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Interaction of Blood Pressure and Body Mass Index With Risk of Incident Atrial Fibrillation in a Japanese Urban Cohort: The Suita Study

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BACKGROUND AND PURPOSE
To prevent stroke, strategies for atrial fibrillation (AF) prevention and an early detection of AF by electrocardiogram are essential. However, only a limited prospective studies have examined the risk factors for AF, even in blood pressure (BP) and body mass index (BMI), which are not clear among general populations. We investigated the impacts of BP and BMI on the risk of incident AF in a general population.

METHODS
A total of 6,906 participants (30–84 years) in the Suita Study were prospectively followed up for incident AF. Participants were diagnosed with AF if AF or atrial flutter was present on an electrocardiogram from a routine health examination (every 2 years) or if AF was indicated as a present illness from health examinations and/or medical records during follow-up. Adjusted Cox proportional hazard ratios (HRs) were calculated.

RESULTS
During the 12.8-year follow-up, 253 incident AF events occurred. Compared with the systolic BP (SBP) < 120 mm Hg and normal-weight, the adjusted HRs (95% confidence intervals; CIs) of incident AF in the systolic hypertension and the overweight (BMI ≥ 25 kg/m^2) groups were 1.74 (1.22–2.49) and 1.35 (1.01–1.80), respectively. Compared with SBP < 120 mm Hg and normal weight, the adjusted HRs (95% CIs) of incident AF in the SBP = 120–139 mm Hg with overweight and the systolic hypertension with normal or overweight were 1.72 (1.01–2.91), 1.66 (1.10–2.50), and 2.31 (1.47–3.65), respectively (*P* for interaction = 0.04).

CONCLUSIONS
Systolic prehypertension and overweight are associated with incident AF in Japanese population. The association between SBP and AF may be evident by overweight.

Keywords: atrial fibrillation; blood pressure; body mass index; hypertension; prospective cohort study; risk factor.

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Atrial fibrillation (AF) is the most common chronic arrhythmia and is a risk factor for all-cause mortality[1] and stroke.[2] To prevent stroke, strategies for AF prevention and an early detection of AF by electrocardiogram are essential. However, only a limited number of prospective studies have examined the risk factors for AF among general populations.

Recent studies have gradually revealed that not only hypertension,[3,4] but also prehypertension is a risk factor for incident AF.[5,6] Pulse pressure is also a risk factor for incident AF.[7] However, it is still difficult to determine which blood pressure (BP) categories associated with incident AF in prospective studies.[8] It may be dependent on the different backgrounds of populations, lifestyles, and/or cardiovascular risk factors[9] such as obesity.

Positive associations with incident AF have been observed for overweight[10] and class 1[11,12] or 3[13] obesity. These different results may depend on the different backgrounds of the study populations. The combined impact of obesity and should also be considered regarding the incidence of AF. However, few prospective studies have examined the combined effect of BP and BMI on the incidence of AF in a general population. Only the Women's Health Study showed no interaction between hypertension and obesity in incident AF.[14] Different populations may have different incidence rate of AF,[15] and therefore possibly different risk factors for AF. Here, we assessed the hypothesis that the combination of BP and BMI categories increases the risk of incident AF in an urban general Japanese population, which has higher BP and less obesity than Westerners.[9]

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METHODS

Study participants

The design and selection criteria of the Suita Study have already been described.[16] As a baseline, 12,200 and 3,000 participants (aged 30–79 years) were randomly selected from the municipality population registry of Suita city and stratified into groups by sex and age in 10-year increments in 1989 and 1996, respectively. Of these, participants attending the baseline examination of the original cohort (n = 6,485; 1989–1996) and the secondary cohort (n = 1,329; 1996–1998) were eligible for the present investigation. In addition, the baseline examination of a volunteer group (n = 546, 1992–2006) was also included in the present study. Informed consent was obtained from all participants. These evaluations are referred to as the baseline examination for the present investigation. This study was approved by the Institutional Review Board of the National Cerebral and Cardiovascular Center, Suita, Japan.

We excluded participants for the following reasons: prior or current illness of AF or atrial flutter (n = 42) at baseline, missing covariate (n = 2), and failure to complete the baseline examination (n = 2) or the follow-up health surveys (n = 1,408), resulting in a sample of 6,906 participants.

Blood pressure and physical measurement

In National Cerebral and Cardiovascular Center, well-trained physicians measured each participant's BP 3 times using a mercury column sphygmomanometer, an appropriate-size cuff, and a standard protocol.[16] Before the initial BP reading was obtained, participants were seated at rest for at least 5 minutes. BP values were taken as the average of the second and third measurements, which were recorded more than 1-minute apart. At the time of the baseline examination, each participant was classified into 1 of 3 BP categories (normal BP (<120/80 mm Hg), prehypertension (120–139/80–89 mm Hg), and hypertension (≥140/90 mm Hg and/or antihypertensive drug use)). If the systolic BP (SBP) and diastolic BP (DBP) readings for participants were in different categories, the participants were categorized into the higher of the 2 BP categories. SBP and DBP alone categories were as follows: normal (<120/80 mm Hg), systolic and diastolic prehypertension (120–139/80–89 mm Hg), and hypertension (≥140/90 mm Hg including antihypertensive drug users), respectively. Categories of body mass index (BMI), calculated as weight (kg) divided by height (m) squared, were defined by the following criteria: underweight (<18.5 kg/m^2), normal weight (18.5 to <25 kg/m^2), and overweight (≥25 kg/m^2).[17]

Biochemical measurement and questionnaire

At the baseline examination, we performed routine blood tests that included serum total cholesterol and glucose levels. Hypercholesterolemia was defined as total cholesterol levels ≥5.7 mmol/L or current use of antihyperlipidemic medications. Diabetes (DM) was defined as fasting serum glucose ≥7.0 mmol/L, nonfasting serum glucose ≥11.1 mmol/L, or medications for DM. Physicians and nurses administered questionnaires covering personal habits and present illness. Past/present illness of stroke included cerebral infarction, intracerebral hemorrhage, and subarachnoid hemorrhage. Past/present illness of heart disease included coronary heart disease, valvular disease, and chronic heart failure. Premature contractions consisted of frequent atrial, junctional, and/or ventricular premature beats (Minnesota Code 8-1-1, 8-1-2, or 8-1-3) without AF/flutter at the baseline. The glomerular filtration rate (mL/min/1.73 m^2) of each participant was calculated using the Modification of Diet in Renal Disease equation modified by the Japanese coefficient (0.881), as follows: glomerular filtration rate = $0.881 \times 186 \times (\text{age})^{-0.203} \times (\text{serum creatinine})^{-1.154}$ (×0.742 for women).[18] Chronic kidney disease was defined as an estimated glomerular filtration rate <60 mL/min/1.73m.[2]

Definition of AF and follow-up

Standard 12-lead electrocardiograms were obtained from all participants in the supine position. Each record was coded independently using the Minnesota Code by 2 well-trained physicians. Participants were diagnosed with AF if AF (Minnesota Code 8-3-1) or atrial flutter (Minnesota Code 8-3-2) was present (n = 170) on an electrocardiogram from the routine Suita health check-up examination (every 2 years) or if AF was indicated as a present illness by the health check-up examination (n = 46), and hospital medical records (n = 33), and/or death records (n = 4) during follow-up. The end point of the follow-up period for each participant was whichever of the following options occurred first: (i) the date of the first AF event, (ii) date of the last health examination and medical records, and (iii) May 31, 2013 (censored).

Statistical analysis

We examined the association between BP or BMI categories and the risk of incident AF using multivariable-adjusted Cox proportional hazard regressions after adjusting for sex and age in 5-year increments as stratified variables, BMI (underweight, normal weight, and overweight) or BP (normal BP, prehypertension, and hypertension), and other potential confounding factors at the baseline survey: namely, hypercholesterolemia, DM, and current smoking and drinking, respectively (Model 1). For Model 2, further confounding variables were used for cohort groups, chronic kidney disease, histories of stroke, coronary heart disease, chronic heart failure, and premature contractions in addition to those in Model 1 (Model 2). The Cox proportional hazard ratios (HRs) and 95% confidence intervals (95% CI) were fitted to the combination of the BP and BMI categories after adjusting for sex and age in 5-year increments as stratified variables and other potential confounding factors. We tested for interactions term generated by SBP × BMI strata in the Cox model adjusting for Model 2. We tested for interactions between follow-up year and BP or BMI to determine whether the assumption of proportional hazards for prediction of AF was valid. All analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

The baseline characteristics of the study participants grouped according to the SBP and BMI categories are presented in Table 1. At the baseline survey, the participants with systolic hypertension and overweight tend to be older, had higher prevalence of DM and hyperlipidemia, histories of stroke and heart disease, and had a lower frequency of current smoking compared to the participants with normal SBP and underweight, for both men and women. The baseline characteristics according to the 3 cohort groups (original, secondary, and volunteer) are shown in Supplementary Table I, which provides the details of participants' baseline characteristics.

During the 12.8 years of follow-up, 253 incident AF events occurred. There was no interaction between follow-up year and BP for prediction of AF in the primary Cox model, suggesting that the proportional hazards assumption was appropriate. Compared with the normal ranges of BP categories and pulse pressure <40 mm Hg, the risks of incident AF were increased in the participants with systolic and diastolic hypertension, hypertension, and pulse pressure ≥60 mm Hg, respectively adjusting for age and sex, and Models 1 and 2 (Table 2). Systolic hypertension is a risk factor of AF in men (HR = 1.65 and 95% CI = 1.07–2.56) and women (HR = 1.93 and 95% CI = 1.03–3.65, data not shown). After further adjustment by DBP, a significant association was still observed in systolic hypertension (HR = 1.74 and 95% CI = 1.12–2.69). However, the influences of DBP and pulse pressure were attenuated after adjustment for SBP. When antihypertensive medication users were classified into their BP levels, the risks of AF according to BP categories were similar (Model 2-adjusted HRs and 95% CI in SBP: 1.72 and 1.20–2.48 for systolic hypertension, data not shown).

Compared with the normal weight participants, the adjusted risks of incident AF were increased in the overweight participants after adjustment for age and sex, in Models 1 and 2, and even after adjustment for both SBP and DBP (Table 3).

After adjustment in Model 2, each 1-unit increase in SBP, DBP, pulse pressure, and BMI were associated with increases in the risk of AF. Among BP variables, after the further adjustment by BPs, only SBP was observed to increase the risk of AF (Table 4). The results in men and women separately were weak due to the small sample sizes, but were marginally or statistically significant (Supplementary Table II). After the adjustment by Model 2 plus SBP and DBP, a 5% increased risk of incident AF by 1 kg/m^2 increase in BMI was revealed.

Compared with SBP < 120 mm Hg and normal weight, the adjusted HRs (95% CIs) of incident AF in the SBP = 120–139 mm Hg participants with overweight and the systolic hypertension participants with normal weight or overweight were 1.72 (1.01–2.91), 1.66 (1.10–2.50), and 2.31 (1.47–3.65),

Table 1. Baseline characteristics according to categories of systolic blood pressure and body mass index

	Systolic BP categories[a]			Body mass index categories[b]		
	Normal SBP	Systolic prehypertension	Systolic hypertension	Underweight	Normal weight	Overweight
Number, *n*	2,697	2,201	2,008	548	4,960	1,398
Sex (men, %)	42.3	50.7	50.3	36.2	46.8	51.9
Age, year	49.3±12.3	56.9±11.7	63.5±9.6	56.2±14.8	55.6±12.7	56.7±12.0
Systolic BP, mm Hg	107.1±7.8	128.8±5.7	153.2±16.8	119.4±23.1	126.3±21.1	134.6±21.3
Diastolic BP, mm Hg	69.0±7.8	79.9±8.3	87.8±11.7	71.4±11.9	77.2±11.6	83.2±12.0
Body mass index, kg/m^2	21.7±2.8	22.8±2.9	23.4±3.3	17.4±1.0	21.9±1.7	27.1±2.1
Hypertension, %	0.3	10.9	100.0	20.0	29.7	48.1
Diabetes mellitus, %	2.6	5.0	8.3	3.1	4.1	8.9
Hyperlipidemia, %	28.8	40.5	44.9	36.5	36.4	44.4
Chronic kidney disease, %	6	9	13	9	8	10
Current smoking, %	31.7	27.9	23.7	30.1	28.4	26.4
Current drinking, %	49.7	53.1	49.5	41.6	51.5	51.6
History of stroke, %	0.4	1.0	3.5	1.6	1.4	2.0
History of heart disease, %	0.7	2.1	4.0	1.3	2.1	2.7
Premature contractions, %[c]	1.6	2.3	3.6	3.6	2.3	2.2

Abbreviation: SBP, systolic blood pressure.

[a]Normal SBP, SBP < 120 mm Hg; systolic prehypertension, SBP = 120–139 mm Hg; systolic hypertension, SBP ≥ 140 mm Hg and/or antihypertensive drug users.

[b]Body mass index was categorized by the following criteria: underweight, <18.5 kg/m^2; normal weight, 18.5 to <25 kg/m^2; and overweight, ≥25 kg/m^2.

[c]Premature contractions consist of premature atrial and/or ventricular contractions without atrial fibrillation/flatter at the baseline.

Table 2. Multivariable-adjusted hazard ratios (95% confidence intervals) of incident atrial fibrillation according to the various blood pressure categories

SBP	Normal SBP	Systolic prehypertension	Systolic hypertension	Trend *P*
Person years	37,548	28,607	22,503	
Cases	53	83	117	
Incidence/1,000 person-year	1.41	2.90	5.20	
Age- and sex-adjusted	1	1.34 (0.94–1.91)	1.90 (1.34–2.69)	<0.001
Model 1 adjusted	1	1.31 (0.92–1.87)	1.80 (1.26–2.57)	<0.001
Model 2 adjusted	1	1.29 (0.91–1.85)	1.74 (1.22–2.49)	0.002
Model 2 and DBP adjusted	1	1.29 (0.88–1.90)	1.74 (1.12–2.69)	0.010

DBP	Normal DBP	Diastolic prehypertension	Diastolic hypertension	Trend *P*
Person years	48,459	20,950	19,249	
Cases	98	63	92	
Incidence/1,000 person-year	2.02	3.01	4.78	
Age- and sex-adjusted	1	1.22 (0.88–1.68)	1.64 (1.23–2.20)	<0.001
Model 1 adjusted	1	1.18 (0.85–1.64)	1.51 (1.11–2.05)	0.008
Model 2 adjusted	1	1.16 (0.84–1.61)	1.47 (1.08–1.99)	0.014
Model 2 and SBP adjusted	1	1.03 (0.73–1.46)	1.14 (0.77–1.69)	0.513

Blood pressure category	Normal BP	Prehypertension	Hypertension	Trend *P*
Person years	33,751	29,093	25,814	
Cases	50	81	122	
Incidence/1,000 person-year	1.48	2.78	4.73	
Age- and sex-adjusted	1	1.25 (0.87–1.80)	1.70 (1.20–2.40)	0.002
Model 1 adjusted	1	1.22 (0.84–1.75)	1.58 (1.11–2.26)	0.008
Model 2 adjusted	1	1.20 (0.83–1.73)	1.53 (1.07–2.19)	0.016

Pulse pressure	<40 mm Hg	40–59 mm Hg	≥60 mm Hg	Trend *P*
Person years	27,639	44,938	16,053	
Cases	41	121	90	
Incidence/1,000 person-year	1.48	2.69	5.61	
Age- and sex-adjusted	1	1.25 (0.87–1.81)	1.78 (1.19–2.67)	0.003
Model 1 adjusted	1	1.29 (0.89–1.86)	1.78 (1.18–2.67)	0.004
Model 2 adjusted	1	1.29 (0.90–1.87)	1.75 (1.17–2.64)	0.005
Model 2 and SBP adjusted	1	1.14 (0.76–1.70)	1.28 (0.73–2.25)	0.399

Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure.
Model 1: Adjusted by age, sex, body mass index, hypercholesterolemia and diabetes, current smoking and drinking status.
Model 2: Adjusted by Model 1 factors, cohort groups, chronic kidney disease, and histories of stroke, coronary heart disease, chronic heart failure, and premature contractions.

respectively (*P* for interaction between SBP and BMI = 0.04, Figure 1).

DISCUSSION

Our findings indicated that systolic and/or diastolic hypertension, higher pulse pressure, and overweight are risk factors for incident AF. Among these various components of BPs, only systolic hypertension was an independent predictor of incident AF after further adjustment. Overweight also remained a significant factor after further adjustment by both SBP and DBP. Interaction of SBP and BMI with risk of incident AF was observed. Hence, to our knowledge, this is the first positive association on the interaction of SBP and BMI with risk of incident AF in a general population.

In the Women's Health Study, high-normal SBP and DBP were associated with incident AF,[5] and after further

Table 3. Multivariable-adjusted hazard ratios (95% confidence intervals) of incident atrial fibrillation according to body mass index categories

Body mass index category	Underweight	Normal weight	Overweight	Trend *P*
Person years	6,631	64,641	17,385	
Cases	16	166	71	
Incidence/1,000 person-year	2.41	2.57	4.08	
Age- and sex-adjusted	0.98 (0.58–1.65)	1	1.43 (1.08–1.90)	0.023
Model 1 adjusted	1.02 (0.61–1.73)	1	1.35 (1.02–1.80)	0.075
Model 2 adjusted	1.02 (0.60–1.72)	1	1.35 (1.01–1.80)	0.081
Model 2 and systolic and diastolic BPs adjusted	1.05 (0.62–1.78)	1	1.34 (1.01–1.79)	0.096

Abbreviation: BP, blood pressure.

Model 1: Adjusted by age, sex, normal BP, prehypertension, hypertension, hypercholesterolemia and diabetes, current smoking and drinking status.

Model 2: See Table 2 footnote.

Table 4. Cox proportional hazards models for various types of blood pressure and body mass index as predictors of development of atrial fibrillation

Blood pressure component	Units	Age-adjusted HRs (95% CI)	Model 1-adjusted HRs (95% CI)	Model 2-adjusted HRs (95% CI)	Model 3-adjusted HRs (95% CI)
Systolic BP	per 20 mm Hg	1.25 (1.11–1.39)	1.22 (1.08–1.37)	1.22 (1.08–1.37)	1.24 (1.06–1.47)[a]
Diastolic BP	per 10 mm Hg	1.15 (1.04–1.28)	1.11 (1.00–1.24)	1.10 (0.99–1.24)	0.97 (0.84–1.12)[b]
Pulse pressure	per 10 mm Hg	1.13 (1.04–1.22)	1.13 (1.04–1.22)	1.12 (1.04–1.22)	1.03 (0.89–1.19)[b]
BMI	per 1 kg/m^2	1.27 (1.02–1.11)	1.06 (1.01–1.10)	1.05 (1.01–1.10)	1.05 (1.01–1.10)

Abbreviations: BMI, body mass index; BP, blood pressure; CIs, confidence intervals; HR, hazard ratio.

Model 1: age, sex, (body mass index or BP categories (normal BP, prehypertension, and hypertension)), smoking, drinking, hyperlipidemia, diabetes mellitus, and impaired fasting glucose.

Model 2: See Table 2 footnote.

Model 3: [a]Model 2 and diastolic BP adjustment; [b]Model 2 and systolic BP adjustment.

adjustment of both SBP and DBP, systolic hypertension was still associated with incident AF, but DBP was attenuated, which was similar to the current study. In a healthy Norwegian cohort study in men, the upper-normal SBP (128–138 mm Hg) and DBP (≥80 mm Hg) had 2- and 1.7-folds increased risk of incident AF, however, did not reveal an association between pulse pressure and incident AF.[6]

The Framingham Heart Study showed that a 20-mm Hg increase in pulse pressure was associated with a 1.26-increased risk of AF.[7] SBP was positively associated with AF, but when DBP was added in this model, only pulse pressure still consistent with AF. In our study, high pulse pressure (≥60 mm Hg) and diastolic hypertension were associated with incident AF. However, after further adjustment for SBP, the associations were attenuated. The difference in these results might be due in part to the body composition (mean BMI = 22.5 and 25.7 kg/m^2 in Japanese and U.S. populations, respectively). Increasing BMI is a strong risk factors for ventricular diastolic dysfunction[19] and increasing pulse pressure.[20] Previous Japanese prospective studies show that SBP is the highest important predictor of incident cardiovascular disease, among the various BP variables, but pulse pressure is less important.[21] Compared with normal BP group, controlled hypertension group does not increased risk of incident AF. However, uncontrolled hypertension is a risk factor for incident AF (HR = 1.53, 95% CI = 1.06~2.21, Supplemental Table III). Controlled hypertension is important to prevent AF. Arterial stiffness, left ventricular hypertrophy, and increased left atrial size are important mediators of the relationship between BP and incident AF.[22]

We found that overweight was linked to a 1.35-fold increased risk of incident AF in this population, and still associated with incident AF after adjustment for both SBP and DBP. In previous cohort studies, the increased risks of incident AF were observed in obese,[11,12,14] and in overweight.[14] The previous cohort studies showed around 4–5% increased risk of each 1-kg/m^2 increase in BMI,[11,12,14] which is compatible with the results of our study. Recently, a Japanese cohort study has shown that obesity was a 2.2-fold increased risk of incident AF,[23] but nonassociations with overweight and hypertension were observed. Due to the low frequency of our obese subjects (1.6%), we could not calculate the risk of AF associated with obesity.

Increasing body weight has an important association with left atrial enlargement,[24] because it causes left ventricular hypertrophy[25] and elevated blood flow volume,[26] and increases the vulnerability of the atrium that triggers

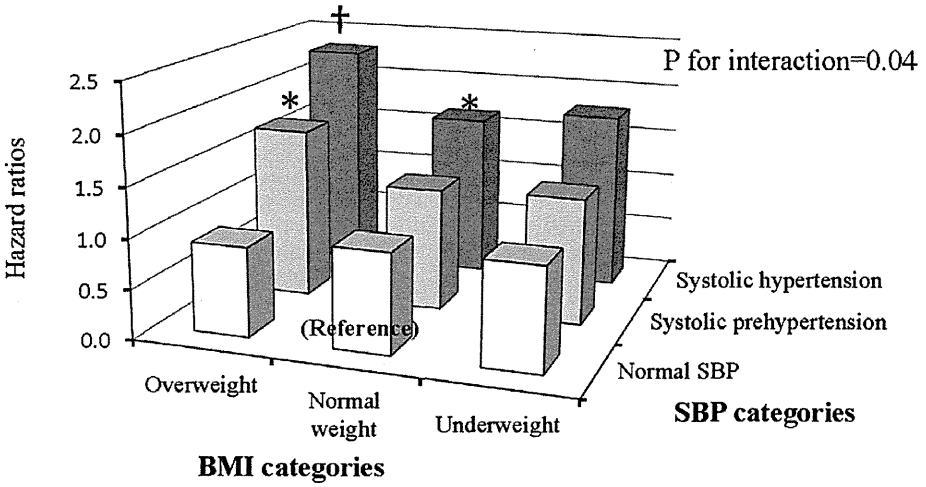

Figure 1. Multivariable-adjusted hazard ratios of incident atrial fibrillation according to combinations of systolic blood pressure (normal SBP and systolic prehypertension and hypertension) and body mass index (underweight, normal weight, and overweight) categories. Data are expressed as multivariable-adjusted hazard ratios adjusted for Model 2. *$P < 0.05$, compared with normal weight and normal SBP as references. †$P < 0.001$, compared with normal weight and normal SBP as a reference. Abbreviations: BMI, body mass index; SBP, systolic blood pressure.

AF.[27] The pathogenesis of increasing body weight is related to increasing BP,[28] and involves metabolic dysregulation,[29] the sympathetic nervous system,[30] renin-angiotensin-aldosterone system activity,[31] and renal sodium reabsorption.[32] In the present study, we observed an interaction between BMI and BP for incident AF. Weight gain has previously been associated with left ventricular hypertrophy[25] and an increased risk of hypertension.[28] Participants with both higher SBP and overweight may experience the mutual exacerbation of left ventricular hypertrophy and hypertension, and consequently, a synergistically increased risk of AF.

Our study has several limitations. The primary limitation is a dilution bias; this study was based on a baseline survey of BP and BMI, which may have led to a misclassification of these risk factors for AF. A previous study has suggested, however, that BP measurements taken on a single day are accurate.[33] Second, we did not perform Holter electrocardiography cyclopedically, even if we perform Holter electrocardiography, we may have missed participants with paroxysmal AF. Third, even with the moderate sample size and 12.8-year follow-up, the numbers of incident AF were limited. A study with a larger sample size is required to validate to the associations. Fourth, 1,408 participants without follow-up were excluded from our baseline data. Compared with the followed-up subjects, the subjects without follow-up had higher percentage of men and smoking and higher prevalence of hypertension, DM, and hyperlipidemia. However, the prevalence of AF at the baseline was not significant in the 2 groups (Supplemental Table IV). Fifth, we did not use follow-up data of BMI and BP, but baseline data. We can predict the future AF by healthy examinations as a baseline in this study. Near future, we will conduct the different study using updated measures to account for changes in BMI over time frame and to characterize the short-term impact that BMI could have on AF risk, and even how weight-loss and -gain influence on BP level. Finally, we did not have types of antihypertensive agents at the baseline.

In conclusion, hypertension and overweight are important risk factors for incident AF. Interaction of these 2 risk factors with risk of incident AF was observed. For AF prevention, it is important to not have these 2 risks. For early detection of AF, it is also important for a person with those 2 risk factors to take an electrocardiogram regularly.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

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DISCLOSURE

The authors declared no conflict of interest.

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Effect of Plasma Fibrinogen, High-Sensitive C-Reactive Protein, and Cigarette Smoking on Carotid Atherosclerosis: The Suita Study

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Background: Few studies have reported on the association between inflammatory markers and atherosclerosis by smoking status. We investigated the effect of plasma levels of fibrinogen and high-sensitive C-reactive protein (hsCRP) on subclinical atherosclerosis stratified by smoking in a general urban population. *Methods:* From participants of the Suita study without a history of cardiovascular diseases, a total of 2502 subjects (805 men, median age 64 years) who underwent carotid ultrasonography were enrolled. Subjects were divided into current smokers (n = 566) and never-smokers. Ex-smokers were not included in the study. Each group was subdivided according to the median levels of markers (plasma fibrinogen [2.99 g/L] and hsCRP [.51 mg/L]) and the smoking amounts. We compare the adjusted maximum and mean intima–media thickness (IMT). *Results:* In men and women, maximum IMT and mean IMT of the high fibrinogen and high hsCRP (Fib(H) CRP(H)) with smoking were thicker than those of the low fibrinogen and low hsCRP (Fib(L)CRP(L)) without smoking, the Fib(L)CRP(L) with smoking, and the Fib(H) CRP(H) without smoking after adjusting for covariates. The Fib(L)CRP(L) with smoking had thicker IMTs than the Fib(L)CRP(L) without smoking. There was a dose-dependent smoking effect on IMT in men. These trends were similar in age 60, 65, and 70. *Conclusions:* Plasma fibrinogen and hsCRP levels were related to multivariate-adjusted IMT, and smoking was associated with IMT in men. The combination of plasma fibrinogen and hsCRP levels could be a potential marker on subclinical carotid atherosclerosis in urban people. **Key Words:** Intima–media thickness—plaque—risk factors—inflammatory marker.

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Introduction

Cigarette smoking is strongly associated with atherothrombotic diseases.[1] In smokers, an inflammatory process or impaired endogenous fibrinolysis caused by endothelial dysfunction is thought to be involved in the pathogenesis of atherothrombotic diseases.[2,3]

Some studies reported that either plasma fibrinogen or C-reactive protein (CRP) was associated with atherosclerosis.[4-6] There are, however, limited studies surveying the combined effect of fibrinogen and CRP on subclinical atherosclerosis.

Therefore, we conducted a cross-sectional population-based study to examine the effect of fibrinogen and high-sensitive CRP (hsCRP) on subclinical carotid atherosclerosis by smoking habits.

Methods

The informed consent was obtained from all participants, and the institutional review board approved this study. The details of the Suita study have been described elsewhere.[7] Briefly, a total of 3655 participants of the Suita study underwent carotid ultrasonography from 2002 to 2003. Participants underwent questioner, which included alcohol intake and smoking habit, evaluation by a medical doctor, blood pressure measurement, and blood test. Of these, 396 were excluded because of histories of coronary artery disease and/or stroke (n = 271) and missing data (n = 125). The remaining 3259 participants consisted of 1936 never-smokers, 757 ex-smokers, and 566 current smokers. Because we did not get the information of the smoking history in detail, the group of ex-smokers could involve subjects with various smoking history such as long years after smoking secession and just given up smoking. Therefore, ex-smokers were not included in the present study to examine the effect of smoking on carotid atherosclerosis.

Blood pressure was measured 3 times at least 1 minute apart by a mercury column sphygmomanometer in a sitting position after at least 5 minutes rest. Systolic and diastolic blood pressures were taken to be the average of the second and the third measurements.[7] A blood test was performed to measure the levels of lipids, glucose, plasma fibrinogen, and hsCRP. Diabetes mellitus was defined as fasting blood glucose of 6.1 mmol/L or more and/or the current use of medications for diabetes mellitus. Total cholesterol and high-density lipoprotein were measured directly. Plasma fibrinogen was measured by the Clauss method. Circulating hsCRP was measured by the latex turbidimetric immunoassay.

The details of the ultrasonic carotid artery examination have been published elsewhere.[8] We used a high-resolution ultrasonography with a 7.5-MHz transducers at .2-mm axial resolution (apparatus, Toshiba SSA-250A, Toshiba, Japan). Carotid atherosclerosis was evaluated by high-resolution ultrasonography with atherosclerotic indexes of intima–media thickness (IMT) in the common carotid artery, carotid artery bulb, and internal and external carotid arteries. Max-IMT was defined as the maximum IMT in the entire scanned area. Mean-IMT was defined as the mean of the IMT of the proximal and distal walls for both sides of the common carotid artery at a point 10 mm proximal to the beginning of the dilation of each carotid artery bulb. Plaques were defined as protrusions with more than 1.1 mm of the IMT in the bilateral carotid arteries in the scanning area. When plaques were detected, examiner calculated the area of stenosis.

Smoking index was used for smoking amount quantification and was defined as the number of cigarettes smoked per day multiplied by years smoked. To investigate the dose–response relationship between IMT and smoking, we categorized the participants into 3 groups by smoking index: never-smokers (smoking index = 0); light-to-moderate smokers (smoking index = 1-799 for men and 1-399 for women); and heavy smokers (smoking index ≥800 for men and 400 for women).

Subjects were divided into 2 groups: never-smokers (n = 1936) and current smokers (n = 566). To clarify the association of max-IMT and mean-IMT with either a coagulation abnormality or an inflammation that was caused by a smoking habit, subjects of each group were subdivided into 4 groups according to the median levels of plasma fibrinogen (2.99 g/L) and hsCRP (.51 mg/L): low fibrinogen and low hsCRP (Fib(L)CRP(L)); high fibrinogen and low hsCRP (Fib(H)CRP(L)); low fibrinogen and high hsCRP (Fib(L)CRP(H)); and high fibrinogen and high hsCRP (Fib(H)CRP(H)).

To investigate the clinical characteristics, univariate tests were performed using the Student *t* test for continuous variables and the chi-square test for categorical variables. Values were shown as a number, percentage, or mean ± standard deviations.

Multiplicity of tests was adjusted in accordance with the following closed procedure using the Bonferroni and Dunnett methods. Analysis of variance was done for the comparison of max-IMT and mean-IMT adjusted for the clinical covariates (age, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, and body mass index) of each subgroup divided by plasma fibrinogen and hsCRP levels in men and women using the Bonferroni method. Only if there were significant differences, adjusted IMTs of never-smokers and current smokers in men and women were compared with that of the Fib(L)CRP(L) in never-smokers with the Dunnett method. Additionally, only if there were significant differences, we compared adjusted IMTs in current smokers with the Fib(L)CRP(L) with the Dunnett method. Finally, only if there were significant differences, adjusted IMTs in Fib(H)CRP(H) with smoking were compared with that without smoking. Adjusted max-IMT and mean-IMT fixed to age 60, 65, and 70 years were calculated.

Table 1. *Baseline characteristics by smoking*

Characteristics	Men			Women		
	Never-smokers	Current smokers	*P* value	Never-smokers	Current smokers	*P* value
Subjects, n	347	458		1589	108	
Age, y	65.2 ± 12.0	61.7 ± 11.3	<.01	63.6 ± 10.9	56.8 ± 10.8	<.01
Current alcohol intake, %	59	72	<.01	26	44	<.01
SBP, mm Hg	130 ± 19	126 ± 18	<.01	129 ± 20	117 ± 16	<.01
DBP, mm Hg	81 ± 9	78 ± 11	<.01	77 ± 10	72 ± 10	<.01
Diabetes mellitus, %	11	12	.49	6	2	.06
Total cholesterol, mmol/L	5.21 ± .80	5.13 ± .87	.13	5.60 ± .80	5.52 ± .85	.34
High-density lipoprotein, mmol/L	1.44 ± .36	1.38 ± .37	.01	1.69 ± .39	1.60 ± .40	.03
Fibrinogen, g/L	2.9 ± 0.6	3.1 ± 0.6	<.01	3.1 ± 0.5	3.0 ± 0.6	.10
log hsCRP, mg/L	6.3 ± 1.1	6.7 ± 1.1	<.01	6.2 ± 1.1	6.2 ± 1.2	.62
Body mass index, kg/m^2	23.6 ± 2.9	23.1 ± 3.1	.04	22.3 ± 3.2	22.0 ± 3.6	.41
Max-IMT, mm	1.42 ± .59	1.43 ± .68	.76	1.20 ± .11	1.15 ± .43	.26
Mean-IMT, mm	.84 ± .13	.83 ± .13	.66	.80 ± .13	.78 ± .12	.02
Presence of plaque, %	76	74	.58	61	49	.01
Stenosis ≥25%, %	16	19	.38	7	8	.67

Abbreviations: DBP, diastolic blood pressure; IMT, intima–media thickness; hsCRP, high-sensitive C-reactive protein; SBP, systolic blood pressure.

Values were shown as a number, percentage, or mean ± SD.

To know the dose dependency of smoking, max-IMT and mean-IMT adjusted to the same covariates earlier mentioned were evaluated by smoking index categories (never-smokers, light-to-moderate smokers, and heavy smokers).

Two-tailed *P* values less than .05 were considered statistically significant. However, *P* values less than .0125 following the Bonferroni method (.05 of 4) was used only in the analysis for adjusted IMTs by plasma fibrinogen and hsCRP levels. All calculations were performed with the JMP 11 software (SAS Institute Inc., Cary, NC).

Results

Baseline Characteristics

The number of enrolled participants was 2502 (805 men, median age 64 years, 566 current smokers). In both male and female current smokers, there was a larger number of younger participants, and current alcohol consumption was involved, and systolic blood pressure, diastolic blood pressure, and high-density lipoprotein were lower than those in never-smokers (Table 1). Male current smokers had higher fibrinogen concentration, higher log hsCRP, and lower body mass index than never-smokers. The mean-IMT and the percentage of plaque presence in female current smokers were lower than that in never-smokers.

Adjusted IMTs by Plasma Fibrinogen and hsCRP Levels

In both men and women and both current smokers and never-smokers, the thickest adjusted max-IMT and mean-IMT group was Fib(H)CRP(H), followed by Fib(H)CRP(L) or Fib(L)CRP(H), and Fib(L)CRP(L) in descending order (Table 2).

Max-IMT and mean-IMT adjusted to age 65 of the Fib(H)CRP(H) with smoking were thicker than those of the Fib(L)CRP(L) without smoking, the Fib(L)CRP(L) with smoking, and the Fib(H)CRP(H) without smoking in both men and women after adjusting for covariates ($P < .0125$). The Fib(L)CRP(L) with smoking had thicker max-IMT and mean-IMT adjusted to age 65 than the Fib(L)CRP(L) without smoking in both sex and smoking status ($P < .0125$). Max-IMT and mean-IMT adjusted to age 60 and 70 had the similar trends to those adjusted to age 65 (Supplemental Table 1).

Effects of Smoking Amounts on IMT

In men, heavy smokers significantly had the thickest max-IMT and mean-IMT, followed by light-to-moderate smokers and never-smokers in descending order (Table 3). In women, max-IMT and mean-IMT of heavy smokers were thicker than that of never-smokers but were not significantly thicker than that of light-to-moderate smokers. These trends were the same in age 60, 65, and 70 (Supplemental Table 2).

Discussion

We have shown 2 findings; first, the combination of plasma fibrinogen and hsCRP levels was related to multivariate adjusted max-IMT and mean-IMT in people (both men and women and current and never-smokers) without known cardiovascular diseases, and second,

Table 2. *IMTs by plasma fibrinogen/hsCRP level, adjusted to age 65 years*

		Men				Women			
		Never-smokers		Current smokers		Never-smokers		Current smokers	
		Fib(L)	Fib(H)	Fib(L)	Fib(H)	Fib(L)	Fib(H)	Fib(L)	Fib(H)
n	CRP(L)	135	37	112	54	536	336	50	15
	CRP(H)	80	95	117	175	188	529	13	30
Max-IMT, mm, mean ± SD	CRP(L)	1.37 ± .07	1.43 ± .09*	1.44 ± .07*	1.49 ± .07*,†	1.18 ± .08	1.24 ± .09*	1.24 ± .07*	1.28 ± .09*
	CRP(H)	1.40 ± .09	1.45 ± .09*	1.46 ± .08*	1.54 ± .08*,†,‡	1.21 ± .08*	1.27 ± .09*	1.25 ± .08	1.32 ± .07*,†,‡
Mean-IMT, mm, mean ± SD	CRP(L)	.82 ± .02	.84 ± .03*	.84 ± .02*	.85 ± .01*,†	.80 ± .02	.81 ± .02*	.81 ± .02*	.82 ± .03*
	CRP(H)	.83 ± .02*	.85 ± .02*	.85 ± .02*,†	.86 ± .02*,†,‡	.81 ± .02*	.83 ± .02*	.82 ± .03*	.84 ± .02*,†,‡

Abbreviations: Fib(L)CRP(L), low fibrinogen and low hsCRP; Fib(H)CRP(L), high fibrinogen and low hsCRP; Fib(L)CRP(H), low fibrinogen and high hsCRP; Fib(H)CRP(H), high fibrinogen and high hsCRP.

*P value <.0125 (Bonferroni) versus never-smoker/Fib(L)CRP(L) of men or women (Dunnett).

†P value <.0125 (Bonferroni) versus current smoker/Fib(L)CRP(L) of men or women (Dunnett).

‡P value <.0125 (Bonferroni) versus never-smoker/Fib(H)CRP(H) of men or women.

there was a dose-dependent smoking effect on IMTs in men. Resemblance among the IMTs adjusted to different ages provided robustness in our results.

Effect of inflammatory markers on IMT disaccorded among studies. Population-based study showed that both fibrinogen and CRP had a negative association with IMT in middle-aged white, Japanese-American, and Japanese.[9] In young healthy adults, IMT was correlated with fibrinogen but not with hsCRP.[10] Meta-analysis of 20 studies of individual progression of carotid intima–media thickness as a surrogate for vascular risk (the PROG-IMT), which enrolled participants without a history of cardiovascular disease, indicated that the number of elevated markers (hsCRP, fibrinogen, and leukocyte count) was related to baseline IMT.[6] In the present study, plasma fibrinogen and hsCRP independently affected max-IMT in men and in women (data not shown), and the combination of these 2 factors revealed these findings as listed in Table 2. We think that the combination of coagulation abnormalities and inflammation would contribute to the development of carotid atherosclerosis associated with cigarette smoking.

Many studies showed that high CRP level was associated with the risk of cardiovascular event, but recent studies suggest that serum CRP level is defined by genotype.[11,12] Studies are limited about genetic effect on CRP in the Japanese population. In 489 Japanese health checkup examinees, there were no significant associations of serum CRP levels with the genotypes of CRP,[13] but a significant association of ischemic stroke with the CRP genotype rs1800947 was found in 152 Japanese patients.[14]

Reduction of plasma fibrinogen, hsCRP, and IMT was reported by increasing nitric oxide production obtained by L-arginine supplementation in the presence of a reductive state by supplementation of *N*-acetylcysteine in hypertensive patients with diabetes mellitus.[15] The Third National Health and Nutrition Examination Survey showed that markers, including fibrinogen, CRP, and other traditional risk factors, improved with a decreased intensity of smoking.[16] These findings suggest that reducing free radical formation, such as cessation of smoking, could play a role in the antiatherosclerotic effects. Quit smoking is a key lifestyle modification to prevent carotid atherosclerosis from a preventive medical point of view.

Some limitations exist in the present study. Some participants might have reported lighter smoking or drinking status than usual. The longitudinal impact remains unclear in the present cross-sectional study but warrants examination. The power to estimate the gender differences would not be adequate because of the small sample size.

In conclusion, our findings suggest that the concomitant of plasma fibrinogen and hsCRP is useful as a potential marker on subclinical carotid atherosclerosis and that smoking is related to IMT in men.

Table 3. *IMTs by smoking index, adjusted to age 65 years*

	Men				Women			
	Never, mean ± SD	Light to moderate, mean ± SD	Heavy, mean ± SD	*P* value	Never	Light to moderate, mean ± SD	Heavy, mean ± SD	*P* value
Max-IMT, mm, mean ± SD	1.41 ± .09	1.47 ± .08*	1.50 ± .08*,†	<.01	1.23 ± .10	1.25 ± .08	1.28 ± .08*	<.01
Mean-IMT, mm, mean ± SD	.83 ± .02	.85 ± .02*	.85 ± .02*,†	<.01	.81 ± .03	.82 ± .03	.83 ± .03*	<.01

*P value <.01 versus never-smokers of men or women.
†P value <.01 versus light-to-moderate smokers of men or women.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2015.06.039.

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Original Article

Risk of Hypercholesterolemia for Cardiovascular Disease and the Population Attributable Fraction in a 24-year Japanese Cohort Study

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Aims: The population-attributable fraction (PAF) is an indicator of the disease burden. In Western countries, the PAF of hypercholesterolemia in cardiovascular disease (CVD) is the highest among that for traditional risk factors; however, data for Asian populations are limited.
Methods: A 24-year cohort study was conducted among 9,209 randomly selected participants who were not taking statins. We estimated the hazard ratio (HR) after adjusting for covariates and PAF associated with the serum total cholesterol (TC) levels in relation to CVD mortality.
Results: The TC level was found to be positively associated with an increased risk of CVD, coronary heart disease (CHD) and cardiac death (CHD plus heart failure), with an HR of 1.08 (95% confidence interval [CI]: 1.00-1.16), 1.33 (95% CI: 1.14-1.55) and 1.21 (95% CI: 1.08-1.35) for a 1-SD increment in the serum TC level, respectively. Similar positive associations between the TC level and both CHD and cardiac death were observed after classifying the patients by age and sex. Furthermore, the highest serum TC level (≥6.72 mmol/L) was positively associated with CVD death, with an HR of 1.76 (95% CI: 1.25-2.47), as well as both CHD death and cardiac death. In contrast, no significant relationships were observed between the serum TC level and stroke. Meanwhile, the PAF for CVD, CHD, and cardiac deaths due to hypercholesterolemia (serum TC level ≥5.69 mmol/L, defined by the Japan Atherosclerosis Society) was 1.7%, 10.6% and 5.6%, respectively.
Conclusions: The estimated PAF of CVD death due to hypercholesterolemia is moderately high, but lower than that for other risk factors, such as hypertension.

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Key words: Total cholesterol, Cardiovascular disease, Population-attributable fraction, Cohort study, NIPPON DATA80

Introduction

Cardiovascular diseases (CVDs), such as coronary heart disease (CHD); are common causes of death in developed countries, including Japan[1], and a high serum total cholesterol (TC) level is an established risk factor for CVD. The population attributable fraction (PAF) is the proportional reduction in mortality that would occur if the exposure to a risk factor were to be reduced to an alternative ideal level, a parameter that can be used in the management of CVD patients. In studies from Western countries, the PAF of CVD mortality due to hypercholesterolemia is highest among that for traditional risk factors[2-4]; however, evidence of this relationship in Asian countries, including Japan, in which the CHD incidence is low, is scarce[5]. Specifically, the effects of a high serum TC level on the health of the general Japanese population in the context of disease burden is unknown.

Several Japanese studies have estimated the PAF of CVD death based on established CVD risk factors[6, 7], such as smoking and hypertension. However, to the best of our knowledge, no observational studies with a long-term follow-up period of >20 years estimating the PAF for CVD death due to hypercholesterolemia have been conducted in Japan or other Asian countries. We previously reported the relationship between TC and CVD based on the findings of the National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged, 1980 (NIPPON DATA80), trial, with follow-up examinations conducted at 14 and 19 years after study initiation (until 1994 and 1999, respectively)[8, 9]. However, we did not calculate the PAF of CVD caused by hypercholesterolemia.

Therefore, in the current study, we investigated the relationship between the serum TC level and mortality due to CVD in the NIPPON DATA80 cohort with a longer follow-up period than that used in the former study[8, 9] and estimated the PAF of death due to CVD attributable to a high serum TC level.

Methods

Population

The subjects in this cohort were also participants of the National Survey on Circulation Disorders 1980. A total of 10,546 residents ≥30 years of age from 300 randomly selected areas in Japan participated in the follow-up study, the NIPPON DATA80, for a participation rate of 76.6% (10,546/13,771). The details of the NIPPON DATA80 study have been previously reported[9-15]. The subjects were followed up until 2004.

Of the 10,546 participants, a total of 1,337 were excluded for the following reasons: a history of CHD or stroke (n=280), missing information (n=186) and the absence of a permanent address, which was needed to link the patient to their vital statistical records (n=871). We analyzed the remaining 9,209 participants (4,029 men and 5,180 women). The data collected from these participants were not influenced by the effects of statins, as these drugs were not available at the time of the survey. There were no significant differences in the mean serum TC level between the participants included in this study and those who did not provide their address.

Endpoint Determination

As previously reported[9-15], we confirmed which participants died in each area using computer matching of the area, sex, date of birth and death of the subject with data obtained from the National Vital Statistics database. Information regarding the cause of death, which was coded according to the Ninth International Classification of Death (ICD-9) until the end of 1994 and the Tenth International Classification of Disease (ICD-10) from 1995 onward, was also obtained from the National Vital Statistics database. ICD-coding was carried out by specialists at the Ministry of Health and Welfare who were independent of the NIPPON DATA research group. The details of classification are described elsewhere[9-11]. We defined all deaths due to CVD (ICD-9: 393-459/ICD-10: I00 to I99), CHD (ICD-9: 410-414/ICD-10: I20 to I25), stroke (ICD-9: 430-438/ICD-10: I60 to I69) or cerebral infarction (ICD-9:433, 434 and 437.8a-8b/ICD-10:I63 and I69.3) as the primary endpoint. Furthermore, in the present study, we defined "cardiac death" as death due to CHD or heart failure (HF, ICD-9: 428/ICD-10: I50) and treated HF as a cause of death among CHD survivors because HF is the final outcome of CHD. The use of the National Vital Statistics data was permitted by the Management and Coordination Agency, Japan. Approval for this study was obtained from the Institutional Review Board of Shiga University of Medical Science (Nos. 12-18, 2000).

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Table 1. Participant characteristics recorded during the baseline survey in 1980, NIPPON DATA80

	Baseline serum total cholesterol level (mmol/L)							
	<4.14	4.14-4.65	4.66-5.17	5.18-5.68	5.69-6.20	6.21-6.71	6.72-	*p*-values*
Women								
No. of participants	951	1183	1142	925	527	275	177	
Age (years, mean ± SD)	44.7 ± 12.9	47.3 ± 13.0	50.6 ± 12.8	53.1 ± 12.9	54.8 ± 12.0	56.3 ± 11.5	57.0 ± 11.7	<0.001
Albumin (g/dL, mean ± SD)	4.3 ± 0.2	4.3 ± 0.2	4.4 ± 0.3	4.4 ± 0.2	4.4 ± 0.2	4.4 ± 0.3	4.4 ± 0.2	<0.001
BMI (kg/m^2, mean ± SD)	22.1 ± 3.1	22.4 ± 3.2	22.8 ± 3.4	23.3 ± 3.5	23.6 ± 3.4	23.8 ± 3.3	24.3 ± 4.0	<0.001
Hypertension (%)	29.0%	35.2%	41.2%	50.7%	59.2%	60.7%	61.6%	<0.001
Diabetes (%)	1.1%	2.0%	1.8%	4.1%	2.8%	6.5%	5.1%	<0.001
Current smoker (%)	7.9%	7.9%	10.1%	9.2%	11.0%	5.5%	9.0%	0.07
Heavy smoker (%) (>20 cigaretts/day)	0.7%	0.3%	0.8%	0.6%	1.5%	0.7%	0.6%	0.29
Daily drinker (%)	3.2%	2.6%	3.0%	2.7%	3.2%	1.8%	2.8%	0.92
Men								
No.of participants	848	999	936	648	354	167	77	
Age (years, mean ± SD)	51.0 ± 14.0	50.0 ± 13.4	49.3 ± 13.1	48.8 ± 12.6	48.9 ± 11.8	50.2 ± 12.2	49.5 ± 10.9	0.04
Albumin (g/dL, mean ± SD)	4.3 ± 0.3	4.4 ± 0.3	4.4 ± 0.3	4.5 ± 0.3	4.5 ± 0.3	4.6 ± 0.3	4.6 ± 0.3	<0.001
BMI (kg/m^2, mean ± SD)	21.6 ± 2.7	22.0 ± 2.8	22.6 ± 2.8	23.2 ± 2.9	23.5 ± 2.7	23.9 ± 2.8	24.2 ± 2.5	<0.001
Hypertension (%)	47.2%	47.5%	52.6%	53.1%	58.5%	58.7%	87.0%	<0.001
Diabetes (%)	3.7%	3.8%	5.4%	6.2%	7.6%	7.8%	10.4%	<0.01
Current smoker (%)	66.9%	66.4%	63.9%	56.3%	59.3%	57.5%	54.5%	<0.001
Heavy smoker (%) (>20 cigaretts/day)	21.1%	24.6%	25.0%	23.3%	30.5%	29.3%	28.6%	0.02
Daily drinker (%)	46.5%	49.4%	48.9%	49.4%	48.3%	36.5%	50.6%	0.02

SD: standard deviation
*Analysis of variance for continuous variables, chi-square test for categorical variables

Baseline Examinations

The baseline surveys were conducted at public health centers using criteria from a standardized manual. Non-fasting blood samples were drawn and centrifuged within 60 minutes of collection and stored at −70℃ until the analyses. As previously reported, the serum TC and albumin levels were analyzed at a single central laboratory (present name: Osaka Medical Center for Health Science and Promotion) using an autoanalyzer (SMA12/60; Technicon, Tarrytown, USA). Since April 1975, the precision and accuracy of the cholesterol measurements obtained in this laboratory have been certified by the CDC-NHLBI Lipid Standardization Program of the Center for Diseases Control and Prevention[16]. Trained research nurses measured the blood pressure of the seated subject using a standard mercury sphygmomanometer on the right arm after five minutes of rest. Hypertension was defined as a systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 mmHg, use of antihypertensive drugs, history of hypertension or any combination of these findings. The serum glucose level was measured using the cupric-neocuproine method. Since the serum glucose level is more commonly measured using the hexokinase method, this parameter was adjusted using the following formula: [0.047× (glucose concentration in mg/dL) − 0.541][17]. Diabetes was defined as a non-fasting serum glucose level of ≥11.1 mmol/L, history of diabetes or both. Height in stocking feet and weight in light clothing were measured. Questionnaires responses regarding smoking and drinking habits and medical history were analyzed by public health nurses.

Statistical Analysis

The serum TC levels were categorized into the following seven categories: <4.14, 4.14-4.65, 4.66-5.16, 5.17-5.68, 5.69-6.20, 6.21-6.71 and ≥6.72 mmol/L. The participants with a serum TC level of 4.14-4.65 mmol/L formed the reference group. These categories were determined based on the results of our previous study, which provided key evidence for the guidelines for the diagnosis and prevention of atherosclerosis and CVD in the Japanese population issued by the Japan Atherosclerosis Society (JAS)[8]. Cox proportional hazard models were used to estimate the relative risk as the hazard ratio (HR) for death due to CVD according to one standard deviation (SD), i.e., a

Table 2. Number of deaths and multivariable-adjusted HRs for CVD, CHD, cardiac death, stroke and cerebral infarction during the 24-year follow-up period

	1SD increment of serum TC (per 0.87 mmol/L increment)	Category of baseline serum TC level (mmol/L)						
		<4.14	4.14-4.65	4.66-5.17	5.18-5.68	5.69-6.20	6.21-6.71	6.72-
No of Persons	9209	1799	2182	2078	1573	881	442	254
Person-Years	193021.5	37099.5	46260.5	43685.5	33025.5	18612.5	9199	5139
CVD								
No deaths	884	152	189	181	169	101	48	44
HR	1.08	1.04	1.00	1.03	1.15	1.06	1.00	1.76
95%CI	1.00-1.16	0.84-1.30	-	0.84-1.26	0.93-1.43	0.83-1.36	0.73-1.39	1.25-2.47
CHD								
No deaths	172	23	34	28	36	24	11	16
HR	1.33	0.87	1.00	0.89	1.42	1.39	1.25	3.52
95%CI	1.14-1.55	0.51-1.48	-	0.54-1.47	0.87-2.31	0.81-2.39	0.62-2.51	1.89-6.57
Cardiac Death								
No deaths	348	52	69	72	68	42	22	23
HR	1.21	0.99	1.00	1.16	1.35	1.25	1.33	2.68
95%CI	1.08-1.35	0.68-1.42	-	0.83-1.62	0.95-1.91	0.84-1.86	0.81-2.17	1.64-4.38
Stroke								
No deaths	411	72	94	81	82	48	18	16
HR	1.01	0.99	1.00	0.90	1.08	1.00	0.74	1.25
95%CI	0.90-1.12	0.72-1.34	-	0.67-1.22	0.79-1.47	0.70-1.43	0.44-1.24	0.73-2.15
Cerebral infarction								
No deaths	241	40	59	45	48	35	8	6
HR	1.02	0.83	1.00	0.84	1.03	1.20	0.55	0.81
95%CI	0.89-1.18	0.55-1.25	-	0.57-1.25	0.69-1.54	0.77-1.85	0.26-1.16	0.35-1.92

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

The HRs were adjusted according to age, sex, the serum albumin level, body mass index, hypertension, diabetes, smoking status and drinking status.

0.87 mmol/L increment in the baseline serum TC level. We evaluated the HRs for total CVD, CHD, cardiac death, stroke and cerebral infarction. In the Cox regression model, age, serum albumin, body mass index (BMI), hypertension, diabetes, smoking status (never-smoker as the reference, ex-smoker, current smoker ≤20 and smoker ≥20 cigarettes/day) and drinking status (never-drinker as the reference, ex-drinker, occasional drinker and daily drinker) were adjusted. We also estimated the HRs according to a Cox model assessing the serum TC level with reference to the seven categories described above. Violation of the proportional hazard assumption was determined using Schoenfeld residuals. Tests for interactions between sex, age (<65 or ≥65 years), hypertension, current smoking and BMI (<25 or ≥25 kg/m^2) were conducted with an interaction term generated by multiplying the continuous serum TC level by the cardiovascular risk factors described above. Tests for interactions were performed for death due to CVD, CHD and cardiac death. In addition, multivariable HRs were calculated following the classification of the subjects into the following groups: age (<65 or ≥65 years), sex, hypertension, current smoking and BMI (<25 or ≥25 kg/m^2).

The PAFs of CVD, CHD and cardiac death were calculated using the formula below[18]:

[Proportion of cases exposed to risk factor × (Adjusted HR-1)/Adjusted HR].

The PAFs for the TC categories ≥5.69 mmol/L (according to the definition of "hypercholesterolemia" provided by the JAS)[19] and ≥6.21 mmol/L (according to the definition provided by the adult treatment panel III by the National Cholesterol Education Program, United States: ATP III) were estimated as the excess death fractions due to a high TC level. We recalculated the adjusted HR for each hypercholesterolemia case in order to estimate the PAFs.

All statistical analyses were performed using the

Table 3. The multivariable-adjusted HRs for CVD, CHD and cardiac death, classified according to age and sex, over the 24-year follow-up period

A. Age

Age <65 years old	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	7713	172219	363	1.09	0.98-1.22	83	1.29	1.04-1.61	132	1.22	1.03-1.46
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	1527	33848	60	1.04	0.75-1.46	12	1.05	0.49-2.23	19	1.04	0.57-1.88
4.14-4.65	1856	41724	79	1.00		16	1.00		26	1.00	
4.66-5.17	1751	39145	65	0.79	0.57-1.10	10	0.60	0.27-1.33	22	0.82	0.46-1.45
5.18-5.68	1302	29144	73	1.20	0.86-1.66	18	1.36	0.68-2.73	26	1.28	0.73-2.25
5.69-6.20	724	16092	48	1.21	0.83-1.75	14	1.54	0.73-3.23	22	1.60	0.89-2.88
6.21-6.71	353	7897	19	0.88	0.52-1.47	5	0.99	0.35-2.79	8	1.06	0.47-2.42
6.72-	200	4369	19	1.63	0.97-2.74	8	3.00	1.23-7.35	9	2.22	1.01-4.89
Age >=65 years old	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	1496	20803	521	1.06	0.96-1.16	89	1.32	1.05-1.65	216	1.17	1.01-1.36
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	272	3252	92	1.13	0.85-1.50	11	0.80	0.37-1.74	33	1.03	0.65-1.65
4.14-4.65	326	4537	110	1.00		18	1.00		43	1.00	
4.66-5.17	327	4541	116	1.25	0.95-1.63	18	1.22	0.63-2.38	50	1.41	0.93-2.14
5.18-5.68	271	3882	96	1.15	0.87-1.54	18	1.46	0.73-2.93	42	1.38	0.88-2.16
5.69-6.20	157	2521	53	0.98	0.70-1.37	10	1.15	0.52-2.55	20	0.98	0.57-1.70
6.21-6.71	89	1302	29	1.16	0.76-1.77	6	1.63	0.63-4.25	14	1.61	0.86-2.99
6.72-	54	770	25	1.92	1.23-3.02	8	3.93	1.62-9.52	14	2.96	1.58-5.57

R version 2.15 software program (R Foundation for Statistical Computing, Vienna, Austria). All confidence intervals (CIs) were estimated at the 95% level, and statistical significance was defined as a p value of <0.05.

Results

The means and prevalence of the baseline characteristics of all subjects in each TC category based on sex are summarized in **Table 1**. The mean serum TC level was 4.88±0.87 mmol/L overall (mean±SD), 4.93±0.88 mmol/L in women and 4.81±0.85 mmol/L in men. The mean age of the subjects in this study was 50.0±13.2 years overall, 50.1±13.3 years in women and 49.7±13.1 years in men. Age, the serum albumin level, BMI and the prevalence of hypertension and diabetes were statistically different in each TC category for both sexes, whereas the proportion of current smokers and drinkers was significantly different among the TC categories only in men.

The total person-years were 193,022 years and the mean follow-up period was 21.0±5.8 years (mean±SD). During the follow-up period, there were 2,566 total deaths (1,365 men and 1,201 women), with 884 deaths due to CVD (34%), including 172 deaths due to CHD (7%), 176 deaths due to heart failure (7%) and 411 deaths due to stroke (16%). The deaths due to stroke also included 241 cerebral infarction-related deaths (9%).

The number of deaths, person-years and multivariable adjusted HRs for the CVD-related deaths according to a 1-SD increment in the serum TC level and the seven TC level categories in all subjects are summarized in **Table 2**. Consequently, a 1-SD increment in the serum TC level was found to be positively associated with an increased risk of CVD death (HR: 1.08, 95% CI: 1.00-1.16). The positive relationships between a 1-SD increment in the serum TC level and an increased risk of CHD death and cardiac death

(Cont Table 3)

B. Sex

Women	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	5180	111018	449	1.06	0.96-1.17	86	1.23	0.99-1.53	187	1.18	1.02-1.37
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	951	20689	56	1.24	0.88-1.75	10	1.05	0.47-2.35	22	1.27	0.73-2.20
4.14-4.65	1183	25878	81	1.00		16	1.00		32	1.00	
4.66-5.17	1142	24431	88	0.94	0.70-1.28	12	0.67	0.32-1.44	35	1.00	0.62-1.62
5.18-5.68	925	19585	100	1.05	0.78-1.41	21	1.19	0.61-2.31	42	1.20	0.75-1.93
5.69-6.20	527	11164	63	1.03	0.74-1.45	12	1.05	0.49-2.25	26	1.20	0.71-2.03
6.21-6.71	275	5752	28	0.92	0.59-1.43	3	0.51	0.15-1.79	12	1.15	0.58-2.27
6.72-	177	3521	33	1.76	1.16-2.67	12	3.48	1.60-7.58	18	2.79	1.54-5.06
Men	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	4029	82004	435	1.08	0.97-1.20	86	1.37	1.11-1.71	161	1.19	1.01-1.40
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	848	16411	96	1.02	0.77-1.35	13	0.91	0.44-1.87	30	0.97	0.59-1.58
4.14-4.65	999	20383	108	1.00		18	1.00		37	1.00	
4.66-5.17	936	19255	93	1.10	0.83-1.46	16	1.07	0.54-2.11	37	1.28	0.81-2.03
5.18-5.68	648	13441	69	1.32	0.97-1.81	15	1.63	0.80-3.32	26	1.52	0.91-2.56
5.69-6.20	354	7449	38	1.09	0.74-1.59	12	1.73	0.81-3.67	16	1.25	0.69-2.29
6.21-6.71	167	3447	20	1.16	0.72-1.89	8	2.41	1.02-5.69	10	1.71	0.84-3.50
6.72-	77	1619	11	1.65	0.88-3.11	4	2.83	0.92-8.71	5	2.14	0.82-5.57

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

The HRs were adjusted based on the following factors:

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

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

were also noted. The HRs for CHD and cardiac death were 1.33 (95% CI: 1.14-1.55) and 1.21 (95% CI: 1.08-1.35), respectively. Alternatively, deaths due to stroke and cerebral infarction were not associated with a 1-SD increment in the serum TC level. In the analyses of the seven TC level categories, we found a positive association between the highest TC level category and an increased risk of CVD death (HR: 1.76, 95% CI: 1.25-2.47). An increased risk of CHD death and cardiac death was also observed in the highest TC level category. The HR was 3.52 (95% CI: 1.89-6.57) for CHD death and 2.68 (95% CI: 1.64-4.38) for cardiac death. Meanwhile, death due to stroke and/or cerebral infarction was not associated with any TC level category. There were no significant violations of the proportional hazard assumption in these models.

The results obtained after classifying the subjects by age and sex are summarized in **Table 3**. The relationship between a 1-SD increment in the serum TC level and death due to CHD and/or cardiac death was very similar to that observed in the overall analysis. Specifically, we showed that a 1-SD increment in the serum TC level was positively associated with cardiac death in both age categories (<65 or ≥65 years) and sexes (women and men). There were no interactions with age or sex in the associations between the TC level and each endpoint, i.e., CVD, CHD and cardiac death. In **Table 4**, we present the multivariable HRs obtained after stratifying the patients by other risk factors, including hypertension, smoking and BMI. The results of these analyses were similar to those of the over analysis and the analyses performed following classification based on age or sex. There were no interactions with hypertension or BMI in the associations

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

A. Hypertension

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

B. Current Smoking

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

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

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