

Plasma vitamin C concentrations predict risk of incident stroke over 10 y in 20 649 participants of the European Prospective Investigation into Cancer–Norfolk prospective population study^{1–3}

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ABSTRACT

Background: The relation between plasma vitamin C and risk of stroke remains unclear. Although clinical trials showed no significant benefit of vitamin C supplementation in reducing stroke risk, they were not able to examine the relation between plasma vitamin C concentrations and stroke risk in a general population.

Objective: The objective was to examine the relation between baseline plasma vitamin C concentrations and risk of incident stroke in a British population.

Design: A population-based prospective study was conducted in 20 649 men and women aged 40–79 y without prevalent stroke at baseline and participating in the European Prospective Investigation into Cancer–Norfolk prospective population study. The participants completed a health questionnaire and attended a clinic during 1993–1997 and were followed up for incident strokes through March 2005.

Results: Over 196 713 total person-years (average follow-up: 9.5 y), 448 incident strokes occurred. In a Cox proportional hazards model, persons in the top quartiles of baseline plasma vitamin C concentrations had a 42% lower risk (relative risk: 0.58; 95% CI: 0.43, 0.78) than did those in the bottom quartile, independently of age, sex, smoking, body mass index, systolic blood pressure, cholesterol, physical activity, prevalent diabetes and myocardial infarction, social class, alcohol consumption, and any supplement use. Similar results were obtained after exclusion of persons with illnesses, users of ascorbic acid–containing supplements, and persons with a history of early strokes during the initial 2 y of follow-up.

Conclusions: Plasma vitamin C concentrations may serve as a biological marker of lifestyle or other factors associated with reduced stroke risk and may be useful in identifying those at high risk of stroke. *Am J Clin Nutr* 2008;87:64–9.

KEY WORDS Plasma vitamin C, epidemiology, fruit and vegetables, stroke

INTRODUCTION

The main source of vitamin C (ascorbic acid) in humans is from the consumption of fruit, vegetables, and plant foods because they cannot synthesize ascorbic acid in the body (1). Furthermore, ascorbic acid in the food is easily destroyed (eg, via cooking in water, roasting, or grilling) (1) and has a short half-life (≈ 30 min) in the blood (2, 3). Therefore, plasma concentrations of vitamin C in a random blood sample are most likely to be related to an individual's habitual dietary pattern and method of food preparation. For example, fresh fruit and vegetables are a

richer source of ascorbic acid than are cooked or boiled fruit and vegetables. As a result of antioxidant activity, higher levels of ascorbic acid are thought to be associated with reduced cardiovascular disease risk (4). Although randomized trials indicate that supplementation with antioxidant vitamins, including β -carotene, vitamin E, and vitamin C, do not reduce cardiovascular disease risk (5, 6), prospective studies indicate that high fruit and vegetable intakes, of which plasma vitamin C is a good biomarker, are associated with lower stroke risk (7–9). However, few prospective studies have examined the relation between plasma vitamin C concentrations and stroke (8) (10), and such studies have been limited in their ability to account for potential confounding factors. We explored the relation between baseline plasma vitamin C concentrations and future stroke risk in British participants in the European Prospective Investigation into Cancer (EPIC)–Norfolk.

SUBJECTS AND METHODS

Participants

Participants were drawn from the EPIC–Norfolk prospective population study, which recruited men and women aged 40–79 y at the study baseline during 1993–1997 from residents in Norfolk, United Kingdom. The detailed recruitment method and study protocol of EPIC–Norfolk were described previously (11). Briefly, all eligible community-dwelling adults from 35 participating general practices were invited to participate. A total of 30 445 ($\approx 40\%$ response) persons provided written consent to participate in the study (99.6% white British persons). The Norwich Local Research Ethics Committee approved the study.

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Measurements

At the time of the baseline survey, participants completed a detailed health and lifestyle questionnaire. The participants were asked about their medical history with the question, "Has a doctor ever told you that you have any of the following?", in reference to the following conditions: stroke, heart attack, diabetes, and cancer. Smoking history was obtained by asking the following yes or no questions: "Have you ever smoked as much as one cigarette a day for as long as a year?" and "Do you smoke cigarettes now?". Participants who took any supplements or supplements containing vitamin C were identified from the question, "Have you taken any vitamins, minerals, or other food supplements regularly during the past year (such as vitamin C, vitamin D, iron, calcium, fish oils, primrose oil, β -carotene)?", which was followed by "name/brand and dose."

Trained nurses examined individuals at a baseline clinic visit. Height and weight were measured, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m). Blood pressure (BP) was measured with an Accutorr monitor (Datascop, Huntingdon, United Kingdom) after the participant had been seated for 5 min. We used the mean of 2 measurements for analysis. Nonfasting venous blood samples were taken into plain and citrate bottles. After overnight storage in a dark box at 4–7 °C, the sample bottles were centrifuged at 2100 \times g for 15 min at 4 °C. About 1 y after the start of the study, when funding became available, extra blood samples from participants were taken for ascorbic acid assays. Plasma vitamin C was measured from blood collected into citrate bottles, and plasma was stabilized in a standardized volume of metaphosphoric acid stored at –70 °C. We estimated plasma vitamin C concentrations with a fluorometric assay within 1 wk of sampling (12). The CV was 5.6% at the lower end of the range (mean: 33.2 μ mol/L) and 4.6% at the upper end (mean: 102.3 μ mol/L). Other blood samples for assay were stored at 4 °C and assayed at the Department of Clinical Biochemistry (University of Cambridge, Cambridge, United Kingdom) within 1 wk after samples were collected. We measured serum total cholesterol, HDL cholesterol, and triacylglycerol with the RA 1000 (Bayer Diagnostics, Basingstoke, United Kingdom) and calculated LDL-cholesterol concentrations with the Friedewald formula (13).

Social class was classified according to the Registrar General's occupation-based classification scheme (14). Social class I consists of professionals, social class II includes managerial and technical occupations, social class III is subdivided into non-manual skilled workers and manual skilled workers, social class IV consists of partly skilled workers, and social class V comprises unskilled manual workers (15). Social class was also re-categorized as nonmanual (I, II, and III nonmanual) and manual (III manual, IV, and V) social classes. Alcohol consumption was derived from a food-frequency questionnaire (FFQ) collected at baseline. For the "drinks" category, responses ranging from never to >6 times/d were given for 4 types of alcoholic drinks. An in-house computer program, CAFE, was developed for data entry and analysis (16). A 4-level physical activity index was derived from the validated EPIC short physical activity questionnaire designed to assess combined work and leisure activity. Participants were categorized into inactive, moderately inactive, moderately active, and active categories. The validity and repeatability of this scoring system was detailed elsewhere (17).

We further categorized these into inactive (inactive and moderately inactive) and active (moderately active and active) categories.

Case ascertainment

Incident stroke cases were ascertained by using death certificate data and hospital record linkage. All participants are flagged for death at the UK Office of National Statistics. Death certificates are coded by trained nosologists using International Classification of Disease (ICD), revisions 9 and 10. Participants are also linked to National Health Service hospital information systems so that admissions anywhere in the United Kingdom are reported to EPIC-Norfolk through routine annual record linkage. Stroke death was defined as ICD-9 430–438 or ICD-10 60–69 anywhere on the death certificate. Incident stroke was defined as stroke death or hospital discharge code ICD-9 430–438 or ICD-10 60–69. The current study is based on follow-up through March 2005.

Statistical analysis

We excluded participants with a history of stroke at the baseline and who had any missing value for the variables included in the analyses. Statistical analyses were performed by using SPSS for WINDOWS (version 14.0; SPSS, Chicago, IL). We used Cox proportional hazards model to determine the independent association between plasma vitamin C concentrations at baseline and risk of stroke during the follow-up. To estimate the independent contribution of vitamin C concentration to stroke risk, multivariate Cox regression models were constructed for quartile of ascorbic acid concentration with control for 1) age and sex; 2) age, sex, and smoking; 3) age, sex, smoking, BMI, systolic BP (every 10-mm Hg increase), cholesterol concentration, physical activity, history of myocardial infarction, and diabetes mellitus; 4) the preceding factors plus social class and alcohol consumption; and 5) the preceding factors plus control for any supplement use. Analyses were repeated (as per final model) after additional exclusion of 1) those with prevalent myocardial infarction and cancer, 2) users of vitamin C-containing supplements, 3) those with a history of incident stroke occurring during the initial 2 y of follow-up, and 4) additionally adjusted for average fruit and vegetable consumption.

We further examined the relative risk of stroke stratified by 1) sex (men and women), 2) age (<70 and \geq 70 y), 3) smoking status (never, former, and current smokers) 4) BMI (<27 and \geq 27), 5) systolic BP (<150 and \geq 150 mm Hg), 6) cholesterol concentration (<6 and \geq 6 mmol/L), 7) social class (manual and nonmanual), 8) physical activity (inactive and active), and 9) supplement use (users and nonusers) for every 20- μ mol/L increase (\approx 1 SD) in plasma vitamin C concentration.

RESULTS

A total of 20 649 participants were included in the current study after exclusion of those who reported a stroke at the baseline survey ($n = 455$) and those with missing data for any of the variables included in the main model ($n = 9341$). There were a total of 448 incident strokes over 196 713 total person-years of follow-up (average: 9.5 y). Of these, 147 (33%) were fatal (main cause of death).



The distribution of sample characteristics by quartiles of plasma vitamin C concentration by sex-combined and then sex-specific analyses are shown in **Table 1**. Quartile 1 represents the lowest quartile, whereas quartile 4 represents the highest plasma vitamin C concentration. Men and women who were in the top quartile category were younger and had a lower BMI, systolic BP, and cholesterol concentration. Participants in the highest quartile were less likely to be smokers, were more likely to be in nonmanual occupations, were physically more active, were more likely to take supplements, consumed more fruit and vegetables, and had a lower prevalence of myocardial infarction and diabetes than did those in the lower quartile categories. Of those variables included in the analyses, vitamin C \times sex interaction terms were significant only for BMI and vitamin C-containing supplement use ($P < 0.0001$ for both).

The relative risks (RRs) and corresponding 95% CIs for incident stroke are shown in **Table 2**. With increasing adjustments for covariates, RR estimates were slightly attenuated. In model E, participants who were in the top quartile had a 42% lower RR (0.58; 95% CI: 0.43, 0.78) than did those who were in the bottom quartile of plasma vitamin C, independently of age, sex, smoking, BMI, systolic BP, cholesterol, physical activity, prevalent diabetes, myocardial infarction, social class, alcohol consumption, and any supplement use. The results were similar after the exclusion of participants with prevalent myocardial infarction and cancer (model F), those who used vitamin C-containing supplements (model G), and after additional adjustment for fruit and vegetable consumption (model H) and after exclusion of incident strokes occurring within the first 2 y of follow-up (model I). There appeared to be an inverse dose-response linear relation

TABLE 1

Distribution of sex-combined and selected sex-specific sample characteristics by plasma vitamin C quartiles in 20 649 men and women of the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort at baseline (1993-1997)¹

| | Plasma vitamin C concentration quartile | | | | <i>P</i> for trend |
|--|---|------------------------------|------------------------------|-----------------------------------|----------------------|
| | 1 (< 41 $\mu\text{mol/L}$) | 2 (41-53 $\mu\text{mol/L}$) | 3 (54-65 $\mu\text{mol/L}$) | 4 (≥ 66 $\mu\text{mol/L}$) | |
| No. of subjects | 5298 | 5184 | 4824 | 5343 | |
| Men | 3403 | 2767 | 1893 | 1386 | |
| Women | 1895 | 2417 | 2931 | 3957 | |
| Age (y) | 59.5 \pm 9.4 ² | 58.4 \pm 9.4 | 57.8 \pm 9.0 | 58.0 \pm 9.1 | <0.0001 |
| Height (cm) | 169.0 \pm 9.1 | 168.1 \pm 9.2 | 166.4 \pm 8.9 | 164.8 \pm 8.4 | <0.0001 |
| BMI (kg/m ²) | 26.9 \pm 4.0 | 26.7 \pm 3.8 | 26.1 \pm 3.7 | 25.4 \pm 3.5 | <0.0001 |
| Men | 26.8 \pm 3.4 | 26.7 \pm 3.1 | 26.2 \pm 3.0 | 25.6 \pm 2.9 | <0.0001 |
| Women | 27.1 \pm 4.9 | 26.7 \pm 4.4 | 26.0 \pm 4.1 | 25.3 \pm 3.7 | <0.0001 |
| Systolic blood pressure (mm Hg) | 138 \pm 18 | 136 \pm 18 | 134 \pm 18 | 132 \pm 18 | <0.0001 |
| Cholesterol concentration (mmol/L) | 6.2 \pm 1.2 | 6.2 \pm 1.2 | 6.2 \pm 1.2 | 6.2 \pm 1.2 | 0.095 |
| Daily alcohol intake (g) | 8.9 \pm 14.4 | 9.0 \pm 13.1 | 8.3 \pm 11.9 | 8.6 \pm 12.1 | 0.037 |
| Vitamin C concentration ($\mu\text{mol/L}$) | 28.2 \pm 9.7 | 48.5 \pm 3.7 | 59.8 \pm 3.1 | 78.1 \pm 13.1 | <0.0001 |
| Daily fruit and vegetable intake (mg) | 354 \pm 203 | 444 \pm 221 | 492 \pm 251 | 527 \pm 276 | <0.0001 |
| Smoking status [<i>n</i> (%)] | | | | | <0.0001 ³ |
| Current smoker | 1084 [20.5] | 490 [9.5] | 379 [7.9] | 371 [6.9] | |
| Former smoker | 2379 [44.9] | 2312 [44.6] | 1955 [40.5] | 2087 [39.1] | |
| Never smoker | 1835 [34.6] | 2382 [45.9] | 2490 [51.6] | 2885 [54.0] | |
| Occupational social class [<i>n</i> (%)] | | | | | <0.0001 ³ |
| I | 284 [5.4] | 351 [6.8] | 370 [7.7] | 452 [8.5] | |
| II | 1658 [31.3] | 1861 [35.9] | 1890 [39.2] | 2224 [41.6] | |
| III, nonmanual | 805 [15.2] | 880 [17.0] | 844 [17.5] | 901 [16.9] | |
| III, manual | 1461 [27.6] | 1256 [24.2] | 991 [20.5] | 1030 [19.3] | |
| IV | 838 [15.8] | 672 [13.0] | 583 [12.1] | 614 [11.5] | |
| V | 252 [4.8] | 164 [3.2] | 146 [3.0] | 122 [2.3] | |
| Physical activity level [<i>n</i> (%)] | | | | | <0.0001 ³ |
| Inactive | 1803 [34] | 1453 [28.0] | 1198 [24.8] | 1241 [23.2] | |
| Moderately inactive | 1423 [26.9] | 1522 [29.4] | 1479 [30.7] | 1648 [30.8] | |
| Moderately active | 1142 [21.6] | 1194 [23.0] | 1158 [24.0] | 1338 [25.0] | |
| Active | 930 [17.6] | 1015 [19.6] | 989 [20.5] | 1116 [20.9] | |
| Prevalent myocardial infarction [<i>n</i> (%)] | 253 [4.8] | 152 [2.9] | 121 [2.5] | 82 [1.5] | <0.0001 |
| Prevalent diabetes [<i>n</i> (%)] | 177 [3.3] | 129 [2.5] | 80 [1.7] | 51 [1.0] | <0.0001 |
| Any supplement user [<i>n</i> (%)] | 1685 [31.8] | 2136 [41.2] | 2325 [48.2] | 3222 [60.3] | <0.0001 |
| Vitamin C containing-supplement user [<i>n</i> (%)] | 101 [1.9] | 216 [4.2] | 262 [5.4] | 559 [10.5] | <0.0001 |
| Men | 49 [1.4] | 104 [3.8] | 99 [5.2] | 161 [11.6] | <0.0001 |
| Women | 52 [2.7] | 112 [4.6] | 163 [5.6] | 398 [10.1] | <0.0001 |

¹ A general linear model was used for continuous variables, and a chi-square test was used for categorical variables. Sex-specific data are reported only for those variables with a significant interaction between vitamin C and sex.

² $\bar{x} \pm$ SD (all such values).

³ Overall *P* value.



TABLE 2

Relative risks (and 95% CIs) for risk of stroke by quartile of plasma vitamin C concentration at baseline in the European Prospective Investigation into Cancer (EPIC)–Norfolk population (1993–1977 to 2005)¹

| | No. of events | Plasma vitamin C quartile | | | | P |
|---------|---------------|---------------------------|-------------------|-------------------|-------------------|---------|
| | | 1 (< 41 μmol/L) | 2 (41–53 μmol/L) | 3 (54–65 μmol/L) | 4 (≥ 66 μmol/L) | |
| Model A | 448 | 1.00 | 0.76 (0.61, 0.96) | 0.57 (0.43, 0.75) | 0.49 (0.37, 0.64) | <0.0001 |
| Model B | 448 | 1.00 | 0.80 (0.63, 1.01) | 0.60 (0.45, 0.79) | 0.51 (0.39, 0.68) | <0.0001 |
| Model C | 448 | 1.00 | 0.83 (0.66, 1.05) | 0.63 (0.48, 0.83) | 0.57 (0.43, 0.76) | <0.0001 |
| Model D | 448 | 1.00 | 0.84 (0.67, 1.07) | 0.64 (0.48, 0.84) | 0.58 (0.44, 0.78) | 0.001 |
| Model E | 448 | 1.00 | 0.84 (0.66, 1.07) | 0.64 (0.48, 0.84) | 0.58 (0.43, 0.78) | 0.001 |
| Model F | 381 | 1.00 | 0.91 (0.70, 1.17) | 0.67 (0.50, 0.91) | 0.61 (0.45, 0.84) | 0.006 |
| Model G | 428 | 1.00 | 0.80 (0.63, 1.02) | 0.62 (0.46, 0.82) | 0.58 (0.43, 0.78) | 0.001 |
| Model H | 448 | 1.00 | 0.83 (0.65, 1.05) | 0.67 (0.47, 0.83) | 0.57 (0.42, 0.76) | <0.0001 |
| Model I | 427 | 1.00 | 0.91 (0.72, 1.16) | 0.69 (0.52, 0.92) | 0.60 (0.44, 0.81) | 0.003 |

¹ A Cox-proportional hazards model was used. Model A was adjusted for age and sex. Model B was adjusted for age, sex, and smoking status. Model C was adjusted for age, sex, smoking status, BMI, systolic blood pressure (by 10-mm Hg increase), cholesterol, physical activity, and prevalent myocardial infarction and diabetes. Model D was adjusted as in model C and for social class and alcohol consumption. Model E was adjusted as in model D and for any supplement use. Model F was adjusted as in model E after exclusion of prevalent myocardial infarction and cancer. Model G was adjusted as in model E after exclusion of vitamin C supplement users. Model H was adjusted as in model E and for fruit and vegetable consumption. Model I was adjusted as in model E after exclusion of strokes occurring within 2 y of follow-up.

between baseline plasma vitamin C concentration and stroke risk across the sample population.

With a Cox-proportional hazards model, RRs for every 20-μmol/L increase in plasma vitamin C concentration were estimated in stratified analyses categorized by 1) sex (male and female), 2) age (<70 y and ≥70 y), 3) smoking status (never, former, and current smokers), 4) BMI (<27 and ≥27), 5) systolic BP (<150 and ≥150 mm Hg), 6) cholesterol concentration (<6 and ≥6 mmol/L), 7) occupational social class (manual and non-manual), 8) physical activity (active and inactive), and 9) supplement use (user and nonuser). The covariates adjusted in these models (except in the model in which the variable is of interest) were age, sex, smoking status, BMI, 10-mm Hg increase in systolic BP, cholesterol concentration, physical activity, social class, diabetes, myocardial infarction, alcohol consumption, and any supplement use (data not shown). An increasing plasma vitamin C concentration was associated with a reduced stroke risk in all stratified analyses. The effects appeared to be somewhat attenuated in men, within manual social class, and among participants with a cholesterol concentration <6 mmol/L. We reported the main effect only because the interaction terms for each of the subgroups were not significant. There was an overall 17% reduction in incident stroke (RR: 0.83; 95% CI: 0.75, 0.92) for every 20- μmol/L increase in plasma vitamin C concentration.

DISCUSSION

Consistent with previous population-based studies (8–10, 18, 19), we found a significant inverse relation between plasma vitamin C concentrations and subsequent stroke risk in a general population who had no history of stroke at baseline. This result was independent of known biological, social, and lifestyle risk factors for stroke: age, sex, smoking, BMI, systolic BP, cholesterol concentration, physical activity, prevalent diabetes and myocardial infarction, social class, alcohol consumption, and any supplement use.

Nearly 10 y ago, Simon et al (20) showed a cross-sectional relation between serum ascorbic acid concentration and prevalence of stroke in 6624 US men and women enrolled in the second National Health and Nutrition Examination Survey. Although previous population-based prospective studies (8–10) confirmed a relation between plasma vitamin C concentration and incident stroke risk, these studies have generally been based on small sample sizes with a lower number of incident strokes (<250) and often were not able to examine many covariates. The current study is a large prospective population-based study (>20 000 participants) that was able to take into account biological (age, BMI, BP, and cholesterol), social (occupational social class), and lifestyle behavior (smoking, alcohol consumption, physical activity level, and supplement use) factors that are associated with risk of stroke and had the additional ability to take into account prevalent illnesses at baseline, including myocardial infarction, diabetes, and cancer.

In an observational study, confounding and reverse causality issues require attention. We addressed these issues in this study in several ways. First, we adjusted for possible confounders that potentially relate to both ascorbic acid concentration and known stroke risk factors (21). Second, we examined the multivariate-adjusted relation after excluding those with prevalent illness, those who took supplements, and those with a history of stroke within the first 2 y of follow-up. Third, we performed analyses stratifying by age, sex, BMI, systolic BP, cholesterol concentration, occupational social class, physical activity level, and supplement use; the results did not differ significantly between the various subgroups.

Naturally, our study had limitations. Because of the requirement of subjects to provide detailed health and lifestyle information and to be able to undergo health checks, the initial response rate was modest (≈40%). This may have introduced a healthy responder bias. Nevertheless, the baseline characteristics of the study population were similar to those of other UK population samples, except for a slightly lower prevalence of smokers (11). Moreover, the truncation of distribution because of

healthy responders likely attenuated the associations, but this should not have affected the relation between vitamin C and stroke observed within the study participants; if anything, truncation of the distribution is likely to reduce the power of any associations. We used death certification and a hospital record linkage system using ICD coding to identify stroke cases. Although follow-up with the use of these methods is virtually complete, this approach may underestimate incident nonfatal stroke cases that are not admitted to the hospital. The use of self-reported stroke to exclude prevalent cases may have missed some prevalent strokes. We were not able to separately examine stroke subtypes. Nevertheless, the primary focus of the study was to assess the risk prediction of clinical stroke event severe enough to lead to hospitalization or death regardless of stroke subtype. In any case, the misclassification of strokes was likely to only attenuate any associations.

Only single measurements of plasma vitamin C and other covariates, such as cholesterol and BP, were made at baseline. These measures as well as lifestyle behaviors, which may affect vitamin C concentration, may have changed over the follow-up period. Moreover, the blood sample taken was a nonfasting sample and was therefore less standardized for some of the variables (eg, cholesterol concentration) than was a fasting blood sample. Nevertheless, random measurement error was likely only to attenuate any relations observed between plasma vitamin C and stroke.

Plasma vitamin C concentration was a good biomarker of plant food, namely fruit and vegetable intakes, in our cohort. The 20- μ mol or 1-SD increase in plasma vitamin C concentration is associated with approximately one additional serving of fruit and vegetables daily (22). This agrees with more recently published literature on the relation between higher dietary fruit and vegetable intake and reduced stroke risk (23, 24). However, observational studies are not all consistent (25, 26), and antioxidant supplementation including vitamin C did not produce a substantial benefit in clinical trial settings in high risk individuals (6, 27). The recent Women's Health Initiative also reported no reduction in cardiovascular disease in the group allocated to a low-fat and higher fruit and vegetable target diet (28).

There are some plausible explanations why the discrepancy exists between cohort studies and trials. Many of the supplementation trials, such as the Heart Protection Study and the Finnish Alpha-Tocopherol Beta-Carotene Cancer Prevention Study, were conducted in high-risk or highly selected rather than general populations (27, 29, 30). Additionally, combinations of antioxidants, some of them in pharmacologic doses, such as vitamin E, may have unpredicted biological effects.

Moreover, the lack of benefit of vitamin C in clinical trials could be explained by the relation between vitamin C dose and plasma concentration. At doses <100 mg/d, there is a large change in plasma concentration for small changes in dose. Above 100 mg/d, there is little change in plasma concentration despite large changes in dose. If the control group (ie, lowest quartile) consumed 100 mg/d, then further increases in dose would be predicted to cause little change in concentration. The outcome, therefore, may not be affected at higher doses. This problem was first pointed out by Levine et al in 1999 (31).

The association appeared to be independent of the most plausible confounders (32), such as smoking, physical activity, and social class, as we previously highlighted (22, 33). The mean height of men and women by quartile of vitamin C concentrations

did not show material differences (Table 2); hence, life-course factors are unlikely to be significant confounders. An intriguing possibility is that the plasma vitamin C concentration is a good marker of a wider range of health behaviors, such as fruit and vegetable consumption, that may be protective against stroke. Even then, it appears that the relation was independent of fruit and vegetable consumption. It is also possible that the relation could reflect measurement error related to the dietary instruments. It is also plausible that vitamin C may biochemically affect stroke risk. Given the current evidence, it is unlikely that long-term randomized controlled trials of isolated vitamin C supplementation and cardiovascular disease endpoints will be conducted. Nevertheless, the magnitude of the association between plasma vitamin C and subsequent stroke is substantial and independent of known major risk factors for stroke.

We believe that these findings are of interest for several reasons. First, the strong inverse association between plasma vitamin C and stroke suggests that plasma vitamin C is likely to be a good biomarker of whatever causal factors affect stroke risk, most plausibly the dietary intake of plant foods. However, identification of the relevant factors may lead to better stroke prevention. Second, irrespective of any causal associations, plasma vitamin C appears to be a good predictive risk indicator of stroke, independent of known risk factors such as age, BP, smoking, lipids, diabetes, and BMI. Given that about half of the risk of stroke is unexplained by conventional cardiovascular disease risk factors (34) and that the predictive validity of traditional cardiovascular disease risk factors appears to diminish with age (35, 36), risk markers that may help to identify those persons at greatest risk of stroke for targeted preventive interventions with established therapies, such as BP reduction, may be of interest.

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