BP category on carotid atherosclerosis, we analyzed the association between BP category and the carotid atherosclerosis index in subjects with and without CKD, using ANCOVA and logistic regression analysis, adjusting covariates (age, smoking and drinking status, blood glucose category, total and HDL cholesterol and body mass index). A P value <0.05 was considered significant for all comparisons. All analyses were performed with SAS statistical software (version 8.2; SAS Institute, Cary, NC, USA).

Supplemental Table I. Characteristics of study subjects according to eGFR category

	GFR in men, mL/min/1.73m^2 (n = 1602)				GFR in women, mL/min/1.73m^2 (n = 1844)			
	≤90	60-89	50-59	<50	≤90	60-89	50-59	<50
Patients, n	236	1106	174	86	436	1214	137	57
Age, y	57±11	67±11	72±8	78±7	59±9	64±11	71±9	74±8
BP category								
Optimal BP, %	31	20	14	8	35	31	21	9
Normal BP, %	18	19	19	11	18	19	10	5
High normal BP, %	13	17	12	11	15	13	14	11
Hypertension, %	38	44	55	70	32	37	55	75
Diabetes mellitus, %	14	11	13	15	7	5	8	9
Total cholesterol, mg/dL	199±33	199±31	200±30	196±35	215±32	217±31	216±32	207±33

HDL cholesterol, mg/dL	59±16	55±14	52±13	51±13	66±16	65±15	61±13	63±16
Body mass index, kg/m^2	23±3	23±3	23±3	23±3	22±3	22±3	23±3	22±4
Current smoking, %	43	29	20	17	8	6	6	4
Current drinking, %	74	68	58	50	32	27	20	16

Values are the means±standard deviation or percent.
GFR, glomerular filtration rate; BP, blood pressure; HDL, high-density lipoprotein

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Original Article

Effect of Age on the Association Between Waist-to-Height Ratio and Incidence of Cardiovascular Disease: The Suita Study

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ABSTRACT

Background: Waist-to-height ratio (WHtR) has been shown to be a useful screening tool for metabolic syndrome and cardiovascular disease (CVD). We investigated the association of WHtR with CVD incidence by age group.
Methods: We conducted a 13.0-year cohort study of Japanese adults (2600 men and 2888 women) with no history of CVD. WHtR was calculated as waist circumference (cm) (WC) divided by height (cm). We stratified participants by sex and age group (30–49, 50–69, ≥70 years). Using the Cox proportional hazards model, we calculated hazard ratios (HRs) and 95% CIs for CVD in relation to WHtR quartile for participants aged 50 to 69 years and 70 years or older.
Results: Men aged 50 to 69 years in the highest quartile had significantly increased risks of CVD and coronary heart disease as compared with the lowest quartile; the HRs (95% CI) were 1.82 (1.13–2.92) and 2.42 (1.15–5.12), respectively. Women aged 50 to 69 years in the highest quartile had a significantly increased risk of stroke (HR, 2.43; 95% CI, 1.01–5.85). No significant results were observed in men or women aged 70 years or older. The likelihood ratio test showed that the predictive value of WHtR was greater than that of WC among men aged 50 to 69 years.
Conclusions: The association between WHtR and CVD risk differed among age groups. WHtR was useful in identifying middle-aged Japanese at higher risk of CVD and was a better predictor than WC of CVD, especially in men.

Key words: waist-to-height ratio; age difference; cardiovascular disease

INTRODUCTION

Obesity and central obesity are closely tied to metabolic risks.[1,2] Waist circumference (WC) is an index of central obesity[3] and is an important component in the diagnostic criteria for metabolic syndrome.[4] Several meta-analyses have reported an association of WC with cardiovascular disease (CVD) and mortality.[5,6] Recently, waist-to-height ratio (WHtR) was shown to be a useful global clinical screening tool for cardiometabolic risk and CVD.[7,8]

WHtR is easy to measure, and the cut-off point for WHtR is subject to less ethnic variation.[7,8] However, WHtR could differ among age groups because whole-body fat distribution and WC change considerably with age[9,10] and because height differs among generations.[11] It is thus important to consider age in assessing the association between WHtR and CVD risk, but few previous studies have done so.[12,13] Therefore, in this long-term prospective cohort study of a Japanese urban population, we investigated the effect of WHtR on CVD risk among participants classified by age group.

METHODS

Study population

The Suita Study is a prospective population-based cohort study of an urban area of Japan and was established in 1989. The details of this study have been described elsewhere.[14–16] Briefly, 6407 men and women aged 30 to 83 years underwent

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a baseline survey at the National Cerebral and Cardiovascular Center between September 1989 and March 1994. Among them, a total of 919 were excluded due to past history of CVD ($n = 208$), loss to follow-up ($n = 535$), and missing data ($n = 176$). The remaining 5488 participants (2600 men and 2888 women) were included in the analysis. This cohort study was approved by the Institutional Review Board of the National Cerebral and Cardiovascular Center.

Baseline examination

Blood samples were centrifuged immediately after collection, and a routine blood examination was performed, including measurement of serum levels of total cholesterol and glucose. About 96% of participants had fasted for at least 8 hours before the blood test. Well-trained physicians used a standard mercury sphygmomanometer to measure blood pressure in triplicate on the right arm after 5 minutes of rest. Hypertension was defined as systolic blood pressure of at least 140 mm Hg, diastolic blood pressure of at least 90 mm Hg, or use of antihypertensive agents. Diabetes was defined as a fasting plasma glucose level of at least 7.0 mmol/L (126 mg/dL), a non-fasting plasma glucose level of at least 11.1 mmol/L (200 mg/dL), or use of antidiabetic agents. Hypercholesterolemia was defined as a total cholesterol level of at least 5.7 mmol/L (220 mg/dL) or use of antihyperlipidemic agents. Participants were wearing light clothing during height and weight measurement. WC was measured at the umbilical level, with the participant in a standing position. WHtR was defined as WC (cm) divided by height (cm). Body mass index (BMI) was defined as weight (kg) divided by the height (m) squared. Public-health nurses obtained information on participants' smoking, drinking, and medical histories.

Endpoint determination

The endpoint determination has been previously reported.[14–16] The endpoints of the present study were (1) date of first coronary heart disease (CHD) or stroke event; (2) date of death; (3) date of departure from Suita city; or (4) December 31, 2007. The first step in the survey of CHD and stroke was checking the health status of all participants by means of clinical visits every 2 years and a yearly questionnaire (by mail or telephone). For the second step, in-hospital medical records of participants suspected of having CHD or stroke were reviewed by registered hospital physicians, who were blinded to the baseline information. In addition, to complete the survey, we also conducted a systematic search of death certificates to identify cases of fatal CHD and stroke. In Japan, all death certificates are forwarded to the Ministry of Health, Welfare, and Labour and coded for the National Vital Statistics. The criteria for myocardial infarction were based on the World Health Organization Monitoring of Trends and Determinants in Cardiovascular Disease projects.[17] In addition to myocardial infarction, we also evaluated coronary angioplasty, coronary artery bypass grafting, and sudden cardiac death, all of which were included in the definition of CHD. Stroke was defined according to criteria from the US National Survey of Stroke and was confirmed by computed tomography.[18] Classification of stroke was based on examination of computed tomography scans, magnetic resonance images, and autopsy findings.

Statistical analysis

To assess the association between age and WHtR, we analyzed mean WC, height, and WHtR according to age in men and women. Pearson product-moment correlation coefficients between height and waist were calculated by sex and age group (30–49, 50–69, ≥70 years). Participants were categorized based on quartiles of WHtR by sex and age group. To compare baseline characteristics among WHtR quartiles, analysis of variance was used for continuous variables and the χ^2 test was used for dichotomous and categorical variables.

The Cox proportional hazards model was used to investigate the association between WHtR and CVD risk only among participants aged 50 to 69 years and 70 years or older, because there were too few CVD cases (men: 17, women: 11) for statistical analysis among those aged 30 to 49 years. Interaction terms were added to the models to assess the interaction between age and WHtR quartile for the risk of CVD. Hazard ratios (HRs) and 95% CIs were computed, and the lowest quartile of WHtR was defined as the reference group. To adjust for confounding factors, we included age, smoking status (current, quit, or never), and drinking status (current, quit, or never) in the model. Cardiometabolic risk factors such as hypertension, diabetes, and hypercholesterolemia were not included in the model because central obesity is upstream in the "metabolic domino".[19] However, in sensitivity analysis, we adjusted for hypertension, diabetes, and hypercholesterolemia to confirm that WHtR was an independent risk factor. The same analysis was performed for WC. In addition, to further assess cut-off points for WHtR, the highest quartile was dichotomized by median WHtR (ie, upper Q4 and lower Q4), and HRs and 95% CIs were estimated. The likelihood ratio test was used to compare the predictive values of WHtR with WC, as follows. First, we calculated the −2 logarithm likelihood for the model including the confounding factors, age, smoking, and drinking status ($-2\,ln[L_c]$). Second, we calculated the −2 logarithm likelihood for the model including the confounding factors plus WHtR ($-2\,ln[L_{c+WHtR}]$). The difference, ie, $(-2\,ln[L_c] - (-2\,ln[L_{c+WHtR}]))$, had an approximate χ^2 distribution with 1 degree-of-freedom. The same analysis was performed for WC.

All P values were 2-tailed, and a P value less than 0.05 was considered statistically significant. All statistical analyses were performed with SPSS (Version 20.0J; Japan IBM, Tokyo, Japan).

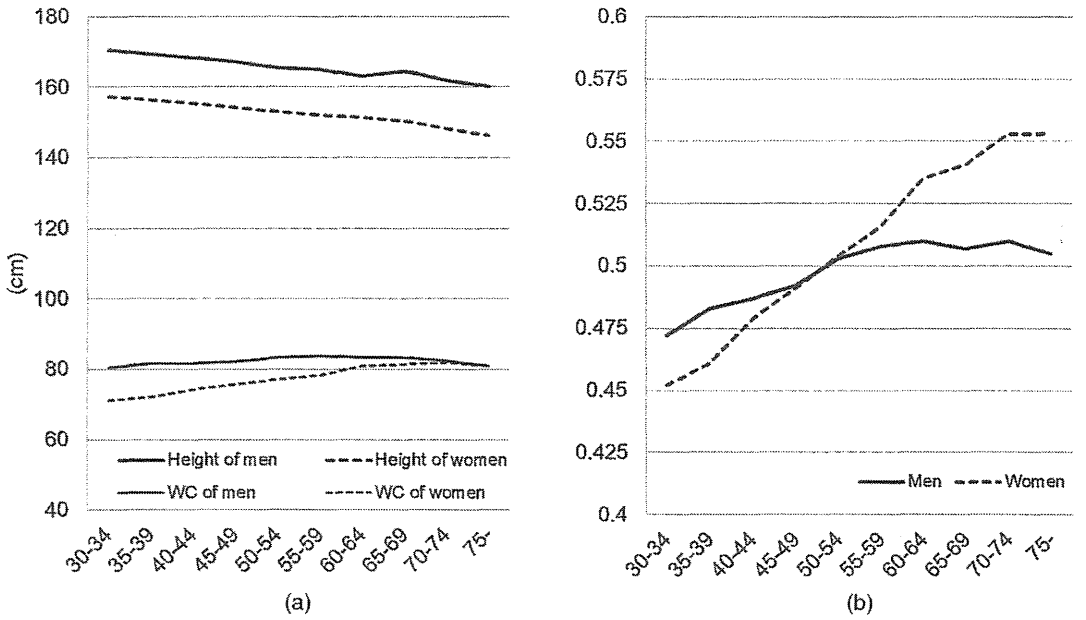

Figure. (a) Average WC (waist circumference), height, and (b) waist-to-height ratio according to age (The Suita Study, Japan)

RESULTS

During the follow-up period (mean, 13.0 years), 428 CVD events (184 CHD and 244 strokes) were observed. The Figure shows average WC, height, and WHtR by sex and age. WC in men increased up to age 50 years, remained almost unchanged from age 50 to 69 years, and decreased at age 70 years or older. WC in women younger than 75 years increased with advancing age and decreased in women aged 75 years or older, as compared with women aged 70 to 74 years. Height decreased with advancing age in both sexes. WHtR in men increased until approximately age 60 years. WHtR in women younger than 75 years increased with advancing age. The Pearson product-moment correlation coefficients (95% CI) between height and WC were 0.16 (0.09–0.22), 0.24 (0.19–0.30), and 0.13 (0.04–0.22) among men aged 30 to 49, 50 to 69, and 70 years or older, respectively, and 0.07 (0.01–0.13), 0.07 (0.02–0.13), 0.09 (−0.003–0.19) among women in the respective age groups.

Tables 1 and 2 summarize the baseline characteristics according to WHtR quartile (results among men and women aged 30–49 years are shown in eTable 1.) The prevalence of hypertension significantly differed by WHtR quartile, except among men aged 70 years or older. The prevalence of hypercholesterolemia and diabetes significantly differed by WHtR quartile among men and women aged 50 to 69 years.

Table 3 shows multivariable-adjusted HRs and 95% CIs for CVD and its subtypes according to WHtR quartile. A significant interaction was observed between age and WHtR for CVD among men (P for interaction = 0.02). Men aged 50 to 69 years in the highest quartile had significantly higher risks of CVD and CHD as compared with men in the lowest quartile; the HRs (95% CI) were 1.82 (1.13–2.92) and 2.42 (1.15–5.12), respectively. There were significant linear increases in the HRs for CVD, CHD, and ischemic stroke in men aged 50 to 69 years. After further adjustment for hypertension, diabetes, and hypercholesterolemia, the HRs (95% CI) were 1.46 (0.90–2.36) and 1.89 (0.89–4.03), respectively (eTable 3). Women aged 50 to 69 years in the highest quartile had a significantly higher risk of stroke than did those in the lowest quartile; the HR (95% CI) was 2.43 (1.01–5.85). There were significant linear increases in the HRs of CVD and stroke in women aged 50 to 69 years. After further adjustment for hypertension, diabetes, and hypercholesterolemia, the HR (95% CIs) was 2.06 (0.84–5.04) (eTable 3).

When men aged 50 to 69 years in the highest quartile were dichotomized by median WHtR (0.56), the HR (95% CI) for CVD was 1.37 (0.76–2.46) for those in the lower WHtR group and 2.34 (1.38–3.97) for those in the upper WHtR group (eTable 2). When women aged 70 years or older in the highest quartile were dichotomized by median WHtR (0.65), the HR for CVD was 1.42 (0.63–3.18) for those in the lower WHtR group and 2.33 (1.10–4.94) for those in the upper WHtR group. After adjustment for hypertension, diabetes, and hypercholesterolemia, the HRs in the upper WHtR decreased but remained significant, ie, 1.78 (1.04–3.05) among men aged 50 to 69 years and 2.16 (1.02–4.61) among women aged 70 years or older.

Table 4 shows the HRs and 95% CIs for CVD in relation to WC quartile. Among men aged 50 to 69 years in the highest quartile, the HR for CVD was 1.63 (1.03–2.59), although the HRs of CVD did not show a significant linear increase in this group. Among women aged 50 to 69 years, a significant linear

Table 1. Baseline characteristics of men, according to age group and quartile of waist-to-height ratio: The Suita Study, Japan

	Q1 (low)	Q2	Q3	Q4 (high)	*P*-value
Age 50–69 years					
No. of subjects	308	304	304	308	
Waist-to-height ratio	0.374–0.475	0.476–0.508	0.509–0.536	0.537–0.761	
Waist, cm	74.0 ± 4.3	81.2 ± 2.9	85.7 ± 3.1	92.8 ± 5.5	<0.01
Height, cm	165.0 ± 5.3	164.9 ± 5.6	164.4 ± 5.4	163.7 ± 5.3	0.01
Age, years	59.0 ± 5.3	59.1 ± 5.2	59.1 ± 5.5	59.4 ± 5.3	0.77
Body mass index, kg/m^2	20.1 ± 1.7	22.1 ± 1.5	23.7 ± 1.5	25.9 ± 2.3	<0.01
Hypertension, %	31	35	45	51	<0.01
Diabetes, %	6	7	9	11	0.045
Hypercholesterolemia, %	23	28	40	35	<0.01
Smoking status (current/quit/never), %	58/25/17	50/31/19	46/35/19	44/38/19	0.01
Drinking status (current/quit/never), %	79/2/19	74/4/22	79/4/17	76/4/21	0.58
Age ≥70 years					
No. of subjects	120	120	124	119	
Waist-to-height ratio	0.352–0.472	0.473–0.508	0.509–0.543	0.544–0.688	
Waist, cm	70.6 ± 5.0	79.8 ± 3.4	84.9 ± 3.3	92.2 ± 5.6	<0.01
Height, cm	162.5 ± 6.0	162.2 ± 5.7	161.3 ± 5.3	159.3 ± 6.0	<0.01
Age, years	74.0 ± 3.0	73.5 ± 2.7	74.1 ± 2.7	73.7 ± 2.9	0.40
Body mass index, kg/m^2	18.5 ± 1.7	21.3 ± 1.7	22.7 ± 1.4	25.6 ± 2.0	<0.01
Hypertension, %	42	44	51	57	0.07
Diabetes, %	4	7	7	8	0.70
Hypercholesterolemia, %	23	29	26	31	0.46
Smoking status (current/quit/never), %	37/48/16	42/41/18	38/47/15	30/50/19	0.66
Drinking status (current/quit/never), %	58/8/33	62/11/28	62/6/32	65/8/28	0.73

Continuous data with a normal distribution were analyzed with analysis of variance: mean ± SD.
Dichotomous and categorical data were analyzed with the χ^2 test.
Q, quartile; hypertension was defined as systolic blood pressure/diastolic blood pressure ≥ 140/90 mm Hg or current use of antihypertensive medications; diabetes was defined as a fasting plasma glucose level ≥ 7.0 mmol/L, a non-fasting plasma glucose level ≥ 11.1 mmol/L, or current use of antidiabetic medications; hypercholesterolemia was defined as a total serum cholesterol level ≥ 5.7 mmol/L or current use of antihyperlipidemic medications.

increase was observed in the HRs for CVD (P for trend = 0.04). However, after further adjustment for hypertension, diabetes, and hypercholesterolemia, these associations were no longer significant among men or women.

The χ^2 values for the likelihood ratio test were 6.49 ($P = 0.01$) for WHtR and 3.63 ($P = 0.06$) for WC among men aged 50 to 69 years, and 4.45 ($P = 0.03$) for WHtR and 4.54 ($P = 0.03$) for WC among women aged 50 to 69 years.

DISCUSSION

Our main findings were that WHtR was significantly positively associated with CVD and CHD risk among men aged 50 to 69 years and with stroke risk among women aged 50 to 69 years. Among men, there was a significant interaction between age and WHtR for CVD incidence. Among women aged 50 to 69 years, there was a borderline association between a WHtR in the highest quartile and increased CVD risk. In addition, among women aged 70 years or older, a WHtR in the upper level of the highest quartile was associated with significantly elevated CVD risk. These findings suggest that the association between WHtR and CVD incidence differs according to age and sex.

Two previous studies, in the United States and China, reported that the association between WHtR and CVD risk was stronger among younger adults as compared with elderly adults.[12,13] We too observed a significantly stronger association between WHtR and CVD risk among relatively young adults (age 50–69 years) as compared with elderly adults (age ≥70 years), which supports the results of previous studies. Consequently, these findings suggest that age stratification is important in estimating the association between WHtR and CVD risk.

In this population, physical frame, eg, WC and height, differed by age group. It has been reported that WC and the ratio of abdominal fat to whole-body fat differ by age.[9,10] In addition, the National Health and Nutrition Examination Survey in Japan noted that height clearly differed by generation.[11] This generational difference in physical frame, as well as aging, could lead to age differences in the association between WHtR and CVD risk.

A recent meta-analysis reported an optimal cut-off point of 0.50 for WHtR in both sexes.[7] However, the present findings suggest that, regardless of age or sex, a cut-off of 0.50 is somewhat low for identifying individuals at higher risk for CVD. The association with CVD risk was of at least

Table 2. Baseline characteristics of women, according to age group and quartile of waist-to-height ratio: The Suita Study, Japan

	Q1 (low)	Q2	Q3	Q4 (high)	*P*-value
Age 50–69 years					
No. of subjects	337	340	335	339	
Waist-to-height ratio	0.348–0.472	0.473–0.520	0.521–0.568	0.569–0.838	
Waist, cm	67.3 ± 4.1	75.4 ± 3.3	82.7 ± 3.4	92.1 ± 6.6	<0.01
Height, cm	153.0 ± 4.7	151.8 ± 4.9	152.1 ± 5.1	150.3 ± 5.2	<0.01
Age, years	57.6 ± 5.3	58.5 ± 5.3	59.5 ± 5.2	60.5 ± 5.4	<0.01
Body mass index, kg/m^2	19.8 ± 2.0	21.7 ± 2.0	23.1 ± 2.3	25.9 ± 3.3	<0.01
Hypertension, %	21	32	36	52	<0.01
Diabetes, %	2	3	5	9	<0.01
Hypercholesterolemia, %	49	57	57	62	0.01
Smoking status (current/quit/never), %	11/2/86	11/3/86	9/3/88	12/5/84	0.43
Drinking status (current/quit/never), %	26/2/73	29/2/69	28/2/71	31/1/68	0.75
Postmenopausal, %	90	94	95	94	0.06
Age ≥70 years					
No. of subjects	103	103	103	103	
Waist-to-height ratio	0.379–0.496	0.497–0.554	0.556–0.602	0.603–0.812	
Waist, cm	68.1 ± 4.4	77.3 ± 4.1	85.6 ± 3.6	95.2 ± 6.4	<0.01
Height, cm	148.4 ± 5.5	147.7 ± 6.1	148.1 ± 5.1	145.8 ± 5.1	<0.01
Age, years	73.8 ± 2.9	73.4 ± 2.7	73.8 ± 2.7	74.0 ± 2.6	0.56
Body mass index, kg/m^2	19.1 ± 2.1	21.3 ± 2.3	23.1 ± 2.1	26.2 ± 2.9	<0.01
Hypertension, %	53	44	50	64	0.03
Diabetes, %	2	5	6	4	0.54
Hypercholesterolemia, %	42	51	53	52	0.32
Smoking status (current/quit/never), %	12/6/83	9/4/87	6/5/89	7/5/88	0.78
Drinking status (current/quit/never), %	22/5/73	18/2/81	19/1/80	19/4/77	0.62
Postmenopausal, %	100	100	100	100	1.00

Continuous data with a normal distribution were analyzed with analysis of variance: mean ± SD.
Dichotomous and categorical data were analyzed with the χ^2 test.
Q, quartile; hypertension was defined as systolic blood pressure/diastolic blood pressure ≥ 140/90 mm Hg or current use of antihypertensive medications; diabetes was defined as a fasting plasma glucose level ≥ 7.0 mmol/L, a non-fasting plasma glucose level ≥ 11.1 mmol/L, or current use of antidiabetic medications; hypercholesterolemia was defined as a total serum cholesterol level ≥ 5.7 mmol/L or current use of antihyperlipidemic medications.

borderline significance for a WHtR in the fourth quartile, except among men aged 70 years or older. Additional analyses showed that the risks markedly increased, particularly in the upper level of the fourth WHtR quartile, among men aged 50 to 69 years and women aged 70 years and older. These results suggest the presence of a threshold rather than a dose-response relation for WHtR, although the present sample was too small to confirm this hypothesis. Additionally, we think that cut-offs should be set in relation to age and sex. On the basis of our results, we propose the following cut-offs (which do not include men aged 70 years or older): 0.560 for men aged 50 to 69 years, 0.569 for women aged 50 to 69 years, and 0.647 for women aged 70 years or older.

The risk of CVD among men aged 50 to 69 years, and women aged 70 years, in the upper level of the highest quartile was significantly elevated even after adjustment for hypertension, hyperlipidemia, and diabetes. We believe that there are 2 possible explanations for this finding. First, an extremely high WHtR might actually be an independent risk factor ie, separate from classical cardiometabolic risks. It has been reported that abdominal obesity is related to increased levels of plasminogen activator inhibitor-1, which can lead to blood coagulation.[20] Such background mechanisms might be important. Second, our findings could be due to insufficient adjustment for confounders in the Cox regression model. Irrespective of the reason, men aged 50 to 69 years, and women aged 70 years or older, with extremely high WHtRs have a considerably higher risk for CVD and should be closely monitored.

We previously investigated the association between WC and CVD risk without age stratification[21] and found a significant association between WC and the risks of CVD and stroke among women but no significant association among men. However, the present age-stratified analysis of WC suggests that our previous results were substantially influenced by age. Therefore, we compared WHtR and WC in relation to CVD in analysis stratified by age group and found that the HRs associated with the highest quartile of WHtR were higher than those associated with WC among middle-aged men and that the predictive value of WHtR was greater than that of WC. Several previous studies reported similar results[12,22–24]; therefore our findings are consistent with those

Table 3. Multivariable-adjusted hazard ratios for cardiovascular disease according to sex, age group, and quartile of WHtR: The Suita Study, Japan

	Q1 (low)	Q2	Q3	Q4 (high)	*P* for trend
Men					
Age 50–69 years					
Person-years	4070	3069	3879	3842	
CVD, no. of cases	28	31	32	47	
HRs	1	1.14 (0.68–1.90)	1.23 (0.74–2.05)	1.82 (1.13–2.92)	0.01
CHD, no. of cases	10	16	16	23	
HRs	1	1.57 (0.71–3.47)	1.72 (0.77–3.80)	2.42 (1.15–5.12)	0.02
Stroke, no. of cases	18	15	16	24	
HRs	1	0.91 (0.46–1.81)	0.95 (0.48–1.87)	1.56 (0.84–2.89)	0.16
Ischemic stroke, no. of cases	10	9	15	18	
HRs	1	0.99 (0.40–2.43)	1.59 (0.71–3.56)	2.06 (0.94–4.49)	0.04
Age ≥70 years					
Person-years	1055	1128	1193	1155	
CVD, no. of cases	21	29	27	30	
HRs	1	1.36 (0.77–2.39)	1.09 (0.62–1.93)	1.36 (0.78–2.38)	0.45
CHD, no. of cases	13	11	10	15	
HRs	1	0.87 (0.39–1.97)	0.63 (0.28–1.45)	1.09 (0.52–2.30)	0.99
Stroke, no. of cases	8	18	17	15	
HRs	1	2.09 (0.90–4.81)	1.79 (0.77–4.15)	1.84 (0.78–4.35)	0.29
Ischemic stroke, no. of cases	4	12	10	11	
HRs	1	2.84 (0.91–8.83)	2.22 (0.69–7.07)	2.71 (0.86–8.53)	0.18
Women					
Age 50–69 years					
Person-years	4811	4863	4477	4470	
CVD, no. of cases	16	18	21	33	
HRs	1	1.09 (0.56–2.14)	1.32 (0.69–2.54)	1.80 (0.98–3.32)	0.04
CHD, no. of cases	9	4	4	13	
HRs	1	0.47 (0.14–1.51)	0.47 (0.14–1.54)	1.35 (0.56–3.22)	0.43
Stroke, no. of cases	7	14	17	20	
HRs	1	1.85 (0.75–4.60)	2.35 (0.97–5.70)	2.43 (1.01–5.85)	0.04
Ischemic stroke, no. of cases	3	7	9	10	
HRs	1	2.09 (0.54–8.10)	2.78 (0.75–10.33)	2.35 (0.63–8.77)	0.22
Age ≥70 years					
Person-years	1095	1259	1164	1094	
CVD, no. of cases	15	15	13	24	
HRs	1	1.00 (0.48–2.08)	0.91 (0.43–1.93)	1.83 (0.95–3.53)	0.08
CHD, no. of cases	6	7	5	9	
HRs	1	1.23 (0.40–3.77)	0.98 (0.29–3.32)	1.78 (0.62–5.14)	0.34
Stroke, no. of cases	9	8	8	15	
HRs	1	0.85 (0.32–2.23)	0.88 (0.34–2.29)	1.92 (0.83–4.45)	0.11
Ischemic stroke, no. of cases	5	4	4	9	
HRs	1	0.83 (0.22–3.16)	0.77 (0.21–2.91)	1.99 (0.66–6.04)	0.21

Multivariable adjustment was performed for age, smoking, and drinking status. Parentheses indicate 95% CIs for HRs.
Abbreviations: WHtR, waist-to-height ratio; Q, quartile; CVD, cardiovascular disease; CHD, coronary heart disease; HR, hazard ratio.

of previous studies. In contrast, WHtR and WC had similar predictive values for CVD among women in the present study. Many previous studies found that WHtR was similar to WC in predicting CVD risk among women.[12,22,24–26] The effect of dividing WC by height might be limited because the correlation of WC with height is weaker among women than among men. Consequently, we believe that WHtR is a better predictor than WC, particularly among middle-aged men.

The superiority of WHtR might be explained by the fact that WHtR, as measured by computed tomography, was more closely correlated than WC with intra-abdominal fat,[27] and a previous study reported that intra-abdominal fat was positively associated with number of cardiometabolic risk factors.[28] In addition, shorter adults tend to have more cardiometabolic risk factors than do taller individuals with a similar WC.[29] This suggests that WHtR, ie, dividing WC by height, is more strongly related than WC to cardiometabolic risk factors. Thus, we believe that WHtR better reflects the accumulation of cardiometabolic risks and leads to superior prediction of CVD.

BMI, along with indices of central obesity, has been an important obesity index in predicting CVD incidence,[30] although a meta-analysis reported that the predictive power of WHtR for CVD was higher than that of BMI.[7] Another report found a significant association between BMI and CVD after adjustment for WHtR[12] and suggested that WHtR and BMI are independently associated with CVD risk. Therefore, it might be better to use both BMI and WHtR to assess obesity.

Table 4. Multivariable-adjusted hazard ratios for cardiovascular disease according to sex, age group, and quartile of WC: The Suita Study, Japan

	Q1 (low)	Q2	Q3	Q4 (high)	*P* for trend
Men					
Age 50–69 years					
Person-years	4078	4004	3872	3806	
CVD, no. of cases	32	33	29	44	
HRs	1	1.07 (0.66–1.75)	0.97 (0.58–1.61)	1.63 (1.03–2.59)	0.06
CHD, no. of cases	13	17	12	23	
HRs	1	1.28 (0.62–2.63)	0.96 (0.44–2.12)	2.02 (1.02–4.02)	0.07
Stroke, no. of cases	19	16	17	21	
HRs	1	0.97 (0.50–1.88)	0.96 (0.49–1.86)	1.43 (0.76–2.67)	0.31
Ischemic stroke, no. of cases	13	9	13	17	
HRs	1	0.80 (0.34–1.87)	1.07 (0.49–2.31)	1.64 (0.79–3.41)	0.15
Age ≥70 years					
Person-years	999	1208	1200	1124	
CVD, no. of cases	25	28	27	27	
HRs	1	0.94 (0.55–1.62)	0.91 (0.53–1.58)	1.06 (0.61–1.84)	0.87
CHD, no. of cases	14	11	12	12	
HRs	1	0.67 (0.30–1.47)	0.65 (0.30–1.43)	0.82 (0.38–1.78)	0.60
Stroke, no. of cases	11	17	15	15	
HRs	1	1.29 (0.60–2.77)	1.21 (0.55–2.66)	1.36 (0.62–2.99)	0.52
Ischemic stroke, no. of cases	5	10	10	12	
HRs	1	1.70 (0.58–4.98)	1.82 (0.62–5.37)	2.26 (0.79–6.47)	0.14
Women					
Age 50–69 years					
Person-years	4669	4685	5046	4221	
CVD, no. of cases	15	18	25	30	
HRs	1	1.19 (0.60–2.36)	1.43 (0.75–2.71)	1.87 (1.00–3.51)	0.04
CHD, no. of cases	7	5	5	13	
HRs	1	0.74 (0.24–2.34)	0.65 (0.21–2.08)	1.86 (0.73–4.72)	0.18
Stroke, no. of cases	8	13	20	17	
HRs	1	1.56 (0.65–3.77)	2.06 (0.90–4.70)	1.93 (0.82–4.54)	0.11
Ischemic stroke, no. of cases	4	6	9	10	
HRs	1	1.44 (0.41–5.10)	1.70 (0.52–5.54)	2.00 (0.62–6.52)	0.23
Age ≥70 years					
Person-years	1175	1234	1046	1157	
CVD, no. of cases	16	16	15	20	
HRs	1	1.05 (0.52–2.11)	1.11 (0.54–2.25)	1.45 (0.74–2.83)	0.28
CHD, no. of cases	8	6	7	6	
HRs	1	0.85 (0.29–2.49)	1.21 (0.43–3.43)	0.88 (0.30–2.59)	0.98
Stroke, no. of cases	8	10	8	14	
HRs	1	1.24 (0.49–3.14)	1.10 (0.41–2.93)	2.00 (0.83–4.87)	0.15
Ischemic stroke, no. of cases	5	4	4	9	
HRs	1	0.85 (0.23–3.21)	0.93 (0.25–3.47)	1.86 (0.61–5.61)	0.24

Multivariable adjustment was performed for age, smoking, and drinking status. Parentheses indicate 95% CIs for HRs.
Abbreviations: WC, waist circumference; Q, quartile; CVD, cardiovascular disease; CHD, coronary heart disease; HR, hazard ratio.

Our study has several limitations. First, the number of cases of CVD among participants aged 30 to 49 years was insufficient for statistical analysis. Further study is required to confirm an association between WHtR and CVD risk among younger adults. Second, the effect of visceral fat could not be estimated because we did not use computed tomography to measure abdominal fat distribution. Third, changes in WHtR during the follow-up period were not considered in the present study. Finally, because WC was measured once, the estimated risks might have been underestimated because of regression dilution bias.[31]

In conclusion, the present findings suggest that WHtR is useful in identifying middle-aged Japanese at higher risk of CVD and is more predictable than WC in determining CVD risk, especially among men. In addition, the data indicate that WHtR cut-off points should be set according to sex and age. This study enrolled a limited Japanese population, and further studies with larger and more ethnically diverse samples are required to confirm our findings.

ONLINE ONLY MATERIALS

eTable 1. Baseline characteristics and CVD incidence among men and women aged 30–49 years according to quartile of waist-to-height ratio: the Suita Study, Japan.

eTable 2. Multivariable-adjusted hazard ratios for cardiovascular disease in the upper and lower fourth quartile of WHtR according to sex and age group: the Suita Study, Japan.

eTable 3. Multivariable-adjusted hazard ratios for cardiovascular disease according to sex, age group, and quartile of WHtR: the Suita Study, Japan.
Abstract in Japanese.

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Conflicts of interest: None declared.

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Original Article

Small Dense Low-Density Lipoproteins Cholesterol can Predict Incident Cardiovascular Disease in an Urban Japanese Cohort: The Suita Study

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Aim: Several lines of evidence indicate that small dense low-density lipoproteins (sd-LDL) are more atherogenic than large buoyant LDL; however, few prospective studies have addressed the role of sd-LDL in cardiovascular disease (CVD). We therefore examined the association between sd-LDL cholesterol (sd-LDL-C) and CVD in a Japanese cohort.
Methods: An 11.7-year prospective study was performed using a general population aged 30-79 without a history of cardiovascular disease. Direct LDL-C and sd-LDL-C were measured in samples from 2034 participants (968 men and 1066 women).
Results: During the follow-up period, there were 116 incident cases of CVD. The multivariable-adjusted hazard ratios (HRs) of sd-LDL-C for CVD were calculated using a proportional hazards regression model after adjusting for age, hypertension, diabetes, use of lipid-lowering drugs, body mass index, and current smoking and alcohol drinking, and found that increasing quartiles of sd-LDL-C were associated with increased risk of CVD. We also determined that age and sex-adjusted HRs per 10 mg/dL of sd-LDL-C and HRs for CVD, stroke, cerebral infarction, and coronary artery disease 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.
Conclusions: It was demonstrated that sd-LDL-C was significantly associated with CVD in a Japanese population, providing evidence of sd-LDL-C as an important biomarker to predict CVD.

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Key words; Cardiovascular disease, Lipoproteins, Lipids, Risk factors, Epidemiology

Introduction

The causal relationship between high levels of serum low-density lipoprotein cholesterol (LDL-C) and cardiovascular disease (CVD) has been well established in previous cohort studies[1-5]. Recent clinical trials have also indicated significant event reduction by statins in the primary and secondary prevention of CVD[6-8]; therefore, LDL-C is one of the most important risk factors of CVD and many guidelines, including ours, recommend certain target LDL-C goals for risk management to prevent the development of CVD[5].

Although we use LDL-C as the primary target for cholesterol-lowering therapy, LDL particles are heterogeneous with respect to size and density. Compared to large, buoyant LDL, small dense LDL (sd-LDL) particles exhibit a prolonged plasma residence time, increased penetration into the arterial wall, lower affinity for the LDL receptor, and increased sus-

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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

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

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.

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)**

(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-

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