**Table 2. HRs and 95% CIs of Smoking for Incidence of CVD (Stroke+MI), Stroke, Ischemic Stroke, and MI**

| | Never-smoker | Ex-smoker | Current-smoker | |
|---|---|---|---|---|
| | | | ≤20 cigarettes/day | >20 cigarettes/day |
| Men (n) | 338 | 583 | 524 | 373 |
| Person-years | 4,147 | 6,837 | 5,965 | 4,343 |
| CVD (stroke+MI) | | | | |
| Cases (n) | 11 | 29 | 40 | 16 |
| Incidence (/1,000 person-years) | 2.65 | 4.24 | 6.71 | 3.68 |
| Multivariate-adjusted HR (95%CI) | 1.00 | 1.34(0.67–2.69) | 2.65(1.35–5.21) | 2.31(1.06–5.05) |
| Stroke | | | | |
| Cases (n) | 8 | 18 | 30 | 12 |
| Incidence (/1,000 person-years) | 1.93 | 2.63 | 5.03 | 2.76 |
| Multivariate-adjusted HR (95%CI) | 1.00 | 1.07(0.46–2.48) | 2.47(1.12–5.45) | 2.48(1.00–6.20) |
| Ischemic stroke | | | | |
| Cases (n) | 4 | 16 | 24 | 8 |
| Incidence (/1,000 person-years) | 0.96 | 2.34 | 4.02 | 1.84 |
| Multivariate-adjusted HR (95%CI) | 1.00 | 1.94(0.64–5.86) | 4.06(1.40–11.83) | 3.37(1.00–11.41) |
| MI | | | | |
| Cases (n) | 3 | 11 | 10 | 4 |
| Incidence (/1,000 person-years) | 0.72 | 1.61 | 1.68 | 0.92 |
| Multivariate-adjusted HR (95%CI) | 1.00 | 2.21(0.61–8.00) | 2.74(0.80–10.90) | 1.89(0.41–8.70) |
| Women (n) | 1,790 | 67 | 209 | 23 |
| Person-years | 21,881 | 727 | 2,363 | 240 |
| CVD (stroke+MI) | | | | |
| Cases (n) | 45 | 0 | 10 | 1 |
| Incidence (/1,000 person-years) | 0.21 | – | 4.23 | 4.17 |
| Multivariate-adjusted HR (95%CI) | 1.00 | – | 2.70(1.34–5.45) | 2.80(0.36–21.55) |
| Stroke | | | | |
| Cases (n) | 37 | 0 | 5 | 1 |
| Incidence (/1,000 person-years) | 1.69 | – | 2.12 | 4.17 |
| Multivariate-adjusted HR (95%CI) | 1.00 | – | 1.60(0.62–4.16) | 2.70(0.34–21.68) |
| Ischemic stroke | | | | |
| Cases (n) | 19 | 0 | 4 | 1 |
| Incidence (/1,000 person-years) | 0.87 | – | 1.69 | 4.17 |
| Multivariate-adjusted HR (95%CI) | 1.00 | – | 3.00(1.00–8.97) | 7.15(0.84–60.64) |
| MI | | | | |
| Cases (n) | 8 | 0 | 5 | 0 |
| Incidence (/1,000 person-years) | 0.37 | – | 2.12 | – |
| Multivariate-adjusted HR (95%CI) | 1.00 | – | 8.35(2.64–26.48) | – |

Multivariate-adjusted HR (95%CI): age, BMI, systolic blood pressure, blood glucose, non-HDL-cholesterol, glomerular filtration rate, and alcohol drinking were adjusted.
HRs, hazard ratios; CIs, confidence intervals; CVD, cardiovascular disease; MI, myocardial infarction. Other abbreviations see in Table 1.

Well-trained physicians measured blood pressure (BP) 3 times in the right arm using a standard mercury sphygmomanometer while the participant was seated after a 5-min rest. The average of the 2nd and 3rd measurements was used in the analyses. Height in stockings and weight in light clothing were measured. Trained public health nurses or technicians measured waist circumference at the umbilical level while the participant was standing.

Blood samples were collected at the NCVC after the participants had fasted for at least 12 h. The samples were centrifuged immediately, and a routine blood examination, which included serum total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), triglycerides and glucose levels, was then carried out. Non-HDLC was calculated by subtracting the HDL from the TC. Serum creatinine (Cre) was measured by the non-compensated kinetic Jaffe method. The glomerular filtration rate (GFR: $ml \cdot min^{-1} \cdot 1.73 m^{-2}$) was calculated using the MDRD equation modified by the Japanese coefficient (0.881): $186 \times (Cre\ (mg/dl))^{-1.154} \times (age\ (years))^{-0.203} \times 0.881 \times (0.742\ if\ female)$.[15,16]

## Definition of MetS

In the present study, MetS was defined using the criteria recommended in the National Cholesterol Education Program on Adult Treatment Panel III guideline with a modification (modified NCEP-ATP III criteria).[17,18] Specifically, abdominal obesity was defined as a waist circumference ≥90 cm in men and ≥80 cm in women according to the International Obesity Task Force central obesity criteria for Asia.[17] High BP was defined as average systolic/diastolic BPs ≥130/85 mmHg and/or current medication for hypertension. High triglyceride was defined as a serum level ≥1.68 mmol/L. Low HDLC was defined as a serum level <1.03 mmol/L in men and <1.29 mmol/L in women. High blood glucose was defined as fasting blood glucose (FBG) ≥6.10 mmol/L and/or current use of insulin or oral medication for diabetes. MetS was defined as the presence of 3 or more of these components.

## Follow-up and Endpoints

The method of follow-up has been described elsewhere.[2,11–14] Briefly, the participants were followed until December 31, 2005. The first step in the survey involved checking the health status of all participants by repeat visits to NCVC every 2 years and yearly questionnaires conducted by mail or telephone interview. The in-hospital medical records of the participants who were suspected of having had a myocardial infarction (MI) or stroke were reviewed

**Table 3. Risk of Smoking and MetS for CVD (Stroke+MI)**

| | MetS (−) | | MetS (+) | |
|---|---|---|---|---|
| | Non-smoker | Smoker | Non-smoker | Smoker |
| Men | | | | |
| n | 732 | 730 | 189 | 171 |
| Person-years | 8,721 | 8,506 | 2,263 | 1,835 |
| CVD (stroke+MI) cases (n) | 26 | 41 | 14 | 16 |
| CVD incidence (/1,000 person-years) | 2.98 | 4.82 | 6.19 | 8.72 |
| Multivariate-adjusted HR (95%CI)† | Reference | 2.03(1.24–3.33) | 2.11(1.10–4.04) | 3.39(1.81–6.33) |
| Multivariate-adjusted HR (95%CI)‡ | Reference | 2.07(1.26–3.40) | 2.09(1.08–4.04) | 3.56(1.89–6.72) |
| PAF | | 21.8 | 7.5 | 11.9 |
| Women | | | | |
| n | 1,424 | 174 | 433 | 58 |
| Person-years | 17,684 | 2,027 | 4,925 | 577 |
| CVD (stroke+MI) cases (n) | 23 | 6 | 22 | 5 |
| CVD incidence (/1,000 person-years) | 1.30 | 2.96 | 4.47 | 8.67 |
| Multivariate-adjusted HR (95%CI)† | Reference | 2.64(1.07–6.51) | 2.58(1.42–4.69) | 5.40(2.04–14.25) |
| Multivariate-adjusted HR (95%CI)‡ | Reference | 2.67(1.07–6.65) | 2.33(1.25–4.34) | 4.84(1.81–12.97) |
| PAF | | 6.7 | 22.4 | 7.1 |

Multivariate-adjusted HR (95%CI): †adjusted for age.
Multivariate-adjusted HR (95%CI): ‡adjusted for age, alcohol drinking (never-, ex-, current-), glomerular filtration rate and non-HDL-cholesterol.
PAF, population attributable fraction. Other abbreviations see in Tables 1,2.

by registered hospital physicians or research physicians who were unaware of the baseline information.

The criteria for definite and probable MI were defined according to the criteria of the Monitoring Trends and Determinants of Cardiovascular Disease (MONICA) project,[19] which requires evidence from an ECG, cardiac enzymes, and/or autopsy. Stroke was defined according to the National Survey of Stroke criteria,[20] which require rapid onset of a constellation of neurological deficits lasting at least 24 h or until death. Strokes were classified as ischemic stroke (thrombotic or embolic), intracerebral hemorrhage, subarachnoid hemorrhage, or undetermined type. A definite stroke was defined by autopsy or diagnostic imaging, such as computed tomography or magnetic resonance imaging. In the present study, cases of definite MI or stroke were used in the analysis.

### Statistical Analysis

To compare baseline risk characteristics among the 4 groups classified by the combination of MetS and smoking status, analysis of variance was used for continuous variables, and the chi-squared test was used for dichotomous variables. In this analysis, ex-smoker and never-smoker were classified as non-smokers.

Sex-specific analyses were performed. First, the Cox proportional hazards model was used to estimate the hazard ratios (HR) of smoking status for the incidence of CVD (stroke+MI) and its subtypes. Smoking status was classified as never-, ex-, or current smoker (≤20 cigarettes/day and >20 cigarettes/day). In this analysis, age, body mass index (BMI), systolic BP, FBG, non-HDLC,[11] GFR, and alcohol drinking (never-, ex-, and current drinker) were included as confounding factors.

Second, the source population was divided into 4 groups according to the combination of smoking and the presence of MetS. In this analysis, ex-smoker and never-smoker were also classified as non-smokers. The 2 models were used for estimating the HRs of the combinations for CVD incidence. To adjust for the confounding factors, only age was included in model 1, and alcohol drinking (never, ex-, and current drinker), GFR and non-HDLC were also included in model 2. To express the impact of smoking on CVD incidence in the participants, the PAF (%) was estimated as $Pe\times(HR-1)/HR$, in which Pe is the proportion of incident cases in each category.[21]

All statistical analyses were performed using SPSS statistical software, version 15.0 J (SPSS, Tokyo, Japan). $P<0.05$ (2-tailed) was considered statistically significant.

## Results

### Baseline Characteristics

Among the participants, 901 of the 1,822 men and 232 of 2,089 women were current smokers (smoking rate: men, 49.5%; women, 11.1%). Similarly, 360 men and 491 women had MetS (prevalence: men, 19.8%; women, 23.5%). **Table 1** summarizes the baseline characteristics of the participants classified into 4 groups according to the combination of current smoking and MetS by sex. All variables, except for alcohol drinking in men, were significantly different among the 4 groups.

### Risk of Smoking for CVD Incidence

In the present study, the mean follow-up period was 11.9 years, and 42 definite cases of MI and 111 of definite stroke occurred.

**Table 2** shows the multivariate-adjusted HRs and 95% confidence intervals (CI) of smoking status for the incidence of CVD and its subtypes. In men, the HR of current smokers who were smoking ≤20 cigarettes/day compared with never smokers was 2.65 (95%CI 1.35–5.21) for CVD, 2.47 (95%CI 1.12–5.45) for stroke, 4.06 (95%CI 1.40–11.83) for ischemic stroke, and 2.74 (95%CI 0.80–10.90) for MI. Similarly in women, the HR was 2.70 (95%CI 1.34–5.45) for CVD, 1.60 (95%CI 0.62–4.16) for stroke, 3.00 (95%CI 1.00–8.97) for ischemic stroke, and 8.35 (95%CI 2.64–26.48) for MI. Among the participants who were smoking >20 cigarettes/day, the HRs for CVD incidence were similar to those who were smoking ≤20 cigarettes/day, although in both men and women most of them did not reach to statistical significance because of the small sample size.

Among the ex-smokers, the HR was 1.34 (95%CI 0.67–

2.69) for CVD incidence, 1.07 (95%CI 0.46–2.48) for stroke, 1.94 (95%CI 0.64–5.86) for ischemic stroke, and 2.21 (95%CI 0.61–8.00) for MI in men. In women, there was no case of CVD among ex-smokers.

**Risk of Smoking and MetS for CVD Incidence**

**Table 3** shows the multivariate-adjusted HRs of the combination of smoking and MetS for CVD incidence.

In men, the multivariate-adjusted HRs were 2.07 (95%CI 1.26–3.40) for participants with smoking without MetS, 2.09 (95%CI 1.08–4.04) for those with MetS without smoking, and 3.56 (95%CI 1.89–6.72) for those with both, compared with those both smoking and MetS. In women, the multivariate-adjusted HRs were 2.67 (95%CI 1.07–6.65) for participants with smoking without MetS, 2.33 (95%CI 1.25–4.34) for those with MetS without smoking, and 4.84 (95%CI 1.81–12.97) for those with both, compared with those without both smoking and MetS. When we excluded the ex-smokers among women in this analysis, the HRs were almost similar to the results shown in **Table 3**. And these results were not substantially affected when TC instead of non-HDLC was included as a confounding factor in the Cox proportional hazard models.

In men the PAF for CVD incidence was 21.8% because of smoking, 7.5% because of MetS, and 11.9% because of both. In women, the respective PAFs were 6.7%, 22.4%, and 7.1%.

## Discussion

To our knowledge, this is the first report of a comparison of the CVD risk of smoking, MetS, and both. The magnitude of the HR of smoking or MetS was almost equal. As expected, the risk for the participants with both was the highest. The PAF for CVD incidence among men with smoking alone was much higher than that among those with MetS alone. In women, the PAF among those with MetS was higher than that among those with smoking.

Furthermore, this is also the first report to show the risk of smoking for CVD in an urban area of Japan. In the present study, the prevalence of smoking was 49.5% in men and 11.1% in women. Compared with the data from the National Health and Nutrition Survey conducted in 1989 (men aged 40–69 years in 1989, 50.4–59.5%; women aged 40–69 years in 1988, 6.8–10.6%)[22] and several large collaborative cohort studies in Japan,[8,9,23,24] the prevalence of smoking in the present study was lower in men and higher in women, but is most consistent with the current Japanese prevalence of smoking (men: 39.9%; women: 10.0%). The present study might reflect the prevalence of smoking in urban Japanese communities around the 1990s. In addition, the high smoking prevalence in women and low prevalence in men in the present study is consistent with that in most of the Asia-Pacific region.

Our study showed that smoking is a prominent risk factor for CVD in an urban Japanese cohort, as shown in previous studies in Japanese rural populations.[9,23,24] Similarly, as previously reported,[1,25–27] MetS was a risk factor for CVD in our cohort.[2] The association between MetS and CVD has been reported in several Japanese cohort studies; however, the number of participants was fewer than in the present study,[1] or non-fasting blood samples and BMI were used instead of waist circumference for the analysis.[25] These points are another important strength of our study.

MetS has been reported as associated with high percent plaque volume and abnormal plaque quality in coronary arteries,[28] and chronic subclinical inflammation.[29] As for smoking, Howard et al reported that smoking is associated with progression of an index of atherosclerosis expressed as the intima–medial thickness of the carotid artery.[30] Antoniades et al also stated that smoking induces both functional and structural abnormalities in the vascular wall, by mechanisms involving endothelial dysfunction and impairment of vascular smooth muscle cells in the human arterial tree.[31] They also stated that smoking must be approached within the context of the overall lifestyle: smoking coexists with a pro-atherogenic metabolic profile.[31] The reason for the elevated CVD risk among the present participants with both MetS and smoking is unclear, but the concurrent effect on plaque formation by MetS and smoking, and the additional abnormality in function of vascular smooth muscle cells because of smoking, might be associated with the highest CVD risk among the participants with both risk factors in the present study. Individuals with both smoking and MetS are inevitably in the highest risk group for CVD and should be targeted for intervention.

We compared the HRs of these important CVD risk factors, and the HRs of smoking or MetS for CVD incidence were almost consistent. Accordingly, we calculated the PAF, which shows the impact on CVD incidence. As the result, the PAF of smoking was higher than that of MetS in men, and that of MetS was higher than that of smoking in women, a result that may reflect the higher smoking rate in men. Our study results offer a simple key to solving the problem of "which risk factor should we intervene on first for the population to improve their health outcome". Recently, the smoking rate has been decreasing in Japanese men; however, compared with the United States for example,[10] it remains still high. As well as countermeasures against MetS, we need to continue considering smoking as an important public health problem and promoting antismoking campaigns in Japan.

In Western developed countries such as the United States, evaluating the risk of MetS under a high prevalence of smoking is difficult because the prevalence of smoking is much lower[10] than in Japan. Although the data of the present study are limited to 1 city in Japan, it might offer evidence of the risk of MetS under a high prevalence of smoking.

There has been controversy about defining the optimal diagnostic criteria for MetS. We have already compared the predictive value between the Japanese criteria and the modified NCEP-ATPIII criteria.[2] The results suggested that the modified NCEP-ATPIII criteria are suitable for predicting CVD in the Japanese community setting, as well as in the Hisayama study.[1] Accordingly, in the present study MetS was defined using the modified NCEP-ATPIII criteriai[17,18] Some investigators consider that MetS is an adipose tissue disease different from obesity. If it is an adipose tissue disease, it would be characterized by inflammation detected through high-sensitivity C-reactive protein (hs-CRP) and insulin resistance, reflecting histological changes in adipose tissue.[32] Thus, inflammation-related factors such as hs-CRP might be a candidate for 1 of the components of MetS.[33] Furthermore, according to the Japanese MetS criteria, the prevalence of MetS tends to be very low in women because obesity is a required component and the definition of obesity is waist circumference ≥90 cm. In addition, because some previous studies showed that the prevalence of non-obese individuals with several metabolic risk factors is high

and their CVD risk is also high, the simple exclusion of non-obese participants from the diagnosis of MetS may overlook their potential risk for CVD.[25–27] We might misclassify participants with a high risk for CVD if we adopt the Japanese MetS criteria.

### Study Limitations

First, we could not assess the risk of smoking on the incidence of hemorrhagic stroke because of the small number of cases. Second, the measurement of single MetS components and the questionnaire for smoking in the baseline survey may have underestimated the relationship between these risk factors and CVD because of a regression dilution bias.

In conclusion, smoking is still an important risk factor for CVD in urban areas of Japan, and the combination of smoking and MetS worsens the risk for CVD. Lifestyle modification for not only MetS but also smoking continues to be important in populations with a high PAF for CVD because of a high prevalence of smoking.

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## Disclosure

None.

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# Triglycerides and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort: The Suita study

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ABSTRACT

*Objective:* The impact of elevated triglycerides (TG) and non-high density lipoprotein cholesterol (non-HDLC) on the incidence of stroke and myocardial infarction (MI) has not been well evaluated in Asian populations such as in Japan, which have a lower incidence of myocardial infarction, but a higher risk of stroke than Western populations.
*Methods:* The authors conducted an 11.7-year prospective study ending in 2005 of 5098 Japanese aged 30–79 living in an urban population, initially free of stroke or MI. The relationship between serum lipids and the risk for stroke and MI was determined by dividing the participants into four groups stratified by the combination of serum levels of TG and non-HDLC. The cut-off value was 1.7 mmol/L for TG and 4.9 mmol/L for non-HDLC.
*Results and conclusion:* The total person-years were 59,774 (27,461 for men and 32,313 for women). During the follow-up period, there were 113 cases of MI and 180 of stoke (with 116 cerebral infarctions). Compared with the low TG/low non-HDLC group, the hazard ratio (95% confidence interval) for MI in the high TG/high non-HDLC group was 2.55 (1.53–4.24) after adjustment for other cardiovascular risk factors. The hazard ratio for cerebral infarction in the high TG alone group was 1.63 (1.03–2.56); however, the risk of cerebral infarction was not significantly increased in the other groups. High serum levels of TG and non-HDLC are both important targets for the prevention of cardiovascular disease in Japan.


## 1. Introduction

Previous studies suggested that high levels of serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) are causal risk factors for coronary artery disease (CAD) [1–4] and possibly for ischemic stroke [5]. However, less attention has been paid to high serum levels of triglycerides (TG) [6–8]. Furthermore, although the US National Cholesterol Education Program Adult Treatment Panel guideline III (NCEP-ATP III) has set goals for non-high-density lipoprotein cholesterol (non-HDLC) after the achievement of LDL-C goals in patients with elevated TG [9], the impact of TG and non-HDLC on the incidence of cardiovascular disease (CVD) has not been evaluated in the Japanese population, which has a lower incidence of CAD but a higher risk of stroke than Western populations [10].

Therefore, our a priori hypothesis was that the coexistence of high serum TG and non-HDLC increases the risk of CAD and stroke in the Japanese population. To investigate this hypothesis, we performed a long-term prospective study in an urban, community-dwelling Japanese population.

## 2. Methods

### 2.1. Populations

The Suita study, a cohort study for CVD of urban residents was established in 1989. The details of this study have been described elsewhere [4,11–14]. Briefly, 6485 men and women aged 30–79 years had a baseline survey at the National Cardiovascular Center between September 1989 and March 1994. Of these, a total of 1387 were excluded for the following reasons: past history of coronary heart disease or stroke ($n=210$), lack of participation in the baseline survey ($n=79$), non-fasting visit ($n=166$), use of lipid-lowering agents ($n=125$), missing data ($n=109$), and lost to follow-up ($n=698$). Data from the remaining 5098 participants (2404 men and 2694 women) were included in the analysis. This

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cohort study was approved by the Institutional Review Board of the National Cardiovascular Center.

### 2.2. Baseline examination

Blood samples were collected after the participants had fasted for at least 10 h. The samples were centrifuged immediately and a routine blood examination was performed that included serum total cholesterol (TC), HDL cholesterol, TG and glucose levels.

Blood pressure was measured in triplicate on the right arm after 5 min of rest by well-trained physicians using a standard mercury sphygmomanometer. The average of the second and third measurements was used for analysis. Hypertension was defined as either a systolic blood pressure (SBP) ≥ 140 mmHg, a diastolic blood pressure (DBP) ≥ 90 mmHg or the use of antihypertensive agents. Diabetes was defined as a fasting serum glucose ≥ 7.0 mmol/L (126 mg/dL), the use of anti-diabetic agents, or both. Height with bare feet and weight in light clothing were measured. Waist circumference (WC) was measured at umbilical level in a standing position. Metabolic syndrome (MetS) was defined using modified NCEP-ATP III criteria [13], of which abdominal obesity was defined according to the International Obesity Task Force central obesity criteria for Asia [15].

Public health nurses obtained information on the smoking, drinking and medical histories.

### 2.3. Endpoint determination

The endpoint determination was previously reported [4,11–14]. The endpoints of the present study were: (1) the first myocardial infarction (MI) or stroke event; (2) death; (3) leaving Suita city; or (4) December 31, 2005.

The first step in the survey for MI and stroke involved checking the health status of all participants by repeated clinical visits every two years and yearly questionnaires by mail or telephone. In the second step, in-hospital medical records of participants who were suspected of having an MI or stroke were reviewed by registered hospital physicians or research physicians, who were blinded to the baseline information. The criteria for stroke were defined according to the US National Survey of Stroke criteria [16]. For each stroke subtype [i.e., cerebral infarction, intracerebral hemorrhage, and subarachnoid hemorrhage], a definite diagnosis was established based on the computed tomography, magnetic resonance imaging, or autopsy. The criteria for definite and probable MI were defined according to the criteria of the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project [17]. Sudden deaths of unknown origin that occurred within 24 h of the onset were classified as MI in the present study.

### 2.4. Statistical analysis

The relationship between serum lipids and the risk of MI and stroke was described by dividing the participants into four groups stratified by the combination of serum levels of TG and non-HDLC. We used 1.7 mmol/L (150 mg/dL) of serum TG as a cut-off point for high serum TG according to the classification of NCEP-ATP III [9] and that of the Japan Atherosclerosis Society [3]. The category of non-HDLC ≥4.9 mmol/L (190 mg/dL) was defined as a high serum non-HDLC, which was equivalent to 6.2 mmol/L (240 mg/dL) of TC or 4.1 mmol/L (160 mg/dL) of LDL-C, because non-HDLC was usually 0.8 mmol/L (30 mg/dL) higher than LDL-C [9,18–19].

Continuous variables between groups were compared by analysis of variance and categorical variables were compared by a chi-square test. The hazard ratio (HR) for MI or stroke was calculated using a proportional hazards model adjusted for age, hypertension (dichotomous variable), diabetes, HDL-C, body mass index (BMI), smoking (never-smoked; ex-smoker; current smoker) and drinking (never-drank; ex-drinker; regular drinker) (model 1). Sex-combined analysis with further adjustment for sex was also performed. Another statistical model after replacement of BMI and hypertension with WC and SBP level (continuous variable) was also performed (model 2).

All confidence intervals were estimated at the 95% level and significance was set at a $P$ value of <0.05. The Statistical Package for the Social Sciences (SPSS Japan Inc. version 15.0J, Tokyo, Japan) was used for all the analyses.

## 3. Results

The median and interquartile range of serum TG in the baseline survey was 1.29 mmol/L (0.90, 1.90) in men and 0.98 mmol/L (0.73, 1.41) in women. The mean baseline serum non-HDLC was 3.93 ± 0.91 mmol/L in men and 4.03 ± 1.03 mmol/L in women.

The means or prevalence of major cardiovascular risk factors in each group stratified by the combination of serum levels of TG and non-HDLC are summarized in Table 1. There was no significant difference in mean age and the prevalence of smoking among the TG and non-HDLC groups for men. There were significant differences in all other variables. Mean BMI, waist circumference and the prevalence of hypertension and diabetes were highest in the high-TG/high non-HDLC group, whereas the values of these parameters were lowest in the low-TG/low-non-HDLC group for both sexes. The prevalence of Mets was much higher in the high-TG groups than in the low-TG groups irrespective of non-HDLC level.

The total person-years were 59,774 (27,461 for men and 32,313 for women), with a mean follow-up period of 11.7 years. During the follow-up period, there were 113 first MIs and 180 first strokes. The strokes consisted of 28 intracerebral hemorrhages, 116 cerebral infarctions, 21 subarachnoid hemorrhages and 15 unclassified cases.

Table 2 shows the number of cases, age and multivariable-adjusted HRs for MI stratified by TG and non-HDLC. Compared with the low TG/low non-HDLC group, the HR for MI in the high TG/high non-HDLC group was 2.05 (95% confidence interval, CI, 1.08–3.90) in men, 3.79 (95% CI, 1.58–9.14) in women and 2.55 (95% CI, 1.53–4.24) in both sexes combined in multivariable adjusted model 1. We did not observe a significant increase in the HR for MI in the other groups. Similar results were observed after replacement of BMI and hypertension with WC and SBP level (model 2).

Table 3 shows the multivariable-adjusted HRs for cerebral infarction stratified by levels of TG and non-HDLC. Compared with the low TG/low non-HDLC group, the HR for cerebral infarction in the high TG alone group (high TG/low non-HDLC group) was 1.45 (95% CI, 0.84–2.50) in men, 2.09 (95% CI, 0.92–4.73) in women and 1.63 (95% CI, 1.03–2.56) in both sexes combined in statistical model 1. There was no significant increase of cerebral infarction in the other groups. Similar results were also observed in statistical model 2.

The incidence of total stroke, intracerebral hemorrhage and subarachnoid hemorrhage was not related to TG and non-HDLC levels in either sex. When the participants were divided into two groups by age (<60 and ≥60), the results of all the analyses listed above were similar in both age groups (data not shown).

## 4. Discussion

To our knowledge, this is the first cohort study in Japan to clarify the risk for MI and ischemic stroke of high serum level of TG, non-HDLC and both. The risk for MI of both high serum TG and non-HDLC was considerably higher than the risk without both or with only one. This relationship was similarly observed in both men and

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**Table 1**
Means and prevalence of major cardiovascular risk factors in each group stratified by the combination of serum levels of triglycerides (TG) and non-high-density lipoprotein cholesterol (non-HDLC).

| Variables | Low TG/low Non-HDLC | | Low TG/high Non-HDLC | | High TG/low Non-HDLC | | High TG/high Non-HDLC | | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| Men | | | | | | | | | |
| No. of subjects | 1532 | | 117 | | 550 | | 205 | | |
| Non-HDLC (stratum mean), mmol/L | 3.6 | (0.7) | 5.4 | 0.4 | 4.0 | 0.6 | 5.5 | 0.5 | |
| Triglycerides (stratum median), mmol/L | 1.0 | (0.8, 1.3)* | 1.3 | (1.0, 1.5)* | 2.2 | (1.9, 2.9)* | 2.4 | (2.0, 3.7)* | |
| Age, years | 55.8 | (13.5) | 57.4 | (12.9) | 54.8 | (12.7) | 54.8 | (11.8) | 0.16 |
| HDLC, mmol/L | 1.4 | (0.3) | 1.3 | (0.3) | 1.1 | (0.3) | 1.1 | (0.2) | <0.01 |
| BMI, kg/m$^2$ | 22.2 | (2.8) | 23.1 | (3.1) | 23.8 | (2.6) | 24.2 | (2.6) | <0.01 |
| Waist circumference, cm | 80.8 | (7.9) | 82.7 | (8.6) | 85.7 | (7.0) | 86.3 | (6.9) | <0.01 |
| Systolic blood pressure, mmHg | 127 | (21) | 129 | (20) | 130 | (20) | 132 | (21) | <0.01 |
| Diastolic blood pressure, mmHg | 78 | (12) | 79 | (12) | 81 | (11) | 82 | (11) | <0.01 |
| Hypertension, % | 30.0 | | 35.0 | | 36.4 | | 38.0 | | 0.01 |
| Diabetes, % | 4.8 | | 4.3 | | 7.5 | | 9.3 | | 0.02 |
| Metabolic syndrome, %** | 4.5 | | 4.3 | | 45.1 | | 47.8 | | <0.01 |
| Smoking, % | | | | | | | | | |
| Current smoker | 49.9 | | 43.6 | | 53.5 | | 47.3 | | 0.51 |
| Ex-smoker | 30.3 | | 35.0 | | 28.4 | | 32.7 | | |
| Never-smoker | 19.8 | | 21.4 | | 18.2 | | 20.0 | | |
| Drinking, % | | | | | | | | | |
| Current drinker | 76.0 | | 63.2 | | 76.4 | | 69.3 | | 0.02 |
| Ex-drinker | 3.6 | | 6.0 | | 2.9 | | 5.4 | | |
| Never-drinker | 20.4 | | 30.8 | | 20.7 | | 25.4 | | |
| Women | | | | | | | | | |
| No. of subjects | 1956 | | 290 | | 256 | | 192 | | |
| Non-HDLC (stratum mean), mmol/L | 3.6 | (0.7) | 5.5 | (0.5) | 4.2 | (0.5) | 5.8 | (0.8) | |
| Triglycerides (stratum median), mmol/L | 0.9 | (0.7, 1.1)* | 1.2 | (0.9, 1.4)* | 2.0 | (1.8, 2.4)* | 2.4 | (2.0, 3.0)* | |
| Age, years | 51.5 | (12.9) | 59.3 | (9.6) | 57.9 | (11.2) | 60.7 | (8.8) | <0.01 |
| HDLC, mmol/L | 1.5 | (0.3) | 1.4 | (0.3) | 1.2 | (0.3) | 1.1 | (0.3) | <0.01 |
| BMI, kg./m2 | 21.7 | (3.1) | 22.9 | (3.1) | 23.6 | (3.3) | 24.2 | (3.1) | <0.01 |
| Waist circumference, cm | 75.5 | (9.8) | 79.8 | (9.7) | 82.7 | (10.0) | 83.5 | (9.7) | <0.01 |
| Systolic blood pressure, mmHg | 121 | (21) | 131 | (21) | 132 | (21) | 137 | (21) | <0.01 |
| Diastolic blood pressure, mmHg | 73 | (12) | 79 | (12) | 79 | (12) | 80 | (13) | <0.01 |
| Hypertension, % | 20.4 | | 37.9 | | 37.1 | | 48.4 | | <0.01 |
| Diabetes, % | 2.4 | | 4.5 | | 6.6 | | 7.8 | | <0.01 |
| Metabolic syndrome, %** | 7.5 | | 19.3 | | 66.8 | | 74.5 | | <0.01 |
| Smoking, % | | | | | | | | | |
| Current smoker | 11.8 | | 8.6 | | 14.5 | | 16.1 | | 0.04 |
| EX-smoker | 3.5 | | 2.8 | | 2.7 | | 6.3 | | |
| Never-smoker | 84.7 | | 88.6 | | 82.8 | | 77.6 | | |
| Drinking, % | | | | | | | | | |
| Current drinker | 34.9 | | 29.3 | | 28.5 | | 24.5 | | <0.01 |
| Ex-drinker | 1.8 | | 0.3 | | 0.8 | | 4.2 | | |
| Never-drinker | 63.3 | | 70.3 | | 70.7 | | 71.4 | | |

TG, triglycerides; non-HDLC, non-high-density lipoprotein cholesterol; BMI, body mass index. Brackets indicate standard deviation.
Analysis of variance was used for comparisons of multiple group means and the chi-square test was used to compare proportions.

* Inter-quartile range.

** MetS was defined using modified NCEP-ATP III. Abdominal obesity was defined as a waist circumference ≥0.90 m in men and ≥ 0.80 m in women. High blood pressure was defined as average systolic/diastolic blood pressures of ≥ 130/85 mm Hg and/or current medication for hypertension. High triglyceride was defined as serum triglycerides of ≥1.7 mmol/L. Low HDL cholesterol was defined as serum HDL cholesterol levels of <1.03 mmol/L in men and of <1.29 mmol/L in women. High blood glucose was defined as fasting blood glucose of ≥6.1 mmol/L and/or current use of anti-diabetic medication. MetS was defined as the presence of three or more of these components.

women. In contrast, the risk for ischemic stroke was highest in the participants with high TG alone.

TG-rich lipoproteins have been shown to be atherogenic, and thus, they are associated with coronary atherosclerosis [9,19–20]. As NCEP-ATP III pointed out [9], elevated non-HDLC is a good therapeutic target in patients with high TG, because the serum concentration of non-HDLC reflects not only LDL-C but also the cholesterol content of all other TG-rich and apolipoprotein B containing lipoproteins, such as very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), small dense LDL particles and their remnant lipoproteins [19–20]. In the Helsinki Heart study [21], most of the risk for coronary heart disease (CHD) was confined to participants with high levels of both TG and LDL-C. In the West of Scotland Coronary Prevention Study [22], a higher incidence of CHD was observed in men in both the pravastatin and placebo groups when TG was at or above the median level. Pischon et al. suggested that TG added significant information to non-HDLC for CAD risk prediction in a nested case-control study [23]. Our findings are consistent with previous studies.

Similar to previous studies in Japan [4,10], we found no association between non-HDLC and cerebral infarction even in the presence of high serum TG, which may be due to a lower prevalence of atherothrombotic infarction than in Western populations. The ARIC study indicated that TC was associated with increased risk of non-lacunar, non-embolic stroke (atherothrombotic infarction), but not with lacunar or embolic stroke [24]. A recent report from a Japanese rural population showed that LDL-C is a risk factor for only atherothrombotic infraction [25]. Unfortunately, due to the relatively small stroke cases in our study, we were not able to demonstrate an association between any subtype of cerebral infarction and non-HDLC.

It is not clear why participants with high TG alone showed the increased risk for cerebral infarction in the present study. In a meta-analysis of 26 cohort studies in Asia-Pacific area, partici-

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**Table 2**
Age and multivariable-adjusted hazard ratios (95% confidence intervals) for myocardial infarction stratified by TG and non-HDLC groups in an 11.7-year prospective study of 5098 Japanese men and women.

| | Low TG/low Non-HDLC | Low TG/high Non-HDLC | High TG/low Non-HDLC | High TG/high Non-HDLC |
|---|---|---|---|---|
| Men | | | | |
| Person-years | 17410 | 1288 | 6358 | 2404 |
| Case, *n* | 45 | 6 | 11 | 14 |
| Age adjusted | 1.00 | 1.63 (0.70-3.83) | 0.76 (0.39-1.48) | 2.74(1.50-5.02) |
| Model 1[a] | 1.00 | 1.48 (0.62-3.49) | 0.63 (0.32-1.26) | 2.05(1.08-3.90) |
| Model 2[b] | 1.00 | 1.55 (0.66-3.66) | 0.64 (0.32-1.29) | 2.10 (1.10-3.98) |
| Women | | | | |
| Person-years | 23652 | 3455 | 2936 | 2270 |
| Case, *n* | 14 | 5 | 6 | 12 |
| Age adjusted | 1.00 | 1.59 (0.57-4.40) | 2.28 (0.88-5.94) | 4.88 (2.25-10.6) |
| Model 1[a] | 1.00 | 1.63 (0.58-4.26) | 1.99 (0.71-5.57) | 3.79 (1.58-9.14) |
| Model 2[b] | 1.00 | 1.55 (0.55-4.38) | 1.92 (0.69-5.34) | 3.18 (1.34-7.52) |
| Men and women | | | | |
| Person-years | 41062 | 4743 | 9294 | 4674 |
| Case, *n* | 59 | 11 | 17 | 26 |
| Age adjusted | 1.00 | 1.51 (0.79-2.89) | 1.04 (0.60-1.78) | 3.42 (2.15-5.44) |
| Model 1[a] | 1.00 | 1.42 (0.74-2.74) | 0.86 (0.49-1.53) | 2.55 (1.53-4.24) |
| Model 2[b] | 1.00 | 1.45 (0.75-2.79) | 0.87 (0.49-1.54) | 2.48 (1.49-4.10) |

TG, triglycerides; non-HDLC, non high-density lipoprotein cholesterol.
[a] Multivariable adjusted for age, body mass index, hypertension, diabetes, HDL (high-density lipoprotein) cholesterol, cigarette smoking and alcohol intake by a Cox proportional hazard model. Sex was also adjusted in the men and women combined model.
[b] Replacement of body mass index and hypertension as covariates in model 1 with waist circumference and systolic blood pressure level.

pants grouped in the highest fifth of serum TG had a 50% increased risk of stroke compared with those in the lowest fifth [26]. Recent reviews have also concluded that hypertriglyceridemia seems to be a causal risk factor for ischemic stroke [7–8]. However, abovementioned findings were not able to explain the low incidence of cerebral infarction in the high TG/high non-HDLC group in the present study. An elevated risk for MI might mask the relationship between TG and cerebral infarction; because there would be no further follow-up after a first MI. Another large study concerning about the relationship between serum TG and stroke should be needed.

Recently, we have reported that high serum LDLC and non-HDLC are both associated with an increased risk of MI; and the predictive value of non-HDLC for MI is almost similar to that of LDL-C [4]. However, we did not use serum TG as a covariate to avoid overadjustment, because difference between serum level of LDLC and non-HDLC was automatically determined by serum TG level when serum LDLC value was calculated by the Friedewald formula [27]. Considering all the findings together, non-HDLC and TG may be recommended as beneficial screening markers for primary prevention of CAD in the Japanese community, as they are less expensive and more convenient because non-HDLC can be calculated irrespective of serum TG level.

The present study has some limitations. First, the single TG and non-HDLC measurement at the baseline survey may have underestimated the relationship between these lipids and cardiovascular disease due to regression dilution bias. Furthermore, we did not evaluate longitudinal trend for each risk factor and its medication status after baseline survey. Especially, hypertriglyceridemia is associated with not only present existence of metabolic components, such as hypertension and diabetes, but also new onset

**Table 3**
Age and multivariable-adjusted hazard ratios (95% confidence intervals) for cerebral infarction stratified by TG and non-HDLC groups in an 11.7-year prospective study of 5098 Japanese men and women.

| | Low TG/low Non-HDLC | Low TG/high Non-HDLC | High TG/low Non-HDLC | High TG/high Non-HDLC |
|---|---|---|---|---|
| Men | | | | |
| Person-years | 17410 | 1288 | 6358 | 2404 |
| Case, *n* | 46 | 2 | 22 | 5 |
| Age adjusted | 1.00 | 0.53 (0.13-2.19) | 1.51 (0.91-2.52) | 0.99 (0.39-2.51) |
| Model 1[a] | 1.00 | 0.54 (0.13-2.25) | 1.45 (0.84-2.50) | 0.92 (0.35-2.38) |
| Model 2[b] | 1.00 | 0.56 (0.14-2.31) | 1.48 (0.86-2.56) | 0.75 (0.26-2.14) |
| Women | | | | |
| Person-years | 23652 | 3455 | 2936 | 2270 |
| Case, *n* | 20 | 8 | 10 | 3 |
| Age adjusted | 1.00 | 1.77 (0.78-4.02) | 2.62 (1.23-5.60) | 0.81 (0.24-2.72) |
| Model 1[a] | 1.00 | 1.52 (0.66-3.50) | 2.09 (0.92-4.73) | 0.69 (0.20-2.44) |
| Model 2[b] | 1.00 | 1.54 (0.67-3.54) | 2.10 (0.93-4.73) | 0.77 (0.22-2.71) |
| Men and women | | | | |
| Person-years | 41062 | 4743 | 9294 | 4674 |
| Case, *n* | 66 | 10 | 32 | 8 |
| Age adjusted | 1.00 | 1.14 (0.58-2.23) | 1.82 (1.19-2.79) | 0.94 (0.45-1.95) |
| Model 1[a] | 1.00 | 1.12 (0.57-2.20) | 1.63 (1.03-2.56) | 0.79 (0.37-1.69) |
| Model 2[b] | 1.00 | 1.12 (0.57-2.21) | 1.62 (1.03-2.55) | 0.69 (0.62-1.88) |

TG, triglycerides; non-HDLC, non high-density lipoprotein cholesterol.
[a] Multivariable adjusted for age, body mass index, hypertension, diabetes, HDL (high-density lipoprotein) cholesterol, cigarette smoking and alcohol intake by a Cox proportional hazard model. Sex was also adjusted in the men and women combined model.
[b] Replacement of body mass index and hypertension (prevalence) as covariates in model 1 with waist circumference and systolic blood pressure levels.

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of them in the future [28,29]. Second, we did not measure serum apolipoprotein B (apoB) [22], apolipoprotein A1 (ApoA1) and LP(a) [30], which some previous studies have shown to be strong risk factors for CAD [22]. Third, a recent study indicated that non-fasting TG is a better predictor of CAD than fasting TG [31]. However, in a large individual based meta-analysis in the Asia-Pacific region [26], most blood samples were collected during fasting, and there was a significant positive relationship between serum TG and CAD or stroke.

In conclusion, a combination of higher serum levels of TG and non-HDLC is associated with an increased risk of MI in a Japanese population. Furthermore, the risk for ischemic stroke was highest in the participants with high TG alone; however, further research should be needed. High serum levels of TG and non-HDLC are both important targets for the prevention of cardiovascular disease, which requires evidence-based guidelines for management in the primary care setting.

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CARDIOVASCULAR DISEASES

# Elevated serum creatine kinase predicts first-ever myocardial infarction: a 12-year population-based cohort study in Japan, the Suita study

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**Background** In myocardial infarction (MI), it is well known that serum creatine kinase (s-CK) increases after onset, but it is unclear whether s-CK elevates before MI onset. The present analysis examined whether elevated s-CK levels predicted first-ever MI or stroke.

**Methods** This study was a population-based cohort study in a Japanese urban area. Study subjects were comprised of 5026 initially healthy Japanese (2370 men and 2656 women, mean age: 54.5 years) without a history of MI or stroke. They were followed-up for 11.8 years on average, and 103 MIs (definite: 45; probable: 58) and 168 strokes (definite: 126; probable: 42) were observed. There was no subject who developed MI just at baseline (the follow-up period among those with definite MI was, at earliest, 0.20 years).

**Results** The adjusted hazard ratio for definite MI was 4.18 (95% confidence interval 1.66–10.53) with s-CK levels of $\geqslant$200 IU/l, compared with the reference category (s-CK levels of $\leqslant$99 IU/l), whereas no relationship was observed between s-CK levels and the risk for stroke. With regard to definite MI, an interaction between s-CK levels and dyslipidaemia was observed. Among subjects with hypercholesterolaemia, the hazard ratio linearly elevated with increased s-CK levels. On the other hand, no linear elevation was observed among subjects without hypercholesterolaemia ($P$ for interaction = 0.011).

**Conclusions** The present study suggested that screening for elevated s-CK levels in initially healthy Japanese subjects was useful to predict first-ever MI in the future, especially in subjects with dyslipidaemia.

**Keywords** Creatine kinase, myocardial infarction, stroke, cohort studies, urban population

## Introduction

Creatine kinase is included mainly in skeletal muscle, cardiac muscle and brain, and is involved in energy production. Serum creatine kinase (s-CK) levels in healthy individuals are influenced by age, sex, race, pregnancy, muscle mass and physical activity, and is well known to be elevated following any damage to or disease of the above-mentioned organs.[1]

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In myocardial infarction (MI), s-CK levels usually increase within 4–8 h after MI onset and return to normal 48–72 h later.[2] It is unclear, however, whether s-CK elevation occurs before MI onset. If s-CK elevates before MI onset, s-CK elevation might predict MI incidence, and three possible hypotheses for background mechanism can be considered. First, small amounts of intramyocardial proteins such as creatine kinase might leak into serum by micro-infarction or ischaemia in myocardium.[3,4] Secondly, s-CK might be a surrogate marker of hypothyroidism, as recent meta-analyses reported the relationship between hypothyroidism and a modest increased risk for coronary heart diseases.[5,6] Thirdly, medication for hyperlipidaemia that can occasionally cause rhabdomyolysis with s-CK elevation might be involved.[7,8]

To our knowledge, there has been no previous cohort study that elucidated the association between s-CK levels at baseline and a risk of cardiovascular diseases (CVDs). Therefore, as a first step of testing the above-stated hypotheses, the present analysis examined whether elevated s-CK levels predicted first-ever MIs or strokes in a 12-year population-based cohort study of a Japanese urban area.

# Methods

## Study design and samples

The Suita study is a population-based cohort study in an urban area located in the mid-west part of Japan.[9–11] In 1989, the Suita study randomly sampled 12 200 Japanese men and women, aged 30–79 years, from the Suita city residents by sex and age class (10-year increments). From this sample, 6406 men and women participated in a baseline survey (participation rate: 52.5%) at the National Cardiovascular Center from September 1989 to March 1994, and were enrolled as study subjects. They have been examined every 2 years and followed-up to December 2005.

A total of 1380 subjects were excluded from this analysis for the following reasons: past or present history of MI or stroke at baseline ($n=208$); abnormal Q wave on standard 12-lead electrocardiogram (ECG) ($n=3$, corresponding to 1-1 in Minnesota code); missing data for s-CK or any other explanatory variables at baseline ($n=330$); ST depression on baseline electrocardiogram (corresponding to 4-1 in Minnesota code) or angina on treatment ($n=139$); and lost to follow-up ($n=700$). Consequently, 5026 initially healthy subjects (2370 men and 2656 women, mean age: 54.5 years) were included in this analysis. Informed consent was obtained from all subjects. This study was approved by the institutional review board at the National Cardiovascular Center.

## Baseline data collection

A baseline survey included questionnaires, anthropometric measurements, a standard 12-lead ECG and blood sample testing with overnight fasting ($\geqslant 10$ h). Height and weight were measured in light clothing, and body mass index was calculated as weight (kilograms) divided by height (square metres). Blood pressure of subjects was measured three times by well-trained physicians in a sitting position after $\geqslant 5$ min of rest, using a standard mercury sphygmomanometer. Blood sample tests included serum total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, creatinine, creatine kinase and fasting plasma glucose (FPG), and all samples were immediately centrifuged after blood sampling and analysed by an automatic analyser at the laboratory of the National Cardiovascular Center. Creatine kinase was measured in serum with the Japan Society of Clinical Chemistry (JSCC) method.

The standard 12-lead ECGs were double-checked and coded with Minnesota code by well-trained physicians.[12,13] Physicians or nurses administrated questionnaires for life styles and past medical history. Current cigarette use was defined as smoking at least one cigarette a day. Current alcohol consumption was defined as drinking alcohol at least once a week.

## Ascertainment of MI or stroke events

Details for the ascertainment of MI or stroke events were previously described elsewhere.[9–11] To detect MI or stroke events, each subject's health status was checked by physicians or nurses at clinical visits to the National Cardiovascular Center every 2 years. In addition, yearly questionnaires by mail or telephone were also completed for all participants. Those who reported to have developed MI or stroke were confirmed by a review of medical records after informed consent was obtained from each individual. Medical record review was performed by physicians at either the registered hospitals or the administration office of this cohort study. The percentage of informed consents obtained for medical record review was 86.2%.

With regard to MI, definite and probable MI was defined according to the criteria set out by the MONICA project, which requires evidence from ECGs, cardiac enzymes and/or autopsy.[14] Strokes were defined according to the National Survey of Stroke criteria, which require the rapid onset of a series of neurological deficits lasting $\geqslant 24$ h or until death. For each stroke subtype [i.e. cerebral infarction (thrombotic or embolic infarction), intracerebral haemorrhage and subarachnoid haemorrhage], a definite diagnosis was established, based on examination of computed tomographic scans, magnetic resonance images or autopsies.[15]

In order to complete the surveillance for fatal stroke and MI, we also conducted a systematic search of death certificates (all death certificates in Japan are forwarded to the Ministry of Health, Welfare and Labour and coded for National Vital Statistics) for the following subjects: (i) those without informed

consent for medical records survey to confirm MI or stroke events that were detected at regular visits to the National Cardiovascular Center or by annual questionnaires; and (ii) those with death certificates who had not been in our register of MI or stroke events. These subjects were defined as probable stroke or MI.

### Statistical analysis

s-CK levels were divided into four categories with 100 IU/l increments (i.e. ⩽99, 100–199, 200–299, ⩾300) to compare baseline characteristics and calculate overall and sex-specific crude incidence rates (per 1000 person-years) by the following subtypes of CVD: all MI (including probable and definite MI), definite MI, all strokes, cerebral infarction and haemorrhagic or unclassified strokes. Hypertension was defined as systolic blood pressure ⩾140 mmHg, diastolic blood pressure ⩾90 mmHg or use of anti-hypertensive medication. Impaired fasting glucose or diabetes was defined as FPG ⩾6.1 mmol/l (110 mg/dl) or use of anti-diabetic medication. Hypercholesterolaemia was defined as total cholesterol ⩾5.7 mmol/l (220 mg/dl) or use of anti-hypercholesterolemic medication. Hypertriglyceridaemia was defined as triglycerides ⩾1.7 mmol/l (150 mg/dl) or use of anti-hypertriglyceridemic medication. Low HDL-cholesterolaemia was defined as HDL cholesterol ⩽1.0 mmol/l (39 mg/dl).

s-CK levels of ⩾200 IU/l were unified into one category (i.e. creating three categories with 100 IU/l increments: ⩽99, 100–199 and ⩾200) to estimate age- and multiple-adjusted hazard ratios (HRs), using a Cox regression model with a reference category of s-CK of ⩽99 IU/l. This is because the number of subjects and events in s-CK levels of ⩾300 IU/l was substantially smaller than other categories and because crude incidence rates of s-CK levels of ⩾300 IU/l were also similar with those of s-CK of 200–299 IU/l. Categories of s-CK levels were entered into models as dummy variables. Men and women were combined in estimating HRs with 95% confidence interval (95% CI) because there was no interaction between sex and s-CK levels. Each model adjusted for the following confounding factors: Model 1 adjusted for age; Model 2 adjusted for age, sex, body mass index, hypertension, impaired fasting glucose or diabetes, hypercholesterolaemia, hypertriglyceridaemia, low HDL-cholesterolaemia, creatinine, current cigarette use and current alcohol consumption. Three additional analyses were conducted in Model 2 for definite MI: one excluded 111 further subjects with 4-2, 4-3 in Minnesota code ($n=4915$, number of MI events = 45), another further adjusted for regular physical activity habits (yes or no; 224 subjects were excluded because of missing data, $n=4802$, number of MI events = 45) and the other excluded 51 subjects in the 99th percentile or higher of s-CK ($n=4975$, number of MI events = 44).

The follow-up period of each participant was divided into four categories (0.20–3.30 years, 3.31–4.99 years, 5.00–8.40 years and 8.41–13.89 years), each of which included an equal number of subjects with definite MI. In each category, the median baseline s-CK levels were compared between subjects with and without definite MI in order to examine whether a specific period of follow-up was involved in the present results.

With regard to definite MI, interactions between s-CK levels and each of several confounding factors (i.e. hypercholesterolaemia, hypertriglyceridaemia, low HDL-cholesterolaemia, hypertension, impaired glucose tolerance or diabetes, current cigarette use and current alcohol consumption) were tested by adding the interaction term to Model 2. HRs by s-CK levels and confounding factors were estimated, adjusting for the same factors as Model 2 except for the stratifying factor of each interaction.

The *P*-value for trend was calculated to test for linearity of HRs. For blood pressure, a third measurement was adopted in all analyses. All *P*-values were two-tailed.

## Results

The overall distribution of baseline s-CK levels (minimum, 25th percentile, median, 75th percentile, maximum) for men was 30, 101, 132, 176 and 3547 IU/l, respectively, and for women it was 25, 87, 110, 141 and 1820 IU/l, respectively. There were 1.0% of men and 0.3% of women with s-CK levels of ⩾500 IU/l.

Baseline characteristics are shown by s-CK levels in Table 1. Regardless of sex, body mass index and creatinine tended to increase with elevation in s-CK levels, and the prevalence of current cigarette smoker decreased with elevation in s-CK levels. In women, higher s-CK levels were associated with an increased prevalence of hypertension, hypercholesterolaemia and hypertriglyceridaemia.

Study subjects were followed for 11.8 years on average, and 103 MIs (definite: 45; probable: 58) and 168 strokes (definite: 126; probable: 42) were observed. Strokes were comprised of 27 intracerebral haemorrhages, 106 cerebral infarctions, 20 subarachnoid haemorrhages and 15 unclassified strokes. The follow-up period among those with definite MI was from 0.20 to 13.89 years.

Median baseline s-CK levels were higher among subjects with definite MI than among subjects without definite MI, regardless of the follow-up period (Figure 1).

Overall and sex-specific crude incidence rates were presented by s-CK levels and CVD subtype (Table 2). Overall, incidence rates of MI or stroke were higher in men than in women, and rates of stroke were higher than MI. Overall or sex-specific crude incidence rates of definite MI tended to be higher in subjects with

**Table 1** Baseline characteristics by s-CK levels in a cohort study of a Japanese urban area, 1989–2005

| | Men | | | | Women | | | |
|---|---|---|---|---|---|---|---|---|
| | Creatine kinase (IU/l) | | | | Creatine kinase (IU/l) | | | |
| | ⩽99 | 100–199 | 200–299 | ⩾300 | ⩽99 | 100–199 | 200–299 | ⩾300 |
| Number of subjects | 566 | 1396 | 306 | 102 | 1024 | 1420 | 176 | 36 |
| Age (years) | 58.1 (12.4) | 54.2 (13.1) | 55.4 (13.3) | 57.4 (14.4) | 51.1 (12.5) | 54.8 (12.4) | 57.6 (12.3) | 58.8 (10.3) |
| Body mass index (kg/m$^2$) | 22.5 (2.8) | 22.9 (2.9) | 23.2 (3.1) | 22.9 (2.8) | 21.8 (3.0) | 22.4 (3.3) | 22.8 (3.4) | 23.2 (2.5) |
| Hypertension (%)[a] | 32.9 | 32.3 | 34.6 | 36.3 | 21.8 | 27.6 | 34.7 | 50.0 |
| IFG or diabetes (%)[b] | 21.2 | 13.8 | 8.8 | 13.7 | 8.6 | 8.7 | 10.2 | 11.1 |
| Hypercholesterolaemia (%)[c] | 30.6 | 26.8 | 34.6 | 26.5 | 33.2 | 46.0 | 48.3 | 63.9 |
| Hypertriglyceridaemia (%)[d] | 38.2 | 30.7 | 26.8 | 28.4 | 16.4 | 16.7 | 19.9 | 30.6 |
| Low HDL-cholesterolaemia (%)[e] | 29.0 | 24.1 | 18.6 | 21.6 | 9.9 | 7.8 | 9.7 | 5.6 |
| Creatinine (mmol/l) | 78.1 (19.7) | 80.4 (17.7) | 80.9 (17.1) | 85.7 (18.7) | 58.7 (17.3) | 62.2 (15.1) | 62.6 (15.6) | 63.6 (16.5) |
| **Medication** | | | | | | | | |
| Hypertension (%) | 11.1 | 10.0 | 9.2 | 12.8 | 7.8 | 10.2 | 11.4 | 27.8 |
| Diabetes (%) | 3.2 | 2.3 | 0.0 | 1.0 | 1.4 | 1.1 | 1.1 | 0.0 |
| Hypercholesterolaemia (%) | 1.6 | 1.2 | 1.6 | 2.9 | 1.1 | 2.6 | 4.6 | 8.3 |
| Hypertriglyceridaemia (%) | 1.2 | 0.4 | 0.3 | 2.0 | 0.2 | 0.3 | 1.1 | 2.8 |
| Current cigarette use (%)[f] | 53.0 | 51.0 | 46.1 | 36.3 | 15.3 | 9.7 | 8.5 | 5.6 |
| Current alcohol consumption (%)[g] | 73.1 | 75.7 | 75.2 | 77.5 | 35.3 | 32.0 | 29.6 | 30.6 |

Means in continuous variables are shown with standard deviation in parentheses.
[a]Hypertension was defined by systolic blood pressure ⩾140 mm Hg or diastolic blood pressure ⩾90 mm Hg or use of anti-hypertensive medication.
[b]IFG or diabetes were defined by FPG ⩾6.1 mmol/l (110 mg/dl) or use of anti-diabetic medication.
[c]Hypercholesterolaemia was defined by total cholesterol ⩾5.7 mmol/l (220 mg/dl) or use of anti-hypercholesterolaemic medication.
[d]Hypertriglyceridaemia was defined by triglycerides ⩾1.7 mmol/l (150 mg/dl) or use of anti-hypertriglyceridaemic medication.
[e]Low HDL-cholesterolaemia was defined by HDL cholesterol ⩽1.0 mmol/l (39 mg/dl).
[f]Current cigarette use was defined as smoking at least one cigarette a day.
[g]Current alcohol consumption was defined as drinking alcohol at least once a week.
IFG = impaired fasting glucose.

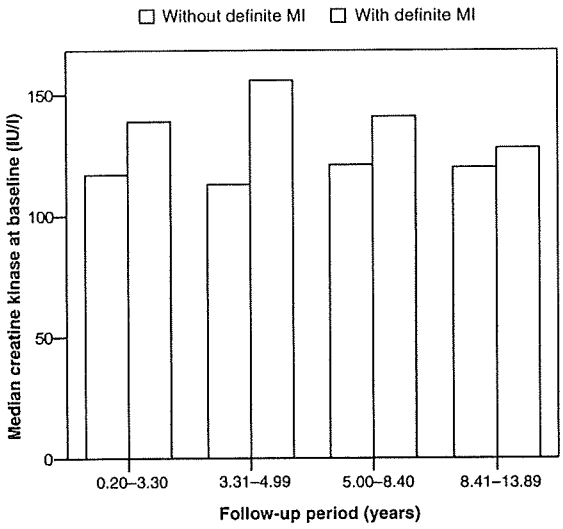


**Figure 1** Comparison of median s-CR levels between subjects with and without definite MI by follow-up periods of 0.20–3.30 years, 3.31–4.99 years, 5.00–8.40 years and 8.41–13.89 years

s-CK levels of ⩾200 IU/l, compared to subjects with levels of ⩽199 IU/l. Regarding all strokes, this relationship was unclear, although the incidence rate of cerebral infarction in subjects with s-CK levels of ⩾200 IU/l was slightly higher than in subjects with levels of ⩽199 IU/l.

Age- and multiple-adjusted HRs are shown in Table 3. Compared with the reference group (⩽99 IU/l), the adjusted HRs for definite and all MI in subjects with s-CK levels of ⩾200 IU/l were 4.18 (95% CI 1.66–10.53) and 2.06 (95% CI 1.15–3.70), respectively, in Model 2, and were also linearly elevated with increased s-CK levels, regardless of the model.

In Model 2 for definite MI, excluding further subjects with ST depression on the standard 12-lead ECG, corresponding to 4-2, 4-3 and 4-1 in Minnesota code, or adjusting regular physical activity habits had little effect on the results. In addition, excluding 51 subjects in the 99th percentile or higher of s-CK from the analysis also hardly altered the results. No relationships were observed between s-CK levels and risk for all strokes or cerebral infarction.

For definite MI, interactions between lipid status (hypercholesterolaemia, hypertriglyceridaemia, low HDL-cholesterolaemia) and s-CK levels were shown in Table 4. Interactions in hypercholesterolaemia were observed ($P$ for interaction = 0.011). Among those subjects with hypercholesterolaemia, HRs were linearly elevated, and the HR in s-CK levels of ⩾200 IU/l was 10.05 (95% CI 2.74–36.81). Similar interaction and linear elevations of HRs were observed among subjects with hypertriglyceridaemia ($P$ for interaction = 0.033) or low-HDL cholesterolaemia ($P$ for interaction = 0.036). Interactions between other confounding factors (hypertension, impaired glucose tolerance or diabetes, current cigarette use and current alcohol consumption) and s-CK levels were not observed: $P$ for interaction in hypertension, 0.94; for impaired glucose tolerance or diabetes, 0.85; for current cigarette smoking, 0.87; and for current alcohol drinking, 0.96 (data not shown).

There were 45 subjects with definite MI during follow-up: 31 subjects with s-CK of ⩽199 IU/l, and 14 subjects with s-CK of ⩾200 IU/l (maximum s-CK was 421 IU/l). Among these 45 definite MI subjects, there was one subject with medication for hypercholesterolaemia and s-CK of ⩽199 IU/l, one with medication for hypertriglyceridaemia and s-CK of ⩾200 IU/l, nine with medication for hypertension (seven with s-CK of ⩽199 IU/l, two with s-CK of ⩾200 IU/l), and four with medication for diabetes and s-CK of ⩽199 IU/l (data not shown).

Baseline s-CK distribution or other CVD risk factor profiles did not differ so much between 700 individuals (360 men and 340 women) excluded for loss to follow-up and 5026 study subjects as follows, respectively; s-CK distribution (IU/l) (25th percentile, median, 75th percentile): 88, 118, 157 vs 92, 119, 158, mean age (years): 55.0 vs 54.5, hypertension (%): 32.9 vs 29.3, hypercholesterolaemia (%): 33.6 vs 35.4.

## Discussion

This is the first report of a prospective cohort study in which elevated s-CK levels at baseline predicted first-ever MI. Elevated s-CK levels were associated with a moderately increased risk for MI. On the other hand, there was no association observed between s-CK and a risk for stroke. Therefore, elevated s-CK was a specific predictor for MI in the present analysis. In addition, an interaction between s-CK levels and dyslipidaemia (hypercholesterolaemia, hypertriglyceridaemia, low-HDL cholesterolaemia) was observed. S-CK elevation might be a stronger predictor for MI among subjects with dyslipidaemia.

On the basis of present results, following three possible hypotheses could be considered. One hypothesis is the involvement of hypothyroidism that often accompanies both s-CK elevation and dyslipidaemia including hypercholesterolaemia.[16] On the basis of this hypothesis, s-CK elevation is supposed to be a surrogate marker of hypothyroidism. Although the association between hypothyroidism and coronary heart disease is still controversial,[17–20] recent meta-analyses suggested an association between hypothyroidism and a modest increased risk for coronary heart disease.[5,6] On the other hand, there were few studies demonstrating causal relationship between hypothyroidism and stroke.[19,21,22] Present results also demonstrated no relationship between stroke and s-CK elevation, and were consistent with that. Influence of hypothyroidism on stroke might not be as strong as coronary heart disease. It might be partly

**Table 2** Crude incidence rates of CVD by s-CK levels in a cohort study of a Japanese urban area, 1989–2005

| | Creatine kinase (IU/l) | | | |
|---|---|---|---|---|
| | ⩽99 | 100–199 | 200–299 | ⩾300 |
| **Men and Women** | | | | |
| *N* | 1590 | 2816 | 482 | 138 |
| Person-years | 18 684 | 33 392 | 5514 | 1490 |
| **Number of events (crude incidence rate per 1000 person-years)** | | | | |
| All MIs | 23 (1.2) | 57 (1.7) | 18 (3.3) | 5 (3.4) |
| Definite MI | 7 (0.4) | 24 (0.7) | 10 (1.8) | 4 (2.7) |
| All strokes | 52 (2.8) | 89 (2.7) | 21 (3.8) | 6 (4.0) |
| Cerebral infarction | 33 (1.8) | 55 (1.6) | 13 (2.4) | 5 (3.4) |
| Haemorrhagic or unclassified stroke | 19 (1.0) | 34 (1.0) | 8 (1.5) | 1 (0.7) |
| **Men** | | | | |
| *N* | 566 | 1396 | 306 | 102 |
| Person-years | 6403 | 16 184 | 3424 | 1123 |
| **Number of events (crude incidence rate per 1000 person-years)** | | | | |
| All MIs | 16 (2.5) | 39 (2.4) | 12 (3.5) | 4 (3.6) |
| Definite MI | 4 (0.6) | 18 (1.1) | 6 (1.8) | 3 (2.7) |
| All strokes | 28 (4.4) | 47 (2.9) | 17 (5.0) | 5 (4.5) |
| Cerebral infarction | 19 (3.0) | 33 (2.0) | 11 (3.2) | 4 (3.6) |
| Haemorrhagic or unclassified stroke | 9 (1.4) | 14 (0.9) | 6 (1.8) | 1 (0.9) |
| **Women** | | | | |
| *N* | 1024 | 1420 | 176 | 36 |
| Person-years | 12 281 | 17 208 | 2090 | 366 |
| **Number of events (crude incidence rate per 1000 person-years)** | | | | |
| All MIs | 7 (0.6) | 18 (1.0) | 6 (2.9) | 1 (2.7) |
| Definite MI | 3 (0.2) | 6 (0.3) | 4 (1.9) | 1 (2.7) |
| All strokes | 24 (2.0) | 42 (2.4) | 4 (1.9) | 1 (2.7) |
| Cerebral infarction | 14 (1.1) | 22 (1.3) | 2 (1.0) | 1 (2.7) |
| Haemorrhagic or unclassified stroke | 10 (0.8) | 20 (1.2) | 2 (1.0) | 0 (0.0) |

because of weak relation between dyslipidaemia and stroke in the Japanese population.[23]

A second possible hypothesis is that very small infarction or vulnerability of myocardium might precede clinical MI onset.[3,4] That is, a small amount of intramyocardial protein leakage might occur after micro-MI, or it may follow reversible disturbance of myocardium because permeability of the myocardial cell membrane is metabolically controlled. s-CK elevation might be an indicator that can detect micro-MI or vulnerability before any symptoms or ECG change appears. In this hypothesis, it might be reasonable that s-CK elevation does not predict stroke because s-CK elevation is due to leakage from myocardium.

A third hypothesis is that medication for hyperlipidaemia can occasionally cause rhabdomyolysis of various degrees with s-CK elevation,[7,8] and is prescribed to individuals with hyperlipidaemia who are possibly at high risk for MI. Therefore, it could become a confounder for an association between s-CK and MI. However, we thought that this hypothesis had little influence on the present results because there was just one subject medicated for hyperlipidaemia at baseline among those with both definite MI and s-CK of ⩾200 IU/l.

We have no information for creatine kinase isozyme that is important to elucidate the background mechanism. Since s-CK in the present study was just one of screening measurements for CVD in the health check-up to healthy individuals that was conducted almost 20 years before, we did not measure creatine kinase isozyme at baseline. Therefore, we can neither infer a further mechanism nor positively support any of above-mentioned hypotheses.

A cross-sectional study demonstrated a positive association between s-CK and blood pressure, and hypothesized that elevated s-CK levels reflected high tissue creatine kinase activity, which might cause

**Table 3** Adjusted HRs (95% CIs) for CVDs by s-CK levels in a cohort study of a Japanese urban area, 1989–2005

| | | | Creatine kinase (IU/l) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | ⩽99 | 100–199 | | ⩾200 | | |
| | *N* | Number of events | HR | HR | 95% CI | HR | 95% CI | *P* for trend |
| **All MIs** | | | | | | | | |
| Model 1 | 5026 | 103 | 1 (reference) | 1.29 | (0.79–2.09) | 2.10 | (1.17–3.74) | 0.015 |
| Model 2 | 5026 | 103 | 1 (reference) | 1.35 | (0.83–2.20) | 2.06 | (1.15–3.70) | 0.018 |
| **Definite MI** | | | | | | | | |
| Model 1 | 5026 | 45 | 1 (reference) | 1.83 | (0.79–4.24) | 4.56 | (1.84–11.31) | 0.001 |
| Model 2 | 5026 | 45 | 1 (reference) | 1.75 | (0.75–4.09) | 4.18 | (1.66–10.53) | 0.002 |
| **All strokes** | | | | | | | | |
| Model 1 | 5026 | 168 | 1 (reference) | 0.89 | (0.63–1.25) | 1.10 | (0.69–1.75) | 0.89 |
| Model 2 | 5026 | 168 | 1 (reference) | 0.91 | (0.64–1.28) | 1.09 | (0.68–1.75) | 0.90 |
| **Cerebral infarction** | | | | | | | | |
| Model 1 | 5026 | 106 | 1 (reference) | 0.87 | (0.56–1.33) | 1.13 | (0.63–2.00) | 0.87 |
| Model 2 | 5026 | 106 | 1 (reference) | 0.91 | (0.59–1.41) | 1.13 | (0.63–2.02) | 0.83 |

Model 1: adjusting for age.
Model 2: adjusting for age, sex, body mass index, creatinine, hypertension, impaired fasting glucose or diabetes, hypercholesterolaemia, low HDL-cholesterolaemia, hypertriglyceridaemia, current cigarette use and current alcohol consumption.
Men and women were combined.

**Table 4** Adjusted HRs (95% CIs) for definite acute MI stratified by lipid status and s-CK levels in a cohort study of a Japanese urban area, 1989–2005

| | | | Creatine kinase (IU/l) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | ⩽99 | 100–199 | | ⩾200 | | | |
| *N* | Number of events | | HR | HR | 95% CI | HR | 95% CI | *P* for trend | *P* for interaction |
| **Total cholesterol <5.7 mmol/l (220 mg/dl)** | | | | | | | | | |
| 3245 | 22 | | 1 (reference) | 1.90 | (0.63–5.76) | 0.87 | (0.16–4.89) | 0.85 | 0.011 |
| **Total cholesterol ⩾5.7 mmol/l (220 mg/dl) or use of anti-hypercholesterolaemic medication** | | | | | | | | | |
| 1781 | 23 | | 1 (reference) | 1.39 | (0.36–5.31) | 10.05 | (2.74–36.81) | <0.0001 | |
| **Triglycerides <1.7 mmol/l (150 mg/dl)** | | | | | | | | | |
| 3819 | 28 | | 1 (reference) | 1.21 | (0.47–3.11) | 1.69 | (0.53–5.35) | 0.39 | 0.033 |
| **Triglycerides ⩾1.7 mmol/l (150 mg/dl) or use of anti-hypertriglyceridaemic medication** | | | | | | | | | |
| 1207 | 17 | | 1 (reference) | 5.32 | (0.61–46.15) | 24.02 | (2.79–207.05) | 0.0002 | |
| **HDL-cholesterol ⩽1 mmol/l (39 mg/dl)** | | | | | | | | | |
| 809 | 17 | | 1 (reference) | 4.08 | (0.49–33.86) | 23.37 | (2.78–196.53) | 0.0002 | 0.036 |
| **HDL-cholesterol >1 mmol/l (39 mg/dl)** | | | | | | | | | |
| 4217 | 28 | | 1 (reference) | 1.24 | (0.48–3.19) | 1.88 | (0.59–5.98) | 0.30 | |

All HRs adjusting for age, sex, body mass index, creatinine, hypertension, impaired fasting glucose or diabetes, hypercholesterolaemia, low HDL-cholesterolaemia, hypertriglyceridaemia, current cigarette use and current alcohol consumption except for each stratifying factor.

higher blood pressure levels.[24] The present analysis of baseline characteristics also demonstrated the possibility of a positive association between hypertension and s-CK levels in women. If this hypothesis is correct, s-CK levels might also be considered a risk factor for stroke. However, results from the present study were not consistent with this hypothesis.

To assure validity of our analysis, it was very important to exclude MI with a past or present history of MI. First, this analysis excluded the subjects with a medical history of MI, abnormal *Q*-wave or ST depression on ECG at the baseline survey. Additionally, no subject who had developed MI just at baseline was included (the follow-up period among those with definite MI was, at earliest, 0.20 years). Secondly, regardless of the follow-up period duration, median baseline s-CK levels among subjects with definite MI were higher than among those without

definite MI. As seen in Figure 1, even with a follow-up period of $\geqslant$8.41 years, median s-CK level among subjects with definite MI was higher than among those without definite MI. This suggested that an association between s-CK levels and MI in a specific period did not influence the present results. Therefore, the possibility that any obviously biased data collection for MI events affected the present results seemed to be low.

The present study had several limitations except for the above-mentioned creatine kinase isozyme. First, since we did not measure thyroid function including thyroid-stimulating hormone at baseline, we could not estimate the involvement of hypothyroidism with the present results. Secondly, we did not collect medical history, such as muscle diseases, thyroid diseases, medications causing rhabdomyolysis or recent history of severe physical training, all of which might affect s-CK levels. Therefore, we could not completely rule out the existence of unknown confounding factors. Thirdly, the single s-CK measurement at baseline may have underestimated the relationship between s-CK levels and CVD due to regression dilution bias.[25] Fourthly, the participation rate at baseline was not so high, but those of other population-based cohort studies in Japan with baseline clinical laboratory data were similar,[26–29] except for large cohort studies with baseline survey by questionnaires.[30,31] Especially in urban areas, the participation rates in cohort studies tend to be lower because the urban residents generally have more opportunities for health check-up than the rural population.[29] That is, the present participation rate was not always low. Moreover, previous reports in the present cohort showed reasonable causal relationship among blood pressure, low-density lipoprotein cholesterol or metabolic syndrome and CVD,[9–11] which were similar with previous findings from the Japanese cohort studies.[23] This indirectly suggested that the results from the present cohort and analysis were not extremely biased by participation rates or loss to follow-up.

In conclusion, the present study suggested that screening for elevated s-CK levels in initially healthy Japanese subjects was useful to predict first-ever MI in the future, especially in subjects with dyslipidaemia. However, since the present study was conducted in a limited Japanese population with a relatively low MI incidence rate and had several limitations, these findings should be interpreted carefully and examined by further studies in various populations, races and areas.

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**Conflict of interest:** None declared.

**KEY MESSAGES**

- Slight elevation in s-CK is associated with increased risk of MI, not stroke, in population-based cohort study of a Japanese urban area.
- Especially in individuals with dyslipidaemia, measurement of s-CK might be more useful to predict MI.
- However, since present findings seemed to be the first report, they should be examined by further studies in various populations, races and areas.

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# Relationship Between Blood Pressure Category and Incidence of Stroke and Myocardial Infarction in an Urban Japanese Population With and Without Chronic Kidney Disease

## The Suita Study

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***Background and Purpose***—Chronic kidney disease (CKD) is increasingly recognized as an independent risk factor for stroke and myocardial infarction (MI). Few studies, however, have examined the relationship between blood pressure (BP) category and these diseases in subjects with and without CKD.

***Methods***—We studied 5494 Japanese individuals (ages 30 to 79, without stroke or MI at baseline) who completed a baseline survey and received follow-up through December 2005. The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease study equation modified by the Japanese coefficient. CKD was defined as an estimated GFR <60 mL/min/1.73m$^2$. BP categories were defined by the European Society of Hypertension and European Society of Cardiology 2007 criteria.

***Results***—In 64 395 person-years of follow-up, we documented 346 incidences of cardiovascular diseases (CVD; 213 strokes and 133 MI events). Compared with the GFR (≥90 mL/min/1.73m$^2$) group, the hazard ratios (95% confidential intervals) for stroke were 1.9 (1.3 to 3.0) in the GFR 50 to 59 mL/min/1.73m$^2$ group and 2.2 (1.2 to 4.1) in the GFR <50 mL/min/1.73m$^2$ group. Results for cerebral infarction were similar. Compared with the optimal BP subjects without CKD, the normal BP, high-normal BP, and hypertensive subjects without CKD showed increased risks of CVD and stroke; however the impact of each BP category on CVD (*P* for interaction: 0.04 in men, 0.49 in women) and stroke (0.03 in men, 0.90 in women) was more evident in men with CKD.

***Conclusions***—CKD may increase the association of BP and CVD in a Japanese urban population. (***Stroke*. 2009;40:2674-2679.)**

**Key Words:** chronic kidney disease ■ blood pressure category ■ stroke ■ myocardial infarction ■ epidemiology ■ prospective studies ■ general population

Recently, chronic kidney disease (CKD) has become a major public health problem and a risk factor for all-causes mortality, stroke, and myocardial infarction (MI).[1] In end-stage renal disease, the cardiovascular disease (CVD) mortality rate is more than 10 times as high as that in the general population.[2] In asymptomatic general populations or outpatients, a severely or moderately decreased glomerular filtration rate (GFR) has been shown by most but not all studies to be an independent risk factor for stroke and MI.[1] However, in low-risk or general populations, the relationship between levels of kidney function and clinical outcomes has not been as clear. Some studies have demonstrated no association between CKD and CVD,[3,4] whereas others have shown CKD as an independent risk factor for CVD.[5–8] These inconsistencies may be attributable to differences between the selected study populations as well as the severity of the CKD.

The frequency of hypertension is relatively higher in Japanese than in Western countries.[9] Hypertension is one of the major risk factors for both CVD and CKD. Recently, a larger prospective study has indicated that CKD increased the association between blood pressure (BP) categories and CVD, although the relevant data were gathered from 10 rural areas with different methods

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for the measurement of creatinine.[10] A few studies in general population have demonstrated a stronger association between BP and CVD in subjects with CKD.[5,10] We examined the association between BP category and incidence of stroke and MI subjects with and without CKD in a Japanese urban population.

## Methods

### Study Subjects

Suita city is located adjacent to Osaka city, which is the second largest metropolitan area in Japan. The Suita Study,[11–13] an epidemiological study of cerebrovascular and cardiovascular diseases, was based on a random sampling of 12 200 Japanese urban residents. As a baseline, participants (aged 30 to 79 years) were randomly selected from the municipality population registry and stratified into groups by sex and age in 10-year increments in 1989. Of these, 6485 people underwent regular health checkups between September 1989 and March 1994.

Cohort members in the study population were excluded from these analyses if they had a past or present history of CVD at baseline (n=208), were missing data (n=170), attended health checkups after April 1994 (n=79), or failed to complete the follow-up health surveys or questionnaires after the baseline examination (n=534). After applying these exclusions, a total of 5494 participants aged 30 to 79 years old were selected. Informed consent was obtained from all participants. This study was approved by the Institutional Review Board of the National Cardiovascular Center.

### Measurement of Blood Pressure and Covariates

Well-trained physicians measured BP 3 times using a mercury column sphygmomanometer, an appropriate-size cuff, and a standard protocol. Before the initial BP reading was obtained, participants were seated at rest for at least 5 minutes. First, systolic blood pressure (SBP) was measured for the purpose of obtaining approximate SBP levels. SBP and diastolic blood pressures (DBP) were taken as the average of the second and third measurements, which were recorded more than 1 minute apart.

At the time of the baseline examination, subjects were classified into 1 of the 5 BP categories based on the European Society of Hypertension and European Society of Cardiology (ESH-ESC) 2007 criteria[14]: optimal (SBP <120 mm Hg and DBP <80 mm Hg), normal (SBP 120 to 129 mm Hg or DBP 80 to 84 mm Hg), high-normal BP (SBP 130 to 139 mm Hg or DBP 85 to 89 mm Hg), and hypertensive (SBP ≥140 mm Hg or DBP ≥90 mm Hg). Antihypertensive drug users were classified according to their BP levels at the baseline survey. If the SBP and DBP readings for a subject were in different categories, the subjects were categorized into the higher of the two BP categories.

At the baseline examination, we performed routine blood tests that included serum total cholesterol, HDL cholesterol, and glucose levels. Physicians or nurses administered questionnaires covering personal habits and present illness. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Hypercholesterolemia was defined as total cholesterol levels ≥5.7 mmol/L or current use of antihyperlipidemic medications. Diabetes was defined as a fasting plasma glucose level ≥7.0 mmol/L, a nonfasting plasma glucose level ≥11.0 mmol/L, or current use of antidiabetic medications.

### Definition of CKD

Serum creatinine (Cre) was measured by noncompensated kinetic Jaffé methods. The glomerular filtration rate (GFR) of each participant was calculated from the Cre value and the age, using the MDRD equation modified by the Japanese coefficient (0.881), as follows[15]:

$$\text{GFR (ml/min/1.73 m}^2) = 0.881 \times 186 \times \text{age}^{-0.203} \times \text{Cre}^{-1.154} \text{ (for men)}$$

$$\text{and GFR (ml/min/1.73 m}^2) = 0.881 \times 186 \times \text{age}^{-0.203} \times \text{Cre}^{-1.154} \times 0.742 \text{ (for women).}$$

CKD was defined as an estimated GFR <60 mL/min/1.73m$^2$.

### Confirmation of Stroke and MI and End Point Determination

The confirmation of stroke and MI in the Suita Study has been described elsewhere.[11–13] In brief, the 5 hospitals in this area, where acute stroke and MI patients were admitted, were all capable of performing computed tomographic scans or MRI. Medical records were reviewed by registered hospital physicians or research physicians who were blinded to the baseline data. Strokes were defined according to the U.S. National Survey of Stroke criteria.[16] For each stroke subtype (ie, cerebral infarction [thrombotic or embolic infarction], intracerebral hemorrhage, and subarachnoid hemorrhage), a definite diagnosis was established based on examination of computed tomographic scans, magnetic resonance images, or autopsies. Definite and probable MIs were defined according to the criteria set out by the MONICA project.[17] Sudden deaths of unknown origin were deaths that occurred within 24 hours from the onset of symptoms, and were also classified as MI. In this study CVD was defined as stroke or MI.

To detect MI and stroke occurrences, each participant's health status was checked at clinical visits to the National Cardiovascular Center every 2 years. Yearly questionnaires by mail or telephone were also completed for all participants. In addition, to complete our surveillance for fatal strokes and MIs, we conducted a systematic search for death certificates. All the data (health check-ups, questionnaires, and death certificates) were checked against medical records to confirm the incidence of CVD. We identified possible strokes or MIs using data from (1) the health examination and questionnaires from the stroke and MI registries without informed consent for medical records survey; and (2) death certificates bearing a diagnosis of probable stroke or MI without registration of CVD incidence.

The end points of the current follow-up study were (1) date of the first MI or stroke event (2); date of death (3); date of leaving Suita; and (4) December 31, 2005 (censored).

### Statistical Analysis

Analyses of variances and $\chi^2$ tests were used to compare mean values and frequencies. The Cox proportional-hazard ratios (HRs) were fitted to the GFR categories and CKD after adjusting for sex and age in 5-year increments as stratified variables and other potential confounding factors at the baseline survey: namely, present illness of hypertension, hypercholesterolemia and diabetes, smoking status (never, quit, and current smoker), and drinking status (never, quit, and current drinker). The Cox proportional HRs were fitted to the combination of the BP categories and CKD (positive or negative) after adjusting for sex and age in 5-year increments as stratified variables and other potential confounding factors including an interactive term for CKD and BP categories. The fit of the proportional hazards model was evaluated by examining discrete regression models and by permitting the proportionality assumption to vary with time, and assessments of nonlinearity involving associations with blood pressure and GFR categories were made. The probability values for the model of interaction between CVD incidence and log (person year) were 0.38 in men and 0.81 in women. Proportionality was also checked by log-log survival plot.

To express the impact of CKD on CVD occurrence in the participants, we estimated the population attributable fraction (PAF, %). PAF was estimated as follows:

$$Pe \times (HR-1)/HR,$$

in which $Pe$ is the proportion of incident cases in CKD, and $HR$ is the multiple-adjusted hazard ratio.[18] All statistical analyses were conducted using the SAS statistical package software (release version 8.2, SAS Institute Inc).

## Results

Figure 1 shows that the frequency of CKD increases with age in both men and women. At the baseline survey, both men and women with CKD (8.9% for men and 11.3% for women)