Antioxidant vitamin status and carotid atherosclerosis in the elderly[1–3]

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ABSTRACT

Background: The oxidative modification of LDL is thought to play a crucial role in the initiation of atherosclerosis. Antioxidant vitamins can protect LDL from oxidation, and high intakes or blood concentrations of these vitamins have been linked with a reduced risk of cardiovascular disease. Few data are available on the importance of antioxidant vitamins in earlier stages of atherogenesis.

Objective: We investigated the cross-sectional relation between antioxidant vitamin status and carotid atherosclerosis in a group of elderly persons.

Design: The study sample comprised 468 men and women aged 66–75 y living in Sheffield, United Kingdom. Duplex ultrasonography was used to measure intima-media thickness and the degree of stenosis in the extracranial carotid arteries. Antioxidant vitamin status was assessed by measuring fasting plasma concentrations of vitamin C, vitamin E, and β-carotene.

Results: In the men, after adjustment for age and cardiovascular disease risk factors, a 20% higher plasma vitamin C concentration was associated with a 0.004-mm smaller intima-media thickness; a 20% higher β-carotene concentration was associated with a 0.005-mm smaller intima-media thickness. Compared with men with high blood concentrations of β-carotene or cholesterol-adjusted vitamin E, those with low blood concentrations of these vitamins were 2.5 times as likely to have carotid stenosis of >30%. We found no significant trends between plasma concentrations of antioxidant vitamins and either measure of carotid atherosclerosis in the women.

Conclusion: A high antioxidant vitamin status may help to prevent the initiation and progression of early atherosclerotic lesions in men. *Am J Clin Nutr* 2001;74:402–8.

KEY WORDS Carotid artery disease, atherosclerosis, dietary antioxidants, vitamin C, vitamin E, β-carotene, elderly, low-density lipoprotein, LDL, oxidation

INTRODUCTION

The oxidative modification of LDL is thought to play a crucial role in the initiation of atherosclerosis (1). Oxidized LDL is rapidly taken up by macrophages, causing lipid accumulation and the formation of foam cells; it is chemotactic for monocytes and T lymphocytes, causes increased production of inflammatory cytokines and growth factors, promotes procoagulant activity, and impairs arterial vasomotor responses (2, 3). Findings of a strong association between measurements of lipoprotein oxidation and accelerated thickening of the carotid artery wall in middle-aged men provide direct evidence of the importance of lipoprotein oxidation in atherogenesis (4).

Over the past decade, interest has grown in the possibility that foods rich in antioxidant vitamins may reduce the risk of atherosclerotic disease by protecting LDL from oxidative modification. Supplements of vitamin E, vitamin C, β-carotene, or a combination of antioxidants were shown to inhibit lipid peroxidation ex vivo (5–8). Higher intakes or blood concentrations of these vitamins were associated with a reduced risk of stroke or coronary artery disease in several observational investigations (9–15), but few studies examined the link between antioxidant vitamin status and earlier stages of atherosclerosis.

Duplex ultrasonography is a noninvasive, specific, and sensitive technique for detecting atherosclerosis in the extracranial carotid arteries. Increases in carotid intima-media thickness are associated with the presence of atherosclerotic plaque at other sites in the arterial system and predict myocardial infarction and stroke in asymptomatic adults (16–18). We assessed intima-media thickness and degree of stenosis in a group of elderly men and women. Our aim was to investigate the relation between plasma concentrations of antioxidant vitamins and the extent of carotid atherosclerosis.

SUBJECTS AND METHODS

Subjects

In recent years, the MRC Environmental Epidemiology Unit has carried out several studies in cohorts of persons born in the Jessop Hospital for Women in Sheffield, United Kingdom. These

persons were traced by using the National Health Service Central Register, and those still living in the city were invited to take part in research into the processes by which environment in early life influences adult disease.

We examined the cross-sectional relation between antioxidant vitamin status and carotid atherosclerosis by using 2 data sets from these MRC studies. The participants were aged 66–75 y. In the first data set, 288 men and women were invited to participate and 212 (74%) agreed to be interviewed at home by a fieldworker. Of these, 146 (69%) were willing to attend a clinic. In the second data set, 606 men and women were invited to participate and 353 (58%) agreed to be interviewed. Of these, 334 (95%) were willing to attend a clinic. The study was approved by the North Sheffield Local Research Ethics Committee and all participants gave written, informed consent.

Measurements

During the interview, the fieldworker administered the Rose/WHO Cardiovascular Questionnaire (19) and inquired about history of cardiovascular disease, smoking habits, current medication use, use of vitamin supplements, and the most recent occupation of the participant or her husband. Height, weight, and blood pressure were measured.

A 12-lead electrocardiogram was then recorded at the clinic and fasting venous blood samples were taken for measurement of plasma concentrations of vitamin C, vitamin E, β-carotene, and fibrinogen and serum concentrations of total, HDL, and LDL cholesterol. Plasma samples for vitamin C analysis were stabilized in 5% metaphosphoric acid immediately after collection. All samples were stored at $-70\,°C$ for later analysis. Plasma total vitamin C was measured fluorometrically by using a Cobas Bioautoanalyser (Roche Products, Welwyn Garden City, United Kingdom) (20). Plasma concentrations of vitamin E (as α-tocopherol) and β-carotene were measured by HPLC (21). All measurements had an interbatch precision of <8%, with the exception of β-carotene, for which the interbatch precision was 23%. In-house quality controls were used for all analytes.

The participants underwent a color duplex ultrasonographic examination of the carotid arteries with an HDI3000 high-resolution, real-time scanner equipped with a 10-5 MHz linear array probe (Advanced Technology Laboratories, Letchworth, United Kingdom). The ultrasonographer, who had not seen the data on plasma antioxidant concentrations or other risk factors, examined the common, internal, and external carotid arteries and the carotid bifurcation in the longitudinal and transverse planes and estimated the maximum degree of stenosis as a percentage of lumen diameter loss on each side. Four categories were defined: no plaques, ≤30% stenosis, 31–50% stenosis, and >50% stenosis. The intima-media thickness of the far wall was measured 3 times in the common carotid artery (as far proximally as possible, 1 cm proximal to the bifurcation and midway between these 2 sites) and twice in the internal carotid artery (the proximal internal carotid artery and 1 cm distal to the bifurcation) on each side. Areas of obvious plaque were avoided.

Statistical analysis

We analyzed both data sets together using analysis of variance, the chi-square test, and linear and logistic regression (SPSS for WINDOWS, version 9; SPSS Inc, Chicago). Intima-media thickness was defined as the average of all measurements made in the right and left common carotid and internal carotid arteries. Degree of stenosis was defined as the maximum percentage of stenosis observed on either side. Participants in the first data set tended to have more severe carotid atherosclerosis than did those in the second data set. We adjusted for this difference in the analysis. If the participants reported a history of stroke or transient ischemic attack, this was confirmed from the general practitioners' records. The presence of coronary artery disease was defined by electrocardiography (Minnesota codes Q and QS as developed by the University of Minnesota, Minneapolis), by the participant's medical history (coronary artery bypass grafting or coronary angioplasty), or by a positive response on the Rose/WHO Cardiovascular Questionnaire.

Because plasma vitamin E concentrations are influenced by lipid concentrations, all values for vitamin E vitamin are expressed per unit of total cholesterol. Use of supplementary vitamin C and vitamin E was defined as use of preparations containing either vitamin C or vitamin E alone or in combination with other nutrients. Distributions of blood concentrations of antioxidant vitamins and HDL cholesterol were skewed and were log-transformed. We excluded 12 men who were taking high doses of antioxidant vitamin supplements as part of a trial of the effectiveness of these vitamins in secondary prevention (the MRC/BHF Heart Protection Study) or as self-medication after a heart attack. The analysis is therefore based on 468 men and women.

RESULTS

The characteristics of the study population are shown in **Table 1**. There were significant differences between the sexes in blood pressure, smoking habits, alcohol intake, and concentrations of plasma fibrinogen and serum total, HDL, and LDL cholesterol. The women tended to come from a higher socioeconomic class than did the men, they were more likely to be taking supplements containing vitamin E, and they had significantly higher plasma concentrations of β-carotene and vitamin C. Carotid atherosclerosis, whether assessed by intima-media thickness or degree of stenosis, was more severe in the men.

The relation between intima-media thickness and conventional cardiovascular disease risk factors as determined by linear regression is shown in **Table 2**. In the men, there were significant associations between intima-media thickness and age, systolic blood pressure, pulse pressure, hypertension (systolic blood pressure ≥160 mm Hg or current treatment with antihypertensive drugs), smoking status, and serum total and LDL cholesterol. In the women, age, systolic blood pressure, pulse pressure, and hypertension were the only risk factors significantly associated with intima-media thickness, although there was some evidence that mean intima-media thickness was smaller in women who drank alcohol than in those who did not. When we examined the relation between degree of stenosis and cardiovascular disease risk factors, the pattern of risk factors was the same, with the exception of smoking status in the women: although there was no significant association between smoking and intima-media thickness, the women who were current or former smokers were more likely to have carotid arteries that were narrowed by plaque than were the women who were nonsmokers.

In the men, there were significant trends in intima-media thickness corresponding to plasma concentrations of antioxidant vitamins (**Table 3**). Intima-media thickness rose as concentrations of vitamin C, β-carotene, and vitamin E (relative to total cholesterol) decreased. After adjustment for age and other cardiovascular

TABLE 1
Characteristics of the study population[1]

Characteristic	Men (n = 264)	Women (n = 204)	P
Age (y)	70.2 ± 2.0	70.2 ± 2.1	0.987
BMI (kg/m^2)	26.8 ± 3.9	27.5 ± 5.2	0.104
Systolic blood pressure (mm Hg)	145.8 ± 20.6	141.2 ± 21.7	0.019
Diastolic blood pressure (mm Hg)	81.6 ± 10.1	78.6 ± 9.8	0.002
Pulse pressure (mm Hg)	64.0 ± 15.2	62.6 ± 16.0	0.302
Current or former smoker [n (%)]	206 (78)	117 (57)	<0.001
Total cholesterol (mmol/L)	5.9 ± 1.2	6.4 ± 1.2	<0.001
LDL cholesterol (mmol/L)	4.0 ± 1.1	4.3 ± 1.1	0.002
HDL cholesterol (mmol/L)[2]	1.2 ± 1.3	1.3 ± 1.3	<0.001
Fibrinogen (μmol/L)	9.1 ± 1.7	9.9 ± 1.7	<0.001
Vitamin C (μmol/L)[2]	28.7 ± 2.7	39.0 ± 2.4	0.001
Vitamin E/cholesterol (μmol/L)[2]	5.5 ± 1.3	5.4 ± 1.4	0.457
β-Carotene (μmol/L)[2]	0.18 ± 2.3	0.28 ± 2.2	<0.001
User of vitamin C supplements [n (%)]	30 (11)	29 (14)	0.357
User of vitamin E supplements [n (%)]	49 (19)	56 (28)	0.022
Alcohol intake [n (%)][3]			
None	43 (16)	29 (14)	—
≤14 units/wk	129 (49)	147 (72)	—
>14 units/wk	92 (35)	28 (14)	<0.001
Manual social class [n (%)]	132 (50)	79 (38)	0.011
History of stroke or TIA [n (%)]	18 (7)	10 (5)	0.380
History of coronary artery disease [n (%)]	65 ± 25	45 ± 22	0.521
Intima-media thickness (mm)	0.867 ± 0.156	0.788 ± 0.133	<0.001
Degree of stenosis [n (%)]			
None	35 (13)	50 (25)	<0.001
≤30%	129 (49)	92 (45)	—
31–50%	89 (34)	56 (27)	—
>50%	11 (4)	6 (3)	—

[1] $\overline{x}$ ± SD unless stated otherwise. TIA, transient ischemic attack.
[2] Geometric $\overline{x}$ ± SD.
[3] 1 unit = 8 g ethanol.

disease risk factors, the relation between intima-media thickness and vitamin E was of borderline significance, but the associations with plasma vitamin C and β-carotene persisted. A 20% higher plasma vitamin C concentration was associated with a 0.004-mm lower intima-media thickness (P = 0.023). A 20% higher plasma β-carotene concentration was associated with a 0.005-mm lower intima-media thickness (P = 0.028). These associations remained significant after the exclusion of those taking supplements either of multivitamins or of vitamins E or C alone.

Because men with symptomatic cardiovascular disease might have changed their diets in response to their illness, we examined the relations between intima-media thickness and antioxidant

TABLE 2
Relation between carotid intima-media thickness (mm) and conventional cardiovascular disease risk factors

Risk factor	Men		Women	
	Regression coefficient (95% CI)	P	Regression coefficient (95% CI)	P
Age (y)	0.021 (0.01, 0.03)	<0.001	0.013 (0.005, 0.022)	0.002
BMI (kg/m^2)	0.001 (−0.004, 0.006)	0.637	0.001 (−0.002, 0.005)	0.421
Systolic blood pressure (mm Hg)	0.002 (0.001, 0.003)	<0.001	0.002 (0.001, 0.003)	<0.001
Diastolic blood pressure (mm Hg)	0.001 (−0.001, 0.003)	0.368	0.001 (−0.001, 0.003)	0.372
Pulse pressure (mm Hg)	0.003 (0.002, 0.004)	<0.001	0.003 (0.002, 0.004)	<0.001
Hypertension[1]	0.077 (0.041, 0.114)	<0.001	0.079 (0.040, 0.115)	<0.001
Current or former smoker	0.085 (0.040, 0.129)	<0.001	0.011 (−0.026, 0.049)	0.549
Total cholesterol (mmol/L)	0.017 (0.002, 0.033)	0.029	−0.007 (−0.022, 0.009)	0.393
LDL cholesterol (mmol/L)	0.017 (0.002, 0.035)	0.052	−0.006 (−0.023, 0.011)	0.482
HDL cholesterol (mmol/L)[2]	0.009 (−0.056, 0.074)	0.783	−0.032 (−0.099, 0.035)	0.353
Fibrinogen (μmol/L)	0.0003 (−0.00005, 0.007)	0.090	0.0001 (−0.0002, 0.0005)	0.412
Alcohol intake (units/wk)				
1–14	−0.04 (−0.09, 0.02)	0.158	−0.07 (−0.13, –0.02)	0.013
>14	−0.04 (−0.10, 0.02)	0.165	−0.06 (−0.14, 0.01)	0.099
Manual social class	0.024 (−0.013, 0.062)	0.203	0.0002 (−0.039, 0.039)	0.989

[1] Defined as systolic blood pressure ≥160 mm Hg or current treatment with antihypertensive drugs.
[2] Log-transformed variable.

TABLE 3
Relation between carotid intima-media thickness (mm), plasma concentrations of antioxidant vitamins, and the use of vitamin supplements

	Regression coefficient (95% CI), unadjusted	P	Regression coefficient (95% CI), multivariate-adjusted[1]	P	Regression coefficient (95% CI), multivariate-adjusted, excluding supplement users[1]	P
Men						
Vitamin E/cholesterol (μmol/mmol)[2]	−0.069 (−0.134, −0.004)	0.038	−0.053 (−0.115, 0.010)	0.097	−0.144 (−0.295, 0.008)	0.063
Vitamin C (μmol/L)[2]	−0.020 (−0.039, −0.001)	0.039	−0.021 (−0.039, −0.003)	0.023	−0.026 (−0.046, −0.006)	0.012
β-Carotene (μmo/L)[2]	−0.026 (−0.050, −0.002)	0.035	−0.027 (−0.051, −0.003)	0.028	−0.028 (−0.055, −0.001)	0.042
Vitamin C supplement use	0.009 (−0.051, 0.069)	0.770	−0.002 (−0.057, 0.053)	0.934	—	—
Vitamin E supplement use	−0.020 (−0.068, 0.028)	0.406	−0.017 (−0.062, 0.029)	0.477	—	—
Women						
Vitamin E/cholesterol (μmol/mmol)[2]	0.023 (−0.038, 0.085)	0.450	0.015 (−0.043, 0.073)	0.613	0.004 (−0.075, 0.084)	0.915
Vitamin C (μmol/L)[2]	−0.010 (−0.031, 0.010)	0.322	−0.006 (−0.026, 0.014)	0.562	0.003 (−0.022, 0.028)	0.809
β-Carotene (μmol/L)[2]	−0.012 (−0.038, 0.015)	0.378	−0.006 (−0.031, 0.020)	0.655	−0.0004 (−0.034, 0.034)	0.981
Vitamin C supplement use	−0.058 (−0.112, −0.004)	0.035	−0.036 (−0.090, 0.018)	0.191	—	—
Vitamin E supplement use	−0.064 (−0.105, −0.024)	0.002	−0.043 (−0.084, −0.002)	0.038	—	—

[1] Adjusted for age, pulse pressure, hypertension, alcohol intake, smoking status, total cholesterol, and LDL cholesterol (similar results were obtained when systolic blood pressure was used instead of pulse pressure). Total cholesterol was excluded from the multivariate analysis of cholesterol-adjusted vitamin E.
[2] Log-transformed variable.

vitamin status after excluding this group. In this analysis, the associations between intima-media thickness and plasma vitamin C and β-carotene became slightly stronger (data not shown). Plasma concentrations of vitamin C and β-carotene were significantly correlated (r = 0.29, $P < 0.001$). Among men who were not taking supplements, when both vitamin C and β-carotene were included in the same multivariate model, both variables remained independently associated with intima-media thickness (for vitamin C, regression coefficient = −0.025, 95% CI: −0.047, −0.003, $P = 0.024$; for β-carotene, regression coefficient = −0.029, 95% CI: −0.045, 0.000, $P = 0.052$).

We found no significant trends between plasma concentrations of antioxidant vitamins and intima-media thickness in the women (Table 3). The use of supplements containing vitamin E, however, was associated with a reduction of 0.043 mm in intima-media thickness ($P = 0.038$), after adjustment for age and other cardiovascular disease risk factors. There was no evidence in either sex that the relation between plasma β-carotene and intima-media thickness differed according to smoking status (data not shown).

In the men, the risk of having stenosis of >30% was lowest in those whose plasma concentration of β-carotene or cholesterol-adjusted vitamin E was in the top one-third of the distribution (**Table 4**). Although the men with the highest vitamin C concentrations tended to have a slightly reduced risk of carotid stenosis, this trend was not significant. After adjustment for age and other cardiovascular disease risk factors, the men whose plasma β-carotene concentration was >0.278 μmol/L had an odds ratio (OR) for stenosis of 0.4 (95% CI: 0.2, 0.9) compared with those whose β-carotene concentrations were ≤0.132 μmol/L. This association was little changed when men taking supplements of either multivitamins or vitamins E or C alone were excluded. Exclusion of men with symptomatic cardiovascular disease strengthened the association (OR: 0.3; 95% CI: 0.1, 0.8). After adjustment for age and other cardiovascular disease risk factors, the men whose cholesterol-adjusted vitamin E concentration was >6.02 μmol/mmol had an OR for stenosis of 0.4 (95% CI: 0.2, 0.8) compared with those whose cholesterol-adjusted vitamin E concentration was ≤4.86 μmol/mmol. Men who were taking supplements of vitamin E also had a reduced risk of stenosis.

To examine whether the reduction in risk associated with a high plasma vitamin E concentration was concentrated among supplement users, we repeated the analysis after excluding those taking either multivitamins or vitamins E or C alone. As shown in Table 4, the relation between carotid stenosis and cholesterol-adjusted vitamin E was weakened but remained of borderline significance ($P = 0.060$) and the size of the OR was little changed. Exclusion of men with symptomatic cardiovascular disease strengthened the relation between carotid stenosis and cholesterol-adjusted vitamin E: men whose cholesterol-adjusted vitamin E concentration was >6.02 μmol/mmol had an OR for stenosis of 0.3 (95% CI: 0.1, 0.7) compared with those whose cholesterol-adjusted vitamin E concentration was ≤4.86 μmol/mmol.

The women with more severe carotid stenosis differed little in plasma concentrations of antioxidant vitamins from the women whose arteries were free of plaque (Table 4). The use of vitamin E supplements, however, was associated with a reduced risk of stenosis (OR: 0.5; 95% CI: 0.2, 1.1; NS). There was no evidence in either sex that the relation between plasma β-carotene and carotid stenosis differed according to smoking status (data not shown).

DISCUSSION

One difficulty in interpreting these results stems from the cross-sectional design of the study. The associations observed between plasma concentrations of antioxidant vitamins and carotid atherosclerosis could have arisen not because low concentrations of these vitamins promote atherogenesis, but because the men with symptomatic cardiovascular disease consumed less of these vitamins as a result of their illness. However, it seems unlikely that the latter explains our findings because the associations became slightly stronger when such persons were excluded. It is possible that other aspects of our participants' diets or lifestyles, such as their intakes of fiber or saturated fat or their physical activity, might help to explain these associations, but we had no data on these potentially confounding factors.

The men and women in this community-based study are not representative of all elderly persons in Sheffield because they have continued to live in the city in which they were born. All

TABLE 4
Odds ratios (95% CIs) for carotid stenosis of >30% according to plasma concentrations of antioxidant vitamins and use of vitamin supplements

	Unadjusted	P for trend	Multivariate adjusted[1]	P for trend	Multivariate adjusted, excluding supplement users[1]	P for trend
Men						
Vitamin E/cholesterol (μmol/mmol)						
≤4.86 (n = 88)	1.0		1.0		1.0	
4.87–6.02 (n = 87)	0.6 (0.3, 1.2)		0.8 (0.4, 1.7)		0.8 (0.4, 1.8)	
>6.02 (n = 89)	0.4 (0.2, 0.7)	0.011	0.4 (0.2, 0.8)	0.013	0.5 (0.2, 1.1)	0.060
Vitamin C (μmol/L)						
≤21.87 (n = 87)	1.0		1.0		1.0	
21.88–53.62 (n = 89)	0.9 (0.5, 1.6)		0.8 (0.4, 1.7)		0.8 (0.4, 1.7)	
>53.62 (n = 88)	0.8 (0.5, 1.5)	0.430	0.6 (0.3, 1.4)	0.162	0.7 (0.3, 1.5)	0.356
β-Carotene (μmol/L)						
≤0.132 (n = 88)	1.0		1.0		1.0	
0.133–0.278 (n = 88)	0.8 (0.4, 1.6)		0.6 (0.3, 1.2)		0.5 (0.2, 1.1)	
>0.278 (n = 88)	0.5 (0.2, 0.9)	0.079	0.4 (0.2, 0.9)	0.049	0.4 (0.1, 1.0)	0.059
Vitamin E supplement use						
No (n = 215)	1.0		1.0			
Yes (n = 49)	0.6 (0.3, 1.2)	0.139	0.4 (0.2, 1.0)	0.041	—	—
Vitamin C supplement use						
No (n = 234)	1.0		1.0			
Yes (n = 30)	0.9 (0.4, 2.1)	0.884	1.0 (0.4, 2.4)	0.978	—	—
Women						
Vitamin E/cholesterol (μmol/mmol)						
≤4.84 (n = 68)	1.0		1.0		1.0	
4.85–5.91 (n = 68)	1.1 (0.5, 2.5)		1.0 (0.4, 2.5)		1.5 (0.6, 4.1)	
>5.91 (n = 67)	1.4 (0.7, 3.1)	0.503	1.1 (0.4, 2.5)	0.889	1.5 (0.6, 3.9)	0.513
Vitamin C (μmol/L)						
≤28.71 (n = 68)	1.0		1.0		1.0	
28.72–63.81 (n = 67)	1.0 (0.5, 2.3)		1.3 (0.5, 3.0)		1.2 (0.4, 3.2)	
>63.81 (n = 69)	1.7 (0.8, 3.8)	0.464	1.9 (0.9, 4.5)	0.362	2.6 (1.0, 6.4)	0.272
β-Carotene (μmol/L)						
≤0.218 (n = 68)	1.0		1.0		1.0	
0.219–0.380 (n = 66)	0.9 (0.4, 2.2)		1.0 (0.4, 2.3)		0.9 (0.3, 2.2)	
>0.380 (n = 68)	1.6 (0.7, 3.8)	0.695	2.4 (0.9, 6.0)	0.285	2.4 (0.9, 6.9)	0.466
Vitamin E supplement use						
No (n = 148)	1.0		1.0			
Yes (n = 56)	0.5 (0.2, 1.1)	0.074	0.5 (0.2, 1.1)	0.083	—	—
Vitamin C supplement use						
No (n = 175)	1.0		1.0			
Yes (n = 29)	0.7 (0.3, 1.7)	0.433	0.7 (0.3, 1.8)	0.415	—	—

[1] Adjusted for age, pulse pressure, hypertension, alcohol intake, smoking status, total cholesterol, and LDL cholesterol (similar results were obtained when systolic blood pressure was used instead of pulse pressure). Total cholesterol was excluded from the multivariate analysis of cholesterol-adjusted vitamin E.

comparisons, however, were made within the study sample. Unless the relations observed between antioxidant vitamin status and carotid atherosclerosis differ in nonresponders or in persons who died or moved away, no bias will have been introduced.

Little evidence exists to date on the links between antioxidant vitamin status and carotid atherosclerosis, particularly in the elderly. The Atherosclerosis Risk in Communities (ARIC) Study examined the relation between dietary intake of antioxidant vitamins and intima-media thickness in middle-aged men and women (22). Intima-media thickness decreased with increasing intake of vitamin E or C, although these associations were present only in those aged >55 y. Intima-media thickness also tended to be smaller in older men who ate more carotenoids. In a subsequent case-control study of 231 matched pairs from the ARIC cohort, no significant association was observed between intima-media thickness and blood concentrations of either β-carotene or vitamin E, although cases did have lower concentrations of the carotenoids β-cryptoxanthin and lutein/zeaxanthin than did controls (23). Another report on the ARIC population showed that the prevalence of carotid plaques was lower in men and women with a higher intake of carotenoids, but the relation between plaques and consumption of vitamins C or E was not examined (24). In the EVA Study of men and women aged 59–71 y, those with higher concentrations of erythrocyte vitamin E had a significantly smaller intima-media thickness (25). There was a similar, although nonsignificant, trend between plasma carotenoid concentrations and intima-media thickness. No associations were found between the prevalence of plaques and either vitamin E or carotenoids, but the prevalence of atherosclerosis in this partially self-selected cohort was very low. In our elderly male study participants, in whom the extent to which their carotid arteries had been narrowed by plaque ranged from 0% to 100%, we found significant trends between both plasma vitamin E and β-carotene and the severity of stenosis.

We found no significant associations between plasma concentrations of antioxidants and either measure of carotid atherosclerosis in women. In contrast with our findings in men, some evidence suggested that higher plasma concentrations of antioxidants, particularly vitamin C, may be linked with an increased risk of carotid stenosis in women. The reasons for this finding are unclear. Some investigators have suggested that high doses of vitamin C may have a prooxidant effect in some circumstances (26), but in the present study the increase in risk was most apparent in women who obtained their vitamin C from diet alone. However, the CIs around our risk estimates in women were wide. It is possible that we had insufficient statistical power to examine these associations in this group.

Women who were using supplements of vitamin E had a smaller mean intima-media thickness and a lower risk of stenosis than did nonusers. An association between supplementary vitamin E and reduced risk of stenosis was also seen in the men. Supplement users differed from nonusers not merely in vitamin E status, but also in plasma concentrations of vitamin C and β-carotene, social class, and smoking habits. Whether the lower risk of carotid atherosclerosis in these persons is merely a reflection of the healthier lifestyle of supplement users (27) or is attributable in part to the effects of vitamin E is unclear. Results from a longitudinal study of vitamin E supplement users suggest that higher intakes of this antioxidant may indeed be protective: in the Cholesterol Lowering Atherosclerosis Study, a randomized controlled trial of colestipol-niacin, men in the placebo group who had been taking ≥100 IU (67 mg) supplementary vitamin E/d experienced significantly less progression in carotid intima-media thickness over a 2-y period than did those whose supplement intake was lower (28).

Most intervention trials designed to investigate the effect of supplementary antioxidant vitamins have used clinical events as the endpoint. The results of such trials published so far have been disappointing, particularly for β-carotene. Two trials found no evidence of a protective effect (29, 30), whereas the results from 2 studies in long-term smokers suggested that supplementation with β-carotene might increase the risk of cardiovascular disease (31–33). In a large trial in China, persons taking a combination of β-carotene, vitamin E, and selenium had slightly lower mortality from cerebrovascular disease than did those not taking the supplemental vitamins, but it is impossible to know which antioxidant contributed the most to the reduced risk (34). The same trial found no evidence of reduced mortality in persons who received a combination of vitamin C and molybdenum.

Results from trials of vitamin E are conflicting. In 29 000 long-term male smokers, vitamin E reduced the incidence of angina and fatal coronary artery disease by ≈9%, but had no effect on nonfatal myocardial infarction (35, 36); subsequent analysis showed that the men supplemented with this vitamin had a significantly reduced risk of cerebral infarction after 6 y of follow-up, but an increased likelihood of fatal hemorrhagic stroke (33), possibly because of the antiplatelet action of vitamin E (37). In the Cambridge Heart Antioxidant Study, patients with coronary atherosclerosis had a significantly reduced risk of nonfatal myocardial infarction after 17 mo of vitamin E supplementation, but there was a slight increase in fatal cardiovascular events (38). A secondary prevention trial in Italy found that 3 y of supplementation with vitamin E had little effect on the composite endpoint of death and nonfatal myocardial infarction and stroke, although it did reduce the risk of fatal cardiovascular events by 20% (39). A

recent report from a trial in >9000 men and women with a history of coronary artery disease, stroke, or diabetes found no evidence that vitamin E had an effect on cardiovascular events (40).

The negative findings of some of these trials suggest that the reduced risk of atherosclerotic disease in persons who have higher intakes or blood concentrations of antioxidant vitamins may in fact be due to some other characteristic of these persons' diets or lifestyles. Fruit and vegetables, for example, are rich in antioxidants but also contain other substances that may help to inhibit atherogenesis (41). Additionally, the associations found in the present study and in other observational investigations may well result from a decades-long dietary pattern. A few years of antioxidant supplementation may not be long enough to affect rates of myocardial infarction and stroke, particularly in trial participants whose atherosclerosis is already severe (31, 32, 39, 40). Findings in the present study that elderly men with higher plasma concentrations of vitamin E, vitamin C, and β-carotene have thinner artery walls and little or no plaque in their carotid arteries suggest that antioxidant vitamins are important in the first phases of atherogenesis. Perhaps more emphasis should be given to interventions that monitor the effects of these vitamins on the initiation and progression of early atherosclerotic lesions. ❧

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