Serum Vitamin C Concentration Was Inversely Associated With Subsequent 20-Year Incidence of Stroke in a Japanese Rural Community

The Shibata Study

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Background and Purpose—Epidemiological evidence suggests that vitamin C may decrease the risk of stroke. The purpose of the present study was to examine the association of serum vitamin C concentration with the subsequent incidence of stroke.

Methods—In a Japanese rural community, a cohort of 880 men and 1241 women aged 40 years and older who were initially free of stroke was examined in 1977 and followed until 1997. The baseline examination included a measurement of serum vitamin C concentration. The incidence of stroke was determined by annual follow-up examinations and registry.

Results—During the 20-year observation period, 196 incident cases of all stroke, including 109 cerebral infarctions and 54 hemorrhagic strokes, were documented. Strong inverse associations were observed between serum vitamin C concentration and all stroke (sex- and age-adjusted hazard ratios were 0.93, 0.72, and 0.59, respectively, for the second, third, and fourth quartiles compared with the first quartile; P for trend=0.002), cerebral infarction (0.71, 0.59, and 0.51; P for trend=0.015), and hemorrhagic stroke (0.89, 0.75, and 0.45; P for trend=0.013). Additional adjustments for blood pressure, serum total cholesterol, body mass index, physical activity, smoking, alcohol drinking, antihypertensive medication, atrial fibrillation, and history of ischemic heart disease did not attenuate these associations markedly.

Conclusions—Serum vitamin C concentration was inversely related to the subsequent incidence of stroke. This relationship was significant for both cerebral infarction and hemorrhagic stroke. Additional mechanistic hypotheses may be required to explain our findings. **(*Stroke*. 2000;31:2287-2294.)**

Key Words: ascorbic acid ■ cerebrovascular disorders ■ risk factors ■ Japan

There has been a growing interest in the potential role of antioxidative vitamins for the prevention of cardiovascular disease because epidemiological evidence suggests that an increased consumption of fruit and vegetables may decrease the risk of this disease.[1] For example, several cohort studies found a significant protective association of the consumption of fruit and vegetables or surrogate nutrients (particularly vitamin C and potassium) with stroke.[2–7] Such a protective association has been reported not only for dietary intake but also for serum or plasma concentration of vitamin C. Two large cross-sectional studies in the United States (National Health and Nutrition Examination Survey [NHANES] II and III) demonstrated the association of higher serum ascorbic acid levels with a decreased prevalence of stroke.[8,9] In addition, 2 prospective cohort studies have examined the association between plasma vitamin C concentration and mortality from stroke. Gale et al[6] reported a significantly increased risk at lower levels of plasma vitamin C, and Gey et al[10] found an increased risk among those whose plasma levels of vitamin C and β-carotene were simultaneously low. However, the detailed mechanisms of these associations have not been well revealed.

There are a number of mechanistic hypotheses about the potential protective effects of antioxidative vitamins against cardiovascular disease. One of the most common of these hypotheses may be the antioxidant hypothesis, that is, since oxidative modification of LDL is important, and possibly obligatory, in the pathogenesis of atherosclerotic lesions, antioxidative vitamins are protective against cardiovascular disease through their defensive effect on LDL oxidation.[11,12]

Received April 27, 2000; final revision received July 17, 2000; accepted July 19, 2000.

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Presented in part at the 4th International Conference on Preventive Cardiology, Montreal, Canada, June 30, 1997, and the 15th International Scientific Meeting of the International Epidemiological Association, Florence, Italy, September 2, 1999.

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Another hypothesis may be that the effect of vitamin C is mediated by a lowering of blood pressure[13] because several cross-sectional studies reported a weak but statistically significant inverse relationship between dietary intake or plasma (serum) concentration of vitamin C and blood pressure.[14–16] Alternatively, the protective association of vitamin C with stroke may be explained by confounding because a high intake of fruit and vegetables is associated with other healthy behaviors.[17]

Could vitamin C decrease the risk of stroke through its antioxidative effect, a lowering of blood pressure, other confounding effects on established cardiovascular risk factors, or through a combination of these mechanisms? Although it may be difficult to clearly answer this question, an analysis of the association between vitamin C and stroke according to subtype may advance the knowledge that is necessary to investigate the plausibility of these hypotheses. For example, if a decreased risk was observed for cerebral infarction but not for hemorrhagic stroke, a hypothesis linked with the prevention of atherosclerosis (eg, the antioxidant hypothesis) would be plausible. By contrast, if decreased risks were observed for any subtype, a combination of several mechanistic hypotheses may be required to explain these risk reductions. To gain the knowledge that would allow a more advanced discussion of these hypotheses, we analyzed the association of serum vitamin C concentration and fruit and vegetable intake with a subsequent 20-year incidence of stroke according to subtype using data based on a prospective cohort study conducted in a rural community in Japan.

Subjects and Methods

Study District

This study was performed in the Akadani-Ijimino district of Shibata located in the northern part of Niigata Prefecture, Japan. Shibata has a commercial and residential area at its center and is surrounded by an agricultural area. The Akadani-Ijimino district is in the agricultural area and consists of open fields where rice and fruit are produced on a relatively large scale and a mountainous area several hundred meters above sea level where small-scale farming is carried out. Details of the study area have been described.[18,19]

Baseline Examination

All residents aged 40 years or older (1182 men and 1469 women) were considered the eligible population. A baseline examination was conducted in July 1977. Details of the methods were described previously.[18]

The baseline examination included a serum vitamin C measurement and other blood studies; a dietary survey using a semiquantitative food frequency questionnaire (FFQ); a general health questionnaire (demographic characteristics, personal history of disease, smoking, alcohol consumption, and use of antihypertensive medication); measurements of height, weight, and systolic and diastolic blood pressure (SBP and DBP, respectively); ECG; and a physical activity survey.

The serum vitamin C concentration was determined in venous blood under nonfasting conditions by the 2,3-dinitrophenylhydrazine method with calorimetric analysis.[20] Immediately after separation by centrifugation, serum was deproteinized, and the supernatant of the serum was stored at −20°C on the basis of our stability study.[21] Measurements were completed within 10 days. Precision was reconfirmed by measuring serum vitamin C 15 times from a randomly selected sample in each run. The coefficient of variation was within the range of 5.0% for any run.[21]

Intake of vitamin C–rich products was assessed with the use of a FFQ, details of which were reported previously.[22] This FFQ had been developed to assess the usual food and nutrient intake of individuals over the most recent year. A long list was derived from the Japanese Food Composition Table[23] and systematically reduced with the help of an experienced staff dietitian. Finally, the FFQ consisted of 66 food items. Of these food items, vitamin C–rich products were only fruits and vegetables. Trained dietitians interviewed the participants on the frequency of consumption of each food, as follows: (1) almost never, (2) 1 to 2 d/wk, (3) 3 to 5 d/wk, and (4) 6 to 7 d/wk. The typical portion size was also determined for most food items by this interview with an aid of food models, household measures, and food photograph booklets. However, fruit and vegetable intake was assessed without regard to portion size, and therefore we simply used the frequency of consumption of these foods for this report. Because so few subjects answered "almost never" for fruit and vegetable intake, we combined the "almost never" and "1 to 2 d/wk" categories for the analysis.

Serum total cholesterol (TC) was measured with an auto analyzer in a nonfasting blood sample, and standardization was achieved by participation in the Lipid Standardization Program of the Centers for Disease Control (Atlanta, Ga) through the Osaka Medical Center for Cancer and Cardiovascular Diseases (Osaka, Japan). The blood pressure at rest was measured by nurses specifically trained for this study using a Riva-Rocci sphygmomanometer according to standard procedure.[24] Mean blood pressure (MBP) was calculated as (SBP+2×DBP)/3. Body mass index (BMI) ([body weight in kg]/[height in m]^2) was calculated to express the degree of obesity. To evaluate the usual degree of physical activity, we used a simplified method we had previously developed for estimating energy expenditure.[25] This method was validated by comparison with a traditional time and motion study in estimating energy expenditure (correlation coefficient, 0.64; $P<0.01$). The physical activity index was calculated as a multiple of daily basal metabolism. In the present study the lower 25%, medium 50%, and upper 25% of the physical activity index for the subject's sex and age group based on 5-year increments were categorized as inactive, moderate, and active, respectively. ECG at rest was recorded in 12 leads with equipment from Nihon Koden, Fukuda ME, Fukuda Denshi, and the findings were classified according to the Minnesota code.[24] Of the ECG findings, atrial fibrillation (Minnesota code 8-3) was used in the current analysis. Smoking habits, alcohol consumption, history of ischemic heart disease (IHD), and use of antihypertensive medication were assessed by trained interviewers using a standardized questionnaire. More complete details of the methodology were described previously.[18,19]

Follow-Up and Determination of Stroke

The cohort members were followed for 20 years (July 1977 through June 1997). To identify the occurrence of stroke, a surveillance and registration system was incorporated with the local administration and the regional medical association. Three of the hospitals in the study area were equipped with CT scanners, and their findings since 1980 were available. To further ensure an accurate determination of the incidence of stroke, follow-up examinations were conducted annually during the observation period. Stroke was defined as the occurrence of rapidly developing clinical signs of focal or global disturbances of cerebral function that lasted >24 hours or resulted in death, for which there was no apparent cause other than a vascular accident. According to the standard clinical criteria,[26] cases of stroke were classified into the following subtypes: intracerebral hemorrhage (ICH), cerebral infarction, subarachnoid hemorrhage (SAH), and undetermined. If the clinical diagnosis conflicted with CT findings, the subtype was determined by the latter. Details of follow-up methods and diagnostic criteria for each subtype were described in our previous reports.[19,27,28]

Statistical Analyses

Means and SDs of serum vitamin C concentration were calculated to determine variations between sex and age groups. Pearson's correlation analysis or ANCOVA was used to examine the relationships between serum vitamin C concentration and selected factors.

Fisher's z transformation[29] was used to test the homogeneity of correlation coefficients between sexes. Cox proportional hazards model[30] was used to examine the association of risk factors with occurrence of all stroke, cerebral infarction, and hemorrhagic stroke (ICH plus SAH). Although ICH and SAH were etiologically different and we separately identified ICH and SAH cases, combined analyses for hemorrhagic stroke were done because, as initially mentioned, we had a strong interest in whether vitamin C was associated with a reduced risk of hemorrhagic stroke as well as cerebral infarction. Dummy variables were created to calculate the hazard ratios for the first (referent), second, third, and fourth quartiles of serum vitamin C concentration and for the 0 to 2 (referent), 3 to 5, and 6 to 7 d/wk categories of fruit and vegetable intake. Probability values for trends were calculated by entering these variables into the models as a continuous variable (serum vitamin C concentration) or as codes 0, 1, and 2 (for fruit and vegetable intake representing 0 to 2, 3 to 5, and 6 to 7 d/wk, respectively). All risk estimates were adjusted for sex and age stratified in 5-year increments (or for the latter when analyzed by sex). Additional adjustments for potential confounders were achieved by including them as covariates, in which MBP, BMI, and TC were used as continuous variables; the presence of atrial fibrillation and personal history of IHD were coded as definite or none; the use of antihypertensive medications and cigarette smoking were coded as yes or no; and alcohol consumption was categorized as 0, 0.1 to 2.0, 2.1 to 4.0, and >4.0 drinks per day using dummy variables, where 1 drink is approximately 12 g of ethanol. The amount of alcohol consumed was originally recorded using the Japanese traditional unit "Go" and then converted to "drinks," where 1 Go equals 2 drinks. The homogeneity of hazard ratios between sexes was tested by including an interaction term of sex×serum vitamin C concentration (continuous variable), sex×fruit intake, or sex×vegetable intake (codes 0, 1, and 2 as defined above) into the model. There was no evidence that proportional hazards assumptions were violated[31] for the analyses of the 20-year observation period. All analyses were done with the use of the SAS statistical package (version 6.12, SAS Institute).

Results

Baseline Examination

From the eligible population, 998 men (response rate, 84%) and 1360 women (93%) underwent the baseline examination. Of these, a measurement of serum vitamin C concentration was completed for 919 men and 1266 women. When we excluded those with a previous history of stroke, 880 men and 1241 women were recruited for the current analyses.

The distribution of serum vitamin C concentration was approximately normal. No one in the cohort had been taking vitamin supplements. As shown in Table 1, the mean serum vitamin C concentration was higher in women than in men and lower in the elderly. Associations between serum vitamin C concentration and other selected factors are shown in Table 2. Weak but significant inverse correlations were observed between serum vitamin C concentration and blood pressure variables even after adjustments for age and sex. These correlations were slightly stronger in men than in women (P values for homogeneity between sexes were 0.039, 0.120, and 0.054 for SBP, DBP, and MBP, respectively). The correlation between serum vitamin C concentration and TC was very weak ($P=0.152$ for homogeneity between sexes). The sex- and age-adjusted least square means of serum vitamin C concentration were higher in those who frequently ate vegetables or fruit, drank less alcohol, were physically active, and were not using antihypertensive medication. Although some of these associations were unclear when analyzed separately for men and women, none of the interactions between sex and these factors was statistically significant, that is, there was no evidence of these associations being different between sexes. Details of the association between serum vitamin C concentration and blood pressure have been reported.[16]

TABLE 1. Mean Serum Vitamin C Concentration by Sex and Age

Age Group, y	No.	Serum Vitamin C Concentration, μmol/L Mean	SD
Men			
40–49	300	44.8	16.9
50–59	257	45.2	17.3
60–69	212	40.9	19.2
≥70	111	35.4	21.2
All	880	42.8	18.4
Women			
40–49	395	57.7	14.7
50–59	370	59.9	15.6
60–69	279	57.7	16.1
≥70	197	49.0	19.0
All	1241	57.0	16.4

Risk Analyses

During the 20-year observation period, 196 incident cases of all stroke, including 109 cerebral infarction, 54 hemorrhagic stroke (38 ICH and 16 SAH), and 33 undetermined types, were documented (Table 3). Over this period, 295 men and 285 women and 40 men and 97 women were censored due to death and emigration, respectively.

As shown in Table 4, sex- and age-adjusted risks of all stroke and cerebral infarction were lower at higher serum vitamin C levels, showing clear inverse dose-response relationships (P for trend=0.002 and 0.015, respectively). Interestingly, such an inverse relationship was also observed for hemorrhagic stroke (P for trend=0.013). These inverse relationships were observed similarly in both men and women (P values for homogeneity of hazard ratios between sexes were 0.644, 0.477, and 0.886 for all stroke, cerebral infarction, and hemorrhagic stroke, respectively) although some were not significant because of the small sample size. Additional multivariate adjustments for MBP, BMI, TC, presence of atrial fibrillation, personal history of IHD, use of antihypertensive medications, cigarette smoking, and alcohol drinking slightly attenuated these associations, but the associations remained significant (for all stroke) or marginally significant (P for trend=0.079 and 0.059 for cerebral infarction and hemorrhagic stroke, respectively). To examine which variable most greatly contributed to such attenuations, we also calculated hazard ratios with an adjustment for each of these potential confounders step by step (data not shown) and found that MBP was the sole variable that attenuated the hazard ratios for all stroke and cerebral infarction and that both MBP and physical activity had such an effect on

TABLE 2. Baseline Relationships Between Serum Vitamin C Concentration and Selected Factors

	Serum Vitamin C Concentration								
	Both Sexes			Men			Women		
Factors	r†		P	r†		P	r†		P
SBP	−0.110		<0.001	−0.161		<0.001	−0.071		0.013
DBP	−0.091		<0.001	−0.130		<0.001	−0.062		0.031
MBP	−0.107		<0.001	−0.155		<0.001	−0.071		0.013
TC	0.054		0.014	0.017		0.621	0.080		0.005
BMI	−0.013		0.545	−0.021		0.532	−0.011		0.705
	n*	LSM±SE‡ μmol/L	P	n	LSM±SE‡ μmol/L	P	n	LSM±SE‡ μmol/L	P
Vegetable intake, d/wk									
0–2	55	47.3±2.3		32	39.2±3.2		23	53.1±3.4	
3–5	91	47.9±1.8		52	39.0±2.5		39	54.5±2.6	
6–7	1975	51.3±0.4	0.042	796	43.2±0.6	0.147	1179	57.1±0.5	0.306
Fruit intake, d/wk									
0–2	469	46.3±0.8		296	38.4±1.0		173	52.3±1.2	
3–5	697	50.9±0.6		296	42.8±1.0		401	56.6±0.8	
6–7	955	53.6±0.6	<0.001	288	47.3±1.1	<0.001	667	58.4±0.6	<0.001
Physical activity									
Inactive	553	49.8±0.7		227	41.8±1.2		326	55.3±0.9	
Moderate	1029	51.3±0.5		410	43.1±0.9		619	56.9±0.7	
Active	485	53.0±0.8	0.011	198	43.8±1.3	0.495	287	59.3±1.0	0.009
Smoking									
No	1438	51.7±0.5		252	43.8±1.1		1186	57.1±0.5	
Yes	683	49.8±0.8	0.083	628	42.4±0.7	0.273	55	53.4±2.2	0.099
Alcohol drinking, drinks/d									
0	1352	52.3±0.5		229	44.5±1.2		1123	57.4±0.5	
0.1–2.0	401	50.3±0.9		286	44.2±1.1		115	53.2±1.5	
2.1–4.0	239	48.5±1.3		237	41.6±1.2		2	§	
>4.0	129	45.7±1.6	0.002	128	38.7±1.6	0.013	1	§	0.037
Antihypertensive medication									
No	1658	51.6±0.4		674	43.6±0.7		984	57.2±0.5	
Yes	463	49.3±0.8	0.015	206	40.2±1.3	0.023	257	55.9±1.0	0.246
Atrial fibrillation									
None	2095	51.1±0.4		864	42.7±0.6		1231	57.0±0.5	
Definite	26	51.9±3.4	0.813	16	45.4±4.6	0.566	10	55.2±5.1	0.735
History of IHD									
None	2095	51.1±0.4		870	42.9±0.6		1225	57.0±0.5	
Definite	26	49.3±3.3	0.583	10	36.1±5.8	0.242	16	58.3±4.1	0.743

*Because of missing values for some factors, the number of subjects does not always add up to 2121.

†Pearson's correlation coefficients adjusted for age (and sex).

‡Least square mean (LSM) ± SE adjusted for age (and sex) by ANCOVA. *P* values are for homogeneity between the categories.

§Values were not shown because the number of subjects was very small in these categories.

hemorrhagic stroke. These attenuations were a little larger in men than in women. The adjustment for other variables virtually unchanged these relationships.

As for dietary intake, the sex- and age-adjusted risks of all stroke and cerebral infarction were less than half in those who consumed vegetables 6 to 7 times per week than in those consuming vegetables 0 to 2 times per week, indicating a significant inverse trend (Table 5). Such relationships were observed only in men, although the sex difference was not significant (*P* values for homogeneity of hazard ratios be-

TABLE 3. Number of Events During the 20-Year Follow-up Period, 1977–1997

	No. of Events	
	Men	Women
Cohort members	880	1241
Stroke events		
All stroke	91	105
Cerebral infarction	58	51
Hemorrhagic stroke	18	36
ICH	14	24
SAH	4	12
Undetermined type	15	18
Censored because of death*	295	285
Censored because of emigration	40	97

*Excluded those with a stroke event.

tween sexes were 0.139 and 0.530 for all stroke and cerebral infarction, respectively). The risk reduction for hemorrhagic stroke was not significant. Similar to the results observed for serum vitamin C concentration, these relationships were slightly attenuated after multivariate adjustments but remained significant for all stroke. The frequency of fruit intake was inversely associated with cerebral infarction in women, but the sex difference was not significant.

Other significant risk factors (data not shown) were higher DBP and MBP (for all stroke, cerebral infarction, and hemorrhagic stroke); higher SBP and BMI, use of antihypertensive medication, and presence of atrial fibrillation (for all stroke and cerebral infarction); being physically inactive (for all stroke and hemorrhagic stroke) or active (for all stroke) compared with the moderate level; and a history of IHD (for cerebral infarction). No marked sex differences were found for these associations.

Discussion

To the best of our knowledge, this is the first prospective cohort study to examine the relationship between serum vitamin C concentration and the incidence of stroke. Compared with previous cohort studies based on mortality data,[6,10] our study based on incidence data may have some advantages, such as the relatively accurate diagnosis of disease outcomes and the inclusion of nonfatal cases. Serum vitamin C concentration was inversely associated with the subsequent incidence of all stroke during the 20-year observation period. Although a previous study that examined the association between plasma vitamin C concentration and stroke mortality[6] had a potential weakness that arose from generally known inaccuracies in death certification, particularly in elderly people,[6] our similar results based on incidence data would support their findings and emphasize the importance of the serum (or plasma) vitamin C concentration in predicting the occurrence of stroke.

Most interestingly, an inverse association of serum vitamin C concentration was observed not only with cerebral infarc-

TABLE 4. Hazard Ratios of Stroke Incidence According to Serum Vitamin C Concentration Adjusted for Potential Confounders

Serum Vitamin C Concentration, μmol/L*	Adjusted for Age (and Sex)						Adjusted for All Variables†					
	All Stroke		CI		HS		All Stroke		CI		HS	
	HR	(95% CL)	HR	(95% CL)	HR	(95% CL)	HR	(95% CL)	HR	(95% CL)	HR	(95% CL)
Both sexes												
≤40	1		1		1		1		1		1	
41–51	0.93	(0.64–1.36)	0.71	(0.42–1.19)	0.89	(0.43–1.83)	0.89	(0.60–1.32)	0.66	(0.38–1.13)	1.02	(0.49–2.15)
52–63	0.72	(0.48–1.08)	0.59	(0.34–1.02)	0.75	(0.36–1.55)	0.84	(0.55–1.29)	0.69	(0.39–1.22)	0.91	(0.43–1.94)
≥64	0.59	(0.38–0.93)	0.51	(0.28–0.92)	0.45	(0.19–1.11)	0.71	(0.45–1.14)	0.63	(0.34–1.18)	0.59	(0.24–1.46)
P for trend		0.002		0.015		0.013		0.017		0.079		0.059
Men												
≤28	1		1		1		1		1		1	
29–45	0.93	(0.56–1.54)	0.98	(0.53–1.81)	0.71	(0.24–2.09)	0.91	(0.52–1.56)	0.96	(0.49–1.88)	1.01	(0.31–3.28)
46–57	0.46	(0.22–0.95)	0.50	(0.21–1.20)	0.35	(0.07–1.71)	0.50	(0.24–1.06)	0.55	(0.22–1.35)	0.52	(0.10–2.69)
≥58	0.59	(0.31–1.15)	0.57	(0.25–1.30)	0.36	(0.07–1.74)	0.80	(0.40–1.59)	0.75	(0.31–1.82)	0.64	(0.12–3.38)
P for trend		0.051		0.142		0.103		0.218		0.346		0.459
Women												
≤45	1		1		1		1		1		1	
46–57	0.63	(0.38–1.04)	0.39	(0.18–0.86)	0.88	(0.39–1.99)	0.66	(0.39–1.12)	0.36	(0.16–0.84)	1.00	(0.43–2.31)
58–68	0.69	(0.42–1.14)	0.62	(0.31–1.23)	0.70	(0.29–1.68)	0.67	(0.39–1.12)	0.55	(0.26–1.16)	0.72	(0.29–1.78)
≥69	0.43	(0.21–0.88)	0.39	(0.14–1.06)	0.40	(0.11–1.45)	0.47	(0.23–0.96)	0.44	(0.16–1.22)	0.46	(0.13–1.69)
P for trend		0.014		0.037		0.062		0.021		0.061		0.119

CI indicates cerebral infarction; HS, hemorrhagic stroke; HR, hazard ratio; and CL, confidence limit.

*Categorized by quartiles.

†Adjusted for (sex), age, MBP, TC, BMI, presence of atrial fibrillation, use of antihypertensive medication, personal history of IHD, physical activity, smoking, and alcohol drinking.

TABLE 5. Hazard Ratios of Stroke Incidence According to Vegetable and Fruit Intake Adjusted for Potential Confounders

	Adjusted for Age (and Sex)						Adjusted for All Variables*					
	All Stroke		CI		HS		All Stroke		CI		HS	
	HR	(95% CL)	HR	(95% CL)	HR	(95% CL)	HR	(95% CL)	HR	(95% CL)	HR	(95% CL)
Vegetable intake, d/wk												
Both sexes												
0–2	1		1		1		1		1		1	
3–5	0.56	(0.23–1.38)	0.72	(0.23–2.32)	0.51	(0.07–3.63)	0.59	(0.23–1.49)	0.83	(0.24–2.92)	0.42	(0.06–3.10)
6–7	0.42	(0.22–0.80)	0.42	(0.17–1.06)	0.56	(0.14–2.34)	0.46	(0.23–0.92)	0.56	(0.20–1.56)	0.51	(0.12–2.18)
P for trend	0.008		0.003		0.562		0.027		0.157		0.562	
Men												
0–2	1		1		1		1		1		1	
3–5	0.54	(0.19–1.55)	0.71	(0.19–2.69)	1.02	(0.09–11.4)	0.61	(0.21–1.84)	0.96	(0.22–4.15)	1.26	(0.10–16.0)
6–7	0.31	(0.14–0.66)	0.36	(0.13–1.03)	0.51	(0.07–3.88)	0.33	(0.15–0.77)	0.49	(0.15–1.65)	0.67	(0.08–5.78)
P for trend	0.001		0.023		0.313		0.004		0.106		0.479	
Women												
0–2	1		1		1		1		1		1	
3–5	0.71	(0.12–4.30)	0.86	(0.08–9.56)	0.00	†	0.72	(0.12–4.45)	0.85	(0.07–10.1)	0.00	†
6–7	0.80	(0.20–3.27)	0.65	(0.09–4.76)	0.60	(0.08–4.45)	0.89	(0.22–3.71)	0.83	(0.11–6.25)	0.54	(0.07–4.12)
P for trend	0.886		0.575		0.858		0.944		0.867		0.865	
Fruit intake, d/wk												
Both sexes												
0–2	1		1		1		1		1		1	
3–5	0.78	(0.53–1.14)	0.67	(0.41–1.09)	0.58	(0.27–1.27)	0.84	(0.57–1.25)	0.74	(0.45–1.23)	0.61	(0.28–1.36)
6–7	0.81	(0.57–1.16)	0.62	(0.39–1.01)	0.88	(0.44–1.72)	0.85	(0.58–1.23)	0.68	(0.41–1.12)	0.92	(0.46–1.85)
P for trend	0.306		0.062		0.923		0.426		0.142		0.978	
Men												
0–2	1		1		1		1		1		1	
3–5	0.58	(0.33–1.01)	0.51	(0.26–1.01)	0.14	(0.02–1.13)	0.68	(0.38–1.22)	0.63	(0.31–1.28)	0.15	(0.02–1.28)
6–7	1.08	(0.67–1.73)	0.87	(0.48–1.58)	1.55	(0.59–4.09)	1.14	(0.69–1.91)	0.98	(0.52–1.86)	1.53	(0.55–4.27)
P for trend	0.775		0.589		0.314		0.626		0.922		0.390	
Women												
0–2	1		1		1		1		1		1	
3–5	0.94	(0.54–1.64)	0.80	(0.38–1.68)	0.78	(0.30–2.05)	0.88	(0.49–1.58)	0.78	(0.35–1.70)	0.72	(0.27–1.94)
6–7	0.66	(0.39–1.14)	0.46	(0.22–0.97)	0.66	(0.27–1.60)	0.70	(0.40–1.20)	0.51	(0.24–1.10)	0.71	(0.28–1.76)
P for trend	0.077		0.024		0.354		0.150		0.070		0.520	

Abbreviations are as defined in Table 4.

*Adjusted for (sex), age, MBP, TC, BMI, presence of atrial fibrillation, use of antihypertensive medication, personal history of IHD, physical activity, smoking, and alcohol drinking.

†Unstable estimation because of small sample size.

tion but also with hemorrhagic stroke, which would not be explained by the antioxidant hypothesis alone. If the antioxidative effect on LDL was the sole mechanism whereby vitamin C decreased the risk of stroke, little or no association with hemorrhagic stroke should be observed because the prevention of atherosclerosis would mainly result in a decreased risk of cerebral infarction rather than of hemorrhagic stroke. Therefore, additional mechanistic hypotheses should be considered to explain the simultaneously reduced risks of cerebral infarction and hemorrhagic stroke.

It is plausible that the preventive effect of vitamin C against stroke is partly mediated by lowering blood pressure because (1) serum vitamin C concentration was inversely correlated to blood pressure in this cohort as well as in other populations[14,15]; (2) elevated blood pressure increased the risks of both cerebral infarction and hemorrhagic stroke; and (3) adjustment for blood pressure slightly attenuated the hazard ratios of cerebral infarction and hemorrhagic stroke. The larger attenuation of the hazard ratio by the adjustment for blood pressure in men is probably due to the stronger correlation between serum vitamin C concentration and blood pressure in men than in women. On the other hand, the association between serum vitamin C concentration and hemorrhagic stroke may be partly confounded by the physical

activity level because (1) serum vitamin C concentration was lower in those who were physically inactive; (2) physically inactive persons had a significantly increased risk of hemorrhagic stroke; and (3) adjustment for the physical activity level slightly attenuated the hazard ratio of hemorrhagic stroke. However, these attenuations were so slight that the large risk reduction of stroke was not completely explained.

A number of hypotheses have been proposed to explain the protective association of vitamin C with stroke. Ascorbic acid promotes endothelial prostacyclin,[32,33] which decreases vascular tone and inhibits platelet aggregation.[34] Oxidized LDL–induced increases in leukocyte-platelet aggregation may be prevented by ascorbic acid.[35] In several hypotheses, it is speculated that serum vitamin C per se does not have a protective effect against stroke but is a marker of other preventive factors or healthy behaviors, by which the simultaneous reduction of risks for cerebral infarction and hemorrhagic stroke may be partly explained. For example, serum vitamin C concentration may be lower among those who are sedentary, heavy smokers, or heavy drinkers because the intake of fruits and vegetables, the major sources of vitamin C, is lower among such persons.[17] The confounding effect of physical activity on hemorrhagic stroke, as mentioned above, would be one of such phenomena. However, such confounding by smoking or alcohol consumption was not detected in this study. Serum vitamin C concentration may be a marker of intake of other nutrients abundant in fruit and vegetables such as potassium, magnesium, calcium, fiber, and carotene, and these nutrients may be preventive against stroke.[10,36,37] Whether causal or confounding, none of the individual effects of each mechanism seems to explain sufficiently the simultaneous large reduction of risks for cerebral infarction and hemorrhagic stroke. After all, vitamin C may reduce the risk of stroke through a combination of several mechanisms, including an antioxidative effect on LDL, lowering of blood pressure, being a marker of other preventive factors or healthy behaviors such as physically active lifestyles, and, possibly, as yet unknown mechanisms.

Although the association of serum vitamin C concentration with fruit intake was stronger than that with vegetable intake, decreased risks of all stroke and cerebral infarction were observed only for those who frequently consumed vegetables. This paradoxical difference may suggest a preventive effect on stroke due to other nutrients that are abundant in vegetables but relatively sparse in fruit (eg, β-carotene). However, the comparison between the effects of fruit and vegetable intake may not be valid in our study because the frequency distributions of fruit and vegetable intake were markedly different. Since the vitamin C concentration in serum or plasma reflects dietary intake for several previous months,[38] it may be a more accurate marker of usual intake than dietary assessment by the FFQ and hence may be preferred when such an association with cardiovascular diseases is examined.[9]

Serum vitamin C concentration varies seasonally,[39] and the variation over a period of years has not been well established. Thus, a potential weakness of our study may be that the serum vitamin C concentration and fruit and vegetable intake were measured only at the baseline examination, and intraindividual changes during the 20-year observation period could not be taken into account for the analysis. However, we had an opportunity to reexamine 862 of the cohort members 4 years after the baseline examination using a comparable protocol. The correlation coefficients between the 2 measurements were 0.54 for serum vitamin C concentration, 0.21 and 0.27 (rank correlation) for frequencies of fruit and vegetable intake, respectively, 0.53 for TC, 0.64 for SBP, and 0.59 for DBP, indicating that the reproducibility of serum vitamin C concentration was similar to that of TC. Although a high degree of reproducibility does not always ensure validity, correlations on the order of 0.5 to 0.7 among subjects who live freely in the community over a period of years indicate that only one measurement of a variable would provide a fairly good measure of its long-term level.[40] The lower reproducibility of fruit and vegetable intake may be one of the reasons why their association with stroke was not as clear as that of serum vitamin C concentration. Furthermore, there was no marked change of hazard ratio during the 20-year observation period when graphically examined.[31] Thus, a cohort study with a single measurement of serum vitamin C concentration at the baseline examination could be useful to examine its association with long-term risk of stroke.

In conclusion, a higher serum vitamin C concentration was strongly associated with a reduced risk of subsequent incidence of cerebral infarction and hemorrhagic stroke. Only a small part of these associations was explained by lowering blood pressure and confounding by the physical activity level. Further mechanistic hypotheses are required to explain the simultaneously reduced risk of both cerebral infarction and hemorrhagic stroke. Finally, we would like to emphasize that all of the cohort members had been getting vitamin C from natural foods. Although vitamin C supplements can increase serum vitamin C concentration, we have no evidence of its preventive effect on stroke. Since the risk of stroke is markedly higher among people with low levels of serum vitamin C concentration, a mass screening for such high-risk people may be effective to decrease the occurrence of stroke if appropriate control measures were developed. Until then, we must remember that the effect of controlling risk factors can be determined only by an intervention study, and these do not always show the expected benefits.[41–43]

Acknowledgments

This study was supported by the following grants: cardiovascular diseases, No. 9A-3, from the Ministry of Health and Welfare, Japan; nervous and mental disorders, No. 3A-3, from the National Center of Neurology and Psychiatry, Japan; and cardiovascular diseases, No. 3C-2, from the National Cardiovascular Center, Japan. Appreciation is extended to Drs Torahiko Kon (former mayor of Shibata City), Ken-ichi Baba, Masuro Togashi, Kazuo Kawai, Makoto Kumakura, Shun Mayama, Hatiki Sobue, and many other collaborators in Shibata City, as well as our colleagues.

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