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Association between Dietary Manganese Intake and Mortality from Cardiovascular Disease in Japanese Population: The Japan Collaborative Cohort Study

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Aim: Manganese (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:95% CI, 0.76: 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.

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Key words: Manganese, Cardiovascular disease, Mortality, Cohort studies, Japan

Introduction

Manganese (Mn) is an essential trace element obtained mainly from the diet. It is absorbed through the alimentary tract and then transported to organs enriched with mitochondria to be concentrated rapidly[1]. The Mn content in water and inhaled air is considered negligible compared with dietary Mn from plant foods, such as pineapple, cabbage, spinach, beans, seeds, nuts, whole grains, soy products, and green tea[2]. However, the average dietary Mn intake varies across populations. Thus, the estimated average nutritional Mn intake in the United States is 2.25 mg/day for men and 1.74 mg/day for women[3], 2.6 mg/day for Belgian men[4], 2.37 mg/day for Spanish men[5], and 4.0 mg/day for Korean men and 3.5 mg/day for Korean women[6]. In Japan, the daily intake was estimated at 5.1 and 4.9 mg/day for men and women, respectively[7]. A recent cohort study has suggested that the Japanese diet has a positive effect on preventing

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CVD[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[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[10]. By preventing the LDL oxidation in endothelial cells[11], Mn can curb vascular thickening and narrowing[12-14]. In conjunction with vitamin K, Mn helps in blood clotting and hemostasis[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[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[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)[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)[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 ± SD) was 5.32 ± 2.09 mg/d, and that for the DR was 4.81 ± 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/m^2, 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 >69.0 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.

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/m^2	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	7124	7123	7124	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/m^2	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 csalculated 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[20]; however, due to insufficient scientific evidence, there is no exact recommended dietary allowance (RDA) for dietary Mn intake among adults[21]. 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[9, 22, 23]. 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)	0.113
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)	0.242
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)	0.217
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)	0.343
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)	0.280
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)	0.328
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)	0.551
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)	0.559
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)	0.599
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)	0.82 (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)	0.95 (0.61-1.50)	0.762
Multivariable HR[§§§]	1	0.84 (0.52-1.35)	1.17 (0.75-1.82)	0.79 (0.49-1.27)	0.93 (0.59-1.47)	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)	0.107
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)	0.091
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)	0.102
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)	0.170
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)	0.140
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)	0.140
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)	0.001
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)	0.001
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)	0.001
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)	0.017
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)	0.004
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)	0.005
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)	0.512
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)	0.436
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)	0.431

(Cont. Table 2)

	Quintiles of dietary manganese intake					*P* for trend
	Q1(low)	Q2	Q3	Q4	Q5(high)	
Intraparenchymal hemorrhage						
Cases, *n*	45	35	48	35	38	
Multivariable HR[§]	1	0.82 (0.52-1.29)	1.00 (0.65-1.53)	0.68 (0.43-1.09)	0.62 (0.39-0.99)	0.030
Multivariable HR[§§]	1	0.81 (0.51-1.27)	1.00 (0.65-1.54)	0.69 (0.43-1.11)	0.62 (0.39-0.99)	0.034
Multivariable HR[§§§]	1	0.77 (0.49-1.23)	0.96 (0.62-1.49)	0.68 (0.42-1.09)	0.60 (0.37-0.96)	0.030
Coronary heart disease						
Cases, *n*	65	60	67	57	61	
Multivariable HR[§]	1	1.13 (0.79-1.63)	1.18 (0.80-1.73)	1.03 (0.69-1.54)	0.91 (0.61-1.36)	0.368
Multivariable HR[§§]	1	1.05 (0.73-1.51)	1.11 (0.75-1.63)	1.00 (0.67-1.50)	0.84 (0.56-1.26)	0.261
Multivariable HR[§§§]	1	0.95 (0.65-1.37)	1.05 (0.71-1.55)	0.99 (0.66-1.48)	0.83 (0.55-1.25)	0.351
Total cardiovascular disease						
Cases, *n*	346	325	354	329	380	
Multivariable HR[§]	1	1.06 (0.91-1.24)	1.00 (0.85-1.18)	0.91 (0.77-1.07)	0.84 (0.72-0.99)	0.004
Multivariable HR[§§]	1	1.02 (0.87-1.19)	0.99 (0.84-1.16)	0.90 (0.76-1.06)	0.81 (0.69-0.96)	0.002
Multivariable HR[§§§]	1	0.98 (0.84-1.15)	0.96 (0.81-1.13)	0.89 (0.75-1.05)	0.79 (0.67-0.94)	0.002

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 interaction with sex was 0.13 for total stroke, 0.37 for ischemic stroke, 0.89 for subarachnoid hemorrhage, 0.20 for intraparenchymal hemorrhage, 0.86 for coronary heart disease, and 0.83 for total cardiovascular disease.

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]. 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

patients with dyslipidemia[34].

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[35-37]. 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[38].

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[35]. 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[39, 40]. 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[41, 42]. 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[41]. 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)[7]. 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[43]; 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[44-46]. However, these factors were highly correlated with other potential confounding factors such as fiber (Spearman correlation coefficients=0.81 with vitamin E) and polyunsaturated fatty acid (Spearman correlation coefficients=0.81 with sodium); 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[18]. 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

UL.

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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

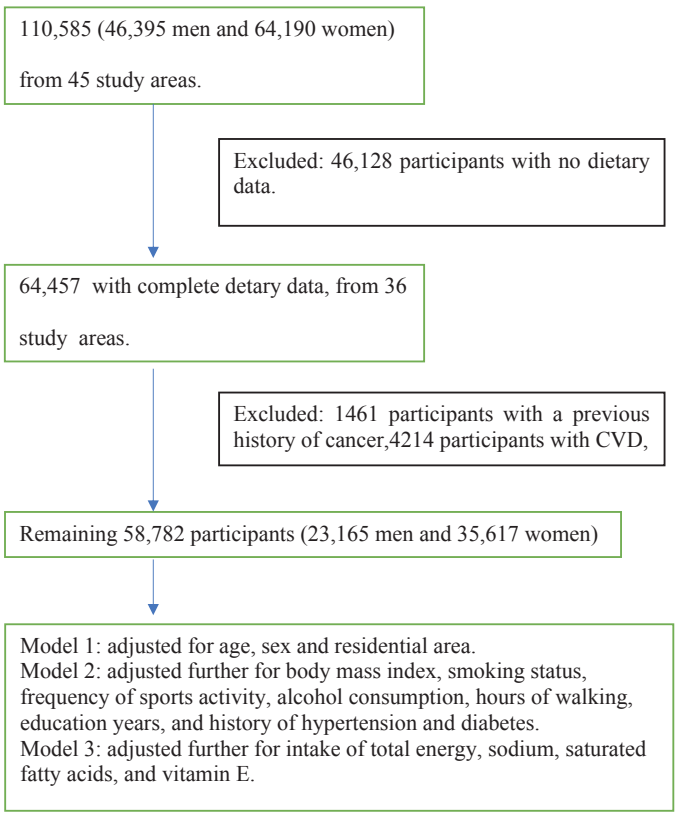
This study was approved by the ethics committees of the Graduate Schools of Medicine in Hokkaido and Osaka Universities.

References

1) Deng Q, Liu J, Li Q, Chen K, Liu Z, Shen Y, Niu P, Yang Y, Zou Y and Yang X: Interaction of occupational manganese exposure and alcohol drinking aggravates the increase of liver enzyme concentrations from a cross-sectional study in China. Environmental health: a global access science source, 2013; 12: 30-30
2) Azadmanesh J and Borgstahl GEO: A Review of the Catalytic Mechanism of Human Manganese Superoxide Dismutase. Antioxidants (Basel, Switzerland), 2018; 7: 25
3) Trumbo P, Yates AA, Schlicker S and Poos M: Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Am Diet Assoc, 2001; 101: 294-301
4) Robberecht HJ, Hendrix P, Van Cauwenbergh R and Deelstra HA: Daily dietary manganese intake in Belgium, using duplicate portion sampling. Zeitschrift für Lebensmittel-Untersuchung und Forschung, 1994; 199: 446-448
5) Rubio C, Gutierrez AJ, Revert C, Reguera JI, Burgos A and Hardisson A: Daily dietary intake of iron, copper, zinc and manganese in a Spanish population. Int J Food Sci Nutr, 2009; 60: 590-600
6) Choi MK and Bae YJ: Relationship between dietary magnesium, manganese, and copper and metabolic syndrome risk in Korean adults: the Korea National Health and Nutrition Examination Survey (2007-2008). Biol Trace Elem Res, 2013; 156: 56-66
7) Yamada M, Asakura K, Sasaki S, Hirota N, Notsu A, Todoriki H, Miura A, Fukui M and Date C: Estimation of intakes of copper, zinc, and manganese in Japanese adults using 16-day semi-weighed diet records. Asia Pac J Clin Nutr, 2014; 23: 465-472
8) Yoshida H: Is the Japan diet instrumental in preventing cardiovascular diseases? J Atheroscler Thromb, 2021; 28: 1023-1024
9) Zhou B, Su X, Su D, Zeng F, Wang MH, Huang L, Huang E, Zhu Y, Zhao D, He D, Zhu X, Yeoh E, Zhang R and Ding G: Dietary intake of manganese and the risk of the metabolic syndrome in a Chinese population. Br J Nutr, 2016; 116: 853-863
10) Keen CL, Ensunsa JL, Watson MH, Baly DL, Donovan SM, Monaco MH and Clegg MS: Nutritional aspects of

manganese from experimental studies. Neurotoxicology, 1999; 20: 213-223
11) Fang X, Weintraub NL, Rios CD, Chappell DA, Zwacka RM, Engelhardt JF, Oberley LW, Yan T, Heistad DD and Spector AA: Overexpression of human superoxide dismutase inhibits oxidation of low-density lipoprotein by endothelial cells. Circ Res, 1998; 82: 1289-1297
12) Szentmihályi K, Vinkler P, Fodor J, Balla J and Lakatos B: The role of manganese in the human organism. Orv Hetil, 2006; 147: 2027-2030
13) Kerr S, Brosnan MJ, McIntyre M, Reid JL, Dominiczak AF and Hamilton CA: Superoxide anion production is increased in a model of genetic hypertension: role of the endothelium. Hypertension, 1999; 33: 1353-1358
14) Tanito M, Nakamura H, Kwon YW, Teratani A, Masutani H, Shioji K, Kishimoto C, Ohira A, Horie R and Yodoi J: Enhanced oxidative stress and impaired thioredoxin expression in spontaneously hypertensive rats. Antioxid Redox Signal, 2004; 6: 89-97
15) Aschner JL and Aschner M: Nutritional aspects of manganese homeostasis. Mol Aspects Med, 2005; 26: 353-362
16) Tamakoshi A, Yoshimura T, Inaba Y, Ito Y, Watanabe Y, Fukuda K and Iso H: Profile of the JACC study. J Epidemiol, 2005; 15 Suppl 1: S4-8
17) Tamakoshi A, Ozasa K, Fujino Y, Suzuki K, Sakata K, Mori M, Kikuchi S, Iso H, Sakauchi F, Motohashi Y, Tsuji I, Nakamura Y, Mikami H, Kurosawa M, Hoshiyama Y, Tanabe N, Tamakoshi K, Wakai K, Tokudome S, Hashimoto S, Wada Y, Kawamura T, Watanabe Y, Miki T, Date C, Kurozawa Y, Yoshimura T, Shibata A, Okamoto N and Shio H: Cohort profile of the Japan Collaborative Cohort Study at final follow-up. J Epidemiol, 2013; 23: 227-232
18) Date C, Fukui M, Yamamoto A, Wakai K, Ozeki A, Motohashi Y, Adachi C, Okamoto N, Kurosawa M, Tokudome Y, Kurisu Y, Watanabe Y, Ozasa K, Nakagawa S, Tokui N, Yoshimura T and Tamakoshi A: Reproducibility and validity of a self-administered food frequency questionnaire used in the JACC study. J Epidemiol, 2005; 15 Suppl 1: S9-23
19) Council for Science and Technology; Ministry of Education C and Sports SaT: Standard Tables of Food Composition in Japan, fifth revised and enlarged edition. 2005;
20) Sasaki S: Dietary Reference Intakes (DRIs) in Japan. J Asia Pacific, 2008; 17: 420-444
21) Haugen M, Frøyland L, Henjum S, Løvik M, Stea T, Strand T, Parr C and Holvik K: Assessment of dietary intake of manganese in relation to tolerable upper intake level. European Journal of Nutrition & Food Safety, 2019; 91-93
22) Choi M-K and Kim E-Y: Evaluation of Dietary Manganese Intake in Korean Men and Women over 20 Years Old. J Korean Soc Food Sci Nuterr, 2007; 36: 447-452
23) Li Y, Guo H, Wu M and Liu M: Serum and dietary antioxidant status is associated with lower prevalence of the metabolic syndrome in a study in Shanghai, China. Asia Pac J Clin Nutr, 2013; 22: 60-68
24) Eshak ES, Muraki I, Imano H, Yamagishi K, Tamakoshi A and Iso H: Manganese intake from foods and beverages is associated with a reduced risk of type 2 diabetes. Maturitas, 143, 127–131.. Maturitas, 2021; 143: 127-131
25) Li L and Yang X: The Essential Element Manganese, Oxidative Stress, and Metabolic Diseases: Links and Interactions. Oxid Med Cell Longev, 2018; 2018: 7580707
26) Bresciani G, Cruz IB, de Paz JA, Cuevas MJ and González-Gallego J: The MnSOD Ala16Val SNP: relevance to human diseases and interaction with environmental factors. Free Radic Res, 2013; 47: 781-792
27) Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D and Degoul F: The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenetics, 2003; 13: 145-157
28) Lee S-H, Jouihan HA, Cooksey RC, Jones D, Kim HJ, Winge DR and McClain DA: Manganese supplementation protects against diet-induced diabetes in wild type mice by enhancing insulin secretion. Endocrinology, 2013; 154: 1029-1038
29) Panee J: Monocyte Chemoattractant Protein 1 (MCP-1) in obesity and diabetes. Cytokine, 2012; 60: 1-12
30) Peluso I, Morabito G, Urban L, Ioannone F and Serafini M: Oxidative stress in atherosclerosis development: the central role of LDL and oxidative burst. Endocr Metab Immune Disord Drug Targets, 2012; 12: 351-360
31) Rains JL, Kanikarla-Marie P and Jain SK: Hyperketonemia induces upregulation of LFA-1 in monocytes, which is mediated by ROS and P38 MAPK activation. Can J Physiol Pharmacol, 2012; 90: 1642-1646
32) Burlet E and Jain SK: Manganese supplementation increases adiponectin and lowers ICAM-1 and creatinine blood levels in Zucker type 2 diabetic rats, and downregulates ICAM-1 by upregulating adiponectin multimerization protein (DsbA-L) in endothelial cells. Mol Cell Biochem, 2017; 429: 1-10
33) Burlet E and Jain SK: Manganese supplementation reduces high glucose-induced monocyte adhesion to endothelial cells and endothelial dysfunction in Zucker diabetic fatty rats. J Biol Chem, 2013; 288: 6409-6416
34) Maruyama C, Shijo Y, Kameyama N, Umezawa A, Sato A, Nishitani A, Ayaori M, Ikewaki K, Waki M and Taramoto T: Effects of nutrition education program for the Japan diet on serum LDL-cholesterol concentration in patients with dyslipidemia: a randomized controlled trial. J Atheroscler Thromb, 2021; 28: 1035-1051
35) Finley JW: Manganese absorption and retention by young women is associated with serum ferritin concentration. Am J Clin Nutr, 1999; 70: 37-43
36) Williams M, Todd GD, Roney N, Crawford J, Coles C, McClure PR, Garey JD, Zaccaria K and Citra M: Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles. In: Toxicological Profile for Manganese, Agency for Toxic Substances and Disease Registry (US), Atlanta (GA), 2012
37) Oulhote Y, Mergler D and Bouchard MF: Sex-and age-differences in blood manganese levels in the US general population: national health and nutrition examination survey 2011–2012. Environ Health, 2014; 13: 87

38) Finley JW, Johnson PE and Johnson LK: Sex affects manganese absorption and retention by humans from a diet adequate in manganese. Am J Clin Nutr, 1994; 60: 949-955
39) Dees WL, Hiney JK and Srivastava VK: Influences of manganese on pubertal development. The Journal of endocrinology, 2017; 235: R33-R42
40) Pine M, Lee B, Dearth R, Hiney JK and Dees WL: Manganese acts centrally to stimulate luteinizing hormone secretion: a potential influence on female pubertal development. Toxicol Sci, 2005; 85: 880-885
41) Wang N, Zhang K, Han B, Li Q, Chen Y, Zhu C, Chen Y, Xia F, Zhai H, Jiang B, Shen Z and Lu Y: Follicle stimulating hormone, its novel association with sex hormone binding globulin in men and postmenopausal women. Endocrine, 2017; 56: 649-657
42) Pugeat M, Moulin P, Cousin P, Fimbel S, Nicolas MH, Crave JC and Lejeune H: Interrelations between sex hormone-binding globulin (SHBG), plasma lipoproteins and cardiovascular risk. J Steroid Biochem Mol Biol, 1995; 53: 567-572
43) Davidsson L, Cederblad A, Lönnerdal B and Sandström B: Manganese retention in man: a method for estimating manganese absorption in man. Am J Clin Nutr, 1989; 49: 170-179
44) Kubota Y, Iso H, Date C, Kikuchi S, Watanabe Y, Wada Y, Inaba Y, Tamakoshi A and Group JS: Dietary intakes of antioxidant vitamins and mortality from cardiovascular disease: the Japan Collaborative Cohort Study (JACC) study. Stroke, 2011; 42: 1665-1672
45) Ikehara S, Iso H, Date C, Kikuchi S, Watanabe Y, Inaba Y and Tamakoshi A: Salt preference and mortality from stroke and coronary heart disease for Japanese men and women: the JACC study. Prev Med, 2012; 54: 32-37
46) Yamagishi K, Iso H, Yatsuya H, Tanabe N, Date C, Kikuchi S, Yamamoto A, Inaba Y and Tamakoshi A: Dietary intake of saturated fatty acids and mortality from cardiovascular disease in Japanese: the Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC) Study. Am J Clin Nutr, 2010; 92: 759-765



Supplemental Fig. 1. Flow chart of the research

Supplemental 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/m^2	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					*P* for trend
	Q1 (low)	Q2	Q3	Q4	Q5 (high)	
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 HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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

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.

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					*P* for trend
	Q1(low)	Q2	Q3	Q4	Q5(high)	
Postmenopausal women, *n*	4667	4667	4667	4667	4667	
Person-years	76913	67665	72388	77313	83875	
Total stroke						
Cases, *n*	142	122	135	122	122	
Multivariable HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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	2169	
Person-years	43884	48623	43390	42121	38690	
Total stroke						
Cases, *n*	30	27	30	26	30	
Multivariable HR[§]	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 HR[§§]	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 HR[§§§]	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 HR[§]	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 HR[§§]	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 HR[§§§]	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)

	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					*P* for trend
	Q1 (low)	Q2	Q3	Q4	Q5 (high)	
≤ 1 cup green tea/day (average intake = 0.36 cup/day)						
Median value of Mn intake (mg/day)	2.45	2.87	3.15	3.45	4.03	
Number of subjects	2580	2581	2580	2581	2580	
Total stroke. Cases, *n*	67	56	63	53	66	
Multivariable HR[§]	1	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
Multivariable HR[§§]	1	1.00 (0.67-1.47)	1.09 (0.73-1.64)	0.91 (0.60-1.40)	1.28 (0.84-1.95)	0.265
Multivariable HR[§§§]	1	0.95 (0.63-1.42)	0.99 (0.64-1.53)	0.79 (0.49-1.28)	1.10 (0.69-1.76)	0.633
Ischemic stroke. Cases, *n*	32	28	37	31	35	
Multivariable HR[§]	1	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
Multivariable HR[§§]	1	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
Multivariable HR[§§§]	1	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
Subarachnoid hemorrhage. Cases, *n*	10	6	10	7	11	
Multivariable HR[§]	1	0.50 (0.18-1.41)	0.78 (0.31-1.95)	0.53 (0.19-1.46)	0.95 (0.37-2.43)	0.905
Multivariable HR[§§]	1	0.62 (0.20-1.89)	1.04 (0.37-2.95)	0.64 (0.21-2.01)	1.18 (0.41-3.39)	0.604
Multivariable HR[§§§]	1	0.50 (0.15-1.63)	0.81 (0.25-2.60)	0.47 (0.13-1.74)	0.92 (0.27-3.15)	0.758
Intraparenchymal hemorrhage. Cases, *n*	23	17	14	12	18	
Multivariable HR[§]	1	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	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	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	15	
Multivariable HR[§]	1	1.09 (0.69-1.74)	0.93 (0.57-1.52)	1.01 (0.62-1.64)	0.45 (0.24-0.85)	0.022
Multivariable HR[§§]	1	1.30 (0.79-2.16)	1.22 (0.70-2.10)	1.23 (0.70-2.16)	0.54 (0.27-1.08)	0.078
Multivariable HR[§§§]	1	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
Total CVD. Cases, *n*	156	142	141	138	125	
Multivariable HR[§]	1	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
Multivariable HR[§§]	1	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	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
Over 1 cup green tea/day (average intake = 4.00 cups/day)						
Median value of Mn intake (mg/day)	4.45	5.23	5.98	6.82	7.62	
Number of subjects	6427	6428	6428	6428	6427	
Total stroke. Cases, *n*	146	165	157	154	173	
Multivariable HR[§]	1	1.03 (0.82-1.29)	0.89 (0.71-1.12)	0.86 (0.69-1.09)	0.82 (0.66-1.04)	0.028
Multivariable HR[§§]	1	1.03 (0.82-1.30)	0.88 (0.70-1.11)	0.87 (0.69-1.10)	0.80 (0.63-1.01)	0.018
Multivariable HR[§§§]	1	1.00 (0.80-1.26)	0.85 (0.67-1.07)	0.85 (0.67-1.07)	0.74 (0.58-0.94)	0.004
Ischemic stroke. Cases, *n*	73	81	77	96	92	
Multivariable HR[§]	1	1.00 (0.72-1.37)	0.83 (0.60-1.15)	1.05 (0.77-1.42)	0.82 (0.59-1.13)	0.329
Multivariable HR[§§]	1	0.99 (0.71-1.38)	0.82 (0.59-1.13)	1.05 (0.76-1.43)	0.76 (0.55-1.06)	0.169
Multivariable HR[§§§]	1	0.98 (0.71-1.36)	0.78 (0.56-1.09)	1.03 (0.75-1.41)	0.70 (0.50-0.98)	0.077
Subarachnoid hemorrhage . Cases, *n*	25	27	26	17	30	
Multivariable HR[§]	1	0.94 (0.54-1.64)	0.91 (0.52-1.59)	0.57 (0.30-1.06)	0.87 (0.50-1.52)	0.291
Multivariable HR[§§]	1	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	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
Intraparenchymal hemorrhage. Cases, *n*	42	51	46	36	48	
Multivariable HR[§]	1	1.18 (0.78-1.78)	0.97 (0.64-1.48)	0.74 (0.47-1.16)	0.89 (0.58-1.36)	0.146
Multivariable HR[§§]	1	1.17 (0.77-1.78)	0.95 (0.62-1.46)	0.74 (0.47-1.17)	0.89 (0.57-1.38)	0.154
Multivariable HR[§§§]	1	1.11 (0.73-1.69)	0.93 (0.60-1.42)	0.71 (0.45-1.13)	0.80 (0.51-1.26)	0.074

(Cont. Supplemental Table 4)

	Quintiles of dietary manganese intake					*P* for trend
	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.