A Randomized Factorial Trial of Vitamins C and E and Beta Carotene in the Secondary Prevention of Cardiovascular Events in Women

Results From the Women's Antioxidant Cardiovascular Study

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Background: Randomized trials have largely failed to support an effect of antioxidant vitamins on the risk of cardiovascular disease (CVD). Few trials have examined interactions among antioxidants, and, to our knowledge, no previous trial has examined the individual effect of ascorbic acid (vitamin C) on CVD.

Methods: The Women's Antioxidant Cardiovascular Study tested the effects of ascorbic acid (500 mg/d), vitamin E (600 IU every other day), and beta carotene (50 mg every other day) on the combined outcome of myocardial infarction, stroke, coronary revascularization, or CVD death among 8171 female health professionals at increased risk in a $2 \times 2 \times 2$ factorial design. Participants were 40 years or older with a history of CVD or 3 or more CVD risk factors and were followed up for a mean duration of 9.4 years, from 1995-1996 to 2005.

Results: A total of 1450 women experienced 1 or more CVD outcomes. There was no overall effect of ascorbic acid (relative risk [RR], 1.02; 95% CI, 0.92-1.13 [*P*=.71]), vitamin E (RR, 0.94; 95% CI, 0.85-1.04 [*P*=.23]), or beta carotene (RR, 1.02; 95% CI, 0.92-1.13 [*P*=.71]) on the primary combined end point or on the individual secondary outcomes of myocardial infarction, stroke, coronary revascularization, or CVD death. A marginally significant reduction in the primary outcome with active vitamin E was observed among the prespecified subgroup of women with prior CVD (RR, 0.89; 95% CI, 0.79-1.00 [*P*=.04]; *P* value for interaction, .07). There were no significant interactions between agents for the primary end point, but those randomized to both active ascorbic acid and vitamin E experienced fewer strokes (*P* value for interaction, .03).

Conclusion: There were no overall effects of ascorbic acid, vitamin E, or beta carotene on cardiovascular events among women at high risk for CVD.

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OXIDATIVE DAMAGE MAY play a role in the development of cardiovascular disease (CVD), particularly through its effect on lipid peroxidation and DNA damage.[1] In addition, free radicals may damage arterial endothelium, encourage thrombosis, and alter vasomotor function.[2] Antioxidants scavenge free radicals and limit the damage they can cause.[3] Diets high in fruit and vegetable intake, and thus rich in such antioxidants, have been associated with reduced rates of coronary heart disease and stroke.[4]

Vitamins C and E and beta carotene are potential mediators of the apparent protective effect of a plant-based diet on CVD. Observational epidemiologic studies of dietary intake of these vitamins provide some support for this hypothesis, particularly for vitamin E,[5-8] although results are not consistent. Some studies found lower event rates among those with higher plasma carotenoid levels,[9,10] but others found no beneficial effects.[11,12]

Several randomized trials have now examined the effects of these antioxidants, and results have generally been disappointing.[13] However, to our knowledge, no trials have examined the effect of ascorbic acid (vitamin C) on CVD, except as part of an antioxidant cocktail. The Women's Antioxidant Cardiovascular Study (WACS) was designed to test the effect of 3 antioxidant agents, ascorbic acid, vitamin E, and beta carotene, on prevention of CVD among women at high risk. It used a factorial design, enabling a comparison for each agent alone as well as interactions among them.
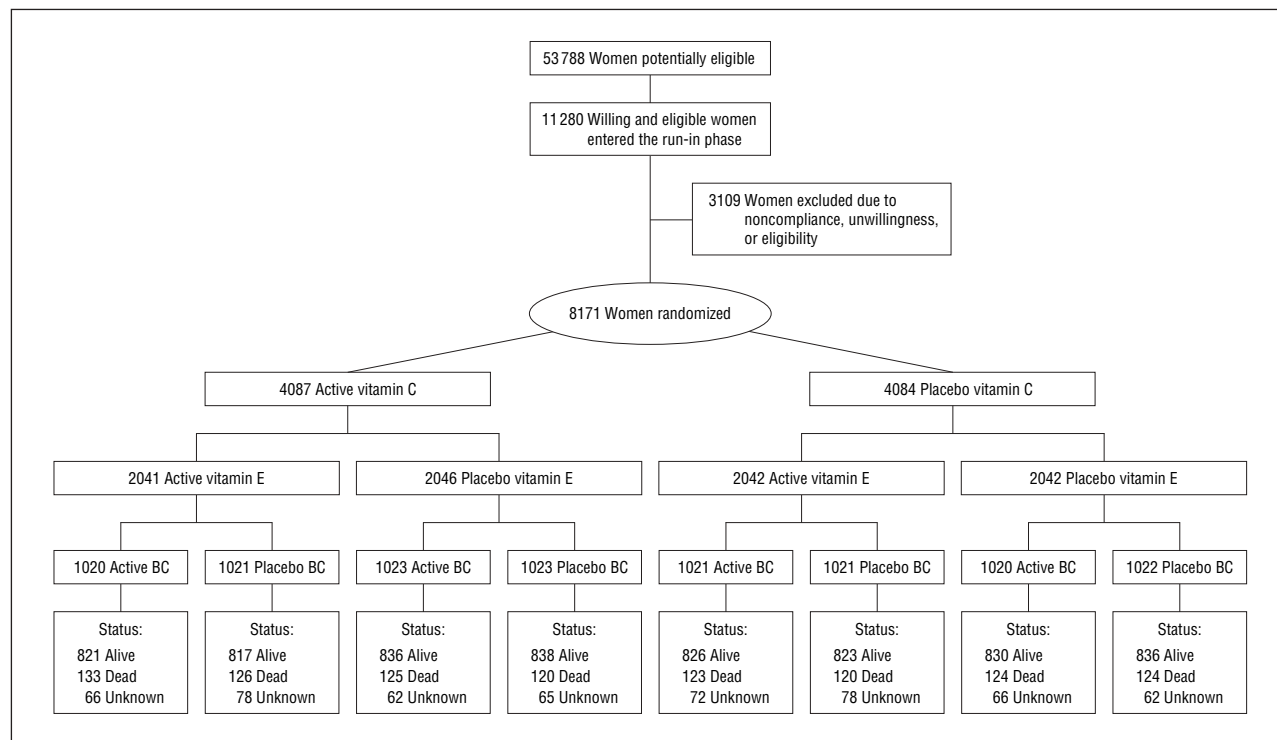
Figure 1. Enrollment, randomization, and follow-up of participants in the Women's Antioxidant Cardiovascular Study. BC indicates beta carotene.

METHODS

STUDY DESIGN AND PARTICIPANTS

The WACS is a randomized, double-blind, placebo-controlled trial evaluating the effects of vitamin C (500 mg/d of synthetic vitamin C [ascorbic acid], provided by BASF Corporation [Mount Olive, New Jersey]), vitamin E (600 IU of natural vitamin E [*d*-alpha tocopherol acetate] every other day, provided by Cognis Corporation [La Grange, Illinois]), and beta carotene (50 mg of Lurotin every other day, provided by BASF Corporation) in the prevention of important vascular events in a $2 \times 2 \times 2$ factorial design among women at high risk with either a history of vascular disease or at least 3 cardiovascular risk factors. In 1998, approximately 2 to 3 years following randomization to the antioxidant arms, a folic acid–vitamin B_6/B_{12} component was added to the trial, expanding it to a 4-arm factorial trial. This report describes the results of the 3 antioxidant interventions.

Details of the design have been reported previously.[14] Briefly, women were eligible if they were 40 years or older, postmenopausal, or had no intention of becoming pregnant, had a self-reported history of CVD, or had at least 3 cardiac risk factors. The cardiac risk factors determining eligibility were self-reported diagnosis of hypertension, high cholesterol level, or diabetes mellitus; parental history of premature myocardial infarction (MI) (before age 60 years); obesity (body mass index [BMI] $\geq$30 [calculated as weight in kilograms divided by height in meters squared]), current cigarette smoking; and inconsistent report of prior CVD. Women were excluded if they had a self-reported history of cancer (excluding nonmelanoma skin cancer) within the past 10 years, any serious non-CVD illness, or were currently using warfarin sodium or other anticoagulants. Potential participants also had to be willing to forgo individual supplements of vitamins A, C, E, and beta carotene at levels beyond the US recommended daily allowance during the trial. A total of 8171 women were willing and eligible, were compliant during a 12-week run-in, and were randomized into the trial from June 1995 through October 1996 (**Figure 1**). The trial was approved by the institutional review board of the Brigham and Women's Hospital, Boston, Massachusetts, and was monitored by an external data and safety monitoring board.

Following randomization, every 6 months for the first year and then annually, the women were sent monthly calendar packs containing active agents or placebos, along with questionnaires on compliance, adverse effects, and medical events. Study medications and end point ascertainment were continued in a blinded fashion until the scheduled end of the trial. Pill taking for women not enrolled in the folic acid component ended as scheduled on January 31, 2005; women in the folic acid component completed pill taking on July 31, 2005. The mean follow-up from randomization to the end of study was 9.4 years (range, 8.3-10.1 years). Follow-up and validation of reported end points were completed in July 2006. A search of the National Death Index was conducted for all participants through December 2003; thus, mortality information was virtually complete through 2003. As of the scheduled end of the trial in January or July 2005, mortality follow-up was 93% complete. In terms of person-time, mortality information was complete for over 99% of person-years of follow-up. Morbidity status was known as of 8 years for 93% of survivors, and response to the final follow-up questionnaire among surviving women was 89%.

Compliance was assessed through self-report and defined as taking at least two-thirds of study pills. Reported compliance was, on average, 76% at 4 years and 68% at 8 years of follow-up for each antioxidant, with no significant difference between active and placebo groups at these times except for ascorbic acid at 8 years (70% vs 67% in the active vs placebo group; P=.01). Mean compliance over follow-up was approximately 73% for all active and placebo agents. In 1999, blood samples were obtained from 30 local participants to evaluate biomarkers for compliance. Blood levels were elevated in each active vs placebo group (ascorbic acid, 1.88 vs 1.26 mg/dL [P=.007]; vitamin E, 20.19 vs 12.15 µg/mL [P=.007]; and beta carotene: 54.39 vs 19.52 µg/mL [P=.003]).

Table 1. Comparison of Baseline Characteristics by Randomized Groups in the Women's Antioxidant Cardiovascular Study[a]

Baseline Factor	Total No. of Participants	Vitamin C (Ascorbic Acid)		Vitamin E		Beta Carotene	
		Active (n = 4087)	Placebo (n = 4084)	Active (n = 4083)	Placebo (n = 4088)	Active (n = 4084)	Placebo (n = 4087)
Age, mean ± SD, y	8171	60.6 ± 8.8	60.6 ± 8.8	60.6 ± 8.9	60.6 ± 8.8	60.6 ± 8.9	60.6 ± 8.8
Age, y							
40-54	2400	29.4	29.4	29.3	29.4	29.4	29.4
55-64	2993	36.6	36.7	36.7	36.6	36.6	36.6
65	2778	34.0	34.0	34.0	34.0	34.0	34.0
Health history[b]							
Prior CVD	5238	64.1	64.1	64.7	63.5	63.8	64.4
≥3 Risk factors	2933	35.9	35.9	35.3	36.5	36.2	35.6
History of hypertension							
Yes	6137	75.4	74.8	74.4	75.8	75.3	74.9
No	2034	24.6	25.2	25.6	24.2	24.7	25.1
History of high cholesterol level							
Yes	5950	72.6	73.0	72.7	73.0	71.5	74.1[d]
No	2221	27.4	27.0	27.3	27.0	28.5	25.9
History of diabetes							
Yes	1564	19.4	18.9	18.7	19.6	19.2	19.1
No	6607	80.6	81.1	81.3	80.4	80.8	80.9
Parental history of MI[c]							
Yes	3032	38.2	36.4	36.7	37.9	37.1	37.5
No	5097	61.8	63.6	63.3	62.1	62.9	62.5
Smoking status							
Current	1269	15.1	15.9	15.5	15.6	15.3	15.8
Past	3405	42.0	41.4	41.7	41.6	41.6	41.8
Never	3497	42.9	42.7	42.8	42.8	43.1	42.4
Alcohol use in past y							
Never	4514	56.1	54.4	54.7	55.8	55.6	54.9
0-<1 drinks/wk	1008	12.3	12.4	12.1	12.6	12.3	12.4
1-6 drinks/wk	1964	23.9	24.2	24.9	23.2	23.7	24.4
Daily	685	7.8	9.0	8.3	8.5	8.4	8.4
BMI, mean ± SD	8164	30.3 ± 6.7	30.3 ± 6.7	30.3 ± 6.6	30.3 ± 6.7	30.2 ± 6.7	30.4 ± 6.6
BMI							
<25	1906	23.2	23.5	23.0	23.7	24.2	22.4
25 to <30	2339	29.1	28.2	29.2	28.1	28.4	28.9
≥30	3919	47.8	48.2	47.8	48.2	47.4	48.6
Menopause and HT use							
Premenopausal	653	8.1	8.2	8.2	8.0	7.8	8.5
Uncertain	1167	14.4	14.7	14.5	14.6	14.5	14.6
Postmenopausal, current HT	3083	39.2	37.7	38.8	38.1	38.7	38.2
Postmenopausal, no HT	3121	38.4	39.4	38.5	39.3	39.0	38.8
Current multivitamin use							
Yes	2192	26.5	27.5	27.1	26.9	26.9	27.1
No	5927	73.5	72.5	72.9	73.1	73.1	72.9

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CVD, cardiovascular disease; HT, hormone therapy; MI, myocardial infarction.

[a] Data are given as percentage of participants unless otherwise specified.

[b] "Prior CVD" is history of MI, stroke, coronary revascularization, angina pectoris, or transient ischemic attack; "≥3 risk factors" denotes women with no prior CVD but with at least 3 of the following: hypertension, high cholesterol level, diabetes mellitus, parental history of premature MI (before age 60 years), obesity (BMI ≥30), current cigarette smoking, and inconsistent report of prior CVD.

[c] Before age 60 years.

[d] $P = .007$ for active vs placebo.

Information was obtained on outside supplements of study medications for at least 4 days per month ("drop-ins"). Use of vitamin C supplements was approximately 10% at both 4 and 8 years and did not differ by intervention group. Use of vitamin E supplements was 13% at 4 years and 12% at 8 years, with no difference by randomized group. Use of supplements of beta carotene or vitamin A averaged 2% at 4 and 8 years, and did not differ by beta carotene assignment. When use of multivitamins containing more than 100% of the recommended daily allowance was included in the definition of outside use, rates were approximately 3% to 5% higher for ascorbic acid, 4% to 8% higher for vitamin E, and 1% to 3% higher for beta carotene, but again, did not differ by randomized assignment.

END POINT DEFINITION

The primary outcome was a combined end point of CVD morbidity and mortality, including incident MI, stroke, coronary revascularization procedures (coronary artery bypass grafting or percutaneous transluminal coronary angioplasty), and cardiovascular mortality. The individual components of MI, stroke,

Table 2. Relative Risk (RR) of Cardiovascular Outcomes by Randomized Antioxidant Intervention Group in the Women's Antioxidant Cardiovascular Study

Outcome	Vitamin C (Ascorbic Acid)				Vitamin E				Beta Carotene			
	Active, No.	Placebo, No.	RR (95% CI)	P Value	Active, No.	Placebo, No.	RR (95% CI)	P Value	Active, No.	Placebo, No.	RR (95% CI)	P Value
Major CVD[a]	731	719	1.02 (0.92-1.13)	.71	708	742	0.94 (0.85-1.04)	.23	731	719	1.02 (0.92-1.13)	.71
MI, stroke, CVD death	419	415	1.01 (0.88-1.16)	.87	399	435	0.90 (0.78-1.03)	.12	435	399	1.09 (0.95-1.25)	.21
MI[b]	140	134	1.05 (0.83-1.33)	.70	131	143	0.91 (0.72-1.15)	.44	135	139	0.97 (0.77-1.23)	.82
Fatal	15	19	0.79 (0.40-1.55)	.49	18	16	1.11 (0.57-2.18)	.76	10	24	0.42 (0.20-0.87)	.02
Nonfatal	125	115	1.09 (0.85-1.41)	.50	113	127	0.88 (0.69-1.14)	.34	125	115	1.09 (0.85-1.40)	.51
Revascularization[b]	446	443	1.01 (0.89-1.15)	.88	438	451	0.96 (0.85-1.10)	.59	438	451	0.98 (0.86-1.11)	.71
Total CHD	510	489	1.05 (0.93-1.19)	.46	491	508	0.96 (0.85-1.09)	.52	500	499	1.01 (0.89-1.14)	.92
Stroke[b]	138	160	0.86 (0.69-1.08)	.21	137	161	0.84 (0.67-1.05)	.12	161	137	1.17 (0.93-1.47)	.17
Ischemic	123	148	0.83 (0.66-1.06)	.13	121	150	0.79 (0.62-1.01)	.06	143	128	1.12 (0.88-1.42)	.37
Hemorrhagic	13	12	1.09 (0.50-2.39)	.83	15	10	1.47 (0.66-3.27)	.35	17	8	2.13 (0.92-4.93)	.08
Fatal	15	18	0.84 (0.42-1.67)	.63	18	15	1.15 (0.58-2.28)	.69	22	11	1.98 (0.96-4.07)	.07
Nonfatal	123	142	0.87 (0.68-1.10)	.25	119	146	0.80 (0.63-1.02)	.08	139	126	1.10 (0.87-1.40)	.42
TIA	203	218	0.93 (0.77-1.13)	.49	205	216	0.94 (0.78-1.14)	.54	201	220	0.91 (0.75-1.10)	.35
CVD death[b]	206	189	1.10 (0.90-1.33)	.37	193	202	0.94 (0.77-1.15)	.56	211	184	1.14 (0.94-1.39)	.18
Total mortality	504	491	1.03 (0.91-1.17)	.62	502	493	1.00 (0.89-1.14)	.95	505	490	1.03 (0.91-1.17)	.65

Abbreviations: CHD, coronary heart disease (includes MI, revascularization, and CHD death); CI, confidence interval; CVD, cardiovascular disease; MI, myocardial infarction; TIA, transient ischemic attack.
[a] Primary outcome, includes MI, stroke, revascularization procedure or death due to cardiovascular cause.
[b] Secondary outcome.

coronary revascularization, and CVD death were prespecified secondary end points. Information on transient ischemic attack and total mortality was also collected and reviewed.

Women reported relevant end points through questionnaire, letter, or telephone call. Deaths were reported by family members or postal authorities or through the National Death Index. Written permission for medical records was sought from the participant or the next of kin in case of death. These were reviewed by an end points committee of physicians blinded to randomized treatment assignment. An MI was confirmed if symptoms met World Health Organization criteria,[15] and cardiac enzymes or diagnostic electrocardiograms were abnormal. Coronary revascularization (coronary artery bypass grafting or percutaneous transluminal coronary angioplasty) was confirmed by medical record review. Confirmed stroke was defined as a new neurologic deficit of sudden onset that persisted for more than 24 hours or until death within 24 hours. Clinical information, computed tomographic scans, and magnetic resonance images were used to distinguish hemorrhagic from ischemic events. A confirmed transient ischemic attack was defined as a neurologic deficit of sudden onset that lasted less than 24 hours. Death due to a cardiovascular cause was confirmed by examination of autopsy reports, death certificates, medical records, and information obtained from the next of kin or other family members. Death from any cause was confirmed by the end points committee or on the basis of a death certificate. Only confirmed end points were included in these analyses, except for total mortality, which included 66 reported deaths with no death certificate.

STATISTICAL ANALYSIS

Primary analyses were performed on an intent-to-treat basis, including all randomized women. Baseline characteristics were compared by randomized groups using 2-sample t tests, χ^2 tests for proportions, and tests for trend for ordinal categories. Kaplan-Meier curves were used to estimate cumulative incidence over time by randomized group, and the log-rank test was used to compare curves. The Cox proportional hazards model was used to estimate the relative risk (RR), expressed as a hazards ratio, along with the 95% confidence interval (CI). Models included main effect terms for each of the 3 antioxidants along with age. Tests of proportionality of the hazards ratio over time used an interaction term for treatment times the logarithm of time. To examine the impact of lack of compliance, a post hoc sensitivity analysis censored women if and when they stopped taking at least two thirds of their study medication, reported taking outside supplements containing study agents, or were missing compliance information. Statistical analyses were conducted using SAS version 9 (SAS Institute, Cary, North Carolina), using 2-sided tests with a significance level of $P < .05$.

Interaction terms were used to test for additivity of the 3 antioxidant agents. All 2-way interactions as well as a 3-way interaction were tested, using multiplicative terms in the Cox model. In addition, we conducted subgroup analyses according to the presence or absence of major cardiovascular risk factors, including age, prior CVD, smoking, alcohol use, BMI, history of hypertension, high cholesterol level or diabetes, parental history of MI before age 60 years, menopausal status and hormone therapy use, and current multivitamin use. Tests for effect modification by subgroup used interaction terms between subgroup indicators and randomized assignment, with a test for trend for ordinal subgroup categories, or a multidegree of freedom test for unordered categories. Any significant modification of effect is described in the "Results" section.

To convert ascorbic acid (vitamin C) to micromoles per liter, multiply by 56.78; to convert vitamin E to micromoles per liter, multiply by 23.22; to convert beta carotene to micromoles per liter, multiply by 0.01863.

RESULTS

A total of 8171 women were randomized into the antioxidant arms of the trial, with a mean±SD age of 60.6±8.8 years. Of these women, 5238 (64%) had a prior cardio-
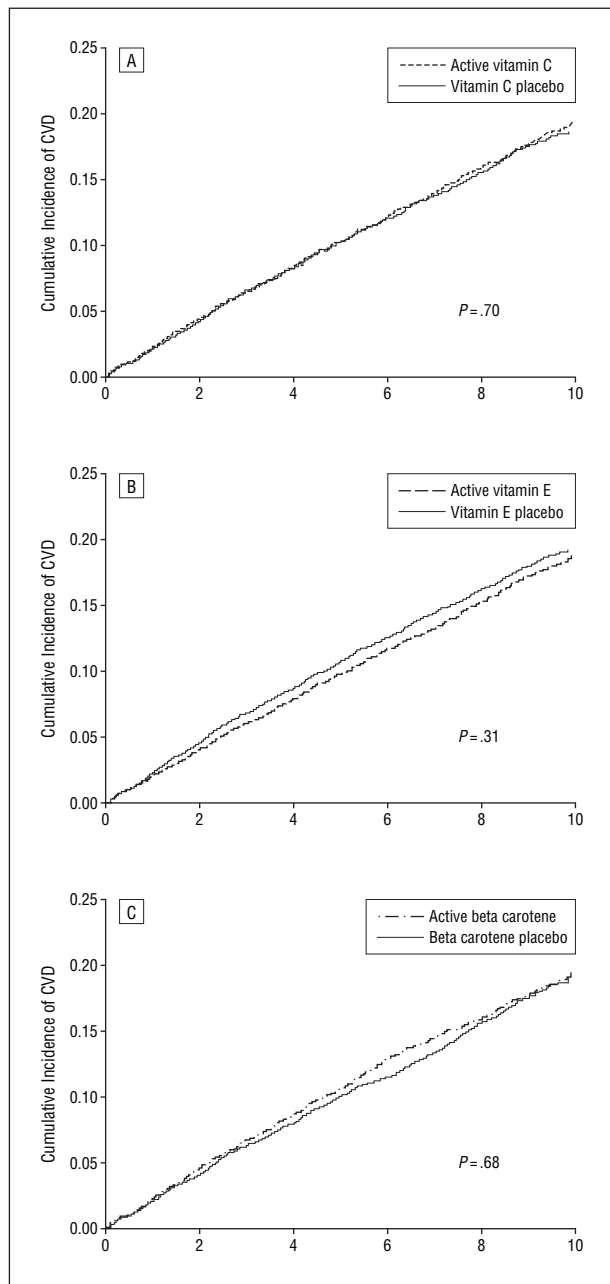
Figure 2. Cumulative incidence of major vascular disease (myocardial infarction, stroke, coronary revascularization, or cardiovascular death) by randomized antioxidant intervention (A, active vitamin C [ascorbic acid] vs placebo; B, active vitamin E vs placebo; and C, active beta carotene vs placebo) in the Women's Antioxidant Cardiovascular Study. *P* value is from the log-rank test. CVD indicates cardiovascular disease.

vascular event, and 2933 (36%) had 3 or more CVD risk factors. Mean ± SD BMI was 30.3 ± 6.7, and 1269 women (16%) were current smokers, 1564 (19%) had diabetes, 6137 (75%) had a history of hypertension, and 5950 (73%) had a history of high cholesterol level at baseline. There were no statistically significant differences in baseline characteristics between the randomized groups, except history of high cholesterol level for the beta carotene groups (**Table 1**).

During the mean 9.4-year follow-up, 1450 women experienced a confirmed CVD event, including 274 MIs, 298 strokes, 889 coronary revascularization procedures, and 395 cardiovascular deaths, with some experiencing more than 1 event. A total of 995 women died during follow-up.

ASCORBIC ACID

There was no effect of ascorbic acid on the primary combined end point, with a RR of 1.02 (95% CI, 0.92-1.13) (*P*=.71) (**Table 2**). Cumulative incidence curves showed no variation of the effect over time (**Figure 2**), and the test result for proportionality of the RR over time was not significant. Individual components of the primary end point also did not differ significantly, although there was some suggestion of a benefit for ischemic stroke. When participants were censored on noncompliance, results were similar (RR, 0.95; 95% CI, 0.83-1.09 [*P*=.47]). There were no significant effects of randomized ascorbic acid on the primary end point in any cardiovascular risk factor subgroup considered (**Table 3**).

VITAMIN E

No differences were seen in the primary end point by randomized vitamin E assignment (RR, 0.94; 95% CI, 0.85-1.04 [*P*=.23]) (Table 2 and Figure 2), with no significant variation in the relative risk over time. We found a nonsignificant 16% reduction in total stroke, with a 21% reduction in ischemic stroke (*P*=.06) and an increase in hemorrhagic stroke based on small numbers. There was an overall 10% reduction in the combination of MI, stroke, and CVD death, with a nonsignificant decrease (*P*=.08) in benefit over time. No difference in total mortality by vitamin E group was found.

Censoring participants on noncompliance led to a significant 13% reduction in the primary end point (RR, 0.87; 95% CI, 0.76-0.99 [*P*=.04]). Reductions in secondary study end points were also stronger, with a 22% reduction in MI (RR, 0.78; 95% CI, 0.58-1.06 [*P*=.11]), a 27% reduction in stroke (RR, 0.73; 95% CI, 0.54-0.98 [*P*=.04]), and a 9% reduction in CVD mortality (RR, 0.91; 95% CI, 0.66-1.25 [*P*=.55]). There was a 23% reduction in the combination of MI, stroke, or CVD death (RR, 0.77; 95% CI, 0.64-0.92 [*P*=.005]). Among those with prior CVD, the active vitamin E group experienced fewer major CVD events (RR, 0.89; 95% CI, 0.79-1.00 [*P*=.04]; *P* value for interaction, .07) (Table 3).

BETA CAROTENE

For beta carotene, there was no difference in the primary end point (RR, 1.02; 95% CI, 0.92-1.13 [*P*=.71]) (Table 2 and Figure 2), with no variation over time. There were no significant treatment effects on individual secondary end points. There was a nonsignificant 14% increase in CVD mortality in the active group, with a significant decline over time in the effect on CVD deaths (*P*=.04) but no difference in total mortality. When participants were censored on noncompliance, the effect on the primary end point remained nonsignificant (RR for major vascular disease, 1.09; 95% CI, 0.96-1.24 [*P*=.18]), but an increase in CVD mortality appeared to emerge (RR, 1.48; 95% CI, 1.08-2.02 [*P*=.02]).

Table 3. Relative Risk (RR) of Major Cardiovascular Outcomes by Randomized Intervention Within Subgroups in the Women's Antioxidant Cardiovascular Study[a]

Baseline Factor	Vitamin C (Ascorbic Acid)				Vitamin E				Beta Carotene			
	Active, No.	Placebo, No.	RR (95% CI)	P Value	Active, No.	Placebo, No.	RR (95% CI)	P Value	Active, No.	Placebo, No.	RR (95% CI)	P Value
Age, y												
40-54	111	123	0.90 (0.70-1.17)	.44	117	117	1.01 (0.78-1.30)	.95	115	119	0.97 (0.75-1.25)	.82
55-64	269	244	1.12 (0.94-1.33)	.20	246	267	0.91 (0.77-1.09)	.30	252	261	0.96 (0.81-1.15)	.68
≥65	351	352	0.99 (0.85-1.15)	.91	345	358	0.94 (0.81-1.09)	.39	364	339	1.08 (0.93-1.25)	.31
Health history												
Prior CVD	592	567	1.05 (0.93-1.18)	.43	558	601	0.89 (0.79-1.00)	.04	580	579	1.02 (0.91-1.14)	.77
≥3 Risk factors	139	152	0.93 (0.74-1.16)	.51	150	141	1.13 (0.89-1.42)	.32	151	140	1.06 (0.84-1.34)	.60
Smoking status												
Current	139	158	0.92 (0.73-1.16)	.48	151	146	1.05 (0.84-1.32)	.68	152	145	1.12 (0.89-1.40)	.34
Past or never	592	561	1.05 (0.94-1.18)	.38	557	596	0.91 (0.81-1.02)	.12	579	574	1.00 (0.89-1.12)	.98
Alcohol use in past y												
Never	443	442	0.97 (0.85-1.11)	.68	427	458	0.95 (0.83-1.08)	.43	461	424	1.08 (0.95-1.23)	.24
Ever	288	277	1.08 (0.92-1.28)	.35	281	284	0.94 (0.79-1.11)	.44	270	295	0.92 (0.78-1.09)	.35
BMI												
<25	192	170	1.17 (0.95-1.44)	.14	167	195	0.88 (0.71-1.08)	.22	197	165	1.09 (0.88-1.34)	.43
25-<30	229	225	0.98 (0.81-1.18)	.82	233	221	0.98 (0.81-1.18)	.81	210	244	0.89 (0.74-1.07)	.21
≥30	310	323	0.97 (0.83-1.14)	.73	307	326	0.94 (0.80-1.10)	.43	323	310	1.08 (0.93-1.27)	.32
History of hypertension												
Yes	583	591	0.98 (0.87-1.09)	.68	570	604	0.95 (0.84-1.06)	.35	595	579	1.01 (0.90-1.13)	.87
No	148	128	1.21 (0.95-1.53)	.12	138	138	0.93 (0.73-1.18)	.54	136	140	1.03 (0.82-1.31)	.78
Diabetes												
Yes	232	235	0.96 (0.80-1.15)	.63	222	245	0.92 (0.77-1.11)	.38	244	223	1.09 (0.91-1.31)	.34
No	499	484	1.04 (0.92-1.18)	.52	486	497	0.95 (0.84-1.08)	.45	487	496	0.99 (0.87-1.12)	.82
Menopause and HT use												
Pre	26	30	0.92 (0.54-1.55)	.74	31	25	1.26 (0.74-2.13)	.40[b]	24	32	0.82 (0.48-1.40)	.47
Uncertain	77	65	1.16 (0.83-1.61)	.39	60	82	0.70 (0.50-0.98)	.04	74	68	1.06 (0.77-1.48)	.71
Post, current HT	277	252	1.07 (0.90-1.27)	.45	279	250	1.10 (0.93-1.31)	.27	280	249	1.10 (0.92-1.30)	.29
Post, no HT	337	359	0.96 (0.83-1.11)	.59	328	368	0.88 (0.76-1.02)	.08[c]	338	358	0.96 (0.83-1.11)	.60
Current multivitamin use												
Yes	173	194	0.89 (0.72-1.09)	.26	179	188	0.92 (0.75-1.13)	.42	183	184	0.99 (0.81-1.22)	.94
No	551	518	1.07 (0.95-1.21)	.28	525	544	0.96 (0.85-1.08)	.46	540	529	1.02 (0.90-1.15)	.76

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CI, confidence interval; CVD, cardiovascular disease; HT, hormone therapy; Post, postmenopausal; Pre, premenopausal.
[a] Baseline factors are defined as in Table 1.
[b] P value for interaction, <.05.
[c] Among postmenopausal women, P<.05 for interaction comparing HT users vs nonusers.

No statistically significant subgroup effects were seen for the primary end point. In particular, in the prespecified subgroup of smokers, we did not find any elevation in the risk of the primary end point (Table 3) or any of the individual components (data not shown).
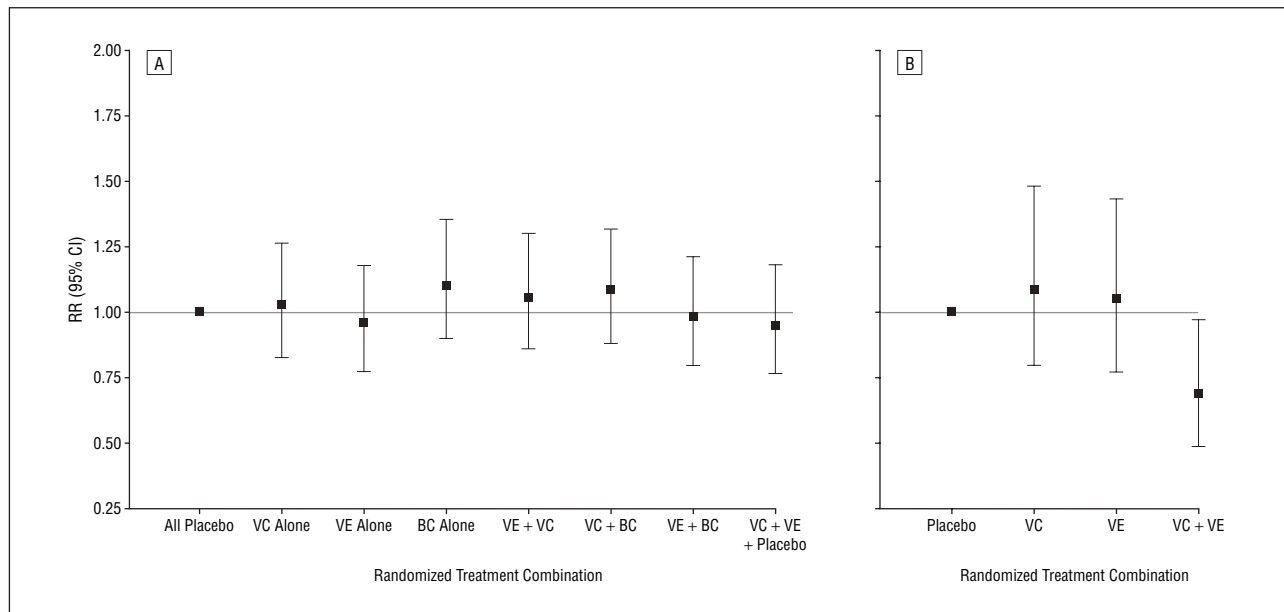
COMBINATIONS OF ANTIOXIDANTS

There were no significant 2- or 3-way interactions among the agents for the primary end point. The effects for each of the combinations of active agents compared with the group with all 3 placebos is shown in **Figure 3**. There were also no interactions for the secondary end points of MI or cardiovascular death. For stroke, we found a significant 2-way interaction between ascorbic acid and vitamin E (P=.03). Those in the active groups for both agents experienced fewer strokes compared with those in the placebo group for both agents (RR, 0.69; 95% CI, 0.49-0.98 [P=.04]) (Figure 3).

ADVERSE EFFECTS

We examined reports of bleeding (including gastrointestinal bleeds, hematuria, easy bruising, and epistaxis), of gastrointestinal symptoms (including peptic ulcer, gastric upset, nausea, constipation, and diarrhea), or of fatigue or drowsiness. There were no statistically significant differences by any of the randomized antioxidant groups except for a small increase in reports of symptoms suggestive of gastric upset among those in the active beta carotene group (2785 vs 2717 reports; RR, 1.06; 95% CI, 1.00-1.11 [P=.05]).

COMMENT

In this large-scale randomized trial among women at high risk for CVD, we found no overall effects of vitamin E, ascorbic acid, or beta carotene on the primary

Figure 3. Relative risks (RRs) and 95% confidence intervals (CIs) of major cardiovascular disease (CVD) by 8 combinations of all 3 active antioxidant assignments relative to the all placebo group (A) or of stroke by combinations of active vitamin C (ascorbic acid) (VC) and vitamin E (VE) assignments relative to the groups with placebo VC and VE (B). BC indicates beta carotene.

end point of major vascular disease over a long-term follow-up of more than 9 years. These null results are consistent with the majority of trials of these antioxidants in both primary and secondary prevention. When combinations of agents were examined, there were no significant interactions, except for a possible reduction in stroke among those taking both active ascorbic acid and active vitamin E. In contrast to a recent meta-analysis of antioxidant supplements,[16] we found no detrimental effects of any of these agents on total or CVD mortality.

To our knowledge, no previous trial has considered the effect of ascorbic acid alone on CVD prevention. A few trials, however, have considered ascorbic acid as one component of an antioxidant cocktail but have found no benefit for CVD either in primary[17,18] or in secondary[19,20] prevention. Studies of atherosclerotic progression among patients with coronary disease[21] or hypercholesterolemia[22] have shown inconsistent results for combinations of vitamins C and E. The null results for the primary end point seen in WACS further limit enthusiasm for the role of ascorbic acid in cardiovascular risk protection.

For vitamin E, the lack of effect in primary prevention is consistent with results from 3 previous intervention trials of vitamin E as a single agent.[23-25] As in the Women's Health Study,[25] there was some suggestion of an early effect of vitamin E in WACS (Figure 2), but this diminished over time and was null overall. We also found no evidence of an increase in total mortality at the 600-IU-every-other-day dose tested, as suggested for higher doses in a prior meta-analysis.[26] We did find a significant reduction in the primary end point in the subgroup of women with prior CVD. Previous trials of vitamin E in secondary prevention have been inconsistent, with significant decreases in CVD found in 2 trials of shorter 1.4 years duration.[27,28] Later and longer trials

have generally not upheld this effect.[27] Some found a reduction in secondary outcomes only,[29,30] and another reported no effect at all in a combined high-risk primary and secondary prevention population.[31,32] Meta-analyses of patients with prior CVD enrolled in these trials may provide additional insights as to whether there may still be a role for vitamin E in the secondary prevention of CVD.

For beta carotene, we found no overall benefit or harm, a lack of effect that is consistent with studies using a similar 50-mg-alternate-day regimen.[33,34] The Alpha-Tocopherol Beta Carotene Cancer Prevention Study[23] and the Beta-Carotene and Retinol Efficacy Trial[35] found some increase in total and cardiovascular deaths among smokers or those exposed to asbestos, but we found little difference in these end points. Compliance-adjusted analyses found some increase in CVD mortality, but these were not adjusted for time-varying risk factors associated with compliance and are more prone to bias than intent-to-treat analyses. Previous studies in secondary prevention had been small and inconsistent,[30,36] and we found no evidence for benefit or harm in this prespecified subgroup.

For vitamin E, there have been suggestions that gamma tocopherol is a more powerful antioxidant. Supplementation with alpha tocopherol depletes gamma tocopherol, which may explain the lack of effect seen in vitamin E trials.[37] Single antioxidants may not reflect the complex vitamins and nutrients found in foods, which may explain the discrepancies between most intervention trials and studies of fruits and vegetables.[38] Trials using an antioxidant combination, however, also have not shown a clear benefit for CVD.[17-20] Questions concerning the complex nature of dietary effects on lipid peroxidation, however, are deserving of further study.

Limitations of the trial include the lack of complete follow-up and compliance. Mortality information fol-

low-up was virtually complete through 2003 and then 93% complete for the remaining 2 years. Overall, however, mortality follow-up as a percentage of person-time was over 99% complete. Although suboptimal, compliance in WACS over time is relatively comparable to several other trials of vitamin supplementation with at least a 4-year duration in secondary[20,30] as well as primary[18,39,40] prevention.

In summary, results from WACS and other antioxidant trials have not found consistent preventive effects on CVD. Overall, we found no benefit on the primary combined end point for any of the antioxidant agents tested, alone or in combination. We also found no evidence for harm. While additional research into combinations of agents, particularly for stroke, may be of interest, widespread use of these individual agents for cardiovascular protection does not appear warranted.

REFERENCES

1. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med*. 1989;320(14):915-924.
2. Diaz MN, Frei B, Vita JA, Keaney JFJ. Antioxidants and atherosclerotic heart disease. *N Engl J Med*. 1997;337(6):408-416.
3. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence. *Lancet*. 1994;344(8924):721-724.
4. Dauchet L, Amouyel P, Hercberg S, Dallongeville J. Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. *J Nutr*. 2006;136(10):2588-2593.
5. Stampfer MJ, Hennekens CH, Manson JE, et al. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med*. 1993;328(20):1444-1449.
6. Rimm EB, Stampfer MJ, Asherio A, et al. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med*. 1993;328(20):1450-1456.
7. Kushi LH, Folsum AR, Prineas RJ, Mink PJ, Wu Y, Bostick RM. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med*. 1996;334(18):1156-1162.
8. Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the Iowa Women's Health Study. *Am J Clin Nutr*. 1996;64(2):190-196.
9. Hak AE, Ma J, Powell CB, et al. Prospective study of plasma carotenoids and tocopherols in relation to risk of ischemic stroke. *Stroke*. 2004;35(7):1584-1588.
10. Buijsse B, Feskens JM, Schlettwein-Gsell D, et al. Plasma carotene and α-tocopherol in relation to 10-y all-cause and cause-specific mortality in European elderly: the Survey in Europe on Nutrition and the Elderly, a Concerted Action (SENECA). *Am J Clin Nutr*. 2005;82(4):879-886.
11. Hak AE, Stampfer MJ, Campos H, et al. Plasma carotenoids and tocopherols and risk of myocardial infarction in a low-risk population of US male physicians. *Circulation*. 2003;108(7):802-807.
12. Evans RW, Shaten BJ, Day BW, et al. Prospective association between lipid soluble antioxidants and risk of myocardial infarction in the elderly: the Multiple Risk Factor Intervention Trial. *Am J Epidemiol*. 1998;147(2):180-186.
13. Bassuk SS, Manson JE, Gaziano JM. Antioxidant vitamins. In: Manson JE, Buring JE, Ridker PM, Gaziano JM, eds. *Clinical Trials in Heart Disease*. Philadelphia, PA: Elsevier; 2004.
14. Bassuk SS, Albert CM, Cook NR, et al. The Women's Antioxidant Cardiovascular Study: design and baseline characteristics of participants. *J Womens Health (Larchmt)*. 2004;13(1):99-117.
15. Nomenclature and criteria for diagnosis of ischemic heart disease: report of the Joint International Society and Federation of Cardiology/World Health Organization Task Force on Standardization of Clinical Nomenclature. *Circulation*. 1979;59(3):607-609.
16. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA*. 2007;297(8):842-857.
17. Blot WJ, Li JY, Taylor PR. Nutritional intervention trials in Linxian, China: supplementation with specific vitamin/mineral combination, cancer incidence and disease-specific mortality in the general population. *J Natl Cancer Inst*. 1993;85(18):1483-1492.
18. Hercberg S, Galan P, Preziosi P, et al. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. *Arch Intern Med*. 2004;164(21):2335-2342.
19. Brown BG, Zhao XQ, Chait A, et al. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med*. 2001;345(22):1583-1592.
20. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20 536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002;360(9326):23-33.
21. Waters DD, Alderman EL, Hsia J, et al. Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in postmenopausal women: a randomized controlled trial. *JAMA*. 2002;288(19):2432-2440.
22. Salonen RM, Nyyssönen K, Kaikkonen J, et al. Six-year effect of combined vita-

min C and E supplementation on atherosclerotic progression: the Antioxidant Supplementation in Atherosclerosis Prevention Study (ASAP). *Circulation.* 2003; 107(7):947-953.

23. Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med.* 1994;330(15):1029-1035.

24. de Gaetano G; Collaborative Group of the Primary Prevention Project. Low-dose aspirin and vitamin E in people at cardiovascular risk: a randomised trial in general practice. *Lancet.* 2001;357(9250):89-95.

25. Lee IM, Cook NR, Gaziano JM, et al. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. *JAMA.* 2005;294(1):56-65.

26. Miller ER, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med.* 2005;142(1):37-46.

27. Stephens NG, Parsons A, Schofield PM, et al. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet.* 1996;347(9004):781-786.

28. Boaz M, Smetana S, Weinstein T, et al. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomized placebo-controlled trial. *Lancet.* 2000;356(9237):1213-1218.

29. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet.* 1999; 354(9177):447-455.

30. Rapola JM, Virtamo J, Ripatti JK, et al. Randomized trial of α-tocopherol and β-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. *Lancet.* 1997;349(9067):1715-1720.

31. Yusuf S, Dagenais G, Pogue J, et al; The Heart Outcomes Prevention Evaluation Study Investigators. Vitamin E supplementation and cardiovascular events in high-risk patients. *N Engl J Med.* 2000;342(3):154-160.

32. Lonn E, Bosch J, Yusuf S, et al; HOPE and HOPE-TOO Trial Investigators. Effects of long-term vitamin E supplementation on cardiovascular events and cancer. *JAMA.* 2005;293(11):1338-1347.

33. Hennekens CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med.* 1996;334(18):1145-1149.

34. Lee IM, Cook NR, Manson JE, et al. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. *J Natl Cancer Inst.* 1999;91(24):2102-2106.

35. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med.* 1996;334(18):1150-1155.

36. Gaziano JM, Manson JE, Ridker PM, Buring JE, Hennekens CH. Beta carotene therapy for chronic stable angina [abstract 0796]. *Circulation.* 1990;82(suppl 3): 201.

37. Devaraj S, Jialal I. Failure of vitamin E in clinical trials: is gamma-tocopherol the answer? *Nutr Rev.* 2005;63(8):290-293.

38. Blomhoff R. Dietary antioxidants and cardiovascular disease. *Curr Opin Lipidol.* 2005;16(1):47-54.

39. Jackson RD, LaCroix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med.* 2006;354(7):669-683.

40. Greenberg ER, Baron JA, Karagas MR, et al. Mortality associated with low plasma concentration of beta carotene and the effect of oral supplementation. *JAMA.* 1996;275(9):699-703.