended the medical examination and were followed until the end of 2007. Of these, 403 participants were excluded due to the following reasons: history of CAD or stroke (n=106), lost to follow-up (n=132), and other reasons such as missing data (n=165). Data from the remaining 2,034 participants (968 men and 1,066 women) were included in the analysis. Informed consent was obtained from all participants. This cohort study was approved by the Institutional Review Board of the National Cerebral and Cardiovascular Center.

Baseline Examination

Blood samples were collected after the participants had fasted for at least 10 hours. The samples were centrifuged immediately. Blood pressure was measured in triplicate on the right arm after 5 min of rest by well-trained physicians using a standard mercury sphygmomanometer. The average of the second and third measurements was used for analysis. At baseline examination, subjects were classified into one of the 5 blood pressure categories based on the criteria of ESH-ESC 2007: optimal (SBP < 120 mmHg and DBP < 80 mmHg), normal (SBP 120-129 mmHg or DBP 80-84 mmHg), high-normal blood pressure (SBP 130-139 mmHg or DBP 85-89 mmHg), hypertension grade 1 (SBP 140-159 mmHg or DBP 90-99 mmHg), or hypertension grade ≥2 (SBP ≥160 mmHg or DBP ≥100 mmHg). Antihypertensive drug users were classified according to their blood pressure at the baseline survey. Diabetes was defined as fasting serum glucose ≥7.0 mmol/L (126 mg/dL) or current use of medications for diabetes. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Well-trained health nurses obtained information on smoking, drinking, and medical histories.

Laboratory Measurements

Serum total cholesterol, triglyceride, and HDL cholesterol (HDL-C) were determined by standard enzymatic methods. Serum glucose was also measured. For the purposes of this study, we used archived plasma samples that had been frozen at −80℃ and never previously thawed for the assessment of direct LDL-C and sd-LDL-C by homogeneous methods on a Hitachi 7180 automated analyzer (Hitachi, Tokyo, Japan)[18, 19]. The kits used for these tests (LDL-C and sd-LDL-C) were provided by Denka Seiken (Tokyo, Japan). Assays

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for direct LDL-C and sd-LDL-C were previously calibrated and directly compared with concentrations obtained after isolation of LDL and sd-LDL by ultracentrifugation.

Endpoint Determination

As previously reported, the endpoints of the present study were (1) date of first CAD or stroke event; (2) date of death; (3) date of leaving Suita city; and (4) the end of December 2007. The first step in the survey for CAD and stroke involved checking the health status of all participants by repeated clinical visits every two years and yearly questionnaires by mail or telephone. In the second step, in-hospital medical records of participants who were suspected of having CAD were reviewed by registered hospital physicians or research physicians who were blinded to the baseline information. The criteria for a diagnosis of CAD included first-ever acute myocardial infarction, sudden cardiac death within 24 h after the onset of acute illness, or coronary artery disease followed by coronary artery bypass surgery or angioplasty. The criteria for definite and probable MI were defined according to the criteria of the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project[23]. The criteria for stroke were defined according to the US National Survey of Stroke criteria[24]. Classification of patients into stroke subtypes was based on examination of computed tomography, magnetic resonance imaging, or autopsy.

Statistical Analysis

Continuous variables between groups were compared by analysis of variance and categorical variables were compared by a chi-square test. Triglyceride levels were logarithmically transformed to improve the skewed distribution. The hazard ratio (HR) for MI or stroke was calculated using a proportional hazards model adjusted for age, sex, hypertension (dichotomous variable), diabetes, HDL-C, BMI, smoking (never-smoked; ex-smoker; current smoker) and drinking (never-drank; ex-drinker; regular drinker). All confidence intervals were estimated at the 95% level and significance was set at $p<0.05$. All statistical analyses were conducted using the SAS statistical software package (release version 8.2; SAS Institute, Cary, NC, USA).

Results

Baseline Clinical Characteristics According to sd-LDL-C Quartiles

To study the role of sd-LDL in the incidence of CVD, we divided the cohort into quartiles according to the basal level of sd-LDL-C. **Table 1** shows the clinical characteristics and cardiovascular risk factors of the study population according to the quartiles of sd-LDL-C. BMI, total cholesterol, LDL-C, and triglyceride significantly increased across the sd-LDL-C quartiles both in men and women, while HDL-C decreased in both genders. A significant trend was observed across the quartiles for the severity of high blood pressure, lipid-lowering drug use, and prevalence of diabetes at baseline both in men and women; however, a significant trend for age was only found in women, not in men.

Incidence of CVD According to sd-LDL-C Quartiles

To confirm our previous study, the association between LDL-C and CAD was examined by dividing the cohort into quartiles according to the baseline LDL-C. It was found that age- and multivariable-adjusted HRs for CAD were statistically significant only in men, not in women or the total cohort. The HR of the 4th quartile in men was 3.53 (95% confidence intervals (CIs): 1.31-9.54) in an age-adjusted model and 3.56 (95% CIs: 1.28-9.86) in a multivariable-adjusted model, consistent with our previous report[1]. We then performed analysis to examine the effect of sd-LDL-C. During the observation period, 116 cases of CVD, 53 cases of stroke, 36 cases of cerebral infarction, and 63 cases of CAD were reported. As shown in **Table 2**, increasing quartiles of sd-LDL-C were significantly associated with increased risks of CVD (stroke + CAD), stroke, cerebral infarction, and CAD after age and multivariable adjustment. Age and sex-adjusted HRs per 10 mg/dL of sd-LDL-C for CVD, stroke, cerebral infarction, and CAD were 1.21 (95% CI: 1.12-1.31), 1.17 (95% CI: 1.05-1.30), 1.15 (95% CI: 1.00-1.33), and 1.29 (95% CI: 1.14-1.45), respectively. HRs after multivariable adjustment were almost the same. When we analyzed each gender, age-adjusted HRs per 10 mg/dL of sd-LDL-C for CVD, stroke, cerebral infarction, and CAD were significant in women, while those for CVD and CAD were significant in men. HR for CAD of the fourth quartile was almost 4 after age and multivariable adjustment in men.

After putting LDL-C into the multivariable adjusted-models (Model A), sd-LDL-C was still associated with increased risk for CVD, stroke, cerebral infarction, and CAD in the total cohort, for CVD in men, and CVD, stroke, and cerebral infarction in women. After further putting logarithmically transformed triglyceride and HDL-C variables into Model A (Model B), sd-LDL-C was still associated with

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Table 1. Baseline characteristics of cardiovascular risk factors according to small dense LDL cholesterol quartiles

	Small dense LDL Cholesterol				*p* value for Trend
	Q1	Q2	Q3	Q4	
Men					
Number of subjects	241	243	242	242	
Small dense LDL, range (mean), mg/dL	6.3-27.8 (21.1)	27.9-38.2 (32.7)	38.3-53.4 (45.3)	53.5-119.6 (67.3)	
Age, year	60.9±13.1	59.7±12.5	59.1±12.3	59.4±11.3	0.421
Body mass index, kg/m^2	21.5±2.5	22.4±2.8	23.4±2.4	24.0±2.7	<0.001
TC, mg/dL	170±25	189±24	199±25	220±27	<0.001
HDL-C, mg/dL	60±15	57±14	51±11	48±11	<0.001
LDL-C, mg/dL	86±20	111±21	124±23	140±26	<0.001
Triglyceride, (median) mg/dL	66	87	112	167	<0.001
Large-LDL-C, mg/dL	65±17	78±21	79±22	72±24	<0.001
Sd-LDL-C/LDL-C ratio	0.25±0.05	0.31±0.07	0.38±0.08	0.50±0.11	<0.001
Blood pressure category, %					0.002
Optimal blood pressure	31	26	25	19	
Normal blood pressure	30	24	19	26	
High-normal blood pressure	16	30	25	29	
Hypertension grade 1-3	19	26	29	28	
Antilipidemic drug use, %	1	4	5	8	0.003
Diabetes, %	3	5	7	9	0.023
Current Smoking, %	44	41	41	44	0.021
Current Drinking, %	66	71	72	74	0.577
Women					
Number of subjects	266	267	266	267	
Small dense LDL, range (mean), mg/dL	7.5-23.9 (18.7)	24.0-33.0 (28.6)	33.1-44.6 (38.5)	44.7-136.6 (59.7)	
Age, year	51.7±13.0	57.3±11.9	60.2±11.2	60.4±9.1	<0.001
Body mass index, kg/m^2	21.0±2.5	21.8±3.2	22.5±3.1	23.2±2.8	<0.001
TC, mg/dL	175±23	200±22	216±25	234±32	<0.001
HDL-C, mg/dL	67±13	64±12	60±13	54±12	<0.001
LDL-C, mg/dL	83±17	109±17	130±18	153±30	<0.001
Triglyceride, (median) mg/dL	61	78	97	140	<0.001
Large-LDL-C, mg/dL	64±14	81±15	92±17	93±25	<0.001
Sd-LDL-C/LDL-C ratio	0.23±0.04	0.27±0.04	0.30±0.05	0.40±0.08	<0.001
Blood pressure category, %					<0.001
Optimal blood pressure	34	27	22	17	
Normal blood pressure	25	24	26	25	
High-normal blood pressure	16	29	20	35	
Hypertension grade 1-3	16	21	31	32	
Antilipidemic drug use, %	4	5	6	12	0.002
Diabetes, %	0	1	3	6	<0.001
Current Smoking, %	13	10	6	7	0.056
Current Drinking, %	34	30	22	23	0.014

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Large LDL-C, LDL-C-Sd-LDL-C. Hypertension was defined as described in methods. Diabetes was defined as fasting serum glucose ≥7.0mmol/L (126 mg/dL), the use of anti-diabetic agents, or both.

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Table 2. Age- and multivariable-adjusted hazard ratios and 95% confidence intervals for the incidence of cardiovascular disease according to small dense LDL cholesterol quartiles

	Small dense LDL Cholesterol, mg/dL				
	Q1 (Lower)	Q2	Q3	Q4 (Higher)	per 10 mg/dL
Men and women, range (mean)	6.3-25.5 (19.7)	25.6-35.3 (30.5)	35.4-49.0 (41.4)	49.1-136.6 (63.9)	
Person-years	5,576	5,789	5,527	5,741	
Cardiovascular disease					
Case	21	23	29	43	
Age and sex-adjusted HR	1	0.75 (0.43-1.29)	1.11 (0.68-1.83)	**1.64 (1.04-2.60)**	**1.21 (1.12-1.31)**
Model 1-adjusted HR	1	0.81 (0.45-1.42)	1.08 (0.65-1.81)	1.60 (0.99-2.60)	**1.21 (1.11-1.32)**
Stroke					
Case	14	13	10	16	
Age and sex-adjusted HR	1	0.58 (0.30-1.14)	0.80 (0.43-1.48)	1.21 (0.69-2.12)	**1.17 (1.05-1.30)**
Model 1-adjusted HR	1	0.63 (0.32-1.23)	0.79 (0.41-1.50)	1.19 (0.65-2.16)	**1.18 (1.04-1.33)**
Cerebral infarction					
Case	8	10	6	12	
Age and sex-adjusted HR	1	1.08 (0.45-2.57)	1.14 (0.47-2.73)	1.74 (0.77-3.90)	**1.15 (1.00-1.33)**
Model 1-adjusted HR	1	1.18 (0.48-2.88)	1.16 (0.46-2.89)	1.85 (0.77-4.40)	**1.18 (1.00-1.39)**
Coronary artery disease					
Case	7	10	19	27	
Age and sex-adjusted HR	1	1.36 (0.49-3.77)	2.26 (0.89-5.73)	**3.35 (1.38-8.13)**	**1.29 (1.14-1.45)**
Model 1-adjusted HR	1	1.44 (0.51-4.08)	2.17 (0.83-5.66)	**3.26 (1.29-8.20)**	**1.28 (1.13-1.46)**
Men, range (mean)	6.3-27.8 (21.1)	27.9-38.2 (32.7)	38.3-53.4 (45.3)	53.5-119.6 (67.3)	
Person-years	2,499	2,615	2,519	2,608	
Cardiovascular disease					
Case	19	19	22	36	
Age-adjusted HR	1	1.06 (0.56-2.01)	1.31 (0.70-2.44)	**2.03 (1.16-3.57)**	**1.15 (1.04-1.28)**
Model 1-adjusted HR	1	1.17 (0.61-2.24)	1.36 (0.70-2.62)	**2.12 (1.16-3.86)**	**1.16 (1.04-1.30)**
Stroke					
Case	14	13	10	16	
Age-adjusted HR	1	1.03 (0.48-2.21)	0.87 (0.38-1.99)	1.43 (0.69-2.97)	1.06 (0.92-1.23)
Model 1-adjusted HR	1	1.13 (0.51-2.47)	0.98 (0.40-2.38)	1.55 (0.70-3.41)	1.08 (0.92-1.28)
Cerebral infarction					
Case	8	10	6	12	
Age-adjusted HR	1	1.33 (0.52-3.39)	0.85 (0.29-2.48)	1.81 (0.73-4.48)	1.08 (0.91-1.29)
Model 1-adjusted HR	1	1.43 (0.54-3.78)	0.90 (0.29-2.80)	1.93 (0.70-5.29)	1.10 (0.90-1.36)
Coronary artery disease					
Case	5	6	12	20	
Age-adjusted HR	1	1.24 (0.37-4.07)	2.48 (0.87-7.07)	**3.89 (1.45-10.42)**	**1.27 (1.10-1.47)**
Model 1-adjusted HR	1	1.27 (0.38-4.29)	2.34 (0.78-6.97)	**4.03 (1.42-11.40)**	**1.28 (1.09-1.50)**
Women, range (mean)	7.5-23.9 (18.7)	24.0-33.0 (28.6)	33.1-44.6 (38.5)	44.7-136.6 (59.7)	
Person-years	3,077	3,174	3,008	3,133	
Cardiovascular disease					
Case	7	12	13	23	
Age-adjusted HR	1	1.01 (0.39-2.60)	0.99 (0.39-2.50)	1.73 (0.74-4.06)	**1.31 (1.16-1.47)**
Model 1-adjusted HR	1	1.04 (0.40-2.72)	0.91 (0.35-2.35)	1.52 (0.63-3.68)	**1.29 (1.13-1.48)**
Stroke					
Case	5	8	6	16	
Age-adjusted HR	1	0.95 (0.30-2.94)	0.64 (0.19-2.11)	1.72 (0.62-4.74)	**1.31 (1.13-1.52)**
Model 1-adjusted HR	1	0.98 (0.31-3.14)	0.64 (0.18-2.19)	1.66 (0.58-4.76)	**1.33 (1.12-1.59)**

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(Cont Table 2)

	Small dense LDL Cholesterol, mg/dL				
	Q1 (Lower)	Q2	Q3	Q4 (Higher)	per 10 mg/dL
Cerebral infarction					
Case	0	5	4	7	
Age-adjusted HR	1	–	–	–	**1.31 (1.05-1.63)**
Model 1-adjusted HR	1	–	–	–	**1.37 (1.05-1.80)**
Coronary artery disease					
Case	2	4	7	7	
Age-adjusted HR	1	1.22 (0.22-7.76)	1.90 (0.39-9.24)	1.84 (0.38-8.91)	**1.32 (1.08-1.61)**
Model 1-adjusted HR	1	1.27 (0.22-7.33)	1.83 (0.35-9.45)	1.54 (0.30-7.83)	1.23 (0.99-1.53)

Model 1: adjusted for age, (sex), body mass index, smoking, drinking, blood pressure category (optimal, normal, and high-normal blood pressure, hypertension grade 1 and 2+3), diabetes, and lipid-lowering drug user
Bold numbers: statistically significant

increased risks of CVD and stroke in the total cohort and in women, but not in men (**Table 3**).

Discussion

This study clearly indicates an increased risk of CVD, stroke, cerebral infarction, and CAD attributed to elevated sd-LDL-C concentrations in a Japanese population without a previous history of CVD. We also showed that HR was significant after multivariable adjustment and by analysis including LDL-C, log-transformed triglyceride, and HDL-C in the same model. Thus, sd-LDL-C measurement with the new test is promising as a new biomarker to predict the risk of CVD.

In addition to traditional risk factors for CVD, such as hypertension, diabetes, and dyslipidemia, other biomarkers are required to better define the risk and refine therapeutic decisions. There is scientific evidence that sd-LDL particles are highly atherogenic and can be a biomarker of CVD[15, 20, 21]. Our data provide additional evidence to show the role of sd-LDL-C as a CVD risk in the general population. Furthermore, measuring sd-LDL-C with this test has an advantage because it is more user-friendly and more applicable than specialized tests such as gradient gel electrophoresis, nuclear magnetic resonance, and gradient ultracentrifugation.

Until now, there have been no target goals of sd-LDL-C to prevent CAD. In this study the HR of the 4th quartile was statistically significant, suggesting that the cutoff of sd-LDL-C is approximately 50 mg/dL, although significance was not obtained in women probably due to the low event rate; therefore, a larger study should be performed to define an appropriate cutoff for sd-LDL-C. Because statins, fibrates, and ezetimibe have been shown to reduce the amount of sd-LDL[25-28], a randomized control study is required to address whether lowering sd-LDL-C to a certain goal by these drugs can prevent the development of CAD.

In this study we found that sd-LDL-C was significantly associated with traditional risk factors, such as hypertension and diabetes. BMI and the prevalence of diabetes increased and HDL-C decreased across the sd-LDL-C quartiles, and more hypertensive subjects were found in second to fourth quartiles than in the first quartile in both genders. We also found that age-adjusted partial correlation coefficients between sd-LDL-C and BMI, log-transformed triglyceride, LDL-C, and HDL-C (Pearson) were 0.305, 0.636, 0.554, and −0.346 ($p<0.0001$), respectively. Thus these data suggest that increased concentrations of sd-LDL-C may be associated with metabolic disorders and that lifestyle modification, such as exercise and weight control, would be effective to reduce sd-LDL in patients with diabetes and metabolic syndrome. Furthermore, we should address whether sd-LDL-C can be used to identify a very high-risk patient with type 2 diabetes, metabolic syndrome, and other metabolic disorders. In contrast to the association with metabolic disorders, an age-related change in sd-LDL-C was found only in women, consistent with the trend of increased atherogenic dyslipidemia in postmenopausal women. Ai *et al.* also found that postmenopausal women had higher levels of sd-LDL-C than premenopausal women in the Framingham Offspring Study[20].

In addition to type 2 diabetes and metabolic syn-

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Table 3. Relationship between major lipid variables and cardiovascular disease

	Cardiovascular disase	Stroke	Cerebral infarction	Coronary artery disease
Men and women				
Age and sex-adjusted	**1.21 (1.12-1.31)**	**1.17 (1.05-1.30)**	**1.15 (1.00-1.33)**	**1.29 (1.14-1.45)**
Multivariable-adjusted Sd-LDL-C/10 mg/dL	**1.21 (1.11-1.32)**	**1.18 (1.04-1.33)**	**1.18 (1.00-1.39)**	**1.28 (1.13-1.46)**
Model A				
Sd-LDL-C/10 mg/dL	**1.26 (1.11-1.43)**	**1.26 (1.06-1.50)**	**1.29 (1.02-1.62)**	**1.29 (1.07-1.55)**
LDL-C/10 mg/dL	0.96 (0.89-1.04)	0.94 (0.85-1.04)	0.93 (0.81-1.06)	0.99 (0.88-1.11)
Model B				
Sd-LDL-C/10 mg/dL	**1.20 (1.01-1.42)**	**1.35 (1.07-1.71)**	1.31 (0.96-1.78)	1.05 (0.81-1.36)
LDL-C/10 mg/dL	0.98 (0.90-1.06)	0.93 (0.83-1.03)	0.92 (0.80-1.07)	1.05 (0.93-1.19)
ln_TG	1.15 (0.71-1.86)	0.76 (0.40-1.46)	0.86 (0.37-1.96)	1.82 (0.87-3.81)
HDL-C/10 mg/dL	0.94 (0.81-1.08)	1.00 (0.84-1.20)	0.93 (0.73-1.18)	0.80 (0.61-1.04)
Men				
Age-adjusted	**1.15 (1.04-1.28)**	1.06 (0.92-1.23)	1.08 (0.91-1.29)	**1.27 (1.10-1.47)**
Multivariable-adjusted Sd-LDL-C/10 mg/dL	**1.16 (1.04-1.30)**	1.08 (0.92-1.28)	1.10 (0.90-1.36)	**1.28 (1.09-1.50)**
Model A				
Sd-LDL-C/10 mg/dL	**1.17 (1.00-1.38)**	1.17 (0.92-1.48)	1.20 (0.90-1.60)	1.18 (0.94-1.48)
LDL-C/10 mg/dL	0.99 (0.89-1.09)	0.94 (0.82-1.08)	0.93 (0.79-1.09)	1.07 (0.93-1.24)
Model B				
Sd-LDL-C/10 mg/dL	1.10 (0.88-1.38)	1.28 (0.92-1.77)	1.28 (0.87-1.90)	0.96 (0.70-1.31)
LDL-C/10 mg/dL	1.01 (0.90-1.13)	0.92 (0.78-1.07)	0.91 (0.76-1.10)	1.14 (0.97-1.33)
ln_TG	1.23 (0.66-2.26)	0.75 (0.32-1.76)	0.86 (0.31-2.38)	1.87 (0.75-4.62)
HDL-C/10 mg/dL	0.96 (0.80-1.14)	1.05 (0.85-1.28)	1.08 (0.94-1.40)	0.72 (0.50-1.03)
Women				
Age-adjusted	**1.31 (1.16-1.47)**	**1.31 (1.13-1.52)**	**1.31 (1.05-1.63)**	**1.32 (1.08-1.61)**
Multivariable-adjusted Sd-LDL-C/10 mg/dL	**1.29 (1.13-1.48)**	**1.33 (1.12-1.59)**	**1.37 (1.05-1.80)**	1.23 (0.99-1.53)
Model A				
Sd-LDL-C/10 mg/dL	**1.44 (1.17-1.77)**	**1.48 (1.13-1.94)**	**1.62 (1.08-2.43)**	1.33 (0.94-1.89)
LDL-C/10 mg/dL	0.92 (0.81-1.04)	0.92 (0.79-1.08)	0.88 (0.69-1.11)	0.94 (0.75-1.16)
Model B				
Sd-LDL-C/10 mg/dL	**1.35 (1.03-1.77)**	**1.47 (1.04-2.08)**	1.33 (0.78-2.29)	1.12 (0.70-1.79)
LDL-C/10 mg/dL	0.93 (0.81-1.07)	0.92 (0.78-1.09)	0.92 (0.72-1.19)	0.98 (0.78-1.24)
ln_TG	1.19 (0.53-2.69)	0.91 (0.31-2.68)	0.86 (0.17-4.25)	1.84 (0.47-7.15)
HDL-C/10 mg/dL	0.92 (0.72-1.19)	0.92 (0.67-1.26)	0.56 (0.31-1.00)	0.92 (0.60-1.41)

Multivariable adjusted for age, sex, body mass index, smoking, drinking, blood pressure category (optimal, normal, and high-normal bloodpressure, hypertension grade 1 and 2 + 3), diabetes, and antilipidemic drug user
Model A: sd-LDL-C per 10 mg/dL and LDL-C per 10 mg/dL in the same model
Model B: sd-LDL-C per 10 mg/dL, LDL-C per 10 mg/dL, ln(TG), and HDL-C per 10 mg/dL in the same model
Sd-LDL-C, small dense LDL cholesterol; ln_TG, logarithmical transformed TG
Bold numbers: statistically significant

drome, sd-LDL-C is increased in familial combined hyperlipidemia and postprandial hyperlipidemia[29, 30]. Hirano *et al.* demonstrated that sd-LDL-C determined by this simple precipitation method is useful for screening familial combined hyperlipidemia in large populations[13]. Because the prevalence of familial combined hyperlipidemia is high in the general population and the increase of sd-LDL particles as well as large VLDL particles is a characteristic feature of familial combined hyperlipidemia, this assay would be quite useful for its diagnosis. Although sd-LDL is decreased by lipid-lowering drugs, such as statins and fibrates, the effect of adequate combination therapy on sd-LDL-C has not yet been confirmed; therefore, this assay would be also useful in determining the therapeutic strategy for patients with a high serum level of sd-LDL-C.

There are some limitations in our study. First, we

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used plasma stored at −80℃, and there is no guarantee that we would have obtained the same results if we had used fresh serum; however, our results are consistent with those reported by Hirano *et al.*, who measured sd-LDL-C in a Japanese general population with the same method, and comparison studies performed in Japan indicate virtually identical results with the use of fresh vs. frozen plasma for sd-LDL-C[13]. Second, the single measurement of sd-LDL-C at the baseline survey and the fact we did not evaluate the longitudinal trend for each risk factor including lipid-lowering agents may have caused us to underestimate the relationship between these conditions and CAD due to regression dilution bias, although we statistically adjusted for the use of lipid-lowering agents at the baseline survey. Third, serum LDL-C was measured by the direct homogeneous assay, which failed to meet the National Cholesterol Education Program total error goals for diseased individuals, although it met these goals in non-diseased individuals[31]. However, the present study is a cohort study of community-dwelling citizens without a history of CVD. Furthermore, the serum levels of LDL-C determined by direct homogeneous assay are almost consistent with those calculated by the Friedewald formula in a large Japanese cohort.

Conclusions

In this large urban cohort study conducted in Japan, we demonstrated that sd-LDL-C is significantly associated with the development of CVD, providing evidence of sd-LDL-C as an important biomarker to predict CVD. A large intervention study is required to determine the appropriate target level of sd-LDL-C.

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Disclosures

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ORIGINAL ARTICLE

Alcohol consumption and cardiovascular disease incidence in men with and without hypertension: the Suita study

This article has been corrected since Advance Online Publication, and a corrigendum is also printed in this issue.

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The relationship between alcohol consumption and the risk for cardiovascular disease (CVD) is U-shaped, whereas alcohol drinking is linearly associated with blood pressure, and the CVD risk also increases linearly according to blood pressure level. Accordingly, we investigated the net effect of alcohol consumption and hypertension on CVD and its subtypes in this study. A 13-year prospective study of 2336 Japanese men who were free from CVD was performed; ex-drinkers were excluded. The participants were divided into eight groups classified by the combination of the presence of hypertension (systolic/diastolic blood pressure ⩾140/90 mm Hg) and alcohol consumption (never-, current- (light, moderate and heavy) drinkers). Multivariate-adjusted hazard ratios (HRs) for the incidence of CVD, coronary artery disease (CAD) and stroke due to the combination of hypertension and alcohol consumption were calculated and compared with non-hypertensive never-drinkers. The HRs for CVD and its subtypes were higher in hypertensives than those in non-hypertensives; in hypertensives without medication for hypertension, the relationship between alcohol consumption and the risks for CVD and CAD was U-shaped, with the highest and most significant increase in never-drinkers. The risk for total stroke was the highest in heavy-drinkers, which was significant. In non-hypertensives, there was no evident increase or decrease in the HRs for CVD and its subtypes in drinkers. Accordingly, controlling blood pressure is important to prevent CVD. In hypertensives, heavy drinking should be avoided to prevent CVD, although light-to-moderate drinking could be protective for CAD. Furthermore, in non-hypertensives, drinkers may need to continuously monitor their blood pressure.
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Keywords: epidemiological study; hazard ratio; prospective study

INTRODUCTION

The relationship between alcohol consumption and the risk for cardiovascular disease (CVD) has been reported to be U-shaped in previous studies.[1,2] However, drinking alcohol is also well known to be positively associated with the development of hypertension.[3] Alcohol consumption is linearly related to increased blood pressure,[4,5] and the CVD risk also linearly increases according to the blood pressure level.[6] Thus, several previous studies have investigated the relationships among alcohol consumption, hypertension and CVD risk in hypertensive patients,[7–9] but few studies were performed in the general population including both hypertensives and non-hypertensives.

Japanese men have been reported to drink more alcohol,[10] have a higher prevalence of hypertension,[11,12] and have a higher prevalence of stroke[13] than Westerners. Therefore, an investigation of the net effect of hypertension and alcohol consumption on the risk for CVD and its subtypes is important in Asian populations, including the Japanese.

To investigate the relationships among alcohol consumption, hypertension and the risk for CVD and its subtypes, a 13-year cohort study of an urban Japanese male population was conducted.

METHODS

Study participants

The Suita study,[14,15] a cohort study of CVD, was established in 1989. In this study, 6485 participants who were randomly selected from the municipal population registry participated in a baseline survey at the National Cardiovascular Center (NCVC, currently, National Cerebral Cardiovascular Center) between September 1989 and February 1994. The present study excluded 821 participants who had a past history of CVD at the baseline survey or who were

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lost to follow-up, as well as 3093 female participants because the alcohol consumption of women was much less than that of men (prevalence of drinking alcohol >23 g ethanol per day in women: 6.3%). In addition, 235 men were excluded for the following reasons: non-fasting visit ($n=83$), missing information at the baseline survey ($n=58$) and being an ex-drinker ($n=94$). The data for the remaining 2336 men aged 30–79 years were then analyzed. Informed consent was obtained from all participants. This study was approved by the Institutional Review Board of the NCVC.

Baseline examination

Well-trained nurses obtained information on smoking, alcohol consumption and the medical histories of the participants. The assessment of alcohol consumption was previously reported.[16] Briefly, current drinkers were asked about the frequency of alcohol consumption during a typical week and the total alcohol intake on each occasion, and the alcohol intake per week was calculated. This value was then divided by seven to obtain the average alcohol intake per day. The usual daily intake of alcohol was assessed in units of 'gou' (a traditional Japanese unit of measurement, corresponding to 23 g of ethanol) and then converted to grams of ethanol per day. In the present study, half a gou was defined as one drink (11.5 g of ethanol), a value nearly equal to a 'standard drink' in other countries.[17] According to the guidelines for lifestyle changes in Japan (Health Japan 21), the recommended amount of alcohol consumption for men was not more than two drinks per day.[18] Thus, the participants were classified as never-drinkers, light-drinkers (≤2.0 drinks per day), moderate-drinkers (>2.0 and ≤4.0 drinks per day) and heavy-drinkers (>4.0 drinks per day).

Well-trained physicians measured the participants' blood pressure in the right arm three times with the participant in a seated position after 5 min rest using a standard mercury sphygmomanometer. The average of the second and third measurements was used in the analyses. Height in socks and weight in light clothing were measured. The body mass index was calculated as weight (kg) divided by the square of height (m^2). Blood samples were collected at the NCVC after the participants had fasted for at least 8 h. The samples were centrifuged immediately, and a routine blood examination that included serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglyceride and glucose levels was then conducted.

Follow-up and endpoint determination

The follow-up method has been described elsewhere.[14,15] Briefly, the endpoints of the present study were as follows: (1) the date of the first stroke or coronary artery disease (CAD) event; (2) the date of death; (3) the date of leaving Suita city; and (4) 31 December 2007. The survey for the stroke and CAD events involved checking the health status of the participants by repeated clinical visits to the NCVC or interview by mail or telephone, followed by checking the in-hospital medical records of the participants who were suspected of having had a stroke or CAD. The criteria for stroke were defined according to the US National Survey of Stroke criteria.[19] For each stroke subtype (cerebral infarction (thrombotic or embolic), intracerebral hemorrhage and subarachnoid hemorrhage), a definitive diagnosis was established based on computed tomography, magnetic resonance imaging or autopsy. In the present study, cerebral infarction was defined as an ischemic stroke, and intracerebral hemorrhage and subarachnoid hemorrhage were defined as hemorrhagic strokes. The criteria for myocardial infarction were defined according to the criteria of the Monitoring Trends and Determinants of Cardiovascular Disease (MONICA) project,[20] which requires evidence from an electrocardiogram, cardiac enzymes and/or autopsy. In addition to acute myocardial infarction, the criteria for a diagnosis of CAD included sudden cardiac death within 24 h after the onset of acute symptoms or CAD followed by coronary artery bypass or angioplasty. Furthermore, to complete the surveillance for fatal strokes and myocardial infarctions, a systematic search for death certificates was conducted.

Statistical analyses

Hypertension was defined as an average systolic/diastolic blood pressure ≥140/90 mm Hg.[21] Dyslipidemia was defined as total cholesterol ≥5.69 mmol l^{-1} (220 mg dl^{-1}) and/or HDL-C <1.03 mmol l^{-1} (40 mg dl^{-1}) and/or triglyceride ≥1.69 mmol l^{-1} (150 mg dl^{-1})[22] and/or current use of oral medication for dyslipidemia. Diabetes was defined as a fasting blood glucose ≥7.06 mmol l^{-1} (126 mg dl^{-1})[23] and/or current use of insulin or oral medication for diabetes.

To show the baseline risk characteristics of the six groups classified by alcohol drinking status (never, light, moderate and heavy) and the presence of hypertension (absent and present), the mean or median was calculated for continuous variables, and the percentage was calculated for dichotomous variables.

The Cox proportional hazards model was used to estimate the age- and multivariate-adjusted hazard ratios (HRs) and 95% confidence intervals of alcohol consumption in those with and without hypertension for the incidence of CVD, CAD, stroke and stroke subtypes after adjustment for age, body mass index, the presence of dyslipidemia and diabetes (absent or present) and smoking status (current or non-current). When the HRs were calculated, never-drinkers without hypertension were defined as the 'reference' group. The estimation of the HRs was also performed after excluding the participants with medication for hypertension at the baseline survey.

All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) statistical software version 15.0 J (SPSS, Tokyo, Japan), and $P<0.05$ (two-tailed) was considered significant.

RESULTS

The mean age of the participants was 55 ± 13 years. Table 1 summarizes the baseline characteristics of the participants divided into eight groups classified by the combination of the presence of hypertension and alcohol consumption. The participants with hypertension were older than those without hypertension, and current drinkers were younger than never-drinkers. The percentage of current smoking was the highest among heavy-drinkers both in those with and without hypertension. In those with hypertension, the triglyceride median increased according to alcohol consumption.

The mean follow-up period was 13 years, and 109 CAD, 78 ischemic stroke and 29 hemorrhagic stroke events occurred. Table 2 shows the age- and multivariate-adjusted HRs (95% confidence intervals) for CVD and its subtypes of the eight groups classified by the combination of alcohol consumption and the presence of hypertension compared with never-drinkers without hypertension in all participants. In non-hypertensives, the HRs for CVD and CAD in current drinkers were consistently lower than that in the reference group. Additionally, the HRs for total and ischemic stroke were similar or slightly higher in the light-drinkers and lower in the moderate- and heavy-drinkers than those in the reference group. However, there was no evident increase or decrease in the HRs for CVD and its subtypes. Among hypertensives, the HRs for CVD and CAD were consistently higher than those in the reference group, and the relationship between alcohol consumption and the risks for CVD and CAD was U-shaped, with the highest and most significant increase in never-drinkers. The HRs for total and ischemic stroke were also consistently higher than those in the reference group, with the highest and most significant increase in heavy-drinkers for total stroke and in light-drinkers for ischemic stroke. For hemorrhagic stroke, the risk associated with alcohol consumption could not be assessed because of the small number of these events (data not shown in the table).

Table 3 shows the age- and multivariate-adjusted HRs (95% confidence intervals) for CVD and its subtypes of the eight groups in the participants without medication for hypertension at the baseline survey. For CVD and CAD, the results were similar to those in Table 2. For total and ischemic stroke, the results in non-hypertensives were also similar to those in Table 2; in hypertensives, the HRs were consistently increased in all groups compared with those in the reference group, and an increase in the HR for both total and ischemic stroke was statistically significant and the highest in heavy-drinkers.

Table 1 Baseline characteristics of the participants; the Suita study: 1989–2007

	Hypertension (−)				*Hypertension (+)*			
		Current drinkers				*Current drinkers*		
	Never-drinkers	*Light (⩽2.0 drinks per day)*	*Moderate (>2.0 and ⩽4.0 drinks per day)*	*Heavy (⩽4.0 drinks per day)*	*Never-drinkers*	*Light (⩽2.0 drinks per day)*	*Moderate (>2.0 and ⩽4.0 drinks per day)*	*Heavy (⩽4.0 drinks per day)*
Number of participants	380	684	348	214	141	276	178	115
Age (years)	56±14	54±13	52±12	47±11	65±10	61±12	59±11	56±10
Body mass index ($kg\,m^{-2}$)	22.3±3.0	22.5±2.7	22.6±2.7	23.0±2.6	23.7±3.6	23.2±3.0	23.4±2.6	23.9±3.0
Systolic blood pressure (mm Hg)	116±13	118±12	119±12	118±11	153±15	152±16	150±16	154±19
Diastolic blood pressure (mm Hg)	72±9	74±9	76±8	75±8	88±10	92±10	91±10	93±10
Total cholesterol ($mmol\,l^{-1}$)	5.20±0.88	5.17±0.85	5.12±0.85	5.09±0.85	5.38±1.03	5.33±0.88	5.30±0.85	5.25±0.98
HDL-cholesterol ($mmol\,l^{-1}$)	1.16±0.31	1.27±0.31	1.34±0.36	1.34±0.36	1.14±0.28	1.27±0.31	1.40±0.36	1.34±0.39
Triglycerides ($mmol\,l^{-1}$)	1.30	1.24	1.24	1.37	1.29	1.34	1.39	1.56
Dyslipidemia (%)	59.5	52.3	47.1	54.2	69.5	60.1	63.5	61.7
Diabetes (%)	2.4	4.1	5.5	5.1	9.9	6.9	7.3	13.9
Current smoking (%)	53.2	47.4	62.1	65.0	34.8	33.0	52.2	61.7

Abbreviation: HDL, high-density lipoprotein.
Values are mean ± s.d. unless specified otherwise. Values of triglycerides are median.
Hypertension: average systolic/diastolic blood pressures of ⩾140/90 mm Hg.
Diabetes: fasting blood glucose of ⩾7 $mmol\,l^{-1}$ and/or current use of insulin or oral medication for diabetes.
Dyslipidemia: fasting serum total cholesterol of ⩾5.69 $mmol\,l^{-1}$ and/or HDL-C <1.03 $mmol\,l^{-1}$ and/or TG ⩾1.69 $mmol\,l^{-1}$ and/or current use of oral medication for dyslipidemia.

When we additionally adjusted for pulse pressure in the estimation of the HRs presented in Tables 2 and 3, the results were equivalent, although the HRs for CAD in hypertensives were slightly attenuated (data not shown).

DISCUSSION

In the present study, the multivariate-adjusted HRs for CVD and its subtypes were consistently higher in the hypertensive participants compared with the non-hypertensive never-drinkers, irrespective of alcohol consumption. In hypertensives without medication for hypertension, the relationship between alcohol consumption and the risks for CVD and CAD was U-shaped, with the highest and most significant increase in never-drinkers. The risk for total and ischemic stroke was the highest in heavy-drinkers, which was significant. In non-hypertensives, there was no evident increase or decrease in the HRs for CVD and its subtypes in drinkers.

One of the strengths of this study was that we compared the risk for CVD and its subtypes due to alcohol consumption among those with and without hypertension. Another strength was that we also estimated the HRs only among the individuals without medication for hypertension at the baseline survey, although the number of events was small. Furthermore, this study is the first to show the relationships among alcohol consumption, hypertension diagnosed by the current definition and the risk for CAD in an Asian population. Although Kiyohara *et al.*[24] investigated the net effect of alcohol consumption on ischemic and hemorrhagic stroke stratified by the presence of hypertension in a Japanese population (Hisayama study), they did not investigate the risk for CAD, and their diagnosis of hypertension was defined as 160/95 mm Hg.

For CAD, the results in the present study were similar to those in previous studies involving hypertensive Western populations. In the previous studies, light-to-moderate alcohol consumption in hypertensives was associated with a reduced risk for CVD mortality or a reduced incidence of myocardial infarction.[7–9] A possible mechanism of reduced risk for CAD in hypertensive drinkers in the present study might be as follows: although they were under high risk for hypertension because of lineally increasing blood pressure due to alcohol drinking[4,5] and high risk for CAD due to hypertension,[6] there might be cardio-protective effects, such as decreased platelet aggregation[25] and increased fibrinolytic activity.[26] An increase in the serum level of HDL-C may be another cardio-protective effect of alcohol.[27] Such cardio-protective effects of alcohol drinking and the relatively higher incidence of CAD compared with that in the previous study in Japan[28] might explain the clear U-shaped relationship between alcohol consumption and the risk for CVD among hypertensives in the present study.

With respect to stroke, Kiyohara *et al.*[24] investigated the combined effects of alcohol drinking and hypertension on stroke in a prospective study of the general Japanese population. The participants were classified as non-drinkers, light-drinkers (<34 g of ethanol per day) and heavy-drinkers (⩾34 g of ethanol per day) and were followed up for 26 years. Among the hypertensive subjects (⩾160/95 mm Hg), the risk for cerebral hemorrhage was significantly increased in heavy-drinkers compared with non-drinkers; the relationship between alcohol consumption and the risk for cerebral infarction was U-shaped, with a significant increase in heavy-drinkers compared with light-drinkers. However, an increase in the risk for hemorrhagic and cerebral stroke was not shown in any drinkers among the non-hypertensives (<160/95 mm Hg). It was observed both in the previous and present studies that the highest risk for stroke was in hypertensive heavy-drinkers, although there was no U-shaped pattern for cerebral infarction herein.

Table 2 Risk for CVD and its subtypes according to the presence of hypertension and alcohol drinking in all participants; the Suita study: 1989–2007

	Hypertension (−)				*Hypertension (+)*			
		Current drinkers				*Current drinkers*		
	Never-drinkers	*Light (⩽2.0 drinks per day)*	*Moderate (>2.0 and ⩽4.0 drinks per day)*	*Heavy (>4.0 drinks per day)*	*Never-drinkers*	*Light (⩽2.0 drinks per day)*	*Moderate (>2.0 and ⩽4.0 drinks per day)*	*Heavy (>4.0 drinks per day)*
Number of participants	380	684	348	214	141	276	178	115
Person-years	4869	8698	4564	2916	1496	3244	2140	1361
CVD								
Number of cases	34	42	17	9	35	46	23	16
Age-adjusted HR (95% CI)	1.00	0.77 (0.49–1.21)	0.76 (0.42–1.36)	0.82 (0.39–1.73)	2.13 (1.32–3.42)	1.55 (0.99–2.41)	1.28 (0.76–2.18)	1.90 (1.05–3.44)
Multivariate-adjusted HR (95% CI)[a]	1.00	0.80 (0.51–1.26)	0.74 (0.41–1.33)	0.79 (0.37–1.66)	2.14 (1.32–3.47)	1.62 (1.03–2.54)	1.23 (0.72–2.10)	1.68 (0.92–3.09)
CAD								
Number of cases	19	17	8	6	24	20	9	6
Age-adjusted HR (95% CI)	1.00	0.55 (0.29–1.06)	0.61 (0.27–1.41)	0.92 (0.36–2.34)	2.72 (1.48–5.01)	1.23 (0.65–2.30)	0.90 (0.41–1.99)	1.25 (0.50–3.14)
Multivariate-adjusted HR (95% CI)[a]	1.00	0.58 (0.30–1.11)	0.63 (0.27–1.44)	0.91 (0.36–2.32)	2.72 (1.46–5.08)	1.28 (0.68–2.43)	0.88 (0.40–1.96)	1.18 (0.47–2.99)
All stroke								
Number of cases	15	25	9	3	11	26	14	10
Age-adjusted HR (95% CI)	1.00	1.05 (0.55–2.00)	0.95 (0.41–2.18)	0.67 (0.19–2.32)	1.46 (0.67–3.18)	1.94 (1.03–3.67)	1.77 (0.85–3.66)	2.73 (1.23–6.10)
Multivariate-adjusted HR (95% CI)[a]	1.00	1.08 (0.57–2.06)	0.88 (0.38–2.02)	0.61 (0.18–2.15)	1.47 (0.66–3.24)	2.03 (1.07–3.88)	1.67 (0.80–3.49)	2.28 (1.01–5.18)
Ischemic stroke								
Number of cases	9	18	6	2	8	21	7	7
Age-adjusted HR (95% CI)	1.00	1.27 (0.57–2.83)	1.05 (0.37–2.98)	0.72 (0.15–3.38)	1.76 (0.68–4.59)	2.61 (1.19–5.71)	1.49 (0.56–4.01)	3.18 (1.18–8.55)
Multivariate-adjusted HR (95% CI)[a]	1.00	1.33 (0.59–2.97)	0.96 (0.34–2.75)	0.63 (0.13–2.98)	1.66 (0.63–4.38)	2.69 (1.21–5.95)	1.33 (0.49–3.61)	2.43 (0.88–6.68)

Abbreviations: CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio.
Hypertension: average systolic/diastolic blood pressures of ⩾140/90 mm Hg.
Dyslipidemia: fasting serum total cholesterol of ⩾5.69 mmol l^{-1} and/or high-density lipoprotein cholesterol <1.03 mmol l^{-1} and/or triglyceride ⩾1.69 mmol l^{-1} and/or current use of oral medication for dyslipidemia.
[a]Multivariate-adjusted HR was calculated after adjustment for age, body mass index, presence of dyslipidemia and diabetes, and smoking status (current or non-current).

Table 3 Risk for CVD and its subtypes according to the presence of hypertension and alcohol drinking in men without medication for hypertension at baseline; the Suita study: 1989–2007

	Hypertension (−)				*Hypertension (+)*			
		Current drinkers				*Current drinkers*		
	Never-drinkers	*Light (⩽2.0 drinks per day)*	*Moderate (>2.0 and ⩽4.0 drinks per day)*	*Heavy (>4.0 drinks per day)*	*Never-drinkers*	*Light (⩽2.0 drinks per day)*	*Moderate (>2.0 and ⩽4.0 drinks per day)*	*Heavy (>4.0 drinks per day)*
Number of participants	368	663	335	207	99	193	133	90
Person-years	4767	8449	4431	2834	1110	2341	1641	1077
CVD								
Number of cases	28	38	15	8	23	28	14	11
Age-adjusted HR (95% CI)	1.00	0.85 (0.52–1.39)	0.82 (0.44–1.54)	0.90 (0.41–2.00)	2.24 (1.28–3.90)	1.59 (0.94–2.69)	1.37 (0.72–2.60)	2.14 (1.06–4.31)
Multivariate-adjusted HR (95% CI)[a]	1.00	0.88 (0.54–1.44)	0.80 (0.42–1.51)	0.86 (0.39–1.92)	2.10 (1.19–3.71)	1.62 (0.96–2.76)	1.26 (0.66–2.42)	2.05 (1.01–4.15)
CAD								
Number of cases	17	16	7	5	15	14	6	4
Age-adjusted HR (95% CI)	1.00	0.59 (0.30–1.17)	0.62 (0.25–1.50)	0.89 (0.33–2.46)	2.48 (1.23–5.00)	1.32 (0.65–2.69)	0.96 (0.38–2.43)	1.27 (0.43–3.80)
Multivariate-adjusted HR (95% CI)[a]	1.00	0.63 (0.32–1.25)	0.66 (0.27–1.61)	0.89 (0.32–2.45)	2.33 (1.13–4.77)	1.39 (0.68–2.85)	0.89 (0.35–2.28)	1.25 (0.42–3.74)
All stroke								
Number of cases	11	22	8	3	8	14	8	7
Age-adjusted HR (95% CI)	1.00	1.27 (0.61–2.61)	1.14 (0.45–2.85)	0.90 (0.25–3.27)	1.92 (0.77–4.79)	2.01 (0.91–4.43)	2.10 (0.81–5.00)	3.49 (1.35–9.06)
Multivariate-adjusted HR (95% CI)[a]	1.00	1.25 (0.60–2.58)	1.02 (0.40–2.56)	0.83 (0.23–3.02)	1.79 (0.70–4.56)	1.98 (0.89–4.41)	1.86 (0.74–4.70)	3.25 (1.24–8.51)
Ischemic stroke								
Number of cases	6	15	5	2	5	10	5	5
Age-adjusted HR (95% CI)	1.00	1.58 (0.61–4.07)	1.26 (0.38–4.17)	1.02 (0.20–5.14)	2.26 (0.69–7.46)	2.68 (0.97–7.38)	2.33 (0.71–7.62)	4.45 (1.35–14.66)
Multivariate-adjusted HR (95% CI)[a]	1.00	1.57 (0.60–4.06)	1.09 (0.33–3.64)	0.88 (0.17–4.48)	1.93 (0.57–6.53)	2.59 (0.93–7.23)	2.02 (0.61–6.71)	3.78 (1.13–12.61)

Abbreviations: CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio.
Hypertension: average systolic/diastolic blood pressures of ⩾140/90 mm Hg.
Dyslipidemia: fasting serum total cholesterol of ⩾5.69 mmol l^{-1} and/or high-density lipoprotein cholesterol <1.03 mmol l^{-1} and/or triglyceride ⩾1.69 mmol l^{-1} and/or current use of oral medication for dyslipidemia.
[a]Multivariate-adjusted HR was calculated after adjustment for age, body mass index, presence of dyslipidemia and diabetes, and smoking status (current or non-current).

However, these results in Japanese hypertensives were not consistent with those in Western populations,[7,8] that is, the risk for stroke was lower in any drinkers compared with never-drinkers in Westerners.[7,8] This inconsistency between the Western and Japanese populations might be due to a difference in the incidence of stroke events and in the percentage of stroke subtypes. Specifically, the incidence of hemorrhagic stroke, which could be affected by heavy alcohol drinking,[2,29] and hypertension[30,31] has been much lower than that of ischemic stroke in Western populations.[32] Furthermore, for ischemic stroke, the frequency of cortical infarction or cerebral embolism was high in Western populations,[33,34] and a pathological study revealed that moderate alcohol intake has a weak inverse association with atherosclerosis in large, cerebral arteries.[35] Thus, even in a hypertensive condition, the risk for stroke associated with alcohol consumption could be low or not evidently increased in Western populations. In contrast, in a Japanese population, the incidence of hemorrhagic stroke is considered to be higher than that in Western populations.[2,31] Additionally, lacunar infarction due to small-vessel disease was the most common among Japanese individuals.[36] Moreover, a pathological study revealed that moderate alcohol intake did not have an inverse association with atherosclerosis in small cerebral arteries.[35] Thus, these factors might have an influence on the additive effect of alcohol and hypertension on stroke in the Japanese population.[24]

In non-hypertensive participants, neither an evident increase nor decrease was shown in the risk for CAD and stroke with increased alcohol consumption. As the numbers of non-hypertensive participants and CVD event cases were small in the present study, the risk for CVD and its subtypes of alcohol drinking in non-hypertensives should be investigated in other large-scale prospective studies.

As shown in the present study, hypertension is the key to determine the risk for CVD and its subtypes. As heavy drinking was associated with a significant increase in the risk for both CAD and stroke in hypertensives, individuals with hypertension should avoid heavy alcohol drinking. In addition, lowering high levels of alcohol consumption is associated with a reduction in blood pressure.[37] Thus, a reduction in alcohol consumption is expected to be followed by both a decrease in blood pressure and particularly a decrease in the risk for stroke, although the association between the reduction of alcohol consumption in hypertensives and the risk for stroke incidence should be examined in future studies among Asian populations. In non-hypertensives, drinkers need to pay attention to their blood pressure and avoid heavy drinking, not only for the prevention of CVD, but also for the prevention of other alcohol-induced diseases.

The present study had several limitations. First, the relationships among alcohol drinking, hypertension and hemorrhagic stroke could not be assessed because of the small number of cases. In addition, we could not assess the risk for CVD and its subtypes in moderate- and heavy-drinkers separately with and without hypertension due to the small number of events. Second, single blood pressure measurements and a single questionnaire for alcohol consumption at the baseline survey might have underestimated the relationships among alcohol drinking, hypertension and CVD due to regression dilution bias.[38] Third, the effects of the type of alcoholic beverage[17] and genetic differences, such as acetaldehyde dehydrogenase genotypes,[39] could not be investigated. Fourth, we potentially could not fully remove the influence of age differences among the groups at the baseline, although we adjusted for age in the estimation of the HRs.

In conclusion, compared with never-drinkers without hypertension, the risks for CVD, CAD, stroke and ischemic stroke were increased in those with hypertension, irrespective of alcohol consumption. The risk for CAD was the highest in hypertensive never-drinkers, whereas the risk for stroke was the highest in hypertensive heavy-drinkers. In non-hypertensives, there was no evident increase or decrease in the HRs for CVD and its subtypes in drinkers. Accordingly, controlling blood pressure is important to prevent CVD. In hypertensives, heavy drinking should be avoided to prevent CVD, although light-to-moderate drinking could be protective for CAD. Furthermore, in non-hypertensives, drinkers may need to continuously monitor their blood pressure.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1. 虚血性心疾患例における頸動脈・頭蓋内動脈狭窄性病変および無症候性脳梗塞について

上原　敏志

要　旨

我々は，MR angiography および MRI を用いて，日本人における冠動脈硬化と頸動脈・頭蓋内動脈の動脈硬化，無症候性脳梗塞（SCI）との関連性の有無を検討した．その結果，冠動脈硬化と頸動脈硬化との間には強い関連性が見られ，病変の頻度，重症度，さらには進行の有無にも関連性があることが明らかとなった．一方，頭蓋内動脈病変と冠動脈病変との間の関連性は弱いが，頭蓋内動脈を部位別にみると，内頸動脈および椎骨動脈と虚血性心疾患（IHD）との間に関連性があることが示された．IHD 例における SCI については，大脳白質と基底核の両方に見られる混在型が多かった．そして，年齢や高血圧と関連して大脳白質の SCI が出現し，さらに基底核の SCI を合併することが頸動脈や冠動脈の動脈硬化の指標のひとつになることが示唆された．

（脳循環代謝　23：142～146，2012）

キーワード：虚血性心疾患，頸動脈狭窄，頭蓋内動脈狭窄，無症候性脳梗塞，MR angiography

1. はじめに

一般的に，脳血管の動脈硬化は大動脈や冠動脈の動脈硬化に遅れて発生・進展すると言われており，実際の日常臨床においても，虚血性心疾患（IHD）が先行し，その後に虚血性脳血管障害を発症する例は少なくない[1]．Framingham Study の 24 年間の追跡調査によると，IHD 例からの脳血管障害発症率は，狭心症例では IHD のない例の 1.6～2.4 倍，心筋梗塞例では 2.7～3.7 倍，両者合併例では 3.8～5.5 倍高いと言われている[2]．

従来，欧米人の脳血管病変は主として頸動脈にみられ，一方，日本人では主として頭蓋内動脈にみられることが特徴とされてきた．そして，欧米人においては，冠動脈硬化と頸動脈硬化との間に強い関連性があることが既に良く知られていた[3,4]．近年，日本人においても，食生活をはじめとする生活様式の欧米化に伴って頸動脈病変の頻度が増加し，冠動脈病変との関連性も注目されるようになってきた．これまでに我々は，日本人における冠動脈硬化と頸動脈・頭蓋内動脈の動脈硬化および無症候性脳梗塞（SCI）との関連性の有無を明らかにするために，MR angiography（MRA）および MRI を用いて IHD 例における無症候性の頸動脈・頭蓋内動脈狭窄性病変および SCI について検討してきたのでその結果を国内外の文献と合わせて紹介したい．

2. 頸動脈狭窄性病変

頸動脈狭窄性病変の非侵襲的検査法として，頸部超音波検査や MRA 検査などがあるが，頸部超音波検査を用いて IHD 例の頸動脈病変の評価を行った報告が数多くみられる．頸動脈病変の程度を IHD の有無により比較した欧米での検討では，IHD を有する群で頸動脈病変が強いことが報告されている[3,4]．田中ら[5]は，冠動脈造影上 75% 以上の狭窄を有する症例の 50.9% に頸動脈の atheromatous plaque が認められ，冠動脈病変の重症度と頸動脈病変の重症度との間に関連性があったと述べている．冠動脈バイパス（coronary artery bypass graft；CABG）術前に頸動脈病変のスクリーニングを行った研究によると，頸動脈狭窄病変の合併頻度は 2.8～22.3% とされている[6,7]．

IHD により冠動脈造影検査が施行された患者のうち脳血管障害の既往のない 67 例を対象に MRA を用

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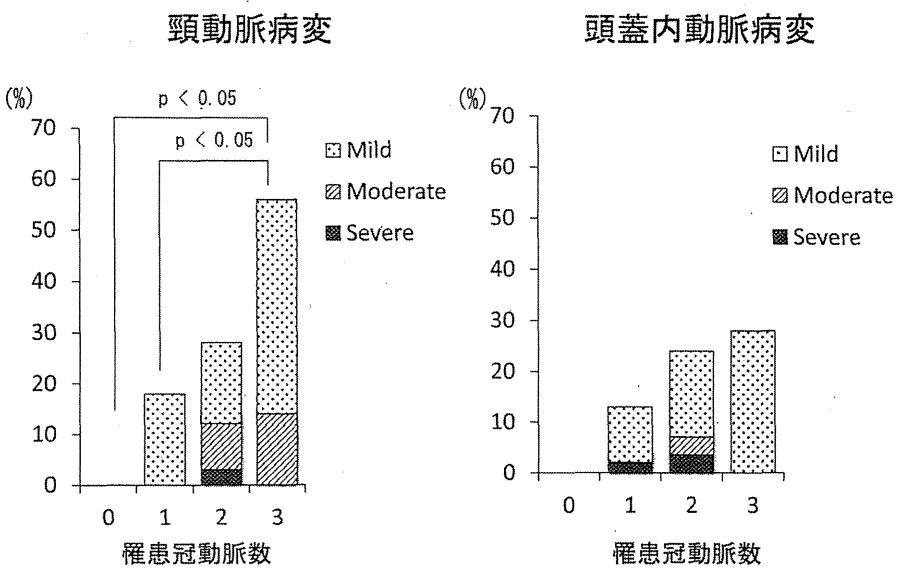

図1. 罹患冠動脈数別にみた無症候性脳血管狭窄性病変の頻度 —虚血性心疾患例での検討—（文献10より改変引用）

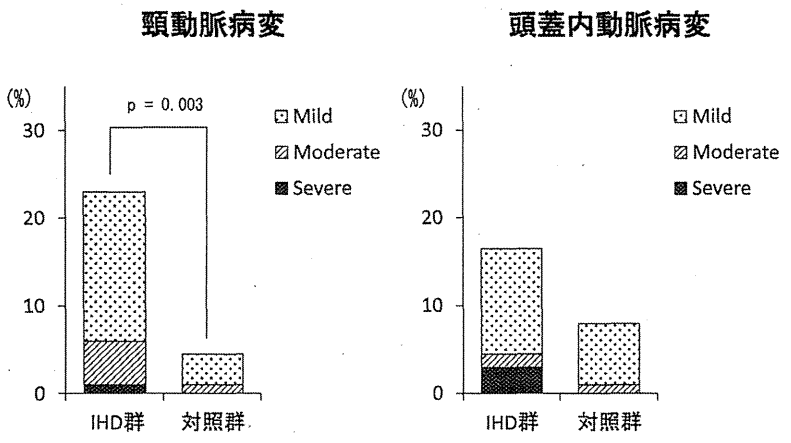

図2. 無症候性脳血管狭窄性病変の頻度 —虚血性心疾患（IHD）群と対照群との比較—（文献11より改変引用）

いて評価した（MRAの撮像方法や狭窄度分類の詳細は既報[8,9]のとおりである）我々の検討では，頸動脈（頭蓋外内頸動脈）に25%以上の無症候性狭窄病変を認める頻度は22.4%であり，冠動脈病変の重症度と頸動脈病変の頻度との間に関連性を認めた[10]（図1）．頭重感，めまいなどを主訴に神経内科外来を受診したIHDの既往がない患者のうち，IHD群と年齢および性別を合わせた64例を対照群として狭窄病変の頻度を比較すると，頸動脈病変の頻度は対照群に比してIHD群で有意に高かった[11]（図2）．IHD群67例中40例に5年後のfollow-up MRAを実施したところ，5例（12.2%）に頸動脈狭窄病変の進行を認めた．多変量解析の結果，頸動脈病変の進行に対する有意な関連因子は，年齢(70歳以上)，登録時の狭窄病変の存在，追跡期間中の冠動脈イベントであった[12]．心血管疾患の高リスク患者を対象に，頸動脈超音波検査で検出された無症候性頸動脈狭窄病変の進行と心血管イベント（心筋梗塞，経皮的冠動脈もしくは末梢動脈インターベンション，冠動脈もしくは血管手術，肢切断術，脳卒中，全死亡）との関連性を検討した報告では，頸動脈狭窄の進行が心血管イベントの予測になったと述べられている[13]．

これらの検討結果より，欧米人のみならず日本人においても，冠動脈硬化と頸動脈硬化の間には強い関連性が見られ，病変の頻度，重症度，さらには進行の有無にも関連性があることが示された．

3. 頭蓋内動脈狭窄性病変

IHD例における頭蓋内動脈狭窄性病変について検

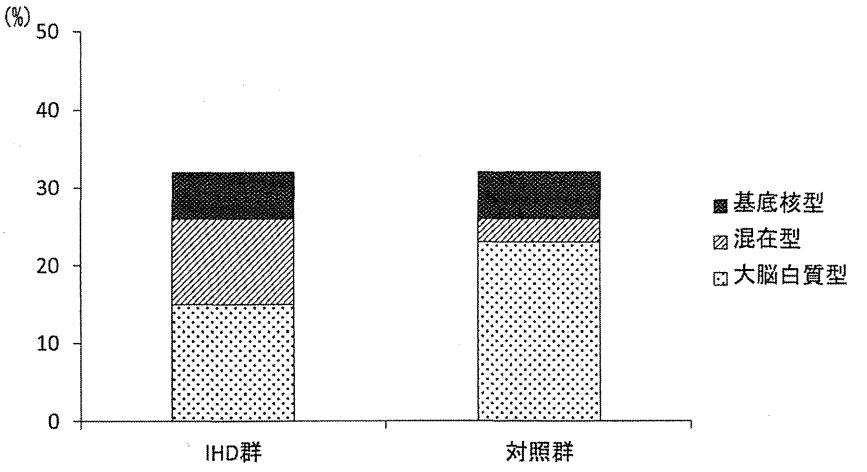

図3. 無症候性脳梗塞の頻度とその分布　—虚血性心疾患（IHD）群と対照群との比較—（文献11より改変引用）

討した報告は少ない．前述のMRAを用いた我々の検討では，頭蓋内動脈（頭蓋内内頸動脈，中大脳動脈，脳底動脈）に25%以上の無症候性狭窄病変を認める頻度は16.4%であり，年齢が有意な危険因子であった．罹患冠動脈数が増えるにしたがって頭蓋内動脈病変の頻度は増加する傾向がみられた[10]（図1）．IHDの既往がない対照群と比較すると，頭蓋内動脈病変の頻度はIHD群で高いものの両群間で統計学的に有意差はなかった[11]（図2）．蔵本ら[14]は剖検例を用いて冠動脈，大動脈，脳動脈（頭蓋内動脈），総頸動脈および大腿動脈の動脈硬化の相互関係を検討した結果，脳動脈（頭蓋内動脈）と冠動脈との間には相関関係が認められるが最も相関係数が低かったと述べている．すなわち，冠動脈硬化と頸動脈硬化との関連性に比べて，冠動脈硬化と頭蓋内動脈の動脈硬化との関連性は弱いものと思われる．しかし，CABG術適応例における頭蓋内動脈病変の頻度は低くない．MRAを用いた我々のCABG術前評価では，頭蓋内動脈に50%以上の狭窄病変を認める頻度は21.2%であった[6]．牧野らの報告[15]では約20%の症例に高度狭窄病変を認めている．CABG術前に超音波検査法もしくはMRAを用いて頭蓋内外動脈の狭窄性病変について検討した韓国の報告では，頭蓋内動脈狭窄病変の頻度は30.3%（50%以上の狭窄病変は8%）であり，頭蓋内動脈狭窄病変が術後の中枢神経合併症の独立した危険因子であったと述べている[16]．頭蓋内MRAによる過大評価の可能性に注意をする必要はあるが，CABGを要するほど重度のIHD例においては，頭蓋内の動脈硬化性病変も進行している可能性が考えられた．

頭蓋内動脈の無症候性狭窄病変に関する危険因子を血管部位別に検討してみると，頭蓋内内頸動脈と脳底動脈については高血圧と糖尿病が，中大脳動脈と頭蓋内椎骨動脈については高血圧が有意な危険因子であり，頭蓋内内頸動脈および椎骨動脈とIHDとの間に有意な関連性が見られた[17]．急性期虚血性脳卒中で入院して脳血管造影検査およびMultislice cardiac computed tomographyを施行した1,304例を対象とした韓国からの報告では，32.3%に無症候性の冠動脈病変（50%以上狭窄）が認められ，冠動脈病変と頭蓋内外の内頸動脈，頭蓋内外の椎骨動脈および脳底動脈病変との間に関連性が示された[18]．頭蓋内動脈の中でも，頭蓋内内頸動脈病変と冠動脈病変との関連性を認めたとする報告は多く[17~20]，頭蓋内内頸動脈病変が全身の動脈硬化の指標のひとつになるという報告も見られる[20]．

4. 無症候性脳梗塞（SCI）

IHD群と対照群とで無症候性病変を比較した我々の検討[11]では，SCIの頻度は両群とも31.3%だった（図3）．SCIはすべて径が15mm以下で大脳白質，基底核にみられた．SCIを認めた症例における梗塞個数の平均は，IHD群が3.3個で対照群の2.3個に比べて多かったが有意差はなかった．病変分布について大脳白質型，基底核型，混在型（大脳白質＋基底核）に分類すると，IHD群では対照群に比して混在型が多い傾向が見られた．SCI（＋）群ではSCI（－）群に比して，年齢が有意に高く，頸動脈狭窄病変の頻度が有意に高かった．IHD患者92例を対象にして頭部CTを用いて無症候性脳梗塞について検討したTanakaらの報告では，26例（28.3%）に無症候性脳梗塞を認め，年齢および冠動脈病変の重症度との間に関連性があっ

たと述べられている[21].

脳病変の精査を希望して神経内科外来を受診した症例のうち，脳血管障害の既往がない219例（平均年齢63.2歳）を対象にした我々の検討[22]では，88例(40.2%)にSCIを認め，大脳白質型が50例，基底核型が6例，混在型が32例であった．多変量解析の結果，大脳白質のSCIに関しては年齢，女性，高血圧が，基底核のSCIに関しては年齢，IHDの既往および頸動脈狭窄病変が有意な関連因子であった．基底核にSCIを有する症例の大部分は大脳白質にもSCIを有していたことから，年齢や高血圧と関連して大脳白質のSCIが出現し，さらに基底核のSCIを合併することが頸動脈や冠動脈にみられる全身性の動脈硬化の指標のひとつになることが示唆された．

5. おわりに

IHDと虚血性脳血管障害は関連性が強く，今後，両疾患の合併例はますます増加することが予想される．IHD例における脳動脈病変の状態を無症候性のうちに把握し，症候性への移行を防ぐことは臨床上，重要な課題のひとつと考えられる．

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Abstract

Asymptomatic stenotic lesions of carotid and intracranial arteries, and silent cerebral infarctions in patients with ischemic heart disease

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We investigated the association of asymptomatic occlusive lesions in carotid and intracranial arteries, and silent cerebral infarcts (SCIs) detected on brain MR angiography and MRI with ischemic heart disease (IHD) in Japanese.

In our study, asymptomatic occlusive lesions in the carotid and intracranial arteries were fairly common in Japanese patients with IHD, although the degree of stenosis was relatively mild. The incidence of extracranial carotid stenosis and severity of coronary atherosclerosis showed a significant correlation. The mean age of the patients with intracranial arterial lesions was statistically higher than those without intracranial lesions. Atherosclerosis of the intracranial vertebral artery and internal carotid artery was related to IHD as was the case for the extracranial carotid artery.

In addition, our studies demonstrated that age, female sex, and hypertension were significant and independent predictors of SCIs in the white matter (WM), and that age, a history of IHD, and carotid artery stenosis were significant and independent predictors of SCIs in the basal ganglia (BG). These results suggested that SCIs were prone to first appear in the WM in association with aging and hypertension, and the additional appearance of SCIs in the BG predicted a progression of generalized atherosclerosis that was manifested in the carotid and coronary arteries.

Key words : ischemic heart disease, carotid arterial stenosis, intracranial arterial stenosis, silent cerebral infarction, MR angiography