Vitamin C status and mortality in US adults[1,2]

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ABSTRACT

Background: Low vitamin C status may increase the risk of mortality from cancer and cardiovascular disease.

Objective: The objective was to test whether an association existed between serum ascorbate concentrations and mortality and whether the association was modified by cigarette smoking status or sex.

Design: Serum ascorbate concentrations were measured in adults as part of the second National Health and Nutrition Examination Survey (1976–1980). Vital status was ascertained 12–16 y later.

Results: The relative risk (RR) of death, adjusted for potential confounders, was estimated by using Cox proportional hazards models. Men in the lowest (<28.4 μmol/L) compared with the highest (≥73.8 μmol/L) serum ascorbate quartile had a 57% higher risk of dying from any cause (RR: 1.57; 95% CI: 1.21, 2.03) and a 62% higher risk of dying from cancer (RR: 1.62; 95% CI: 1.01, 2.59). In contrast, there was no increased risk among men in the middle 2 quartiles for these outcomes and no increased risk of cardiovascular disease mortality in any quartile. There was no association between serum ascorbate quartile and mortality among women. These findings were consistent when analyses were limited to nonsmokers or further to adults who never smoked, suggesting that the observed relations were not due to cigarette smoking.

Conclusions: These data suggest that men with low serum ascorbate concentrations may have an increased risk of mortality, probably because of an increased risk of dying from cancer. In contrast, serum ascorbate concentrations were not related to mortality among women. *Am J Clin Nutr* 2000;72:139–45.

KEY WORDS Ascorbic acid, antioxidants, mortality, cancer, cardiovascular disease, smoking, NHANES II, second National Health and Nutrition Examination Survey, adults

INTRODUCTION

Vitamin C may play a role in the prevention of cardiovascular disease (CVD) and cancer, the 2 leading causes of mortality in the United States (1). By scavenging free radicals, vitamin C may protect LDLs and DNA from oxidative damage and, consequently, prevent atherogenesis (2–5) and carcinogenesis (6–8). Vitamin C may also prevent atherogenesis through its role in the synthesis of collagen (9) and prostacyclin (10). Likewise, vitamin C may protect against cancer through other mechanisms, such as the detoxification of carcinogens and enhancement of immune function (6–8). Although findings from prospective cohort studies that examined the association between vitamin C and total mortality suggest an inverse relation (11–13), reviews of studies investigating CVD and cancer outcomes indicate less-consistent findings (14, 15).

We used data from a nationally representative sample of US adults to test whether serum ascorbate concentrations were associated with the risk of mortality from all causes, CVD, and cancer. Cigarette smoking decreases serum concentrations of vitamin C (16–18) and increases the risk of cancer and CVD, possibly by generating oxygen-derived free radicals (9, 19). Because of the strong potential for cigarette smoking to confound the association between vitamin C and mortality, we tested whether the effect of vitamin C was modified by smoking. Furthermore, we examined whether sex modified this association because sex differences were found in previous studies (13, 20).

SUBJECTS AND METHODS

Subjects

This study used data from the NHANES II Mortality Study (21), a prospective study of participants examined in the second National Health and Nutrition Examination Survey (NHANES II) (1976–1980) (22). The NHANES II collected extensive demographic, medical history, nutritional, clinical, and laboratory data on a multistage, probability sample of the civilian, noninstitutionalized US population. The examination response rate was 73%. The vital status of NHANES II participants who were 30–75 y of age at examination was ascertained as of 31 December 1992, resulting in 12–16 y of follow-up data. Vital status was assessed by searching for deaths in the National Death Index and the Social Security Administration Death Master File (21). Cause of death was obtained from the National Center for Health Statistic Multiple Cause of Death file (21) or from death certificates and coded according to the ninth revision of the *Inter-*

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national Classification of Diseases, Injuries, and Causes of Death (ICD-9) (23). Deaths from CVD were defined as those with underlying causes-of-death codes 402, 410–414, 427.5, 428.9, 429.2, 430–438, 440–444; deaths from cancer were defined as those with underlying causes of death codes 140–239.

Baseline variables

Blood was drawn from participants at baseline in mobile examination centers, stabilized with metaphoshoric acid, and shipped on dry ice to the Centers for Disease Control and Prevention for processing. Plasma was analyzed for total serum vitamin C by using the 2,4-dinitrophenylhydrazine method (24, 25). Serum ascorbate concentrations were classified into sex-specific quartiles.

Systolic blood pressure was measured twice in the sitting position by a physician using a mercury sphygmomanometer (26). The average of the 2 blood pressure readings was used in our analyses. Body mass index (BMI) was calculated as weight (kg)/height^2 (m). Diabetes status was positive if a participant reported having been told by a physician that he or she had diabetes, was using insulin at baseline, had a plasma glucose concentration ≥11.1 mmol/L (200 mg/dL) 2 h after an oral glucose challenge, or had a fasting plasma glucose concentration (with no challenge) ≥7.8 mmol/L (140 mg/dL). Total serum cholesterol was determined by using a Lieberman-Burchard reagent (27). Participants were asked about their consumption of beer, wine, and other alcoholic beverages. Responses to these questions were summed to calculate the total frequency per week. Participants were asked about their smoking status (current, former, or never) and, if they ever smoked, the intensity (number of cigarettes smoked) and duration (years as a smoker) of the habit.

Dietary vitamin C intakes from a 24-h recall were available from the public use data set (28). Dietary vitamin E intakes were calculated from the 24-h recall data by using food-composition data obtained from the National Cancer Institute (29). Supplements used during the week before examination were categorized according to vitamin content; data for participants taking more than one product were recoded so that the content of all supplements was recorded instead of just the first listed, as released previously by the National Center for Health Statistics (28). We calculated total vitamin E intakes by summing estimated intakes from supplements taken daily or almost daily and dietary vitamin E intakes. Because the amount of vitamin E contained in the supplements was not determined, we categorized supplements as either a multivitamin or a specialized product and assumed that the multivitamins contained 10 mg vitamin E [the US recommended dietary allowance (RDA) for men] and that the specialized vitamin E products contained 25 mg (250% of the US RDA) (30). Sex-specific tertiles of total vitamin E intake were used in the analyses (<5.9, 5.9–11.1, and >11.1 mg for men; <4.5, 4.5–8.9, and >8.9 mg for women). A food-frequency questionnaire was administered that contained 2 items concerning consumption of fruit and vegetables rich in vitamins C and A during the 3 mo before the interview. Responses to these questions were summed to estimate total weekly fruit and vegetable consumption.

Analytic sample

Serum ascorbate concentrations were missing for 7% of the eligible 9250 NHANES II Mortality Study participants. There were few differences in the selected baseline characteristics between participants with missing and those with available serum ascorbate data (data not shown). However, mean systolic blood pressure was significantly higher in women with missing data. On average, men with missing serum ascorbate data reported smoking fewer cigarettes at baseline than did men with available data. An additional 2% of participants were excluded because of missing data for established CVD and cancer risk factors. About 16% of the remaining 8451 participants were excluded because of a history of heart disease or cancer at baseline. Participants with a history of physician-reported stroke or heart attack or who reported symptoms of angina, as determined by a modified Rose questionnaire, were considered to have heart disease. Participants with a history of physician-reported cancer were considered to have cancer. After all exclusions, there were 3347 men and 3724 women available for the main analyses. The effect of the duration of cigarette smoking on the relation between serum ascorbate concentrations and mortality was analyzed by using data from 3112 men; 5% of the 3347 men had missing data on smoking duration.

Data analysis

The relative risks (RRs) of CVD, cancer, and total mortality were estimated from hazards ratios derived from Cox proportional hazards models. Person-years of follow-up for each participant were calculated from baseline examination to the date of death or 31 December 1992. The inclusion of time-dependent variables in the initial Cox models confirmed that the proportional hazards assumption was met. RR estimates were initially adjusted for age (y) and sex. Multivariate models also included race (African American versus other), educational level (<12 versus ≥12 y), smoking intensity (number of cigarettes smoked at baseline), alcohol consumption (weekly consumption frequency of beer, wine, and liquor), diabetes status (yes or no), serum total cholesterol concentrations (mmol/L), systolic blood pressure (mm Hg), and BMI.

Statistical analyses were performed after participants with a history of CVD or cancer were excluded. We performed tests for trend by fitting serum ascorbate concentration as a continuous variable in the Cox models. All analyses were run by using SUDAAN (31) to account for the complex sample design (22), except the analyses involving time-dependent variables, which were run by using SAS (32). A P value < 0.05 indicated statistical significance.

Interactions between serum ascorbate concentrations and selected covariates were assessed by including interaction terms in preliminary multivariate models. There were no significant interactions between serum ascorbate concentrations and age, sex, or race. Neither smoking intensity nor smoking duration significantly affected the relation between serum ascorbate and total mortality; however, smoking status (current versus former and never smokers) did. For this reason, we also present analyses stratified by smoking status. Interactions between serum ascorbate and smoking status were not significant when CVD or cancer mortality were examined among men or for any outcomes among women.

The addition of smoking duration to the models did not substantially change the risk estimates for serum ascorbate quartiles (data not shown). Likewise, the addition of recreational exercise level (light, moderate, or heavy), fasting status before venipuncture, seasonality of examination, number of times per week that fruit and vegetables were consumed, and tertile of total vitamin E intake to the final models did not substantially change the risk estimates for serum ascorbate quartiles (data not shown). Therefore, these variables were not included in the final models. Finally, the results did not change substantially when the analyses were repeated with the exclusion of deaths occurring during

TABLE 1
Distribution of selected baseline characteristics by quartile (Q) of serum ascorbate concentration (μmol/L) among participants aged 30–75 y with no history of cardiovascular disease or cancer at baseline: NHANES II, 1976–1980[1]

	Men				Women			
Characteristic	Q1 (n = 814)	Q2 (n = 698)	Q3 (n = 963)	Q4 (n = 872)	Q1 (n = 785)	Q2 (n = 981)	Q3 (n = 947)	Q4 (n = 1011)
Age (y)	47.5 ± 0.5	46.6 ± 0.5	47.3 ± 0.6	49.3 ± 0.4[2]	46.0 ± 0.6	47.2 ± 0.4	49.4 ± 0.4	51.6 ± 0.4[2]
White (%)	84.2 ± 2.4	86.0 ± 2.8	89.7 ± 1.9	92.1 ± 2.3[3]	82.7 ± 2.5	83.0 ± 2.4	91.5 ± 1.4	94.4 ± 0.8[3]
Diabetes (%)	3.0 ± 0.5	3.7 ± 0.7	5.2 ± 0.8	5.2 ± 0.9[3]	5.2 ± 0.9	7.2 ± 1.0	5.2 ± 0.8	4.9 ± 0.6
BMI (kg/m^2)	25.9 ± 0.2	26.6 ± 0.2	26.1 ± 0.1	25.5 ± 0.2[2]	26.1 ± 0.3	26.6 ± 0.2	25.4 ± 0.2	24.7 ± 0.2[2]
Serum total cholesterol (mmol/L)	216.8 ± 2.5	222.0 ± 2.0	219.7 ± 2.2	220.0 ± 1.8[2]	219.6 ± 2.4	219.2 ± 1.9	226.6 ± 1.9	230.4 ± 1.6[2]
Systolic blood pressure (mm Hg)	133.0 ± 1.1	131.7 ± 0.9	131.9 ± 0.9	131.6 ± 0.9	128.5 ± 1.3	128.4 ± 0.9	128.3 ± 1.0	129.0 ± 1.2
Current smoker (%)	63.3 ± 2.1	42.1 ± 2.4	32.0 ± 1.6	28.6 ± 1.3[3]	54.0 ± 2.5	31.1 ± 1.5	26.1 ± 1.9	21.5 ± 2.1[3]
Cigarettes smoked (number/d)	16.2 ± 0.7	10.6 ± 0.8	7.4 ± 0.5	7.3 ± 0.5[2]	11.8 ± 0.7	5.8 ± 0.4	4.7 ± 0.4	4.1 ± 0.5[2]
Duration of smoking (y)	23.3 ± 0.6	17.9 ± 0.6	16.1 ± 0.5	16.8 ± 0.6[2]	14.5 ± 0.7	10.3 ± 0.5	8.9 ± 0.5	9.3 ± 0.6[2]
Alcohol consumption (drinks/wk)	3.7 ± 0.2	3.5 ± 0.2	4.0 ± 0.2	3.8 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	1.8 ± 0.1	1.9 ± 0.2[2]
High school education or more (%)	45.7 ± 2.5	63.0 ± 2.5	73.5 ± 1.9	73.2 ± 1.7[3]	52.6 ± 2.0	60.2 ± 2.4	69.1 ± 1.8	73.8 ± 1.7[3]
Dietary vitamin C intake (mg)	52.4 ± 3.1	70.1 ± 2.4	117.1 ± 4.4	146.9 ± 4.5[2]	49.2 ± 2.6	75.5 ± 3.1	112.8 ± 4.0	135.0 ± 4.1[2]
Dietary vitamin E intake (mg)	8.2 ± 0.3	8.8 ± 0.3	11.9 ± 0.4	15.9 ± 0.6[2]	6.8 ± 0.3	8.4 ± 0.3	10.6 ± 0.4	13.8 ± 0.4[2]
Vitamin C supplement user (%)	0.2 ± 0.1	0.1 ± 0.1	5.4 ± 1.0	19.0 ± 1.4[3]	0.4 ± 0.2	3.3 ± 0.7	9.0 ± 1.3	20.4 ± 1.4[3]
Vitamin E supplement user (%)	0.8 ± 0.3	0.9 ± 0.4	4.4 ± 0.8	10.6 ± 1.0[3]	1.7 ± 0.6	4.1 ± 0.6	6.2 ± 0.9	13.9 ± 1.3[3]
Multivitamin user (%)	0.8 ± 0.3	2.7 ± 0.6	17.2 ± 1.7	30.6 ± 1.7	4.9 ± 0.8	10.7 ± 1.4	20.1 ± 1.7	33.8 ± 2.3
Vitamin C–rich fruit or vegetable consumption (times/wk)	2.4 ± 0.1	3.7 ± 0.2	5.7 ± 0.3	6.6 ± 0.2[2]	2.8 ± 0.1	5.0 ± 0.2	6.6 ± 0.2	7.3 ± 0.2[2]
Vitamin A–rich fruit or vegetable consumption (times/wk)	1.3 ± 0.1	1.4 ± 0.1	1.8 ± 0.1	2.0 ± 0.1[2]	1.5 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.1 ± 0.1[2]

[1] $\bar{x}$ ± SE. Quartiles of serum ascorbate concentration were derived from the entire cohort: men (<28.4, 28.4–51.0, 51.1–73.7, and ≥73.8 μmol/L) and women (<39.7, 39.7–68.0, 68.1–85.1, and ≥85.2 μmol/L). NHANES II, second National Health and Nutrition Examination Survey.
[2,3] Quartiles significantly different from each other: [2] $P < 0.05$ (ANOVA), [3] $P < 0.05$ (chi-square test).

the first 3 y of follow-up to assess the effect of preexisting morbidity at baseline (data not shown).

RESULTS

Median serum ascorbate concentrations were lower among men (49.4 μmol/L; 95% CI: 47.7, 51.7) than among women (64.2 μmol/L; 95% CI: 61.9, 65.9). Sex-specific quartiles, defined as serum ascorbate concentrations <28.4, 28.4–51.0, 51.1–73.7, and ≥73.8 μmol/L for men and <39.7, 39.7–68.0, 68.1–85.1, and ≥85.2 μmol/L for women were used in subsequent analyses. The proportion of participants in each quartile of serum ascorbate concentration for selected baseline characteristics is presented in **Table 1**. At higher serum ascorbate quartiles, men were more likely to be white, have at least a high school education, have diabetes, be nonsmokers, smoke fewer cigarettes at baseline, and have smoked fewer years ($P < 0.05$). Furthermore, men in the highest quartile were significantly older than were men in other quartiles. BMI and serum total cholesterol differed significantly among quartiles, although the relation was not graded among men. At higher serum ascorbate quartiles, women were more likely to be older, have lower BMIs, be nonsmokers, smoke fewer cigarettes at baseline, have smoked fewer years, and have at least a high school education ($P < 0.05$). Serum total cholesterol and weekly consumption of alcohol were higher in the 2 highest serum ascorbate quartiles. Among both sexes, dietary intakes of vitamins C and E, use of supplements containing vitamins C and E, and consumption frequency of vitamin C– and vitamin A–rich fruit and vegetables were higher at higher serum ascorbate quartiles.

During the 12–16 y of follow-up, 791 men and 566 women died. Men in the lowest serum ascorbate quartile had almost twice the risk of dying than did men in the highest quartile after adjustment for age (RR: 1.91; 95% CI: 1.50, 2.42). After further adjustment for race, educational level, number of cigarettes smoked at baseline, serum total cholesterol, systolic blood pressure, BMI, diabetes status, and alcohol consumption, men in the lowest serum ascorbate quartile had a 57% higher risk of dying (RR: 1.57; 95% CI: 1.21, 2.03) than did men in the highest quartile. In contrast, there was no significantly higher risk among men in the middle 2 quartiles than among those in the highest quartile (**Table 2**).

Men in the lowest serum ascorbate quartile had a 73% higher risk of dying from CVD than did men in the highest quartile after adjustment for age (RR: 1.73; 95% CI: 1.10, 2.74). However, this association was attenuated after adjustment for established CVD risk factors (Table 2). Men in the lowest serum ascorbate quartile had a 2-fold higher risk of dying from cancer than did men in the highest quartile after adjustment for age (RR: 2.05, 95% CI: 1.31, 3.22). After further adjustment for the factors listed above, men in the lowest serum ascorbate quartile had a 62% higher risk of dying from cancer (RR: 1.62; 95% CI: 1.01, 2.59) than did men in the highest quartile. Although the risk among men in the middle 2 quartiles was not significantly higher than that of men in the highest quartile (Table 2), the risk was lower with lower serum ascorbate concentrations ($P = 0.001$ for trend).

Women in the lowest serum ascorbate quartile had a 46% higher risk of dying than did women in the highest quartile after adjustment for age (RR: 1.46; 95% CI: 1.09, 1.95). However, this risk was attenuated and was no longer significant after further adjustment for race, educational level, number of cigarettes smoked at baseline, serum total cholesterol, systolic blood pressure, BMI, diabetes status, and alcohol consumption. No association was

TABLE 2
Relative risks and 95% CIs of total, cardiovascular disease (CVD), and cancer mortality by quartile (Q) of serum ascorbate concentration (μmol/L) among participants aged 30–75 y with no history of CVD or cancer at baseline: NHANES II Mortality Study, 1976–1992[1]

Serum ascorbate quartile[1]	Men					Women				
	No. of deaths	Age-adjusted		Multivariate-adjusted[2]		No. of deaths	Age-adjusted		Multivariate-adjusted[2]	
		RR	(95% CI)	RR	(95% CI)		RR	(95% CI)	RR	(95% CI)
Total mortality										
Q1	242	1.91	(1.50, 2.42)	1.57	(1.21, 2.03)	127	1.46	(1.09, 1.95)	1.19	(0.86, 1.66)
Q2	141	1.06	(0.82, 1.38)	1.07	(0.83, 1.39)	134	1.14	(0.87, 1.49)	1.10	(0.82, 1.47)
Q3	204	0.97	(0.79, 1.19)	0.99	(0.81, 1.22)	138	1.01	(0.78, 1.31)	1.01	(0.78, 1.31)
Q4	203	1.00	—	1.00	—	167	1.00	—	1.00	—
P for trend[3]	—	0.001	—	0.03	—	—	0.31	—	0.97	—
CVD mortality										
Q1	79	1.73	(1.10, 2.74)	1.45	(0.90, 2.32)	43	1.22	(0.77, 1.94)	0.93	(0.57, 1.53)
Q2	47	0.96	(0.62, 1.49)	0.95	(0.61, 1.46)	47	1.02	(0.67, 1.56)	0.92	(0.60, 1.40)
Q3	93	1.31	(0.87, 1.96)	1.33	(0.91, 1.96)	54	0.90	(0.58, 1.40)	0.89	(0.58, 1.36)
Q4	74	1.00	—	1.00	—	69	1.00	—	1.00	—
P for trend[3]	—	0.52	—	0.84	—	—	0.95	—	0.32	—
Cancer mortality										
Q1	73	2.05	(1.31, 3.22)	1.62	(1.01, 2.59)	34	1.21	(0.74 1.98)	1.06	(0.60, 1.90)
Q2	47	1.36	(0.86, 2.15)	1.35	(0.85, 2.16)	39	1.09	(0.62, 1.94)	1.15	(0.68, 1.97)
Q3	51	0.82	(0.53, 1.26)	0.84	(0.54, 1.31)	37	1.09	(0.62, 1.92)	1.13	(0.64, 1.98)
Q4	57	1.00	—	1.00	—	45	1.00	—	1.00	—
P for trend[3]	—	0.001	—	0.001	—	—	0.85	—	0.66	—

[1] Quartiles of serum ascorbate concentration were derived from the entire cohort: men (<28.4, 28.4–51.0, 51.1–73.7, and ≥73.8 μmol/L) and women (<39.7, 39.7–68.0, 68.1–85.1, and ≥85.2 μmol/L). NHANES II, second National Health and Nutrition Examination Survey; RR, relative risk.

[2] Adjusted for age at baseline examination, race (African American versus other), highest attained educational level (<12 or ≥12 years), number of cigarettes smoked at baseline, weekly frequency of alcohol consumption, diabetes (yes or no), serum total cholesterol, systolic blood pressure, and body mass index.

[3] Serum ascorbate concentration was entered as a continuous variable. Cox proportional hazards regression models were used.

observed between quartiles of serum ascorbate concentration and mortality from CVD or cancer among women (Table 2).

When the analyses were stratified by smoking status (**Table 3**), the results for nonsmokers were similar to those in the unstratified analyses. In contrast, the associations among male smokers were generally attenuated and not significant. When we limited the analysis to men who had never smoked, those in the lowest serum ascorbate quartile had twice the risk of dying as did men in the highest quartile after adjustment for the above risk factors (RR: 2.02; 95% CI: 1.15, 3.54). In this same group, there were too few cancer deaths to produce reliable estimates of RR of cancer mortality.

Cancer deaths among men were clustered in the respiratory and digestive organs, whereas among women, deaths were more evenly distributed among sites (**Table 4**). There were generally too few deaths for specific sites to produce stable risk estimates, except for lung cancer among men. Men in the lowest quartile had a higher risk of dying from lung cancer (RR: 2.97; 95% CI: 1.46, 6.08) than did men in the highest quartile. After adjustment for age, number of cigarettes smoked, BMI, and educational level, however, the risk was attenuated and was no longer significant (RR: 1.88; 95% CI: 0.88, 4.02).

DISCUSSION

Using data from a nationally representative sample followed for 12–16 y, we found a higher risk of dying from any cause and from cancer among men in the lowest than in the highest serum ascorbate quartile. Although the trend of lower risk with higher serum ascorbate concentrations was significant for both outcomes, risk estimates for the middle quartiles were not significantly different from those for the highest quartile. We observed a modest association between serum ascorbate quartiles and CVD mortality among men. In contrast, serum ascorbate quartiles among women were not related to any of the 3 outcomes studied. Our findings were consistent when analyses were limited to nonsmokers or even further to those who never smoked, suggesting that the observed relations were not due to the effects of cigarette smoking. Furthermore, there was no association between serum ascorbate quartile and any of the 3 outcomes among current smokers at baseline. Thus, the protective effect of vitamin C may not be strong enough to counteract the increased oxidative stress from cigarette smoking (9, 19, 33, 34).

Our data indicated that the relation between vitamin C intakes and mortality differs by sex. Because women have higher serum ascorbate concentrations than do men, the cutoff for the lowest quartile was higher among women. If very low serum ascorbate concentrations confer the most risk, it might explain why we did not observe a relation between vitamin C intakes and mortality among women. However, in post hoc analyses among women, we found no association between serum ascorbate concentrations below those necessary for preventing clinical symptoms of vitamin C deficiency (<22.7 μmol/L) and any of the outcomes examined (data not shown). Another explanation for the difference between sexes may lie in the differential distribution of cancer sites. Findings from previous studies indicate that vitamin C may be most protective against non-hormone-dependent cancers (6, 8, 15). In our study, there was a much higher percentage of hormone-dependent cancers in women (30%) than in men (7%). If most of the increased total mortality risk is due to an increased cancer mortality risk, the distribution among sites would also account for the difference in total mortality between sexes.

TABLE 3
Relative risk and 95% CIs of total, cardiovascular disease (CVD), and cancer mortality by quartile (Q) of serum ascorbate concentration (μmol/L) stratified by sex and smoking status among participants aged 30–75 y with no history of CVD or cancer at baseline: NHANES II Mortality Study, 1976–1992[1]

	Men						Women					
	Smokers			Nonsmoker			Smokers			Nonsmokers		
Serum ascorbate quartile	No. of deaths	RR[2]	(95% CI)	No. of deaths	RR[2]	(95% CI)	No. of deaths	RR[2]	(95% CI)	No. of deaths	RR[2]	(95% CI)
Total mortality												
Q1	140	1.20	(0.81, 1.78)	102	2.05	(1.53, 2.75)	65	1.05	(0.63, 1.74)	62	1.18	(0.75, 1.85)
Q2	54	0.75	(0.45, 1.25)	87	1.34	(0.97, 1.86)	30	0.80	(0.47, 1.38)	104	1.22	(0.91, 1.63)
Q3	77	0.97	(0.67, 1.39)	127	1.05	(0.82, 1.35)	37	0.98	(0.57, 1.69)	101	0.99	(0.72, 1.35)
Q4	82	1.00	—	122	1.00	—	35	1.00	—	132	1.00	—
CVD mortality												
Q1	50	1.47	(0.81, 2.66)	29	1.30	(0.77, 2.21)	21	1.17	(0.61, 2.26)	22	0.75	(0.37, 1.51)
Q2	11	0.55	(0.26, 1.16)	36	1.23	(0.70, 2.15)	11	1.11	(0.49, 2.51)	36	0.84	(0.51, 1.39)
Q3	31	1.52	(0.79, 2.90)	62	1.28	(0.79, 2.06)	13	1.09	(0.45, 2.63)	41	0.79	(0.50, 1.25)
Q4	25	1.00	—	49	1.00	—	10	1.00	—	59	1.00	—
Cancer mortality												
Q1	46	1.49	(0.74, 3.01)	27	1.85	(0.99, 3.46)	17	0.73	(0.31, 1.76)	17	1.42	(0.64, 3.16)
Q2	22	1.09	(0.48, 2.49)	25	1.65	(0.89, 3.04)	11	0.81	(0.30, 2.22)	29	1.37	(0.85, 2.22)
Q3	23	0.88	(0.38, 2.04)	28	0.86	(0.50, 1.47)	14	1.10	(0.42, 2.89)	23	1.10	(0.61, 2.00)
Q4	26	1.00	—	32	1.00	—	13	1.00	—	32	1.00	—

[1] Quartiles of serum ascorbate concentration were derived from the entire cohort: men (<28.4, 28.4–51.0, 51.1–73.7, and ≥73.8 μmol/L) and women (<39.7, 39.7–68.0, 68.1–85.1, and ≥85.2 μmol/L). NHANES II, second National Health and Nutrition Examination Survey; RR, relative risk.

[2] Adjusted for age at baseline examination, race (African American versus other), highest attained educational level (<12 or ≥12 y), number of cigarettes smoked at baseline (in the model for smokers only), weekly frequency of alcohol consumption, diabetes (yes or no), serum total cholesterol, systolic blood pressure, and body mass index.

Our finding that total mortality was related to serum ascorbate concentrations in men is consistent with findings from previous studies. The only other prospective cohort study to examine the relation between vitamin C blood concentrations and total mortality found a decreased risk associated with higher quintiles of plasma vitamin C concentrations in 725 adults followed for 12 y (12). Separate risk estimates were not presented by sex so we were unable to confirm the sex differences that we found. Our results were consistent with those of 2 other studies that measured vitamin C intakes. Vitamin C intakes were associated with total mortality among participants in the NHANES I Epidemiologic Follow-up Study (NHEFS) followed for an average of 14 y (35). On the other hand, when risk was estimated by sex, vitamin C intakes >50 mg/d plus regular supplement use were protective compared with intakes <50 mg/d in men, but apparently not in women (13, 35). In a study of middle-aged men, Pandey et al (11) found an inverse association between vitamin C intakes and total mortality.

Our findings concerning CVD and cancer mortality were less consistent than were those of previous studies examining these same outcomes. Sahyoun et al (12) found a lower risk of CVD mortality in subjects in the highest and combined middle quintiles than in those in the lowest plasma ascorbate quintile and found no association with cancer mortality. However, the subjects in that study were older and probably healthier (12) than our representative sample of the US population. In another nationally representative cohort, the NHEFS, adults with the highest intakes also had a significantly lower risk of dying from CVD than did

TABLE 4
Distribution of cancer deaths according to site among men and women aged 30–75 y with no history of cardiovascular disease or cancer at baseline: NHANES II Mortality Study, 1976–1992[1]

	Men		Women	
Cancer site and ICD-9 code	No. of deaths	Percentage[2]	No. of deaths	Percentage[2]
		%		%
All sites	228	100	155	100
Digestive organs: 150–159.9	52	19.9	48	27.2
Larynx, lung, and pleura: 161–162.9	90	46.9	23	18.5
Lymphatic and hematopoietic tissue: 200–208.9	20	7.3	16	9.7
Prostate: 185	24	7.1	—	—
Breast: 174.9	0	0	24	20.1
Uterus and ovary: 179–183.9	—	—	16	9.9
Other: all other[3]	42	18.8	28	14.7

[1] ICD-9, International Classification of Diseases, Ninth Revision (23); NHANES II, second National Health and Nutrition Examination Survey.

[2] Weighted distribution.

[3] For all other cancer sites with <13 deaths reported for this cohort.

those with the lowest intakes (35). Yet, the NHEFS findings for cancer were consistent with ours; men, but not women, with the highest intakes had a significantly lower risk of dying from cancer than did their counterparts with the lowest intakes (13). The inverse relation found between vitamin C intake and cancer mortality risk in a study of middle-aged men (11) is also consistent with our findings. In another study, plasma ascorbate concentrations <22.7 μmol/L were associated with an increased risk of cancer mortality in a 17-y study of Swiss men (36). In contrast with our findings, vitamin C intakes were inversely associated with cancer mortality risk among elderly women but not men (20). Nevertheless, our findings support Carr and Frei's (15) conclusion that evidence from prospective studies generally suggests that low vitamin C intakes increase the risk of cancer. Carr and Frei also concluded that low vitamin C intakes increase the risk of CVD (15). Although our findings did not support this conclusion, vitamin C may actually have a protective effect against CVD. In our cohort, men with low serum ascorbate concentrations died from cancer, particularly from lung cancer, and thus may not have survived long enough for a protective effect of vitamin C on CVD mortality to be observed. Additionally, vitamin C may have had an effect on CVD morbidity that we were unable to detect because this study only assessed mortality.

One limitation of this study was that our measure of vitamin C status was based on a single serum ascorbate concentration. Because vitamin C is water soluble and thus is not stored for long periods in body tissues, a single measurement of serum ascorbate may indicate only the short-term (1–4 wk) vitamin C status of an individual (37). However, the vitamin C status of our cohort may have changed during the follow-up period, potentially leading to the misclassification of the long-term vitamin C status of some individuals. Such misclassification would have weakened the associations found in this study. Additionally, serum concentrations of other antioxidants were not measured in NHANES II; thus, we cannot be sure that the observed associations were due entirely to vitamin C. Indeed, serum ascorbate quartiles were directly associated with dietary vitamin E intakes, use of vitamin E supplements, and consumption of fruit and vegetables; adjustment of the risk estimates for total vitamin E intakes or fruit and vegetable intakes did not change our findings. This lack of change suggests that vitamin C has an independent effect on mortality. Another limitation of our study was its passive nature. Mortality was probably underestimated in the NHANES II Mortality Study (21), resulting in the misclassification of some participants. Because the serum ascorbate concentration was unlikely to be related to misclassification of vital status, risk estimates were probably unaffected; however, the statistical power may have been reduced (38).

Nonetheless, using a nationally representative sample with a wide range of serum ascorbate concentrations and a relatively long follow-up period, we found that men with serum ascorbate concentrations <28.4 μmol/L had a 57% higher risk of dying from any cause and a 62% higher risk of dying from cancer than did men with a concentration >73.8 μmol/L. Serum ascorbate concentrations considered to indicate the highest risk were at or below those corresponding to intakes of ≈60 mg vitamin C/d (15), the US RDA. Discussion concerning the role of antioxidants in chronic disease prevention has often focused on whether high antioxidant intakes are protective. Our findings as well as those of other studies suggest that, instead, we need to focus on the potential adverse effects of vitamin C intakes that are at or below those currently considered to be adequate.

REFERENCES

1. Peters KD, Kochanek KD, Murphy SL. Report of final mortality statistics, 1996. Vol 47. Hyattsville, MD: National Center for Health Statistics, 1998. (National Vital Statistics report no. 9.)
2. Diplock AT. Antioxidant nutrients and disease prevention: an overview. Am J Clin Nutr 1991;53(suppl):189S–93S.
3. Luc G, Fruchart JC. Oxidation of lipoproteins and atherosclerosis. Am J Clin Nutr 1991;53(suppl):206S–9S.
4. Frei B. Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage. Am J Clin Nutr 1991; 54(suppl):1113S–8S.
5. Schwartz CJ, Valente AJ, Sprague EA. A modern view of atherogenesis. Am J Cardiol 1993;71:9B–14B.
6. van Poppel G, van den Berg H. Vitamins and cancer. Cancer Lett 1997;114:195–202.
7. Rock CL, Jacob RA, Bowen PE. Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E, and the carotenoids. J Am Diet Assoc 1996;96:693–702.
8. Block G. Vitamin C and cancer prevention: the epidemiologic evidence. Am J Clin Nutr 1991;53(suppl):270S–82S.
9. Jacob RA. Vitamin C. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea and Febiger, 1994:432–48.
10. Simon JA. Vitamin C and cardiovascular disease: a review. J Am Coll Nutr 1992;11:107–25.
11. Pandey DK, Shekelle R, Selwyn BJ, Tangney C, Stamler J. Dietary vitamin C and β-carotene and risk of death in middle-aged men: The Western Electric Study. Am J Epidemiol 1995;142:1269–78.
12. Sahyoun NR, Jacques PF, Russell RM. Carotenoids, vitamins C and E, and mortality in an elderly population. Am J Epidemiol 1996;144:501–11.
13. Enstrom JE, Kanim LE, Klein MA. Vitamin C intake and mortality among a sample of the United States population. Epidemiology 1992;3:194–202.
14. Enstrom JE. Vitamin C in prospective epidemiological studies. In: Packer L, Fuchs J, eds. Vitamin C in health and disease. New York: Marcel Dekker, Inc, 1997:381–98.
15. Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. Am J Clin Nutr 1999;69:1086–107.
16. Pelletier O. Smoking and vitamin C levels in humans. Am J Clin Nutr 1968;21:1259–67.
17. Pelletier O. Vitamin C status of cigarette smokers and nonsmokers. Am J Clin Nutr 1970;23:520–4.
18. Kallner AB, Hartmann D, Hornig DH. On the requirements of ascorbic acid in man: steady-state turnover and body pool in smokers. Am J Clin Nutr 1981;34:1347–55.
19. Machlin LJ, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. FASEB J 1987;1:441–5.
20. Shibata A, Paganini-Hill A, Ross RK, Henderson BE. Intake of vegetables, fruits, beta-carotene, vitamin C and vitamin supplements and cancer incidence among the elderly: a prospective study. Br J Cancer 1992;66:673–9.
21. Loria CM, Sempos CT, Vuong C. Plan and operation of the NHANES II Mortality Study, 1992. Vital Health Stat 1 1999;38:1–16.
22. McDowell A, Engle A, Massey JT, Maurer K. Plan and operation of the second National Health and Nutrition Examination Survey 1976–1980. Vital Health Stat 1 1981;15:1–144.
23. World Health Organization. Manual of the international statistical classification of diseases, injuries, and cause of death. 9th rev. Geneva: World Health Organization, 1977.
24. Fulwood R, Johnson CL, Bryner JD. Hematological and nutritional biochemistry reference data for persons 6 months–74 years of age: United States, 1976–80. Vital Health Stat II 1982;232:1–173. Statistics, 1982.
25. Roe JH, Kuether CA. Determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J Biol Chem 1943;147:399–407.

26. National Center for Health Statistics. HANES II examination staff procedures: manual for the Health and Nutrition Examination Survey, 1976–1979. Part 15a. Hyattsville, MD: National Center for Health Statistics, 1976.
27. Carroll M, Sempos C, Briefel R, Gray S, Johnson C. Serum lipids of adults 20–74 years: United States, 1976–80. Vital Health Stat 11 1993;242.
28. National Center for Health Statistics. Public use data tape documentation. Total nutrient intake, food frequency, and other related dietary data. National Health and Nutrition Examination Survey, 1976–80. Hyattsville, MD: National Center for Health Statistics, 1989. (Tape no. 5701.)
29. Murphy SP, Subar AF, Block G. Vitamin E intakes and sources in the United States. Am J Clin Nutr 1990;52:361–7.
30. Levy AS, Schucker RE. Patterns of nutrient intake among dietary supplement users: attitudinal and behavioral correlates. J Am Diet Assoc 1987;87:754–60.
31. Shah BV, Barnwell BG, Bieler GS. SUDAAN user's manual, release 7.0. Research Triangle Park, NC: Research Triangle Institute, 1996.
32. SAS Institute Inc. SAS language and procedures: usage, version 6. 1st ed. Cary, NC: SAS Institute, Inc, 1989.
33. Weber C, Erl W, Weber K, Weber P. Increased adhesiveness of isolated monocytes to endothelium is prevented by vitamin C intake in smokers. Circulation 1996;93:1488–92.
34. Heitzer T, Just H, Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. Circulation 1996;94:6–9.
35. Enstrom JE. Vitamin C intake and mortality among a sample of United States population: new results. Stuttgart, Germany: Hippokrates Verlag, 1994.
36. Eichholzer M, Stahelin HB, Gey KF, Ludin E, Bernasconi F. Prediction of male cancer mortality by plasma levels of interacting vitamins: 17-year follow-up of the prospective Basel study. Int J Cancer 1996;66:145–50.
37. Bates CJ, Rutishauser HE, Black AE, Paul AA, Mandal AR, Patnaik BK. Long-term vitamin status and dietary intake of healthy elderly subjects 2. Vitamin C. Br J Nutr 1979;42:43–56.
38. Howe GR. Use of computerized record linkage in cohort studies. Epidemiol Rev 1998;20:112–21.

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