Table 3 – Adjusted HR for type 2 diabetes mellitus according to quintiles of GI, GL, total energy intake, and total fiber intake in 1995 Japanese men

	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)
GI					
n	402	396	401	402	394
Total person-years	1786	1778	1766	1796	1862
Incident cases (n)	18	28	24	29	34
Rate per 1000 person-years	10.1	15.7	13.6	16.1	18.3
Adjusted HR (95% CI) model 1	1.00 (reference)	1.62 (0.89-2.93)	1.50 (0.81-2.77)	1.68 (0.93-3.03)	1.80 (1.01-3.18)
Adjusted HR (95% CI) model 2	1.00 (reference)	1.68 (0.92-3.04)	1.56 (0.84-2.89)	1.73 (0.96-3.13)	1.88 (1.06-3.35)
Adjusted HR (95% CI) model 3	1.00 (reference)	1.71 (0.94-3.10)	1.66 (0.89-3.10)	1.86 (1.01-3.44)	1.96 (1.04-3.67)
GL					
n	400	401	398	400	396
Total person-years	1733	1735	1739	1856	1924
Incident cases (n)	23	26	34	23	27
Rate per 1000 person-years	13.3	15.0	19.5	12.4	14.0
Adjusted HR (95% CI) model 1	1.00 (reference)	1.07 (0.61-1.88)	1.48 (0.87-2.52)	0.95 (0.53-1.70)	0.98 (0.56-1.72)
Adjusted HR (95% CI) model 2	1.00 (reference)	1.14 (0.65-2.02)	1.54 (0.89-2.65)	1.07 (0.58-1.96)	1.23 (0.67-2.28)
Adjusted HR (95% CI) model 3	1.00 (reference)	1.16 (0.66-2.06)	1.56 (0.89-2.71)	1.07 (0.57-1.99)	1.24 (0.65-2.34)
Total energy intake (range, kcal/d)	(<1703)	(1703-1971)	(1972-2246)	(2247-2641)	(>2641)
n	399	399	399	399	399
Total person-years	1790	1776	1748	1758	1917
Incident cases (n)	24	24	32	24	26
Rate per 1000 person-years	13.4	14.6	18.3	14.2	13.6
Adjusted HR (95% CI) model 1	1.00 (reference)	1.13 (0.65-1.96)	1.49 (0.88-2.54)	1.11 (0.63-1.95)	1.00 (0.57-1.74)
Adjusted HR (95% CI) model 2	1.00 (reference)	1.10 (0.63-1.92)	1.44 (0.84-2.48)	1.06 (0.60-1.87)	0.97 (0.55-1.71)
Adjusted HR (95% CI) model 3	1.00 (reference)	1.12 (0.64-1.97)	1.45 (0.84-2.49)	1.07 (0.60-1.91)	0.97 (0.55-1.72)
Total fiber intake (range, g/1000 kcal)	(<3.7)	(3.8-4.5)	(4.6-5.2)	(5.3-6.0)	(>6.0)
n	400	450	391	370	384
Total person-years	1938	2016	1781	1590	1663
Incident cases (n)	35	26	17	23	32
Rate per 1000 person-years	18.1	12.9	9.5	14.5	19.2
Adjusted HR (95% CI) model 1	1.00 (reference)	0.73 (0.44-1.22)	0.56 (0.31-1.01)	0.80 (0.47-1.35)	0.99 (0.61-1.60)
Adjusted HR (95% CI) model 2	1.00 (reference)	0.73 (0.44-1.23)	0.59 (0.32-1.05)	0.83 (0.48-1.43)	0.98 (0.59-1.64)
Adjusted HR (95% CI) model 3	1.00 (reference)	0.72 (0.43-1.21)	0.59 (0.33-1.06)	0.84 (0.49-1.45)	0.99 (0.59-1.66)

Model 1: adjusted for age and BMI; model 2: adjusted for age, BMI, family history of diabetes, smoking, alcohol intake, habitual exercise, and presence of hypertension and hyperlipidemia at baseline; model 3: adjusted for variables used in model 2 and dietary total energy (for the GI, GL, and total fiber intake) and dietary total fiber intake (for the GI, GL, and total energy intake). CI indicates confidence interval.

those previously reported in Japan [10,14] and higher than the values (range, 48-60) reported in US and European studies [15-19]. Furthermore, both obese and lean Asians who have lower B-cell function are at high risk for developing type 2 diabetes mellitus [4-6]. Our study indicates that the high prevalence of type 2 diabetes mellitus in Asian populations may be explained by high-GI diets in people with lower B-cell function. Thus, an evaluation of the risk of type 2 diabetes mellitus in Asian people must consider lifestyle and food intake as well as genetic background.

Individuals at high risk for diabetes are encouraged to increase their dietary fiber intake and to eat foods containing whole grains [41]. The consumption of such foods is associated with decreased dietary GI. However, the use of GI is recommended as an additional method for management of diabetes in an American Diabetes Association position statement [41] and a recommendation of the American Dietetic Association [42] because the effects of lower-GI diets on glucose metabolism were conflicting [42]. In our study, total fiber intake was not associated with the incidence of diabetes. Furthermore, a higher GI was associated with a higher risk for diabetes, despite a lower total energy intake; and there was no association between total energy intake and the incidence of diabetes. The appropriate energy intake of each person is important for maintaining body weight and preventing obesity and diabetes. However, appropriate energy intake is influenced by many factors, including body composition and physical activity. It is difficult to evaluate the association between total energy intake itself with diabetes; and indices of the quality of food intake such as GI, rather than the quantity of food intake, would be more useful for a population approach.

The strengths of this study include a large sample size, foods contributing to the dietary GI that differed from those in US and European populations, and the fact that it was the first study of the relationship between GI and the incidence of diabetes conducted in Japanese men. Moreover, several previous cohort studies used information collected from self-administered questionnaires, whereas our conclusions are based on more reliable data obtained from medical examinations and fasting blood glucose and insulin levels, HOMA-IR, and HOMA-B. In addition, GI and GL were calculated using responses to a validated questionnaire [11]. A limitation of the present study is that the sample included only people who

Table 4 – Incidence and adjusted HRs[a] for type 2 diabetes mellitus according to GI tertiles of BMI, HOMA-IR, and HOMA-B in 1995 Japanese men

	GI tertiles (range)			P for trend[b]
	T1 (<68.0)	T2 (68.0-71.0)	T3 (≥71.1)	
BMI (kg/m^2)				
<22.0				
Incident cases n/N	3/203	11/227	15/206	
Crude rate per 1000 person-years	3.2	10.4	15.1	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	4.09 (1.13-14.9)	5.78 (1.63-20.5)	.005
22.0-24.9				
Incident cases n/N	14/278	14/257	18/272	
Crude rate per 1000 person-years	11.5	12.4	14.4	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.10 (0.52-2.34)	1.20 (0.59-2.44)	.608
≥25.0				
Incident cases n/N	19/196	20/169	19/187	
Crude rate per 1000 person-years	21.9	28.8	22.5	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.41 (0.75-2.66)	1.11 (0.58-2.11)	.719
HOMA-IR tertiles				
<0.85				
Incident cases n/N	4/217	8/207	16/219	
Crude rate per 1000 person-years	4.1	8.5	15.4	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	2.07 (0.61-6.95)	3.67 (1.21-11.2)	.015
0.85-1.43				
Incident cases n/N	10/222	9/232	21/240	
Crude rate per 1000 person-years	10.2	8.6	18.6	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.78 (0.31-1.94)	1.58 (0.73-3.41)	.221
≥1.44				
Incident cases n/N	22/238	28/214	15/206	
Crude rate per 1000 person-years	20.5	31.4	16.3	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.73 (0.98-3.05)	0.83 (0.43-1.62)	.472
HOMA-B tertiles				
<48.4				
Incident cases n/N	16/227	23/230	31/226	
Crude rate per 1000 person-years	16.1	23.0	30.0	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.64 (0.86-3.13)	1.86 (1.01-3.44)	.049
48.4-79.3				
Incident cases n/N	10/218	11/205	12/224	
Crude rate per 1000 person-years	10.3	11.8	11.5	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.34 (0.56-3.20)	1.26 (0.53-3.00)	.600
≥79.4				
Incident cases n/N	10/232	11/218	9/215	
Crude rate per 1000 person-years	9.4	11.6	8.9	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.39 (0.58-3.31)	0.93 (0.37-2.34)	.922

[a] Adjusted for age, BMI, family history of diabetes, smoking, alcohol intake, habitual exercise, and presence of hypertension and hyperlipidemia at baseline.

[b] Linear regression was used for continuous variables based on ordinal variables containing the median value for each GI tertile.

were employed. Poor health may exclude some individuals from working; thus, the prevalence of obesity may be lower in our sample than in the general Japanese population. Another limitation is that we did not measure waist circumference at baseline, which might have provided more information about abdominal fat accumulation and insulin resistance than measuring BMI did. A further limitation of the present study is that we did not determine whether the diabetes mellitus that developed was type 1 or type 2. However, the study participants were middle-aged men; and as the condition was detected in an annual medical checkup, with relatively mild diabetes mellitus being found, it is most likely that the cases were type 2.

In conclusion, our results indicate that dietary GI is associated with the incidence of diabetes in middle-aged Japanese men. Dietary GI and pancreatic B-cell function were independently associated with the incidence of diabetes. Dietary GI is higher and pancreatic B-cell function is lower in Asian people, as compared with Western people; and these may result in a higher prevalence of diabetes in Asian populations. Our findings suggest that a low-GI diet may be beneficial in preventing type 2 diabetes mellitus in Asian people.

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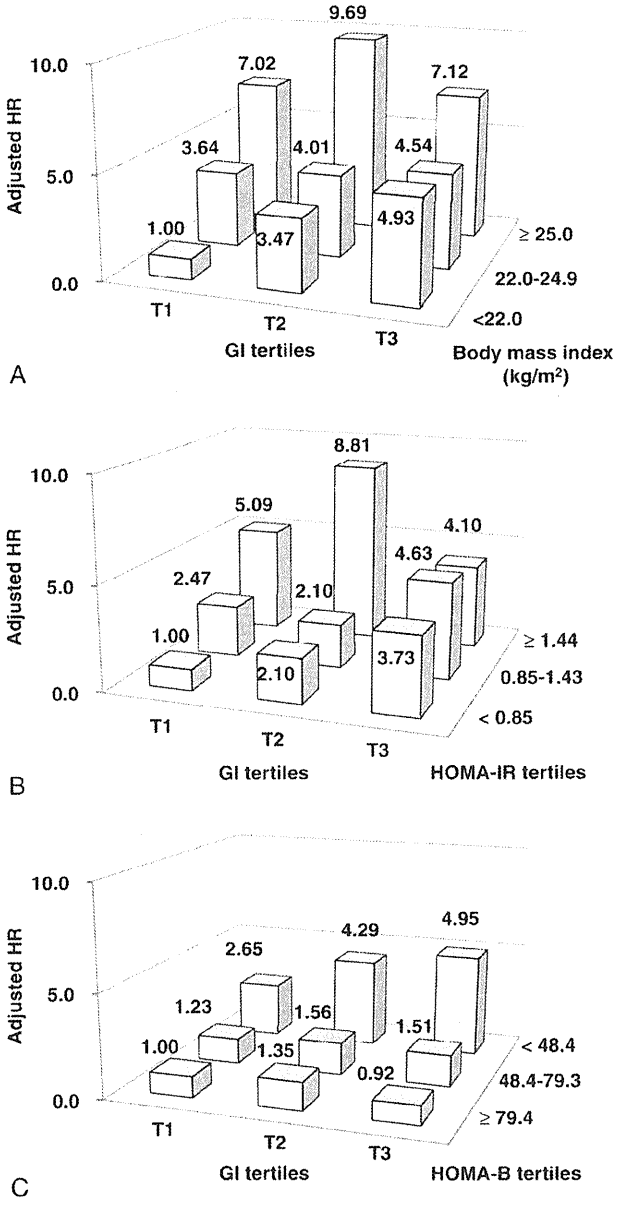

Fig. 1 – Adjusted HRs for type 2 diabetes mellitus by different levels of GI and BMI (A), HOMA-IR (B), and HOMA-B (C) in 1995 Japanese men. The HRs were adjusted for age, BMI, family history of diabetes, smoking, alcohol intake, habitual exercise, and presence of hypertension and hyperlipidemia at baseline.

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Conflict of interest disclosure

None.

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Sex-Specific Threshold Levels of Plasma B-Type Natriuretic Peptide for Prediction of Cardiovascular Event Risk in a Japanese Population Initially Free of Cardiovascular Disease

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Elevated plasma B-type natriuretic peptide (BNP) levels have been reported to be related to a high risk for cardiovascular (CV) disease in the general population. However, there has been no accurate determination of the threshold levels of plasma BNP that indicate an increased potential for the development of general CV events (i.e., heart failure, stroke, and myocardial infarction) and the validity of these levels for predicting CV events compared to classic risk markers. To establish gender-specific thresholds of plasma BNP levels associated with increased risk for CV disease in the general population, baseline BNP levels were determined in community-dwelling adults (n = 13,209, mean age 62 ± 10 years,) and CV events in the cohort were captured prospectively. The cohort was divided by deciles of plasma BNP level in each gender. A Cox proportional-hazards model was used to determine the relative hazard ratios among the deciles. In addition, to compare the utility of plasma BNP to the Framingham 10-year risk score for predicting general CV events, receiver-operating characteristic analysis was performed. During follow-up, CV events were identified in 429 patients in the cohort. Compared to the reference decile level (first to fourth), the hazard ratio was significantly increased from the ninth decile in men (greater than approximately 37 pg/ml) and the highest decile in women (greater than approximately 55 pg/ml). The area under the curve generated on receiver-operating characteristic analysis of plasma BNP testing was comparable to that for the Framingham risk scoring system (0.67 vs 0.68 in men, 0.63 vs 0.68 in women; p = NS for both). In conclusion, within a community-based general population with no CV history, plasma BNP levels higher than defined thresholds show increased risk for general CV events, and the predictive ability for CV events occurring within several years may be comparable to that of an established long-standing risk score. © 2011 Elsevier Inc. All rights reserved. **(Am J Cardiol 2011;108:1564–1569)**

In the present study, we measured plasma B-type natriuretic peptide (BNP) in a large-scale population-based sample of >13,000 men and women. This cohort was followed prospectively for >5 years to ascertain the incidence of cardiovascular (CV) events, including heart failure, stroke, and myocardial infarction. To determine gender-specific threshold levels of plasma BNP, the relation between plasma BNP deciles and risk for CV events was determined. In addition, to validate plasma BNP testing for the prediction of general CV events, its predictive ability was compared to an established CV risk scoring system.

Methods

This study is part of the Iwate-KENCO study, a population-based prospective cohort study to investigate heath status and CV risks in Japanese residents living in the Iwate prefecture, northeast Honsyu, Japan. Details about this cohort are provided elsewhere.[1] In brief, the original cohort (n = 26,469) was recruited from April 2002 and January 2005 in 3 districts (Ninohe, Kuji, and Miyako in the Iwate prefecture). The baseline survey included routine anthropometric measurements, blood pressure measurement, electrocardiography, routine laboratory assessment, and a self-administered lifestyle 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 (96.6%) agreed to provide additional blood samples for the measurement of

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plasma BNP levels, and these are designated as the BNP cohort in the present study. Subjects were excluded from this cohort on the basis of the following characteristics: age <40 years (n = 575) or >80 years (n = 330), serum creatinine level ≥2.0 mg/dl (n = 10), and missing data for blood pressure (n = 3), anthropometrics (n = 47), and/or routine blood tests (n = 4). The final statistical analysis was therefore performed on 13,209 subjects (4,365 men, 8,844 women; mean age 62.1 years).

A follow-up survey assessing mortality, migration, and the incidence of CV events was carried out after the baseline study. We defined CV events as stroke, congestive heart failure, and myocardial infarction requiring hospitalization. Hospital admissions for congestive heart failure and myocardial infarction in the cohort were identified by accessing data from the Northern Iwate Heart Disease Registry Consortium, which has been collecting data since 2002. Heart failure was defined by Framingham criteria,[2] and registration of myocardial infarction was based on criteria used in the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) study.[3] Stroke events were identified by accessing the prefecture stroke registration program conducted by the Iwate Medical Association. Stroke diagnostic criteria in this registry are based on those published by the World Health Organization and defined as the sudden onset of neurologic symptoms.[4] To ensure that nearly all appropriate cases had been identified, researchers in each registration study periodically retrieved and reviewed medical charts and/or discharge summaries for patients admitted to the cardiology, neurology, neurosurgery, and internal medicine wards of all hospitals located within the study district.

In the baseline survey, all participants underwent routine anthropometric measurements, electrocardiography, blood pressure measurements, and laboratory assessments. In addition, a self-administered questionnaire was used to ascertain lifestyle factors such as smoking habits and medical history, including stroke, congestive heart failure, and myocardial infarction. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Systolic and diastolic blood pressure were determined with an automatic device with the subject in a sitting position for ≥5 minutes before measurement. Measurement was performed twice, with the mean value used for statistical analysis. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg and/or current antihypertensive therapy. Diabetes was defined as a nonfasting glucose concentration ≥200 mg/dl, and/or a glycosylated hemoglobin value ≥6.5%, and/or current antidiabetic therapy. Hypercholesterolemia was defined as total cholesterol level ≥240 mg/dl and/or current lipid-lowering therapy. Enzymatic methods were used to measure serum total cholesterol levels, serum creatinine, and blood glucose. Glycosylated hemoglobin was measured quantitatively using high-performance liquid chromatography. Smoking was defined as current smoking. Estimated glomerular filtration rate was calculated using an equation (estimated glomerular filtration rate [ml/min/1.73 m^2] = 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$) from the Modification of Diet in Renal Disease (MDRD) study for the Japanese population.[5] The 10-year risk for general CV disease was calculated using the Framingham 10-year risk

Table 1
Baseline characteristics according to plasma B-type natriuretic peptide deciles in men

Variable	Total	D1–D4	D5	D6	D7	D8	D9	D10
Number	4,365	1,741	441	441	434	436	436	436
BNP (pg/ml)	14.2 (6.3–28.3)	5 (2.1–7.6)	12.3 (11.4–13.2)	16.3 (15.3–17.5)	21.3 (19.8–22.8)	28.3 (26.5–30.5)	41.4 (37.5–46.5)	76.5 (63.4–116.7)
Age (years)	63.3 ± 9.8	58.3 ± 10.0	62.9 ± 9.0	65.5 ± 8.4	65.8 ± 8.1	67.6 ± 7.2	68.1 ± 7.4	69.7 ± 6.2
BMI (kg/m^2)	23.9 ± 2.9	24.1 ± 2.9	24.0 ± 3.0	23.9 ± 2.8	23.7 ± 2.9	23.6 ± 2.8	23.5 ± 2.9	23.7 ± 3.0
Hypertension	43.8%	35.1%	41.0%	46.5%	45.6%	49.8%	56.6%	57.6%
Diabetes mellitus	9.6%	9.8%	8.2%	11.6%	9.0%	10.1%	8.9%	9.2%
Smoker	33.9%	39.1%	33.1%	30.2%	32.7%	31.7%	28.0%	27.1%
Hypercholesterolemia	10.5%	14.5%	9.1%	9.3%	7.1%	8.5%	7.6%	5.7%
eGFR (ml/min/1.73 m^2)	77.2 ± 15.3	80.0 ± 15.3	77.3 ± 15.1	76.4 ± 15.6	76.5 ± 15.0	74.8 ± 14.7	75.8 ± 15.2	71.3 ± 13.3
Antihypertensive drugs	23.3%	15.8%	21.8%	25.9%	26.0%	27.5%	32.8%	35.3%
Framingham risk score	13.8 ± 4.4	12.8 ± 4.5	13.7 ± 4.3	14.5 ± 4.3	14.5 ± 4.1	14.8 ± 4.1	14.9 ± 4.2	15.1 ± 4.1

Data are expressed as median (interquartile range), as mean ± SD, or as percentages.
D = decile; eGFR = estimated glomerular filtration rate.

Table 2
Baseline characteristics according to plasma B-type natriuretic peptide deciles in women

Variable	Total	D1–D4	D5	D6	D7	D8	D9	D10
Number	8,844	3,539	880	880	893	882	885	885
BNP (pg/ml)	16.9 (8.8–29.8)	7.3 (3.8–10.4)	15.0 (14.1–15.9)	18.7 (17.8–19.7)	23.5 (22.2–25.0)	29.8 (28.0–31.9)	40.4 (37.1–43.8)	66.1 (55.1–88.0)
Age (years)	61.6 ± 9.7	58.1 ± 9.5	60.7 ± 9.4	60.9 ± 9.6	63.2 ± 9.0	64.3 ± 8.7	65.3 ± 8.3	68.7 ± 7.2
BMI (kg/m^2)	24.2 ± 3.4	24.2 ± 3.4	24.0 ± 3.3	24.0 ± 3.4	24.1 ± 3.4	24.0 ± 3.3	24.0 ± 3.5	24.4 ± 3.7
Hypertension	38.2%	29.5%	35.1%	36.0%	43.8%	43.2%	47.2%	59.0%
Diabetes mellitus	5.4%	5.3%	4.7%	4.8%	5.0%	6.2%	5.2%	7.2%
Smoker	2.5%	3.5%	1.7%	2.5%	1.9%	2.0%	2.3%	0.8%
Hypercholesterolemia	20.3%	23.3%	18.0%	18.9%	21.2%	19.7%	14.5%	17.2%
eGFR (ml/min/1.73 m^2)	75.8 ± 15.0	78.6 ± 14.9	76.5 ± 14.8	76 ± 13.9	74.7 ± 14.1	73.9 ± 15.2	73.2 ± 14.7	69.5 ± 14.8
Antihypertensive drugs	23.8%	16.8%	22.8%	21.5%	28.4%	27.4%	29.9%	41.2%
Framingham risk score	11.9 ± 4.6	10.8 ± 4.6	11.2 ± 4.5	11.7 ± 4.5	12.4 ± 4.5	12.6 ± 4.4	13.1 ± 4.4	14.3 ± 4.0

Data are expressed as median (interquartile range), as mean ± SD, or as percentages.
D = decile; eGFR = estimated glomerular filtration rate.

Figure 1. Crude incidence of CV events per 1,000 person-years among baseline plasma BNP deciles in men *(closed bars)* and women *(open bars)*.

score, including age, gender-specific cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diabetes, and cigarette smoking.[6]

Blood samples for routine laboratory testing were drawn from the antecubital vein with the subject in a sitting position. While blood samples were being collected into vacuum tubes, an additional 2-ml sample of venous blood was collected into a test tube containing ethylenediaminetetraacetic acid sodium. Tubes were stored immediately after sampling in an icebox and were transported to the laboratory <8 hours after collection. They were then centrifuged at 1,500g for 10 minutes. After separation, the 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 (Shionogi, Osaka, Japan) <4 months after blood sampling. Cross-reactivity of the antibodies was 100% for human BNP and 0.001% for human atrial natriuretic peptide. Intra- and interassay coefficients of variation were 5% and 6%, respectively. The lower detection limit of the assay was 0.05 pg/ml.

Continuous variables are expressed as mean ± SD. The cohort was divided into deciles according to baseline plasma BNP levels. To compare baseline data among the BNP deciles, 1-way analysis of variance and chi-square tests were used as appropriate. Differences in clinical characteristics between men and women were tested using unpaired Student's *t* test or Mann-Whitney U tests. We defined the end point as general CV events (i.e., a composite of stroke, heart failure, and myocardial infarction). The association between baseline plasma BNP levels and the end point was evaluated using a Cox proportional-hazards regression model. The gender-specific hazard ratios (HR) for each BNP decile's end point were assessed. In this multivariate regression model, adjustments were made in the analysis for age, BMI, diabetes, hypertension, hypercholesterolemia, atrial fibrillation, estimated glomerular filtration rate, and current smoking. For analyses of CV incidence, person-years were censored at the date of CV events, the date of emigration from the study area, the date of death, or the end of the follow-up period, whichever came first. To compare the predictive abilities of plasma BNP testing to the Framingham 10-year risk scoring system, receiver-operating-characteristic curves were constructed. The area under the curve (AUC) and 95% confidence interval (CI) for each ROC curve were calculated to provide a measure of the overall diagnostic accu-

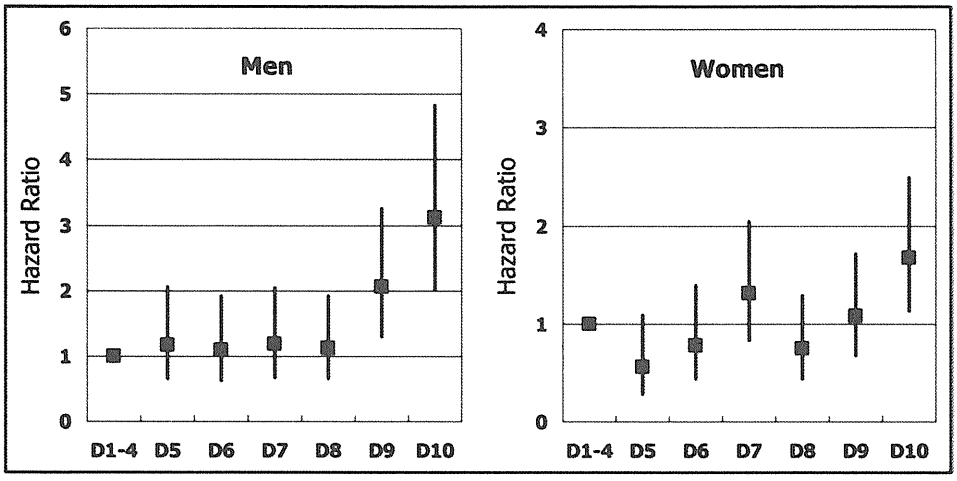

Figure 2. Multivariate-adjusted HRs and 95% CIs for risk for CV events according to plasma BNP decile in men *(left)* and women *(right)*.

racy of the test. The follow-up survey for congestive heart failure, stroke, and myocardial infarction was carried out after the baseline study through to March 2009. Migrations were confirmed by official resident registration data issued by the local government offices (October 2009). All statistical analyses were performed using SPSS version 11.0.1J (SPSS, Inc., Chicago, Illinois). A significant difference was defined as $p < 0.05$.

Results

Mean ages were 63.3 ± 9.8 years in men and 61.6 ± 9.7 years in women (Tables 1 and 2). The number of women was approximately twice the number of men. Plasma BNP levels and BMI were higher in women than in men (median BNP 16.9 vs 14.2 pg/ml, $p < 0.001$; mean BMI 24.2 ± 3.4 vs 23.9 ± 2.9 kg/m^2, $p < 0.001$). The prevalence of hypertension (44% vs 38%), atrial fibrillation (2.9% vs 0.6%), diabetes (9.6% vs 5.4%), and current smoking (33.9% vs 2.5%) was higher in men. The incidence of hypercholesterolemia was higher in women (10.5% vs 20.3%). The administration rates for hypertensive drugs was 23.3% in men and 23.8% in women ($p = 0.232$). The mean Framingham risk score in men was higher than that in women (13.8 ± 4.4 vs 11.9 ± 4.6).

During the mean follow-up period of 5.8 years, 430 CV events (215 in men, 215 in women) were recorded. When the lowest 4 (first to fourth) plasma BNP deciles were set to the reference, the crude incidence of CV events per 1,000 person-years increased with deciles in both genders (Figure 1). As shown in Figure 2, after adjustment for potential confounding factors in the Cox regression model, the relative HR for CV events increased according to deciles (p for trend <0.01 in men, p for trend <0.001 in women). Compared to the reference, the HR was significantly elevated in the ninth (HR 2.06, 95% CI 1.30 to 3.27) and tenth (HR 3.15, 95% CI 2.03 to 4.88) deciles in men and in the tenth decile only in women (HR 1.68, 95% CI 1.13 to 2.50). The thresholds for increased CV risk were greater than approximately 37 pg/ml in men and greater than approximately 55 pg/ml in women.

The overall power for predicting general CV events was comparable between plasma BNP level and Framingham risk score. The areas under the curve for plasma BNP were 0.669 (95% CI 0.629 to 0.710) in men and 0.634 (95% CI 0.593 to 0.676) in women. The areas under the curve did not differ significantly from those for the Framingham risk score (men 0.676, 95% CI 0.640 to 0.712; women 0.681, 95% CI 0.649 to 0.713).

Discussion

The present study has demonstrated that in the general population with no CV history or renal dysfunction, plasma BNP levels signaling increased CV risk are greater than the 80th percentile in men and the 90th percentile in women. The predictive ability of plasma BNP testing for general CV events is similar to that of the established total CV risk scoring system. The present study has therefore shown for the first time that increased plasma BNP levels higher than these gender-specific thresholds are a simple and useful marker for elevated risk for CV events in a community-based middle-aged and elderly population.

Several previously published studies have shown a significant association between plasma BNP and N-terminal pro-BNP (NT-proBNP) levels and CV events in the general population.[7–11] The Framingham study conventionally applied a single cutoff point (the 80th percentile) to examine the association between "high" BNP levels and CV events.[7] Linssen et al[10] recently reported that in a selected population mainly with urinary albumin excretion >10 mg/L, multivariate HRs for the risk for all-cause mortality increased gradually with increasing levels of plasma NT-proBNP, with no clear cut-off level in both genders. However, no studies have explored the threshold levels of BNP that indicate an increased risk for the future development of CV events.

Several studies have shown that median plasma BNP and NT-proBNP levels are higher in women,[12,13] although the incidence of CV events in the general population is usually lower in women than in men. This suggests that a gender-stratified analysis should be incorporated when determining cut-off levels of plasma BNP and NT-proBNP for predicting the future onset of CV events in the general population. However, no reports to date have shown which levels of plasma BNP increase the risk for CV events in either gender.

The present study has shown for the first time in an unselected general population that the adjusted HR was significantly increased from the ninth plasma BNP decile in men and the tenth decile in women. The association between plasma BNP and the future development of CV events may be because elevated plasma BNP is a significant biomarker for asymptomatic structural heart disease such as impaired left ventricular function, left ventricular hypertrophy, atrial dilatation and fibrillation, and myocardial ischemia. In accord with this concept, Struthers and Lang[14] suggested that BNP and NT-proBNP testing could be used to identify "pancardiac" target organ damage and may become to the heart what albuminuria is to the kidneys, that is, a useful biomarker for targeting organ damage in the CV system. In our previous cross-sectional study applying transthoracic echocardiography in the general population, plasma BNP concentrations >50 pg/ml showed sensitivity and specificity for several select phenotypes of structural heart disease that are prone to progress into several types of CV events.[15] The threshold plasma BNP levels that increased the HR (greater than approximately 37 pg/ml in men and greater than approximately 55 pg/ml in women) in the present study are lower compared to the previously reported cut-off level for detection of structural heart disease. This apparent discord may be due to the present study being longitudinal and the cut-off level being gender specific.

The present study suggests that the usefulness in terms of sensitivity and specificity of plasma BNP testing for predicting CV events differs little from the Framingham 10-year risk score for general CV events. This finding may indicate that the predictive ability of BNP testing is equivalent to that of the established risk calculation. However, such a conclusion may be premature, because the mean follow-up period of this study was shorter (<6 years) than the Framingham study (10 years). In fact, the established risk scoring includes lipids, blood pressure, smoking, and diabetes, which are long-standing risk factors for CV events. In contrast, plasma BNP may be unique in that it is instead identifying the end process of several types of cardiac damage itself. In view of this, plasma BNP testing could be useful for identifying subjects at high risk for several types of CV events within a few years. BNP may thus be a direct or novel biomarker for various types of intrinsic cardiac abnormalities rather than an additional biomarker for assessing long-term risk.

The present study had several strengths. This study included the largest general population sample in whom plasma BNP levels have been reported. The plasma BNP measurement was performed in fresh plasma samples without long-term freezing and repeated thawing. CV events were captured prospectively according to previously determined standard epidemiologic criteria and confirmed by the research staff at medical chart review. Baseline data including clinical characteristics and biochemical data were determined well before the start of the follow-up study.

Despite these merits, several limitations must be considered when interpreting the results. First, because echocardiographic evaluation was not included in the baseline data, the utility of the BNP testing could not be compared to that of echocardiography. However, several previous studies have reported that BNP testing remained independently predictive of future CV events after adjusting for echocardiographic variables.[7,11] Second, mean BMI was lower in our study than in previous general population reports.[7,9,10] Plasma BNP levels have been reported to be lower in obese subjects than in the lean population,[16,17] with 1 previous study demonstrating that each standard deviation increase in BMI was associated with a 16% to 18% decrement in plasma BNP.[16] It follows that threshold BNP levels may be slightly lower in predominantly obese populations. Third, according to population-based studies, the Japanese population has a lower incidence of CV events than Western countries. Thus, care must be taken before these data can be generalized to other ethnic groups. Fourth, McKie et al[18] recently demonstrated that the use of NT-proBNP as a CV risk predictor is worthless in healthy subjects, as verified by close clinical examination including echocardiography. This observation may not validate our results, because the present population comprises entirely healthy subjects and may include a substantial part of subjects with CV risk factors, as listed in Tables 1 and 2. Finally, the age range of our population may have been relatively narrow, with no subjects aged <40 years or >80 years.

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Original Article

Serum Low-Density Lipoprotein to High-Density Lipoprotein Ratio as a Predictor of Future Acute Myocardial Infarction Among Men in a 2.7-Year Cohort Study of a Japanese Northern Rural Population

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Aim: To examine and compare the predictive value of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TC/HDL-C and LDL-C/HDL-C ratios for future cardiovascular outcomes in the general Japanese population.
Methods: A total of 24,566 eligible participants aged 18 years or older, without cardiovascular disease, were enrolled through multiphase health screening and divided into quartile groups based on lipoprotein levels or ratios. Primary endpoints of the study were definitive acute myocardial infarction (AMI) and ischemic stroke, and cases of sudden death with unknown causes were not included. We used Cox proportional hazard models to examine the relationships between the quartiles and incidences of AMI or ischemic stroke, adjusting for traditional risk factors.
Results: Mean age was 63.7 years for males and 60.7 years for females. Mean follow-up period was 2.7 years, and 40 cases of AMI and 182 cases of ischemic stroke were recorded. The hazard ratio (HR) for AMI was significantly higher in the top quartile of the LDL-C/HDL-C ratio and LDL-C levels, and third quartile of TC among male participants. The HR of male participants with a LDL-C/HDL-C ratio of 2.6 or higher was significantly higher than other quartiles. No association between lipoprotein levels or their ratio quartiles and ischemic stroke was seen for either sex after adjusting for risk factors.
Conclusions: Our results indicated that the LDL-C/HDL-C ratio is an independent predictor for AMI, and the importance of better management of cardiovascular risks among people with high LDLC/HDLC ratios for the prevention of future cardiovascular disease.

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Key words; Epidemiology, Acute myocardial infarction, Atherosclerosis, Risk factor, Cholesterol

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Introduction

According to the World Health Organization, cardiovascular disease was the most common cause of death worldwide in 2005, accounting for approximately 30% of all deaths[1]. Among individuals 60 years of age or older, the main cause of death was isch-

emic heart disease, followed by cerebrovascular disease[2]. Prevention of cardiovascular disease (CVD), which includes cardiovascular and cerebrovascular disorders, is emphasized in both developed and developing countries[1, 2].

Dyslipidemia is an independent risk factor that contributes to the increase of CVD and death[3, 4]. Epidemiological studies[5, 6] have conclusively linked high levels of low-density lipoprotein cholesterol (LDL-C) and low levels of high-density lipoprotein cholesterol (HDL-C) with CVD incidence and mortality. The positive association between the LDL-C level and risk has been confirmed in a lipid-lowering randomized trial[7]. Current guidelines for the prevention of atherosclerotic disorders recommend specific target levels of lipid profiles to determine CVD risk and to evaluate the effectiveness of lipid-lowering therapies[8, 9]. Accordingly, LDL-C and HDL-C measurements are included in the standard health check-up supported by the Japanese government.

In recent years, additional indicators of CVD based on standard serum lipid profiles have been introduced[5, 10]. For instance, the total cholesterol (TC)/HDLC ratio is a useful and simple index of CVD risk[11]. Furthermore, the LDL-C/HDL-C ratio more accurately predicts CVD risk than LDL-C or HDL-C levels[5, 12]. In a large-scale intervention trial, a change in this ratio was a better indicator of successful CVD risk reduction[13]. Although previous studies have investigated the association between levels and the ratios of various lipoproteins and CVD risk[10, 12-14], only a few reports have evaluated the clinical utility of various lipid measures to predict CVD in Japan.

The aims of our study were to examine and compare the relationships between levels of TC, HDL-C, LDL-C, ratios of TC/HDL-C and LDL-C/HDL-C, and future cardiovascular outcomes among rural Japanese residents.

Methods

Study Participants

This study was part of the large population-based prospective Iwate Kenpoku Cohort study (Iwate-KENCO study) and a government-sponsored, multiphase health check-up program in the northern part of the Japanese main island. Survey methods have been described in detail previously[15, 16].

The baseline survey was conducted from April 2002 to January 2005. Participants were recruited from the community-dwelling population living in the Ninohe, Kuji and Miyako districts of Iwate prefecture, which include 17 municipalities. During the survey period, individuals in these municipalities were invited to participate in multiphase health screening. A total of 31,318 residents (11,003 males and 20,315 females) aged 18 years or older participated in the annual health check-up. We obtained written informed consent from 26,469 of these residents. After excluding 1,903 participants due to a self-reported history of CVD, medical history of CVD confirmed by the Northern Iwate Heart Disease Registry Consortium (NIHDRC) database and the Iwate Stroke Registry (ISR) database[17], taking lipid-lowering medications, and missing data for lipid-related items, we included 24,566 (8,714 males and 15,852 females) in the present analysis.

The study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the Declaration of Helsinki[18].

Measurements

We measured height and weight, and calculated body mass index (BMI) using the following equation: BMI = body weight (kg)/height $(m)^2$. Blood pressure was measured twice in a sitting position after urination and a five-minute rest. Measurements were performed by well-trained staff using automatic devices, and the average of the two measurements was reported for systolic and diastolic blood pressures (SBP and DBP).

Self-administered questionnaires were used in the baseline survey to obtain demographic characteristics, history of cardiovascular disease, cerebrovascular disease, medication use, alcohol consumption, tobacco smoking and exercise habits.

Biochemical Analysis

Fasting or casual blood samples were collected from the antecubital vein, transferred to a laboratory and analyzed the same day. Serum total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) levels were measured by enzymatic methods. Serum low-density lipoprotein cholesterol (LDL-C) was measured by the enzymatic homogenous assay, Cholestest-LDL (Daiichi Chemicals Co. Ltd, Tokyo, Japan). These lipid profiles were measured by Iwate Health Service Association. These measurements have been standardized by the Osaka Medical Center for Health Science and Promotion, a member of the Cholesterol Reference Method Laboratory Network (CRMLN) controlled by the CDC (Centers for Disease Control and Prevention, Atlanta, USA), and have met all criteria for both the precision and accuracy of lipid measurements.

We measured plasma glucose concentrations by the hexokinase ultraviolet method using an automated analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). Glycosylated hemoglobin A1c (HbA1c) levels were determined by high-performance liquid chromatography using an automated analyzer (Tosoh Corporation, Tokyo, Japan).

We determined serum levels of high-sensitivity C-reactive protein (hs-CRP) using the latex-enhanced immunonephelometric method (Dade Behring, Germany). The detection limit of the hs-CRP assay is 0.1 mg/L and results below the limit of detection were reported as 0.1 mg/L.

Outcome Measures

The primary endpoints of this study were the incidence of definitive acute myocardial infarction (AMI) or ischemic stroke, while cases of sudden death of unknown cause were not included as cardiovascular endpoints. Investigators reviewed the population register of each local government and confirmed dates of death and locations to which participants had relocated. Persons known to be alive at the end of follow-up and those who had moved away from the study areas were treated as censored cases[16]. We confirmed that approximately 99.9% of participants who were registered at the baseline were alive. Incidences of AMI among participants were confirmed by assessing the Northern Iwate Heart Disease Registry Consortium (NIHDRC) database, in which definitive AMI cases were determined based on the criteria of the MONICA study. Incidences of ischemic stroke were confirmed by accessing the Iwate Stroke Registry (ISR) database[17].

Statistical Analysis

For each sex, we determined the prevalence (for categorical variables) and mean and standard deviation (for continuous variables). Participants were categorized as follows: LDL-C/HDL-C ratio quartiles (Q1 <1.6, Q2 ≥1.6-<2.1, Q3 ≥2.1-<2.6, Q4 ≥2.6), TC level (mg/dL) quartiles (Q1 <180, Q2 ≥180-<200, Q3 ≥200-<220, Q4 ≥220), LDL-C level (mg/dL) quartiles (Q1 <100, Q2 ≥100-<120, Q3 ≥120-<140, Q4 ≥140), HDL-C level (mg/dL) quartiles (Q1 <50, Q2 ≥50-<60, Q3 ≥60-<70, Q4 ≥70) and TC/HDL-C ratio quartiles (Q1 <2.8, Q2 ≥2.8-<3.4, Q3 ≥3.4-<4.1, Q4 ≥4.1). Significance was estimated using the Kruskal-Wallis test for continuous items and the Chi-square test or Fisher's exact test for categorical items among quartiles.

Disease-free survival curves for AMI or ischemic stroke cases based on lipid profiles and their ratio quartiles were determined using Kaplan-Meier methods. Age- and multivariate-adjusted hazard ratios (HR) and 95% confidence intervals (CI) were computed using Cox proportional hazard models. To estimate adjusted HR, we included age (10 years increase), current smoking status (yes or no), SBP, BMI, uric acid and HbA1c in the multivariate adjusted models from our previous report[15].

All significance tests were two-sided and p values <0.05 were considered significant. All data were analyzed using SPSS version 16 (SPSS Inc., Chicago, USA).

Table 1. Characteristics of participants at baseline

	N (%) or mean (SD)	
	Male (n=8,714)	Female (n=15,852)
Age (years)	63.7 (11.5)	60.7 (11.7)
Body mass index	23.9 (3.0)	24.0 (3.5)
Systolic blood pressure (mmHg)	130.6 (19.6)	125.0 (20.2)
Diastolic blood pressure (mmHg)	78.3 (11.1)	73.6 (11.2)
Total cholesterol (mg/dL)	190.7 (32.5)	204.3 (32.5)
Triglyceride (mg/dL)	124.8 (83.5)	111.1 (66.7)
HDL-C (mg/dL)	55.9 (15.2)	61.2 (14.3)
LDL-C (mg/dL)	113.3 (29.2)	122.9 (28.9)
HbA1c (%)	5.14 (0.73)	5.09 (0.64)
Hypertension (%)	2263 (26.0)	3875 (24.4)
Diabetes mellitus (%)	610 (7.0)	551 (3.5)
Current smoking (%)	2714 (31.1)	471 (3.0)
Regular alcohol consumption (%)	5276 (60.5)	1912 (12.1)

Results

Table 1 shows the characteristics of study participants. Mean age was 63.7 years for males and 60.7 years for females. The proportions of hypertension were 26.0% for males and 24.4% for females, and 7.0% and 3.5% for diabetes mellitus. Mean follow-up period was 2.7 years. During the survey period, 35 males and 5 females suffered from AMI, and 114 males and 68 females suffered from ischemic stroke. We could not estimate a survival curve for AMI for females due to its low incidence.

The LDL-C/HDL-C ratio-specific characteristics at baseline among male participants are shown in **Table 2**. BMI, HbA1c, uric acid, TC, TG, LDL-C and hs-CRP were significantly correlated with higher LDL-C/HDL-C ratio quartiles. In contrast, the proportion of participants who regularly consumed alcohol and HDL-C seemed to be inversely correlated

Table 2. LDL-C/HDL-C ratio-specific characteristics among male participants at baseline (n=24566)

LDL-C/HDL-C ratio	N (%) or mean (SD)				p[a]
	Q1 <1.6 (n=2277)	Q2 ≥1.6-<2.1 (n=2125)	Q3 ≥2.1-<2.6 (n=1818)	Q4 ≥2.6 (n=2494)	
Age (years)	63.7 (11.6)	64.1 (11.5)	63.6 (11.6)	63.5 (11.4)	0.13
Body mass index (kg/m^2)	22.7 (2.8)	23.6 (2.9)	24.3 (2.9)	25.0 (2.8)	<0.01
Current smoking	758 (33.3)	624 (29.4)	523 (28.8)	809 (32.4)	<0.01
Regular alcohol consumption	1774 (77.9)	1346 (63.3)	1065 (58.6)	1091 (43.7)	<0.01
Systolic blood pressure (mmHg)	131.0 (20.3)	130.3 (19.4)	130.3 (19.5)	130.8 (19.1)	0.53
Diastolic blood pressure (mmHg)	78.2 (11.5)	78.0 (11.0)	78.2 (10.8)	78.6 (11.0)	0.44
Antihypertensive medication	566 (24.9)	516 (24.3)	453 (24.9)	605 (24.3)	0.27
Hemoglobin A1c (%)	5.02 (0.64)	5.12 (0.70)	5.18 (0.80)	5.23 (0.76)	<0.01
Hypoglycemic medication	87 (3.8)	90 (4.2)	80 (4.4)	115 (4.6)	0.19
Uric acid (mg/dL)	5.6 (1.4)	5.7 (1.3)	5.7 (1.3)	6.0 (1.4)	<0.01
Total cholesterol (mg/dL)	174.8 (29.0)	184.8 (28.5)	192.9 (28.6)	208.7 (32.3)	<0.01
Triglyceride (mg/dL)	94.3 (67.2)	112.8 (81.7)	129.9 (78.7)	158.9 (88.7)	<0.01
LDL-C (mg/dL)	85.7 (19.4)	107.2 (18.5)	119.7 (19.8)	139.0 (25.5)	<0.01
HDL-C (mg/dL)	71.1 (15.5)	58.3 (10.1)	51.4 (8.7)	43.3 (7.9)	<0.01
hs-CRP (mg/dL)	0.13 (0.44)	0.14 (0.57)	0.14 (0.44)	0.16 (0.45)	<0.01

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; SD, standard deviation; hs-CRP, high sensitivity C-reactive protein

[a]Significance was estimated using Kruskal-Wallis test for continuous items and Chi-square test or Fisher's exact test for categorical items.

with LDL-C/HDL-C ratio quartiles.

Fig. 1 shows disease-free survival curves for AMI among males based on LDL-C/HDL-C ratio quartiles. The disease-free survival rate for male participants with a LDL-C/HDL-C ratio of 2.6 or higher (fourth quartile) was significantly different from other quartiles. Multivariate-adjusted HR of Q4 was significantly higher than Q1 (p=0.03) (**Table 3-1**).

Multivariate-adjusted HR of Q3 for TC levels (HR=2.44, p=0.04) and Q4 for LDL-C levels (HR=2.50, p=0.04) were significantly higher and that of Q3 for HDL-C levels (HR=0.20, p=0.03) was significantly lower than Q1; however, a linear and obvious relationship was not observed across all quartiles (**Table 3-1**).

Table 3-2 shows hazard ratios for ischemic stroke based on lipoprotein levels and their ratio quartiles among males. For the LDL-C/HDL-C ratio, no significant relationship was found between quartiles and the risk of ischemic stroke [multivariate-adjusted HR of Q4=0.86 (p=0.56)]. Furthermore, no relationship was found between quartiles of LDL-C levels, HDL-C levels and the TC/HDL-C ratio, and the risk of ischemic stroke [multivariate-adjusted HRs of Q4=0.73 (p=0.27) for LDL-C levels, 0.90 (p=0.73) for HDL-C levels and 0.81 (p=0.47) for TC/HDL-C ratio]. Although multivariate-adjusted HR of Q3 for TC levels was significantly lower than Q1 (HR=0.55, p=0.04), a linear and obvious relationship was not observed across quartiles.

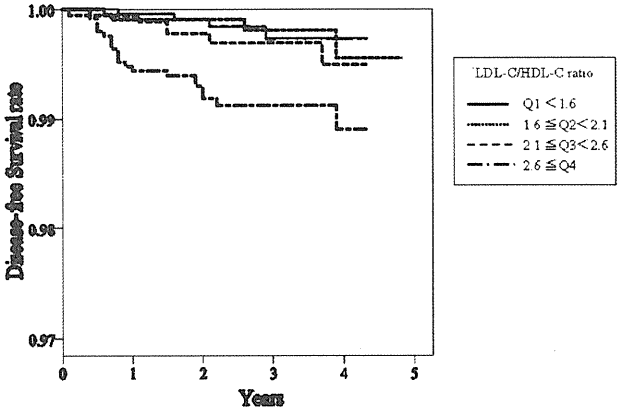

Fig. 1. Acute myocardial infarction-free rate for LDL-C/HDL-C ratio quartiles among male participants (n=8714, Cases=35).

Table 3-3 shows hazard ratios for ischemic stroke for lipoprotein levels or their ratio quartiles among females. We found no significant relationship between quartiles of LDL-C/HDL-C ratio and risk of ischemic stroke [multivariate-adjusted HR of Q4=1.17 (p=0.69)]. In addition, we observed no relationship between the other quartiles [multivariate-adjusted

Table 3-1. Hazard ratios for acute myocardial infarction according to lipid level quartiles among male participants (n=8714, Mean age=63.7±11.5, Mean follow-up years=2.7±0.9)

	Number of events	Incidence rate[a]	Age-adjusted hazard ratios	95% CI	p	Multivariate- adjusted hazard ratios[b]	95% CI	p
LDL-C/HDL-C ratio								
Q1 <1.6	4/2277	0.28	1.00 (Reference)			1.00 (Reference)		
Q2 ≥1.6-<2.1	4/2125	0.32	1.08	0.27-4.33	0.91	0.99	0.25-3.96	0.98
Q3 ≥2.1-<2.6	6/1818	0.67	1.88	0.53-6.67	0.33	1.51	0.42-5.46	0.53
Q4 ≥2.6	21/2494	1.24	5.02	1.72-14.62	<0.01	3.50	1.15-10.64	0.03
TC levels (mg/dL)								
Q1 <180	12/3292	0.41	1.00 (Reference)			1.00 (Reference)		
Q2 ≥180-<200	6/2183	0.46	0.75	0.28-1.99	0.56	0.82	0.34-2.52	0.88
Q3 ≥200-<220	10/1651	1.33	1.63	0.71-3.78	0.25	2.44	1.01-5.91	0.04
Q4 ≥220	7/1588	1.00	1.17	0.46-2.98	0.74	1.81	0.68-4.77	0.23
LDL-C levels (mg/dL)								
Q1 <100	8/2884	0.36	1.00 (Reference)			1.00 (Reference)		
Q2 ≥100-<120	5/2427	0.31	0.72	0.23-2.19	0.56	0.64	0.21-1.96	0.43
Q3 ≥120-<140	9/1813	1.00	1.82	0.70-4.71	0.22	1.30	0.48-3.51	0.60
Q4 ≥140	13/1590	1.85	3.20	1.32-7.72	0.01	2.50	1.02-6.09	0.04
HDL-C levels (mg/dL)								
Q1 <50	24/3253	0.85	1.00 (Reference)			1.00 (Reference)		
Q2 ≥50-<60	7/2346	0.47	0.41	0.18-0.95	0.04	0.48	0.21-1.13	0.09
Q3 ≥60-<70	2/1647	0.27	0.16	0.04-0.67	0.01	0.20	0.05-0.86	0.03
Q4 ≥70	2/1468	0.33	0.19	0.05-0.81	0.02	0.27	0.06-1.19	0.08
TC/HDL-C ratio								
Q1 <2.8	4/2003	0.37	1.00 (Reference)			1.00 (Reference)		
Q2 ≥2.8-<3.4	3/2148	0.24	0.69	0.15-3.08	0.63	0.60	0.13-2.72	0.51
Q3 ≥3.4-<4.1	9/2066	0.77	2.18	0.67-7.07	0.20	1.52	0.45-5.19	0.50
Q4 ≥4.1	19/2497	1.13	4.05	1.38-11.90	0.01	2.82	0.91-8.72	0.07

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol
[a]Unadjusted incidence rate per 1,000,000 per year. [b]Age (10-year increase), current smoking, systolic blood pressure, body mass index, uric acid, and hemoglobin A1c were included in the Cox regression analysis.

HRs of Q4=0.73 (p=0.44) for TC levels, 0.68 (p=0.34) for LDL-C levels, 0.59 (p=0.19) for HDL-C levels and 1.43 (p=0.39) for TC/HDL-C ratio].

Discussion

This community-based, prospective cohort study conducted in a Japanese rural area showed that the LDL-C/HDL-C ratio at baseline was an independent predictor for future AMI among male participants, with a ratio of 2.6 or higher suggesting a risk of disease. Although an association between LDL-C and TC quartiles and the risk was observed, it may be weaker than LDL-C/HDL-C. On the other hand, we observed no obvious association between any lipoprotein level or their ratio quartiles and the risk of ischemic stroke in either sex. To the best of our knowledge, this is the first report to prospectively examine the association between the lipoprotein level or ratio quartiles and cardiovascular events, and to clarify the relationship between the LDL-C/HDL-C ratio and AMI among males in a rural Japanese community.

Several epidemiological studies have reported the LDL-C/HDL-C ratio to be an excellent predictor of coronary heart disease (CHD) risk[10, 12-14, 19]. In the Helsinki Heart Study[12], the LDL-C/HDL-C ratio was a strong predictor of CHD risk among participants with high triglyceride levels during 5-year follow up. Furthermore, the PROSPER study[13], which was a prospective cohort study that examined about 5,800 elderly participants over 3.7 years, suggested that increased CHD risk is associated with an elevated LDL-C/HDL-C ratio. In contrast, the Quebec Cardiovascular Study[11] showed that ratios of LDL-C/HDL-C and TC/HDL-C were associated with ischemic heart disease, and indicated that the TC/HDL-C

Table 3-2. Hazard ratios for ischemic stroke according to lipid level quartiles among male participants (n=8714, Mean age=63.7±11.5, Mean follow-up years=2.7±0.9)

	Number of events	Incidence rate[a]	Age-adjusted hazard ratios	95% CI	p	Multivariate- adjusted hazard ratios[b]	95% CI	p
LDL-C/HDL-C ratio								
Q1 <1.6	33/2277	2.36	1.00 (Reference)			1.00 (Reference)		
Q2 ≥1.6-<2.1	29/2125	2.36	0.93	0.57-1.53	0.78	1.02	0.61-1.71	0.94
Q3 ≥2.1-<2.6	21/1818	2.35	0.79	0.46-1.36	0.39	0.81	0.46-1.44	0.47
Q4 ≥2.6	31/2494	1.84	0.88	0.54-1.43	0.60	0.86	0.50-1.46	0.56
TC levels (mg/dL)								
Q1 <180	53/3292	1.84	1.00 (Reference)			1.00 (Reference)		
Q2 ≥180-<200	25/2183	1.94	0.69	0.43-1.12	0.13	0.69	0.42-1.13	0.15
Q3 ≥200-<220	16/1651	2.13	0.57	0.33-1.00	0.05	0.55	0.31-0.99	0.04
Q4 ≥220	20/1588	2.87	0.74	0.44-1.23	0.25	0.83	0.49-1.42	0.83
LDL-C levels (mg/dL)								
Q1 <100	45/2884	2.04	1.00 (Reference)			1.00 (Reference)		
Q2 ≥100-<120	32/2427	2.00	0.81	0.52-1.28	0.37	0.83	0.52-1.32	0.43
Q3 ≥120-<140	19/1813	2.11	0.66	0.39-1.13	0.13	0.65	0.37-1.12	0.12
Q4 ≥140	18/1590	2.57	0.76	0.44-1.31	0.32	0.73	0.42-1.27	0.27
HDL-C levels (mg/dL)								
Q1 <50	41/3253	1.45	1.00 (Reference)			1.00 (Reference)		
Q2 ≥50-<60	34/2346	2.28	1.17	0.74-1.84	0.51	1.18	0.74-1.87	0.49
Q3 ≥60-<70	23/1647	3.10	1.08	0.65-1.81	0.76	1.12	0.66-1.89	0.69
Q4 ≥70	16/1468	2.70	0.89	0.50-1.58	0.68	0.90	0.49-1.66	0.73
TC/HDL-C ratio								
Q1 <2.8	29/2003	2.68	1.00 (Reference)			1.00 (Reference)		
Q2 ≥2.8-<3.4	32/2148	2.55	0.99	0.60-1.64	0.97	0.99	0.59-1.67	0.97
Q3 ≥3.4-<4.1	22/2066	1.90	0.72	0.41-1.25	0.25	0.70	0.39-1.26	0.23
Q4 ≥4.1	31/2497	1.84	0.89	0.54-1.48	0.65	0.81	0.47-1.42	0.47

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol
[a]Unadjusted incidence rate per 1,000,000 per year. [b]Age (10-year increase), current smoking, systolic blood pressure, body mass index, uric acid, and hemoglobin A1c were included in the Cox regression analysis.

ratio might be a better indicator than the LDL-C/HDL-C ratio in males; however, a population-based cohort study from Framingham, Massachusetts reported that ratios of TC/HDL-C and LDL-C/HDL-C were positively associated with coronary heart disease risk in both sexes[5]. In the present study, multivariate-adjusted analysis showed that while the incidence of AMI was significantly associated with the LDL-C/HDL-C ratio, the magnitude of HR was almost identical in each quartile group of LDL-C/HDL-C and TC/HDL-C. Long-term observation may be needed to reveal an association between the LDL-C/HDL-C ratio or TC/HDL-C ratio and risk of future cardiovascular events.

We also observed a positive association between LDL-C levels and AMI. LDL-C is well known as an important risk factor for CVD. In the Suita study[20], which analyzed about 4,700 participants over a 11.9 years, the risk of myocardial infarction in the highest quartile of LDL-C (≥3.91 mmol/L) was 3.73 times higher than in the lowest quartile (<2.54 mmol/L) in males. Likewise, we showed that the highest quartile of LDL-C was significantly associated with the incidence of AMI. Upon additional analysis, no significant association was found between the LDL-C/HDL-C ratio and AMI after stratifying with the LDL-C median level [multivariate-adjusted HR of Q4 in high LDL-C=3.00 (p=0.56) and in low LDL-C=0.70 (p=0.66)]. The predictive value of LDL-C/HDL-C for AMI incidence may be evident with relatively high serum LDL-C (≥120 mg/L); however, LDL-C quartiles did not show a clear and linear trend compared to the LDL-C/HDL-C ratio. Moreover, TC quartiles did not show a liner a association in this study, although the third quartile of TC levels was significantly associated with the incidence; therefore, the

Table 3-3. Hazard ratios for ischemic stroke according to lipid level quartiles among female participants (n=15852, Mean age=60.7±11.7, Mean follow-up years=2.7±0.9)

	Number of events	Incidence rate[a]	Age-adjusted hazard ratios	95% CI	p	Multivariate-adjusted hazard ratios[b]	95% CI	p
LDL-C/HDL-C ratio								
Q1 <1.6	11/4025	0.26	1.00 (Reference)			1.00 (Reference)		
Q2 ≥1.6-<2.1	18/4408	0.35	1.25	0.59-2.65	0.56	1.13	0.53-2.42	0.76
Q3 ≥2.1-<2.6	16/3599	0.46	1.24	0.58-2.68	0.58	0.92	0.41-2.05	0.83
Q4 ≥2.6	23/3820	0.58	1.66	0.81-3.41	0.17	1.17	0.54-2.49	0.69
TC levels (mg/dL)								
Q1 <180	12/3484	0.38	1.00 (Reference)			1.00 (Reference)		
Q2 ≥180-<200	20/3638	0.56	1.55	0.76-3.17	0.23	1.39	0.66-2.94	0.39
Q3 ≥200-<220	19/3896	0.47	1.38	0.67-2.85	0.38	1.15	0.54-2.48	0.72
Q4 ≥220	17/4834	0.27	0.97	0.46-2.03	0.93	0.73	0.32-1.63	0.44
LDL-C levels (mg/dL)								
Q1 <100	12/3291	0.43	1.00 (Reference)			1.00 (Reference)		
Q2 ≥100-<120	21/4066	0.48	1.15	0.56-2.33	0.71	1.15	0.55-2.42	0.71
Q3 ≥120-<140	16/4301	0.32	0.78	0.37-1.66	0.52	0.74	0.34-1.61	0.45
Q4 ≥140	19/4194	0.39	0.94	0.45-1.94	0.86	0.68	0.31-1.49	0.34
HDL-C levels (mg/dL)								
Q1 <50	22/3345	0.74	1.00 (Reference)			1.00 (Reference)		
Q2 ≥50-<60	21/4453	0.39	0.79	0.43-1.43	0.43	0.85	0.45-1.59	0.61
Q3 ≥60-<70	15/3993	0.35	0.66	0.34-1.28	0.22	0.78	0.39-1.55	0.47
Q4 ≥70	10/4061	0.23	0.46	0.22-0.97	0.04	0.59	0.27-1.29	0.19
TC/HDL-C ratio								
Q1 <2.8	9/3728	0.24	1.00 (Reference)			1.00 (Reference)		
Q2 ≥2.8-<3.4	16/4559	0.29	1.20	0.53-2.73	0.66	1.10	0.48-2.54	0.82
Q3 ≥3.4-<4.1	19/3997	0.44	1.44	0.65-3.19	0.37	1.12	0.49-2.57	0.78
Q4 ≥4.1	24/3568	0.70	2.03	0.94-4.39	0.07	1.43	0.64-3.22	0.39

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol
[a]Unadjusted incidence rate per 1,000,000 per year. [b]Age (10-year increase), current smoking, systolic blood pressure, body mass index, uric acid, and hemoglobin A1c were included in the Cox regression analysis.

LDL-C/HDL-C ratio may have greater clinical potential as a predictor of AMI than LDL-C or TC levels.

HDL-C is a well-known protective factor against CVD, and its levels are inversely associated with CHD[21]. We did not observe a clear association in the present study, which may have been due to the shorter follow-up period. Nevertheless, the LDL-C/HDL-C ratio at baseline was independently associated with future AMI, suggesting that the LDL-C/HDL-C ratio may better predict the outcome than HDL-C alone.

Interestingly, our results showed that the hazard ratio in male participants with a LDL-C/HDL-C ratio of 2.6 or higher was significantly higher than other quartiles. The Munster Heart Study (PROCAM)[14], which included middle-aged German men, showed a continuous and graded relationship between the LDL-C/HDL-C ratio and CHD mortality, with an increase in CHD deaths when the ratio was between 3.7 and 4.3. A clinical study of Japanese patients with suspected ischemic coronary disease evaluated the relationship between plaque formation and lipoprotein levels in coronary arteries using intravascular ultrasonography and found that the mean plaque area was significantly higher if the LDL-C/HDL-C ratio was at least 2.5[22]. It is possible that a LDL-C/HDL-C ratio of 2.6 or higher is a risk factor for AMI among Japanese males, and our results suggest that it is important to maintain an LDL-C/HDL-C ratio lower than 2.6 for primary prevention of AMI.

We did not find an association between the LDL-C/HDL-C ratio and ischemic stroke. Furthermore, there were mostly non-significant associations between other lipid profiles or their indices and ischemic stroke. The Cardiovascular Health study[23] reported a positive association between LDL-C and the risk of ischemic stroke, and the Oyabe study[24]

demonstrated an inverse relationship between HDL-C levels and ischemic stroke incidence; however, the Framingham study[25] and Hisayama study[26] did not report a clear association between the LDL-C level and the risk of ischemic stroke, and HDL-C levels were not associated with the risk of ischemic stroke in the Women's Health study[27]. Furthermore, LDL-C and HDL-C were not associated with ischemic stroke in the Atherosclerosis Risk in Community study[28]. Also, the NIPPON DATA 80[29, 30] reported that there was no relationship between ischemic stroke and TC levels. We propose three possible explanations for these discrepancies. First, these associations were heterogeneous across ischemic stroke subtypes, and lacunar infarction and cardioembolic infarction seem to be less associated with elevated LDL-C levels than atherothrombotic infarction[26]. It is probable that including these subtypes in the analysis masks the true association; therefore, the LDL-C/HDL-C ratio may not be clearly associated with the risk of total ischemic stroke in the present study. Ischemic stroke subtype-specific analysis may be needed to assess the potential relationship with the LDL-C/HDL-C ratio. Second, the follow-up period in our study was relatively short compared to previous studies. Long-term observation may reveal an association between the LDL-C/HDL-C ratio and risk of ischemic stroke. Finally, adjustment for confounding factors, especially blood pressure levels, might be insufficient in multivariate analysis. Ischemic stroke is likely to be influenced by blood pressure levels compared to AMI, and insufficient adjustment for blood pressure levels may mask the true association. To improve the accuracy of our findings, additional analysis stratified by blood pressure levels may be needed.

We found that the LDL-C/HDL-C ratio was associated with cardiovascular risk factors in both sexes. The Hisayama study[26] reported that LDL-C levels were linearly correlated with BMI, fasting blood glucose levels, and systolic and diastolic blood pressures, while HDL-C levels were inversely correlated with LDL-C levels. These factors are components of metabolic syndrome (MetS), which has received considerable attention because it is known to be a condition associated with a high risk for ischemic heart disease[31]. Furthermore, the LDL-C/HDL-C ratio was significantly correlated with hs-CRP levels, which is a circulatory inflammatory marker and well-known predictor of atherosclerotic disorders[32]; therefore, it is probable that the LDL-C/HDL-C ratio can assess the inflammation of blood vessels.

Our study has several limitations. The first is selection bias. Participants were selected from those who attended the annual health check-up, and they may have greater health awareness than the general population. Second, the follow-up period was relatively short (2.7 years) compared to previous cohort studies that observed outcomes for more than five years. Long-term observational studies may be needed to access causal associations between the LDL-C/HDL-C ratio and cardiovascular outcomes. Third, unknown sudden deaths were excluded from analysis, and it is possible that incidences were underestimated. Fourth, the present study investigated the outcome of total ischemic stroke. Ischemic stroke subtype-specific analysis may be needed in the future. Finally, we used LDL-C levels directly measured by a homogeneous assay in this study. It is possible that directly measured LDL-C levels are not as accurate as calculated LDL-C levels.

Conclusions

The LDL-C/HDL-C ratio at baseline was an independent predictor of future AMI among Japanese males. A ratio of 2.6 or higher may indicate non-fatal AMI risk, and might have the potential to assess the inflammation of blood vessels. In addition to other lipid profiles and ratios, our results indicate the utility of the LDL-C/HDL-C ratio as a predictor of AMI among men and the importance of lifestyle modification and better management of cardiovascular risks among people with high LDLC/HDLC ratios for primary prevention of future cardiovascular disease; however, given the relatively short follow-up period of this study, long-term studies may be needed to confirm our findings.

Conflict of Interest

The authors report no conflicts of interest.

Acknowledgements

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