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Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis[1–4]

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ABSTRACT

Background: It has been suggested that a high dietary intake and high circulating concentrations of vitamin C may protect against ischemic heart disease.

Objectives: The objective was to examine the associations between dietary and plasma vitamin C concentrations, fruit and vegetable intakes, and markers of inflammation and hemostasis associated with cardiovascular disease in older men free of cardiovascular disease.

Design: This cross-sectional study examined 3258 men aged 60–79 y with no physician diagnosis of myocardial infarction, stroke, or diabetes and who were drawn from general practices in 24 British towns. Fruit and vegetable intakes and dietary vitamin C were assessed by using a food-frequency questionnaire.

Results: Plasma vitamin C, fruit intake, and dietary vitamin C intake were significantly and inversely associated with mean concentrations of C-reactive protein, an acute phase reactant, and tissue plasminogen activator (t-PA) antigen, a marker of endothelial dysfunction, even after adjustment for confounders. Vegetable intake was correlated significantly (inversely) only with t-PA. For plasma vitamin C (highest versus lowest quartile), the adjusted odds of elevated C-reactive protein and t-PA (highest tertile versus lowest tertile) were 0.56 (95% CI: 0.44, 0.71) and 0.79 (0.62, 1.00); for fruit intake, the corresponding odds ratios were 0.76 (0.60, 0.95) and 0.76 (0.61, 0.95). Plasma (but not dietary) vitamin C also showed inverse associations with both fibrinogen concentrations and blood viscosity. No associations were seen with von Willebrand factor or factor VIII.

Conclusion: The findings suggest that vitamin C has antiinflammatory effects and is associated with lower endothelial dysfunction in men with no history of cardiovascular disease or diabetes. *Am J Clin Nutr* 2006;83:567–74.

KEY WORDS Vitamin C, fruit and vegetable intakes, inflammation, hemostasis

INTRODUCTION

There is increasing evidence that oxidative stress plays an important role in the development and progression of atherosclerosis (1–3). Vitamin C is the main water-soluble nutrient antioxidant in human plasma (4), and it has been hypothesized that it protects against the development of ischemic heart disease (5). Several epidemiologic studies have shown inverse correlations between plasma vitamin C or vitamin C–rich dietary sources (such as fruit and vegetables) and the risk of cardiovascular disease (CVD), cancer, and all-cause mortality (6–10). On the other hand, randomized controlled trials of antioxidant supplements (including vitamin C) have shown no benefit (11, 12), and it has been suggested that the associations of vitamin C with CVD may be confounded by socioeconomic factors operating over the life course (12).

If vitamin C does protect against CVD, proposed possible potential mechanisms include its antioxidant properties and antiinflammatory effects (1–5). A few population studies have shown inverse relations between vitamin C or fruit and vegetable intakes and markers of activated coagulation and inflammation, including C-reactive protein (CRP); interleukin 6; fibrinogen and coagulation factors VII, VIII, and IX; prothrombin fragments F1 + 2; and thrombin-antithrombin complexes (13–17). A recent US intervention study showed that vitamin C supplements can reduce concentrations of CRP (18), which suggested an antiinflammatory effect of vitamin C. However, this was not observed in the Antioxidant Supplementation Atherosclerosis Prevention (ASAP) study in healthy men (19). To clarify the associations between vitamin C status and inflammatory and hemostatic factors, we have examined in a large population study of healthy men aged 60–79 y the relations between markers of vitamin C status (plasma vitamin C, dietary intake of vitamin C, consumption of fruit and vegetables, and use of vitamin C supplements) and several markers of inflammation and hemostasis that have been associated with greater risk of CVD (20). To avoid potential biases, men with diabetes, CVD, or both have been

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excluded from analyses because diagnosis of CVD or diabetes may lead to dietary changes, hemostatic and inflammatory markers are significantly higher in such men (21), and a large proportion of such men regularly take statins and aspirin, which may have antiinflammatory effects.

SUBJECTS AND METHODS

The British Regional Heart Study is a prospective study of CVD involving 7735 men aged 40–59 y who were selected from the age and sex registers of one general practice in each of 24 British towns and screened between 1978 and 1980 (22). Questionnaires measuring medical history and lifestyle changes were mailed to all survivors 5 y after screening (1983–1985), again in 1992, and in 1996. In 1998–2000, all surviving men, now aged 60–79 y, were invited for a 20-y follow-up examination.

At that examination, the men completed a questionnaire (Q20) providing information on their medical history, smoking and drinking habits, physical activity, and occupation and then presented for physical examination. Of the 5565 surviving subjects, 4252 (77%) presented for physical examination. All men were asked to provide a blood sample, which was collected with the use of the Sarstedt Monovette system (Sarstedt, Leicester, United Kingdom). On exclusion of 849 men with a recall of a doctor diagnosis of myocardial infarction, stroke, or diabetes and an additional 145 men who were taking warfarin, 3258 men were available for analyses.

All subjects provided written informed consent to the investigation, which was carried out in accordance with the Declaration of Helsinki. Ethical approval was provided by all relevant local research ethics committees.

Dietary vitamin C

Dietary intake data were obtained from a detailed 7-d-recall food-frequency questionnaire that was developed for use in the World Health Organization's Monitoring Trends and Determinants in Cardiovascular Disease Survey (23) and later for the Scottish Heart Health Study (24) and was validated against weighed intake in a Welsh (25) and a Scottish (26) population and against plasma vitamin C concentrations (24). Study participants were asked to recall their usual intake during the previous 7 d by reporting amounts and frequency of consumption of foods from a list that included 86 different types of food and drink. Nutrient intakes were calculated by using a validated program that multiplied the food frequency by the standard portion sizes for each food and by the nutrient composition of the foods obtained from the UK food-composition tables (27). In addition, men were asked a general question about how often they ate fresh fruit and vegetables in summer and winter and were asked to indicate the number of days that fruit or vegetables (or both) were consumed each week—rarely or never, monthly, or 1, 2, 3, 4, 5, 6, or 7 d/wk. They were also asked whether they regularly took any vitamins or mineral tablets. Information on the amount of vitamin C taken in supplements was not available. Supplement use was not included in the dietary vitamin C intake.

Plasma vitamin C

Plasma vitamin C was measured with HPLC that included ultraviolet and fluorescent detection (28, 29) by using extracts of plasma treated with metaphosphoric acid at the point of collection and then snap-frozen with dry ice. Laboratory-blinded split samples were used to ensure quality control throughout the study.

Hemostatic and inflammatory variables

Hemostatic and inflammatory variables were measured as described previously (30). Blood was anticoagulated with K_2 EDTA (1.5 mg/mL) for measurement of hematocrit, white cell count, and platelet count in an automated cell counter and of plasma viscosity at 37 °C in a semi-automated capillary viscometer (Coulter Electronics, Luton, United Kingdom). Blood viscosity was calculated from hematocrit and plasma viscosity. Blood was also anticoagulated with 0.109 mmol trisodium citrate/L (9:1 by vol:vol) for measurement of clottable fibrinogen (Clauss method); coagulation factors VII, VIII, and IX; and activated partial thromboplastin time and activated protein C resistance in an MDA-180 coagulometer (Organon Teknika, Cambridge, United Kingdom). Plasma concentrations of tissue plasminogen activator (t-PA) antigen and D-dimer were measured by using enzyme-linked immunosorbent assays (Biopool AB, Umea, Sweden) as was von Willebrand factor (vWF) antigen (DAKO plc, High Wycombe, United Kingdom). CRP was assayed by using ultra-sensitive nephelometry (Dade Behring, Milton Keynes, United Kingdom).

Lifestyle factors and medication

The methods of classifying smoking status, physical activity, body mass index (BMI; in kg/m^2), and social class were described in detail elsewhere (21, 30–32). The men were asked about current smoking status at screening and in all follow-up questionnaires. From the combined information at screening (Q1; 1978–80) and follow-up questionnaires, the men were classified at Q20 into 4 smoking groups: those who had never smoked, long-term (>15 y) ex-smokers, recent (≤15 y) ex-smokers, and current smokers (30). A physical activity score was derived for each man at Q20, and the men were grouped into 6 broad activity categories: inactive, occasional, light, moderate, moderately vigorous, and vigorous. In the analyses, the moderately vigorous and vigorous groups were combined (31). The longest-held occupation of each man was recorded and coded in accordance with the Registrar General's occupational classification into 6 social class groups: nonmanual I, II, and III and manual III, IV, and V. The armed forces formed a separate group. The men were asked to report the number of drinks of wine, beer, and spirits consumed each day in the previous 7 d, as part of a dietary assessment. Total alcohol intake was calculated by adding the total number of alcoholic beverages and categorized as none or <1 drink/wk, <1 drink/d, 1–2 drinks/d, 3–4 drinks/d, and ≥5 drinks/d and referred to as none, occasional, light, moderate, and heavy, respectively (32). Height and weight were both measured while the subjects were standing, and BMI was calculated for each subject. The men were asked if they were regularly taking any medication, and they were required to bring any such medications to the examination session. The medication was coded according to the British National Formulary codes (33). Men were considered to be taking antiinflammatory drugs if they used nonsteroidal antiinflammatory drugs, corticosteroids, aspirin, or statins.

TABLE 1
Baseline characteristics in 3258 men aged 60–79 y with no physician diagnosis of myocardial infarction, stroke, or diabetes and not taking warfarin

	Value
Age (y)	68.4 ± 5.49[2]
BMI (kg/m^2)	26.71 ± 3.56
Manual (%)	52.9
Current smokers (%)	13.4
Inactive (none/occasional) (%)	32.1
Heavy drinkers (≥5 drinks/d) (%)	7.9
Supplement vitamin C use (%)	10.0
Antiinflammatory drugs (%)	26.5
Daily fruit intake (%)	37.8
Daily vegetable intake (%)	26.0
Dietary vitamin C (mg/d)	82.1 (37.2)
Plasma vitamin C (μmol/L)	22.2 (14.0–40.1)[3]
C-reactive protein (mg/L)	1.65 (0.78–3.26)
White cell count (10^9/L)	6.75 (5.7–8.0)
Fibrinogen (g/L)	3.24 ± 0.73
Plasma viscosity (mPa s)	1.28 ± 0.08
Blood viscosity (mPa s)	3.40 ± 0.29
Factor VIII (IU/dL)	130.5 ± 31.4
vWF (IU/dL)	136.6 ± 44.9
t-PA (ng/mL)	10.73 ± 4.2
Fibrin D-dimer (ng/mL)	81.5 (48–122)

[1] vWF, von Willebrand disease; *t*-PA, tissue plasminogen activator.
[2] $\bar{x}$ ± SD (all such values).
[3] Geometric $\bar{x}$; interquartile range in parentheses (all such values).

Statistical analysis

The distributions of plasma vitamin C, white cell count, CRP, and fibrin D-dimer were highly skewed, and log transformation was used. The men were divided according to quartiles of the plasma and dietary vitamin C distribution. Analysis of covariance was used to obtain adjusted mean hemostatic and inflammatory markers for the 4 groups by fitting plasma and dietary vitamin C groups as categorical variables. Tests for trend were carried out by fitting plasma (dietary) vitamin C in their original continuous form using multiple regression analyses. Supplement use of vitamin was fitted as a dichotomous variable (yes or no). Logistic regression was used to obtain adjusted relative odds of having elevated concentrations of hemostatic or inflammatory markers, defined as the highest tertile of the distribution. Statistical analyses were performed by using SAS software (version 8.2; SAS Institute Inc, Cary NC).

RESULTS

Data on plasma and dietary vitamin C were not available for 239 and 54 men, respectively. The characteristics of the study sample are given in **Table 1**. Geometric mean (interquartile range) plasma vitamin C was 22.2 (14.0–40.1) μmol/L, and the mean ± SD dietary vitamin C was 82.1 ± 37.2 mg/d in men with no physician-diagnosed myocardial infarction, stroke, or diabetes. The correlation between dietary and plasma vitamin C was 0.26 ($P < 0.0001$).

Plasma and dietary vitamin C and correlates

The mean concentrations of plasma and dietary vitamin C by lifestyle factors and social class are shown in **Table 2**. Smoking, physical inactivity, manual social class, and—to a lesser extent—BMI were significantly associated with both dietary and plasma vitamin C. No association was seen between alcohol and plasma vitamin C, but a U-shaped relation was seen between alcohol and dietary vitamin C; nondrinkers and heavy drinkers had the lowest concentrations.

Plasma and dietary vitamin C and inflammatory and hemostatic markers

Age-adjusted partial correlations between plasma and dietary vitamin C and inflammatory and hemostatic markers are shown in **Table 3**. Both vitamin C measures showed significant inverse correlations with most of these markers. After adjustment for age, smoking, physical activity, alcohol intake, social class, and BMI, plasma vitamin C remained significantly inversely associated with CRP, fibrinogen, t-PA, and blood viscosity. Only CRP and t-PA remained significantly associated with dietary vitamin C. The adjusted mean concentrations of CRP, fibrinogen, t-PA, and blood viscosity by quartiles of plasma and dietary vitamin C are shown in **Table 4**.

Exclusion of men who were taking vitamin C supplements made little difference to the relations seen between dietary or plasma vitamin C and the hemostatic and inflammatory markers in Table 4. There was no difference in plasma vitamin C concentrations between users ($n = 863$) and nonusers ($n = 2395$) of antiinflammatory drugs, but subjects taking antiinflammatory drugs had significantly higher intakes of dietary vitamin C [85.5 (95% CI: 83.0, 88.1) mg/d; 80.9 (79.4, 82.4) mg/d; $P = 0.004$]. Further adjustment for the use of antiinflammatory drugs made little difference to the associations seen in Table 4.

Influence of smoking on vitamin C associations with inflammatory and hemostatic markers

Because smoking is strongly related to inflammatory and hemostatic factors (30), we examined the relations separately in smokers and nonsmokers. No significant interaction was seen between smoking and plasma vitamin C and CRP, fibrinogen, or t-PA. An inverse association with blood viscosity was seen in both smokers and nonsmokers, but the association was stronger in smokers (test for interaction, $P = 0.01$). No significant interaction was seen between dietary vitamin C and smoking and these factors.

Dietary fruit and vegetable intakes

A significant inverse relation was seen between dietary fruit intake and CRP, t-PA, and blood viscosity but not between dietary fruit intake and fibrinogen concentrations (**Table 5**). Vegetable intake was only inversely associated with t-PA. When fruit and vegetable intakes were entered simultaneously in the multivariate model, the relations between vegetable intake and t-PA was attenuated and no longer significant ($P = 0.26$), but the inverse associations seen with fruit intake remained significant ($P = 0.006$). The inverse associations between fruit intake and t-PA or blood viscosity remained significant even after further adjustment for plasma vitamin C ($P = 0.02$ and $P = 0.01$, respectively), but the association with CRP was of marginal significance ($P = 0.05$).

Vitamin supplements

Men taking vitamin C supplements ($n = 327$) had significantly higher geometric mean plasma vitamin C concentrations than did

TABLE 2
Subjects' lifestyle and social factors and mean concentrations of plasma and dietary vitamin C

	Plasma vitamin C	Dietary vitamin C
	μmol/L	*mg/d*
Smoking		
Never ($n = 1011$)	25.5 (16.6, 43.4)[2]	89.3 ± 37.0[3]
Long-term ex-smoker ($n = 1445$)	24.0 (16.2, 40.9)	84.7 ± 36.4
Recent ex-smoker ($n = 360$)	19.1 (10.6, 37.1)	78.4 ± 36.8
Current smoker ($n = 437$)	14.9 (8.4, 30.1)	66.6 ± 32.9
P for trend[4]	<0.0001	<0.0001
Physical activity		
None or occasional ($n = 1010$)	20.3 (12.4, 37.5)	76.4 ± 35.4
Light ($n = 616$)	21.8 (13.5, 39.2)	80.8 ± 36.1
Moderate ($n = 490$)	23.1 (15.5, 42.7)	84.3 ± 35.6
Moderately vigorous or vigorous ($n = 1030$)	25.0 (16.9, 43.4)	90.3 ± 37.9
P for trend[4]	<0.0001	<0.0001
BMI (kg/m^2)		
<25 ($n = 1043$)	23.8 (15.4, 44.2)	81.2 ± 36.1
25–27.4 ($n = 997$)	22.9 (14.4, 38.2)	84.2 ± 35.6
27.5–29.9 ($n = 736$)	20.9 (13.4, 39.7)	81.8 ± 37.2
≥30 ($n = 482$)	21.3 (13.0, 37.7)	87.5 ± 39.9
P for trend[4]	0.02	0.02
Alcohol intake		
None ($n = 666$)	21.1 (13.0, 39.8)	78.8 ± 37.7
Occasional ($n = 700$)	22.9 (15.1, 40.1)	84.7 ± 34.5
Light ($n = 1181$)	23.6 (15.4, 41.9)	86.0 ± 37.9
Moderate ($n = 416$)	22.4 (16.0, 40.3)	81.6 ± 35.5
Heavy ($n = 253$)	20.3 (11.9, 37.6)	76.7 ± 36.4
P for difference between groups[5]	0.14	<0.0001
Social class		
Nonmanual ($n = 1530$)	25.8 (17.5, 43.6)	87.6 ± 36.1
Manual ($n = 1721$)	19.9 (11.5, 37.2)	79.0 ± 37.0
P for difference[5]	<0.0001	<0.0001

[1] Subjects with missing data: smoking, $n = 5$; physical activity, $n = 112$; alcohol intake, $n = 42$; and social class, $n = 7$.
[2] Geometric $\bar{x}$; interquartile range in parentheses (all such values).
[3] $\bar{x} \pm$ SD (all such values).
[4] Test for trend derived from linear regression model. Smoking and physical activity groups were fitted continuously (values 1–4); BMI was fitted in its original continuous form.
[5] ANOVA for differences between groups.

men not taking supplements [35.2 (95% CI: 26.5, 53.9) and 21.1 (13.0, 38.4) μmol/L, respectively; $P < 0.0001$], as would be expected. Men taking vitamin C supplements had significantly lower mean concentrations of CRP than did men not taking supplements, even after adjustment for confounders [adjusted geometric $\bar{x}$: 1.40 (95% CI: 1.23, 1.57) and 1.67 (1.60, 1.72) mg/L, respectively; $P = 0.01$ for difference]. They also showed significantly lower fibrinogen concentrations [3.14 (3.06, 3.22) and 3.24 (3.21, 3.27) mg/L, respectively; $P = 0.03$) and lower blood viscosity [3.35 (3.31, 3.38) and 3.40 (3.39, 3.41) mPa s, respectively; $P = 0.003$], which is consistent with the relations seen for plasma vitamin C. The t-PA concentrations did not differ significantly between the men taking vitamin C supplements [10.77 (10.59, 10.89) ng/mL] and those not taking the supplements [10.74 (10.59, 10.89) ng/mL].

TABLE 3
Age-adjusted Spearman partial correlation coefficients between plasma and dietary vitamin C and hemostatic and inflammatory markers[1]

	Correlation coefficients	
	Plasma vitamin C (μmol/L)	Dietary vitamin C (mg/d)
C-reactive protein (mg/L)[2]	−0.16[3]	−0.10[3]
White cell count (10^9/L)[2]	−0.05[4]	−0.03[4]
Fibrinogen (g/L)	−0.14[5]	−0.05[4]
Plasma viscosity (mPa s)	−0.10[5]	−0.04[4]
Blood viscosity (mPa s)	−0.10[5]	−0.03
Factor VIII (IU/dL)	−0.01	−0.05[4]
vWF (IU/dL)	−0.06[5]	−0.04[4]
t-PA (ng/mL)	−0.08[5]	−0.08[5]
Fibrin D-dimer (ng/mL)[2]	−0.05[4]	−0.03

[1] vWF, von Willebrand factor; t-PA, tissue plasminogen activator.
[2] Log transformed.
[3] $P < 0.001$.
[4] $P < 0.05$.
[5] $P < 0.01$.

Elevated CRP and t-PA

We also examined the odds of being in the highest tertile of CRP, which represents concentrations associated with significantly greater risk of CHD (34). The relative odds of elevated CRP decreased significantly with increasing concentrations of plasma vitamin C and, to a lesser extent, with increasing dietary intakes of vitamin C and fruit (**Table 6**). The relative odds of having elevated t-PA increased significantly with increasing dietary vitamin C and fruit intakes, but the trend for plasma vitamin C was less consistent and not statistically significant.

TABLE 4
Quartiles of vitamin C (plasma and dietary) and mean concentrations C-reactive protein (CRP), fibrinogen tissue plasminogen activator (t-Pa), and blood viscosity adjusted for age, smoking, social class, alcohol intake, and BMI[1]

	Quartiles of plasma vitamin C (μmol/L)					Quartiles of dietary vitamin C (mg/d)				
	<14.44 (*n* = 754)	14.44 to <27.11 (*n* = 755)	27.11 to <40.25 (*n* = 755)	≥40.25 (*n* = 755)	*P* for trend[2]	<54.6 (*n* = 796)	54.6 to <77.2 (*n* = 800)	77.2 to <103.7 (*n* = 804)	≥103.7 (*n* = 802)	*P* for trend
CRP (mg/L)	1.88 (1.73, 2.03)	1.73 (1.60, 1.86)	1.52 (1.40, 1.63)	1.34 (1.23, 1.44)	<0.0001	1.78 (1.65, 1.92)	1.68 (1.56, 1.80)	1.50 (1.39, 1.62)	1.55 (1.45, 1.68)	0.02
Fibrinogen (g/L)	3.30 (3.26, 3.36)	3.29 (3.24, 3.34)	3.18 (3.13, 3.23)	3.12 (3.07, 3.17)	<0.0001	3.23 (3.18, 3.28)	3.25 (3.20, 3.30)	3.21 (3.16, 3.26)	3.23 (3.18, 3.28)	0.75
t-PA (ng/mL)	10.92 (10.63, 11.21)	10.66 (10.38, 10.93)	10.70 (10.42, 10.99)	10.31 (10.03, 10.60)	0.01	11.14 (10.86, 11.43)	10.56 (10.28, 10.84)	10.62 (10.34, 10.91)	10.62 (10.34, 10.90)	0.005
Blood viscosity (mPa s)	3.41 (3.39, 3.44)	3.41 (3.39, 3.43)	3.40 (3.38, 3.43)	3.35 (3.33, 3.37)	<0.0001	3.40 (3.38, 3.42)	3.40 (3.38, 3.42)	3.40 (3.37, 3.41)	3.39 (3.37, 3.41)	0.54

[1] All values are geometric $\bar{x}$; 95% CI in parentheses. $n = 239$ men with missing data on plasma vitamin and 54 men with missing data on dietary vitamin C. BMI measured in kg/m^2.
[2] Derived from multiple regression analysis fitting plasma (dietary) vitamin C in its original continuous form with adjustment for age (in y), smoking (never smoked, ex-smoker for >15 y, ex-smoker for ≤15 y or current smoker), physical activity (none or occasional, light, moderate, or moderately vigorous or vigorous), BMI, alcohol intake (none, occasional, light, moderate, or heavy), and social class (manual or nonmanual).

TABLE 5
Fruit and vegetable intakes, mean plasma vitamin C, and mean concentrations of C-reactive protein (CRP), tissue plasminogen activator (t-PA), and blood viscosity adjusted for age, smoking, social class, alcohol intake, and BMI[1]

	C-reactive protein											
	Fruit intake (d/w)						Vegetable intake (d/w)					
	<1 (*n* = 286)	1–2 (*n* = 436)	3–4 (*n* = 584)	5–6 (*n* = 609)	7 (*n* = 1164)	*P* for trend[2]	<1 (*n* = 95)	1–2 (*n* = 383)	3–4 (*n* = 886)	5–6 (*n* = 947)	7 (*n* = 810)	*P* for trend[2]
Plasma vitamin C (μmol/L)[3]	15.3 (13.7, 16.9)	18.2 (16.8, 19.7)	19.5 (18.0, 20.9)	24.5 (23.3, 26.3)	27.9 (26.6, 29.4)	<0.0001	14.9 (12.3, 18.0)	18.9 (17.5, 20.9)	20.7 (19.7, 22.2)	24.5 (23.3, 26.3)	25.3 (24.1, 27.1)	<0.0001
CRP (mg/L)[3]	1.84 (1.62, 2.08)	1.72 (1.55, 1.90)	1.68 (1.54, 1.82)	1.55 (1.42, 1.68)	1.51 (1.42, 1.62)	0.002	1.73 (1.39, 2.16)	1.55 (1.39, 1.73)	1.69 (1.58, 1.82)	1.63 (1.52, 1.73)	1.51 (1.40, 1.63)	0.15
t-PA (ng/mL)	11.13 (10.65, 11.61)	11.11 (10.73, 11.5)	10.98 (10.65, 11.31)	10.18 (9.86, 10.50)	10.59 (10.36, 10.82)	0.001	11.37 (10.53, 12.20)	11.00 (10.59, 11.40)	10.87 (10.58, 11.11)	10.46 (10.20, 10.71)	10.69 (10.40, 10.96)	0.03
Blood viscosity (mPa s)	3.44 (3.41, 3.49)	3.42 (3.39, 3.44)	3.40 (3.38, 3.43)	3.36 (3.34, 3.39)	3.38 (3.37, 3.40)	0.001	3.39 (3.36, 3.48)	3.43 (3.39, 3.45)	3.39 (3.37, 3.41)	3.39 (3.37, 3.41)	3.40 (3.40, 3.38)	0.35

[1] All values are $\bar{x}$; 95% CI in parentheses (unless indicated otherwise). There are 178 missing data on fruit intake and 137 missing data on vegetable intake. Data on fibrinogen are not shown.
[2] Trend across the groups was derived from multiple regression analysis with the fruit and vegetable intake categories fitted as a continuous ordinal variable (1–5) after adjustment for age (y), smoking [never smoked, long-term (≥15 y) ex-smoker, recent (≤15 y) ex-smoker, and current smoker], physical activity (none or occasional, light, moderate, or moderately vigorous or vigorous), BMI (kg/m^2), alcohol intake (none, occasional, light, moderate, or heavy), and social class (manual or nonmanual).

TABLE 6
Plasma and dietary vitamin C by quartile, fruit intake, and adjusted odds ratio (95% CI) of elevated C-reactive protein (CRP) and tissue plasminogen activator (t-PA) (highest third)

	High CRP (n = 1057)	High t-PA (n = 1072)
Plasma vitamin C (μmo/L)		
<14.44 (n = 754)	1.00	1.00
14.44 to <27.11 (n = 755)	0.87 (0.69,1.09)	0.82 (0.65,1.04)
27.11 to <40.25 (n = 755)	0.79 (0.63,0.99)	0.94 (0.74,1.19)
≥40.25 (n = 755)	0.56 (0.44,0.71)	0.79 (0.62,1.00)
P for trend[1]	<0.0001	0.14
Dietary vitamin C (mg/d)		
<54.6 (n = 796)	1.00	1.00
54.6 to <77.2 (n = 800)	0.99 (0.79,1.24)	0.89 (0.71,1.11)
77.2 to <103.7 (n = 804)	0.77 (0.61,0.97)	0.78 (0.63,0.99)
≥103.7 (n = 802)	0.81 (0.64,1.03)	0.76 (0.60,0.96)
P for trend[1]	0.02	0.01
Fruit intake (d/wk)		
<3 (n = 722)	1.00	1.00
3–4 (n = 584)	0.95 (0.74,1.23)	0.85 (0.66,1.09)
5–6 (n = 609)	0.86 (0.66,1.10)	0.64 (0.49,0.77)
7 (n = 1164)	0.76 (0.60,0.95)	0.76 (0.61,0.95)
P for trend[1]	0.007	0.02

[1] Trend across the groups was derived from multiple logistic regression analysis with adjustment for age (y), smoking [never smoked, long-term (>15 y) ex-smoker, recent (≤15 y) ex-smoker, and current smoker], physical activity (none or occasional, light, moderate, or moderately vigorous or vigorous), BMI (kg/m^2), alcohol intake (none, occasional, light, moderate, or heavy), and social class (manual or nonmanual).

DISCUSSION

In this large random sample of men aged 60–79 y, we confirmed the findings of previous population studies (6, 13–17), most of which were carried out in smaller samples, that plasma vitamin C and dietary intakes of vitamin C are inversely associated with some markers of the acute phase response and hemostasis that have been associated with greater risk of CVD and nonvascular disease (20). The correlation between plasma vitamin C and dietary intakes of vitamin C in the current study, although significant, was modest (r = 0.26) but similar to that observed in the Norfolk European Prospective Investigation of Cancer (r = 0.28; 35) and the Medical Research Council Trial of Assessment and Management of Older People in the Community (r = 0.3; 36). However, both measures showed significant inverse associations with CRP and t-PA, and plasma vitamin C was also significantly associated with fibrinogen and blood viscosity, each of which is a marker of low-grade inflammation (20) after adjustment for age, smoking, physical activity, alcohol intake, BMI, and social class, factors that are associated with inflammation and hemostasis (12, 30–32). Although it is possible that the relation between vitamin C and inflammation is due to residual confounding caused by inadequate adjustment for the complexity of social class acting across the life course (12), adult social class alone had little effect on the relation between vitamin C and inflammation, and adjustment for father's social class in this study had little effect on the relations. We conclude that the inverse associations of vitamin C with low-grade inflammation do not appear to be due to confounding by the effects of CVD risk factors or social class (12). There are known regional variations in diet in the United Kingdom, with significant north-south differences, particularly with respect to vitamin C (37). However, the associations were unchanged after further adjustment for each of the 24 towns.

It has been proposed that low-grade inflammation may promote CVD partly by endothelial disturbance (38), markers of which include vWF and t-PA. Circulating vWF is a carrier of coagulation factor VIII, and we previously showed significant correlations between plasma vWF, factor VIII, and risk of ischemic heart disease (39), whereas inverse associations of factor VIII with serum and dietary vitamin C have been reported (13). In the current study, we observed significant associations between dietary vitamin C and plasma concentrations of vWF and factor VIII after adjustment for age but not after multivariate adjustment. However, we also report significant inverse associations between both dietary and plasma vitamin C and mean concentrations of plasma t-PA antigen, a marker of endothelial disturbance that is also a risk predictor for ischemic heart disease (40) in men with no history of CVD or diabetes. Population studies of vitamin C and t-PA are limited. One small trial reported inverse associations between vitamin C and t-PA in healthy male subjects, and t-PA also tended to be lower with vitamin C supplementation at 2 g/d, although the difference was not significant, possibly because of the small study size and lack of power to detect a difference (41).

Several studies have reported that vitamin C can improve endothelial dysfunction in smokers (42, 43), hypertension patients (44), and patients with coronary artery disease (45). Our finding is therefore consistent with a protective effect of vitamin C on endothelial function, which could be mediated by the effects of vitamin C on low-grade inflammation, effects that may result from its antioxidant properties.

To further study the associations of vitamin C intake with markers of inflammation and hemostasis, we analyzed their associations with dietary intakes of fruit and vegetables. Fruit intakes in particular had significant inverse associations with CRP, blood viscosity, and t-PA. These findings are consistent with those from a previous report (46) in which low fruit intakes were associated with both low-grade inflammation and an edentulous state (which increases CVD risk), but we found that the inverse associations between fruit intakes and t-PA and blood viscosity remained significant even after adjustment for plasma vitamin C, whereas the association with CRP was of marginal significance. This observation raises the possibility that other components of dietary fruit may have protective effects against inflammation and endothelial disturbance. Finally, we observed that men taking vitamin C supplements had significantly lower concentrations of CRP and fibrinogen and blood viscosity (but not t-PA) after adjustment for confounders.

Although this study population is large and broadly representative on a social and geographic basis, it is not strictly a random population sample, because it is influenced by survival and response, both of which tend to lead to underrepresentation of selected groups of persons such as smokers, obese subjects, or persons with ill health (47). However, although this underrepresentation may affect the average concentrations of the inflammatory and hemostatic markers in the population, there is no reason to believe that it should affect the relations between vitamin C and the biological markers studied in men with no CVD or diabetes. A further limitation of the study is the single characterization of vitamin C exposures. Although these exposures

correlate to a degree similar to that in previous reports (35, 36), the measurements of vitamin C (particularly dietary vitamin C) are imprecise, so that the strengths of association with inflammatory and hemostatic markers may well have been underestimated (48).

This study was based on men, who were almost entirely of white origin. We cannot directly generalize our findings to other ethnic groups or to women, although it appears likely from other studies that fruit intakes are related to lower CRP in women (16). Extending the results to subjects with CVD or diabetes is potentially complicated by the problems of dietary change, raised inflammatory markers, and the use of antiinflammatory medications in this group. However, repeating the analyses including men with CVD and diabetes did not materially affect the results observed, which suggests that similar associations may apply in these subjects; however, this cannot be reliably examined in the current study.

Although consistent with a direct antiinflammatory effect of vitamin C, the current study was not a randomized trial. Two published randomized trials of vitamin C have produced conflicting results with respect to a reduction in CRP concentrations (18, 19). Furthermore, larger randomized trials of the effect of vitamin C supplementation on markers of inflammation and hemostasis would help to resolve this uncertainty. The results of the current study suggest that, in addition to CRP, outcome measurements should include fibrinogen, viscosity, and t-PA antigen.

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SGW and GDOL contributed to the study concept and data analysis and drafted the manuscript. PHW was responsible for the 20-y re-screening of the study population and (with KRB) for raising the funds for measurement of plasma vitamin C. GDOL and AR were responsible for the measurements of the inflammatory and hemostatic markers, and KRB was responsible for the measurements of plasma vitamin C. All authors contributed to the writing and revision of the manuscript. None of the authors had a personal or financial conflict of interest.

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