ORIGINAL RESEARCH

Vitamin C Intake, Circulating Vitamin C and Risk of Stroke: A Meta-Analysis of Prospective Studies

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***Background*—**Though vitamin C supplementation has shown no observed effects on stroke prevention in several clinical trials, uncertainty remains as to whether long-term, low-dose intake influences the development of stroke among general populations. Furthermore, the association between circulating vitamin C and the risk of stroke is also unclear. For further clarification of these issues, we conducted a meta-analysis of prospective studies.

***Methods and Results*—**PubMed and EMBASE databases were searched, and the bibliographies of the retrieved articles were also reviewed to identify eligible studies. Summary relative risk (RRs) with corresponding 95% confidence intervals (CIs) were computed with a random-effects model. The summary RR for the high-versus-low categories was 0.81 (95% CI: 0.74 to 0.90) for dietary vitamin C intake (11 studies), and 0.62 (95% CI: 0.49 to 0.79) for circulating vitamin C (6 studies). The summary RR for each 100 mg/day increment in dietary vitamin C was 0.83 (95% CI: 0.75 to 0.93) (10 studies), and for each 20 μmol/L increment in circulating vitamin C was 0.81 (95% CI: 0.75 to 0.88) (5 studies). Few studies reported results for vitamin C supplements (RR for high-versus-low intake=0.83, 95% CI: 0.62 to 1.10, 3 studies).

***Conclusions*—**This meta-analysis suggests significant inverse relationships between dietary vitamin C intake, circulating vitamin C, and risk of stroke. **(*J Am Heart Assoc.* 2013;2:e000329 doi: 10.1161/JAHA.113.000329)**

Key Words: antioxidants • diet • meta-analysis • prevention • stroke

Stroke remains the second leading cause of death globally and the most common cause of disability in adults in most regions. In the United States alone, it is estimated that there are 795 000 people who experience a new or recurrent stroke each year,[1] and the direct and indirect cost of stroke for 2010 is $73.7 billion.[1]

Multiple lines of evidence have demonstrated that a high consumption of fruits and vegetables, which are a major dietary source of antioxidants, is associated with a reduction in the risk of stroke,[2] leading to great interest in the role of antioxidants in the etiology of stroke. Vitamin C is an effective antioxidant shown to have blood pressure-lowering effects.[3,4] While several phase-III randomized controlled trials (RCTs) have suggested that vitamin C supplementation has no effect on stroke prevention,[5] the trials have tended to enroll high-risk populations, have short duration, and use high doses. Therefore, they cannot answer the question of whether long-term and low-dose exposure influences the development of this disease among general populations.

A number of epidemiologic studies to date have provided us with encouraging, but still inconclusive findings that vitamin C intake may reduce risk of stroke. In particular, some have focused on how diet may be associated with a decreased risk of stroke.[6–17] Furthermore, circulating vitamin C, a good indicator of a diet rich in fruits and vegetables,[18] has also been indicated to be inversely associated with stroke risk,[9,19–22] but the reported results so far have not totally been consistent. To better understand these subjects, we took up this meta-analysis of prospective studies in order to characterize the association between vitamin C intake, circulating vitamin C, and risk of stroke.

Methods

Literature Search

We performed a literature search through April 2013 on PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and EMBASE (http://www.embase.com/home) using the search

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terms "*vitamin C* or *ascorbate* or *antioxidant* combined with *stroke* or *cerebrovascular disorders* or *cerebrovascular disease* or *intracranial hemorrhage* or *brain hemorrhage* and *cohort* or *prospective* or *follow-up* or *nested case-control*" with no language restrictions imposed. We also comprehensively reviewed the reference lists of the retrieved publications to identify any additional relevant studies. We also contacted authors of the primary articles for additional information.

Study Selection

Studies were included if they met the following criteria: (1) the study had a prospective design; (2) the exposure of interest was vitamin C intake or circulating vitamin C; (3) the outcome of interest was stroke; and (4) the relative risk (RR) estimates (or odds ratios [OR] in nested case-control studies) with corresponding 95% confidence interval (CI) were provided. When multiple published publications from the same study were available, we included the one with the largest events in the meta-analysis. Studies on multivitamin use only were excluded.

Data Extraction

The following data were extracted from each included eligible study using a standardized data-collection form: the first author's last name, publication year, study location, length of follow-up, sex and age of participants, number of cases and participants, stroke characteristics (fatal or nonfatal), sources of vitamin C, levels of exposure, the maximally adjusted RR or OR of stroke and corresponding 95% CI for each category of exposure, and variables controlled for in the analysis. Literature search and data extraction were conducted independently by 2 authors (Drs. Chen and Liu) with any disagreements resolved by consensus.

Statistical Analysis

We used a DerSimonian and Laird random-effects model,[23] which considers both within- and between-study variation to calculate the summary-risk estimate. The ORs in the nested case-control study were considered approximations of RRs. In case of studies that did not report overall risk estimates, but separately presented results for stroke subtypes[10] or for men and women,[12] we combined the results using a fixed-effects model and then included the pooled RR estimates in the primary analysis. For one study[19] on circulating vitamin C, which presented results by plasma carotene levels, we also combined the results. For better comparability between studies, we pooled RR estimates by sources of vitamin C intake (foods or supplements). We also performed a sensitivity analysis using the results of total vitamin C in 2 studies[6,15] combined with the results of dietary intake in others to test the stability of the summary risk estimates.

We also conducted several subanalyses according to geographic area, length of follow-up, number of cases, characteristics of stroke (fatal or nonfatal), sex, and subtypes of stroke to examine the potential effect modification of these factors when there were sufficient number of studies.

We also conducted a dose-response analysis using the method proposed by Greenland and Longnecker[24] and Orsini et al.[25] This method requires the number of cases/person-years (or controls in nested case-control studies) as well as the risk estimates with their variance estimates for at least 3 quantitative exposure categories. In cases where the studies did not report these data, we contacted relevant authors to request the data. If we did not receive a response, we estimated them according to the reported information. When the risk estimates were reported as a continuous variable,[21] the reported results were used in the analysis. We also examined a potential nonlinear relationship by modeling vitamin C intake or circulating vitamin C using restricted cubic splines with 3 knots at 10%, 50%, and 90% of the distribution.[26] A *P* value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline was equal to zero. Dose-response analysis would be performed when the number of eligible studies was ≥3.

A heterogeneity test was performed by use of Q and I^2 statistics.[27] For the Q statistic, a *P* value of <0.1 was considered statistically significant heterogeneity. Potential publication bias was investigated by use of Egger's regression asymmetry test.[28] This test was conducted when the number of studies was ≥5. All statistical analyses were done using STATA software, version 11.0 (STATA corp.). All *P* values were 2-sided and the level of significance was <0.05, unless explicitly stated.

Results

Literature Search

A flow chart showing the details of study selection is shown in Figure 1. Briefly, 14 articles on vitamin C intake and 7 articles on circulating vitamin C in relation to risk of stroke were identified. One article[29] on vitamin C intake was excluded because it was a duplicate report of another paper[15] with a larger event; 1 publication[30] concerning circulating vitamin C was excluded for the same reason. We further excluded 1 article[31] on multivitamin use. At last, 12 prospective studies[6–17] on vitamin C intake and 6 prospective studies[9,19–22] on circulating vitamin C were included in the meta-analysis.

Study Characteristics

Table 1 shows the characteristics of the 12 prospective studies on vitamin C intake. These studies contained a total of 217 454 participants and 3762 stroke events, and were

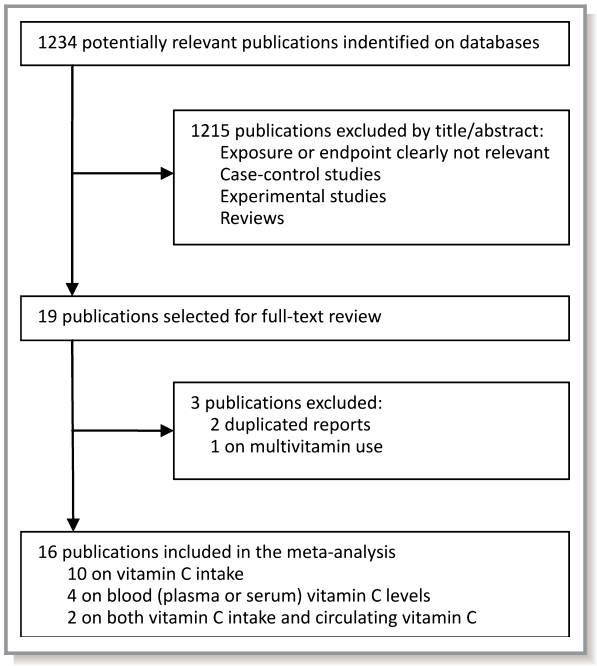

Figure 1. Flow chart of study selection.

published between 1995 and 2011. Half of the 12 studies were conducted in Europe, 3 were carried out in the United States, and the remaining 3 were from Asia. The outcome was fatal stroke only in 4 studies, and was both fatal and nonfatal strokes in 8 studies. Five studies included men only, 5 studies included both men and women, and 2 studies included women only. The study duration ranged between 6.1 and 30 years. The average dietary vitamin C intake in the high categories ranged between 45 and 375.8 mg/day. Two studies also reported results for total vitamin C intake and the average intake in the high categories was 1167 mg/day in the US study by Ascherio et al[6] and 678.7 mg/day in the US study by Yochum et al.[15]

Table 2 presents the characteristics of the 6 prospective studies on circulating vitamin C. These studies involved 989 stroke cases and 29 648 participants. They were published between 1993 and 2008 and followed up for 9.5 to 20 years. Five of the 6 studies were from Europe, and the remaining 1 was carried out in Japan. The exposure of interest was serum vitamin C in 2 studies, and plasma vitamin C in the remaining studies. Four studies included both men and women, and 2 studies consisted entirely of men. All provided multivariable-adjusted risk estimates (Tables 1 and 2).

Dietary Vitamin C

Eleven studies[7–17] examined the association of vitamin C intake from foods with risk of stroke. The summary RR for the high compared with low dietary vitamin C intake was 0.81 (95% CI: 0.74 to 0.90), with no evidence of heterogeneity ($P^{heterogeneity}$=0.71, I^2=0.0%) (Figure 2A). There was little evidence of publication bias with Egger's test ($P^{Egger's}$=0.39).

One study[14] was not eligible for the dose-response analysis. The combined results of the remaining 10 studies suggested a summary RR of 0.83 (95% CI: 0.75 to 0.93) for an increment in dietary vitamin C intake of 100 mg/day, with little heterogeneity ($P^{heterogeneity}$=0.32, I^2=13.4%) (Figure 2B). There was no evidence of a nonlinear association ($P^{nonlinearity}$=0.17).

Supplemental Vitamin C

Three studies[6,14,15] covering 770 stroke cases reported results for supplemental vitamin C intake. The summary RR for the high-versus-low intake was 0.83 (95% CI: 0.62 to 1.10), with no heterogeneity ($P^{heterogeneity}$=0.94, I^2=0.0%). Only 2 studies[6,15] provided adequate data for the dose-response analysis.

Circulating Vitamin C

A pooled analysis of 6 studies showed that participants with a higher level of circulating vitamin C had a 38% lower risk of stroke (RR=0.62, 95% CI: 0.49 to 0.79) compared with those with a lower level, with low heterogeneity ($P^{heterogeneity}$=0.23, I^2=27.6%) (Figure 3A). Further omitting one small study[16] with a very narrow range of exposure, the summary RR was 0.58 (95% CI: 0.48 to 0.71). There was no suggestion of publication bias ($P^{Egger's}$=0.93).

Five studies[9,16,20–22] were included in the dose-response analysis. Combined results suggested that a 20 μmol/L increment in circulating vitamin C was associated with a 19% decreased risk of stroke (RR=0.81, 95% CI: 0.75 to 0.88), with no evidence of heterogeneity ($P^{heterogeneity}$=0.89, I^2=0.0%) (Figure 3B). We observed no evidence of a nonlinear association ($P^{nonlinearity}$=0.13).

Subgroup and Sensitivity Analyses (for Vitamin C Intake)

The observed inverse association between high dietary vitamin C intake and risk of stroke was not significantly modified by geographic area, length of follow-up, number of cases, characteristics of stroke (fatal or nonfatal), sex, and subtypes of stroke (Table 3). In addition, omitting any single study did not remarkably alter the summary risk estimates (RRs ranged between 0.80 and 0.83). Too few studies reported results for women and for hemorrhagic stroke.

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Table 1. Characteristics of the Included Prospective Studies on Vitamin C Intake and Stroke Risk

Study	Year	Location	Duration, Years	Participants	End-Points	Sources	Vitamin C Intake, mg/day		RR (95% CI), High vs Low	Adjustment
							High	Low		
Gale et al[9]	1995	UK	20	730 M/F	124 TS (fatal)	Foods	53.4 (T3)	19.4 (T1)	0.5 (0.3 to 0.8)	Age, sex, DBP, and serum cholesterol.
Keli et al[11]	1996	Netherlands	15	552 M	42 TS (Fatal+ nonfatal)	Foods	131.2 (T3)	59.3 (T1)	1.21 (0.55 to 2.66)	Age, SBP, serum cholesterol, smoking, intakes of fish, alcohol, and energy.
Ross et al[13]	1997	China	8	1470 M	245 TS (fatal)	Foods	45.6 (T3)*	15.2 (T1)*	1.1 (0.7 to 1.6)	BMI, education, marital status, smoking, alcohol, and hypertension.
Daviglus et al[7]	1997	USA	30	1843 M	222 TS (Fatal+ nonfatal)	Foods	258 (Q4)	48 (Q1)	0.71 (0.47 to 1.05)	Age, SBP, smoking, BMI, serum cholesterol, intakes of total energy alcohol, and diabetes.
Ascherio et al[6]	1999	USA	8	43738 M	328 TS, 210 IS, 70 HS (Fatal+ nonfatal)	Total	1167 (Q5)	95 (Q1)	TS: 0.95 (0.66 to 1.35) IS: 1.03 (0.66 to 1.59) HS: 0.82 (0.36 to 1.89)	Age, calendar time, smoking, intakes of total energy and alcohol, hypertension, parental history of MI, profession, BMI and physical activity.
						Supplements	850 (Q5)	0 (Q1)	TS: 0.85 (0.59 to 1.24) IS: 0.93 (0.60 to 1.45) HS: 0.67 (0.28 to 1.60)	
Hirvonen et al[10]	2000	Finland	6.1	26539 M	736 IS, 95 ICH, 83 SAH (Fatal+ nonfatal)	Foods	141 (Q4)	52 (Q1)	IS: 0.89 (0.72 to 1.09) ICH: 0.39 (0.21 to 0.74) SAH: 1.16 (0.62 to 2.18)	Age, supplementation group, SBP, DBP, serum total cholesterol and HDL cholesterol, BMI, height, smoking, history of diabetes or CHD, alcohol intake, and education.
Yochum et al[15]	2000	USA	11	34492 F	215 TS (fatal)	Total	678.7 (Q5)	82.4 (Q1)	1.23 (0.76 to 1.90)	Age, BMI, waist-to-hip ratio, hypertension, diabetes, estrogen replacement therapy, education, marital status, smoking, physical activity, intakes of total energy, cholesterol, alcohol, saturated fat, fish, vitamin E, carotenoids, dietary fiber, and whole grains.
						Foods	247.9 (Q5)	67.2 (Q1)	0.99 (0.58 to 1.72)	
						Supplements	1120 (Q5)	0 (Q1)	0.90 (0.36 to 2.19)	

Continued

Table 1. Continued

Study	Year	Location	Duration, Years	Participants	End-Points	Sources	Vitamin C Intake, mg/day		RR (95% CI), High vs Low	Adjustment
							High	Low		
Voko et al[14]	2003	Netherlands	6.4	5159 M/F	227 (Fatal+ nonfatal)	Foods	T3	T1	0.66 (0.46 to 0.93)	Age, sex, total energy intake, smoking, hypertension, diabetes mellitus, history of CHD, transient ischemic attacks, and, in case of vitamin E, polyunsaturated fatty acid intake.
						Supplements	Yes	No	0.77 (0.47 to 1.26)	
Marniemi et al[16]	2005	Finland	10	755 M/F	70 TS (Fatal+ nonfatal)	Foods	113.8 (T3)†	57.8 (T1)†	0.99 (0.56 to 1.76)	Age, sex, smoking, functional capacity, and weight- adjusted energy intake
Weng et al[17]	2008	Taiwan	10.6	1772 M/F	132 IS (Fatal+ nonfatal)	Foods	375.8 (Q4+Q3)	180.7 (Q1)	0.73 (0.47 to 1.12)	Age, sex, area, smoking, BMI, central obesity, physical activity, diabetes, hypertension, use of antihypertensive drugs, self-reported heart disease, hypercholesterolemia, hypertriglyceridemia, fibrinogen, apolipoprotein B, plasminogen, alcohol
Del Rio et al[8]	2011	Italy	7.9	41620 M/F	194 TS, 112 IS, 48 HS (Fatal+ nonfatal)	Foods	201 (T3)	83 (T1)	TS: 0.89 (0.6 to 1.32) IS: 0.53 (0.31 to 0.89) HS: 1.83 (0.81 to 4.13)	Age, center, sex, hypertension, smoking, education, energy intake, alcohol, waist circumference, obesity, and physical activity.
Kubota et al[12]	2011	Japan	16.5	23119 M/F	1227 TS (fatal)	Foods	Men: 145 (Q5) Women: 150 (Q5)	Men: 52 (Q1) Women: 65 (Q1)	Men: 0.84 (0.62 to 1.13) Women: 0.70 (0.54 to 0.92)	Age, hypertension, diabetes, smoking, BMI, mental stress, walking, sports, education, intakes of total energy, alcohol, cholesterol, saturated fatty acids, n-3 fatty acids, and sodium.

BMI indicates body mass index; CHD, coronary heart disease; CI, confidence interval; DBP, diastolic blood pressure; F, females; HDL, high-density lipoprotein; HS, hemorrhagic stroke; ICH, intracerebral hemorrhagic; IS, ischemic stroke; M, males; MI, myocardial infarction; Q, quartile/quintile; RR, relative risk; SAH, subarachnoid hemorrhagic; SBP, systobic blood pressure; T, tertile; TS, total stroke.

*The midpoint vitamin intake in the lowest and highest tertiles was estimated as the mean intake (30.4 mg/day)±half of the mean intake among noncases.

†The midpoint vitamin intake in the lowest and highest tertiles was estimated as the mean intake (85.8 mg/day)±half of the SD (56 mg/day) among noncases.

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Table 2. Characteristics of the Included Prospective Studies on Blood (Plasma or Serum) Vitamin C Levels and Stroke Risk

Study	Year	Location	Duration, years	Participants	End-points	Sources	Circulating Vitamin C, μmol/L		RR (95% CI)	Adjustment
							High	Low		
Gey et al[19]	1993	Switzerland	12	2974 M	31 (fatal)	Plasma	"Normal"	"Low"	Normal carotene: 0.78 (0.24 to 2.5); Low carotene: 0.24 (0.10 to 0.60)	Age, smoking, BP, cholesterol and carotene.
Gale et al[9]	1995	UK	20	730 M/F	124 TS (fatal)	Plasma	35.77 (T3)	3.96 (T1)	0.7 (0.4 to 1.1)	Age, sex, DBP, and serum cholesterol.
Yokoyama et al[22]	2000	Japan	20	880 M/F	196 TS, 109 IS, 54 HS (nonfatal)	Serum	69.5 (Q4)	35 (Q1)	TS: 0.71 (0.45 to 1.14) IS: 0.63 (0.34 to 1.18) HS: 0.59 (0.24 to 1.46)	Age, sex, BP, serum cholesterol, BMI, presence of a trial fibrillation, use of antihypertensive medication, personal history of IHD, physical activity, smoking, and alcohol drinking.
Kurl et al[20]	2002	Finland	10.4	2419 M	120 TS (Fatal+nonfatal)	Plasma	73.36 (Q4)	18.8 (Q1)	0.48 (0.26 to 0.85)	Age, examination months, BMI, smoking, alcohol, SBP, serum total cholesterol, diabetes, and myocardial ischemia during exercise.
Marniemi et al[16]	2005	Finland	10	755 M/F	70 TS (Fatal+nonfatal)	Serum	6.5 (T3)*	3.6 (T1)*	1.07 (0.59 to 1.93)	Age, sex, smoking, functional capacity.
Myint et al[21]	2008	UK	9.5	20649 M/F	448 TS (Fatal+nonfatal)	Plasma	71.5 (Q4)	35 (Q1)	0.57 (0.42 to 0.76)	Age, sex, smoking, BMI, SBP, cholesterol, physical activity, MI, diabetes, social class, vitamin supplement use, and intakes of alcohol, fruit and vegetable.

BMI indicates body mass index; BP, blood pressure; CI, confidence interval; DBP, diastolic blood pressure; F, females; HS, hemorrhagic stroke; IHD, ischemic heart disease; IS, ischemic stroke; M, males; MI, myocardial infarction; Q, quartile; RR, relative risk; SBP, systobic blood pressure; T, tertile; TS, total stroke.

*The midpoint circulating vitamin C in the lowest and highest tertiles was estimated as the mean value (5.05 μmol/L)±half of the SD (2.9 μmol/L) among noncases.

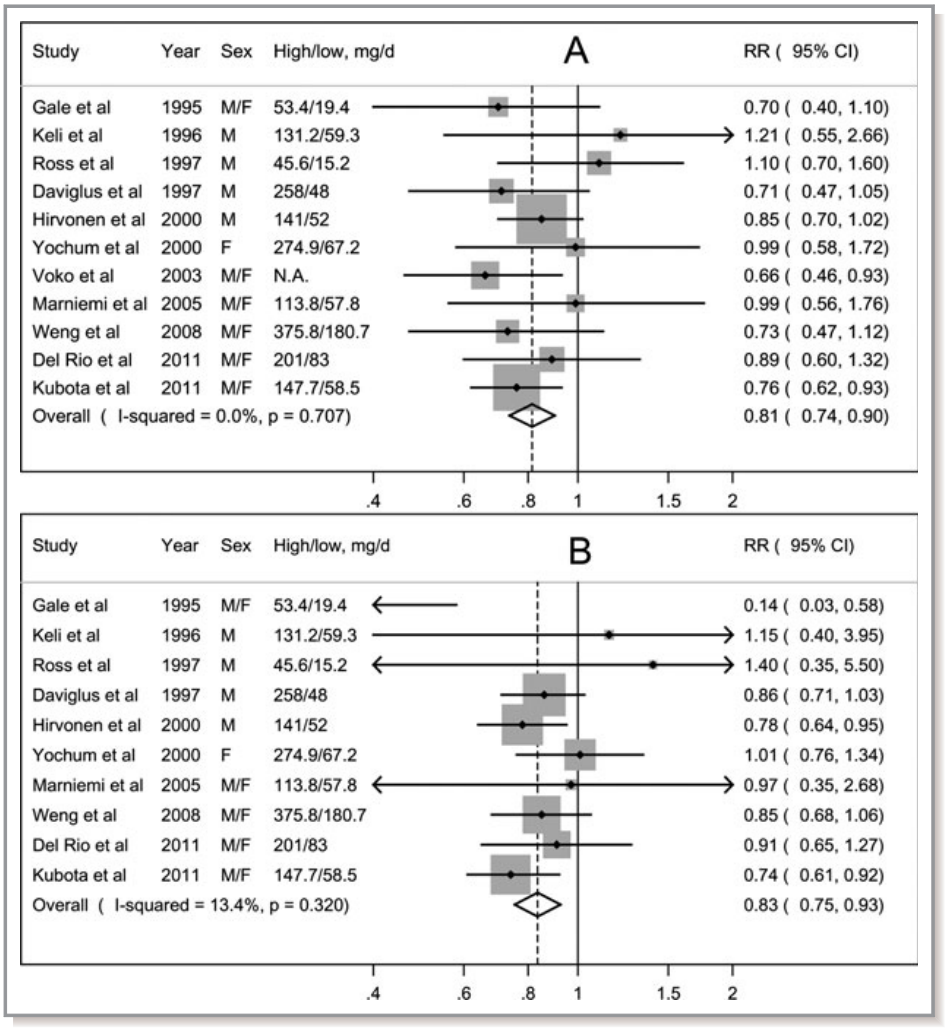

Figure 2. Meta-analysis of dietary vitamin C intake and risk of stroke. A, high vs low analysis; (B) dose-response analysis. F indicates women; M, men; N.A., not available; RR, relative risk.

We conducted a separate analysis using the results of total vitamin C in 2 studies[6,15] and those of dietary vitamin C in other studies. Thus, this separate analysis was based on 12 prospective studies.[6–17] The summary RR for the high- versus-low vitamin C intake was 0.83 (95% CI, 0.76 to 0.92). In the dose-response analysis, an increase in vitamin C intake of 100 mg/day was marginally associated with a reduced risk of stroke (RR=0.91, 95% CI: 0.83 to 1.00), with considerable heterogeneity ($P^{heterogeneity}$=0.001, I^2=66.9%). There was a somewhat U-shaped association between vitamin C intake and risk of stroke ($P^{nonlinearity}$=0.0001) (Figure 4), with the greatest risk reduction observed at an intake of ≈200 mg/day, and remained protective until an intake of ≈550 mg/day. However, data points become especially sparse above intakes of 200 mg/day, and so the results for higher intakes should be treated with caution.

Discussion

The findings from this meta-analysis of prospective studies show that both dietary vitamin C intake and circulating vitamin C are significantly inversely associated with the risk of stroke in a dose-response manner. Supplemental vitamin C intake was not significantly related to a reduced risk of stroke, but the analysis was based on a limited number of studies (3 studies) and stroke cases (770 cases).

Several putative mechanisms whereby vitamin C protects against stroke have been proposed. Vitamin C is a strong antioxidant, and has been shown to reduce the oxidation of low-density lipoproteins, to inhibit the proliferation of smooth muscle, to protect membrane from peroxidation, and ultimately to slow the progression of atherosclerosis.[32–35] There is also growing evidence that systemic inflammation is

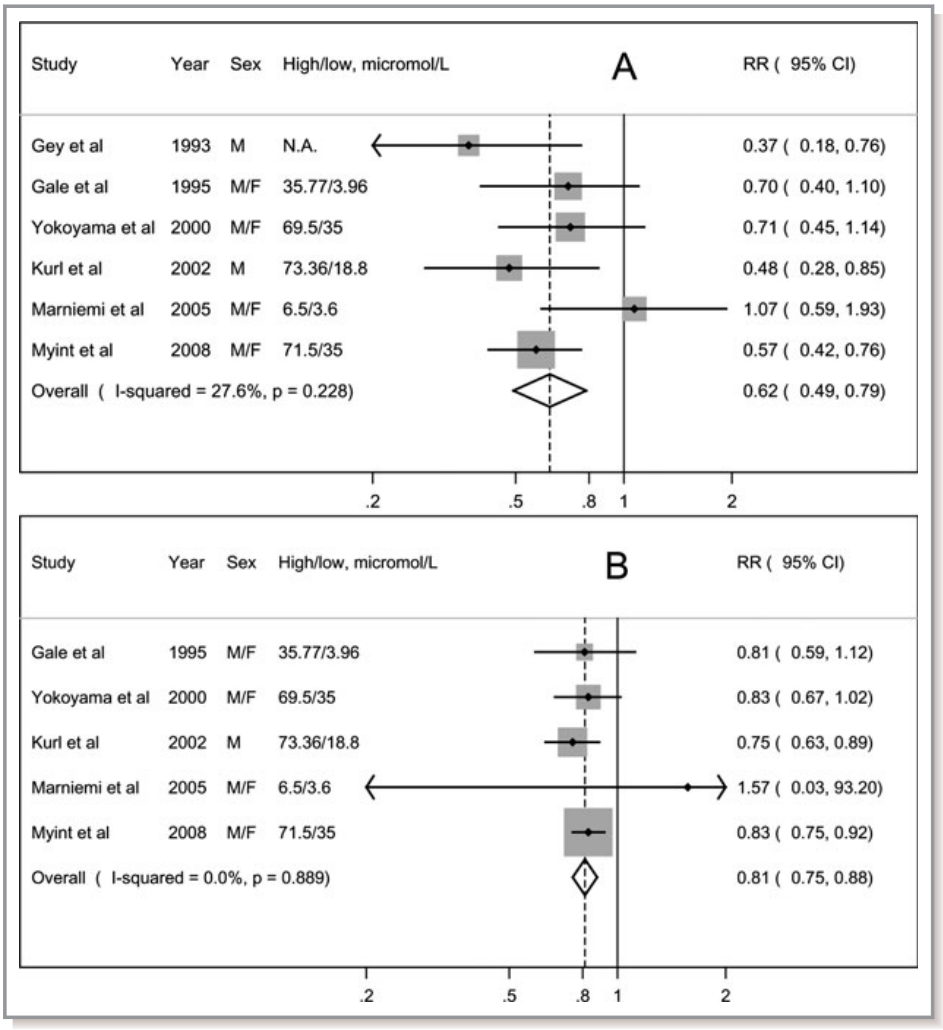

Figure 3. Meta-analysis of circulating vitamin C and risk of stroke. A, high vs low analysis; (B) dose-response analysis. F indicates women; M, men; N.A., not available; RR, relative risk.

involved in stroke etiology and pathology,[36] and plasma or dietary vitamin C has been suggested to have antiinflammatory properties.[37] Vitamin C intake in plasma has also been demonstrated to be inversely associated with blood pressure.[4] Hence, vitamin C may reduce stroke risk through its pressure-lowering effects.

A large body of human clinical trials have been conducted to assess the effect of vitamin C or other traditional antioxidant-related vitamins or minerals on prevention of a multitude of chronic diseases, such as type 2 diabetes,[38] stroke and other cardiovascular diseases,[5] and certain cancers.[39] However, the trials generally failed to produce convincing evidence to justify the effectiveness of these nutrients.

There are only 4 RCTs[40–43] that evaluate the role of vitamin C alone, or in combination with other antioxidants, for primary or secondary prevention of stroke, and all but one[42] of them have reported negative findings. Even then, it is noteworthy that there are several differences between the observational studies and the RCTs which may, to some extent, explain the disparate findings between them. Individuals who took vitamin C supplementation in the RCTs were usually high-risk rather than general populations. Besides, dietary intake represents long-term habitual exposure, whereas supplementation is generally characterized by shorter duration and higher dose. Thus, if the effect of vitamin C is restricted to the early stage of the disease, the trials enrolling high-risk participants or with insufficient follow-up duration may not be able to detect any significant associations.

Furthermore, circulating vitamin C, a more accurate indicator of body vitamin C status, has been shown to be saturable, with a linear increase only observed at vitamin C intakes of <100 mg/day; above 100 mg/day, there is little

Table 3. Subgroup Analyses of Dietary Vitamin C Intake and Stroke, High vs Low Intake

	N	RR (95% CI)	Heterogeneity		$P^{interaction}$
			P Value	I^2 (%)	
All studies	11	0.81 (0.74 to 0.90)	0.71	0.0	
Geographic Area					
Europe	6	0.83 (0.72 to 0.95)	0.63	0.0	0.78
Other areas	5	0.80 (0.69 to 0.93)	0.46	0.0	
Years of Follow-Up					
≥10 years	7	0.78 (0.68 to 0.91)	0.79	0.0	0.49
<10 years	4	0.85 (0.72 to 1.00)	0.32	14.9	
Number of Cases					
>200	6	0.81 (0.72 to 0.91)	0.42	0.0	0.77
<200	5	0.84 (0.67 to 1.05)	0.71	0.0	
Outcome					
Fatal	5	0.81 (0.69 to 0.95)	0.50	0.0	0.99
Fatal and nonfatal	7	0.81 (0.71 to 0.93)	0.69	0.0	
Sex					
Men	5	0.86 (0.75 to 0.99)	0.56	0.0	0.29
Women	2	0.77 (0.57 to 1.03)	0.26	20.6	
Men and women	5	0.76 (0.63 to 0.93)	0.70	0.0	
Stroke Subtypes					
Ischemic stroke	4	0.77 (0.64 to 0.92)	0.30	18.5	0.55
Hemorrhagic stroke	2	1.07 (0.38 to 3.00)	0.03	79.3	

CI indicates confidence interval; RR, relative risk.

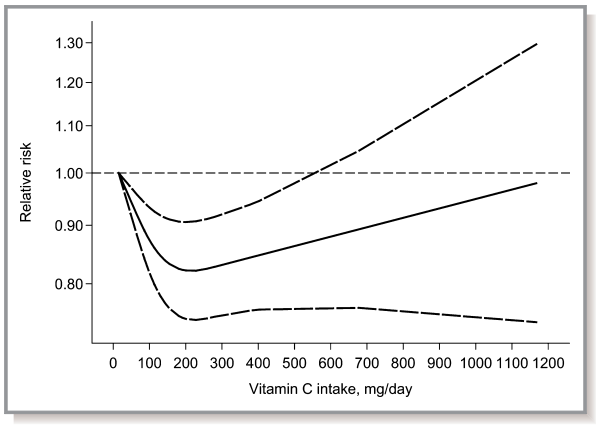

Figure 4. Relative risk (solid line) with 95% confidence interval (long dashed lines) for the association between vitamin C intake (total or dietary intake) and risk of stroke in a restricted cubic spline random-effects meta-analysis. The lowest intake of 15.2 mg/day was used to estimate all relative risks.

change in blood concentration despite large changes in dose.[44] In this respect, for subjects who already have high or saturating vitamin C in their blood (obtained from diet), supplementation may not be expected to provide additional benefits, and those with high concentrations may also be categorized as a placebo group (this is particularly likely to have occurred in the RCTs that tended to recruit health-conscious individuals), which could have limited the statistical power of the trials. Further support for this threshold hypothesis comes from the data of the Linxian General Population Nutrition Intervention Trial.[42] In this trial, a nutritionally deprived population (probably with low concentrations of circulating vitamin C) taking 120 mg/day of vitamin C supplementation (combined with molybdenum of 30 μg/day) had a significantly lower risk of death from stroke (RR=0.92, 95% CI: 0.86 to 0.99). While a recent meta-analysis documented that vitamin C supplementation with a median dose of 500 mg/day may have been effective on reducing blood pressure, the duration of the primary RCTs was very short (median duration was 8 weeks).[3]

On the other hand, it is vitally important to notice that observational studies lack the experimental random allocation of the intervention necessary to test exposure—outcome hypotheses optimally, and so the inverse associations observed in this meta-analysis may be confounded by

unmeasured social and behavioral factors. Those subjects with higher dietary vitamin C intake or higher circulating vitamin C levels would also generally engage in other healthy behaviors; by contrast, those with lower intake or low circulating levels tended to be more likely to have unfavorable lifestyle habits. Although most primary studies had adjusted for multiple variables including smoking, alcohol drinking, dietary cholesterol/circulating cholesterol levels, body mass index/obesity, history of diabetes, and the potential intermediate of hypertension/blood pressure, nearly all studies did not control for many other potential key dietary confounders that may influence the development of stroke, such as dietary fiber, whole grains, nuts, salt, and red and processed meat, etc. Therefore, the observed effect of vitamin C on stroke reduction may simply be a proxy for specific foods (eg, fruits and vegetables) that causally lower stroke, or a proxy for specific foods that themselves are markers for other factors (other dietary or lifestyle habits) that causally lower stroke, but not due to vitamin C per se.

Furthermore, it is clear that dietary or circulating vitamin C marks fruit/vegetable intake.[18,45] In light of the neutral effects of vitamin C on stroke prevention documented in RCTs,[5] even if vitamin C is one of the causal components in fruits and vegetables, it is also plausible that its consumption as part of a matrix of other nutrients in foods may be essential for its benefits.

This meta-analysis has several strengths. All studies included are of a prospective design, which eliminates the possibility of recall and selection biases. In addition, most of the studies included had a long-duration of follow-up. As the single prospective studies were mainly of limited power to prove statistical significance, this meta-analysis involving a large number of stroke cases enhances the statistical power to assess the long-term effects of dietary vitamin C intake and circulating vitamin C on stroke development.

However, apart from the previously described limitations, there are a number of other limitations that merit discussion. First, most included studies assessed exposure of interests only at baseline, and recorded diet intake with a self-administered food frequency questionnaire, which may have led to some misclassification of exposure and, therefore, resulted in an underestimation of the risk estimates. Second, the characteristics of subjects in the primary studies were not always comparable, and the reference categories also varied widely. Third, some of the stratified analyses such as sex-specific and stroke subtypes analyses for dietary vitamin C intake were based on a limited number of studies. Fourth, dietary intake in the high category was in general <250 mg/day in the primary studies, which could limit the generalizability of our findings. Finally, publication bias could be of concern as this meta-analysis was based on published literature. In this meta-analysis, however, little evidence of such bias was found.

Our findings may be of several implications. To date, the recommended dietary allowance for vitamin C has been largely inconsistent among countries, generally ranging from 40 to 110 mg/day.[46] Though current clinical evidence does not recommend vitamin C supplementation to prevent stroke, from a public health point of view, for populations with low intake and for those who are at high risk, increasing consumption of vitamin C-rich foods (eg, fruits and vegetables) and adhering to other healthy dietary habits and lifestyles can lead to substantial reductions in the burden of stroke and other cardiovascular diseases. Furthermore, given that the established risk factors for stroke appear to be responsible for only half of the incident cases,[47] our findings indicate that circulating concentrations of vitamin C may serve as a good predictor of stroke risk and diet status.

Our findings also raised several intriguing questions: Is there a threshold for vitamin C intake to prevent stroke? Is the beneficial effect of vitamin C against stroke (if any) limited to populations with nutritional deficiencies (or with low vitamin C concentrations in the blood)? Future prospective epidemiologic studies conducted among high-intake populations with better adjustment for potential confounders, as well as well-designed RCTs using high-circulating vitamin C concentrations as an exclusion criteria may help to further elucidate these issues.

In summary, findings from this meta-analysis suggest significant inverse dose-response relationships between dietary vitamin C intake, circulating vitamin C, and risk of stroke. In view of evidence from these RCTs, it is clearly premature to recommend supplementation of vitamin C (or other antioxidants) to prevent stroke, and the prevention of this disease should largely lie in the modification of lifestyle habits, as well as effective therapies lowering risk factors for stroke.

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None.

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