Aim: Mn is an essential element in the human body, and it has a significant impact on cardiovascular risk factors such as diabetes, blood pressure, and cholesterol levels. However, no research has been conducted on the association between Mn and cardiovascular disease (CVD), to the best of our knowledge. This study thus examined the association between dietary Mn intake and CVD mortality in the general Japanese population.

Methods: The CVD mortality among 58,782 participants from the Japan Collaborative Cohort Study (JACC) aged 40–79 years was determined during a median follow-up period of 16.5 years. The Mn intake was estimated using a food frequency questionnaire at the baseline (1989–1990), and multivariate-adjusted hazard ratios (HRs) for mortality were computed according to quintiles of energy-adjusted Mn intake.

Results: During the follow-up period, a total of 3408 CVD deaths were recorded. Participants in the highest quintile of Mn intake had a lower risk of mortality from total stroke (HR: 0.76; 95% CI, 0.64–0.90), ischemic stroke (HR: 0.77, 0.61–0.97), ischemic heart disease (HR: 0.76, 0.58–0.98), and total CVD (HR: 0.86, 0.76–0.96) compared with those in the lowest quintile. The reduced risk of mortality from intraparenchymal hemorrhage with high Mn intake was observed among women (HR: 0.60, 0.37–0.96) but not men (HR: 0.93, 0.59–1.47). The observed associations were more robust in postmenopausal than in premenopausal women.

Conclusions: Our study is the first to show the prospective association between dietary Mn intake and reduced risk of mortality from CVD in the Japanese population.
CVD\textsuperscript{8)}, and thus higher dietary Mn intake in the Japanese population might be a contributing factor.

Two cross-sectional studies have shown that a higher dietary Mn intake was associated with a lower risk of metabolic syndrome in Chinese and Korean populations\textsuperscript{6, 9)}. Chinese and Korean men and women with higher Mn intake had higher HDL cholesterol levels and lower prevalence of hypertension, abdominal obesity, and hypertriglyceridemia. Moreover, Mn is vital for the manganese superoxide dismutase (MnSOD) enzyme, which reduces mitochondrial oxidative stress and ameliorates ischemic injury\textsuperscript{10}). By preventing the LDL oxidation in endothelial cells\textsuperscript{11)}, Mn can curb vascular thickening and narrowing\textsuperscript{12-14)}. In conjunction with vitamin K, Mn helps in blood clotting and hemostasis\textsuperscript{15}). All these effects are thus potential mechanisms against cardiovascular morbidity and mortality.

**Aim**

Despite the laboratory evidence that Mn, as an essential cofactor involved in metabolism, has protective effects against CVD, no previous study has examined their direct relationship, to the best of our knowledge. Therefore, this study aimed to investigate the association between dietary Mn intake and the subsequent risk of mortality from CVD in the Japanese population.

**Subjects and Methods**

**Population and Baseline Survey**

The Japanese Ministry of Education, Sports, and Science sponsored the Japan Collaborative Cohort (JACC) Study for Evaluation of Cancer Risks as a prospective cohort study launched in 1988–1990. The study included a total of 110,585 participants (46,395 men and 64,190 women) aged 40–79 years from 45 Japanese communities. Group consent was obtained in 9 of 45 communities, and approval was obtained from the individuals in the remaining communities\textsuperscript{16}). We gathered information from completed self-administered questionnaires, which included questions on lifestyles, histories related to hypertension, diabetes, stroke, CVD, and cancer, and diet under the previously presented methods and protocols\textsuperscript{17}). The food frequency questionnaire (FFQ) that assessed the participants’ diet was not available in some study areas; thus, our starting sample consisted of 64,457 participants from 36 communities, among whom we excluded 1461 and 4214 participants with a previous history of cancer and CVD, respectively. The remaining 58,782 participants (23,165 men and 35,617 women) were considered eligible (Supplementary Fig. 1). This study was approved by the ethics committees of the Graduate School of Medicine in Hokkaido and Osaka Universities.

**Assessment of Diet**

The dietary section of the survey included a 40-item FFQ evaluating the frequency of food and beverage consumption in the previous 12 months as follows: rarely, 1–2 times/month, 1–2 times/week, 3–4 times/week, and almost every day. The portion size of each food item was assessed in a JACC validation study (85 JACC study participants using the median value of each intake frequency determined by the four 3-day weighed dietary records [DR] during 1 year)\textsuperscript{18}). We calculated the total dietary intake of Mn content by multiplying the participants’ frequency scores considering the Mn content in 100g of each food item (according to the Japan Food Composition Tables, Fifth Edition)\textsuperscript{19}). The Spearman correlation coefficient for energy-adjusted Mn intake between the FFQ and DR was 0.41 ($P < 0.001$). The FFQ-estimated Mn intake (mean $\pm$ SD) was 5.32 $\pm$ 2.09 mg/d, and that for the DR was 4.81 $\pm$ 2.5 mg/d ($P = 0.23$).

**Mortality Surveillance**

We obtained the cause of death from death certificates, which were forwarded to the public health center of the respective areas and then centralized at the Ministry of Health and Welfare. Investigators conducted a systematic review of death certificates in each area. The International Classification for Diseases, 10th Revision, was used to identify the causes of death. The endpoints of the current analysis were deaths from total CVD (I01–I99), including coronary heart disease (CHD) (I20–I25), stroke (I60–I69), and its subtypes: cerebral infarction (I63, I69.3), subarachnoid hemorrhage (I60, I69.0), and intracerebral hemorrhage (I61–62, I69.1).

**Data Analysis**

The person-years of follow-up were defined as the duration from the date of response to the baseline survey (1988–1990) to an outcome (death, departure from their original communities, or terminated follow-up). The follow-up was terminated in four areas in 1999, four in 2003, two in 2008, and the rest in 2009. Participants who died after moving from their original communities were treated as censored cases.

In the primary analysis, we divided the sex-specific and the combined men's and women's energy-
adjusted Mn intake into five quintiles. The trend test across Mn intake quintiles was conducted for age-adjusted mean values and proportions of participants’ features and confounding variables using linear and logistic regression analyses. The combined and sex-specific multivariable-adjusted hazard ratios (HRs) and their 95% confidence intervals (CIs) for CVD mortality were calculated using the Cox proportional hazard model, with the lowest quintile of Mn intake as the reference category. In the first model, risk estimates were adjusted for age and residential area, and sex (in the combined analysis). In the second model, we further adjusted for body mass index (BMI, in kg/m², quintiles), smoking status (never, ex-smoker, and current smokers of 1–19 and ≥20 cigarettes/day), alcohol intake (never, ex-drinker, current drinker <23 g/day, 23.0–46.0 g/day, 46.0–49.0 g/day, and 49.0 g/day), history of diabetes (yes or no), history of hypertension (yes or no), education (primary school, junior high school, high school, and college or higher), time spent in sports activity (never, 1–2, 3–4, and ≥5 h/week), and walking time (rarely, 0.5, 0.6–0.9, and ≥1 hour/day). The third model was further adjusted for energy-adjusted quintiles of sodium, saturated fatty acids, vitamin E, and total energy intake. The multicollinearity between dietary factors was not an issue because the Spearman correlation coefficients for dietary intakes of Mn with sodium, saturated fatty acid, and vitamin E were 0.02, −0.07, 0.04, and −0.01, respectively. The linear trend was assessed by including the continuous variable of Mn intake assigned to the median value for each quintile in the model instead of the dummy variables. Stratified analyses by menopausal status in women and green tea consumption were conducted. We tested the correlation of Mn intake with sex and menopausal status (in women) by adding an interaction term generated by multiplying the Mn intake categories by the dichotomous variables of sex and menopausal status. SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA) was used for all analyses. Statistical significance was set at \( p < 0.05 \).

**Results**

The average Mn intake levels were 5.6 mg/day in men and 5.2 mg/day in women. The median value of energy-adjusted Mn intake was 3.0 mg/day in the lowest quintile and 10.0 mg/day in the top quintile for men; for women, it was 2.7 mg/day and 9.2 mg/day, respectively. Table 1 and Supplemental Table 1 show the sex-specific and combined age-adjusted means and prevalence of CVD risk factors at baseline according to the quintiles of dietary Mn intake. Compared with the lowest quintile of Mn intake, men and women in the highest quintile were older, had higher education, lower BMI, and lower alcohol intake. Notably, men with the highest Mn intake were less likely to have hypertension and have more sodium and vitamin E intakes.

On the other hand, women with higher Mn intake exercised more and had lower sodium, saturated fatty acids, and energy intake.

The cohort of 58,782 Japanese men and women was followed up for 16.5 years on average, during which we documented a total of 3408 CVD deaths. A lower risk of CVD mortality was found with the higher Mn intake in both men and women. There was no interaction with sex for the association between Mn intake and CVD mortality endpoints (p-interaction >0.1); however, the association reached the significance level in women, but not men (Table 2). The age, sex, and living area-adjusted and multivariable HRs (95% CIs) of mortality from CVD according to quintiles of Mn intake are presented in Supplemental Table 2. After adjusting for CVD risk factors, including behavioral and dietary factors, Mn intake was associated with the lower mortality from total CVD. The multivariable HRs (95% CIs) in the highest versus the lowest quintiles of Mn intake were 0.76 (0.64–0.90, P-trend = 0.001) for total stroke, 0.77 (0.61–0.97, P-trend = 0.022) for ischemic stroke, 0.76 (0.58–0.98, P-trend = 0.039) for CHD, and 0.86 (0.76–0.96, P-trend = 0.004) for total CVD.

It is important to note that in the sex-specific analysis, the multivariable HR (95% CI) of mortality from intraparenchymal hemorrhage in women with the highest versus the lowest Mn intake was 0.60 (0.37–0.96, P-trend = 0.030) (Table 2). In addition, we observed robust associations between dietary Mn intake and risk of CVD mortality in postmenopausal women (n = 18,668) compared to premenopausal women (n = 16,949) (Supplemental Table 3). However, there was no significant interaction between Mn intake and menopausal status in women for these mortality outcomes (p-interactions >0.1).

In addition, we stratified participants by their daily intake of green tea (<1 cups/day, average intake = 0.36 cup/day, n = 12902 versus >1 cups/day, average intake = 4.00 cups/day, n = 32138) (Supplement Table 4). The inverse association between dietary Mn intake with CVD mortalities was evident for the CHD mortality in the group with a lower green tea intake, while it was mainly attributed to the reduced risk of stroke mortality in the group with a higher green tea intake.
The main finding of this prospective study was that high dietary Mn intake was associated with a lower risk of mortality from total stroke, ischemic stroke, CHD, and total CVD in the Japanese population, independently of a wide range of biological, behavioral, and nutritional factors. These associations were more evident in women. Moreover, a reduced risk of mortality from intraparenchymal hemorrhage was also observed among women with high Mn intake.

The number of nutritional epidemiological studies on dietary Mn intake and its influence on mortality from CVD is limited. According to the dietary reference intakes in Japan, an adequate intake and the tolerable upper intake level are 4 and 11 mg/day respectively; however, due to insufficient scientific evidence, there is no exact recommended dietary allowance (RDA) for dietary Mn intake among adults. This cohort study is, to the best of our knowledge, the first to show an association between dietary Mn intake and mortality from CVD in the general population. Previous epidemiological studies have indicated an association between dietary Mn intake and the risk of metabolic syndrome. Thus, the fifth Chinese National Nutrition and Health Survey (2010–2012) showed that in China,

Table 1. Sex-specific mean values and prevalence of cardiovascular risk factors at baseline according to quintiles of dietary manganese intake

|                           | Quintiles of dietary manganese intake | P for trend* |
|---------------------------|--------------------------------------|-------------|
|                           | Q1 (Low) | Q2 | Q3 | Q4 | Q5 (High) |
| Men, n                   | 4633     | 4633 | 4633 | 4633 | 4633       |
| Manganese intake, mg/day | 3.0 ± 0.4 | 4.2 ± 0.5 | 5.6 ± 0.4 | 7.1 ± 0.4 | 10.0 ± 1.7 | <0.001 |
| Age, years               | 55.6 ± 9.9 | 54.7 ± 10.2 | 55.6 ± 10.1 | 56.2 ± 9.7 | 57.6 ± 9.4 | <0.001 |
| Past history of hypertension, % | 20.6 | 20.1 | 21.6 | 19.1 | 18.7 | 0.04 |
| Past history of diabetes, % | 6.4 | 6.1 | 6.7 | 5.8 | 5.7 | 0.24 |
| Current smoker, %        | 53.9 | 55.2 | 52.5 | 54.5 | 54.4 | 0.75 |
| Body mass index, kg/m²   | 22.8 ± 2.7 | 22.7 ± 2.8 | 22.6 ± 2.8 | 22.7 ± 2.7 | 22.7 ± 2.8 | 0.01 |
| Sports ≥3h/week, %       | 13.3 | 13.4 | 15.0 | 14.9 | 14.0 | 0.10 |
| Walking ≥30mins/day, %   | 68.2 | 66.4 | 68.7 | 70.8 | 72.5 | <0.001 |
| College or higher education, % | 12.4 | 18.6 | 19.2 | 17.9 | 16.8 | <0.001 |
| Alcohol intake, g/day    | 39.8 ± 23.7 | 33.8 ± 22.9 | 33.0 ± 21.7 | 31.9 ± 19.9 | 30.3 ± 21.2 | <0.001 |
| Sodium intake, mg/day    | 2219 ±800 | 2158 ±743 | 2115 ±733 | 2188 ±753 | 2304 ±776 | <0.001 |
| Saturated fatty acid intake, g/day | 9.8 ± 3.3 | 9.6 ± 3.2 | 9.6 ± 3.2 | 9.6 ± 3.2 | 9.3 ± 3.1 | 0.80 |
| Vitamin E intake, mg/day | 5.0 ± 1.6 | 4.9 ± 1.5 | 4.9 ± 1.5 | 5.0 ± 1.5 | 5.2 ± 1.5 | 0.03 |
| Energy intake, kcal/day  | 1834 ±506 | 1662 ±500 | 1684 ±484 | 1751 ±465 | 1781 ±484 | <0.001 |

Women, n | 7123 | 7123 | 7123 | 7123 | 7123

Manganese intake, mg/day 2.7 ± 0.3 | 3.8 ± 0.5 | 5.2 ± 0.3 | 6.6 ± 0.4 | 9.2 ± 1.6 | <0.001 |
Age, years 56.0 ± 9.8 | 55.3 ± 10.0 | 56.3 ± 10.1 | 56.3 ± 9.6 | 57.4 ± 9.6 | <0.001 |
Past history of hypertension, % 20.9 | 21.2 | 19.5 | 18.5 | 19.9 | 0.58 |
Past history of diabetes, % 3.6 | 3.6 | 3.4 | 3.2 | 3.4 | 0.54 |
Current smoker, % 4.5 | 5.6 | 4.5 | 4.2 | 5.6 | 0.15 |
Body mass index, kg/m² 23.1 ± 3.2 | 22.9 ± 3.2 | 22.7 ± 3.0 | 22.9 ± 3.0 | 23.0 ± 3.1 | <0.001 |
Sports ≥3h/week, % 8.7 | 9.5 | 10.1 | 10.4 | 9.6 | 0.01 |
Walking ≥30mins/day, % 70.0 | 70.6 | 71.8 | 73.6 | 74.8 | 0.32 |
College or higher education, % 7.8 | 9.8 | 10.7 | 10.3 | 10.2 | <0.001 |
Alcohol intake, g/day 12.7 ± 17.0 | 10.0 ± 12.6 | 9.0 ± 11.0 | 8.9 ± 10.3 | 9.4 ± 14.0 | <0.001 |
Sodium intake, mg/day 2126 ±708 | 2051 ±658 | 1977 ±646 | 2031 ±660 | 2116 ±661 | <0.001 |
Saturated fatty acid intake, g/day 10.3 ± 3.1 | 9.9 ± 3.2 | 9.9 ± 3.1 | 9.9 ± 2.9 | 9.3 ± 2.9 | 0.03 |
Vitamin E intake, mg/day 5.3 ± 1.4 | 5.2 ± 1.3 | 5.1 ± 1.3 | 5.2 ± 1.3 | 5.3 ± 1.3 | <0.001 |
Energy intake, kcal/day 1506 ±360 | 1365 ±393 | 1403 ±354 | 1462 ±354 | 1448 ±346 | 0.42 |

Nutrient intakes were adjusted for total energy intake by the residual method.
Mean ± SD (all such variables)
*Age-adjusted p-trend was calculated by logistic regression for categorical variables; linear regression for continuous variables.

Discussion

The main finding of this prospective study was that high dietary Mn intake was associated with a lower risk of mortality from total stroke, ischemic stroke, CHD, and total CVD in the Japanese population, independently of a wide range of biological, behavioral, and nutritional factors. These associations were more evident in women. Moreover, a reduced risk of mortality from intraparenchymal hemorrhage was also observed among women with high Mn intake.

The number of nutritional epidemiological studies on dietary Mn intake and its influence on mortality from CVD is limited. According to the dietary reference intakes in Japan, an adequate intake and the tolerable upper intake level are 4 and 11 mg/day respectively; however, due to insufficient scientific evidence, there is no exact recommended dietary allowance (RDA) for dietary Mn intake among adults. This cohort study is, to the best of our knowledge, the first to show an association between dietary Mn intake and mortality from CVD in the general population. Previous epidemiological studies have indicated an association between dietary Mn intake and the risk of metabolic syndrome. Thus, the fifth Chinese National Nutrition and Health Survey (2010–2012) showed that in China,
### Table 2. Sex-specific hazard ratios (HRs) and 95% confidence intervals (CIs) of mortality from total stroke, stroke types, coronary heart disease and total cardiovascular disease according to quintiles of dietary manganese intake

| Quintiles of dietary manganese intake | P for trend |
|--------------------------------------|------------|
| Q1 (low)    | Q2 | Q3 | Q4 | Q5 (high) |
| Men, n 4633 | 4633 | 4633 | 4633 | 4633 |
| Person-years 74866 | 74300 | 73183 | 74770 | 76151 |
| Total stroke |  |  |  |  |
| Cases, n | 156 | 125 | 148 | 137 | 171 |
| Multivariable HR | 1 | 0.88 (0.69-1.12) | 1.06 (0.83-1.35) | 0.82 (0.64-1.05) | 0.84 (0.66-1.06) |
| Multivariable HR$$ | 1 | 0.91 (0.71-1.16) | 1.11 (0.87-1.42) | 0.86 (0.67-1.10) | 0.88 (0.69-1.13) |
| Multivariable HR$$ | 1 | 0.88 (0.67-1.12) | 1.09 (0.85-1.39) | 0.84 (0.66-1.09) | 0.86 (0.68-1.11) |
| Ischemic stroke |  |  |  |  |
| Cases, n | 89 | 77 | 83 | 82 | 103 |
| Multivariable HR | 1 | 0.99 (0.72-1.35) | 1.12 (0.81-1.55) | 0.90 (0.65-1.25) | 0.90 (0.66-1.24) |
| Multivariable HR$$ | 1 | 0.96 (0.70-1.32) | 1.14 (0.82-1.58) | 0.90 (0.64-1.25) | 0.88 (0.63-1.21) |
| Multivariable HR$$ | 1 | 0.96 (0.70-1.32) | 1.14 (0.82-1.59) | 0.91 (0.65-1.26) | 0.89 (0.64-1.23) |
| Subarachnoid hemorrhage |  |  |  |  |
| Cases, n | 19 | 10 | 14 | 13 | 14 |
| Multivariable HR | 1 | 0.62 (0.28-1.35) | 0.92 (0.44-1.95) | 0.76 (0.35-1.65) | 0.71 (0.33-1.52) |
| Multivariable HR$$ | 1 | 0.63 (0.28-1.40) | 0.94 (0.44-2.00) | 0.76 (0.35-1.66) | 0.71 (0.32-1.55) |
| Multivariable HR$$ | 1 | 0.61 (0.27-1.37) | 0.93 (0.44-1.99) | 0.76 (0.35-1.66) | 0.72 (0.33-1.57) |
| Intraparenchymal hemorrhage |  |  |  |  |
| Cases, n | 40 | 33 | 48 | 36 | 49 |
| Multivariable HR | 1 | 0.82 (0.52-1.31) | 0.82 (0.52-1.31) | 0.82 (0.52-1.31) | 0.457 |
| Multivariable HR$$ | 1 | 0.88 (0.55-1.41) | 1.20 (0.77-1.88) | 0.80 (0.50-1.29) | 0.762 |
| Multivariable HR$$ | 1 | 0.84 (0.52-1.35) | 1.17 (0.75-1.82) | 0.79 (0.49-1.27) | 0.764 |
| Coronary heart disease |  |  |  |  |
| Cases, n | 93 | 75 | 73 | 76 | 80 |
| Multivariable HR | 1 | 0.93 (0.68-1.27) | 0.95 (0.68-1.32) | 0.85 (0.61-1.19) | 0.77 (0.55-1.08) |
| Multivariable HR$$ | 1 | 0.87 (0.64-1.20) | 0.93 (0.67-1.31) | 0.86 (0.62-1.20) | 0.74 (0.53-1.04) |
| Multivariable HR$$ | 1 | 0.83 (0.60-1.14) | 0.91 (0.65-1.28) | 0.84 (0.60-1.17) | 0.73 (0.52-1.02) |
| Total cardiovascular disease |  |  |  |  |
| Cases, n | 359 | 294 | 324 | 310 | 387 |
| Multivariable HR | 1 | 0.92 (0.79-1.08) | 1.04 (0.89-1.23) | 0.85 (0.72-1.00) | 0.90 (0.77-1.06) |
| Multivariable HR$$ | 1 | 0.91 (0.77-1.06) | 1.06 (0.90-1.24) | 0.88 (0.74-1.03) | 0.90 (0.77-1.06) |
| Multivariable HR$$ | 1 | 0.88 (0.75-1.03) | 1.04 (0.88-1.23) | 0.86 (0.73-1.05) | 0.89 (0.76-1.05) |
| Women, n 7123 | 7124 | 7123 | 7124 | 7123 |
| Person-years 120797 | 116288 | 115778 | 119434 | 122565 |
| Total stroke |  |  |  |  |
| Cases, n | 172 | 149 | 165 | 148 | 152 |
| Multivariable HR | 1 | 0.99 (0.79-1.24) | 0.97 (0.77-1.23) | 0.83 (0.66-1.06) | 0.69 (0.54-0.88) |
| Multivariable HR$$ | 1 | 0.96 (0.77-1.20) | 0.97 (0.77-1.22) | 0.83 (0.65-1.06) | 0.67 (0.53-0.86) |
| Multivariable HR$$ | 1 | 0.92 (0.73-1.16) | 0.94 (0.74-1.18) | 0.81 (0.64-1.04) | 0.65 (0.51-0.83) |
| Ischemic stroke |  |  |  |  |
| Cases, n | 79 | 79 | 74 | 80 | 80 |
| Multivariable HR | 1 | 1.13 (0.82-1.56) | 0.87 (0.62-1.23) | 0.94 (0.67-1.32) | 0.71 (0.51-1.01) |
| Multivariable HR$$ | 1 | 1.04 (0.75-1.44) | 0.83 (0.59-1.17) | 0.89 (0.63-1.26) | 0.64 (0.45-0.90) |
| Multivariable HR$$ | 1 | 1.02 (0.74-1.42) | 0.82 (0.58-1.16) | 0.89 (0.63-1.25) | 0.63 (0.45-0.90) |
| Subarachnoid hemorrhage |  |  |  |  |
| Cases, n | 35 | 29 | 34 | 29 | 29 |
| Multivariable HR | 1 | 0.99 (0.60-1.66) | 1.20 (0.71-2.04) | 0.96 (0.55-1.67) | 0.87 (0.50-1.52) |
| Multivariable HR$$ | 1 | 1.01 (0.60-1.68) | 1.21 (0.71-2.05) | 0.95 (0.54-1.65) | 0.85 (0.48-1.50) |
| Multivariable HR$$ | 1 | 1.03 (0.61-1.73) | 1.22 (0.72-2.09) | 0.95 (0.55-1.66) | 0.85 (0.48-1.51) |
Controlling oxidative stress and endothelial dysfunction are the most likely causes for low mortality from CVD in individuals with high Mn intake. MnSOD is the primary antioxidant that cleans superoxide from mitochondria\(^2, 25\). Its activity is controlled by both the MnSOD gene and serum Mn status\(^26, 27\). In animal studies, Mn deficiency mediated high oxidative stress, stimulated vascular cells, enhanced blood vessel thickening and narrowing, and induced vasoconstriction and hypertension\(^13, 14\). In addition, in mice whose diet was high in fat, serum Mn content and mitochondrial MnSOD activity in the liver increased 1.7-fold (\(P<0.05\)) and 2-fold (\(P<0.05\)) in the Mn-treated mice compared to controls\(^28\).

In contrast, independently of MnSOD, the Mn supplementation decreased endothelial-dysfunction-related biomarkers, such as intracellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1), and cholesterol, which are associated with monocyte adhesion to endothelial cells\(^29-31\). Mn supplementation upregulated adiponectin multimerization protein (DsbA-L), which resulted in decreased levels of cellular adhesion molecule ICAM-1\(^32\). In addition, Mn supplementation lowered the serum levels of the chemokine MCP-1 responsible for recruiting monocytes to the arterial wall in their way to be engulfed by macrophages, inducing a series of chronic inflammation\(^33\); thus, Mn can lower the risk of hyperlipidemia and inhibit the progression of atherosclerotic lesions. A randomized controlled trial confirmed that a Japanese diet rich in Mn reduced the serum inflammatory parameters such as LDL-cholesterol, triglycerides, and insulin in rice was the primary food source of Mn (>42%). Chinese men in the highest quartile of Mn intake had a significantly lower risk (38%) of metabolic syndrome than those in the lowest quartile. However, there was a tendency toward a higher risk of metabolic syndrome in women with high Mn intake\(^9\). An inverse association was observed for the following metabolic syndrome traits in men: high serum triglycerides (P-trend=0.029) and abdominal obesity (P-trend=0.016), while a higher Mn intake was associated with lower serum HDL levels in both Chinese men and women\(^9\). Another cross-sectional study conducted in China reported a dose-response inverse association between dietary Mn intake and the number of components of metabolic syndrome\(^23\). In South Korea, Choi and Bae et al. reported a cross-sectional association between high Mn intake and low prevalence of hypertension and metabolic syndrome in women\(^6\). Almost 55% of the dietary Mn for Koreans came from cereals\(^22\). In Japan, our prior study found strong inverse associations between dietary Mn intake and the risk of type 2 diabetes in women, which is entirely supportive to our findings\(^24\).

Reducing oxidative stress and endothelial dysfunction are the most likely causes for low mortality from CVD in individuals with high Mn intake. MnSOD is the primary antioxidant that cleans superoxide from mitochondria\(^2, 25\). Its activity is controlled by both the MnSOD gene and serum Mn status\(^26, 27\). In animal studies, Mn deficiency mediated high oxidative stress, stimulated vascular cells, enhanced blood vessel thickening and narrowing, and induced vasoconstriction and hypertension\(^13, 14\). In addition, in mice whose diet was high in fat, serum Mn content and mitochondrial MnSOD activity in the liver increased 1.7-fold (\(P<0.05\)) and 2-fold (\(P<0.05\)) in the Mn-treated mice compared to controls\(^28\).
patients with dyslipidemia. Although we did not find a statistically significant effect modification by sex, our sex-specific analyses showed robust associations between Mn intake and CVD mortality in women. While the association in men was observed, it did not reach statistical significance. We attribute this to the lower Mn absorption and lower blood ferritin diversity in men than in women. Biologically, women can absorb and retain higher levels of dietary Mn than men, with lower serum iron and ferritin levels. In addition, the more pronounced effect of dietary Mn in women could be because female sex hormones may arbitrate a different biological half-life and whole-body retention of Mn in women.

Since there is a large difference in the blood Mn level, female sex hormones, and other physiological changes between postmenopausal and premenopausal women, which might influence the protective effects of Mn on CVD, we stratified our studied women according to the menopausal status. We observed a more robust inverse association between Mn intake and risk of CVD mortality in postmenopausal women than in premenopausal women; however, p-interaction with menopausal status was not statistically significant. A previous report suggested that premenopausal women were more likely to be iron deficient, leading to higher blood Mn levels. Our findings seem to contradict this theory regarding the pronounced effects of Mn in postmenopausal women. However, Mn can elevate serum levels of luteinizing hormone, follicle-stimulating hormone (FSH), and estradiol in mammals. A population-based study indicated that serum FSH levels were positively associated with sex hormone-binding globulin (SHBG) levels alone, which was proven to be a protective factor against CVD by influencing the metabolism and production of HDL and decreasing androgen levels in postmenopausal women. In this case, FSH has shown protective effects against the risk of atherosclerotic CVD and its cardiometabolic risk factors by regulating lipid metabolism and increasing serum SHBG levels in postmenopausal women. For this reason, the effects of Mn on elevated serum levels of female sex hormones and SHBG might explain its specific protection in postmenopausal women.

The strengths of our study include its long-term follow-up of a large sample, the use of a validated FFQ, and documented endpoints. However, this study has some limitations. First, we included only 40 items in our FFQ, which might lead to lower estimates of Mn intake; however, our FFQ covered most of the Mn source items in Japan (rice, green tea, and vegetables). The main sources of Mn in our study were green tea (84%), rice (7%), and oolong tea (4%), and those sources contain other components that can lower CVD mortality, such as polyphenols. However, when we further added green tea intake to our multivariable-adjusted model (data not shown in tables), the reduced risk of CHD mortality (HR = 0.51: 0.27–0.98) was still evident; while those for stroke and CVD were attenuated (HRs: 0.71:0.46–1.10 and 0.80:0.60–1.06, respectively). Moreover, the stratified analysis of green tea intake confirmed the protective effects of Mn against the CVD mortality in both groups of green tea intake. In addition, when we stratified the participants by vegetable intake, the association between Mn intake and risk of CVD was more evident for the lower vegetable intake group than the higher one (p-value of interaction with vegetable consumption was not significant; data not shown in tables). Another issue is that the absorption of Mn is affected by the consumption of other dietary factors; therefore, we adjusted for potential dietary confounding factors such as sodium, saturated fatty acid, and vitamin E, which turned out to be important nutritional factors affecting CVD mortality in the JACC cohort study. However, these factors were highly correlated with other potential confounding factors such as fiber; thus, we did not further adjust for other nutritional factors. There was no material difference in the association and risks of total stroke, stroke types, CHD, and CVD when we replaced vitamin E for fiber in the model. Third, residual confounding by other factors, such as phytic acid intake, could not be ruled out. Fourth, there were only eight male participants in the validation study of the nutrient intakes. This may have led to inaccurate estimation of the Mn intake in the male population and could have attributed to the observed null association between Mn intake and CVD mortality among males. Fifth, the exposure data were collected only at the baseline survey; there is still the possibility that the participants may have altered their diets during the long follow-up period, which may attenuate the association between dietary Mn intake and CVD mortality.

In conclusion, among Japanese men and women, dietary Mn intake was associated with a reduced risk of mortality from total stroke, ischemic stroke, CHD, and total CVD. However, evidence of the impact of dietary Mn on cardiovascular health is still limited, and more research is required to determine the effects of Mn on human health and leading to its RDA and
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Contributors

Hiroyasu Iso and Akiko Tamakoshi designed this research; Ouyang Meishuo conducted the analyses; Ouyang Meishuo prepared the manuscript; Hiroyasu Iso, Ehab S. Eshak, Renzhe Cui, Shirai Kokoro, and Akiko Tamakoshi critically reviewed the manuscript; and Hiroyasu Iso assumed primary responsibility for the final content. All authors approve the manuscript for publication.

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Competing Interests

None declared.

Ethical Approval

This study was approved by the ethics committees of the Graduate Schools of Medicine in Hokkaido and Osaka Universities.

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110,585 (46,395 men and 64,190 women) from 45 study areas.

Excluded: 46,128 participants with no dietary data.

64,457 with complete dietary data, from 36 study areas.

Excluded: 1461 participants with a previous history of cancer, 4214 participants with CVD, 64,457 with complete dietary data, from 36 study areas.

Remaining 58,782 participants (23,165 men and 35,617 women)

Model 1: adjusted for age, sex and residential area.
Model 2: adjusted further for body mass index, smoking status, frequency of sports activity, alcohol consumption, hours of walking, education years, and history of hypertension and diabetes.
Model 3: adjusted further for intake of total energy, sodium, saturated fatty acids, and vitamin E.

Supplemental Fig. 1. Flow chart of the research

Table 1. Mean values and prevalence of cardiovascular risk factors at base line according to quintiles of dietary manganese intake

|                      | Quintiles of dietary manganese intake | P – trend $^\$ |
|----------------------|---------------------------------------|-----------------|
|                      | Q1 (Low) | Q2          | Q3          | Q4          | Q5 (High) |
| Subjects, n          | 11756    | 11757       | 11756       | 11757       | 11756     |
| Males, %             | 44.8     | 40.6        | 36.7        | 38.2        | 36.7      | <0.001     |
| Age, years           | 55.9±9.9 | 55.1±10.1   | 56.0±10.1   | 56.3±9.7    | 57.5±9.6  | <0.001     |
| Manganese intake, mg/day | 2.7±0.4  | 3.9±0.5     | 5.4±0.4     | 6.8±0.4     | 9.6±1.7   | <0.001     |
| Past history of hypertension, % | 20.8 | 19.6        | 19.5        | 19.6        | 20.2      | 0.80       |
| Past history of diabetes, % | 5.0 | 4.6         | 4.6         | 4.1         | 4.2       | 0.53       |
| Current smoker, %    | 27.4     | 26.6        | 22.9        | 23.8        | 24.0      | 0.001      |
| Body mass index, kg/m² | 22.9±3.0 | 22.8±3.0    | 22.7±2.9    | 22.9±2.9    | 22.9±3.0  | <0.001     |
| Sports ≥ 3h/week, %  | 10.8     | 11.0        | 12.0        | 12.1        | 11.2      | 0.03       |
| Walking ≥ 30mins/day % | 68.6     | 68.9        | 71.1        | 72.1        | 74.3      | 0.08       |
| College or higher education, % | 10.1 | 13.4        | 13.9        | 13.2        | 12.5      | <0.001     |
| Alcohol intake, g/day | 33.2±34.9 | 29.1±23.2   | 26.8±22.5   | 25.6±19.9   | 25.0±21.8  | <0.001     |
| Sodium intake, mg/day | 2157±758 | 2078±710    | 2038±688    | 2100±702    | 2196±714  | <0.001     |
| Saturated fatty acid intake, g/day | 10.1±3.3 | 9.6±3.3     | 9.9±3.2     | 9.8±3.2     | 9.3±3.0   | <0.001     |
| Vitamin E intake, mg/day | 5.2±1.6  | 5.0±1.5     | 5.1±1.5     | 5.2±1.5     | 5.3±1.5   | <0.001     |
| Energy intake, kcal/day | 1634±463 | 1486±458    | 1518±434    | 1567±424    | 1581±433  | <0.001     |

Nutrient intakes were adjusted for total energy intake by the residual method.
Mean ± SD (all such variables)
$^\$Age-adjusted p-trend was calculated by logistic regression for categorical variables and linear regression for continuous variables.
### Supplemental Table 2. Hazard ratios (HRs) and 95% confidence intervals (CIs) of mortality from total stroke, stroke types, coronary heart disease and total cardiovascular disease according to quintiles of dietary manganese intake

|                          | Quintiles of dietary manganese intake |                      |                     |                     |                     |
|--------------------------|--------------------------------------|-----------------------|---------------------|---------------------|---------------------|
|                          | Q1 (low) | Q2 | Q3 | Q4 | Q5 (high) | P for trend |
| Number of subjects       | 11756 | 11757 | 11756 | 11757 | 11756 |                |
| Person-years             | 195663 | 190588 | 189961 | 194204 | 198716 |                |
| Total stroke             |                      |                      |                     |                     |                     |
| Cases, n                 | 339 | 268 | 310 | 290 | 316 |                |
| Multivariable HR³        | 1 | 0.90 (0.77-1.07) | 1.00 (0.85-1.18) | 0.83 (0.70-0.99) | 0.75 (0.64-0.89) | 0.001 |
| Multivariable HR³³       | 1 | 0.91 (0.77-1.07) | 1.03 (0.88-1.22) | 0.86 (0.72-1.02) | 0.78 (0.66-0.92) | 0.002 |
| Multivariable HR³³³      | 1 | 0.88 (0.75-1.04) | 1.02 (0.86-1.21) | 0.85 (0.72-1.01) | 0.76 (0.64-0.90) | 0.001 |
| Ischemic stroke          |                      |                      |                     |                     |                     |
| Cases, n                 | 181 | 149 | 150 | 167 | 179 |                |
| Multivariable HR³        | 1 | 0.97 (0.78-1.22) | 0.92 (0.73-1.16) | 0.90 (0.72-1.13) | 0.78 (0.62-0.98) | 0.021 |
| Multivariable HR³³       | 1 | 0.97 (0.78-1.21) | 0.95 (0.75-1.21) | 0.93 (0.74-1.17) | 0.80 (0.63-1.00) | 0.034 |
| Multivariable HR³³³      | 1 | 0.93 (0.74-1.17) | 0.93 (0.74-1.18) | 0.91 (0.72-1.15) | 0.77 (0.61-0.97) | 0.022 |
| Subarachnoid hemorrhage  |                      |                      |                     |                     |                     |
| Cases, n                 | 53 | 41 | 48 | 43 | 41 |                |
| Multivariable HR³        | 1 | 0.88 (0.58-1.33) | 1.07 (0.70-1.65) | 0.89 (0.57-1.39) | 0.75 (0.48-1.19) | 0.216 |
| Multivariable HR³³       | 1 | 0.87 (0.57-1.33) | 1.10 (0.71-1.70) | 0.90 (0.58-1.41) | 0.75 (0.48-1.19) | 0.222 |
| Multivariable HR³³³      | 1 | 0.87 (0.57-1.33) | 1.10 (0.72-1.70) | 0.90 (0.58-1.41) | 0.73 (0.46-1.16) | 0.178 |
| Intraparenchymal hemorrhage |                  |                      |                     |                     |                     |
| Cases, n                 | 85 | 69 | 96 | 71 | 86 |                |
| Multivariable HR³        | 1 | 0.86 (0.62-1.18) | 1.11 (0.83-1.51) | 0.74 (0.53-1.03) | 0.77 (0.56-1.06) | 0.067 |
| Multivariable HR³³       | 1 | 0.89 (0.64-1.22) | 0.89 (0.64-1.22) | 0.89 (0.64-1.22) | 0.89 (0.64-1.22) | 0.146 |
| Multivariable HR³³³      | 1 | 0.85 (0.61-1.18) | 0.85 (0.61-1.18) | 0.85 (0.61-1.18) | 0.85 (0.61-1.18) | 0.127 |
| Coronary heart disease   |                      |                      |                     |                     |                     |
| Cases, n                 | 169 | 135 | 130 | 137 | 136 |                |
| Multivariable HR³        | 1 | 1.00 (0.79-1.26) | 0.96 (0.74-1.23) | 0.92 (0.72-1.18) | 0.79 (0.61-1.02) | 0.039 |
| Multivariable HR³³       | 1 | 0.97 (0.77-1.23) | 0.95 (0.74-1.23) | 0.93 (0.72-1.19) | 0.78 (0.60-1.00) | 0.039 |
| Multivariable HR³³³      | 1 | 0.91 (0.72-1.16) | 0.92 (0.71-1.18) | 0.91 (0.70-1.17) | 0.76 (0.58-0.98) | 0.039 |
| Total cardiovascular disease |              |                      |                     |                     |                     |
| Cases, n                 | 727 | 623 | 658 | 651 | 749 |                |
| Multivariable HR³        | 1 | 0.99 (0.89-1.10) | 0.99 (0.89-1.11) | 0.89 (0.79-1.00) | 0.86 (0.77-0.96) | 0.001 |
| Multivariable HR³³       | 1 | 0.98 (0.88-1.09) | 1.01 (0.90-1.13) | 0.91 (0.81-1.02) | 0.87 (0.77-0.97) | 0.003 |
| Multivariable HR³³³      | 1 | 0.95 (0.85-1.06) | 0.99 (0.89-1.12) | 0.90 (0.80-1.01) | 0.86 (0.76-0.96) | 0.004 |

HR³: adjusted for age, sex and residential area.
HR³³: adjusted further for body mass index, smoking status, frequency of sports activity, alcohol consumption, hours of walking, education years, and past history of hypertension and diabetes.
HR³³³: adjusted further for intake of total energy, sodium, saturated fatty acid, and vitamin E.
### Supplemental Table 3

Hazard ratios (HRs) and 95% confidence intervals (CIs) of mortality from total stroke, stroke types, coronary heart disease and total cardiovascular disease according to quintiles of dietary manganese intake by menopausal status

| Quintiles of dietary manganese intake | Postmenopausal women, n | P for trend |
|--------------------------------------|--------------------------|------------|
|                                      | Q1(low)  | Q2     | Q3     | Q4     | Q5(high) |
| Person-years                         | 4667    | 4667   | 4667   | 4667   | 4667      |
| Total stroke                         | 76913   | 67665  | 72388  | 77313  | 83875     |
| Cases, n                             | 142     | 122    | 135    | 122    | 122       |
| Multivariable HR³                    | 1       | 0.99 (0.77-1.26) | 0.96 (0.74-1.25) | 0.85 (0.65-1.11) | 0.68 (0.52-0.89) | 0.001 |
| Multivariable HR³⁺                   | 1       | 0.96 (0.74-1.23) | 0.97 (0.75-1.25) | 0.84 (0.64-1.10) | 0.66 (0.50-0.86) | 0.001 |
| Multivariable HR³⁺⁺                  | 1       | 0.93 (0.72-1.20) | 0.94 (0.73-1.22) | 0.83 (0.63-1.09) | 0.64 (0.48-0.84) | 0.001 |
| Ischemic stroke                      |         |        |        |        |           |
| Cases, n                             | 70      | 67     | 65     | 69     | 59        |
| Multivariable HR³                    | 1       | 1.08 (0.77-1.52) | 0.85 (0.59-1.23) | 0.93 (0.65-1.35) | 0.61 (0.41-0.89) | 0.004 |
| Multivariable HR³⁺                   | 1       | 1.04 (0.74-1.47) | 0.86 (0.59-1.24) | 0.90 (0.62-1.31) | 0.57 (0.39-0.84) | 0.002 |
| Multivariable HR³⁺⁺                  | 1       | 0.97 (0.68-1.39) | 0.82 (0.56-1.19) | 0.87 (0.60-1.26) | 0.54 (0.37-0.80) | 0.001 |
| Subarachnoid hemorrhage              |         |        |        |        |           |
| Cases, n                             | 28      | 22     | 25     | 23     | 28        |
| Multivariable HR³                    | 1       | 0.95 (0.53-1.69) | 1.05 (0.58-1.92) | 0.91 (0.49-1.68) | 0.98 (0.54-1.79) | 0.923 |
| Multivariable HR³⁺                   | 1       | 0.93 (0.52-1.66) | 1.07 (0.58-1.95) | 0.91 (0.49-1.69) | 0.98 (0.53-1.79) | 0.934 |
| Multivariable HR³⁺⁺                  | 1       | 0.96 (0.54-1.74) | 1.06 (0.58-1.95) | 0.91 (0.49-1.70) | 0.98 (0.53-1.81) | 0.898 |
| Intraparenchymal hemorrhage          |         |        |        |        |           |
| Cases, n                             | 33      | 29     | 38     | 27     | 30        |
| Multivariable HR³                    | 1       | 0.96 (0.58-1.60) | 1.14 (0.69-1.87) | 0.75 (0.44-1.30) | 0.68 (0.39-1.16) | 0.077 |
| Multivariable HR³⁺                   | 1       | 0.94 (0.57-1.58) | 1.15 (0.69-1.90) | 0.76 (0.44-1.31) | 0.67 (0.39-1.15) | 0.074 |
| Multivariable HR³⁺⁺                  | 1       | 0.96 (0.57-1.61) | 1.13 (0.68-1.88) | 0.75 (0.43-1.31) | 0.66 (0.38-1.15) | 0.068 |
| Coronary heart disease               |         |        |        |        |           |
| Cases, n                             | 55      | 52     | 55     | 48     | 50        |
| Multivariable HR³                    | 1       | 1.17 (0.79-1.73) | 1.19 (0.78-1.82) | 1.09 (0.70-1.70) | 0.93 (0.59-1.45) | 0.469 |
| Multivariable HR³⁺                   | 1       | 1.06 (0.71-1.57) | 1.10 (0.72-1.69) | 1.06 (0.67-1.65) | 0.85 (0.54-1.33) | 0.346 |
| Multivariable HR³⁺⁺                  | 1       | 0.95 (0.63-1.42) | 1.03 (0.67-1.58) | 1.01 (0.65-1.59) | 0.82 (0.52-1.29) | 0.386 |
| Total cardiovascular disease         |         |        |        |        |           |
| Cases, n                             | 286     | 269    | 290    | 273    | 310       |
| Multivariable HR³                    | 1       | 1.07 (0.90-1.27) | 1.00 (0.83-1.19) | 0.93 (0.77-1.12) | 0.85 (0.71-1.02) | 0.014 |
| Multivariable HR³⁺                   | 1       | 1.02 (0.86-1.21) | 0.98 (0.82-1.17) | 0.92 (0.76-1.10) | 0.81 (0.68-0.97) | 0.006 |
| Multivariable HR³⁺⁺                  | 1       | 0.98 (0.83-1.17) | 0.95 (0.79-1.14) | 0.90 (0.75-1.09) | 0.79 (0.66-0.95) | 0.005 |
| Premenopausal women, n               | 2456    | 2784   | 2482   | 2383   | 2160      |
| Person-years                         | 43884   | 48623  | 43390  | 42121  | 38690     |
| Total stroke                         |         |        |        |        |           |
| Cases, n                             | 30      | 27     | 30     | 26     | 30        |
| Multivariable HR³                    | 1       | 1.02 (0.60-1.73) | 1.06 (0.62-1.80) | 0.89 (0.51-1.55) | 0.84 (0.49-1.45) | 0.409 |
| Multivariable HR³⁺                   | 1       | 0.94 (0.55-1.60) | 1.08 (0.63-1.85) | 0.91 (0.51-1.60) | 0.84 (0.48-1.45) | 0.484 |
| Multivariable HR³⁺⁺                  | 1       | 0.87 (0.50-1.52) | 1.00 (0.57-1.73) | 0.84 (0.47-1.51) | 0.73 (0.41-1.29) | 0.276 |
| Ischemic stroke                      |         |        |        |        |           |
| Cases, n                             | 9       | 12     | 9      | 11     | 21        |
| Multivariable HR³                    | 1       | 1.55 (0.65-3.70) | 0.99 (0.38-2.54) | 1.12 (0.45-2.80) | 1.46 (0.64-3.34) | 0.498 |
| Multivariable HR³⁺                   | 1       | 1.13 (0.45-2.84) | 0.97 (0.37-2.56) | 1.16 (0.45-2.99) | 1.34 (0.56-3.16) | 0.469 |
| Multivariable HR³⁺⁺                  | 1       | 1.08 (0.42-2.82) | 0.86 (0.32-2.32) | 1.04 (0.39-2.79) | 1.06 (0.43-2.62) | 0.893 |
### Cont. Supplemental Table 3

| Condition                        | Quintiles of dietary manganese intake | $P$ for trend |
|----------------------------------|---------------------------------------|---------------|
|                                  | Q1(low) | Q2 | Q3 | Q4 | Q5(high) |
| **Subarachnoid hemorrhage**      |         |    |    |    |          |
| Cases, $n$                       | 7       | 7  | 9  | 6  | 1        |
| Multivariable HR$^§$             | 1       | 1.21 (0.40-3.61) | 1.92 (0.62-5.97) | 1.31 (0.38-4.49) | 0.22 (0.02-1.91) | 0.216 |
| Multivariable HR$^§§$            | 1       | 0.97 (0.32-2.95) | 1.87 (0.60-5.87) | 1.11 (0.32-3.91) | 0.20 (0.02-1.73) | 0.189 |
| Multivariable HR$^§§§$           | 1       | 1.33 (0.40-4.43) | 2.47 (0.73-8.39) | 1.48 (0.39-5.55) | 0.24 (0.03-2.21) | 0.246 |
| **Intraparenchymal hemorrhage**  |         |    |    |    |          |
| Cases, $n$                       | 12      | 6  | 10 | 8  | 8        |
| Multivariable HR$^§$             | 1       | 0.49 (0.18-1.33) | 0.75 (0.31-1.78) | 0.57 (0.23-1.46) | 0.52 (0.20-1.32) | 0.268 |
| Multivariable HR$^§§$            | 1       | 0.52 (0.19-1.41) | 0.77 (0.32-1.85) | 0.60 (0.24-1.55) | 0.53 (0.21-1.37) | 0.284 |
| Multivariable HR$^§§§$           | 1       | 0.45 (0.16-1.27) | 0.70 (0.28-1.74) | 0.59 (0.22-1.55) | 0.46 (0.17-1.23) | 0.236 |
| **Coronary heart disease**       |         |    |    |    |          |
| Cases, $n$                       | 10      | 8  | 12 | 9  | 11       |
| Multivariable HR$^§$             | 1       | 0.87 (0.33-2.25) | 1.09 (0.45-2.64) | 0.68 (0.26-1.79) | 0.75 (0.30-1.90) | 0.458 |
| Multivariable HR$^§§$            | 1       | 0.75 (0.27-2.04) | 1.18 (0.46-2.99) | 0.66 (0.24-1.81) | 0.68 (0.26-1.79) | 0.397 |
| Multivariable HR$^§§§$           | 1       | 0.64 (0.22-1.89) | 1.18 (0.45-3.12) | 0.65 (0.23-1.84) | 0.72 (0.26-2.01) | 0.579 |
| **Total cardiovascular disease** |         |    |    |    |          |
| Cases, $n$                       | 60      | 56 | 64 | 56 | 70       |
| Multivariable HR$^§$             | 1       | 1.05 (0.72-1.52) | 1.06 (0.73-1.53) | 0.87 (0.59-1.27) | 0.88 (0.61-1.27) | 0.284 |
| Multivariable HR$^§§$            | 1       | 1.01 (0.69-1.47) | 1.07 (0.73-1.55) | 0.86 (0.58-1.27) | 0.83 (0.57-1.22) | 0.204 |
| Multivariable HR$^§§§$           | 1       | 0.96 (0.65-1.43) | 1.03 (0.70-1.51) | 0.84 (0.57-1.26) | 0.81 (0.55-1.20) | 0.207 |

HR$^§$: adjusted for age and residential area.

HR$^§§$: adjusted further for body mass index, smoking status, frequency of sports activity, alcohol consumption, hours of walking, education years, and past history of hypertension, and diabetes.

HR$^§§§$: adjusted further for intake of total energy, sodium, saturated fatty acid and vitamin E.

$P$ value of interaction with menopausal status were 0.72 for total stroke, 0.09 for ischemic stroke, 0.21 for subarachnoid hemorrhage, 0.90 for intraparenchymal hemorrhage, 0.93 for coronary heart disease, and 0.87 for total cardiovascular disease.
### Supplemental Table 4. Hazard ratios (HRs) and 95% confidence intervals (CIs) of mortality from total stroke, stroke types, coronary heart disease and total cardiovascular disease according to green tea intake

| Quintiles of dietary manganese intake | ≤ 1 cup green tea/day (average intake=0.36 cup/day) | Over 1 cup green tea/day (average intake=4.00 cups/day) |
|--------------------------------------|-----------------------------------------------|------------------------------------------------------|
|                                      | Q1 (low) | Q2 | Q3 | Q4 | Q5 (high) | P for trend | Q1 (low) | Q2 | Q3 | Q4 | Q5 (high) | P for trend |
| Median value of Mn intake (mg/day)    | 2.45     | 2.87 | 3.15 | 3.45 | 4.03       |             | 4.45     | 5.23 | 5.98 | 6.82 | 7.62       |             |
| Number of subjects                   | 2580     | 2581 | 2580 | 2581 | 2580       |             | 6427     | 6428 | 6428 | 6428 | 6427       |             |
| Total stroke. Cases, n               | 67       | 56  | 63  | 53  | 66         |             | 146      | 165  | 157  | 154  | 173        |             |
| Multivariable HR                     | 1.00     | 0.84 (0.58-1.20) | 0.85 (0.59-1.21) | 0.73 (0.50-1.07) | 1.04 (0.71-1.50) | 0.901       | 1.00     | 0.91 (0.64-1.29) | 0.89 (0.64-1.28) | 0.87 (0.63-1.09) | 0.82 (0.66-1.04) | 0.028       |
| Multivariable HR                     | 1.00     | 1.00 (0.67-1.47) | 1.09 (0.73-1.64) | 1.00 (0.60-1.40) | 1.00 (0.60-1.40) | 0.265       | 1.00     | 0.99 (0.57-1.73) | 0.93 (0.53-1.62) | 0.59 (0.31-1.10) | 0.91 (0.52-1.60) | 0.347       |
| Multivariable HR                     | 1.00     | 1.00 (0.63-1.42) | 0.99 (0.64-1.53) | 0.79 (0.49-1.28) | 1.10 (0.69-1.76) | 0.633       | 1.00     | 0.95 (0.54-1.67) | 0.91 (0.52-1.59) | 0.56 (0.30-1.05) | 0.86 (0.48-1.52) | 0.252       |
| Ischemic stroke. Cases, n            | 32       | 28  | 37  | 31  | 35         |             | 23       | 17   | 14   | 12   | 18         |             |
| Multivariable HR                     | 1.00     | 0.94 (0.56-1.58) | 1.05 (0.64-1.72) | 0.91 (0.54-1.53) | 1.22 (0.72-2.07) | 0.474       | 1.00     | 0.74 (0.39-1.41) | 0.56 (0.28-1.11) | 0.50 (0.24-1.03) | 0.77 (0.39-1.51) | 0.353       |
| Multivariable HR                     | 1.00     | 1.10 (0.62-1.94) | 1.33 (0.75-2.34) | 1.16 (0.63-2.11) | 1.50 (0.82-2.75) | 0.197       | 1.00     | 0.96 (0.48-1.91) | 0.78 (0.36-1.66) | 0.69 (0.31-1.55) | 1.09 (0.51-2.31) | 0.896       |
| Multivariable HR                     | 1.00     | 1.06 (0.59-1.88) | 1.22 (0.66-2.25) | 1.01 (0.52-1.97) | 1.31 (0.67-2.57) | 0.464       | 1.00     | 0.89 (0.44-1.83) | 0.67 (0.29-1.53) | 0.56 (0.23-1.38) | 0.88 (0.38-2.04) | 0.826       |
| Coronary heart disease. Cases, n     | 36       | 38  | 34  | 37  | 35         |             | 36       | 38   | 34   | 37   | 35         |             |
| Multivariable HR                     | 1.00     | 1.09 (0.69-1.74) | 0.93 (0.57-1.52) | 1.01 (0.62-1.64) | 0.90 (0.37-2.43) | 0.905       | 1.00     | 0.74 (0.39-1.41) | 0.56 (0.28-1.11) | 0.50 (0.24-1.03) | 0.77 (0.39-1.51) | 0.353       |
| Multivariable HR                     | 1.00     | 1.30 (0.79-2.16) | 1.22 (0.70-2.10) | 1.23 (0.70-2.16) | 1.18 (0.41-3.39) | 0.604       | 1.00     | 0.96 (0.48-1.91) | 0.78 (0.36-1.66) | 0.69 (0.31-1.55) | 1.09 (0.51-2.31) | 0.896       |
| Multivariable HR                     | 1.00     | 1.20 (0.71-2.03) | 1.00 (0.55-1.81) | 0.90 (0.48-1.69) | 0.41 (0.19-0.88) | 0.008       | 1.00     | 0.91 (0.71-1.18) | 0.80 (0.60-1.06) | 0.73 (0.54-0.99) | 0.73 (0.53-1.00) | 0.037       |
| Total CVD. Cases, n                  | 156      | 142 | 141 | 138 | 125        |             | 156      | 142  | 141  | 138  | 125        |             |
| Multivariable HR                     | 1.00     | 0.90 (0.72-1.14) | 0.81 (0.64-1.03) | 0.81 (0.64-1.04) | 0.83 (0.64-1.07) | 0.370       | 1.00     | 0.98 (0.76-1.26) | 0.93 (0.72-1.22) | 0.89 (0.68-1.17) | 0.89 (0.67-1.18) | 0.318       |
| Multivariable HR                     | 1.00     | 0.91 (0.71-1.18) | 0.80 (0.60-1.06) | 0.73 (0.54-0.99) | 0.73 (0.53-1.00) | 0.037       | 1.00     | 0.91 (0.71-1.18) | 0.80 (0.60-1.06) | 0.73 (0.54-0.99) | 0.73 (0.53-1.00) | 0.037       |
|                                      | Quintiles of dietary manganese intake |       |       |       |     |       |     |       |       |       |       |     |
|--------------------------------------|---------------------------------------|-------|-------|-------|-----|-------|-----|-------|-------|-------|-------|-----|
|                                      | Q1 (low)                              | Q2    | Q3    | Q4    | Q5  | (high)|     |       |       |       |       |     |
| Coronary heart disease. Cases, n     | 59                                    | 67    | 81    | 74    | 80  |       |     |       |       |       |       |     |
| Multivariable HR                     | 1                                     | 1.12  | (0.78-1.59) | 1.24 | (0.88-1.74) | 1.12 | (0.79-1.59) | 1.12 | (0.79-1.59) | 0.609 |     |
| Multivariable HR§                    | 1                                     | 1.09  | (0.76-1.57) | 1.21 | (0.86-1.70) | 1.11 | (0.78-1.58) | 1.05 | (0.73-1.50) | 0.858 |     |
| Multivariable HR§§                   | 1                                     | 1.08  | (0.76-1.56) | 1.18 | (0.84-1.66) | 1.10 | (0.77-1.57) | 1.01 | (0.70-1.45) | 0.970 |     |
| Total cardiovascular disease. Cases, n| 307                                   | 342   | 361   | 353   | 410 |       |     |       |       |       |       |     |
| Multivariable HR§                    | 1                                     | 1.01  | (0.87-1.18) | 0.98 | (0.84-1.15) | 0.96 | (0.82-1.12) | 0.95 | (0.82-1.11) | 0.113 |     |
| Multivariable HR§§                   | 1                                     | 1.02  | (0.87-1.19) | 0.97 | (0.83-1.13) | 0.96 | (0.82-1.13) | 0.92 | (0.79-1.08) | 0.204 |     |
| Multivariable HR§§§                  | 1                                     | 0.99  | (0.84-1.16) | 0.94 | (0.80-1.10) | 0.94 | (0.80-1.10) | 0.86 | (0.74-1.02) | 0.052 |     |

HR*: adjusted for age, sex and residential area.
HR§: adjusted further for body mass index, smoking status, frequency of sports activity, alcohol consumption, hours of walking, education years, and past history of hypertension and diabetes.
HR§§: adjusted further for intake of total energy, sodium, saturated fatty acid and vitamin E.

P value of interaction with green tea consumption status were 0.25 for total stroke, 0.29 for ischemic stroke, 0.48 for subarachnoid hemorrhage, 0.94 for intraparenchymal hemorrhage, 0.11 for coronary heart disease, and 0.32 for total cardiovascular disease.