31. Nakashita M, Ohkubo T, Hara A, Metoki H, Kikuya M, Hirose T, Tsubota-Utsugi M, Asayama K, Inoue R, Kanno A, Obara T, Hoshi H, Totsune K, Satoh H, Imai Y. Influence of alcohol intake on circadian blood pressure variation in Japanese men: the Ohasama study. *Am J Hypertens* 2009; 22:1171–1176.
32. Barua RS, Ambrose JA, Srivastava S, DeVoe MC, Eales-Reynolds LJ. Reactive oxygen species are involved in smoking-induced dysfunction of nitric oxide biosynthesis and upregulation of endothelial nitric oxide synthase: an ***in vitro*** demonstration in human coronary artery endothelial cells. *Circulation* 2003; 107:2342–2347.
33. Tomiyama H, Yamazaki M, Sagawa Y, Teraoka K, Shirota T, Miyawaki Y, Yamashina A. Synergistic effect of smoking and blood pressure on augmentation index in men, but not in women. *Hypertens Res* 2009; 32:122–126.
34. Kato T, Kikuya M, Ohkubo T, Satoh M, Hara A, Obara T, Metoki H, Asayama K, Hirose T, Inoue R, Kanno A, Totsune K, Hoshi H, Satoh H, Imai Y. Factors associated with day-by-day variability of self-measured blood pressure at home: the Ohasama study. *Am J Hypertens* 2010; 23:980–986.
35. Johansson JK, Niiranen TJ, Puukka PJ, Jula AM. Factors affecting the variability of home-measured blood pressure and heart rate: the Finn-home study. *J Hypertens* 2010; 28:1836–1845.
36. Parati G, Bilo G. Clinical relevance of day-by-day blood pressure and heart rate variability: new information from home self-measurements. *Hypertension* 2008; 52:1006–1008.
37. Lao XQ, Jiang CQ, Zhang WS, Adab P, Lam TH, Cheng KK, Thomas GN. Smoking, smoking cessation and inflammatory markers in older Chinese men: The Guangzhou Biobank Cohort Study. *Atherosclerosis* 2009; 203:304–310.
38. Thuy AB, Blizzard L, Schmidt MD, Luc PH, Granger RH, Dwyer T. The association between smoking and hypertension in a population-based sample of Vietnamese men. *J Hypertens* 2010; 28:245–250.
39. Inoue R, Ohkubo T, Kikuya M, Metoki H, Asayama K, Obara T, Hoshi H, Hashimoto J, Totsune K, Satoh H, Kondo Y, Imai Y. Predicting stroke using 4 ambulatory blood pressure monitoring-derived blood pressure indices: the Ohasama Study. *Hypertension* 2006; 48:877–882.

Original Article

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

Hidenori Arai[1], Yoshihiro Kokubo[2], Makoto Watanabe[2],Tatsuya Sawamura[3], Yasuki Ito[4], Asako Minagawa[4], Tomonori Okamura[5] and Yoshihiro Miyamato[2]

[1]Department of Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan
[2]Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, Suita, Japan
[3]Department of Vascular Physiology, National Cerebral and Cardiovascular Center, Suita, Japan
[4]Research and Development Department, Denka Seiken, Tokyo, Japan
[5]Department of Preventive Medicine and Public Health, School of Medicine, Keio University, Tokyo, Japan

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.**

J Atheroscler Thromb, 2013; 20:195-203.

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-

Address for correspondence: Hidenori Arai, Department of Human Health Sciences, Kyoto University Graduate School of Medicine, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan
E-mail: harai@kuhp.kyoto-u.ac.jp
Received: June 20, 2012
Accepted for publication: September 6, 2012

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

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.

Sources of Funding

The present study was supported by the Intramural Research Fund of the National Cerebral and Cardiovascular Center (22-4- 5), and also supported by grants-in-aid from the Ministry of Health, Labor and Welfare (H22-Seishu-005).

Disclosures

Dr. Arai has received an unrestricted grant from Denka Seiken. Drs Ito and Minagawa are employees of Denka Seiken. The other authors have no conflicts to declare.

References

1) Okamura T, Kokubo Y, Watanabe M, Higashiyama A, Miyamoto Y, Yoshimasa Y, Okayama A: Low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort study: The Suita study. Atherosclerosis, 2009; 203: 587-592
2) Law MR, Wald NJ: An ecological study of serum cholesterol and ischaemic heart disease between 1950 and 1990. Eur J Clin Nutr, 1994; 48: 305-325
3) Law MR, Wald NJ, Rudnicka AR: Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. BMJ, 2003; 326: 1423
4) Prospective Studies C, Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R, Collins R: Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet, 2007; 370: 1829-1839
5) Teramoto T, Sasaki J, Ueshima H, Egusa G, Kinoshita M, Shimamoto K, Daida H, Biro S, Hirobe K, Funahashi T, Yokote K, Yokode M: Executive summary of Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese. J Atheroscler Thromb, 2007; 14: 45-50
6) Nakamura H, Arakawa K, Itakura H, Kitabatake A, Goto Y, Toyota T, Nakaya N, Nishimoto S, Muranaka M, Yamamoto A, Mizuno K, Ohashi Y: Primary prevention of cardiovascular disease with pravastatin in Japan (MEGA Study): a prospective randomised controlled trial. Lancet, 2006; 368: 1155-1163
7) Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ: Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med, 2008; 359: 2195-2207
8) Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Simes R: Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet, 2005; 366: 1267-1278
9) Berneis KK, Krauss RM: Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res, 2002; 43: 1363-1379
10) Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM: Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. J Clin Invest, 1993; 92: 141-146
11) Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PW, D'Agostino RB, Vasan RS, Robins SJ: Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. Circulation, 2006; 113: 20-29
12) Deighan CJ, Caslake MJ, McConnell M, Boulton-Jones JM, Packard CJ: Atherogenic lipoprotein phenotype in end-stage renal failure: origin and extent of small dense

low-density lipoprotein formation. Am J Kidney Dis, 2000; 35: 852-862
13) Hirano T, Nohtomi K, Sato Y, Kamata K, Ito Y: Small dense LDL-cholesterol determined by a simple precipitation assay for screening familial combined hyperlipidemia. Atherosclerosis, 2009; 205: 603-607
14) Hirano T, Ito Y, Koba S, Toyoda M, Ikejiri A, Saegusa H, Yamazaki J, Yoshino G: Clinical significance of small dense low-density lipoprotein cholesterol levels determined by the simple precipitation method. Arterioscler Thromb Vasc Biol, 2004; 24: 558-563
15) St-Pierre AC, Cantin B, Dagenais GR, Mauriege P, Bernard PM, Despres JP, Lamarche B: Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec Cardiovascular Study. Arterioscler Thromb Vasc Biol, 2005; 25: 553-559
16) Swinkels DW, Hak-Lemmers HL, Demacker PN: Single spin density gradient ultracentrifugation method for the detection and isolation of light and heavy low density lipoprotein subfractions. J Lipid Res, 1987; 28: 1233-1239
17) Nichols AV, Krauss RM, Musliner TA: Nondenaturing polyacrylamide gradient gel electrophoresis. Methods Enzymol, 1986; 128: 417-431
18) Hirano T, Ito Y, Saegusa H, Yoshino G: A novel and simple method for quantification of small, dense LDL. J Lipid Res, 2003; 44: 2193-2201
19) Ito Y, Fujimura M, Ohta M, Hirano T: Development of a homogeneous assay for measurement of small dense LDL cholesterol. Clin Chem, 2011; 57: 57-65
20) Ai M, Otokozawa S, Asztalos BF, Ito Y, Nakajima K, White CC, Cupples LA, Wilson PW, Schaefer EJ: Small dense LDL cholesterol and coronary heart disease: results from the Framingham Offspring Study. Clin Chem, 2010; 56: 967-976
21) Koba S, Yokota Y, Hirano T, Ito Y, Ban Y, Tsunoda F, Sato T, Shoji M, Suzuki H, Geshi E, Kobayashi Y, Katagiri T: Small LDL-cholesterol is superior to LDL-cholesterol for determining severe coronary atherosclerosis. J Atheroscler Thromb, 2008; 15: 250-260
22) Mannami T, Baba S, Ogata J: Strong and significant relationships between aggregation of major coronary risk factors and the acceleration of carotid atherosclerosis in the general population of a Japanese city: the Suita Study. Arch Intern Med, 2000: 160: 2297-2303
23) Tunstall-Pedoe H, Vanuzzo D, Hobbs M, Mahonen M, Cepaitis Z, Kuulasmaa K, Keil U: Estimation of contribution of changes in coronary care to improving survival, event rates, and coronary heart disease mortality across the WHO MONICA Project populations. Lancet, 2000; 355: 688-700
24) Walker AE, Robins M, Weinfeld FD: The National Survey of Stroke. Clinical findings. Stroke, 1981; 12: I13-44
25) Mudd JO, Borlaug BA, Johnston PV, Kral BG, Rouf R, Blumenthal RS, Kwiterovich PO Jr: Beyond low-density lipoprotein cholesterol: defining the role of low-density lipoprotein heterogeneity in coronary artery disease. J Am Coll Cardiol, 2007; 50: 1735-1741
26) Otvos JD, Collins D, Freedman DS, Shalaurova I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ: Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation, 2006; 113: 1556-1563
27) Vakkilainen J, Steiner G, Ansquer JC, Aubin F, Rattier S, Foucher C, Hamsten A, Taskinen MR, Group, D: Relationships between low-density lipoprotein particle size, plasma lipoproteins, and progression of coronary artery disease: the Diabetes Atherosclerosis Intervention Study (DAIS). Circulation, 2003; 107: 1733-1737
28) Rizzo M, Rini GB, Spinas GA, Berneis K: The effects of ezetimibe on LDL-cholesterol: quantitative or qualitative changes? Atherosclerosis, 2009; 204: 330-333
29) Veerkamp MJ, de Graaf J, Bredie SJ, Hendriks JC, Demacker PN, Stalenhoef AF: Diagnosis of familial combined hyperlipidemia based on lipid phenotype expression in 32 families: results of a 5-year follow-up study. Arterioscler Thromb Vasc Biol, 2002; 22: 274-282
30) Tsunoda F, Koba S, Hirano T, Ban Y, Iso Y, Suzuki H, Geshi E, Katagiri, T: Association between small dense low-density lipoprotein and postprandial accumulation of triglyceride-rich remnant-like particles in normotriglyceridemic patients with myocardial infarction. Circ J, 2004; 68: 1165-1172
31) Miller WG, Myers GL, Sakurabayashi I, Bachmann LM, Caudill SP, Dziekonski A, Edwards S, Kimberly MM, Korzun WJ, Leary ET, Nakajima K, Nakamura M, Nilsson G, Shamburek RD, Vetrovec GW, Warnick GR, Remaley AT: Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. Clin Chem, 2010; 56: 977-986

Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

ORIGINAL ARTICLE
Preventive Medicine

Prospective Study on Waist Circumference and Risk of All-Cause and Cardiovascular Mortality

– Pooled Analysis of Japanese Community-Based Studies –

Isao Saito, MD, PhD; Yoshihiro Kokubo, MD, PhD; Yutaka Kiyohara, MD, PhD; Yasufumi Doi, MD, PhD; Shigeyuki Saitoh, MD, PhD; Hirofumi Ohnishi, MD, PhD; Yoshihiro Miyamoto, MD, PhD

Background: The aim of the present study was to clarify the association between waist circumference and all-cause and cardiovascular disease (CVD) mortality risk in relatively lean Japanese subjects.

Methods and Results: A total of 3,554 men and 4,472 women who had no history of CVD were examined and their waist circumference measured at baseline. The subjects were aged ≥40 years and were obtained from 3 prospective cohort studies during 1988–1996. Hazard ratios for all-cause and CVD mortality were analyzed over a follow-up period of 14.7 years using a Cox proportional hazards model and penalized spline method, after adjustment for study cohort, age, smoking, alcohol drinking, hypertension, dyslipidemia, and diabetes. Compared with the lowest quintile, the highest quintile of waist circumference in men was associated with a linear reduction in all-cause mortality risk (multivariate-adjusted hazard ratio, 0.73; 95% confidence interval: 0.60–0.89; P for trend=0.001). CVD mortality risk was increased in men aged ≤65 years with a higher waist circumference. This relationship was U-shaped. Waist circumference was not associated with all-cause or CVD mortality risk in women.

Conclusions: Waist circumference was associated inversely with increased risk of all-cause death in men, but not in women. Middle-aged men with a greater waist circumference potentially have an increased risk of CVD mortality. (*Circ J* 2012; **76:** 2867–2874)

Key Words: Cardiovascular disease; Mortality; Prospective study; Waist circumference

Recently, a prospective study in more than 1 million Asian subjects has confirmed that there is a U-shaped association between body mass index (BMI) and the risk of death from cancer, cardiovascular disease (CVD), and other causes.[1] But previous epidemiological studies in Japan suggested that increased BMI was unlikely to influence mortality risk,[2,3] whereas a lower BMI or weight loss markedly raised this risk.[4]

In contrast, it has been reported that Japanese overweight or obese subjects have an increased risk for incident coronary heart disease or ischemic stroke.[5,6] The Hisayama study in Japan documented that increased BMI was associated with an increased risk of stroke in women, but not in men.[7] An enhanced risk for incident CVD and stroke associated with increased waist circumference was also found only in women in the Suita study.[8] Taken together, these findings indicate that overweight or obesity has an impact on incident CVD in Japanese men and women, except for stroke in men.

A large European prospective study documented that abdominal obesity, as indexed by waist circumference, had a J-shaped association with mortality risk.[9] That study showed that overweight or obesity, assessed by BMI or waist circumference, was associated closely with increased risk of CVD mortality in Caucasian subjects. Compared with indices of adiposity, a waist circumference signifying central obesity was a stronger marker for predicting CVD mortality than BMI.[10] A recent meta-analysis in Caucasian subjects found increased all-cause and CVD mortality risks for elderly people (65–74 years old) with an increased waist circumference across BMI categories and for underweight elderly people.[11] The association of waist circumference levels with CVD mortality risk, however, remains to be determined in Japanese adults. Therefore, to better

Received November 6, 2011; revised manuscript received June 19, 2012; accepted July 17, 2012; released online August 8, 2012 Time for primary review: 22 days

Department of Public Health, Social Medicine and Medical Informatics, Ehime University Graduate School of Medicine, Toon (I.S.); Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, Suita (Y. Kokubo, Y.M.); Department of Environmental Medicine (Y. Kiyohara), Department of Medicine and Clinical Science (Y.D.), Graduate School of Medical Sciences, Kyushu University, Fukuoka; and Second Department of Internal Medicine, Sapporo Medical University of Medicine, Sapporo (S.S., H.O.), Japan
Mailing address: Isao Saito, MD, PhD, Department of Public Health, Social Medicine and Medical Informatics, Ehime University Graduate School of Medicine, 454 Shitsukawa, Toon 791-0295, Japan. E-mail: saitoi@m.ehime-u.ac.jp

ISSN-1346-9843 doi:10.1253/circj.CJ-11-1259

Table 1. Subject Characteristics

	Cohorts		
	Tanno/Sobetsu	Suita	Hisayama
Baseline year	1994	1991–1996	1988
n	1,525	4,135	2,366
Follow-up period (years)	11.3	14.2	17.9
No. deaths	226	792	693
No. CVD deaths	71	174	202
Age (years)	62.1±9.7	59.4±10.9	56.5±10.4
Male (%)	40.7	43.2	46.2
WC (cm)	79.0±9.6	81.3±9.1	81.7±9.2
BMI (kg/m^2)	23.5±3.1	22.6±3.0	23.0±3.1
SBP (mmHg)	136.9±20.4	126.6±20.5	132.3±20.6
DBP (mmHg)	79.0±9.8	76.6±11.4	77.9±11.4
Hypertension (%)	47.7	31.3	40.0
Dyslipidemia (%)	41.8	54.1	60.8
Diabetes (%)	6.5	4.3	9.0
Current smoker (%)	25.1	26.4	25.4
Regular alcohol drinker (%)	17.3	59.8	22.9

Data given as mean±SD, n or %.
BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference.

understand the association of abdominal obesity with all-cause and CVD mortality risk, we conducted a 14.7-year prospective study in the general Japanese population.

Methods

Subjects

The subjects consisted of the participants, aged between 40 and 90 years, of 3 large Japanese epidemiological studies: the Tanno and Sobetsu study (n=1,627), the Suita study (n=4,278), and the Hisayama study (n=2,487). We selected 3,554 men and 4,472 women from these studies who had no history of ischemic heart disease (IHD) or stroke and who had their waist circumference measured at baseline.

Briefly, subjects in the Tanno and Sobetsu study were recruited from annual health check-ups carried out in 1994 in the towns of Hokkaido Island, located in the northern part of Japan.[12] The Suita study, which was performed in an urban area of Japan (Osaka), also enrolled individuals for regular health check-ups between 1991 and 1996.[8] The Hisayama study, which was conducted in a suburban town located in Kyushu Island in the southern part of Japan, was established in 1961 for all residents ≥40 years of age, and we used the third cohort, which was established in 1988.[13] Details of recruitment and the procedures for all studies have been described elsewhere.[8,12,13]

Measurements

All blood samples were collected from the cubital vein under fasting conditions (≥8 h since the last meal). Trained technicians measured blood pressure using a standard mercury sphygmomanometer, after the subjects had rested for at least 5 min in the sitting position. Waist circumference was measured at the point of the umbilicus level in the standing position. BMI (kg/m^2) was calculated as weight divided by the square of the height in meters. High-density lipoprotein cholesterol (HDL-C) and triglycerides were measured using conventional methods. Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's formula when the triglyceride level was <4.5 mmol/L.[14]

Each subject completed a self-administered questionnaire at baseline to assess medical history including current medications, smoking habits, and alcohol consumption. Hypertension was defined as a systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or the use of antihypertensive agents. Diabetes was defined as a fasting blood glucose ≥7.0 mmol/L, or the use of anti-diabetic agents. Dyslipidemia was defined as either LDL-C ≥3.6 mmol/L, HDL-C <1.0 mmol/L, triglycerides ≥1.7 mmol/L, or the use of anti-lipemic agents.

Until 1995, the underlying cause of death was determined based on death certificates coded according to the criteria of the International Classification of Diseases, ninth revision (ICD-9). From 1995, the codes were translated into the corresponding ICD-10 codes. Deaths from IHD and stroke were defined as 410–414 and 430–438 (ICD-9); and I20–25 and I60–69 (ICD-10), respectively. IHD and stroke were combined as CVD in the analyses.

Statistical Analysis

The mean duration of follow-up was 14.7 years, during which time there were 1,711 all-cause deaths, including 447 CVD deaths. The person-years studied were calculated as the period from baseline to either the first endpoint (death or emigration) or 31 December 2008.

Sex-specific hazard ratios and 95% confidence intervals (CIs) of all-cause and CVD deaths were calculated using Cox's regression models with data grouped according to waist circumference quintile by sex. The first quintile served as the reference. Model 1 was adjusted for age (continuous) and the study cohort using 2 dummy variables, whereas model 2 was adjusted further for smoking status (current smoker, ex-smoker, or never smoker) and alcohol consumption (regular drinker, ex-drinker, or never drinker). Model 3 was adjusted for the variables in model 2 plus hypertension, diabetes, and dyslipidemia (yes or no). The penalized spline (P-spline) method was used in the Cox regression models[15] to examine non-linear dose-response relationships between waist circumference and the hazard ra-

Table 2. CVD Risk Factors vs. WC

	WC quintile				
	Q1 (low)	Q2	Q3	Q4	Q5 (high)
Men					
n	688	781	723	632	730
WC (cm)	58.0–75.8	76.0–81.0	81.5–85.0	85.5–89.5	90.0–112.0
Median WC (cm)	72.0	79.0	84.0	87.0	93.0
Age (years)	61.6±11.4	58.8±11.1	58.6±10.8	59.0±10.3	59.6±10.4
BMI (kg/m^2)	19.5±1.7	21.7±1.6	23.1±1.5	24.2±2.0	26.4±3.0
SBP (mmHg)	126.4±21.1	128.3±20.1	130.9±19.0	133.8±20.3	136.2±20.0
DBP (mmHg)	74.9±10.9	77.3±10.7	79.8±10.1	80.7±10.6	83.2±11.3
Hypertension (%)	29.9	34.3	34.0	42.4	51.4
Dyslipidemia (%)	33.7	46.5	57.5	64.9	66.0
Diabetes (%)	6.1	6.7	7.5	8.5	10.6
Smoking status (%)					
Current smoker	58.5	51.4	42.2	45.1	40.9
Ex-smoker	24.6	28.0	31.8	33.0	35.8
Never smoker	16.9	20.6	26.1	22.0	23.3
Alcohol consumption (%)					
Regular drinker	60.7	72.5	69.4	68.6	69.6
Ex-drinker	5.5	4.8	5.0	5.6	4.4
Never drinker	33.8	22.7	25.7	25.8	26.0
Women					
n	875	986	817	891	903
WC (cm)	53.0–70.4	71.0–76.0	76.5–81.5	82.0–87.5	88.0–120.0
Median WC (cm)	67.0	74.0	79.0	84.0	92.0
Age (years)	55.9±11.5	57.4±10.8	58.5±10.5	59.7±10.2	61.9±10.2
BMI, kg/m^2	20.0±2.0	21.4±2.0	22.7±2.1	23.9±2.3	26.3±2.9
SBP (mmHg)	122.7±20.5	126.7±20.9	129.5±20.9	132.7±21.0	136.4±20.5
DBP (mmHg)	73.6±10.7	76.0±10.6	77.6±11.3	79.0±10.7	81.0±10.7
Hypertension (%)	23.5	29.7	34.3	40.7	51.2
Dyslipidemia (%)	35.0	47.1	53.0	64.3	70.0
Diabetes (%)	2.5	2.6	3.9	4.9	9.3
Smoking status, %					
Current smoker	10.1	7.9	7.2	8.3	9.1
Ex-smoker	2.3	1.8	2.5	2.8	4.0
Never smoker	87.6	90.3	90.4	88.9	86.9
Alcohol consumption (%)					
Regular drinker	22.5	20.4	19.8	20.7	16.6
Ex-drinker	1.8	1.3	1.1	1.4	1.8
Never drinker	75.7	78.2	79.1	77.9	81.6

Data given as mean±SD, n or (%).
Abbreviations as in Table 1.

tios for all-cause and CVD deaths. The degrees of freedom in the P-spline terms were selected automatically, based on Akaike's information criteria. The P-spline function provided the linear and non-linear components in the model. The P-values for the non-linear trends were therefore determined by the significance of the non-linear P-spline term. Although the general spline curve may be influenced by a skewed distribution and the number of knots specified, these factors had little influence on the curves in our method, with these being more accurate up to the 99th percentile of the distribution (ie, waist circumference of 103 cm in men and 104 cm in women).[15] In the Cox regression models, the linear trends were examined using continuous waist circumference data. The statistical tests were 2-sided, with $P<0.05$ being regarded as statistically significant. S-Plus version 8.1J (TIBCO Software, Palo Alto, CA, USA), was used for statistical analysis.

Results

Table 1 lists the characteristics of each cohort in the study. Mean follow-up was 11.3 years in the Tanno and Sobetsu study, 14.2 years in the Suita study, and 17.9 years in the Hisayama study. There were 1,711 all-cause deaths during the follow-up period in the 3 cohorts combined. Mean waist circumference in the cohorts ranged from 79.0 to 81.7 cm.

Table 2 lists the means and percentages of the cardiovascular risk factors at baseline, grouped according to sex-specific waist circumference quintile. Waist circumference was associated positively with systolic and diastolic blood pressure, hypertension, dyslipidemia, and diabetes. Men with a small waist

Table 3. Multivariate HRs for All-Cause and CVD Mortality vs. WC

	Quintile of WC					P for trend	
	Q1 (low)	Q2	Q3	Q4	Q5 (high)	Linear	Non-linear
Men							
Person-years	9,284	11,288	10,501	9,108	10,223		
All-cause							
No. deaths	279	218	181	177	187		
Model 1	1.00 (Ref.)	0.75 (0.63–0.90)	0.68 (0.56–0.82)	0.79 (0.66–0.96)	0.72 (0.59–0.86)	<0.001	0.087
Model 2	1.00 (Ref.)	0.78 (0.65–0.93)	0.73 (0.60–0.88)	0.84 (0.69–1.01)	0.77 (0.64–0.93)	0.005	0.091
Model 3	1.00 (Ref.)	0.76 (0.63–0.91)	0.72 (0.59–0.87)	0.81 (0.66–0.98)	0.73 (0.60–0.89)	0.001	0.11
CVD							
No. deaths	57	52	47	31	45		
Model 1	1.00 (Ref.)	0.91 (0.62–1.33)	0.91 (0.61–1.34)	0.71 (0.46–1.10)	0.90 (0.60–1.33)	0.29	0.12
Model 2	1.00 (Ref.)	0.96 (0.65–1.40)	0.99 (0.67–1.48)	0.74 (0.47–1.15)	0.97 (0.65–1.45)	0.49	0.13
Model 3	1.00 (Ref.)	0.92 (0.63–1.36)	0.95 (0.64–1.42)	0.67 (0.43–1.06)	0.87 (0.57–1.32)	0.22	0.16
Women							
Person-years	12,863	14,834	12,294	13,749	14,040		
All-cause							
No. deaths	116	115	123	134	181		
Model 1	1.00 (Ref.)	0.76 (0.59–0.98)	0.86 (0.67–1.12)	0.75 (0.59–0.97)	0.83 (0.65–1.05)	0.29	0.029
Model 2	1.00 (Ref.)	0.79 (0.61–1.02)	0.91 (0.70–1.18)	0.76 (0.59–0.98)	0.84 (0.66–1.07)	0.27	0.060
Model 3	1.00 (Ref.)	0.81 (0.62–1.05)	0.94 (0.73–1.22)	0.80 (0.62–1.03)	0.86 (0.67–1.10)	0.36	0.16
CVD							
No. deaths	38	29	38	46	64		
Model 1	1.00 (Ref.)	0.61 (0.37–0.98)	0.83 (0.53–1.30)	0.81 (0.53–1.25)	0.91 (0.61–1.36)	0.73	0.29
Model 2	1.00 (Ref.)	0.63 (0.39–1.02)	0.88 (0.56–1.38)	0.83 (0.54–1.27)	0.91 (0.61–1.37)	0.84	0.35
Model 3	1.00 (Ref.)	0.64 (0.39–1.03)	0.90 (0.57–1.43)	0.85 (0.54–1.32)	0.90 (0.59–1.38)	0.90	0.43

Non-linear trend P was computed using the p-spline terms in the Cox regression models, with adjustment for the covariates in models 1, 2 and 3. Model 1 was adjusted for age and community. Model 2 was adjusted further for smoking and alcohol drinking. Model 3 was adjusted for the variables in model 2 plus hypertension, dyslipidemia, and diabetes.
HR, hazard ratio. Other abbreviations as in Table 1.

circumference were more likely to be current smokers and less likely to be regular alcohol drinkers. The Pearson's correlation coefficient between waist circumference and BMI was 0.85 (P<0.001) in men and 0.74 (P<0.001) in women.

Table 3 lists the sex-specific hazard ratios for all-cause and CVD deaths, grouped according to the sex-specific quintile of waist circumference. We tested the association using 3 models adjusting for age and the study cohort (model 1); model 1 plus smoking status, and alcohol consumption (model 2); and the variables in model 2 plus hypertension, diabetes, and dyslipidemia (model 3). Non-linear trends were tested using the P-spline function, and P-spline curves drawn based on model 3 (**Figure 1**). In men, the hazard ratios for all-cause death were significantly lower in the second to fifth quintile range of waist circumference compared with the first quintile, and showed a significant inverse linear trend in all the models. The hazard ratio of the highest quintile of waist circumference in model 3 was 0.73 (95% CI: 0.60–0.89). The inverse linear association between hazard ratio and waist circumference remained significant after adjustment for confounders in Model 3 (P for trend=0.001), whereas the non-linear dose-response relationship between hazard ratio and waist circumference was not significant in either model (**Figure 1A**). For CVD deaths, although the fourth quintile of waist circumference had the lowest hazard ratio, the ratios changed very little with level of waist circumference in the 3 models, with non-significant linear and non-linear trends (**Figure 1B**).

In women, the hazard ratios for all-cause death in the second and fourth quintiles of waist circumference were significantly decreased in model 1. The non-linear relationship in model 1 was also statistically significant (P for trend=0.029). In models 2 and 3, however, these associations were attenuated after further adjustment for conventional CVD risk factors (**Figure 1C**), with waist circumference showing no linear or non-linear association with CVD mortality risk (**Figure 1D**).

In addition, we analyzed all-cause mortality risk stratified by the presence or absence of hypertension, diabetes, or dyslipidemia in men (**Table S1**). Inverse linear associations were found in individuals with hypertension and individuals without dyslipidemia or diabetes, but we did not find any adverse effects of greater waist circumference regardless of these common diseases in the present analysis.

The multivariate-adjusted hazard ratios for all-cause and CVD mortality in men stratified by age (<65 years and ≥65 years) are given in **Table 4**. **Figure 2** shows the P-spline fitting curves of the hazard ratios. The lowest hazard ratio for CVD was seen in the fourth quintile of waist circumference in men aged <65 years. Although there was no longer a linear association between waist circumference and CVD mortality, the non-linear association was significant (P for non-linear trend <0.001) and the spline curve was U-shaped (**Figure 2C**). Using the lowest risk group (fourth quintile) as the reference group, the hazard ratio of the fifth quintile was increased significantly (hazard ratio=2.45; 95% CI: 1.08–5.57). In contrast, waist circumference had a linear association with decreased risk of all-cause mortality in men ≥65 years. The spline curves in women are

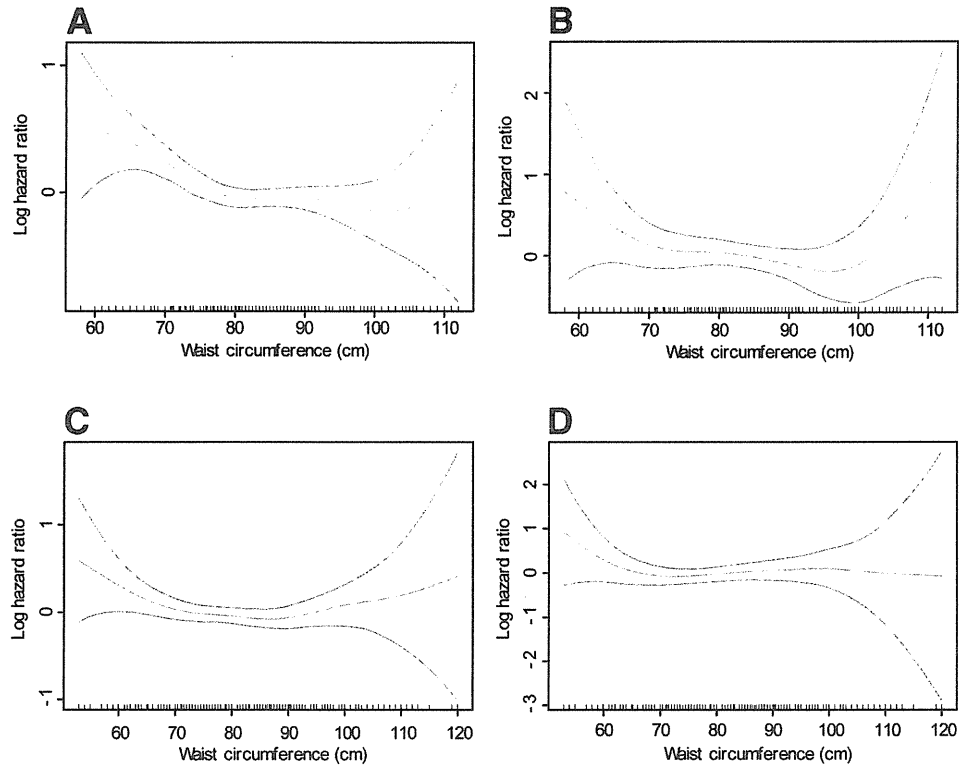

Figure 1. P-spline fitting curves (from Cox regression models) of hazard ratios (blue lines) and 95% confidence intervals (orange lines) for (**A**,**C**) all-cause and (**B**,**D**) cardiovascular disease (CVD) deaths vs. waist circumference in (**A**,**B**) men and (**C**,**D**) women. (**A**) Men, all-cause death (non-linear trend, P=0.11); (**B**) men, CVD death (P=0.16); (**C**) women, all-cause death (P=0.16); (**D**) women, CVD death (P=0.43). The hazard ratios were adjusted for age, study community, smoking habits, alcohol consumption, hypertension, hyperlipidemia, and diabetes mellitus. Actual waist circumference used in the present analysis is marked on x-axis.

Table 4. Multivariate-Adjusted HRs for All-Cause and CVD Mortality in Men vs. WC

	Quintile of WC					P for trend	
	Q1 (low)	Q2	Q3	Q4	Q5 (high)	Linear	Non-linear
<65 years							
Person-years	6,013	8,267	7,570	6,756	7,496		
All-cause							
No. deaths	82	81	76	72	72		
HR	1.00 (Ref.)	0.86 (0.63–1.17)	0.90 (0.65–1.24)	0.86 (0.62–1.19)	0.82 (0.59–1.15)	0.36	0.089
CVD							
No. deaths	15	26	17	8	21		
HR	1.00 (Ref.)	1.61 (0.84–3.09)	1.18 (0.58–2.43)	0.53 (0.22–1.27)	1.30 (0.64–2.64)	0.99	<0.001
≥65 years							
Person-years	3,271	3,021	2,932	2,352	2,727		
All-cause							
No. deaths	197	137	105	105	115		
HR	1.00 (Ref.)	0.72 (0.57–0.90)	0.63 (0.49–0.80)	0.81 (0.63–1.03)	0.71 (0.55–0.90)	0.003	0.11
CVD							
No. deaths	42	26	30	23	24		
HR	1.00 (Ref.)	0.66 (0.40–1.08)	0.91 (0.55–1.48)	0.85 (0.50–1.47)	0.74 (0.43–1.26)	0.23	0.31

The hazard ratios were adjusted for study cohort, age, smoking, alcohol drinking, hypertension, dyslipidemia, and diabetes. Non-linear trend P was computed using the p-spline terms in the Cox regression model adjusted for the same variables.
Abbreviations as in Tables 1,3.

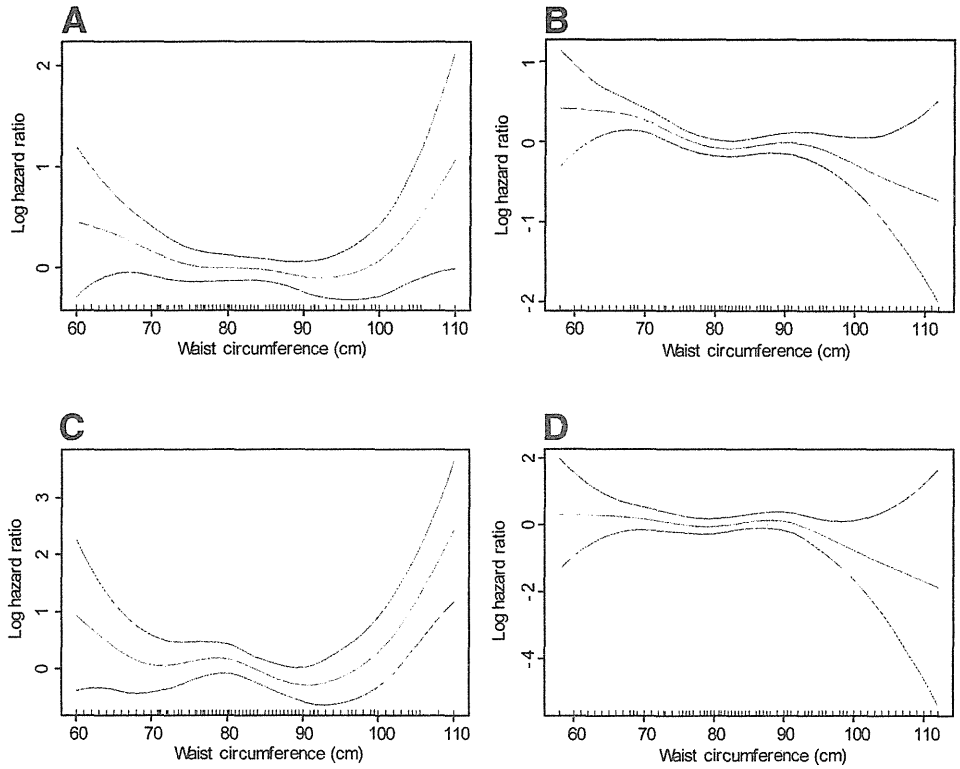

Figure 2. P-spline fitting curves (from Cox regression models) of hazard ratios (blue lines) and 95% confidence intervals (orange lines) for (**A**,**B**) all-cause and (**C**,**D**) cardiovascular disease (CVD) deaths vs. waist circumference in men (**A**,**C**) <65 years and (**B**,**D**) ≥65 years of age. (**A**) All-cause death, age <65 years (non-linear trend, P=0.089); (**B**) all-cause death, age ≥65 years (P=0.11); (**C**) CVD death, age <65 years (P<0.001); (**D**) CVD death, age ≥65 years (P=0.31). The hazard ratios were adjusted for age, study community, smoking habits, alcohol consumption, hypertension, hyperlipidemia, and diabetes mellitus. Actual waist circumference used in the present analysis is marked on x-axis.

shown in **Figure S1**.

Figure 3 shows the dose-response curves between BMI and hazard ratios for all-cause and CVD deaths according to gender. In comparison with the curves for waist circumference in **Figure 1**, the non-linear trend was significant for all-cause and CVD deaths in women, but not in men. Hazard ratios for all-cause and CVD deaths were increased moderately in obese women, although the 95% CIs became wider. When tested for linear trends, BMI levels were associated inversely with all-cause mortality risk (P for trend <0.001).

The analyses were repeated after excluding data from the first 5 years of follow-up (**Table S2**). We confirmed that all the associations were similar, with the non-linear association between waist circumference and CVD mortality in men becoming significant.

Discussion

This 14.7-year follow-up prospective study has shown that waist circumference is inversely associated with increased risk of all-cause death in men. We also showed that the non-linear dose-response relationship between waist circumference and CVD mortality risk is significant only in men aged <65 years. P-spline analysis, however, showed that the relationship between waist circumference and CVD mortality risk in middle-aged men was U-shaped, and that CVD mortality risk was increased only in the highest quintile of waist circumference (≥90 cm). In contrast, increased waist circumference did not influence all-cause or CVD mortality risk in elderly men, whereas a small waist circumference increased this risk.

Despite the close relationship between greater waist circumference and aggregation of CVD risk factors,[16] abdominal obesity did not increase mortality risk. Although we examined this association using 3 regression models that included confounding factors such as smoking, alcohol drinking, and common diseases such as hypertension, the pattern of decreased risk for mortality did not change. Similar findings were seen in a previous Japanese study on the association between weight change and mortality risk.[4] Considering the results of the present study as well as previous studies, the effect of obesity or weight gain on death appears to be considerably smaller in Japanese than Caucasian subjects. In contrast, recent large Japanese prospective studies demonstrated that overweight or obesity assessed using BMI raised the risk of incident stroke only in women,[5] and that of incident coronary heart disease only in men.[17] Another meta-analysis in Japan also found that increased BMI was associated with a higher risk of incident stroke in both men and women, but a higher risk of coronary heart disease only in men.[6] The results of these epidemiological studies suggest that obesity has a much different effect on CVD incidence than on

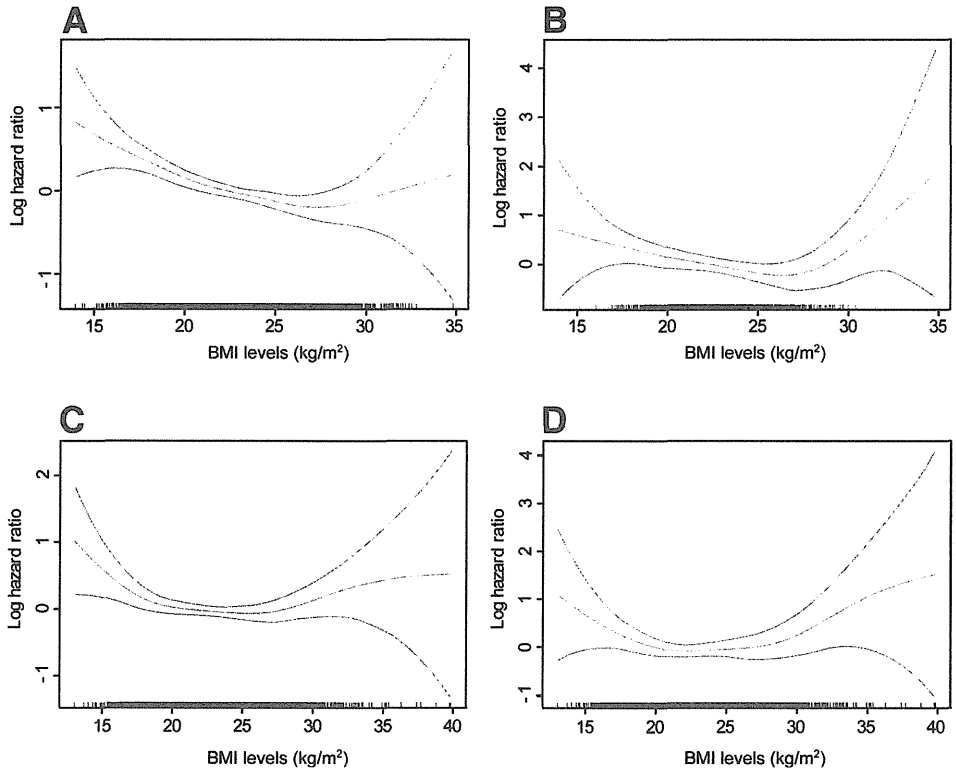

Figure 3. P-spline fitting curves (from Cox regression models) of hazard ratios (blue lines) and 95% confidence intervals (orange lines) for (**A**,**C**) all-cause and (**B**,**D**) cardiovascular disease (CVD) deaths vs. body mass index (BMI) in (**A**,**B**) men and (**C**,**D**) women. (**A**) Men, all-cause death (non-linear trend P=0.17); (**B**) men, CVD death (P=0.079); (**C**) women, all-cause death (P=0.026); (**D**) women, CVD death (P=0.046). The hazard ratios were adjusted for age, study community, smoking habits, alcohol consumption, hypertension, hyperlipidemia, and diabetes mellitus. Actual BMI used in the present analysis is marked on the x-axis.

CVD mortality.

Although it is not clear why the present findings are different from those in Western countries, this may be explained in part by the prevalence of obesity in the population. The prevalence of obesity (BMI ≥30 kg/m²) in the present study was 1.2% in men and 2.3% in women, lower than the approximate 30% prevalence reported in US adults.[18] Furthermore, based on vital statistics, the leading cause of death in Japan is cancer, being responsible for one-third of all deaths. The lower prevalence of obesity in Japan may reduce the effect of obesity on all-cause mortality risk. Furthermore, a previous Japanese prospective study showed that overweight subjects with metabolic syndrome did not have elevated CVD mortality compared with non-overweight subjects who had ≥2 other conventional CVD risk factors.[19,20]

Waist circumference had a non-linear association with CVD mortality risk in middle-aged men, with this association being U-shaped.[10] When compared with the fourth quintile, risk was increased at a waist circumference greater than approximately 90 cm, which corresponded to the upper 20% of the entire distribution. In contrast, abdominal obesity was not associated with greater mortality risk in older men. Although this discrepancy is consistent with the findings of a previous US study,[21] the explanation for the effect of aging on the relationship between obesity and mortality is not clear. We believe that older men who had a small waist circumference tended to lose weight due to nutrient deficiency, heavy smoking, or worsening diabetes.

In this study, we found similar non-linear dose-response relationships between all-cause mortality risk and BMI, which correlated strongly with waist circumference. But we did not determine which parameter was better for predicting cardiovascular endpoints. As mentioned in the UK survey, a new study on this issue is required in Japanese adults.[10]

To the best of our knowledge, this is the first report to show an association between abdominal obesity and mortality risk in a large Japanese population-based study with long-term follow-up. The Tanno and Sobetsu study,[12,22] the Suita study,[23,24] and the Hisayama study[25] are epidemiological studies that have been conducted for decades under high quality control, but several limitations should be noted. First, although we confirmed that the associations remained unaltered after excluding the first 5 years of follow-up data to eliminate the influence of latent disease at baseline, the results do not prove the existence of a cause and effect relationship. The increased risk of mortality was considered to be due to the consequence of ill-health related to weight loss and low body weight.[26] Although the present study demonstrated that the effect of increased waist circumference on mortality was comparable regardless of the presence or absence of hypertension, dyslipidemia, or diabetes, ill-health associated with low body weight was not fully controlled. Second, we did not assess the physical activity of the subjects, which may have confounded the associa-

tion between obesity and mortality risk.[27,28] Third, increased waist circumference may have a different effect on disease onset than on mortality risk, and the present study evaluated only mortality risk. Future studies on the effect of waist circumference on the incidence of CVD are therefore needed to address this issue.[8]

In conclusion, waist circumference was associated inversely with increased risk of all-cause death in men, but not in women. Although a non-linear statistical approach showed increased risk of CVD mortality with larger waist circumference in middle-aged men, a smaller waist circumference was generally associated with an increased risk of mortality in the general Japanese population. Similar findings have been reported in previous studies that measured BMI rather than waist circumference.

Acknowledgments

This study was supported by the Health and Labour Science Research Grants (H19-ShiShu-017, H20-SeiShu-013, and H21-SeiShu-015). We appreciate the work of all the staff in the Tanno and Sobetsu study, the Suita study, and the Hisayama study. We thank Dr. Yasunao Yoshimasa (principal investigator) and all members of the Suita City Health Center and the Suita Medical Association. We also thank all researchers and the staff of the Division of Preventive Cardiology for performing the medical examinations and study follow-up, and Satsuki-Junyukai, the volunteer who organized the Suita Study.

Disclosures

None.

References

1. Zheng W, McLerran DF, Rolland B, Zhang X, Inoue M, Matsuo K, et al. Association between body-mass index and risk of death in more than 1 million Asians. *N Engl J Med* 2011; **364:** 719–729.
2. Tsugane S, Sasaki S, Tsubono Y. Under- and overweight impact on mortality among middle-aged Japanese men and women: A 10-y follow-up of JPHC study cohort I. *Int J Obes Relat Metab Disord* 2002; **26:** 529–537.
3. Cui R, Iso H, Toyoshima H, Date C, Yamamoto A, Kikuchi S, et al. Body mass index and mortality from cardiovascular disease among Japanese men and women: The JACC study. *Stroke* 2005; **36:** 1377–1382.
4. Saito I, Konishi M, Iso H, Inoue M, Tsugane S. Impact of weight change on specific-cause mortality among middle-aged Japanese individuals. *J Epidemiol Community Health* 2009; **63:** 447–454.
5. Saito I, Iso H, Kokubo Y, Inoue M, Tsugane S. Body mass index, weight change and risk of stroke and stroke subtypes: The Japan Public Health Center-based prospective (JPHC) study. *Int J Obes (Lond)* 2011; **35:** 283–291.
6. Yatsuya H, Toyoshima H, Yamagishi K, Tamakoshi K, Taguri M, Harada A, et al. Body mass index and risk of stroke and myocardial infarction in a relatively lean population: Meta-analysis of 16 Japanese cohorts using individual data. *Circ Cardiovasc Qual Outcomes* 2010; **3:** 498–505.
7. Yonemoto K, Doi Y, Hata J, Ninomiya T, Fukuhara M, Ikeda F, et al. Body mass index and stroke incidence in a Japanese community: The Hisayama study. *Hypertens Res* 2011; **34:** 274–279.
8. Furukawa Y, Kokubo Y, Okamura T, Watanabe M, Higashiyama A, Ono Y, et al. The relationship between waist circumference and the risk of stroke and myocardial infarction in a Japanese urban cohort: The Suita study. *Stroke* 2010; **41:** 550–553.
9. Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, et al. General and abdominal adiposity and risk of death in Europe. *N Engl J Med* 2008; **359:** 2105–2120.
10. Czernichow S, Kengne AP, Stamatakis E, Hamer M, Batty GD. Body mass index, waist circumference and waist-hip ratio: Which is the better discriminator of cardiovascular disease mortality risk?: Evidence from an individual-participant meta-analysis of 82 864 participants from nine cohort studies. *Obes Rev* 2011; **12:** 680–687.
11. de Hollander EL, Bemelmans WJ, Boshuizen HC, Friedrich N, Wallaschofski H, Guallar-Castillon P, et al. The association between waist circumference and risk of mortality considering body mass index in 65- to 74-year-olds: A meta-analysis of 29 cohorts involving more than 58 000 elderly persons. *Int J Epidemiol* 2012; **41:** 805–817.
12. Ohnishi H, Saitoh S, Takagi S, Katoh N, Chiba Y, Akasaka H, et al. Incidence of type 2 diabetes in individuals with central obesity in a rural Japanese population: The Tanno and Sobetsu study. *Diabetes Care* 2006; **29:** 1128–1129.
13. Ohmura T, Ueda K, Kiyohara Y, Kato I, Iwamoto H, Nakayama K, et al. Prevalence of type 2 (non-insulin-dependent) diabetes mellitus and impaired glucose tolerance in the Japanese general population: The Hisayama Study. *Diabetologia* 1993; **36:** 1198–1203.
14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18:** 499–502.
15. Govindarajulu US, Spiegelman D, Thurston SW, Ganguli B, Eisen EA. Comparing smoothing techniques in Cox models for exposure-response relationships. *Stat Med* 2007; **26:** 3735–3752.
16. Choi SJ, Keam B, Park SH, Park HY. Appropriate waist circumference cut-offs to predict diabetes in the Korean population: The Korean Genome and Epidemiology Study. *Circ J* 2010; **74:** 1357–1363.
17. Chei CL, Iso H, Yamagishi K, Inoue M, Tsugane S. Body mass index and weight change since 20 years of age and risk of coronary heart disease among Japanese: The Japan Public Health Center-Based Study. *Int J Obes (Lond)* 2008; **32:** 144–151.
18. Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. Increasing prevalence of overweight among US adults. The National Health and Nutrition Examination Surveys, 1960 to 1991. *JAMA* 1994; **272:** 205–211.
19. Saito I, Iso H, Kokubo Y, Inoue M, Tsugane S. Metabolic syndrome and all-cause and cardiovascular disease mortality: Japan Public Health Center-based Prospective (JPHC) Study. *Circ J* 2009; **73:** 878–884.
20. Saito I. Epidemiological evidence of type 2 diabetes mellitus, metabolic syndrome, and cardiovascular disease in Japan. *Circ J* 2012; **76:** 1066–1073.
21. Kuk JL, Ardern CI. Influence of age on the association between various measures of obesity and all-cause mortality. *J Am Geriatr Soc* 2009; **57:** 2077–2084.
22. Chiba Y, Saitoh S, Takagi S, Ohnishi H, Katoh N, Ohata J, et al. Relationship between visceral fat and cardiovascular disease risk factors: The Tanno and Sobetsu study. *Hypertens Res* 2007; **30:** 229–236.
23. Kokubo Y, Kamide K, Okamura T, Watanabe M, Higashiyama A, Kawanishi K, et al. Impact of high-normal blood pressure on the risk of cardiovascular disease in a Japanese urban cohort: The Suita study. *Hypertension* 2008; **52:** 652–659.
24. Kokubo Y, Nakamura S, Okamura T, Yoshimasa Y, Makino H, Watanabe M, et al. Relationship between blood pressure category and incidence of stroke and myocardial infarction in an urban Japanese population with and without chronic kidney disease: The Suita Study. *Stroke* 2009; **40:** 2674–2679.
25. Kubo M, Hata J, Doi Y, Tanizaki Y, Iida M, Kiyohara Y. Secular trends in the incidence of and risk factors for ischemic stroke and its subtypes in Japanese population. *Circulation* 2008; **118:** 2672–2678.
26. Wannamethee SG, Shaper AG, Walker M. Weight change, body weight and mortality: The impact of smoking and ill health. *Int J Epidemiol* 2001; **30:** 777–786.
27. Bellocco R, Jia C, Ye W, Lagerros YT. Effects of physical activity, body mass index, waist-to-hip ratio and waist circumference on total mortality risk in the Swedish National March Cohort. *Eur J Epidemiol* 2010; **25:** 777–788.
28. Patel AV, Bernstein L, Deka A, Feigelson HS, Campbell PT, Gapstur SM, et al. Leisure time spent sitting in relation to total mortality in a prospective cohort of US adults. *Am J Epidemiol* 2010; **172:** 419–429.

Supplementary Files

Supplementary File 1

Table S1. Multivariate-Adjusted HR for All-Cause Mortality in Men

Table S2. Multivariate HRs for All-Cause and CVD Mortality After Exclusion of Data From the First 5 Years of Follow-up

Figure S1. P-spline fitting curves (from Cox regression models) of hazard ratios and 95% CIs for (A,B) all-cause and (C,D) CVD deaths, according to variation in waist circumference in women, stratified by age group.

Please find supplementary file(s);
http://dx.doi.org/10.1253/circj.CJ-11-1259

Original Article

Impact of lower range of prehypertension on cardiovascular events in a general population: the Hisayama Study

Masayo Fukuhara[a,b], Hisatomi Arima[a], Toshiharu Ninomiya[a,b], Jun Hata[a,b], Koji Yonemoto[c], Yasufumi Doi[a,b], Yoichiro Hirakawa[a,b], Kiyoshi Matsumura[b], Takanari Kitazono[b], and Yutaka Kiyohara[a]

Objectives: The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) defined blood pressure (BP) levels of 120–139/80–89 mmHg as prehypertension. The objective of the present analysis was to examine the impact of prehypertension and its population-attributable fraction for development of cardiovascular events in a general Japanese population.

Methods: Two thousand, six hundred and thirty-four residents of the town of Hisayama aged at least 40 years without cardiovascular disease were followed up for 19 years. BP categories were defined using JNC7, and prehypertension was divided into the lower (120–129/80–84 mmHg) and higher ranges (130–139/85–89 mmHg). During the follow-up period, 449 participants developed cardiovascular disease (305 strokes and 187 coronary heart diseases).

Results: The frequencies of normal BP, prehypertension, and stages 1 and 2 hypertension were 24.9, 37.7, 23.8, and 13.6%, respectively. The age and sex-adjusted incidence of cardiovascular disease rose progressively with elevation of BP levels ($P < 0.001$ for trend). The risks of cardiovascular disease in lower and higher ranges of prehypertension were 58% [95% confidence interval (CI) 11–126%] and 70% (95% CI 18–144%) higher than normal BP even after controlling for other cardiovascular risk factors. The population-attributable fraction of prehypertension was 13.2%, which was similar to those of stages 1 and 2 hypertension.

Conclusions: The risks of cardiovascular disease increased significantly from the lower range of prehypertension in a general Japanese population. Approximately one-third of excess cardiovascular events attributable to elevated BP levels were estimated to occur among individuals with prehypertension.

Keywords: blood pressure, cardiovascular disease, population-attributable fraction, prehypertension, prevention, prospective cohort studies, stroke

Abbreviations: BP, blood pressure; CI, confidence interval; CVD, cardiovascular disease; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; JNC7, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; PAF, population-attributable fraction

INTRODUCTION

The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) defined the blood pressure (BP) levels of 120–139/80–89 mmHg as prehypertension based on the evidence of a modest increase in cardiovascular risk among individuals with such BP levels [1]. However, current evidence of increased risks of cardiovascular disease (CVD) associated with prehypertension has mainly been reported for its higher range (130–139/85–89 mmHg) [2,3], and it is still unclear about the cardiovascular risks among individuals with the lower range of prehypertension (120–129/80–84 mmHg), particularly in the Japanese. Because the prevalence of prehypertension has been reported to be as high as 31–43% [4–6], a large portion of the burden of CVD is likely attributable to prehypertension. Although a number of large-scale observational studies have shown population-attributable fractions (PAFs) of this BP category for premature deaths or deaths due to cardiovascular causes [7,8], uncertainty remains surrounding the frequency of 'fatal and nonfatal' cardiovascular events attributable to prehypertension.

The Hisayama Study has demonstrated that the incidence rates of stroke significantly increased from BP levels of 140/90 mmHg among participants recruited in 1961 [5].

Journal of Hypertension 2012, 30:893–900

[a]Department of Environmental Medicine, [b]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka and [c]Biostatistics Center, Kurume University, Kurume, Japan

Correspondence to Hisatomi Arima, MD, PhD, Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel: +81 92 652 3080; fax: +81 92 652 3075; e-mail: harima@envmed.med.kyushu-u.ac.jp

Received 30 June 2011 **Revised** 3 December 2011 **Accepted** 25 January 2012

DOI:10.1097/HJH.0b013e328351d380

However, the effects of BP on the risks of stroke and coronary heart disease might have changed since then because of the substantial changes in lifestyle and the improved awareness, treatment, and control of hypertension [9]. The objective of the present new analysis from the Hisayama Study is to investigate the influence of BP on cardiovascular events among participants recruited in 1988 and to estimate population-attributable risks of prehypertension (lower and higher ranges) and hypertension for incident CVD in a general Japanese population.

METHODS

Study population

The Hisayama Study is a population-based prospective cohort study of CVD established in 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area in Kyushu Island of Japan. Based on data from the national census, the age and occupational distributions in Hisayama have been almost identical to those in Japan since the 1960s [10]. In 1988, a total of 2742 residents aged at least 40 years consented to participate in the screening examination (participation rate 80.9%). After the exclusion of 106 residents with a history of stroke or coronary heart disease and two residents who died before the start of follow-up, the remaining 2634 residents (1107 men and 1527 women) were enrolled in this study. The study design and characteristics of this cohort population have been described in detail elsewhere [11–13].

Follow-up survey

The participants were followed up prospectively for 19 years, from December 1988 to November 2007, by annual health examinations. The health status of any individual who did not undergo a regular examination or who moved out of town was checked yearly by mail or telephone. We also established a daily monitoring system among the study team, local physicians, and members of the town's Health and Welfare Office. Using this system, we gathered information on new events of CVD, including suspected cases. When stroke or coronary heart disease occurred or was suspected, physicians in the study team examined the individual and evaluated his/her detailed clinical information. The clinical diagnosis of stroke or coronary heart disease was based on the patient's history, physical and neurological examinations, and ancillary laboratory examinations. Furthermore, when a patient died, an autopsy was performed at the Departments of Pathology of Kyushu University. During the follow-up period, there was no true loss to follow-up, and 842 patients died, of whom 605 (71.9%) underwent autopsy.

Blood pressure measurements and classification

At the baseline examination, BP was measured three times using a standard mercury sphygmomanometer in the sitting position after rest for at least 5 min. Appropriately-sized cuffs were used for BP assessment. Korotkoff phase 5 was taken as the diastolic BP unless the sound persisted at 0, in which case Korotkoff phase 4 was recorded. The mean of the three measurements was used for the analysis. BP levels were classified into four categories according to JNC7: normal BP (<120/80 mmHg), prehypertension (120–139/80–89 mmHg), stage 1 hypertension (140–159/90–99 mmHg), and stage 2 hypertension (≥160/100 mmHg) [1]. Prehypertension was divided into two subcategories: lower (120–129/80–84 mmHg) and higher (130–139/85–89 mmHg) BP ranges. If systolic and diastolic BP readings for a participant were in different categories, that participant was categorized into the higher of the two BP categories. Antihypertensive drug users were classified according to BP levels at baseline.

Other risk factor measurement

At baseline, each participant completed a self-administered questionnaire covering medical history, treatment for hypertension and diabetes, smoking habits, alcohol intake, and exercise. Smoking habits and alcohol intake were classified into currently habitual or not. The participants engaging in sports or other forms of exertion at least three times a week during their leisure time made up a regular exercise group. Body height and weight were measured in light clothing without shoes, and the body mass index (kg/m^2) was calculated. Electrocardiogram (ECG) abnormalities were defined as left-ventricular hypertrophy (Minnesota code 3–1), ST depression (4–1, 2, 3), or atrial fibrillation (8–3).

Serum total and high-density lipoprotein (HDL) cholesterol levels were determined enzymatically. Hypercholesterolemia was defined as total cholesterol at least 5.7 mmol/l. Blood glucose levels were measured by the glucose oxidase method. Diabetes was determined by medical history, plasma glucose levels (fasting glucose level ≥7.0 mmol/l or postprandial glucose level ≥11.1 mmol/l), or a 75-g oral glucose tolerance test using the 1998 World Health Organization criteria [14]. Serum creatinine was measured by the noncompensated Jaffé method. The Jaffé method value was converted to an enzymatic method value by using the following equation [15]:

$$\begin{aligned}&\text{Serum creatinine (enzymatic method [mg/dl])}\\&\quad = 0.9754\\&\qquad \times \text{serum creatinine (Jaffé method [mg/dl])}\\&\qquad - 0.2802.\end{aligned}$$

Estimated glomerular filtration rate (eGFR) was calculated using the isotope dilution mass spectrometry-traceable 4-variable Modification of Diet in Renal Disease (IDMS-MDRD) Study equation modified with the Japanese correction [16]:

$$\begin{aligned}&\text{eGFR (ml/min per 1.73 m}^2\text{)}\\&\quad = 194\\&\qquad \times \text{serum creatinine (enzymatic method)}^{-1.094}\\&\qquad \times \text{age}^{-0.287} \times 0.742 \text{ (if women)}.\end{aligned}$$

Chronic kidney disease was defined as proteinuria (+ or more using the test paper method) or eGFR below 60 ml/min per 1.73 m^2 according to the National Kidney