were elderly (aged ≥65 years); (2) approximately half of HF cases showed atrial fibrillation at admission; (3) the prevalence of preserved ejection fraction was significantly higher in females than in males; (4) the incidence of HF was less than 100 per 100,000 person-years; and (5) there was seasonal variation with the onset of HF.

The median age of HF cases as a whole was just under 80 years, with a significantly higher mean age in females than in males. This is comparable to reports in other racial populations.[12–14] Approximately half of the HF patients captured by the present study showed atrial fibrillation at admission. No previous community based study in Japan has reported the prevalence of atrial fibrillation in patients with HF. However, a similar rate has been reported in hospital-based studies.[15,16] There is also evidence of racial variation in the prevalence of atrial fibrillation among patients with HF. Ruo et al have demonstrated that African-Americans had a 50% lower incidence of atrial fibrillation than Caucasians.[17] As incidence rates of atrial fibrillation among Caucasian HF patients have been reported to range from 28 to 42%,[18,19] the prevalence of atrial fibrillation in our patients with HF was somewhat higher than that in other racial populations. However, as atrial fibrillation was prevalent in males and the elderly,[20] sex- and age-adjusted analysis would be essential to determine the racial difference.

Of patients with HF who underwent echocardiography, half of the female cohort showed preserved ejection fraction while only a quarter of males did so. Although there are no previous reports of the incidence of preserved ejection fraction among HF patients in community based Japanese populations, the present value is comparable to that reported from other ethnic populations using the same partition value for left ventricular ejection fraction.[21,22] However, of the potentially eligible patients in the present study, only 65 percent had a documented assessment of left ventricular ejection fraction. This might have resulted in a selection bias. The mean age of patients not undergoing echocardiography was higher than those who did undergo echo examination. As the incidence of preserved ejection fraction is greater in the elderly, this might have been underestimated in the present study.

The incidence of HF in our study community was less than 100 per 100,000 person-years for patients aged 20–65 years. The value rose to approximately 300 per 100,000 person-years in those aged ≥65 years, and approximately 700 per 100,000 person-year in those aged ≥80 years. However, these values are clearly lower than that of published data from the USA and European countries using the same definition of HF.[23–26] The reasons for the low incidence of HF in our population remain unknown on the basis of the present study. However, as the main etiology of HF was recognized as coronary artery disease, 1 reason might be the low prevalence of coronary artery disease in the Japanese population.[27–29] Alternatively, health-care systems differ between countries. Specifically, Japan has a universal health insurance system and most Japanese could visit medical facilities at relatively low cost. In contrast, in the USA, 15% of persons aged under 65 years are uninsured.[30] One may argue, however, that the system for capturing HF cases in the present study might have been incomplete, resulting in the underestimation of incidence. However, we did attempt to retrieve and review all medical charts or discharge summaries from cardiology and internal medicine wards of all hospitals located within the survey district. Moreover, to further reduce the potential for missing cases, the study included several remote teaching hospitals and tertiary referral medical centers located within 100 km of the survey area. This makes it unlikely that a significant number of HF cases would have been lost to the present registry.

Our community based study revealed significant seasonal variation in the onset of new HF as well as acute worsening of the condition. The peak in variation was seen in Winter-Spring compared to Summer-Autumn. A similar seasonal variation has been reported from European countries.[31–33] Although the precise reasons for this variation remain unknown, a potential explanation might be the presence of some other condition with a well-known seasonal variation such as respiratory tract infection, myocardial infarction and ischemia, or high blood pressure. Heart rate and systemic blood pressure have been reported to rise in cold environments, thus increasing cardiac oxygen consumption and cardiac afterload. This, in turn, might increase the onset of HF during the Winter-Spring season in a cold climate.

Despite the advantages afforded by our community based study, several limitations must be considered when the results are interpreted. First, registration was restricted to hospitalized patients so that HF patients treated at an outpatient clinic only might be missing from the registry, resulting in an underestimation of the incidence of HF. However, physicians are less likely to treat a severe HF patient without hospitalization as the Framingham criteria used in this study tended to capture relatively advanced HF. Second, this community based study was limited to the Ninohe district, a rural area in Northeast Japan, and might therefore be restricted in its generalizability to other areas in Japan. However, other ethnicities are very rare in the Japanese population (less than 2%), making the genetic background relatively homogeneous. Moreover, the percentage of the population aged ≥65 years in the survey area is identical to the value predicted for the Japanese population in 2020. In light of this, the present study results might assist our understanding of the future epidemiological setting of HF in this country. Third, as the determination of exact etiology of HF (ie, coronary artery disease, hypertensive heart disease, valvular heart disease, cardiomyopathy, myocarditis) by non-invasive examination in an epidemiological setting has been reported to be difficult,[34] we did not attempt to classify the etiology of HF in this study. Specifically, a predominantly elderly population is unlikely to be systematically examined in detail for possible coronary artery disease by coronary angiography or stress myocardial perfusion imaging. Finally, the present study did not evaluate the prognosis of HF, and thus could not compare the prognosis for Japanese patients with HF to that of other racial populations. Further community based studies using a follow-up design would be needed to answer this question.

In conclusion, when compared with USA and European community based studies of HF, the present HF cohort has shown that: (1) mean age, prevalence of preserved ejection fraction, and seasonal variability were comparable; however, (2) the incidence rate was obviously lower. These epidemiological and clinical characteristics should be taken into consideration when establishing therapeutic and preventive strategies for HF.

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Dietary intake of n-3 polyunsaturated fatty acids is inversely associated with CRP levels, especially among male smokers

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Abstract

Objective: To examine whether dietary intake of n-3 polyunsaturated fatty acid (n-3PUFA) is associated with serum C-reactive protein (CRP) levels with regard to smoking status in the Japanese general population in a cross-sectional study.
Methods and results: A total of 14,191 participants aged 40–69 years were enrolled and divided into quartile groups according to their intake of n-3PUFA. Multivariate-adjusted logarithm-transformed CRP levels were compared between the quartile groups with regard to smoking status after adjusting for traditional risk factors and intake of saturated fatty acids. Adjusted CRP levels were inversely associated with dietary intake of n-3PUFA for both the male subjects and female subjects ($p < 0.05$ for trend). A linear trend was not seen between intake of n-3PUFA and adjusted CRP levels in male nonsmokers. Adjusted CRP level in the lowest quartile group of n-3PUFA was significantly higher than the levels in other groups in male smokers.
Conclusion: Sufficient dietary intake of n-3PUFA may attenuate inflammatory reaction and this effect is more evident among high-risk populations such as male smokers although the small numbers of female ex-smokers and nonsmokers limited statistical power to draw strong conclusions about these groups.

Keywords: n-3 Polyunsaturated fatty acid; C-reactive protein; Smoking; Nutrition; Risk factors

Accumulating evidence indicates that fish consumption is inversely correlated with fatal coronary artery disease and other atherosclerotic cardiovascular diseases (CVDs) [1,2]. However, the underlying biochemical mechanism has not been elucidated and the causal inference remains premature. n-3 Polyunsaturated fatty acids (n-3PUFA), which are contained in marine fish and some plants, play a key role in the prevention of CVD [3]. Possible mechanisms by which n-3PUFA lowers CVD mortality and morbidity are its effects on cardiac arrhythmia, hemodynamics, endothelial function, lipid metabolism, and coagulation function [4–8].

Chronic systemic inflammation plays a pivotal role in the development of atherosclerosis [9]. Traditional risk factors for atherosclerotic CVDs are thought to induce an inflammatory reaction and cause the development of atherosclerosis [9,10]. Cigarette smoking is considered a major factor responsible for the promotion and progression of atherosclerosis

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[11,12], and smoking is also thought to induce inflammatory responses [13–15].

n-3PUFA is a precursor of anti-inflammatory eicosanoids, and the anti-inflammatory effects of n-3PUFA may play a key role in the prevention of CVDs. Favorable effects due to the dietary intake of fish with regard to preventing CVD are also evident, especially in high-risk populations, including smokers [1,2,16,17]. This evidence suggests that the anti-inflammatory effects of n-3PUFA attenuate active inflammation, such as that related to smoking.

However, whether dietary intake of n-3PUFA is associated with inflammatory reactions in the general population has not yet been fully elucidated with regard to smoking status. In this cross-sectional study, we examined the association between dietary intake of n-3PUFA and serum CRP level, and we compared the CRP levels in groups in the Japanese general population categorized by smoking status.

1. Methods

1.1. Study subjects

The Iwate-KENCO Study (Iwate KENpoku COhort Study) is a prospective cohort study of 26,472 Japanese men and women who are undergoing annual health check-ups [15]. The baseline survey was carried out between 2002 and 2004. Of these participants, 14,191 participants aged 40–69 years with serum CRP levels less than 10 mg/L completed anthropometrical examinations, blood tests, self-administered questionnaires regarding lifestyle, and food frequency questionnaires. All participants provided written informed consent prior to participation in the study. The study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the guidelines of the Declaration of Helsinki.

1.2. Measurements

Anthropometrical examinations and blood pressure measurements were performed in a unified manner [15]. Self-administered questionnaires about demographic characteristics, history of cardiovascular disease, drug use, alcohol consumption, and smoking were used to collect individual information. Dietary habits during the previous month were assessed using a brief self-administered diet history questionnaire (BDHQ). This was a 4-page structured questionnaire consisting of three sections: general dietary behavior and major cooking methods, frequency and amount of intake of five alcoholic beverages, and frequency of consumption of 50 selected food and nonalcoholic beverage items. The food and beverage items and the standard portion sizes in the BDHQ were derived primarily from a self-administered diet history questionnaire, a 16-page structured questionnaire consisting of seven sections, which was used previously by one of the authors [18,19]. Estimated dietary intake of 48 food and beverage items, energy, and nutrients were calculated using an ad hoc computer algorithm for the BDHQ, which was based primarily on the Standard Tables of Food Composition in Japan [20]. Pearson's correlation coefficients between intakes assessed using the BDHQ and 16-day semi-weighed dietary records in 92 men and 92 women were 0.24 and 0.26 for energy, 0.34 and 0.33 for cholesterol, 0.50 and 0.55 for fat, 0.55 and 0.60 for saturated fatty acid, 0.50 and 0.57 for monounsaturated fatty acid, and 0.38 and 0.40 for polyunsaturated fatty acid (energy density values), respectively (unpublished observations, Sasaki, 2004). In addition, the intake of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) assessed using the BDHQ was significantly and positively correlated with serum concentrations of EPA + DHA: Pearson's correlation coefficients were 0.37 ($p < 0.001$) in 91 men and 0.31 ($p < 0.01$) in 91 women (unpublished observations, Sasaki, 2004).

Serum levels of CRP were determined by the latex-enhanced immunonephelometric method (Dade Behring Diagnostics, Germany) using a threshold of 0.1 mg/L. In this estimation, CRP values under the minimum detectable level were treated as 0.1 mg/L. Methods for measuring total cholesterol (TC) levels, triglyceride (TG) levels, high-density lipoprotein cholesterol (HDLC) levels, low-density lipoprotein cholesterol (LDLC) levels, plasma glucose levels, and glycosylated hemoglobin (HbA_{1c}) levels were previously described in detail [15].

1.3. Classification and definition

The male and female subjects were divided into groups according to their smoking status (current smokers, ex-smokers, and nonsmokers). To examine the extent to which dietary intake of n-3PUFA affects serum lipid levels and CRP levels, we divided the male and female subjects into quartile groups according to their dietary intake of n-3PUFA. Several studies have shown that alcohol intake [21] and exercise [22] are associated with serum CRP levels. Regular drinking was defined as drinking 5 days or more per week, and regular exercise was defined as exercising (at least 60 min) 8 days or more per month.

1.4. Statistical analysis

Student's t-test was used to test for differences in several parameters between two groups. A chi square test was used to compare frequencies between categories. Comparisons of skewed data were performed using a Mann–Whitney U test. To determine confounding factors that could affect the association between dietary intake of n-3PUFA and serum CRP levels, sex-specific multiple linear regression analyses were performed using natural logarithm-transformed CRP (ln CRP) as a dependent variable and smoking status patterns (current smoking and past smoking), regular drinking, regular exercise, age, BMI, SBP, intake of saturated fatty acid,

intake of n-6PUFA, intake of n-3PUFA, HbA_{1c} level, HDLC level, and LDLC level as independent variables.

After adjusting for factors (those significantly related to ln CRP levels in multiple regression analysis), adjusted CRP levels (expressed as geometric means) of the quartile groups were compared using analysis of covariance (ANCOVA). Adjusted CRP levels were also compared between quartile groups according to intake of long-chain n-3PUFA (EPA + DHA) or according to intake of alpha linolenic acid (ALA). Multiple comparisons were performed using Bonferroni's method. Linear trends across quartile groups were confirmed after adjusting for confounding factors both in male subjects and female subjects. Linear trend tests were also performed across quartile groups separately by smoking status. All p values were based on two-sided tests, and p values less than 0.05 were considered statistically significant. The Statistical Package for Social Sciences (SPSS Japan Inc., Tokyo, version 14.0) was used for all analyses.

2. Results

Table 1 shows the demographic, biochemical, lifestyle, and dietary characteristics of the male and female subjects for all smoking status. The proportions of current smokers were 35.5% in the male subjects and 3% in the female subjects. Crude CRP levels in the male subjects were higher than those in the female subjects (mean values: 0.86 in male subjects and 0.71 mg/L in female subjects, $p < 0.05$). Mean dietary intake of n-3PUFA was 4.0 g/day (1.4% of total energy intake) in the male subjects and 3.3 g/day (1.6% of total energy intake) in the female subjects, intake of saturated fatty acid was 15.5 g/day (5.5% of total energy intake) in the male subjects and 13.8 g/day (6.7% of total energy intake) in the female subjects, and the ratio of n-6PUFA to n-3PUFA in the diet was 3.3 in the male subjects and 3.4 in the female subjects. Mean age was higher in nonsmokers than in others both in male and female subjects. The proportion of regular drinkers was higher than that of ex-drinkers or nondrinkers in current smokers both in men and women.

Table 2 shows the demographic, biochemical, and lifestyle characteristics of the subjects by quartile groups created according to the dietary intake of n-3PUFA. Higher intake of n-3PUFA was associated with more advanced of age, higher SBP, lower TG levels, and lower LDLC levels in the male subjects. In the female subjects, a higher intake of n-3PUFA was associated with more advanced age, lower TG levels, and higher HDLC levels. Crude CRP levels in the lowest

Table 1
Demographic, biochemical, lifestyle, and dietary characteristics of the study subjects

	Male subjects			Female subjects		
	Nonsmoker	Ex-smoker	Current smoker	Nonsmoker	Ex-smoker	Current smoker
Subjects (n)	1547	1261	1543	9399	148	293
Age (years)	60.4 (7.2)	59.9 (7.6)	56.6 (8.3)	57.9 (7.7)	24.0 (3.3)	51.3 (7.4)
BMI (kg/m^2)	24.4 (2.9)	24.5 (2.8)	23.7 (2.9)	24.0 (3.3)	24.4 (4.2)	23.4 (3.9)
SBP (mmHg)	129.5 (18.8)	130.2 (18.7)	127.3 (19.6)	123.5 (19.3)	120.0 (19.4)	118.0 (19.1)
TC (mg/dL)	193.8 (32.6)	197.5 (31.7)	192.1 (33.8)	207.0 (32.2)	202.1 (33.8)	205.0 (35.0)
TG (mg/dL)	122.8 (77.5)	137.9 (100.1)	142.2 (95.2)	111.7 (64.4)	114.1 (70.8)	135.9 (155.2)
HDLC (mg/dL)	56.9 (15.2)	56.4 (15.3)	55.4 (15.2)	61.9 (14.3)	65.0 (15.7)	62.7 (15.2)
LDLC (mg/dL)	115.4 (29.0)	118.0 (28.2)	113.6 (31.9)	124.7 (28.9)	117.8 (29.0)	121.0 (32.2)
PG (mg/dL)	112.9 (31.4)	113.4 (33.3)	113.9 (38.9)	105.2 (24.8)	101.0 (20.2)	101.5 (32.4)
HbA_{1C} (%)	5.08 (0.67)	5.15 (0.76)	5.14 (0.77)	5.08 (0.62)	4.98 (0.59)	5.01 (0.71)
CRP (mg/L)	0.75 (1.14)	0.89 (1.24)	0.95 (1.24)	0.71 (1.08)	0.77 (1.26)	0.74 (1.25)
% of drinkers	42.0%	50.5%	58.6%	4.3%	19.6%	18.8%
% of Reg ex	16.9%	20.6%	11.9%	11.5%	13.5%	15.4%
Ex/month	3.68 (8.56)	4.39 (9.29)	2.63 (7.48)	2.30 (6.74)	2.73 (7.05)	3.53 (8.65)
Dietary intake of each variable: expressed as g/day (% of total energy)						
Carbohydrate	358.8 (56.3%)	337.9 (55.0%)	348.3 (54.8%)	260.1 (57.3%)	238.0 (55.6%)	232.2 (55.4%)
Protein	97.4 (15.3%)	93.5 (15.2%)	92.5 (14.5%)	74.2 (16.1%)	65.5 (15.1%)	65.5 (15.2%)
Total fat	65.0 (22.8%)	61.0 (22.1%)	60.1 (21.1%)	53.4 (25.9%)	50.1 (25.5%)	47.4 (24.8%)
SFA	16.3 (5.8%)	15.5 (5.6%)	14.8 (5.2%)	13.8 (6.7%)	13.5 (6.9%)	12.4 (6.5%)
MUFA	21.8 (7.6%)	20.5 (7.4%)	20.4 (7.1%)	17.9 (8.6%)	17.0 (8.6%)	16.1 (8.4%)
PUFA	17.6 (6.2%)	16.4 (5.9%)	16.4 (5.7%)	14.1 (6.8%)	12.8 (6.5%)	12.3 (6.5%)
n-3PUFA	4.2 (1.5%)	3.9 (1.4%)	3.9 (1.4%)	3.3 (1.6%)	2.8 (1.5%)	2.8 (1.5%)
n-6PUFA	13.2 (4.6%)	12.2 (4.4%)	12.3 (4.3%)	10.6 (5.1%)	10.0 (5.1%)	9.5 (5.0%)
EPA + DHA	2.0 (0.7%)	1.8 (0.7%)	1.9 (0.6%)	1.5 (0.7%)	1.1 (0.6%)	1.2 (0.6%)
α linolenic acid	2.2 (0.8%)	2.1 (0.7%)	2.1 (0.7%)	1.8 (0.9%)	1.7 (0.9%)	1.6 (0.8%)
n6/n3 ratio	3.3 (0.9)	3.3 (1.0)	3.3 (1.0)	3.4 (0.9)	3.6 (0.9)	3.6 (1.0)

Data are expressed as means (S.D.s) or percentages. Abbreviations: BMI, body mass index; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; PG, plasma glucose; HbA1c, percentage of glycosylated hemoglobin; CRP, C reactive protein; smokers, current smokers; drinkers, regular drinkers; Reg ex, regular exercise; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n6/n3 ratio, ratio of dietary n-6PUFA to n-3PUFA.

Table 2
Demographic, biochemical, and lifestyle characteristics of the subjects by quartile group (as determined by dietary intake of n-3PUFA)

Q4 groups according to dietary intake of n-3 PUFA (% of total energy)	Q1	Q2	Q3	Q4
	Men (0.15–1.0%) Women (0.24–1.2%)	Men (1.0–1.4%) Women (1.2–1.5%)	Men (1.4–1.7%) Women (1.5–1.9%)	Men (1.7–4.2%) Women (1.9–6.4%)
Male subjects	1088	1087	1088	1088
Age (years)	56.9 (8.6)	58.2 (8.0)	59.5 (7.5)	61.0 (7.0)
BMI (kg/m^2)	24.2 (2.9)	24.1 (2.8)	24.2 (3.0)	24.2 (2.9)
SBP (mmHg)	128.2 (18.9)	129.0 (18.7)	129.1 (19.1)	129.4 (19.8)
TC (mg/dL)	195.7 (33.4)	194.0 (33.4)	195.6 (32.3)	191.8 (32.1)
TG (mg/dL)	144.4 (106)	137.2 (85.6)	134.2 (91.2)	120.7 (78.9)
HDLC (mg/dL)	56.0 (15.2)	55.8 (14.6)	56.9 (15.5)	56.3 (15.6)
LDLC (mg/dL)	116.5 (30.2)	115.7 (30.5)	115.8 (30.0)	113.9 (29.0)
PG (mg/dL)	114.2 (37.8)	111.9 (31.9)	113.0 (31.4)	114.6 (37.4)
HbA_{1C} (%)	5.11 (0.82)	5.10 (0.66)	5.09 (0.63)	5.18 (0.80)
CRP (mg/L)	0.91 (1.25)	0.85 (1.22)	0.84 (1.17)	0.85 (1.19)
Smokers (%)	39.4	37.6	32.8	32.2
Ex-smokers (%)	29.7	28.1	29.4	28.7
Drinkers (%)	54.4	53.4	49.8	43.8
Reg ex (%)	13.2	15.0	18.8	20.6
Female subjects	2459	2460	2460	2461
Age (years)	56.8 (8.1)	56.8 (8.1)	57.7 (7.8)	59.3 (7.1)
BMI (kg/m^2)	24.0 (3.4)	24.0 (3.4)	23.9 (3.3)	24.1 (3.4)
SBP (mmHg)	123.1 (19.3)	123.4 (20.0)	122.6 (18.9)	124.2 (19.1)
TC (mg/dL)	205.6 (32.1)	207.7 (32.5)	206.3 (32.0)	207.9 (32.6)
TG (mg/dL)	116.7 (82.3)	113.5 (65.4)	110.4 (62.3)	109.1 (64.0)
HDLC (mg/dL)	61.4 (14.1)	61.9 (14.5)	62.3 (14.1)	62.4 (14.6)
LDLC (mg/dL)	123.6 (29.2)	125.8 (29.7)	124.1 (28.3)	124.6 (28.8)
PG (mg/dL)	104.8 (26.4)	105.3 (27.3)	104.3 (21.4)	105.7 (24.5)
HBA_{1C} (%)	5.08 (0.67)	5.06 (0.63)	5.06 (0.53)	5.12 (0.65)
CRP (mg/L)	0.74 (1.15)	0.71 (1.10)	0.65 (0.95)	0.75 (1.15)
Smokers (%)	4.1	3.0	2.7	2.1
Ex-smokers (%)	2.0	1.7	1.5	0.9
Drinkers (%)	7.2	4.9	3.9	3.8
Reg ex (%)	9.8	11.0	12.0	13.1

Data are expressed as means (S.D.s) or percentages. Abbreviations are the same as those in Table 1.

Table 3
Standardized regression coefficients by multiple regression analysis predicting logarithm-transformed CRP

	Men (4351)		Women (9840)	
	Standardized coefficient	p value	Standardized coefficient	p value
Age (years)	0.119	<0.001	0.086	<0.001
BMI (kg/m^2)	0.176	<0.001	0.291	<0.001
SBP (mmHg)	0.040	0.008	0.059	<0.001
HDLC (mg/dL)	−0.157	<0.001	−0.131	<0.001
LDLC (mg/dL)	0.057	<0.001	0.043	<0.001
HbA_{1C} (%)	0.084	<0.001	0.091	<0.001
Current smoking	0.149	<0.001	0.013	0.179
Ex-smoking	0.074	<0.001	0.005	0.610
Regular drinking	0.041	0.022	−0.006	0.551
Regular exercise	−0.018	0.216	−0.014	0.138
Carbohydrate intake (%)	−0.017	0.424	−0.037	0.070
SFA intake (%)	0.047	0.014	0.017	0.235
n3 intake (%)	−0.054	0.010	−0.038	0.012
n6 intake (%)	−0.012	0.518	−0.008	0.464

Abbreviations are the same as those in Table 1.

Table 4
Crude means of CRP level and adjusted geometric means of CRP level by groups according to dietary intake of n-3PUFA, by groups according to dietary intake of EPA and DHA or by groups according to dietary intake of α linolenic acid

Dietary intake of n-3 PUFA (% of total energy)	Q1 Men (0.15–1.0%) Women (0.24–1.2%)	Q2 Men (1.0–1.4%) Women (1.2–1.5%)	Q3 Men (1.4–1.7%) Women (1.5–1.9%)	Q4 Men (1.7–4.2%) Women (1.9–6.4%)	Trend *p*
Male participants (*n*)	1088	1089	1089	1088	
CRP (mg/L)	0.91	0.85	0.84	0.85	
Adjusted CRP (mg/L)	0.54 (0.51–0.58)	0.48 (0.46–0.51)	0.48 (0.45–0.51)	0.46 (0.43–0.49)	<0.001
Female participants (*n*)	2461	2461	2461	2461	
CRP (mg/L)	0.74	0.71	0.65	0.75	
Adjusted CRP (mg/L)	0.44 (0.42–0.46)	0.43 (0.41–0.45)	0.41 (0.39–0.43)	0.42 (0.40–0.43)	0.011
Dietary intake of EPA & DHA (% of total energy)	Q1 Men (0.00–0.36%) Women (0.00–0.39%)	Q2 Men (0.36–0.57%) Women (0.39–0.63%)	Q3 Men (0.57–0.85%) Women (0.63–0.91%)	Q4 Men (0.85–3.4%) Women (0.91–4.3%)	Trend *p*
Male participants (*n*)	1088	1089	1089	1088	
CRP (mg/L)	0.89	0.86	0.84	0.85	
Adjusted CRP (mg/L)	0.52 (0.49–0.56)	0.50 (0.47–0.53)	0.48 (0.45–0.51)	0.46 (0.43–0.49)	0.002
Female participants (*n*)	2461	2461	2461	2461	
CRP (mg/L)	0.71	0.70	0.71	0.73	
Adjusted CRP (mg/L)	0.44 (0.42–0.45)	0.43 (0.41–0.44)	0.42 (0.40–0.44)	0.41 (0.40–0.43)	0.044
Dietary intake of α linolenic acid (% of total energy)	Q1 Men (0.13–0.55%) Women (0.18–0.68%)	Q2 Men (0.55–0.72%) Women (0.68–0.85%)	Q3 Men (0.72–0.92%) Women (0.85–1.1%)	Q4 Men (0.92–3.5%) Women (1.1–3.1%)	Trend *p*
Male participants (n)	1088	1089	1089	1088	
CRP (mg/L)	0.93	0.82	0.91	0.80	
Adjusted CRP (mg/L)	0.53 (0.50–0.56)	0.48 (0.46–0.51)	0.49 (0.46–0.52)	0.46 (0.43–0.49)	0.004
Female participants (n)	2461	2461	2461	2461	
CRP (mg/L)	0.76	0.70	0.69	0.70	
Adjusted CRP (mg/L)	0.44 (0.42–0.45)	0.42 (0.41–0.44)	0.42 (0.40–0.43)	0.42 (0.41–0.44)	0.207

Data are expressed as crude means or adjusted geometric means (95% CI). Adjusted geometric means of CRP level for persons aged 60 years with BMI of 24 (kg/m^2), SBP of 128 (mmHg), HDLC of 56.0 (mg/L), LDLC of 117.0 (mg/L) HbA1c of 5.10 (%), intake of saturated fatty acid of 5.5% of total energy, current smoking, ex-smoking, and regular drinking (mean). 95% CI (confidence interval) is based on standard errors from analysis of covariance.

quartile group were higher than those in each of the other three groups for the male subjects ($p < 0.05$, Mann–Whitney U test). On the other hand, there was no difference between mean crude CRP levels in the quartile groups for the female subjects. Higher intake of n-3PUFA was associated with lower percentage of smokers, lower percentage of drinkers, and higher percentage of subjects performing regular exercise.

Table 3 shows the results of multiple linear regression analyses using ln CRP as the dependent variable and smoking status patterns and other factors as independent variables. "Current smoking" and "ex-smoking" were significantly correlated with ln CRP levels in the male subjects but not in the female subjects. Age, BMI, systolic blood pressure, levels of HDLC, LDLC, and HbA1c, intake of SFA, and intake of n-3PUFA were related to ln CRP level in both sexes. Regular drinking was also correlated with ln CRP level, but regular exercise was not associated with ln CRP level. The high levels of correlation among the explanatory variables produce challenges for statistical modelling to ensure the results are not artifacts of collinearity. We also performed multiple regression analyses using the products of pairs of explanatory variables as independent variables to adjust for interactions between explanatory variables. The results were unchanged even after adjusting for interactions between explanatory variables (data not shown).

Non-adjusted and adjusted geometric mean levels of CRP in the quartile groups according to intake of n-3PUFA, according to intake of long-chain n-3PUFA, or according to intake of ALA are shown in Table 4. Multiple comparisons showed significant difference only between the Q1 category and Q4 category according to intake of n-3PUFA (0.54 vs. 0.46, $p < 0.05$) in male subjects. Linear trends across quartile groups according to intake of n-3PUFA or according to intake of long-chain n-3PUFA existed both in male and female subjects. The higher the intake of n-3PUFA was, the lower adjusted CRP level was. The higher the intake of long-chain PUFA was, the lower the adjusted CRP level was. A linear trend across quartile groups according to intake of ALA existed only in male subjects. In female subjects, slightly elevated CRP levels in Q1 and equal levels in Q2, Q3, and Q4 categories were observed.

Table 5 shows adjusted geometric means of CRP level in the quartile groups separately by smoking status. Linear trends across quartile groups were shown in both smokers and ex-smokers in the male subjects. Multiple comparisons showed significant differences in CRP levels between the Q1 and Q2 categories, between the Q1 and Q3 categories, and between the Q1 and Q4 categories in male smokers (0.54 vs. 0.48, 0.48, 0.48, or 0.46, $p < 0.05$). The difference between CRP levels in the Q1 and Q4 categories was also significant in male ex-smokers. A significant difference was not

Table 5
Adjusted geometric means of CRP level in the quartile groups according to dietary intake of n-3PUFA separately by smoking status

Dietary intake of n-3 PUFA (% of total energy)		Q1 Men (0.15-1.0%) Women (0.24-1.2%)	Q2 Men (1.0-1.4%) Women (1.2-1.5%)	Q3 Men (1.4-1.7%) Women (1.5-1.9%)	Q4 Men (1.7-4.2%) Women (1.9-6.4%)	trend p
Male nonsmokers	(n)	336	374	411	426	
Adjusted CRP	(mg/L)	0.43 (0.38-0.48)	0.42 (0.38-0.46)	0.42 (0.38-0.46)	0.39 (0.35-0.43)	0.232
Male ex-smokers	(n)	323	306	320	312	
Adjusted CRP	(mg/L)	0.57 (0.51-0.64)	0.48 (0.43-0.54)	0.47 (0.42-0.52)	0.46 (0.41-0.51)	0.009
			$p < 0.05$			
Male smokers	(n)	429	407	357	350	
Adjusted CRP	(mg/L)	0.54 (0.51-0.58)	0.48 (0.46-0.51)	0.48 (0.45-0.51)	0.46 (0.43-0.49)	0.029
			$p < 0.05$			
				$p < 0.05$		
					$p < 0.05$	
Female nonsmokers	(n)	2,310	2,343	2,358	2,388	
Adjusted CRP	(mg/L)	0.44 (0.42-0.46)	0.43 (0.41-0.44)	0.41 (0.39-0.42)	0.41 (0.39-0.43)	0.005
Female ex-smokers	(n)	48	43	36	21	
Adjusted CRP	(mg/L)	0.43 (0.31-0.60)	0.47 (0.32-0.67)	0.52 (0.36-0.77)	0.68 (0.42-1.09)	0.097
Female smokers	(n)	101	74	66	52	
Adjusted CRP	(mg/L)	0.56 (0.44-0.71)	0.48 (0.37-0.63)	0.41 (0.31-0.55)	0.58 (0.43-0.79)	0.605

Data are expressed as adjusted geometric means (95% CI). Adjusted geometric means of CRP level for persons aged 60 years with BMI of 24 (kg/m^2), SBP of 128 (mmHg), HDLC of 56.0 (mg/L), LDLC of 117.0 (mg/L) HbA1c of 5.10 (%), intake of saturated fatty acid of 5.5% of total energy, and regular drinking (mean). 95% CI (confidence interval) is based on standard errors from analysis of covariance. p values were determined by analysis of covariance. Multiple comparisons were performed using Bonferroni's method.

found in CRP levels between the groups in male nonsmokers. Although differences in CRP levels between groups were small, a significant linear trend across the quartiles was found in female nonsmokers.

3. Discussion

The main findings of this study were (1) adjusted CRP levels were inversely associated with dietary intake of n-3PUFA or dietary intake of long-chain n-3PUFA for both the male subjects and female subjects, (2) the inverse relationship between adjusted CRP levels and dietary intake of n-3PUFA was more evident in smokers than in nonsmokers in the male subjects, and (3) male smokers taking a low dose of n-3PUFA (in the lowest quartile group) had significantly higher levels of adjusted CRP than those in other male subjects.

Although a linear trend between dietary intake of n-3PUFA and serum CRP levels exists, we also plotted the adjusted geometric mean CRP levels in 20 equally partitioned subgroups according to their intake of n-3PUFA in order to show a non-linear effect between CRP levels and intake of n-3PUFA. An interpolation curve obtained by using spline function showed a steep descent between categories 2 and 3 and a gradual descent between C3 and C20. The spline curve suggests that a cutoff point exists at the C3 point (i.e., 0.91% of energy) and this point possibly means n-3PUFA requirement to maintain attenuated inflammatory reaction (see additional figure as supplementary appendix).

This study also revealed gender-based differences in CRP levels. However, when subjects were limited to nonsmokers, the adjusted CRP levels were almost the same in the male and female subjects (0.42 mg/L in male nonsmokers vs. 0.44 mg/L in female nonsmokers). Considering the low rate of smoking among women and the high rate of smoking among men in this study, the sex-based difference in adjusted CRP levels is probably due to the difference in smoking prevalence between men and women.

The results of this study suggest that activated inflammation is independently attenuated by high-dose dietary intake of n-3PUFA, especially in male smokers. Since n-3PUFAs are precursors of anti-inflammatory eicosanoids (such as prostaglandin I3, prostaglandin E3, thromboxane A3, and leukotriene B5), n-3PUFAs are hypothesized to attenuate the inflammatory response [23,24]. Several studies have been carried out to determine the associations between dietary intake of n-3PUFA (or fish) and levels of inflammatory markers. Pischon et al. showed that dietary intake of n-3PUFA was inversely associated with plasma levels of soluble tumor necrosis factor (TNF) receptors and somewhat less with C-reactive protein in healthy men and women [23,25]. Ciubotaru et al. showed that dietary fish oil reduces levels of C-reactive protein and interleukin-6 in postmenopausal women undergoing hormone replacement therapy (HRT) [26]. These studies suggest that intake of n-3PUFA (or fish) decreases the levels of inflammatory cytokines and CRP levels in healthy subjects. The effect of n-3PUFA intake on serum CRP levels is more evident in high-risk subjects, such as women undergoing HRT. Some studies showed that both CRP levels and other inflammation-related markers were influenced by intake of n-3PUFA. However, we did not measure the levels of interleukins, TNF-alfa, or other inflammation-related agents such as matrix metalloproteinase or macrophage colony-stimulating factor, and we therefore can not discuss the possibility of effects on different inflammation-related pathways due to n-3PUFA.

Rodriguez et al. showed that the favorable effects of fish consumption were more evident in male smokers than that in male nonsmokers in Japanese Americans living in Hawaii [17]. Notably, the apparent preventive effects of fish consumption on cardiac events in their study and the apparent anti-inflammatory effect of dietary intake of n-3PUFA in our study are commonly observed in male smokers. Although the underlying biochemical mechanism was not discussed in their report, the anti-inflammatory effects of n-3PUFA are thought to contribute to the mechanisms that decrease cardiac events in heavy-smoking males.

Several limitations to our study should be noted. The cross-sectional design of the present study tolerates uncertainty of causal relationships. A single instance of blood sampling may be susceptible to short-term variation. Because determination of dietary variables, including fatty acid, was based on a self-administered food frequency questionnaire, information on dietary variables might have been overestimated or underestimated. Socio-economical status (SES) is one of the important confounding factors. However, there was little information about SES in this study and this is one of the limitations. Exercise habit is also one of the important factors that are associated with CRP levels. We performed regression analysis using exercise-related variables and other factors as explanatory variables in three ways: regular exercise, times per month, and quartile categories. There was no relationship between exercise habit and CRP levels in any of the three patterns. We thought that information about exercise in this study was not sufficient for adjusting for confounding effects. The small numbers of female ex-smokers (1.5%) and female nonsmokers (3%) limited statistical power to draw strong conclusions about these groups. The small number of individuals who consumed small amount of n-3PUFA was also one of the limitations.

Despite the lack of causal relationships, based on our findings and those of others, it is reasonable to conclude that sufficient dietary intake of n-3PUFA may attenuate inflammatory reaction and that this effect is more evident in high-risk populations such as male smokers.

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M.O. had full access to all data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

Appendix A

A.1. Members of Iwate-KENCO Study

Chairman: Akira Okayama (The First Institute of Health Service, Japan Anti-Tuberculosis Association, Tokyo)

Principal investigators: Akira Ogawa, Motoyuki Nakamura, YasuoTerayama, Kazuyoshi Itai, Toshiyuki Onoda, Masaki Ohsawa, Kozo Tanno, Kiyomi Sakata, Yuki Yoshida (Iwate Medical University, Morioka), Mitsumasa Tazawa (Morioka Public Health Care Center), Kazuko Kawamura (Iwate Health Service Association), Toru Kuribayashi (Iwate University, Morioka)

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2008.01.008.

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Serum C-reactive protein levels can be used to predict future ischemic stroke and mortality in Japanese men from the general population

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ABSTRACT

Background: High C-reactive protein (CRP) levels have been reported to be associated with an increased risk of atherosclerotic cardiovascular events. The relationship of CRP levels to the risk of cerebrovascular events in the Japanese population, which has a lower prevalence of coronary artery disease and a lower CRP level than Western populations, has not been fully clarified. The present study examined the predictive value of serum high sensitivity CRP (hs-CRP) levels for future cerebrovascular events and mortality in the general Japanese population.

Methods: The subjects for this community-based, prospective cohort study were recruited from the general population (n = 7901, male only, mean age = 64.0 years). Serum hs-CRP levels and cardiovascular risk factors were determined at baseline. The mean follow-up period was 2.7 years. After excluding subjects with a cardiovascular history, the relationships between hs-CRP levels and cerebrovascular events and mortality were assessed.

Results: During follow-up, 130 participants had a first stroke (95 ischemic strokes), and 161 participants died. The hs-CRP tertile level was a significant predictor for a first ischemic stroke (3rd tertile, HR = 1.77: 95% CI, 1.04–3.03, compared with the 1st tertile), after adjustment for age and classical cardiovascular risk factors. Similar trends were observed for the prediction of all-cause mortality (3rd tertile, HR = 2.26: 95% CI, 1.49–3.42, compared with the 1st tertile).

Conclusion: CRP levels can be used to predict future ischemic stroke and mortality in Japanese men from the general population, independently from traditional cardiovascular risk factors.

1. Introduction

The degree of systemic inflammation that is represented by elevated high sensitivity C-reactive protein (hs-CRP) levels has been associated with an increased risk of cardiovascular events in studies conducted in the United States and Europe [1–3]. In the prospective Physicians' Health Study (PHS), elevated hs-CRP levels were associated with an approximately twofold increase in the risk of stroke [1].

We previously reported that, in apparently healthy males living in Japan, hs-CRP levels were closely associated with atherosclerotic changes as measured by carotid plaque formation [4]. Thus, the extent of inflammation may reflect the propensity of atherosclerotic lesions to precipitate clinical vascular events. However, the serum hs-CRP levels of the general Japanese population have been reported to be lower than those of other ethnic groups [5,6]. One must clarify whether associations between a future risk of cerebrovascular diseases and elevated hs-CRP levels also exist in a population that has a relatively lower hs-CRP level. Only one study has reported the association between hs-CRP and ischemic stroke in a rural area of Japan [7]. Therefore, we evaluated the ability of hs-CRP levels to predict future cerebrovascular events and mortality in a larger cohort of the general Japanese population.

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2. Methods

2.1. Study subjects

The study subjects were recruited from the community-dwelling population living in the Ninohe, Kuji, and Miyako districts of Iwate in northern Japan (the Iwate-Kenpoku Cohort study). This study was conducted as part of a government-sponsored, multiphasic health checkup program aimed at the general population. Between April 2002 and January 2005, invitations to participate in this health checkup program were issued by government offices in 17 rural municipalities located in these districts; 26,469 individuals (9161 males) took part in the program and agreed to join the present study. Of these, 25,925 subjects (8957 males) had hs-CRP measurements. Subjects aged over 80 years (280 males) and those under 40 years (300 males), as well as those with a history of cardiovascular disease or stroke (527 males), were excluded. Thus, the data of 7901 males (mean age, 64.0 ± 9.7 years) were analyzed. Baseline clinical examinations included a standard 12-lead electrocardiogram, and a self-reported questionnaire was administered to document subjects' medical history and lifestyle. Hospital inpatients, persons who could not walk independently, and persons with recent inflammatory conditions, such as major trauma, surgery, or obvious acute infectious disease, were not included in the present study.

The study was approved by our institutional ethics committee, and all of the participants provided their written informed consent.

2.2. Risk factor definitions

The presence of baseline cardiovascular risk factors, including hypertension, diabetes mellitus, hypercholesterolemia, obesity, and smoking, was determined. Hypertension was defined as at least one of: systolic blood pressure ≥140 mmHg; a diastolic blood pressure ≥90 mmHg; or current antihypertensive therapy. Diabetes mellitus was defined as a history of a random blood glucose level ≥200 mg/dL or an HbA1c level ≥6.5% or current anti-diabetic therapy. Dyslipidemia was defined as a total cholesterol level ≥240 mg/dL or high density lipoprotein cholesterol level <40 mg/dL or current cholesterol-lowering therapy. Obesity was defined as a body mass index ≥25.0 kg/m^2. The estimated glomerular filtration rate (eGFR) was calculated using the modified equation of the Modification of Diet in Renal Disease (MDRD) study [8].

An electrocardiogram was not done in 225 males (2.8%). Body height or body weight was missing in 10 males, and blood pressure data were missing in 2 males. These participants were considered to have no risk factors such as atrial fibrillation, obesity, or hypertension if they had no history of atrial fibrillation or hypertension.

2.3. Blood samples and hs-CRP measurement

Blood samples were collected from an antecubital vein. The samples were collected into vacuum tubes containing EDTA or a serum separator gel (CRP, lipids). After sampling, tubes were stored immediately in an icebox and centrifuged at 1500 × g for 10 min within 8 h of collection. Aliquots of serum were stored at −20 °C, and routine hematology and biochemistry tests, including hs-CRP, were done within a few days after blood sampling. hs-CRP levels were determined using a highly sensitive immunonephelometric method with a coefficient of variation <5% (N Latex CRP, Dade Behring). The detection limit of CRP assay is 0.1 mg/L, and cases with levels below the limit of detection were considered as 0.1 mg/L.

2.4. Outcome measures

In this cohort study, the primary endpoint was all-cause death, as well as any non-fatal cardiovascular events, such as myocardial infarction, cerebral infarction, or other strokes. The dates of death and move-out were confirmed by the investigators reviewing population-register sheets in each local government. Persons who were known to be alive at the end of follow-up and those who had moved away from the study area were treated as censored cases.

Stroke events were identified by accessing the Iwate prefecture stroke registration program, which included the entire area where the subjects lived; details of this registry have been described previously [9]. Since 1991, the stroke registration program has been coordinated by the Iwate prefecture government and the Iwate Medical Association; the medical records of all medical facilities within the survey area are verified to ensure complete capture of all data. Incidents of acute myocardial infarction were identified by accessing data from the Northern Iwate Heart Disease Registry Consortium, which has been collecting data since 2002. The registration of acute myocardial infarction and sudden death was based on the criteria of the MONICA study [10]. To verify the accuracy of the data, a physician or trained research nurse visited and checked the medical records of the referral hospitals.

Females were excluded from the analysis due to a low incidence of ischemic stroke events (59 events in 15,457 females; 0.4%). For the same reason, coronary heart disease events (non-fatal myocardial infarction, 34 events in 7901 males; 0.4%) were also not analyzed.

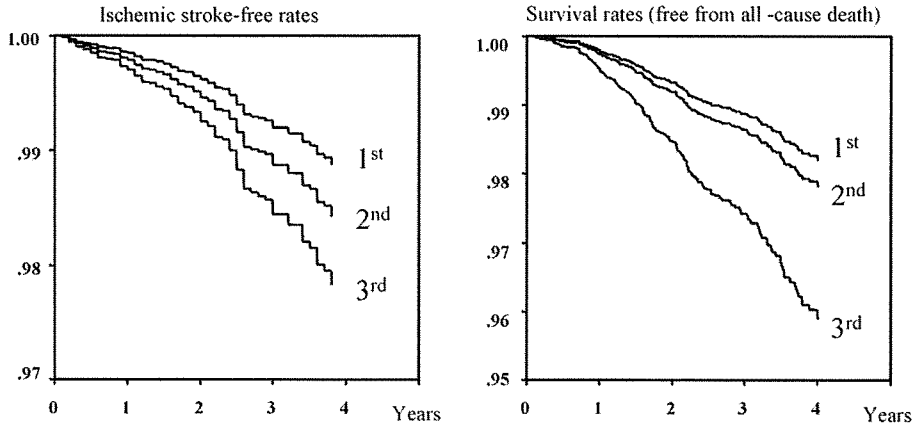

Fig. 1. Cumulative ischemic stroke-free rates and survival rates by age-adjusted Cox regression model for hs-CRP tertiles.

Table 1
Baseline clinical characteristics of all subjects with and without endpoints

	Ischemic stroke			All-cause death		
	(−)	(+)	p	(−)	(+)	p
No. of subjects	7806	95		7740	161	
Age (years)	63.9 ± 9.7	69.6 ± 7.2	<0.001	63.9 ± 9.7	69.8 ± 7.8	<0.001
Body mass index (kg/m^2)	23.9 ± 2.9	23.6 ± 3.0	0.20	24.0 ± 2.9	22.9 ± 3.0	<0.001
Systolic blood pressure (mmHg)	131 ± 19	139 ± 20	<0.001	131 ± 19	132 ± 20	0.31
Diastolic blood pressure (mmHg)	79 ± 11	80 ± 11	0.31	79 ± 11	76 ± 10	0.006
Hemoglobin A1c (%)	5.15 ± 0.74	5.28 ± 0.83	0.09	5.15 ± 0.74	5.30 ± 0.98	0.052
Serum creatinine (mg/dL)	0.82 ± 0.20	0.85 ± 0.16	0.15	0.82 ± 0.19	0.88 ± 0.42	0.18
eGFR (mL/min/1.73 m^2)	73.4 ± 15.1	69.2 ± 13.5	0.006	73.4 ± 15.0	70.2 ± 18.0	0.004
Uric acid (mg/dL)	5.73 ± 1.35	5.45 ± 1.51	0.038	5.72 ± 1.35	5.95 ± 1.59	0.16
Total cholesterol (mg/dL)	191 ± 32	188 ± 35	0.15	192 ± 32	181 ± 35	0.001
Triglyceride (mg/dL)	126 ± 84	123 ± 85	0.41	126 ± 84	121 ± 81	0.39
LDL-cholesterol (mg/dL)	114 ± 29	112 ± 31	0.25	114 ± 29	107 ± 32	0.023
HDL-cholesterol (mg/dL)	56 ± 15	56 ± 16	0.90	56 ± 15	53 ± 17	0.002
hs-CRP (mg/L)	0.54	0.80	<0.001	0.55	1.07	<0.001
Hypertension (%)	45.6	67.4	<0.001	45.7	54.0	0.038
Diabetes mellitus (%)	7.7	11.6	0.17	7.6	14.3	0.004
Dyslipidemia (%)	21.6	18.9	0.61	21.4	26.1	0.17
Obesity (%)	33.3	33.7	0.91	33.5	26.1	0.052
Atrial fibrillation (%)	2.6	15.8	<0.001	2.7	6.2	0.013
Current/past smoking (%)	62.2	75.8	0.007	62.2	68.9	0.085

hs-CRP, high sensitivity C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR: estimated glomerular filtration rate.
Log-transformed values were used for comparisons of CRP levels.
Data are shown as mean ± S.D. hs-CRP are shown as geometric mean.

2.5. Statistical analysis

The cumulative survival curves (free of ischemic stroke or free of all-cause death) by hs-CRP tertile levels were determined according to the age-adjusted Cox model (Fig. 1). The proportionality assumptions of the hazard by hs-CRP tertile were verified by log minus log curves. To determine the relative risks for each hs-CRP tertile level, multivariate Cox proportional hazard models were used. Age and known cardiovascular risk factors were used, and age (10-year increase), systolic blood pressure, total cholesterol, high density lipoprotein cholesterol, uric acid, estimated glomerular filtration rate, body mass index, smoking, and presence of diabetes were forced into the multivariate adjusted model. One rural community (n = 728) was excluded from multivariate analysis because of missing data for serum uric acid, and cases having other missing data as random phenomena were also excluded. This multivariate analysis was finally performed for 7127 subjects. The results are expressed as the hazard ratio (HR) and the corresponding 95% confidence interval (CI). The analyses were performed using the SPSS statistical package, version 11.0.

3. Results

The mean follow-up period was 2.7 years. During follow-up, 130 subjects (1.6%) had a first stroke. Of these, 95 (1.2%) had an ischemic stroke; 161 (2.0%) died due to any cause; and 34 (0.4%) had a new onset, non-fatal myocardial infarction (MI). All of the non-ischemic strokes were the result of intracerebral or subarachnoid hemorrhages.

Baseline characteristics of the participants with and without ischemic stroke or all-cause death are shown in Table 1. Age, systolic and diastolic blood pressures, serum creatinine level, the prevalence of hypertension, atrial fibrillation, and smoking were higher in those with ischemic stroke than in those without. On the other hand, eGFR was lower in those with ischemic stroke than in those without. Similar results were obtained with respect to all-cause death. Some paradoxical relationships were found with respect to the uric acid level in participants with ischemic stroke, and the total cholesterol level and LDL level in those with all-cause death (Table 1).

The median serum hs-CRP level was 0.5 mg/L (95 percentile range: 0.1–4.3 mg/L) in males. This median hs-CRP level was lower than the levels reported in other populations in which hs-CRP levels were measured using the same assay methodology [1–3]. A total of 917 participants showed CRP levels ≤0.1 mg/L. Overall tertile ranges for the hs-CRP levels were: 1st, 0.1–0.3; 2nd, 0.4–0.7; and 3rd, ≥0.8 mg/L. Participants showing CRP > 10.0 mg/L comprised 1.7% of the study population. However, presence of acute infectious condition cannot be judged by CRP level alone, so making a cut-off level for infection is not possible. We therefore ventured to perform analyses without any exclusion criteria for high CRP level.

Table 2
Hazard ratios for first ischemic stroke and all-cause death by hs-CRP tertile levels

	hs-CRP tertile	Incidence of events/no. of subjects, n (%)	Age adjusted hazard ratios (95% CI)	p	Multivariate adjusted hazard ratios (95% CI)[a]	p
Ischemic stroke	1	22/2922 (0.75)	1.00 (reference)		1.00 (reference)	
	2	28/2296 (1.22)	1.41 (0.80–2.48)	0.24	1.30 (0.72–2.33)	0.39
	3	45/2683 (1.68)	1.95 (1.17–3.25)	0.010	1.77 (1.04–3.03)	0.037
All-cause death	1	36/2922 (1.23)	1.00 (reference)		1.00 (reference)	
	2	37/2296 (1.61)	1.22 (0.77–1.93)	0.40	1.15 (0.71–1.88)	0.57
	3	88/2683 (3.28)	2.32 (1.57–3.42)	<0.001	2.26 (1.49–3.42)	<0.001

hs-CRP, high sensitivity C-reactive protein; CI, confidence interval.
[a] Age (10-year increase), systolic blood pressure, total cholesterol, high density lipoprotein cholesterol, uric acid, estimated glomerular filtration rate, body mass index, smoking (current and past), and the presence of diabetes were forced into the Cox regression analysis model.

As shown in Fig. 1, first ischemic stroke-free survival was lower in the higher hs-CRP tertile level when adjusted for age ($p = 0.034$). Similar results were observed for all-cause death-free survival rates ($p < 0.001$). The proportionality assumptions of the hazard by hs-CRP tertiles for these outcomes were satisfied.

In the multivariate Cox regression analysis model adjusted by age, a significantly increased hazard ratio of ischemic stroke was found in the 3rd hs-CRP tertile (HR = 1.95, $p = 0.010$) compared to the 1st hs-CRP tertile. After adjustment for age (10-year increase) and other classical cardiovascular risk factors, such as systolic and diastolic blood pressures, total cholesterol, high density lipoprotein cholesterol, uric acid, eGFR, BMI, smoking (current and past), and the presence of diabetes, the estimated HRs were maintained in the 3rd hs-CRP tertiles (HR = 1.77, $p = 0.037$). The results of the analysis of all-cause death were similar (Table 2). When the presence of atrial fibrillation was included in the multivariate adjusted model for ischemic stroke, the statistical significance of the hs-CRP tertiles declined (3rd hs-CRP tertile, HR = 1.56, $p = 0.10$).

On the other hand, there was no significant association between the hs-CRP tertiles and strokes from any causes (trend $p = 0.19$) in the model adjusted by age and other classical cardiovascular risk factors.

4. Discussion

This prospective cohort study found that baseline serum hs-CRP level was an independent predictor for future ischemic stroke and all-cause mortality in an apparently healthy population. It is interesting that these results were obtained in the Japanese population, which has a lower median hs-CRP level than Western populations [4,5].

The major risk factors for stroke and cardiovascular disease, such as smoking, diabetes, and hypertension, are associated with higher hs-CRP levels [11,12]. These relationships could potentially explain the associations that have been found between hs-CRP level and stroke or mortality. However, since adjustment for such risk factors did not have a large effect on the associations, the traditional risk factors cannot completely explain the relationship between the hs-CRP level and ischemic stroke events.

Carotid plaque formation is a well-established predictor for future ischemic stroke in the general population [13,14]. Our previous data showed a close association between the hs-CRP level and the severity of carotid atherosclerosis as demonstrated by plaque formation in men [6]. The present prospective results show that future stroke events were related to elevated baseline hs-CRP levels; this finding appears to substantiate our previous cross-sectional data. Although a significant association between the hs-CRP level and carotid atherosclerosis was only seen in men in our previous data, the present study could not demonstrate a gender difference for the association between hs-CRP level and the study endpoint.

Atrial fibrillation has been known to be closely related with ischemic stroke due to cardiac thromboembolism. In the present study, the presence of atrial fibrillation was the strongest predictor for ischemic stroke in the same model of multivariate Cox regression analysis with various risk factors (HR = 5.13, 95% CI; 2.82–9.35, $p < 0.001$). It is considered natural that the significance of the hs-CRP tertiles declined when the presence of atrial fibrillation was included in the multivariate adjusted model for ischemic stroke.

In the present cohort, the association between elevated hs-CRP level and stroke was only present when the analysis was limited to the ischemic stroke subtype. In the present study's subjects, all non-ischemic strokes were intracranial hemorrhages, which are known to be caused by rupture of cerebral perforating arteries or an intracranial aneurysm. These pathological conditions develop primarily due to hypertension and small artery hyalinosis [15]. The relationship between cerebral aneurysm and atherosclerosis is not considered to be very strong [16]. Few large-scale prospective cohort studies have addressed stroke subtype.

The major results of our study are completely consistent with the findings of the Hisayama Study [7]. Although the novelty of our study may be lacking, we would raise some unique minor points of difference from the findings and design of the Hisayama Study. First, the presence of atrial fibrillation reduced the predictive power of CRP for ischemic stroke in our study. Second, hs-CRP measurement at baseline was planned a priori and the assay was performed immediately, without long-term cryopreservation. Third, registration of our study population was started in 2002. Compared with the survey in 1988 of the Hisayama study, many new anti-atherosclerotic agents such as strong statins, long-acting anti-hypertensive agents and angiotensin-receptor blockers were likely to be in more frequent use in our study population. Furthermore, our study population comprised older, more obese subjects compared with those in Hisayama Study. All of these characteristics are thought to represent a closer fit with modern Japanese society and community population.

It is possible that the hospital-based follow-up used in the present study was not completely reliable for detecting clinical events. However, an attempt was made to retrieve and view all medical charts from all hospitals and clinics located in the survey area, and the study included several remote teaching hospitals and tertiary referral medical centers. Furthermore, the population of the study district has been stable, with an annual variation rate of only 0.2%. Moreover, participants who developed cerebrovascular and cardiovascular diseases or fatal events had access to only a limited number of medical institutes. Therefore, most major clinical adverse events were likely to have been captured in the present study cohort.

Elevated hs-CRP levels did not reflect the presence of imminent diseases from which stroke events or all-cause deaths had not yet occurred, since the interval between baseline hs-CRP measurement and the ischemic stroke event or death was relatively long: a mean of 1.8 years for ischemic stroke events and a mean of 1.9 years for all-cause death.

Some study limitations should be noted. The results of this study are based on one baseline hs-CRP measurement. Subjects who had recent acute inflammatory conditions, other than a mild "common cold", were not included in the study. However, the subjects were not examined to determine whether any chronic infections, including silent infections such as periodontitis, bladder cystitis, and chronic bronchitis, were present. Chronic infections have been known to have a relationship with carotid atherogenesis [17]. The present study did not assess the use of drugs that can lower hs-CRP levels, such as rennin–angiotensin system inhibitors [18,19], statins [20], and thiazolidinedione [21]. However, it was unlikely that the frequency of the use of these medications was higher in event-free participants. Although imaging was used to verify all stroke cases who visited the hospital with typical symptoms of neurological deficit, patients with events who were not hospitalized or those who were hospitalized at hospitals located outside the area could not be captured in this study design. However, this occurred very infrequently. Finally, this study tested several possible outcomes, including stroke and coronary heart disease in each gender, and then reported the significant findings. The possibility thus remains that chance findings were responsible for the present results.

In conclusion, CRP levels can predict future ischemic stroke and mortality in Japanese males from the general population, independently from traditional cardiovascular risk factors other than atrial fibrillation.

Conflict of interest

The authors report no conflicts of interest.

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Predictive value of plasma B-type natriuretic peptide for ischemic stroke: A community-based longitudinal study

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ABSTRACT

Objective: Structural heart diseases including atrial fibrillation are precursors for ischemic stroke. Plasma B-type natriuretic peptide (BNP) has been reported to be increased in patients with several types of structural heart diseases. However, the predictive value of plasma BNP for ischemic stroke remains unknown. We have studied the predictive ability of plasma BNP for future development of stroke in community dwelling adults.

Methods: Subjects of this community-based study were recruited from the general population (n = 13,466). Plasma BNP levels and cardiovascular risk factors were determined at baseline. The incidence of ischemic stroke in the cohort was identified from regional stroke registry data. A multivariate Cox regression analysis was performed to analyze the relationship between plasma BNP levels and the risk of stroke.

Results: During a mean follow-up period of 2.8 years, 102 participants (65 males, 37 females) experienced a first ischemic stroke. In men, after adjustment for classical cardiovascular risk factors and atrial fibrillation, the hazard ratio (HR) for ischemic stroke was significantly elevated in the highest plasma BNP quartile (HR = 2.38; 95% CI = 1.07–5.29). In women, the relationship between plasma BNP levels and risk of ischemic stroke was of marginal significance after adjusting for the presence or absence of atrial fibrillation (HR = 3.03; 95% CI = 0.84–10.92, P = 0.09).

Conclusion: Elevated plasma BNP levels predict the risk of ischemic stroke within men from the general population.

1. Introduction

B-type natriuretic peptide (BNP) is a cardiac hormone secreted from the myocardium in response to changes in intracardiac volume and pressure [1,2]. Plasma BNP levels are known to be elevated in patients with symptomatic left ventricular systolic dysfunction [3,4] and correlate to New York Heart Association (NYHA) class as well as prognosis [5,6]. In addition, irrespective of the degree of left ventricular dysfunction, plasma BNP levels have been shown to be elevated in patients with various structural heart diseases including previous myocardial infarction, cardiomyopathy, valvular heart disease, hypertensive heart disease, and atrial fibrillation [3,7–13].

These structural heart diseases are precursors not only for heart failure, but also for ischemic stroke, and especially cardioembolic stroke [14]. However, there have been very few reports on the association between plasma BNP levels and the risk of stroke. The Framingham Heart Study [15] has described a 4.9-fold increase in the crude incidence of stroke or transient ischemic attack in the highest tertile of BNP levels compared to the lowest tertile. Kistorp et al. [16] reported that plasma levels of N-amino terminal fragment of the prohormone BNP (NT-proBNP) predicted the risk of stroke or transient ischemic attack, with a 3.6-fold increase in risk of stroke for participants with values above the 80th percentile vs those with values equal to or below the 80th percentile in the general population. However, the association between plasma BNP levels and risk of stroke subtypes remains unclear. The predictive value of plasma BNP measurement for ischemic stroke remains unknown.

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We have studied the predictive ability of plasma BNP for future development of ischemic stroke in community dwelling adults.

2. Methods

2.1. Study population

The Iwate-Kenpoku Cohort (Iwate-KENCO) study was designed to prospectively investigate the risk of cardiovascular diseases including stroke and malignant tumor in the general Japanese adult population as described previously [17,18]. Subjects consisted of residents of the Ninohe, Kuji and Miyako districts in the northern Iwate prefecture, Japan. Between April 2002 and January 2005, 26,469 of these residents (men = 9161, women = 17,308) who were participating voluntarily in a multiphasic health checkup agreed to join the study (original cohort). The baseline survey included routine anthropometrical measurement, blood pressure measurement, ECG, routine laboratory assessment, a self-administered lifestyle questionnaire, and a food-frequency questionnaire. This study protocol was approved by our institutional ethics committee. All participants gave written informed consent.

Of the original cohort living in the Ninohe and Kuji districts (n = 15,927), 15,394 subjects (men = 5288, women = 10,106) underwent BNP measurement (BNP cohort). Subjects were excluded from this cohort on the basis of the following characteristics: age under 40 years (n = 575), history of cardiovascular or cerebrovascular events (n = 507), non-measurement of adjustment factors (n = 846). The final statistical analysis was therefore performed in 13,466 subjects (men = 4527, women = 8939, mean age = 62.7 years).

2.2. Outcome

In this cohort study, the primary endpoint was all-cause death, in addition to any nonfatal cardiovascular events such as myocardial infarction, cerebral infarction, or other strokes. Information about death and emigration was obtained from local government records. Stroke events were identified by accessing the Iwate prefecture stroke registration programme, which has been conducted since 1991 by the Iwate Medical Association with the support of the government of the Iwate prefecture [19]. Registration forms were submitted to the registration office of the Iwate Medical Association by mail when a patient with stroke was discharged from a medical facility. Diagnostic criteria for stroke used by the registry correspond with those published by the World Health Organization, based on a definition of sudden onset of neurological symptoms [20]. For diagnosis of stroke subtypes, computed tomography and/or magnetic resonance imaging were performed within each hospital. In order to improve accuracy of registration, trained research nurses checked medical charts in all hospitals located within these districts. Follow-up was conducted until August 2007.

2.3. Measurement

At the time of baseline survey, participants underwent anthropometrical measurement, ECG, blood pressure measurement, and routine laboratory assessment. In addition, a self-administered questionnaire was used to ascertain family history, symptoms, and lifestyle factors such as smoking habits, alcohol consumption, and exercise habits. A medical history including the status of drugs prescribed for hypertension, hyperlipidemia, diabetes, angina, myocardial infarction, congestive heart failure, and stroke was recorded by trained research staff. Using a 3-channel device, a standard 12-lead ECG was recorded in a supine position. Atrial fibrillation was defined by this 12-lead ECG at the time of baseline survey. Systolic and diastolic blood pressures were determined with an automatic device placed on the right arm of seated subjects who had rested in a sitting position for at least 5 min before measurement. Measurement was performed twice, with the mean value used for statistical analysis. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, and/or current anti-hypertensive therapy. Hyperlipidemia was defined as total cholesterol level ≥240 mg/dL, and/or current lipid lowering therapy. Diabetes was defined as nonfasting glucose concentration ≥200 mg/dL, and/or glycosylated hemoglobin (HbA1c) value ≥6.5%, and/or current anti-diabetic therapy. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Smoking was defined as current smoker. Regular alcohol consumption was defined as drinking alcohol 5 days or more per week. Regular exercise was defined as exercising (at least 60 min) 8 days or more per month.

Venous blood samples for plasma BNP measurement were drawn from the antecubital vein of seated participants with minimal tourniquet use. Samples were collected into vacuum tubes containing ethylenediaminetetraacetic acid sodium. Tubes were stored in an icebox immediately after sampling and were transported to our laboratory within 8 h of collection. These were then centrifuged at 1500 × g for 10 min. After separation, plasma samples were stored frozen at −20 °C until the time of assay. Plasma BNP levels were measured by direct radioimmunoassay using monoclonal antibodies specific for human BNP (ShionoRIA BNP, Shionogi, Japan) within 4 months of separation. The intraassay and interassay coefficients of variation were 5% and 6%, respectively. The lower detection limit of the assay was 0.05 pg/mL. Enzymatic methods were used to measure serum total cholesterol levels, serum creatinine, and blood glucose. HbA1c was measured quantitatively with an HPLC method.

2.4. Statistical analysis

Participants were divided into quartiles according to their baseline plasma BNP levels. Continuous variables were expressed as mean ± SD. Group comparisons were based on the unpaired t-test and multiple group comparisons across BNP quartiles were based on the one-way analysis of variance. Because BNP values were not normally distributed, these were expressed as median and the Mann–Whitney U-test was used for comparison. Categorical parameters were expressed as proportions (percentage) and group comparisons were based on the chi-square test.

The ischemic stroke event free rates according BNP quartiles were estimated using the Kaplan–Meier method, followed by Log-rank test. A multivariate Cox regression analysis was performed to analyze the relationship between plasma BNP levels and risk of stroke. For all models, the hazard ratios were adjusted for age, BMI, blood hemoglobin levels, serum creatinine levels, presence or absence of hypertension, hyperlipidemia, diabetes, smoking, regular alcohol consumption, and regular exercise. The analysis was not adjusted for presence or absence of atrial fibrillation in Model 1 and was adjusted in Model 2. Additional multivariate Cox regression analysis using covariates in Model 1 was performed using 1 SD increments in natural logarithm-transformed BNP values. For the analysis of stroke incidence, person-years were censored at the date of stroke diagnosis, the date of emigration from the study area, the date of death, or the end of the follow-up period, whichever came first. All statistical analysis was performed using SPSS software, version 11.0. A significant difference was defined as $P < 0.05$.

3. Results

Baseline characteristics of participants by sex are shown in Table 1. The mean age of men was higher than that of women. The percentages of hypertension, diabetes, atrial fibrillation, smoking, regular alcohol consumption, regular exercise, and mean values for

Table 1
Comparison of baseline characteristics between men and women.

Characteristic	Men ($N=4527$)	Women ($N=8939$)	P-value
Age (years)	64.1 ± 10.3	62.0 ± 10.0	<0.001
Hypertension (%)	44.4	38.8	<0.001
Hyperlipidemia (%)	10.3	20.3	<0.001
Diabetes (%)	8.0	4.3	<0.001
Body mass index (kg/m^2)	23.9 ± 2.9	24.2 ± 3.4	<0.001
Smoking (%)	33.4	2.5	<0.001
Regular alcohol consumption (%)	47.4	4.2	<0.001
Regular exercise (%)	17.0	10.5	<0.001
Atrial fibrillation (%)	3.0	0.6	<0.001
Hemoglobin (g/dL)	14.6 ± 1.3	13.0 ± 1.1	<0.001
Creatinine (mg/dL)	0.82 ± 0.19	0.63 ± 0.12	<0.001
BNP (median) (pg/mL)	14.8	17.1	<0.001

Continuous variables are expressed as mean ± SD.
Comparison of BNP data are performed using a Mann-Whitney U test.

hemoglobin and serum creatinine were significantly higher in men. The percentage of hyperlipidemia and mean BMI were significantly higher in women. The median value for plasma BNP was higher in women.

Table 2 shows baseline characteristics among the BNP quartiles. In men, mean age and BMI and mean levels of hemoglobin, and serum creatinine were different among the BNP quartiles ($P<0.001$). Although the percentages of hypertension, hyperlipidemia, current smoking, and regular exercise were different ($P<0.001$), the percentages of diabetes and regular alcohol consumption did not differ among the BNP quartiles. In women, although mean age and mean levels of hemoglobin, and serum creatinine were different among the BNP quartiles ($P<0.001$), the mean BMI did not differ among the BNP quartiles. Although the percentages of hypertension, hyperlipidemia, diabetes, current smoking, and regular alcohol consumption were different ($P<0.05$), the percentage undertaking regular exercise did not differ among the BNP quartiles. Subjects with atrial fibrillation were concentrated in the highest BNP quartile in both men and women.

During a mean follow-up period of 2.8 years, 102 participants (65 males, 37 females) had a first ischemic stroke event. Ranges of BNP levels in men and women are shown in Table 2. The crude incidences of ischemic stroke (per 1000 person-years) among BNP quartiles in men and women are shown in Tables 3 and 4. The crude incidence of ischemic stroke in men was 2.76 per 1000 person-years in Q1 (the lowest quartile) and 12.51 per 1000 person-years in Q4 (the highest quartile). The crude incidence of ischemic stroke in women was 0.44 per 1000 person-years in Q1 and 2.95 per 1000 person-years in Q4. The crude incidence of ischemic stroke elevated in the highest quartile in both men and women.

The Kaplan–Meier curves for ischemic stroke event free rates according to BNP quartiles in men and women are shown in Fig. 1. The ischemic stroke event free rates differed significantly among the BNP quartiles in both men and women (men: $P<0.001$; women: $P<0.001$ by log-rank test).

Several studies have demonstrated that blood hemoglobin levels [21], renal function [22] and BMI [23] influence plasma BNP levels. For that reason, after adjustment for classical cardiovascular risk

Table 2
Comparisons of baseline characteristics among BNP quartiles.

BNP quartiles	Q1	Q2	Q3	Q4	P-value
Men					
Number of subjects	1131	1134	1129	1133	
Range of BNP levels (pg/mL)	<6.5	6.5–14.8	14.9–29.9	30.0<	
Age (years)	57.4 ± 10.1	61.9 ± 9.8	66.7 ± 8.3	70.3 ± 7.8	<0.001
Hypertension (%)	33.3	40.0	47.5	56.8	<0.001
Hyperlipidemia (%)	16.5	9.8	7.9	7.1	<0.001
Diabetes (%)	7.6	8.2	8.4	7.6	0.846
Body mass index (kg/m^2)	24.1 ±2.9	24.1 ±2.9	23.8 ± 2.9	23.6 ± 3.0	<0.001
Smoking (%)	40.4	34.9	31.6	26.6	<0.001
Regular alcohol consumption (%)	48.2	45.7	47.3	48.3	0.576
Regular exercise (%)	11.6	17.1	20.0	19.3	<0.001
Atrial fibrillation (%)	0.53	0.00	0.71	10.59	<0.001
Hemoglobin (g/dL)	15.0 ± 1.1	14.8 ± 1.1	14.5 ± 1.2	14.2 ± 1.4	<0.001
Creatinine (mg/dL)	0.80 ± 0.14	0.81 ± 0.16	0.83 ± 0.24	0.84 ± 0.19	<0.001
Women					
Number of subjects	2235	2228	2242	2234	
Range of BNP levels (pg/mL)	<8.9	8.9–17.0	17.1–30.4	30.5<	
Age (years)	57.6 ± 9.5	60.0 ± 9.8	62.8 ± 9.4	67.4 ± 8.7	<0.001
Hypertension (%)	28.6	33.4	40.7	52.4	<0.001
Hyperlipidemia (%)	24.3	20.4	20.2	16.3	<0.001
Diabetes (%)	5.0	3.1	4.1	4.9	0.006
Body mass index (kg/m^2)	24.3 ± 3.4	24.2 ± 3.3	24.0 ± 3.3	24.1 ± 3.5	0.206
Smoking (%)	3.9	2.4	2.2	1.5	<0.001
Regular alcohol consumption (%)	5.0	4.6	3.8	3.4	0.027
Regular exercise (%)	10.2	10.2	10.9	10.9	0.756
Atrial fibrillation (%)	0.09	0.05	0.04	2.24	<0.001
Hemoglobin (g/dL)	13.2 ± 1.1	13.1 ± 1.1	13.0 ± 1.1	12.8 ± 1.1	<0.001
Creatinine (mg/dL)	0.61 ± 0.10	0.63 ± 0.11	0.63 ± 0.10	0.66 ± 0.15	<0.001

Continuous variables are expressed as mean ± SD.

Table 3
The crude incidence and multivariate hazard ratio of ischemic stroke among BNP quartiles in men.

BNP quartiles	Q1	Q2	Q3	Q4	*P* for trend
Observed person-years	3619	3198	3116	3036	
Ischemic stroke					
Crude incidence (/1000 person-years)	2.76	2.19	3.21	12.51	
Multivariate HR (95%CI)					
Model 1	1.0 (ref.)	0.71 (0.27–1.89)	0.85 (0.34–2.12)	2.83 (1.29–6.20)	<0.001
Model 2	1.0 (ref.)	0.71 (0.27–1.88)	0.81 (0.33–2.03)	2.38 (1.07–5.29)	<0.005

For all models, the hazard ratios were adjusted for age, presence or absence of hypertension, hyperlipidemia, diabetes, smoking, regular alcohol consumption, and regular exercise. BMI, blood hemoglobin levels, and serum creatinine levels.
Model 1: The analysis was not adjusted for presence or absence of atrial fibrillation.
Model 2: The analysis was adjusted for presence or absence of atrial fibrillation.

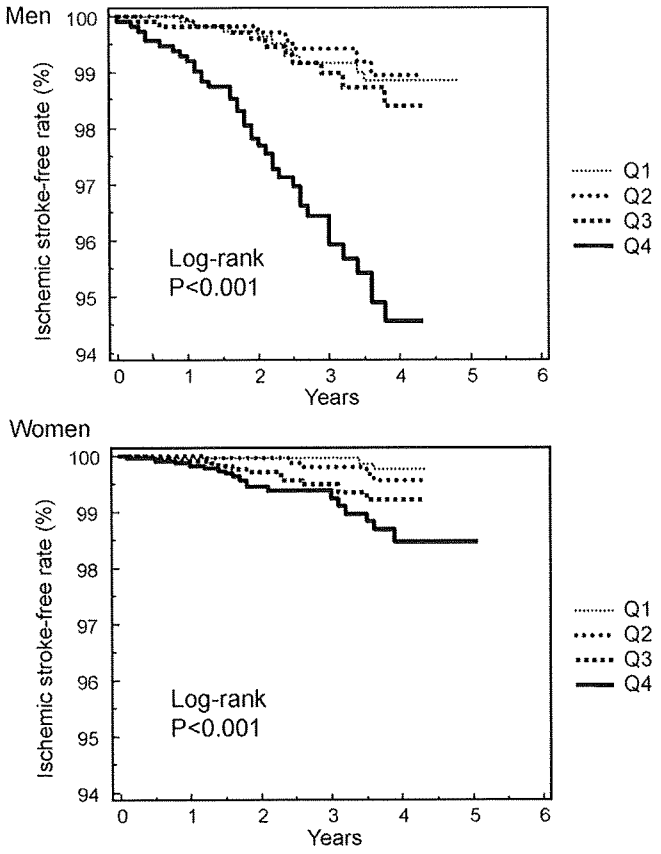

Fig. 1. Kaplan–Meier curves for ischemic stroke event free rate according to BNP quartiles by sex.

factors, blood hemoglobin levels, serum creatinine levels, and BMI, a multivariate Cox regression analysis was performed to analyze the relationship between plasma BNP levels and the risk of stroke. In men, the hazard ratio (HR) obtained from a Cox proportional model for ischemic stroke in the highest BNP quartile was significantly elevated in Model 1 (HR = 2.83; 95% CI = 1.29–6.20; Table 3). After also adjusting for the presence or absence of atrial fibrillation (Model 2), HR in the highest BNP quartile was still significantly elevated (HR = 2.38; 95% CI = 1.07–5.29; Table 3). The risk of incidence of ischemic stroke increased in association with BNP levels ($P < 0.01$). In women, HR for ischemic stroke in the highest BNP quartile was significantly elevated in Model 1 (HR = 3.61; 95% CI = 1.01–12.93; Table 4). After adjusting for the presence or absence of atrial fibrillation (Model 2), the relationship between plasma BNP levels and the risk of ischemic stroke was of marginal significance (HR = 3.03; 95% CI = 0.84–10.92, $P = 0.09$; Table 4).

An additional multivariate Cox regression analysis was performed using 1 SD increments in natural logarithm-transformed BNP values. Elevated plasma BNP levels were associated with an elevated risk of ischemic stroke in both men and women (HR = 1.70; 95% CI = 1.17–2.45 in men: HR = 1.69; 95% CI = 1.04–2.75 in women).

4. Discussion

There have been very few reports on the association between plasma BNP levels and the risk of stroke, [15,16] and the relationship with risk of stroke subtypes therefore remains unclear. The present study suggests that high plasma BNP levels predict the risk of ischemic stroke within the general Japanese population. Ischemic stroke is classified into atherothrombotic infarction, cardiogenic embolic infarction, and lacunar infarction. Several types of structural heart diseases including atrial fibrillation, which are associated with elevated plasma BNP levels, may be an important cause of ischemic stroke, especially cardioembolic stroke. In view of this, elevated plasma BNP levels may be a biomarker for high risk of ischemic stroke.

Cardiac disorders linked with ischemic stroke, especially cardioembolic stroke, are nonvalvular atrial fibrillation, acute

Table 4
The crude incidence and multivariate hazard ratio of ischemic stroke among BNP quartiles in women.

BNP quartiles	Q1	Q2	Q3	Q4	*P* for trend
Observed person-years	6794	6283	6188	6099	
Ischemic stroke					
Crude incidence (/1000 person-years)	0.44	0.80	1.78	2.95	
Multivariate HR (95%CI)					
Model 1	1.0 (ref.)	1.72 (0.41–7.25)	3.07 (0.84–11.16)	3.61 (1.01–12.93)	0.168
Model 2	1.0 (ref.)	1.79 (0.43–7.55)	3.15 (0.87–11.44)	3.03 (0.84–10.92)	0.269

For all models, the hazard ratios were adjusted for age, presence or absence of hypertension, hyperlipidemia, diabetes, smoking, regular alcohol consumption, and regular exercise. BMI, blood hemoglobin levels, and serum creatinine levels.
Model 1: The analysis was not adjusted for presence or absence of atrial fibrillation.
Model 2:The analysis was adjusted for presence or absence of atrial fibrillation.

myocardial infarction, ventricular aneurysm, and valvular heart disease. According to the Cerebral Embolism Task Force [14], nonvalvular atrial fibrillation is the most common cardiac disorder associated with embolic stroke, accounting for 45% of embolic strokes. Several previous studies have suggested that plasma BNP levels were significantly higher in patients with atrial fibrillation than in those without atrial fibrillation [11,13]. The Framingham Heart Study [15] has indicated that higher plasma BNP levels predict risk of atrial fibrillation. It is therefore possible that atrial fibrillation-related high plasma BNP levels are associated with increased risk of ischemic stroke. We therefore analyzed the relationship between plasma BNP levels and risk of ischemic stroke after adjusting for the presence or absence of atrial fibrillation. Even after this adjustment, HR was still significant in men. This suggests that there may be factors other than atrial fibrillation underlying the apparent relationship between plasma BNP levels and risk of ischemic stroke. As the present study did not perform echocardiography as a baseline examination, some subjects may have had asymptomatic structural heart disease (i.e. left ventricular dysfunction, valvular heart disease, or left ventricular hypertrophy) characterized by elevated plasma BNP [12] which would account for the significant relationship between plasma BNP levels and risk of ischemic stroke. This study was therefore unable to show a correlation between plasma BNP levels and risk of stroke independent of the presence of heart disease. However, it is difficult to perform echocardiography routinely for participants in a community-based multiphasic health checkup. A simple blood test for BNP is an ideal approach for selecting males at high risk for ischemic stroke within the general population. In addition, a previous study examining the relationship between traditional and nontraditional risk factors and the incidence of ischemic stroke subtypes has reported that left ventricular hypertrophy increases the risk not only of cardioembolic stroke but also of atherothrombotic stroke [24]. It follows that high plasma BNP levels may be associated with both cardioembolic and atherothrombotic stroke.

The present study has shown a median plasma BNP level of 14.8 pg/mL and the threshold plasma BNP levels associated with elevated risk of ischemic stroke of 30.0 pg/mL in men. The Framingham Heart Study [15] found a median plasma BNP level of 6.2 pg/mL and the threshold plasma BNP levels associated with elevated risk of stroke or transient ischemic attack of 20.0 pg/mL in a community-based male sample. Both studies have shown that excess risk is apparent at plasma BNP levels well below the thresholds currently used to diagnose heart failure [25].

A possible reason for the marginal significance of the relationship between plasma BNP levels and risk of ischemic stroke in women after adjusting for the presence or absence of atrial fibrillation may be the low incidence of stroke in the female cohort. The crude incidences of stroke in women were clearly lower than those in men, and thus, the statistical power to show any relationship between risk and incidence of stroke might be limited in women. As the statistical results concerning the relationship between plasma BNP levels and risk of stroke in women were not so robust, more events should be gathered to investigate the predictive power of plasma BNP with regard to stroke in women.

Although our study was a large, prospective community-based longitudinal study, several limitations must be considered when interpreting the results. Since ECG testing was performed only at the time of baseline survey, paroxysmal atrial fibrillation had not been detected and new incidence of atrial fibrillation was not captured after the baseline survey. Hence the impact of atrial fibrillation on the association between plasma BNP levels and risk of ischemic stroke may not have been accurately estimated. In addition, since the attending physicians participating in the registration survey were not all neurological specialists, the diagnosis of stroke subtypes was occasionally carried out by general physicians. However, since most of the patients registered were diagnosed using computed tomography or magnetic resonance imaging, the differential diagnosis between ischemic stroke and hemorrhagic stroke was made correctly.

In conclusion, this community-based study has shown that elevated plasma BNP levels predict the risk of ischemic stroke within Japanese men from the general population. This suggests that a simple blood test for BNP is an ideal approach for selecting men at high risk for ischemic stroke within the general population.

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