that would significantly reduce risk of COPD mortality to the level similar to never smokers.

The detailed examination of this issue would enable us to formulate more an explicit public health recommendation. Therefore, in this 18-year follow-up cohort study of approximately 95000 Japanese men and women, we examined risk of COPD mortality associated not only with smoking status but time since quitting smoking.

Methods

The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC study) was initiated 1988–1990(Kawado et al., 2005; Ohno and Tamakoshi, 2001). Self-administered questionnaires that included items on lifestyles and medical histories of COPD, cancer, cardiovascular disease and other diseases were completed by 110792 persons (46465 men and 64327 women) aged 40–79 years from 45 communities across Japan. Among them, 44201 men and 55592 women provided valid responses about smoking status. Those who had quit smoking were asked at what age or what year they stopped in order to calculate the years of smoking cessation. We also excluded 2736 men and 2930 women with a reported history of COPD, asthma, other chronic lung diseases, cardiovascular disease or cancer at baseline, leaving 41465 men and 52662 women for the present analysis.

Mortality surveillance was conducted systematically by reviewing death certificates. The underlying causes of death according to the *International Classification of Diseases* (ICD-10) were obtained centrally from the Ministry of Health and Welfare. COPD was defined as ICD-10 codes of J41 to J44 and J47. The present study was approved by the Ethical Committee, Nagoya University and Osaka University.

Statistical analysis

Participants were followed-up until death or they moved away from the original community to the end of 2008. The follow-up of six and five communities ended at the end of 1999 and 2003, respectively. Median follow-up period was 18 years. Sex-specific, age-adjusted means and proportions of selected COPD risk factors were calculated by general linear model.

Sex-specific, age-adjusted and multivariable-adjusted hazard ratios (HRs) and their 95% confidence intervals (95% CIs) were calculated by Cox proportional hazard models. Duration of smoking cessation was divided to three groups (0–4, 5–9 and ≥10 years before the baseline). Variables included in the multivariable-adjusted model were age at baseline, body mass index, ethanol intake, hours of walking, hours of exercise, education, perceived mental stress, and histories of hypertension and diabetes. Number of cigarettes smoked per day and age of smoking initiation were also included in the smoking cessation analysis.

Sensitivity analyses were conducted separately by excluding early deaths from COPD mortality within the first 5-year of follow-up and by excluding those with self-reported persistent phlegm symptom in an attempt to reduce a reverse causal relationship. Interaction for sex-by-smoking status was tested by using cross-product terms of sex with smoking status. In order to evaluate the specificity of association of smoking status or smoking cessation duration with COPD mortality, all-cause mortality was also modeled, and the result was compared with that of COPD. This was done by computing a test statistic: $(b_1-b_2)^2/\{[SE(b_1)]^2+[SE(b_2)]^2\}$, where b_1 is the coefficient for the association with COPD, b_2 is the coefficient for all-cause mortality, $SE(b_1)$ and $SE(b_2)$ are the corresponding standard errors for the association with COPD and all-cause mortality, respectively (Allison, 1995).

The proportional hazard assumption was confirmed graphically by examining the parallelness of the ln (−ln) survival curves for smoking status as well as by a model including the interaction term between follow-up time and smoking status. The follow-up time was first treated as a continuous scale and then dichotomized at year 11 (middle value of follow-up) in the model. We found no violation for the proportional hazard assumption.

All analyses were performed by using SAS version 9.1.3 Service Pack 4 (SAS Institute, Cary, North Carolina). Two-tailed probability values of <0.05 were considered statistically significant.

Results

The proportions of current and former smokers were 54% and 25% in men, and 6% and 2% in women, respectively. Majority of male smokers (68%) smoked 20 or more cigarettes per day, but the corresponding proportion in female current smokers was 31%. Compared with never or current smokers, former smokers were older, more educated, and more likely to have hypertension and diabetes mellitus in both men and women (Table 1).

A total of 251 deaths from COPD among 41465 men and 34 deaths among 52662 women were documented during the 18-year follow-up. Both former smoking and current smoking were significantly associated with increased COPD mortality for both men and women in models adjusted for age and potential confounding variables (Table 2). The multivariable-adjusted HRs (95% CIs) for former and current smokers compared with never smokers were 2.97 (1.76–5.02) and 4.46 (2.72–7.29) in men and 8.57 (2.75–26.7) and 9.26 (4.19–20.5) in women, respectively (Table 2). There appeared dose–response associations between the number of cigarettes smoked daily and age- and multivariable-adjusted risk of COPD mortality among current smokers in both sexes. Although crude COPD mortality rates were higher in men than in women in any smoking status categories at baseline, associations of both former and current smoking with COPD mortality tended to be stronger in women than in men (P for sex-by-smoking status interaction = 0.08).

Compared with current smokers, former smokers at baseline were associated with lower COPD mortality in men but only when cessation duration was five years or more before the baseline (Table 3). Men who had quit smoking more than 10 years before baseline had COPD mortality risk close to never smokers. Quitters for less than five years did not experience the lowering of mortality risk compared to current continuous smokers. The finding did not change materially even after excluding COPD deaths that occurred within five years from the baseline or individuals who reported persistent phlegm symptom at baseline (HRs: 95% CIs were 1.19: 0.80–1.79 and 1.11: 0.71–1.72, respectively). There were too few death cases in female former smokers (n = 4) at each smoking cessation group to yield meaningful results.

The associations of smoking status (P = 0.005 in men, P<0.001 in women) and smoking cessation duration (P<0.001 in men) with COPD mortality were stronger than those with all-cause mortality (Supplemental tables 1 and 2).

Discussion

We observed the excess risk of COPD mortality among current and former smokers of both sexes in this large prospective cohort study of Japanese. Our finding is consistent with the results from previous prospective studies, including US veterans' cohort (Rogot and Murray, 1980), British doctors' cohort (Doll et al., 1980, 2004), Swedish registers' cohort (Carstensen et al., 1987), Copenhagen registers' cohort (Lange et al., 1992), and Washington white registers' cohort (Tockman and Comstock, 1989).

Our study also revealed that the duration of smoking cessation was inversely associated with COPD mortality in men, and the excess risk that would have been observed if they had continuous smoking could be reduced after long-term (≥10 years) cessation before the baseline similar to the level observed in never smokers.

A few studies have evaluated the duration of quitting smoking associated with COPD mortality and morbidity (Lokke et al., 2006; Rogot and Murray, 1980). Our finding that accounted for other smoking-related variables, which previous studies did not address, was similar to that of a 25-year follow-up study in a general population of both sexes (Lokke et al., 2006). That study showed a dose–response relationship between the duration of smoking cessation and cumulative incidence of COPD, and the odds ratio for stage 2 or more COPD in ex-smokers who had quit 25 years or more at the end of follow-up compared to continuous smokers was similar to that in never smokers (Lokke et al., 2006). Another 16-year observation in the US veterans described that crude COPD mortality rate fell to approximately one fifth of continuous smokers if subjects had quit smoking 20 years or more at the end of follow-up (Rogot and Murray, 1980).

Table 1
Sex-specific, age-adjusted means and proportions according to smoking status at baseline, Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study), 1988–2008.

	Men					Women				
	Never	Former	Current	Cigarettes smoked* (no./day)		Never	Former	Current	Cigarettes smoked* (no./day)	
	Smokers	Smokers	Smokers	1–19	≥20	Smokers	Smokers	Smokers	1–19	≥20
No. at risk	8613	10394	22458	7174	14973	48914	853	2895	1932	848
Age (years)	56.6	60.0	55.9	59.0	54.4	57.1	60.0	56.0	56.7	54.2
Body mass index (kg/m^2)	23.0	22.9	22.4	22.0	22.6	22.9	23.3	22.8	22.7	23.2
History of hypertension (%)	19.1	26.0	17.8	21.4	16.0	21.6	27.4	22.2	23.2	20.2
History of diabetes (%)	5.7	8.4	6.2	6.7	6.1	3.5	6.8	5.0	4.8	4.5
Ethanol intake (g/day)	18.2	22.5	27.4	24.4	28.9	1.2	4.7	6.9	5.5	10.5
Walk half an hour or more/day (%)	69.3	68.2	69.8	71.1	69.3	71.9	64.5	69.3	70.8	66.2
Exercise 5 h or more/week (%)	7.2	8.3	6.6	8.0	5.9	4.5	4.3	4.0	4.3	3.2
High perceived mental stress (%)	22.4	21.8	23.9	19.7	25.9	20.0	24.1	26.2	24.1	30.7
College or higher education (%)	18.3	20.2	15.9	15.2	16.3	10.1	12.5	8.5	9.1	7.5

* Information on number of cigarettes smoked per day among current smokers was missing for 311 men and for 115 women.

It is noteworthy that both former and current smokers were more strongly associated with COPD mortality in women than in men, especially in female heavy smokers who currently smoked 20 or more cigarettes per day. The interaction for sex-by-smoking status was found to be of borderline significance ($P=0.08$) in the multivariable model. Similarly, Copenhagen City Heart Study (Lange et al., 1992) and British doctors' cohort study (Doll et al., 1980, 2004) also presented that the COPD mortality ratio associated with smoking in female was higher than that in male, however the number of female deaths from COPD was too small in both studies to confirm the gender difference. The higher age-adjusted relative risks for COPD hospitalization in female smokers compared to the risk in male smokers were also observed in Danish longitudinal population study ($P=0.08$ for the interaction for sex by pack-years categories). Previous prospective studies demonstrated that at comparable levels of smoking exposure, women expressed a faster decline in lung function (FEV_1) (Prescott et al., 1997; Xu et al., 1994). A possible explanation for the faster deterioration is that women have smaller airways and lung volume than dose men, which results in higher exposure in per volume of lung tissues with each cigarette. In addition, estrogen and related compounds have been reported to increase smoking-induced lung damage possibly through up-regulating the expression of cytochrome P450 enzymes in lungs (Benowitz et al., 2006). Cytochrome P450 enzymes metabolize some harmless substances in cigarette smoke into toxic chemicals, for example benzo[*a*]pyrene into benzo[*a*]pyrene-7,8-diol (Ben-Zaken Cohen et al., 2007). A family study of early-onset COPD probands found no differences in lung function between their female and male first-degree relatives. However, smoking female first-degree relatives showed significantly lower lung function than smoking male first-degree relatives, which implied a genetic predisposition for smoking-induced lung damage in women (Silverman et al., 2000). In the current study, the misclassification of smoking status as never smoking in women was smaller than that in men, which may contribute to the stronger association between smoking and mortality in women. In addition, the finding might have been observed by chance due to small number of women who died from COPD.

Potential effects of smoking cessation on pulmonary pathology have been reported. Macroscopic signs of chronic bronchitis (edema, erythema and mucus) disappeared totally after 6 months' smoking cessation (Skold et al., 1992). In addition, after smoking cessation, the number of macrophages in bronchoalveolar lavage fluid (Skold et al., 1992), blood neutrophils and lymphocytes (Jensen et al., 1998) was largely reversed, and

Table 2
Sex-specific, age- and multivariable-adjusted hazard ratios and 95% confidence intervals of mortality from COPD according to smoking status, Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study), 1988–2008.

	Never smokers	Former smokers	Current smokers	Cigarettes smoked (no./day)	
				1–19	≥20
Men					
No. at risk	8613	10394	22458	7174	14973
No. of person-years	138752	157215	346870	106398	235693
No. of death	18	68	165	53	111
Crude death rate*	13	43	48	50	47
Age-adjusted HR	1.0	2.76 (1.64–4.64)	4.84 (2.97–7.88)	3.57 (2.09–6.09)	6.06 (3.67–10.0)
Multivariable HR†	1.0	2.97 (1.76–5.02)	4.46 (2.72–7.29)	3.27 (1.91–5.60)	5.60 (3.38–9.29)
Women					
No. at risk	48914	853	2895	1932	848
No. of person-years	796017	12463	44454	29454	13099
No. of death	20	4	10	6	4
Crude death rate*	3	32	22	20	31
Age-adjusted HR	1.0	8.82 (3.01–25.9)	10.1 (4.71–21.6)	8.35 (3.35–20.8)	19.3 (6.58–56.4)
Multivariable HR†	1.0	8.57 (2.75–26.7)	9.26 (4.19–20.5)	7.54 (2.95–19.3)	18.3 (5.96–56.3)

* Mortality rate was expressed as rate per 100000 person-years.

† *Multivariable adjustment*: age (*continuous*), body mass index (*sex-specific quintiles*), ethanol intake (*never, former, current intake of* 1–22, 23–45, 46–68, and ≥69 g per day), hours of walking (<0.5, 0.5, 0.6–0.9, and ≥1.0 h per day), hours of exercise (<1, 1–2, 3–4, and ≥5 h per week), education (<10, 10–12, 13–15, and ≥16 years), perceived mental stress (low, medium, and high), and histories of hypertension and diabetes.

Table 3
Sex-specific, age- and multivariable-adjusted hazard ratios and 95% confidence intervals of mortality from COPD according to years since quitting, Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study), 1988–2008.

	Current smokers	No. of years since quitting smoking before the baseline*			Never smokers
		0–4	5–9	≥10	
Men					
No. at risk	22458	2599	2360	4964	8613
No. of person-years	346870	39582	35889	74612	138752
No. of death	165	32	9	20	18
Crude death rate**	48	81	25	27	13
Age-adjusted HR	1.0	1.24 (0.85–1.82)	0.41 (0.21–0.80)	0.30 (0.19–0.48)	0.21 (0.13–0.34)
Multivariable HR†	1.0	1.23 (0.83–1.81)	0.44 (0.22–0.87)	0.36 (0.22–0.58)	0.30 (0.16–0.57)
Women					
No. at risk	2895	251	184	315	48914
No. of person-years	44454	3758	2657	4510	796017
No. of death	10	0	2	2	20
Crude death rate**	22	0	75	44	3
Age-adjusted HR	1.0	.....	1.00 (0.31–3.18)		0.10 (0.05–0.21)

* Information on number of years since quitting smoking was missing for 471 men and for 103 women.
** Mortality rate was expressed as rate per 100000 person-years.
† Multivariable adjustment: variables included in multivariable model in table 2 plus number of cigarettes smoked per day (<20, 20–29, and ≥30) and age of smoking initiation (<20, 20–24, 25–29, and ≥30 years).

those in bronchoalveolar lavage fluid normalized at 6, 9, and 15 months, respectively (Skold et al., 1996). These data indicated that the inflammatory changes are reversible rapidly after smoking cessation. However, in the present study, quitters for less than five years did not experience the lowering of COPD mortality risk compared to current continuous smokers. One possible explanation is that the sample in the present study included people who already had preclinical but irreversible emphysema. Indeed, a recent study in Japan showed that only 9.4% of cases with airflow limitation reported a previous diagnosis of COPD (Fukuchi et al., 2004). Even among early stage COPD patients, it takes 11-years or more for sustained quitters to experience the same rate of FEV_1 decline as never smokers (Anthonisen et al., 2002).

We could not clarify the association between the duration of smoking cessation and COPD mortality in women due to the small number of deaths in former smokers. However, one previous intervention study demonstrated that women experienced larger improvements in lung function with smoking cessation than men ($\triangle FEV_1$ change: 3.7% vs. 1.6%) (Connett et al., 2003). The effect of smoking cessation on COPD incidence and mortality in women warrants further investigation.

Since persons who quit smoking years prior to the enrollment were more likely to be unhealthy or had some respiratory symptoms, we conducted analyses by excluding the early deaths of COPD within 5-years of follow-up or those who had persistent phlegm at baseline. This exclusion, however, did not alter our results essentially.

Some limitations in the present study merit discussion. COPD develops in a long-term process and is often undiagnosed (Fukuchi et al., 2004; Mannino et al., 2000). Therefore, the duration of smoking cessation to reduce mortality from COPD (≥10 year before baseline) might be longer than that to reduce the incidence of COPD. Smoking information was assessed only at baseline and was not updated throughout the entire study period in the present study. However, the examination of about one-third of the present sample with 5-year follow-up data indeed showed that the percentage of current smokers had decreased (Kawado et al., 2005) by 5.6 points for men, and 0.4 points for women in the present study sample. This suggests that the beneficial effect for smoking cessation may be underestimated, especially for men.

Our study takes advantages of a long observation period, large population-based samples and the availability of information about potential confounding factors for COPD. We have found that smoking status and smoking cessation duration were more strongly associated with COPD than with all-cause mortality.

Overall, the present study suggests that women may be more susceptible to smoking cigarettes for COPD mortality, and that longer time of smoking cessation was associated with progressively decreased COPD mortality in men. We conclude that smokers should be encouraged to stop smoking as early as possible for the prevention of COPD.

Author contributions

Y.L. analyzed data, and wrote manuscript. H.I. analyzed data, and conducted critical revision of manuscript. K.Y., H.Y. and A.T. conducted critical revision of manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ypmed.2012.09.006.

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Original Article

Associations of Dietary Iron Intake With Mortality From Cardiovascular Disease: The JACC Study

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ABSTRACT

Background: We investigated the relationship between dietary iron intake and mortality from cardiovascular disease (CVD) in a population-based sample of Japanese adults.

Methods: The study cohort consisted of 58 615 healthy Japanese (23 083 men and 35 532 women), aged between 40 and 79 years, who had no history of stroke, coronary heart disease (CHD), or cancer at baseline. Dietary iron intake was assessed at baseline by a validated food frequency questionnaire administered between 1988 and 1990 as part of the Japan Collaborative Cohort (JACC) Study.

Results: We documented 2690 (1343 men and 1347 women) deaths from CVD: 1227 (607 men and 620 women) deaths from total stroke, 651 from ischemic stroke (355 men and 296 women), 459 (196 men and 263 women) from hemorrhagic stroke, and 557 (311 men and 246 women) from CHD. Dietary intake of total iron was positively associated with mortality from total and ischemic stroke and total CVD in men. The multivariable hazard ratio for the highest versus the lowest quintile of total iron intake was 1.43 (95% CI, 1.02–2.00; P for trend = 0.009) for total stroke and 1.27 (1.01–1.58; 0.023) for total CVD in men. Dietary total iron intake was not associated with mortality from other endpoints in men, and was not associated with any endpoints in women.

Conclusions: Dietary intake of total iron was positively associated with mortality from stroke and total CVD in Japanese men.

Key words: dietary iron; mortality; stroke; coronary heart disease; cardiovascular disease; follow-up studies

INTRODUCTION

Iron is an important mineral for humans because it is responsible for oxygen transport, digestion of food, and metabolism of body fat, which is essential for cell renewal.[1] However, iron has been implicated in the development of cardiovascular disease because it induces free-radical damage to tissues.[2] In 1981, Sullivan proposed the "iron hypothesis" (ie, that iron stored in the body increases the risk of cardiovascular disease) after observing myocardial failure in people with iron storage disease, age-related accumulation of stored iron in men, and levels of post-menopausal accumulation of stored iron in women that were similar to those in men.[3] Although this hypothesis has been tested during the subsequent 30 years, the results have been inconsistent.

Among studies that used serum ferritin as an indicator of body iron stores, a cross-sectional study of German women found a significant positive association between serum ferritin levels and carotid atherosclerosis and noted that this association was more evident among women with higher levels of low-density lipoprotein (LDL) cholesterol.[4] A prospective study of Italian men and women reported a positive association between serum ferritin level and development of early carotid atherosclerosis, which supports the iron hypothesis.[5] Another prospective study, of Finnish men, showed that serum ferritin level was positively associated with risk of coronary heart disease,[6] while a prospective study of Dutch postmenopausal women found a positive association with risk of ischemic stroke.[7] However, other studies showed nonsignificant relations.[8–13] Among studies that used food frequency questionnaires (FFQs) to

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evaluate dietary iron intake, the prospective study of Finnish men found a positive association between total iron intake and risk of myocardial infarction.[6] A case-control study of Chinese men and women reported that dietary iron intake was positively associated with risk of ischemic stroke.[14] Other studies found a positive association between dietary intake of heme iron derived mainly from red meat and risk of coronary heart disease,[15,16] whereas others found no, or even inverse, associations.[8,17,18] To our knowledge, no prospective study has examined the association between dietary iron intake and cardiovascular disease incidence or mortality in an Asian population.

Using data from a large prospective study of Japanese men and women, we investigated the association between dietary iron and mortality from cardiovascular disease.

METHODS

Study population

The Japan Collaborative Cohort (JACC) Study for the Evaluation of Cancer Risk, a large-scale cohort study sponsored by the Ministry of Education, Sports and Science, was conducted from 1988 to 1990 as a baseline survey. The population-based sample consisted of 110 792 adults (46 465 men and 64 327 women) from 45 community areas. The sampling methods and protocols of the JACC study have been recently described.[19] Participants were asked to complete self-administered questionnaires concerning their lifestyle behaviors and medical histories of cardiovascular disease and cancer. Subjects were excluded if they reported a history of stroke, coronary heart disease, or cancer ($n = 5864$) at baseline or were unable to provide data for the FFQ ($n = 46\,313$). Data from the remaining 58 615 subjects (23 083 men and 35 532 women) were used in the analyses. There were some differences in baseline characteristics between individuals who responded to the FFQ and those who did not, such as mean age (56.5 vs 59.3 years, respectively), college or higher education (12.6% vs 5.7%), and higher perceived mental stress (20.7% vs 7.7%).

Informed consent was obtained either from participants before they completed the questionnaire or from community leaders (which was a common way to obtain informed consent in Japan at the time the study was conducted). The institutional review boards of Nagoya University, University of Tsukuba, and Osaka University approved the present study.

Assessment of iron intake

Dietary iron intake was assessed at baseline by using a self-administered FFQ, which included 33 food items. Participants were asked to estimate average consumption of certain foods over the previous year. Five frequency responses were provided: rarely, 1 to 2 days per month, 1 to 2 days per week, 3 to 4 days per week, and almost every day. Iron content per 100 g of each food (as determined with the Japan Food Tables, Fifth Revised Edition) and the portion size for each food was estimated in a previous validation study.[20] Iron intake was calculated from the participants' frequency scores for the consumption of each food, ie, 0, 0.38, 1.5, 3.5, and 7 times per week. Iron was adjusted for total energy using the residual method. Supplemental iron intake was not measured because we did not have sufficient data on vitamin supplementation. Four 3-day dietary records were collected over a 1-year period ($n = 85$) to test the validity of the FFQ. The records showed that average dietary iron intake was 9.4 mg/day, while that calculated from the FFQ was 6.8 mg/day. The Pearson correlation coefficient between iron intake as estimated by the FFQ and by the four 3-day dietary records was 0.50 after individual energy adjustment. As for reproducibility, 2 questionnaires were administered, 1 year apart, to 85 participants, and the Spearman correlation coefficient was 0.95.

We also used the FFQ to assess heme iron intake. We adopted the method used in the Netherlands Cohort Study on Diet and Cancer (NLCS) and the Canadian National Breast Screening Study (NBSS) database.[21,22] The food items that contributed to heme iron intake were beef, pork, sausage, chicken, liver, fish, dried fish, and boiled fish paste. Heme iron was estimated by using the percentage of heme iron to total iron in these major food items, namely, 69% for beef, 39% for pork and ham, 26% for chicken, 21% for liver, and 26% for fish, dried fish, and boiled fish paste. Average daily intake of heme iron was calculated by multiplying frequency of consumption by the amount of total iron and the above percentages. Non-heme iron intake was calculated as total iron minus heme iron intake. Heme and non-heme iron intakes were also adjusted for total energy using the residual method.

Mortality surveillance

In each community, investigators conducted a systematic review of death certificates as part of the mortality surveillance. Death certificates were forwarded to the public health departments of the respective areas, and mortality data were then centralized at the Ministry of Health and Welfare. Underlying cause of death was coded for National Vital Statistics using the International Classification of Disease, 9th Revision (ICD-9) from 1988 to 1994 and ICD-10 from 1995 to 2003. All deaths within the cohort were ascertained by means of a death certificate from a public health center, except for subjects who died after moving out of their original communities, which were treated as censored cases ($n = 2956$). Follow-up was conducted until the end of 2006, except in 4 areas where it was terminated in 1999 and 4 areas where it was terminated in 2003. The median follow-up period was 14.7 years.

The primary endpoints for this analysis were death from total cardiovascular disease (ICD-9 codes 390–459 and ICD-10 codes 101–199), including total stroke (codes 430–38 and I50–I69)—which was further subdivided into hemorrhagic

Table 1. Baseline characteristics and risk factors of participants by quintile of total iron intake

	Quintile of total iron intake					*P* for trend
	1 (low)	2	3	4	5 (high)	
Men						
No. of subjects	4616	4617	4617	4617	4616	
Median iron intake (mg/day)[a]	5.12	6.60	7.65	8.78	10.58	
Mean age (SD) (years)	53.4 (9.7)	54.3 (9.6)	55.8 (9.6)	57.1 (9.8)	59.2 (9.8)	<0.001
Mean body mass index (kg/m^2)	22.8 (2.7)	22.7 (2.7)	22.8 (2.6)	22.6 (2.7)	22.6 (2.8)	0.002
Mean energy intake (SD) (kcal/day)	1688 (492)	1775 (480)	1800 (488)	1783 (493)	1666 (496)	<0.001
Mean dietary sodium intake (mg/day)	1324	1929	2286	2584	2872	<0.001
Current smokers (%)	57.8	52.9	51.7	50.9	47.6	<0.001
Mean alcohol intake (g/day of ethanol)	39.2	36.6	34.0	31.3	27.2	<0.001
History of hypertension (%)	18.5	19.3	19.0	19.2	19.0	0.906
History of diabetes mellitus (%)	6.6	6.2	6.5	5.5	5.9	0.234
Sports time ≥3 hours/week (%)	11.9	12.6	13.2	14.7	16.4	0.050
Walking time ≥30 minutes/day (%)	51.8	48.0	47.1	45.3	44.3	<0.001
College or higher education (%)	16.5	16.7	16.8	18.0	16.7	0.178
High perceived mental stress (%)	26.6	24.5	21.7	20.4	18.4	<0.001
Women						
No. of subjects	7106	7107	7106	7107	7106	
Median iron intake (mg/day)[a]	5.14	6.43	7.35	8.31	9.81	
Mean age (SD) (years)	55.0 (10.1)	55.0 (9.7)	55.8 (9.7)	57.1 (9.7)	58.5 (9.7)	<0.001
Mean body mass index (kg/m^2)	23.0 (3.1)	22.8 (3.0)	22.9 (3.0)	22.9 (3.0)	22.9 (3.0)	0.002
Mean energy intake (SD) (kcal/day)	1415 (403)	1451 (367)	1459 (348)	1466 (340)	1398 (358)	<0.001
Mean dietary sodium intake (mg/day)	1353	1858	2134	2368	2595	<0.001
Current smokers (%)	6.9	4.5	3.8	3.4	3.6	<0.001
Mean alcohol intake (g/day of ethanol)	14.3	9.5	9.2	7.8	7.8	<0.001
History of hypertension (%)	21.0	19.6	20.7	21.1	20.3	0.157
History of diabetes mellitus (%)	3.5	2.9	3.2	3.5	3.9	0.019
Sports time ≥3 hours/week (%)	7.8	8.4	9.3	9.9	11.1	<0.001
Walking time ≥30 minutes/day (%)	47.7	46.3	43.6	44.2	43.5	<0.001
College or higher education (%)	9.0	8.9	10.6	10.2	9.9	<0.001
High perceived mental stress (%)	21.1	20.7	19.8	18.4	16.6	<0.001
Menopause (%)	58.8	60.5	63.2	67.3	70.3	<0.001
Hormone-replacement therapy (%)	4.7	4.6	4.2	4.1	4.5	<0.001

stroke (430–431 and I60–I61) and ischemic stroke (433–434 and I63)—as well as coronary heart disease (410–414 and I20–I25) and myocardial infarction (410 and I21–I22).

Data analysis

Data analyses were based on age-adjusted mortality rates for cardiovascular disease endpoints in the 45 study areas during the follow-up period from 1989 to 2006, with the exception of 8 areas (follow-up ended in 1999 in 4 areas and in 2003 in 4 other areas). Duration of follow-up was defined as the period from submission of the initial baseline questionnaire to either death, termination of follow-up, or the departure of a participant from his/her original community. Participants were divided into quintiles of estimated dietary intakes of total, heme, and non-heme iron.

Age-adjusted mean values and proportions of selected CVD risk factors were presented based on the quintiles. We used Cox proportional hazards models to estimate age-adjusted and multivariable-adjusted hazard ratios (HRs) and 95% CIs. We also tested for trends across the quintiles by assigning median values to each quintile. The confounding variables used for adjustment included body mass index (BMI; sex-specific quintiles), smoking status (never, ex-smoker, or current smoker of 1–19 or ≥20 cigarettes per day), ethanol intake (never, ex-drinker, or current drinker of 1–22, 23–45, 46–69, or ≥69 g per day), history of hypertension (yes or no), history of diabetes (yes or no), sports participation time (never, 1–2 hours, 3–4 hours, or ≥5 hours per day), walking time (never, about 30 min, 30–60 min, or ≥60 min per day), educational level (educated until age 12, 13–15, 16–18, or ≥19 years), perceived mental stress (low, medium, or high), and, for women, menopausal status (yes or no) and hormone replacement therapy (yes or no).

All statistical analyses for 2-tailed tests were performed using Statistical Analysis Software (SAS) version 9.13 (SAS Institute Inc., Cary, North Carolina, USA). A *P* value less than 0.05 was considered to indicate statistical significance.

RESULTS

Tables 1, 2, and 3 show the age-adjusted means and prevalences of cardiovascular risk factors at baseline according to quintiles of dietary total iron, heme iron, and non-heme iron intakes, respectively. The median intake of energy-adjusted total iron was 5.12 mg/day in the bottom quintile and 10.58 mg/day in the top quintile among men; the

Table 2. Baseline characteristics and risk factors of participants by quintile of heme iron intake

	Quintile of heme iron intake					*P* for trend
	1 (low)	2	3	4	5 (high)	
Men						
No. of subjects	4616	4617	4617	4617	4616	
Median heme iron intake (mg/day)[a]	0.07	0.16	0.22	0.28	0.44	
Mean age (SD) (years)	56.4 (10.1)	55.4 (9.9)	55.4 (9.9)	55.8 (9.8)	56.7 (9.9)	<0.001
Mean body mass index (kg/m^2)	22.9 (2.8)	22.7 (2.6)	22.7 (2.7)	22.7 (2.7)	22.5 (2.7)	<0.001
Mean energy intake (SD) (kcal/day)	1719 (483)	1768 (485)	1766 (486)	1738 (502)	1722 (506)	<0.001
Current smokers (%)	53.7	51.7	51.8	52.6	53.1	0.054
Mean alcohol intake (g/day of ethanol)	34.2	34.3	33.9	33.8	33.7	0.850
Mean dietary sodium intake (mg/day)	1936	2122	2197	2270	2366	<0.001
History of hypertension (%)	22.1	19.0	17.6	18.7	17.6	<0.001
History of diabetes mellitus (%)	5.0	6.3	5.9	6.5	7.0	0.003
Sports time ≥3 hours/week (%)	11.4	12.5	14.1	14.9	15.9	<0.001
Walking time ≥30 minutes/day (%)	43.0	48.0	48.1	49.8	47.6	<0.001
College or higher education (%)	12.3	16.7	18.5	19.3	17.7	<0.001
High perceived mental stress (%)	19.3	23.0	23.2	24.4	23.4	<0.001
Women						
No. of subjects	7106	7107	7106	7107	7106	
Median heme iron intake (mg/day)	0.06	0.16	0.21	0.28	0.48	
Mean age (SD) (years)	57.7 (10.0)	56.5 (10.0)	55.9 (9.8)	55.5 (9.6)	55.7 (9.8)	<0.001
Mean body mass index (kg/m^2)	23.0 (3.1)	22.8 (3.0)	22.8 (2.9)	22.8 (3.0)	22.8 (3.0)	0.009
Mean energy intake (SD) (kcal/day)	1410 (373)	1470 (358)	1458 (355)	1441 (355)	1410 (378)	<0.001
Mean dietary sodium intake (mg/day)	1873	2021	2073	2107	2128	<0.001
Current smokers (%)	5.2	4.2	3.9	4.2	4.5	0.001
Mean alcohol intake (g/day of ethanol)	11.6	11.1	9.8	9.0	9.5	<0.001
History of hypertension (%)	25.7	21.3	19.2	18.8	17.6	<0.001
History of diabetes mellitus (%)	3.7	3.3	3.1	3.5	3.4	0.605
Sports time ≥3 hours/week (%)	8.0	9.6	9.2	9.4	10.3	<0.001
Walking time ≥30 minutes/day (%)	41.2	45.3	46.3	46.4	46.2	<0.001
College or higher education (%)	5.9	9.8	10.0	11.8	11.2	<0.001
High perceived mental stress (%)	17.4	18.9	20.2	20.5	19.5	<0.001
Menopause (%)	68.2	64.3	63.3	62.0	62.2	<0.001
Hormone-replacement therapy (%)	4.0	4.0	4.3	5.2	4.5	<0.001

respective intakes among women were 5.14 mg/day and 9.81 mg/day. The corresponding values of energy-adjusted heme iron intake were 0.07 mg/day and 0.44 mg/day among men and 0.06 mg/day and 0.48 mg/day among women. The corresponding values of energy-adjusted non-heme iron intake were 3.84 mg/day and 10.19 mg/day among men and 3.81 mg/day and 9.46 mg/day among women. The Spearman correlation between total iron intake and heme iron intake was 0.30 for men and 0.28 for women, while the corresponding correlations between total iron intake and non-heme iron intake were 0.73 and 0.70, respectively.

As compared with subjects in the lowest quintile of total iron intake, those in the higher quintiles were older, less likely to be a current smoker, more likely to have high perceived mental stress, consumed less ethanol, and played sports more. History of hypertension was similar between sexes. Similar trends are shown in Tables 2 and 3. However, as compared with subjects in the lowest quintile of heme iron intake, both man and women in the higher quintiles had lower proportions of hypertension.

During 859 450 person-years of follow-up, we documented 2690 deaths (1343 men and 1347 women) from total CVD. Among men, 607 deaths were due to total stroke (which included 196 hemorrhagic strokes and 355 ischemic strokes), 311 to CHD, and 243 to myocardial infarction. Among women, there were 620 total stroke deaths (including 263 hemorrhagic strokes and 296 ischemic stroke), 246 CHD deaths, and 185 deaths due to myocardial infarction.

As shown in Table 4, dietary intake of total iron showed a tendency to be associated with increased mortality from total and ischemic strokes among men in the age-adjusted model. These associations became more evident after further adjustment for known cardiovascular risk factors: the multivariable HRs for the highest versus lowest quintile of total iron intake were 1.43 (95% CI, 1.02–2.00; *P* for trend = 0.01) for total stroke and 1.27 (1.01–1.58; 0.02) for total CVD. Among women, dietary iron intake showed a tendency to be inversely associated with mortality from CHD, but the multivariable HR for the highest versus lowest quintile of total iron intake did not reach statistical significance.

Table 5 shows the relationship between heme iron intake and mortality from cardiovascular disease in the multivariable model. Heme iron intake was inversely associated with mortality from myocardial infarction in men. The multivariable HR for the highest versus lowest quintile of heme

Table 3. Baseline characteristics and risk factors of participants by quintile of non-heme iron intake

	Quintile of non-heme iron intake					*P* for trend
	1 (low)	2	3	4	5 (high)	
Men						
No. of subjects	4616	4617	4617	4617	4616	
Median non-heme iron intake (mg/day)	3.84	6.05	7.23	8.40	10.19	
Mean age (SD) (years)	54.9 (10.1)	54.0 (9.6)	55.1 (9.6)	56.7 (9.7)	58.1 (9.7)	<0.001
Mean body mass index (kg/m^2)	22.9 (2.7)	22.7 (2.7)	22.7 (2.6)	22.6 (2.7)	22.6 (2.8)	0.002
Mean energy intake (SD) (kcal/day)	1697 (491)	1754 (484)	1796 (488)	1795 (493)	1671 (494)	<0.001
Mean dietary sodium intake (mg/day)	1655	1756	2188	2537	2860	<0.001
Current smokers (%)	56.4	54.6	51.2	51.2	47.4	<0.001
Mean alcohol intake (g/day of ethanol)	37.7	36.9	34.9	31.8	27.2	<0.001
History of hypertension (%)	20.6	18.7	18.5	18.5	18.7	0.053
History of diabetes mellitus (%)	6.2	5.5	6.6	5.7	6.5	0.075
Sports time ≥3 hours/week (%)	12.3	12.3	13.2	14.8	16.1	0.033
Walking time ≥30 minutes/day (%)	46.0	50.5	48.8	45.3	45.0	<0.001
College or higher education (%)	13.5	17.0	18.8	18.2	17.0	<0.001
High perceived mental stress (%)	22.9	26.7	23.4	21.2	19.0	<0.001
Women						
No. of subjects	7106	7107	7106	7107	7106	
Median non-heme iron intake (mg/day)	3.81	5.91	6.96	7.98	9.46	
Mean age (SD) (years)	55.8 (10.0)	54.9 (9.8)	55.4 (9.8)	56.7 (9.7)	58.6 (9.7)	<0.001
Mean body mass index (kg/m^2)	23.3 (3.2)	22.8 (3.0)	22.8 (3.0)	22.8 (3.0)	22.9 (3.0)	<0.001
Mean energy intake (SD) (kcal/day)	1404 (385)	1451 (379)	1459 (354)	1474 (343)	1399 (354)	<0.001
Mean dietary sodium intake (mg/day)	1636	1706	2046	2333	2587	<0.001
Current smokers (%)	6.6	4.7	3.9	3.4	3.5	<0.001
Mean alcohol intake (g/day of ethanol)	13.7	10.4	9.3	8.0	7.7	<0.001
History of hypertension (%)	23.5	19.2	19.1	20.2	20.5	<0.001
History of diabetes mellitus (%)	3.6	3.0	3.2	3.3	4.0	0.493
Sports time ≥3 hours/week (%)	7.5	8.6	9.2	10.0	11.3	<0.001
Walking time ≥30 minutes/day (%)	43.4	47.9	45.5	44.8	43.8	<0.001
College or higher education (%)	7.0	10.0	11.2	10.5	9.9	<0.001
High perceived mental stress (%)	19.2	21.4	20.7	18.6	16.7	<0.001
Menopause (%)	62.3	59.6	61.7	66.0	70.4	<0.001
Hormone-replacement therapy (%)	4.8	4.6	4.3	4.1	4.3	<0.001

iron intake was 0.59 (95% CI, 0.38–0.90; *P* for trend = 0.015). There was no significant association between heme iron intake and mortality from any cardiovascular disease endpoint in women.

As shown in Table 6, non-heme iron intake was associated with mortality from hemorrhagic stroke in men: the multivariable HR for the highest versus lowest quintile of non-heme iron intake was 1.72 (95% CI, 1.02–2.90; *P* for trend = 0.038). The corresponding HRs were 1.77 (0.95–3.31; 0.05) for intraparenchymal hemorrhage (no. of deaths = 141) and 1.74 (95% CI, 0.66–4.59; *P* for trend = 0.36) for subarachnoid hemorrhage (no. of deaths = 55). In women, non-heme iron was not associated with mortality from any cardiovascular disease endpoint.

DISCUSSION

In this large prospective cohort study with a median follow-up of 14.7 years, we found that greater dietary iron intake was associated with a higher risk of mortality from total stroke in Japanese men.

Several studies have examined the association between serum ferritin, an indicator of body iron stores, and stroke risk, but the results have been inconsistent. A prospective study of 11 471 Dutch postmenopausal women aged 49 to 70 years showed that higher serum ferritin concentrations were associated with increased risk of ischemic stroke.[7] In that study, the multivariable risk ratio for the highest versus lowest tertile of serum ferritin concentration was 2.23 (95% CI, 1.05–4.73). However, a 17-year follow-up study in Australia, consisting of 1612 men and women aged 40 to 89 years, reported that serum ferritin was not associated with risk of total stroke: the HRs for the highest versus lowest tertile were 1.71 (0.72–1.49) for men, 0.99 (0.38–2.59) for women, and 1.43 (0.78–2.64) for all subjects.[12]

There is little evidence regarding the association of dietary iron intake with stroke risk. A recent case-control study comprising 374 ischemic stroke cases and 464 hospital-based controls showed that higher iron intake was associated with greater risk of ischemic stroke: the odds ratio for the highest (≥161 mg/wk) versus lowest (≤100 mg/wk) tertile was 2.43 (95% CI, 1.06–5.58; *P* for trend = 0.03). However, that association became insignificant after adjustment for other CVD risk factors.[14] To our knowledge, our study is the first prospective study to show a positive association between dietary iron intake and stroke mortality in men.

Table 4. Sex-specific hazard ratios (95% CI) of mortality from stroke, coronary heart disease, heart failure, and total cardiovascular disease by quintile of total iron intake

	Quintiles of total iron intake					*P* for trend
	1 (low)	2	3	4	5 (high)	
Men						
Number of subjects	4616	4617	4617	4617	4616	
Person-years	65666	66308	67818	67305	65423	
Total stroke						
n	79	93	103	145	187	
Age-adjusted HR	1.00	1.07 (0.79–1.45)	0.93 (0.70–1.25)	1.14 (0.87–1.50)	1.22 (0.94–1.59)	0.066
Multivariable HR[a]	1.00	1.11 (0.81–1.54)	0.99 (0.71–1.37)	1.26 (0.91–1.75)	1.43 (1.02–2.00)	0.009
Hemorrhagic stroke						
n	32	36	35	38	55	
Age-adjusted HR	1.00	1.05 (0.66–1.70)	0.89 (0.55–1.44)	0.89 (0.56–1.43)	1.16 (0.75–1.81)	0.567
Multivariable HR[a]	1.00	1.18 (0.71–1.96)	1.06 (0.62–1.82)	1.12 (0.64–1.96)	1.62 (0.92–2.85)	0.083
Ischemic stroke						
n	40	49	58	88	120	
Age-adjusted HR	1.00	1.11 (0.73–1.68)	0.97 (0.65–1.45)	1.23 (0.85–1.80)	1.34 (0.93–1.92)	0.046
Multivariable HR[a]	1.00	1.13 (0.72–1.76)	0.95 (0.60–1.49)	1.25 (0.80–1.96)	1.40 (0.89–2.21)	0.056
Coronary heart disease						
n	58	58	44	65	86	
Age-adjusted HR	1.00	0.91 (0.63–1.31)	0.56 (0.38–0.82)	0.73 (0.51–1.05)	0.83 (0.60–1.17)	0.346
Multivariable HR[a]	1.00	1.00 (0.67–1.47)	0.62 (0.40–0.96)	0.86 (0.56–1.32)	0.93 (0.60–1.45)	0.959
Myocardial infarction						
n	50	42	34	49	68	
Age-adjusted HR	1.00	0.77 (0.51–1.16)	0.51 (0.33–0.79)	0.66 (0.44–0.98)	0.80 (0.55–1.16)	0.381
Multivariable HR[a]	1.00	0.85 (0.54–1.32)	0.57 (0.35–0.93)	0.76 (0.47–1.23)	0.87 (0.53–1.41)	0.882
Total cardiovascular disease						
n	185	221	241	296	400	
Age-adjusted HR	1.00	1.08 (0.89–1.32)	0.94 (0.78–1.13)	1.00 (0.83–1.21)	1.14 (0.95–1.35)	0.161
Multivariable HR[a]	1.00	1.13 (0.92–1.39)	0.99 (0.80–1.23)	1.11 (0.89–1.38)	1.27 (1.01–1.58)	0.023
Women						
Number of subjects	7106	7107	7106	7107	7106	
Person-years	101715	104019	106270	107404	107568	
Total stroke						
n	106	109	131	124	150	
Age-adjusted HR	1.00	1.04 (0.80–1.36)	1.07 (0.83–1.38)	0.84 (0.65–1.09)	0.88 (0.68–1.13)	0.100
Multivariable HR[a]	1.00	0.98 (0.74–1.30)	0.98 (0.74–1.30)	0.74 (0.55–0.99)	0.77 (0.57–1.04)	0.024
Hemorrhagic stroke						
n	49	46	62	49	57	
Age-adjusted HR	1.00	0.92 (0.62–1.38)	1.12 (0.77–1.62)	0.77 (0.52–1.15)	0.81 (0.55–1.19)	0.166
Multivariable HR[a]	1.00	0.93 (0.61–1.41)	1.11 (0.73–1.68)	0.75 (0.48–1.18)	0.78 (0.50–1.23)	0.162
Ischemic stroke						
n	46	51	59	62	78	
Age-adjusted HR	1.00	1.18 (0.79–1.75)	1.10 (0.75–1.62)	0.92 (0.63–1.35)	0.97 (0.63–1.40)	0.485
Multivariable HR[a]	1.00	1.04 (0.68–1.58)	0.92 (0.60–1.41)	0.74 (0.48–1.13)	0.78 (0.50–1.20)	0.107
Coronary heart disease						
n	49	43	43	54	57	
Age-adjusted HR	1.00	0.90 (0.60–1.36)	0.75 (0.50–1.13)	0.77 (0.52–1.13)	0.69 (0.47–1.01)	0.047
Multivariable HR[a]	1.00	0.98 (0.64–1.51)	0.91 (0.58–1.42)	0.96 (0.61–1.49)	0.89 (0.57–1.41)	0.643
Myocardial infarction						
n	39	33	24	39	50	
Age-adjusted HR	1.00	0.87 (0.55–1.38)	0.53 (0.32–0.88)	0.70 (0.45–1.09)	0.77 (0.50–1.17)	0.224
Multivariable HR[a]	1.00	0.97 (0.60–1.57)	0.64 (0.37–1.11)	0.92 (0.55–1.53)	1.04 (0.62–1.73)	0.745
Total cardiovascular disease						
n	233	232	259	269	354	
Age-adjusted HR	1.00	1.01 (0.84–1.22)	0.95 (0.80–1.14)	0.81 (0.68–0.97)	0.92 (0.78–1.08)	0.086
Multivariable HR[a]	1.00	1.01 (0.83–1.22)	0.97 (0.79–1.17)	0.82 (0.67–0.99)	0.94 (0.77–1.15)	0.301

HR: hazard ratio.

[a]Adjusted further for body mass index, smoking status, ethanol intake, history of hypertension, history of diabetes mellitus, sports time, walking time, educational status, perceived mental stress, dietary sodium intake, and, for women, menopausal status and hormone replacement therapy.

The present study showed no significant association between dietary iron intake and mortality from CHD or myocardial infarction in either sex. A meta-analysis of 12 prospective studies that were conducted before 1998 and involved 7800 CHD cases also showed no association between iron status and CHD risk.[8] A comparison of

Table 5. Hazard ratios (95% CI) of mortality from stroke, coronary heart disease, heart failure, and total cardiovascular disease according by quintile of heme iron intake

	Quintiles of heme iron intake					*P* for trend
	1 (low)	2	3	4	5 (high)	
Men						
Number of subjects	4616	4617	4617	4617	4616	
Person-years	67 446	66 998	67 081	66 607	64 390	
Total stroke						
n	153	111	108	102	133	
Multivariable HR[a]	1.00	0.93 (0.72–1.21)	0.95 (0.73–1.23)	0.84 (0.64–1.09)	1.04 (0.81–1.33)	0.833
Hemorrhagic stroke						
n	40	41	46	26	43	
Multivariable HR[a]	1.00	1.17 (0.71–1.75)	1.29 (0.83–2.01)	0.71 (0.42–1.18)	1.13 (0.72–1.78)	0.988
Ischemic stroke						
n	95	65	53	67	75	
Multivariable HR[a]	1.00	0.97 (0.70–1.35)	0.84 (0.59–1.19)	0.97 (0.70–1.35)	1.03 (0.70–1.35)	0.810
Coronary heart disease						
n	80	56	62	64	49	
Multivariable HR[a]	1.00	0.88 (0.62–1.26)	1.02 (0.72–1.44)	1.02 (0.72–1.45)	0.74 (0.51–1.07)	0.190
Myocardial infarction						
n	69	47	50	43	34	
Multivariable HR[a]	1.00	0.86 (0.58–1.26)	0.94 (0.64–1.38)	0.79 (0.53–1.18)	0.59 (0.38–0.90)	0.015
Total cardiovascular disease						
n	341	247	230	245	280	
Multivariable HR[a]	1.00	0.91 (0.77–1.08)	0.88 (0.74–1.05)	0.89 (0.75–1.06)	0.96 (0.81–1.14)	0.740
Women						
Number of subjects	7106	7106	7106	7106	7106	
Person-years	107 688	105 828	105 477	104 747	103 238	
Total stroke						
n	180	138	114	100	88	
Multivariable HR[a]	1.00	1.01 (0.80–1.27)	0.95 (0.75–1.22)	0.91 (0.70–1.17)	0.80 (0.61–1.04)	0.073
Hemorrhagic stroke						
n	72	57	51	42	41	
Multivariable HR[a]	1.00	0.97 (0.67–1.38)	0.94 (0.64–1.36)	0.81 (0.55–1.21)	0.80 (0.54–1.20)	0.208
Ischemic stroke						
n	88	74	48	48	38	
Multivariable HR[a]	1.00	1.15 (0.83–1.58)	0.88 (0.61–1.27)	0.99 (0.69–1.44)	0.77 (0.52–1.14)	0.156
Coronary heart disease						
n	74	49	33	41	49	
Multivariable HR[a]	1.00	0.88 (0.61–1.28)	0.70 (0.46–1.07)	0.99 (0.67–1.48)	1.18 (0.80–1.72)	0.352
Myocardial infarction						
n	58	35	23	28	41	
Multivariable HR[a]	1.00	0.81 (0.53–1.25)	0.63 (0.38–1.03)	0.87 (0.55–1.40)	1.25 (0.82–1.91)	0.281
Total cardiovascular disease						
n	386	287	236	217	221	
Multivariable HR[a]	1.00	0.97 (0.83–1.13)	0.91 (0.77–1.08)	0.93 (0.78–1.10)	0.94 (0.79–1.12)	0.405

HR: hazard ratio.
[a]Adjusted further for body mass index, smoking status, ethanol intake, history of hypertension, history of diabetes mellitus, sports time, walking time, educational status, perceived mental stress, dietary sodium intake, and, for women, menopausal status and hormone replacement therapy.

subjects in the top versus bottom quintile of baseline variables revealed nonsignificant risk ratios for total iron-binding capacity: the combined risk ratio was 1.0 (95% CI, 0.7–1.5), and the risk ratio was 0.8 (0.7–1.0) for serum iron and 0.8 (0.7–1.1) for total dietary iron.[8] As for serum ferritin levels, in 5 studies involving 570 cases of CHD, a comparison of individuals with baseline values of 200 mg/L or higher versus those with values less than 200 mg/L yielded a combined risk ratio of 1.0 (95% CI, 0.8–1.3). As for transferrin saturation levels, in 5 studies involving 6194 cases of CHD, a comparison of individuals in the top versus bottom tertile of the baseline measurement yielded a combined risk ratio of 0.9 (95% CI, 0.7–1.1).[8] In contrast, a 13-year follow-up study consisting of 4237 residents aged 40 to 74 years showed that serum iron was inversely associated with risk of myocardial infarction: the multivariable relative risk associated with an increase of 5.4 μmol/L in serum iron concentration was 0.82 in women (95% CI, 0.70–0.95) and 0.92 (0.85–1.00) in men.[17]

Because of its high absorption rate, we separated the analysis of dietary heme iron from that of total dietary iron in our investigation of associations with cardiovascular disease mortality. In men with repletion iron stores, 26% of dietary heme iron was absorbed, while only 2.5% of non-heme iron was absorbed.[23] However, only a few studies have focused on

Table 6. Hazard ratios (95% CI) of mortality from stroke, coronary heart disease, heart failure and total cardiovascular disease by quintile of non-heme iron intake

	Quintiles of non-heme iron intake					*P* for trend
	1 (low)	2	3	4	5 (high)	
Men						
Number of subjects	4616	4617	4617	4617	4616	
Person-years	65 974	66 161	67 062	67 666	65 660	
Total stroke						
n	117	82	91	138	179	
Multivariable HR[a]	1.00	0.93 (0.69–1.26)	0.85 (0.63–1.15)	1.01 (0.83–1.46)	1.19 (0.90–1.57)	0.088
Hemorrhagic stroke						
n	33	33	36	39	55	
Multivariable HR[a]	1.00	1.14 (0.69–1.90)	1.20 (0.72–2.00)	1.23 (0.73–2.08)	1.72 (1.02–2.90)	0.038
Ischemic stroke						
n	73	41	48	82	111	
Multivariable HR[a]	1.00	0.85 (0.57–1.29)	0.74 (0.50–1.11)	1.02 (0.71–1.46)	1.03 (0.72–1.48)	0.495
Coronary heart disease						
n	76	49	50	58	78	
Multivariable HR[a]	1.00	0.81 (0.56–1.19)	0.73 (0.49–1.08)	0.75 (0.51–1.11)	0.81 (0.55–1.18)	0.326
Myocardial infarction						
n	63	38	39	41	62	
Multivariable HR[a]	1.00	0.77 (0.50–1.19)	0.71 (0.46–1.09)	0.66 (0.42–1.02)	0.80 (0.52–1.22)	0.312
Total cardiovascular disease						
n	271	189	221	283	379	
Multivariable HR[a]	1.00	0.90 (0.74–1.10)	0.87 (0.72–1.06)	0.96 (0.80–1.17)	1.04 (0.86–1.26)	0.386
Women						
Number of subjects	7106	7106	7106	7106	7106	
Person-years	105 114	101 826	105 246	107 307	107 483	
Total stroke						
n	130	95	116	126	153	
Multivariable HR[a]	1.00	1.01 (0.77–1.34)	1.04 (0.79–1.37)	0.89 (0.67–1.17)	0.86 (0.65–1.12)	0.154
Hemorrhagic stroke						
n	52	43	59	51	58	
Multivariable HR[a]	1.00	1.08 (0.70–1.65)	1.33 (0.88–1.99)	0.97 (0.63–1.50)	0.94 (0.61–1.44)	0.579
Ischemic stroke						
n	62	41	50	64	79	
Multivariable HR[a]	1.00	0.92 (0.60–1.39)	0.88 (0.58–1.33)	0.82 (0.55–1.22)	0.76 (0.52–1.12)	0.155
Coronary heart disease						
n	58	41	37	53	57	
Multivariable HR[a]	1.00	0.95 (0.63–1.45)	0.80 (0.51–1.24)	0.96 (0.63–1.46)	0.85 (0.55–1.29)	0.491
Myocardial infarction						
n	45	32	22	36	50	
Multivariable HR[a]	1.00	0.95 (0.59–1.53)	0.61 (0.35–1.05)	0.87 (0.53–1.41)	0.98 (0.61–1.58)	0.999
Total cardiovascular disease						
n	271	217	230	275	354	
Multivariable HR[a]	1.00	1.09 (0.90–1.31)	1.00 (0.83–1.21)	0.96 (0.79–1.15)	0.99 (0.83–1.19)	0.661

HR: hazard ratio.
[a]Adjusted further for body mass index, smoking status, ethanol intake, history of hypertension, history of diabetes mellitus, sports time, walking time, educational status, perceived mental stress, dietary sodium intake, and, for women, menopausal status and hormone replacement therapy.

the relationship between heme iron intake and CVD. The Health Professionals Follow-up Study found that the risk of fatal CHD or nonfatal myocardial infarction was higher among men in the top quintile of heme iron intake as compared with those in the lowest quintile: the multivariable relative risk was 1.42 (95% CI, 1.02–1.98; *P* for trend = 0.02).[15] The European Prospective Investigation into Cancer and Nutrition also showed that higher dietary heme iron intake was associated with increased CHD risk among women.[16] In that study, the multivariable HR for CHD development among women in the highest versus the lowest quintile of heme iron intake was 1.65 (1.07–2.53; 0.02).

In contrast, the present study showed an inverse association between heme iron intake and mortality from myocardial infarction among men. The reasons for these contradictory trends are unknown. However, 1 reason might be that dietary heme iron intake among Japanese is lower than that among Western people. Mean heme iron intake in the present study was 0.25 mg/day for men and 0.24 mg/day for women, which was much lower than that for European women (1.81 mg/day), even when underestimation of our values was taken into account.[16] Dietary heme iron intake in Japanese adults may not be high enough to develop high body iron stores and increase CVD risk. Non-heme iron

was positively associated with mortality from hemorrhagic stroke in men.

We found no association between dietary iron intake and cardiovascular disease mortality among women. Two studies of European and American women also showed no associations,[17,24] but 1 study of European women showed a positive association between heme iron intake and CHD risk.[16] A reason for the lack of association is that iron stores are lower in women than in men.[3]

The role of iron in the development of stroke and CVD is not established. Iron catalyzes production of highly reactive hydroxyl free radicals and promotes LDL oxidation, leading to atherosclerosis.[25] However, this mechanism is unlikely to explain our findings because the major pathologic process for stroke among Japanese is arteriolosclerosis (in which oxidized LDL has only a limited role) rather than atherosclerosis.[26] Iron-dependent oxidative stress may lead to cell death, ie, necrosis and apoptosis,[27] which may partly explain the excess risk of hemorrhagic stroke (intraparenchymal or subarachnoid hemorrhage) associated with high non-heme iron intake, as the basic pathologic process of hemorrhagic stroke is necrosis or apoptosis of vascular wall cells in cerebral arteries.[28,29] However, our finding that high iron intake was associated with an increased risk of hemorrhagic stroke among men could have been observed by chance and thus requires further investigation. Free radicals that form during brain ischemia and reperfusion may induce vasodilatation and increase blood–brain barrier permeability to macromolecules, which leads to brain damage.[30] Brain damage is promoted by iron-foaming hydroxyl free radicals,[25] which are associated with poor stroke prognosis.[30] An experimental study of rats found that ischemic brain injury was reduced by free radical scavengers.[31] These pathologic processes may in part explain the excess risk of mortality from stroke.

The strengths of the present study include its large population-based sampling from throughout Japan and its prospective design. In addition, the measurement of exposure variable covariates and outcomes was standardized through the use of a uniform questionnaire and surveillance protocol. However, the study also had limitations. First, only approximately 53% of potential participants responded to the FFQ. Respondents were 3 years younger, more educated, and had more perceived mental stress than did nonresponders. Thus, we adjusted these variables in our examination of the associations between iron intake and cardiovascular disease mortality. Second, dietary iron intake may not accurately reflect total body iron stores, because gastrointestinal absorption varies with body iron status, age, and inflammation status, among other factors.[18] Third, the exposure data were collected only once, at the baseline survey, and the subjects were followed over time. It is possible that the subjects changed their diets during the follow-up period, which could have weakened the true association between dietary iron intake and CVD mortality.

In summary, the present study showed that a high dietary intake of iron was associated with increased mortality from total stroke and total CVD in Japanese men. These findings lend support to the hypothesis that excess iron intake increases CVD risk.

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Conflicts of interest: None declared.

ONLINE ONLY MATERIALS

The Japanese-language abstract for articles can be accessed by clicking on the tab labeled Supplementary materials at the journal website http://dx.doi.org/10.2188/jea.JE20120006.

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Original Article

Relation of Serum α- and γ-Tocopherol Levels to Cardiovascular Disease-Related Mortality Among Japanese Men and Women

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ABSTRACT

Background: There is limited evidence regarding the relationship between serum tocopherol levels and cardiovascular disease.

Methods: We conducted a nested case-control study as part of the Japan Collaborative Cohort Study for evaluation of cancer risk (JACC Study). Baseline serum samples were collected from 39 242 participants (age range, 40–79 years) between 1988 and 1990. During the 13-year follow-up, there were 530 stroke deaths (302 ischemic strokes and 210 hemorrhagic strokes) and 211 deaths from coronary heart disease. Controls were matched for sex, age, and area of residence.

Results: Serum α-tocopherol level was not associated with any type of cardiovascular death in men; however, in women, it was inversely associated with total stroke mortality and hemorrhagic stroke mortality. The multivariate odds ratio (95% CI) for the highest versus the lowest quintile of serum α-tocopherol levels among women was 0.35 (0.16–0.77; *P* for trend = 0.009) for total stroke and 0.26 (0.07–0.97; *P* for trend = 0.048) for hemorrhagic stroke. Serum γ-tocopherol was inversely associated with ischemic stroke mortality in men but positively associated with hemorrhagic stroke mortality in women. The respective multivariate odds ratios (95% CI) for the highest versus the lowest quintile and for a 1-standard deviation increment in γ-tocopherol level were 0.48 (0.22–1.06; *P* for trend = 0.07) and 0.77 (0.58–1.02), respectively, for ischemic stroke in men and 3.10 (0.95–10.12; *P* for trend = 0.052) and 1.49 (1.04–2.13) for hemorrhagic stroke in women.

Conclusions: Among women, hemorrhagic stroke mortality was inversely associated with serum α-tocopherol and positively associated with serum γ-tocopherol. These findings are due in part to the antioxidative and antithrombotic activities of these tocopherols.

Key words: α-tocopherol; γ-tocopherol; vitamin E; prospective study; stroke; cardiovascular disease; nested case-control study

INTRODUCTION

Tocopherols are believed to be promising agents for reducing the risk of cardiovascular disease, due of their strong antioxidant activity.[1] Although randomized control trials (RCTs) of tocopherol supplementation have been conducted,[2–7] the results have been inconsistent. Several, but not all, RCTs have shown a beneficial effect of α-tocopherol supplementation on the risk of nonfatal myocardial infarction,[2] subarachnoid hemorrhage,[3] ischemic stroke,[3] hemorrhagic stroke,[4] and cardiovascular disease mortality.[5] RCTs are useful for establishing causality, especially in evaluations of the short- and moderate-term effects of drugs. However, it is difficult to implement RCTs of the effects of long-term dietary exposure. Thus, observational studies are a useful alternative.

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Abbreviations used: JACC Study, The Japan Collaborative Cohort Study for evaluation of cancer risk; OR, odds ratio; CI, confidence interval; HPLC, high-performance liquid chromatography; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells.

Previous observational studies reported an association between intake of tocopherol, either from food or via supplementation, and cardiovascular disease risk.[8–12] However, pharmacokinetic studies suggest that complex mechanisms are involved in the regulation of tocopherol levels.[13] For example, large consumption of vitamin E induces excretion of vitamin E via the pregnane X receptor drug metabolism system activated by vitamin E.[14–16] In contrast, some nutrients, such as sesamin, increase blood tocopherol levels by suppressing tocopherol metabolism.[17] Thus, tocopherol intake is not always reflected in serum tocopherols levels. For these reasons, we estimated serum levels of tocopherols and examined their relationship with cardiovascular disease risk. Although previous studies have examined the relationship of serum levels of α- and γ-tocopherol with cardiovascular disease risk in men,[18–22] no such studies have been conducted in women.

In this nested case-control study using a large prospective cohort of approximately 40 000 men and women, we evaluated the association of serum levels of α- and γ-tocopherol with the risk of cardiovascular disease mortality among Japanese men and women.

METHODS

Survey population

We conducted a nested case-control study as part of the Japan Collaborative Cohort (JACC) Study. The methods of the JACC Study have been previously described. In brief, 110 792 individuals (46 465 men and 64 327 women) aged 40 to 79 years during the baseline period (1988–1990) were enrolled from 45 communities throughout Japan. Using self-administered questionnaires, participants gave information on their lifestyle and medical histories of cardiovascular disease and cancer.[23,24]

Written or explicit verbal informed consent to participate in the study was obtained from the individuals who completed the questionnaire. In several communities, informed consent was obtained at a community level after the purpose and methods of the study and its emphasis on data confidentiality had been explained to community leaders and mayors on behalf of the individual participants. At the time of recruitment for this study, this was a common method of obtaining informed consent in Japan.

A total of 39 242 participants (35.4% of the questionnaire respondents) agreed to provide blood samples and gave individual informed consent.[25,26] After exclusion of 457 men and 627 women with a history of heart disease, stroke, or cancer at the baseline survey, a total of 38 158 participants (13 382 men and 24 776 women) were enrolled in the present study. The ethics committees of the University of Tsukuba and Osaka University approved the present study.

Mortality surveillance

The participants were followed up to determine mortality due to cardiovascular disease until the end of 2003, except for 4 communities in which follow-up ended in 1999.

The study investigators conducted a systematic review of death certificates in each community. Because registration of death is a legal requirement throughout Japan, the investigators were confident of completing the follow-up. Participants who had moved out of their original community were treated as censored cases.

To determine cause-specific mortality of cardiovascular disease, we used the International Classification of Diseases (ICD), 9th and 10th revisions, as follows: total stroke (ICD 9th revision, codes 430–438 and ICD 10th revision, codes I60–I69); coronary heart disease (410–414, I20–I25). Total stroke was subdivided into hemorrhagic stroke (430–431, I60–I61) and ischemic stroke (433–434, I63). For each case, we randomly selected 1 control participant (ie, a person without death from stroke or coronary heart disease) from the participants. Controls were matched for sex, age (±5 years), community, tocopherol measurement method, and year of blood drawing.

Determination of biochemical variables

Venous blood was collected at baseline, and sera were prepared from blood samples as soon as possible after blood collection at laboratories in or near the surveyed municipalities. The serum samples were collected in 0.3-ml tubes and stored at −80°C until further use. Serum α-tocopherol and γ-tocopherol levels were measured using high-performance liquid chromatography (HPLC) at the Osaka laboratory facility of SRL, Inc. (Tokyo, Japan; 246 case-control pairs) and the Public Health Institute of Kochi Prefecture (495 case-control pairs). The analytical protocols for tocopherol estimation were similar at both facilities and included the deproteinization of serum samples, extraction of tocopherols by an organic solvent, and assay by reversed-phase HPLC using a fluorescent detector. However, there were some differences between the SRL and Kochi methods. The SRL method involved deproteinization by ethanol, extraction by n-hexane, and concentration using nitrogen gas, whereas the Kochi institute method involved deproteinization and extraction by 2-propanol, but no concentration step.

The measurements obtained at SRL were adjusted using the results of 30 samples that were measured by both methods. The regression equations for the values obtained by the 2 methods were $y_1 = 2.135x_1 - 1.036$ and $y_2 = 1.483x_2 + 0.203$ for α-tocopherol and γ-tocopherol, respectively, where y_1 and y_2 were Kochi method values and x_1 and x_2 were SRL method values. The correlation coefficients were 0.903 and 0.970 for α-tocopherol and γ-tocopherol, respectively.

Serum total cholesterol was measured by an enzymatic method using an automatic analyzer at Kotobiken Medical Laboratories, Inc. (Tokyo, Japan). The standardization of lipid measurement was performed with the aid of the Osaka

Table 1. Sex-specific mean values or prevalence of cardiovascular risk factors in cases and controls

	Total stroke		Ischemic stroke		Hemorrhagic stroke		Coronary heart disease	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Men								
No. of subjects	257	257	165	165	85	85	114	114
Age, years	66.6 ± 0.5	65.8 ± 0.5	67.9 ± 0.6	67.2 ± 0.5	64.1 ± 1.0	63.1 ± 0.9	64.7 ± 1.0	64.0 ± 0.9
Hypertension, %	40.5***	23.7	43.6***	24.8	32.9	21.2	36.0*	21.1
Diabetes mellitus, %	7.0	5.4	8.5	6.1	4.7	4.7	9.6	7.9
BMI, kg/m^2	22.3 ± 0.2	22.3 ± 0.2	22.2 ± 0.2	22.1 ± 0.2	22.2 ± 0.3	22.6 ± 0.3	22.7 ± 0.3	22.7 ± 0.3
Alcohol intake, g/day	20.7 ± 1.6*	16.6 ± 1.4	18.2 ± 1.7	16.2 ± 1.7	26.3 ± 3.2	18.4 ± 2.7	17.7 ± 2.2	15.5 ± 2.1
Current smoker, %	56.1*	45.9	57.4*	46.8	54.2	42.9	53.2	49.1
Total cholesterol, mg/dl	184.1 ± 2.4	190.1 ± 2.2	187.0 ± 2.9	189.8 ± 2.7	179.4 ± 4.6	189.6 ± 4.0	196.4 ± 3.5	194.2 ± 3.6
HDL cholesterol, mg/dl	44.6 ± 1.0	46.1 ± 1.0	44.6 ± 1.2	47.5 ± 1.3	44.5 ± 1.8	44.3 ± 1.7	44.9 ± 1.5	44.2 ± 1.3
Walking ≥0.5 h/week, %	68.3	77.7	68.4	76.7	70.5	79.0	66.2	66.7
Sports ≥5 h/week, %	10.2	12.4	10.2	11.5	9.4	14.7	11.4	7.3
Vitamin E intake, mg	5.9 ± 0.2	5.6 ± 0.2	6.1 ± 0.2	5.7 ± 0.2	5.6 ± 0.3	5.5 ± 0.3	5.1 ± 0.3	4.8 ± 0.3
Serum α-tocopherol, μg/ml	13.67 ± 0.33	13.78 ± 0.33	13.77 ± 0.43	14.04 ± 0.42	13.57 ± 0.56	13.27 ± 0.55	13.17 ± 0.41	13.51 ± 0.47
Serum γ-tocopherol, μg/ml	1.08 ± 0.04	1.17 ± 0.04	1.04 ± 0.04	1.14 ± 0.05	1.18 ± 0.06	1.21 ± 0.06	1.29 ± 0.07	1.20 ± 0.06
Women								
No. of subjects	273	273	137	137	125	125	97	97
Age, years	68.3 ± 0.6	68.1 ± 0.5	71.9 ± 0.7	71.5 ± 0.6	64.2 ± 0.8	64.2 ± 0.8	68.9 ± 0.8	68.7 ± 0.8
Hypertension, %	40.3	32.2	35.8	36.5	43.2**	26.4	40.2	39.2
Diabetes mellitus, %	5.9	4.0	7.3	5.1	4.8	2.4	17.5**	4.1
BMI, kg/m^2	22.4 ± 0.2	22.9 ± 0.2	22 ± 0.3	22.4 ± 0.3	22.8 ± 0.3	23.2 ± 0.3	23.6 ± 0.4	23.2 ± 0.3
Alcohol intake, g/day	1.1 ± 0.4	1.0 ± 0.3	0.6 ± 0.3	1.1 ± 0.4	1.7 ± 0.9	0.7 ± 0.2	2.2 ± 1.1	0.9 ± 0.5
Current smoker, %	5.6	3.3	7.1	4.2	4.5	2.6	18.2	9.0
Total cholesterol, mg/dl	204.2 ± 2.6**	214.2 ± 2.5	205.7 ± 4.1	213.1 ± 3.5	202.3 ± 3.5*	214.8 ± 3.7	217.5 ± 4.9	213.6 ± 4.3
HDL cholesterol, mg/dl	44.0 ± 0.9	45.1 ± 0.9	45.0 ± 1.3	45.3 ± 1.3	43.1 ± 1.2	44.7 ± 1.3	39.9 ± 1.4*	44.5 ± 1.7
Walking ≥0.5 h/week, %	70.8	75.3	71.7	75.5	73.0	73.1	67.2	76.2
Sports ≥5 h/week, %	5.9	9.1	6.7	11.5	5.5	6.3	1.4	7.9
Vitamin E intake, mg	5.4 ± 0.2	5.3 ± 0.1	5.4 ± 0.2	5.3 ± 0.2	5.4 ± 0.2	5.3 ± 0.2	5.0 ± 0.3	5.4 ± 0.2
Serum α-tocopherol, μg/ml	15.63 ± 0.38	16.21 ± 0.35	15.40 ± 0.57	16.06 ± 0.46	15.78 ± 0.48	16.57 ± 0.56	15.34 ± 0.58	16.46 ± 1.15
Serum γ-tocopherol, μg/ml	1.33 ± 0.04	1.33 ± 0.04	1.28 ± 0.05	1.38 ± 0.05	1.41 ± 0.06	1.27 ± 0.06	1.32 ± 0.08	1.43 ± 0.07

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein.
Serum α- and γ-tocopherol values were adjusted for total cholesterol by using the residual method.
Difference from controls: *P < 0.05, **P < 0.01, ***P < 0.001.

Medical Center for Health Science and Promotion, which is an international member of the US National Cholesterol Reference Method Laboratory Network (CRMLN).[27]

Statistical analysis

Because tocopherols are highly correlated with total cholesterol levels, we used the residual method to adjust α- and γ-tocopherol levels for serum total cholesterol.[28]

To compare the baseline characteristics of mortality cases and control subjects, the paired t-test was used to test mean values, and the McNemar test was used for percentages of cardiovascular risk factors.

Cut-off values in quintile analyses of the distribution of control subjects were determined. Using conditional logistic regression models, odds ratios (ORs) for total stroke, stroke subtype, and coronary heart disease were estimated according to sex-specific quintiles of serum α- and γ-tocopherol levels and 1-SD increments in α-tocopherol levels (5.12 μg/ml for men and 6.90 μg/ml for women) and γ-tocopherol levels (0.61 μg/ml for men and 0.67 μg/ml for women). Linear regression was used to test for linear trends across tocopherol categories by using the median tocopherol level for each tocopherol category. Covariates for adjustment included body mass index (categorized as <18.5 kg/m^2, 18.5–24.9 kg/m^2, and ≥25 kg/m^2), serum total and high-density lipoprotein (HDL) cholesterol levels (mg/dl), cigarette smoking status (never, former, current), alcohol drinking status (never, ex-, and current), walking (≥30 min/day or not), sports (≥5 hour/week or not), and self-reported history of a physician diagnosis of hypertension or diabetes mellitus (yes or no), as well as matching for sex, age, area of residence, tocopherol measurement method, and year of blood drawing.

The data were analyzed with Statistical Analysis Software (SAS; Version 9.1.3 Service Pack 4; SAS Institute, Cary, NC, USA). All probability values for statistical tests were 2-tailed, and a P value less than 0.05 was considered statistically significant.

RESULTS

During the 13-year follow-up, we documented 530 stroke deaths (257 men and 273 women). Of these, 302 deaths (165 men and 137 women) were due to ischemic stroke and 210 (85 men and 125 women) were due to hemorrhagic stroke, whereas 211 deaths (114 men and 97 women) were due to coronary heart disease.

Table 1 shows the sex-specific characteristics of cases and controls. Mean age at death was 67 years among men and 72 years among women for ischemic stroke, 63 years among men and 64 years among women for hemorrhagic stroke, 64 years

Table 2. Sex-specific odds ratios (ORs) and 95% CIs for cardiovascular disease mortality by quintile of total cholesterol-adjusted serum α-tocopherol

	Serum α-tocopherol level									
	Q1 (low)	Q2		Q3		Q4		Q5 (high)		*P* for trend	1-SD increment
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI		
Men											
Serum α-tocopherol											
Median, μg/ml	8.7	11.1		12.8		14.7		19.5			
Range, μg/ml	(1.7–10.2)	(10.2–11.8)		(11.8–13.7)		(13.7–16.3)		(16.3–49.0)			
Total stroke											
No. of cases	63	32		56		50		56			
No. of controls	52	52		48		52		53			
Age-, community-matched OR	1.00	0.50	(0.27–0.91)	0.93	(0.51–1.70)	0.80	(0.43–1.49)	0.92	(0.49–1.70)	0.92	0.98 (0.81–1.17)
Multivariable OR[a]	1.00	0.44	(0.22–0.86)	0.85	(0.44–1.63)	0.73	(0.37–1.42)	0.81	(0.41–1.60)	0.87	0.91 (0.75–1.11)
Ischemic stroke											
No. of cases	38	22		37		30		38			
No. of controls	31	28		30		43		33			
Age-, community-matched OR	1.00	0.63	(0.30–1.33)	0.92	(0.42–1.98)	0.48	(0.21–1.09)	0.84	(0.38–1.85)	0.89	0.95 (0.76–1.18)
Multivariable OR[a]	1.00	0.63	(0.26–1.52)	0.90	(0.38–2.16)	0.46	(0.19–1.14)	0.83	(0.35–2.00)	0.91	0.90 (0.71–1.15)
Hemorrhagic stroke											
No. of cases	23	9		17		19		17			
No. of controls	19	24		15		9		18			
Age-, community-matched OR	1.00	0.23	(0.07–0.78)	0.97	(0.34–2.75)	1.58	(0.55–4.59)	0.89	(0.29–2.74)	0.61	1.08 (0.76–1.52)
Multivariable OR[a]	1.00	0.22	(0.05–0.95)	1.83	(0.47–7.11)	2.11	(0.54–8.18)	0.97	(0.25–3.77)	0.58	1.09 (0.74–1.61)
Coronary heart disease											
No. of cases	28	23		22		20		21			
No. of controls	21	23		26		23		21			
Age-, community-matched OR	1.00	0.76	(0.34–1.70)	0.63	(0.28–1.42)	0.63	(0.26–1.51)	0.71	(0.29–1.78)	0.50	0.91 (0.66–1.25)
Multivariable OR[a]	1.00	0.78	(0.31–1.94)	0.59	(0.24–1.45)	0.65	(0.23–1.83)	0.68	(0.24–1.92)	0.46	0.87 (0.61–1.25)
Women											
Serum α-tocopherol											
Median, μg/ml	9.6	13.0		15.3		17.9		23.0			
Range, μg/ml	(0.0–11.8)	(11.8–14.1)		(14.2–16.2)		(16.2–19.9)		(19.9–103.8)			
Total stroke											
No. of cases	68	57		45		61		42			
No. of controls	52	52		56		53		60			
Age-, community-matched OR	1.00	0.70	(0.39–1.25)	0.48	(0.26–0.92)	0.61	(0.32–1.20)	0.39	(0.20–0.78)	0.01	0.86 (0.68–1.08)
Multivariable OR[a]	1.00	0.70	(0.37–1.34)	0.44	(0.21–0.91)	0.52	(0.24–1.10)	0.35	(0.16–0.77)	0.009	0.85 (0.66–1.10)
Ischemic stroke											
No. of cases	37	31		20		27		22			
No. of controls	30	19		31		25		32			
Age-, community-matched OR	1.00	1.05	(0.43–2.60)	0.36	(0.14–0.95)	0.53	(0.19–1.48)	0.37	(0.14–1.02)	0.03	0.83 (0.59–1.16)
Multivariable OR[a]	1.00	1.19	(0.40–3.57)	0.39	(0.12–1.29)	0.51	(0.15–1.74)	0.47	(0.14–1.52)	0.12	0.97 (0.66–1.43)
Hemorrhagic stroke											
No. of cases	25	25		25		34		16			
No. of controls	19	31		23		25		27			
Age-, community-matched OR	1.00	0.57	(0.25–1.32)	0.76	(0.30–1.91)	0.87	(0.34–2.23)	0.38	(0.14–1.09)	0.15	0.81 (0.58–1.15)
Multivariable OR[a]	1.00	0.61	(0.22–1.74)	0.74	(0.22–2.46)	0.62	(0.18–2.05)	0.26	(0.07–0.97)	0.048	0.68 (0.44–1.05)
Coronary heart disease											
No. of cases	22	25		20		12		18			
No. of controls	21	23		17		21		15			
Age-, community-matched OR	1.00	0.99	(0.40–2.45)	1.01	(0.39–2.59)	0.52	(0.18–1.50)	0.99	(0.36–2.75)	0.88	0.89 (0.68–1.15)
Multivariable OR[a]	1.00	0.66	(0.18–2.33)	0.89	(0.25–3.17)	0.33	(0.08–1.32)	0.97	(0.24–3.95)	0.85	0.92 (0.62–1.36)

[a]Adjusted for body mass index (<18.5, 18.5–24.9, 25≤), cigarette smoking status (never, ex-, and current), alcohol drinking status (never, ex-, and current), history of hypertension and diabetes mellitus, walking (≥30 min/day or not), sports (≥5 h/week or not), total cholesterol, and high-density lipoprotein cholesterol (continuous).

among men and 69 years among women for coronary heart disease. Among cases compared with controls, the prevalence of hypertension was higher in men who had any stroke, ischemic stroke, or coronary heart disease and in women with hemorrhagic stroke; average alcohol intake and prevalence of current smoking were higher in men; the prevalence of current smoking was higher in men with any stroke or ischemic stroke; the prevalence of diabetes mellitus was higher in women with coronary heart disease; mean serum total cholesterol was lower in women with any stroke or hemorrhagic stroke; and mean serum α-tocopherol tended to be lower for all cardiovascular endpoints among both men and women, except for hemorrhagic stroke in men.

In addition, among cases, mean serum γ-tocopherol tended to lower for all cardiovascular endpoints among both men and women, except for coronary heart disease in men and any stroke and hemorrhagic stroke in women.

Table 2 shows age-, sex-, and community-matched ORs and multivariate ORs (95% CIs) for cardiovascular disease mortality according to quintile of serum α-tocopherol levels.

Table 3. Sex-specific odds ratios (ORs) and 95% CIs for cardiovascular disease mortality by quintile of total cholesterol-adjusted serum γ-tocopherol

	Serum γ-tocopherol level									
	Q1 (low)	Q2		Q3		Q4		Q5 (high)		*P* for trend	1-SD increment
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI		
Men											
Serum γ-tocopherol											
Median, μg/ml	0.53	0.83		1.07		1.37		1.96			
Range, μg/ml	(0.01–0.70)	(0.71–0.95)		(0.95–1.21)		(1.21–1.60)		(1.60–4.53)			
Total stroke											
No. of cases	68	48		49		46		46			
No. of controls	52	47		52		56		50			
Age-, community-matched OR	1.00	0.78	(0.45–1.36)	0.69	(0.40–1.20)	0.59	(0.33–1.03)	0.66	(0.38–1.17)	0.15	0.84 (0.68–1.03)
Multivariable OR[a]	1.00	0.73	(0.40–1.34)	0.75	(0.41–1.37)	0.55	(0.30–1.03)	0.68	(0.37–1.25)	0.21	0.83 (0.67–1.04)
Ischemic stroke											
No. of cases	50	34		30		23		28			
No. of controls	37	31		33		32		32			
Age-, community-matched OR	1.00	0.84	(0.44–1.62)	0.64	(0.32–1.26)	0.50	(0.25–1.02)	0.60	(0.30–1.19)	0.12	0.80 (0.62–1.04)
Multivariable OR[a]	1.00	0.81	(0.38–1.69)	0.57	(0.26–1.23)	0.48	(0.21–1.09)	0.48	(0.22–1.06)	0.07	0.77 (0.58–1.02)
Hemorrhagic stroke											
No. of cases	16	14		18		19		18			
No. of controls	12	16		19		21		17			
Age-, community-matched OR	1.00	0.62	(0.21–1.84)	0.67	(0.24–1.89)	0.63	(0.21–1.85)	0.75	(0.26–2.23)	0.78	0.93 (0.66–1.31)
Multivariable OR[a]	1.00	0.34	(0.08–1.58)	0.58	(0.12–2.72)	0.27	(0.05–1.37)	0.88	(0.18–4.31)	0.73	1.03 (0.65–1.62)
Coronary heart disease											
No. of cases	20	20		25		23		26			
No. of controls	22	27		22		18		25			
Age-, community-matched OR	1.00	0.84	(0.34–2.11)	1.32	(0.50–3.45)	1.43	(0.57–3.59)	1.19	(0.47–3.00)	0.64	1.13 (0.89–1.44)
Multivariable OR[a]	1.00	1.02	(0.36–2.93)	1.54	(0.48–4.91)	1.72	(0.60–4.98)	1.54	(0.52–4.62)	0.48	1.20 (0.91–1.57)
Women											
Serum γ-tocopherol											
Median, μg/ml	0.57	0.99		1.30		1.60		2.21			
Range, μg/ml	(0.01–0.81)	(0.81–1.15)		(1.15–1.44)		(1.44–1.80)		(1.80–4.98)			
Total stroke											
No. of cases	54	69		42		53		55			
No. of controls	61	54		54		49		55			
Age-, community-matched OR	1.00	1.48	(0.87–2.51)	0.88	(0.50–1.54)	1.26	(0.71–2.23)	1.13	(0.64–1.97)	0.92	1.01 (0.84–1.22)
Multivariable OR[a]	1.00	1.32	(0.74–2.36)	0.87	(0.48–1.60)	1.21	(0.64–2.26)	1.26	(0.68–2.32)	0.56	1.06 (0.86–1.30)
Ischemic stroke											
No. of cases	29	36		20		29		23			
No. of controls	29	26		26		25		31			
Age-, community-matched OR	1.00	1.46	(0.67–3.17)	0.75	(0.34–1.66)	1.14	(0.52–2.50)	0.70	(0.31–1.58)	0.27	0.81 (0.61–1.08)
Multivariable OR[a]	1.00	1.19	(0.49–2.87)	0.72	(0.29–1.79)	1.00	(0.39–2.60)	0.82	(0.33–2.04)	0.59	0.84 (0.61–1.16)
Hemorrhagic stroke											
No. of cases	20	32		21		22		30			
No. of controls	29	26		26		21		23			
Age-, community-matched OR	1.00	1.76	(0.82–3.80)	1.15	(0.50–2.66)	1.55	(0.63–3.79)	1.99	(0.85–4.65)	0.19	1.26 (0.97–1.65)
Multivariable OR[a]	1.00	1.44	(0.52–4.00)	0.90	(0.30–2.68)	1.82	(0.63–5.27)	3.10	(0.95–10.1)	0.052	1.49 (1.04–2.13)
Coronary heart disease											
No. of cases	25	21		12		16		23			
No. of controls	12	20		20		26		19			
Age-, community-matched OR	1.00	0.52	(0.20–1.33)	0.25	(0.08–0.77)	0.31	(0.12–0.81)	0.56	(0.21–1.48)	0.24	0.85 (0.64–1.13)
Multivariable OR[a]	1.00	0.66	(0.19–2.28)	0.27	(0.06–1.21)	0.23	(0.06–0.82)	0.57	(0.15–2.23)	0.32	0.87 (0.59–1.28)

[a]Adjusted for body mass index (<18.5, 18.5–24.9, 25≤), cigarette smoking status (never, ex-, and current), alcohol drinking status (never, ex-, and current), history of hypertension and diabetes mellitus, walking (≥30 min/day or not), sports (≥5 h/week or not), total cholesterol, and high-density lipoprotein cholesterol (continuous).

In men, there was no association between serum α-tocopherol and cardiovascular disease mortality. In women, serum α-tocopherol was inversely associated with mortality from any stroke and hemorrhagic stroke. The multivariate OR (95% CI) for the highest versus lowest quintile of serum α-tocopherol among women was 0.35 (0.16–0.77; *P* for trend = 0.009) for total stroke, 0.47 (0.14–1.52; *P* for trend = 0.12) for ischemic stroke, 0.26 (0.07–0.97; *P* for trend = 0.048) for hemorrhagic stroke, and 0.97 (0.24–3.95; *P* for trend = 0.85) for coronary heart disease.

In age-, sex-, and community-matched analyses, there was no significant association between serum γ-tocopherol and mortality from any cardiovascular disease outcome among men (Table 3). After adjusting for cardiovascular risk factors, the association between serum γ-tocopherol and ischemic stroke death was of borderline significance in men: the multivariate ORs (95% CI) for the highest versus the lowest quintile and for a 1-SD increment in γ-tocopherol level were 0.48 (0.22–1.06; *P* for trend = 0.07) and 0.77 (0.58–1.02), respectively. In women, serum γ-tocopherol was associated

with increased hemorrhagic stroke mortality. The multivariate OR (95% CIs) for the highest versus the lowest quintile and for a 1-SD increment in γ-tocopherol level were 3.10 (0.95–10.1; *P* for trend = 0.052) and 1.49 (1.04–2.13), respectively.

DISCUSSION

In this prospective, nested case-control study of Japanese men and women from the general population without diagnosed cardiovascular disease at enrolment, higher baseline serum α-tocopherol was associated with lower mortality from total stroke and hemorrhagic stroke among women. Serum γ-tocopherol tended to be associated with lower mortality from ischemic stroke among men and higher mortality from hemorrhagic stroke among women. There was no significant association between α- or γ-tocopherol and coronary heart disease mortality among men or women.

To our knowledge, only 3 studies have investigated the association between blood levels of tocopherols and cardiovascular disease risk among men[18–22]; however, have been no such studies of women. Our study is the first assessment of the relationship between serum tocopherol levels and cardiovascular disease risk in women.

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study found that serum α-tocopherol was associated with a decreased risk of ischemic stroke among men: the multivariate relative risk (95% CI) of ischemic stroke for the highest versus the lowest quartile of α-tocopherol levels was 0.70 (0.55–0.89).[18] The ATBC study also reported that serum α-tocopherol was inversely associated with risk of intracerebral hemorrhage: the multivariate relative risk (95% CI) of intracerebral hemorrhage for the highest versus the lowest quintile of α-tocopherol levels was 0.45 (0.26–0.77).[18]

The Physicians' Health Study (PHS) reported no association between serum α- or γ-tocopherol levels and ischemic stroke risk among men.[20] Serum α-tocopherol was not associated with risk of myocardial infarction in PHS[21] or The Multiple Risk Factor Intervention Trial,[22] while γ-tocopherol was associated with increased risk of myocardial infarction in PHS: the multivariate relative risk (95% CI) for the highest versus the lowest quintile was 2.14 (1.18–3.87; *P* for trend = 0.01).[21] In the present study, we did not observe an association between serum α- or γ-tocopherol and coronary heart disease mortality in men or women. These inconsistent findings regarding serum tocopherol levels and myocardial infarction may be due to a paradoxical effect of tocopherol on oxidation. Although tocopherols are known antioxidants, several in vivo studies have reported that excessive concentrations of tocopherol can cause oxidative stress, leading to lipid peroxidation mediated by the tocopherol radical.[29]

The antioxidant activity of α-tocopherol is a potential pathophysiologic mechanism for the inverse association between serum α-tocopherol and hemorrhagic stroke mortality. A Japanese autopsy study found that 11% of 101 intracerebral hemorrhage cases had cerebral amyloid angiopathy,[30] and the prevalence of amyloid angiopathy tended to be higher in women (28.0%) than in men (18.3%).[31] In a neuroimaging study in the United States, 2.2% of 460 subarachnoid hemorrhage patients aged 60 years or older had amyloid angiopathy.[32] Deposits of the amyloid beta protein cause degeneration of smooth muscle cells of the vascular media.[33] In a mice model of amyloid beta deposition, α-tocopherol supplementation in mice aged 5 to 13 months reduced amyloid beta protein levels and the area of cerebral amyloid deposits in brain tissue, as compared with a control group.[34]

Cerebral aneurysm can occur due to the development of chronic inflammation mediated by nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB),[35] which is induced by oxidative stress.[36] Cerebral aneurysms in antioxidant-treated rats were 54% smaller than in controls, due to inhibition of NF-κB activity.[37]

The reasons for the absence of an association between α-tocopherol and mortality from total stroke and its subtypes among men in the present study are unknown, although the lower prevalence of amyloid angiopathy[31] and lower proportion of subarachnoid hemorrhage[38] among men as compared with women might partially explain this finding.

In addition to acting as antioxidants, tocopherols are also inhibitors of platelet aggregation and thrombus formation.[39,40] In an animal experiment, time to thrombus formation was 25% longer in rats fed α-tocopherol and 58% longer in rats fed γ-tocopherol as compared with controls. Platelet aggregation was 16% lower in rats fed α-tocopherol and 43% lower in rats fed γ-tocopherol as compared with controls.[40] In humans, α-tocopherol supplementation alone reduced serum γ-tocopherol levels,[41] while a supplement with equal amounts of α- and γ-tocopherols raised plasma α- and γ-tocopherol levels.[42] A supplement with mixed tocopherols (100 mg γ-, 40 mg δ-, and 20 mg α-tocopherol; corresponding to a 20 mg α-tocopherol equivalent) reduced platelet aggregation by 14% as compared with pre-supplementation platelet aggregation values. No change in platelet aggregation was observed in subjects receiving only an α-tocopherol supplement.[39] This inhibitory effect of γ-tocopherol on platelet aggregation and thrombus formation could account for the association between serum γ-tocopherol and reduced ischemic stroke mortality, as well as the association between serum γ-tocopherol and increased hemorrhagic stroke mortality, observed in the present study.

The present study has several strengths. It is the first study to evaluate the prospective association between serum α- and γ-tocopherol and cardiovascular disease mortality in