

Effects of vitamin C and vitamin E on in vivo lipid peroxidation: results of a randomized controlled trial¹⁻³

Han-Yao Huang, Lawrence J Appel, Kevin D Croft, Edgar R Miller III, Trevor A Mori, and Ian B Puddey

ABSTRACT

Background: Lipid peroxidation may be important in the pathogenesis of atherosclerosis, particularly in its earliest stages. Evidence predominantly from in vitro studies suggests that antioxidant vitamins can prevent lipid peroxidation and that vitamin C and vitamin E have synergistic effects. However, in vivo evidence in support of these hypotheses is sparse.

Objective: The objective was to determine the effects of vitamin C and vitamin E, alone or in combination, on in vivo lipid peroxidation.

Design: We conducted a placebo-controlled, 2 × 2 factorial trial of vitamin C (500 mg ascorbate/d) and vitamin E (400 IU RRR- α -tocopheryl acetate/d) supplementation in 184 nonsmokers. The mean duration of supplementation was 2 mo. The outcome measures were changes from baseline in urinary 8-iso-prostaglandin F_{2 α} , urinary malondialdehyde + 4-hydroxyalkenals, and serum oxygen-radical absorbance capacity.

Results: The within-group mean changes (and 95% CIs) in urinary 8-iso-prostaglandin F_{2 α} (pg/mg creatinine) were 9.0 (–125.1, 143.1), –150.0 (–275.4, –24.6), –141.3 (–230.5, –52.1), and –112.5 (–234.8, 9.8) in the placebo, vitamin C alone, vitamin E alone, and vitamins C + E groups, respectively. No synergistic effect of these 2 vitamins on urinary 8-iso-prostaglandin F_{2 α} was observed ($P = 0.12$). Neither vitamin had an effect on urinary malondialdehyde + 4-hydroxyalkenals. Vitamin C, but not vitamin E, increased serum oxygen-radical absorbance capacity ($P = 0.01$).

Conclusions: Supplementation with vitamin C or vitamin E alone reduced lipid peroxidation to a similar extent. Supplementation with a combination of vitamins C and E conferred no benefit beyond that of either vitamin alone. *Am J Clin Nutr* 2002;76:549–55.

KEY WORDS Antioxidants, vitamin C, vitamin E, free radicals, lipid peroxidation, malondialdehyde, prostaglandins, oxygen-radical absorbance capacity, F₂-isoprostanes

INTRODUCTION

Lipid peroxidation may be important in the pathogenesis of atherosclerotic cardiovascular disease, particularly in its earliest stages (1). Antioxidant vitamins may prevent or mitigate lipid peroxidation. Most studies that assessed the effect of antioxidant vitamins on lipid peroxidation relied on markers that are not specifically or directly related to in vivo processes. A common approach (thiobarbituric acid–reactive substances test) is to measure certain end products of lipid peroxidation, mainly malondialdehyde

(MDA), which react with thiobarbituric acid under heat and acidic conditions (2). However, the specificity of this approach is limited (3). Another approach is to measure the susceptibility of harvested LDL to oxidation in vitro. The biological relevance of this in vitro approach is questionable (4).

F₂-isoprostanes are produced by free radical–catalyzed peroxidation of arachidonic acid (5). Of the 4 known classes of F₂-isoprostane regioisomers, most researches have focused on 8-iso-prostaglandin F_{2 α} (8-iso-PGF_{2 α}) as an in vivo marker of lipid peroxidation because of its abundance, high specificity, and potent vasoconstriction activity (6). Elevated 8-iso-PGF_{2 α} concentrations have been found in human atherosclerotic lesions (7, 8), cigarette smokers (9, 10), and patients with diabetes (11, 12), hypercholesterolemia (13), and vascular reperfusion (14).

In vivo evidence of the effects of vitamin C and vitamin E on lipid peroxidation is sparse. Vitamin C protects biomembranes against peroxidative damage in the aqueous phase in vitro (15, 16). Vitamin E (α -tocopherol) is considered the predominant lipid-soluble, chain-breaking micronutrient antioxidant. In vitro studies suggest that vitamin C may enhance the effects of vitamin E by reducing tocopheroxyl radicals (17, 18). In some (19, 20) but not all (21, 22) animal studies, a high intake of vitamin C increased tissue vitamin E concentrations. To our knowledge, there is no documentation in humans that vitamin C enhances the antioxidant effect of vitamin E. Despite the paucity of convincing evidence, a positive or supraadditive interaction of vitamin C and vitamin E is commonly cited in the medical literature.

We conducted a double-masked, placebo-controlled, 2 × 2 factorial trial to determine the main and interactive effects of vitamin C and vitamin E on the urinary excretion of 8-iso-PGF_{2 α} and MDA + 4-hydroxyalkenals (MDA and 4-hydroxyalkenals are end products of lipid peroxidation that also have mutagenic and carcinogenic

¹ From the Department of Epidemiology, Bloomberg School of Public Health (H-YH) and the Department of Medicine, School of Medicine (LJA and ERM), Johns Hopkins University, Baltimore, and the Department of Medicine, University of Western Australia, Perth, Australia (KDC, TAM, and IBP).

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³ Address reprint requests to H-Y Huang, Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University; 615 North Wolfe Street, E-6144, Baltimore, MD 21205-2223. E-mail: hyhuang@jhsph.edu.

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properties) and on serum oxygen-radical absorbance capacity (ORAC), which is a global measure of the capacity of serum to resist oxidative damage (23).

SUBJECTS AND METHODS

Study population

The study population consisted of 184 community-dwelling nonsmokers recruited between February 1996 and June 1997 in the greater Baltimore residential area of Maryland. The eligibility criteria were a willingness to provide written, informed consent and to take the study supplements but no other vitamin supplements for 2 mo. The exclusion criteria were regular exposure to passive tobacco smoke for ≥ 1 h/d and consumption of ≥ 14 alcohol beverages/wk. Persons taking vitamin supplements were eligible after a 2-mo period of abstinence. The institutional review boards of the Johns Hopkins Medical Institutions approved the protocol, and all participants provided written, informed consent.

Methods

An in-person screening visit was conducted to ascertain eligibility and obtain baseline data. Participants then made one randomization visit and 2 follow-up visits (1 and 2 mo after randomization) in which 12-h fasting blood samples and 24-h urine samples were obtained. Each participant was randomly assigned to 1 of 4 supplementation groups (placebo, vitamin C alone, vitamin E alone, or vitamins C + E) according to a fixed randomization scheme generated by the Moses-Oakford algorithm (24) with a block size of 8. Group assignment was issued by opening an opaque, sealed envelope that contained a card indicating codes for a supplementation group. Participants, data collectors, and laboratory technicians were masked to group assignment.

The study pills were tablets containing active vitamin C (500 mg ascorbate/tablet) or a corresponding placebo (dicalcium phosphate, 380 mg/tablet), both purchased from Consolidated Midland Co (Brewster, NY), and capsules containing active vitamin E (400 IU *RRR*- α -tocopheryl acetate/capsule) or a corresponding placebo (soybean oil), both from Henkel Co (LaGrange, IL). Participants were instructed to take 2 types of pills—vitamin C or placebo and vitamin E or placebo each day and to avoid taking any vitamin supplement other than the study pills during the study period. Compliance with pill-taking was determined on the basis of average pill counts (observed/expected number of pills consumed \times 100%) at each follow-up visit, changes from baseline in serum concentrations of ascorbic acid and α -tocopherol, and self-reports.

Blood samples were drawn, allowed to clot for ≤ 15 min, and centrifuged at $2000 \times g$ for 15 min at room temperature. Serum specimens were portioned into polypropylene tubes. Participants collected 24-h urine samples before the randomization visit and follow-up visits. To prevent autooxidation, the urine samples were portioned into polypropylene tubes that contained a solution of butylated hydroxytoluene and ethanol at a final concentration of 100 $\mu\text{g}/\text{mL}$. All biological specimens were stored at -70°C until analyzed.

Outcomes

The primary outcome was the change in urinary excretion of creatinine-adjusted 8-iso-PGF_{2 α} . Secondary outcomes were the changes in urinary excretion of creatinine-adjusted MDA + 4-hydroxyalkenals and serum ORAC. Change was the difference between measurements obtained at baseline and at the end of

pill-taking. The reproducibility (intraassay CV) of each measure was assessed in 40 pairs of duplicate samples that were randomly inserted into the array of specimen tubes in a pairwise fashion.

Laboratory assays

Urinary 8-iso-PGF_{2 α} was measured with enzyme immunoassay kits (Assay Designs, Inc, Ann Arbor, MI). Urine samples were treated with potassium hydroxide to hydrolyze 8-iso-PGF_{2 α} esters. After dilution with hydrochloric acid, a polyclonal antibody was used to bind with 8-iso-PGF_{2 α} in the samples on a microtiter plate. After incubation at room temperature, excess reagents were washed away and a buffer solution of *p*-nitrophenyl phosphate substrate was added. The reaction was stopped with diluted sodium hydroxide, and the generated yellow color was read at 405 nm. The intraassay CV of this assay was 9.0%.

Gas chromatography–mass spectrometry (GC-MS) (25) was used to measure urinary F₂-isoprostanes in 88 samples collected from 44 participants with low vitamin E intakes at baseline (less than the median, ie, 6.9×10^{-3} α -tocopherol equivalents/kcal). The intraassay CV of this assay was 8.0%.

Urinary free MDA + 4-hydroxyalkenals were measured by a colorimetric method (Calbiochem-Novabiochem Corp, San Diego) based on a principle similar to that of the thiobarbituric acid–reactive substances assay. Urine samples were mixed with *N*-methyl-2-phenylindole (in acetonitrile) for 3–4 s and then with methanesulfonic acid. The mixture was incubated at 45°C for 40 min and cooled on ice. The absorbance was measured at 586 nm. The intraassay CV of this assay was 21.7%.

Serum ORAC was measured by a system with β -phycoerythrin as fluorescent indicator protein, 2-2'-azo-bis (2-amidinopropane) dihydrochloride as a peroxy radical generator, and the water-soluble vitamin E analogue Trolox (Hoffmann-La Roche, Nutley, NJ) as a reference standard (23). The overall antioxidant capacity was expressed as ORAC units, where one unit equals the net protection by 1 μmol Trolox/L. The intraassay CV of this assay was 3.0%.

Serum ascorbic acid was measured based on the reduction of Fe³⁺ to Fe²⁺ by ascorbic acid, followed by chromogenic chelation of Fe²⁺ with ferrozine (26). The intraassay CV of this assay was 3.2%. Serum α -tocopherol was measured by isocratic HPLC (27). The intraassay CV of this assay was 3.3%. Urinary creatinine was measured by a modified Jaffé reaction (28). The intraassay CV of this assay was 2.7%.

Statistical analysis

The statistical analyses were performed on an intention-to-treat basis. The sample size of 184 had 90% statistical power to detect a 10% change in urinary 8-iso-PGF_{2 α} by either vitamin alone. Multiple linear regression models were used to estimate the main and interactive effects of vitamin C and vitamin E supplements on the outcomes. Post hoc subgroup analyses were performed according to sex, ethnic group, chronic illness (hypertension, diabetes mellitus, or hypercholesterolemia compared with none of the above), baseline oxidative damage to lipids (the highest compared with the lowest one-third of 8-iso-PGF_{2 α} and MDA + 4-hydroxyalkenals), baseline ORAC (the lowest compared with the highest one-third), and baseline serum vitamin C and vitamin E. The statistical analyses were performed with the SAS system for WINDOWS (version 6.12; SAS Institute Inc, Cary, NC). Hypothesis tests were considered statistically significant at a 2-sided α level < 0.05 .

RESULTS

Of 318 individuals screened, 184 participants were randomly assigned to a treatment group. Loss of interest was the main reason for nonenrollment. None of the baseline characteristics were signi-

ificantly different between the 4 groups, except for race ($P = 0.03$) and serum ascorbic acid concentrations ($P = 0.02$) (Table 1).

The percentages of participants who completed the 2 follow-up visits were 91.5%, 89.1%, 93.3%, and 95.6% in the placebo, vitamin C, vitamin E, and vitamins C + E groups; 93% of the participants took $\geq 90\%$ of the study pills. Serum vitamin concentrations in the active vitamin groups were significantly increased compared with the corresponding placebo group ($P = 0.0001$ for both vitamins). Vitamin C supplementation did not affect the change in serum α -tocopherol concentrations by vitamin E supplementation ($P = 0.49$ for the interaction term) and vice versa ($P = 0.92$ for the interaction term) (Table 2). Seventy-five percent of the participants reported perfect compliance, and no differences

in compliance between the vitamin and placebo groups were observed.

Fifty-three percent of the participants correctly guessed whether they were taking a vitamin or placebo pill. There was no difference in the percentage correct between the vitamin and placebo groups. These results are consistent with chance alone, without any evidence of unmasking.

The changes (means and 95% CIs) in urinary 8-iso-PGF_{2 α} (pg/mg creatinine) from baseline to the end of supplementation are shown in Figure 1. The pattern of within-group changes and the results of the regression analyses suggest no synergistic interactive effects of vitamin C and vitamin E ($P = 0.12$). That is, supplementation with a combination of vitamins C and E conferred no

TABLE 1

Baseline characteristics of subjects by group[†]

Characteristic	Group			
	Placebo (n = 47)	Vitamin C (n = 46)	Vitamin E (n = 45)	Vitamin C + E (n = 46)
Age (y)	58.1 \pm 13.8 ²	61.2 \pm 12.3	55.5 \pm 14.5	57.7 \pm 13.8
Women (%)	48.9	56.5	64.4	52.2
Race (%) ³				
African American	48.9	45.6	71.1	34.8
White	46.8	52.2	24.4	58.7
Other	4.3	2.2	4.4	6.5
Education, more than high school (%)	83.0	84.8	68.9	80.4
BMI (kg/m ²)	28.9 \pm 4.8	28.7 \pm 5.4	28.6 \pm 5.1	28.9 \pm 5.9
Regular exercise (%) ⁴	53.2	52.5	62.2	56.5
Alcohol consumption (%)				
None	63.0	56.5	51.1	52.2
<2 drinks/d	37.0	43.5	48.9	47.8
Hypertension (%) ⁵	45.6	34.8	53.3	52.2
Diabetes (%) ⁶	17.0	13.0	8.9	8.7
Hypercholesterolemia (%) ⁷	29.8	39.1	29.5	34.8
Prior antioxidant supplement use (%)	19.1	17.4	22.2	15.2
Dietary intake ⁸				
Vitamin C (mg/d)	149.8 \pm 96.3	127.6 \pm 69.5	129.6 \pm 70.6	143.4 \pm 80.
Vitamin E (α -TE/d)	8.8 \pm 7.1	7.4 \pm 4.2	8.5 \pm 5.7	11.1 \pm 7.7
Fruit and vegetables (servings/d)	3.8 \pm 2.1	4.1 \pm 1.9	3.9 \pm 2.0	3.9 \pm 2.2
Serum concentration				
Ascorbic acid (μ mol/L) ⁹	62.6 \pm 15.3	62.0 \pm 14.7	57.0 \pm 17.8	66.8 \pm 13.2
α -Tocopherol (μ mol/L)	28.3 \pm 8.1	29.3 \pm 7.7	26.6 \pm 7.0	26.8 \pm 7.0
Total cholesterol (mmol/L)	5.69 \pm 1.06	5.78 \pm 1.07	5.65 \pm 1.11	5.62 \pm 0.91
HDL cholesterol (mmol/L)	1.28 \pm 0.41	1.32 \pm 0.44	1.40 \pm 0.54	1.29 \pm 0.33
LDL cholesterol (mmol/L)	3.73 \pm 0.97	3.83 \pm 1.07	3.66 \pm 0.91	3.69 \pm 0.81
Triacylglycerol (mmol/L)	1.47 \pm 0.69	1.37 \pm 0.74	1.25 \pm 0.81	1.38 \pm 0.73
ORAC (μ mol Trolox/L) ¹⁰	5537 \pm 804	5251 \pm 537	5417 \pm 568	5361 \pm 602
Urinary excretion				
Creatinine (μ mol/L)	8363 \pm 4482	7496 \pm 3775	8539 \pm 4137	8035 \pm 4119
8-Iso-PGF _{2α} (pg/mg creatinine) ¹¹	1590 \pm 531	1589 \pm 579	1483 \pm 520	1559 \pm 455
F ₂ -isoprostanes (pg/mg creatinine) ¹²	1072 \pm 298	2081 \pm 1365	1894 \pm 1877	1544 \pm 694
MDA + 4-hydroxyalkenals (nmol/mmol creatinine)	922 \pm 639	1022 \pm 692	881 \pm 564	901 \pm 558

[†]TE, tocopherol equivalents; ORAC, oxygen-radical absorbance capacity; PGF_{2 α} , prostaglandin F_{2 α} ; MDA, malondialdehyde.

² $\bar{x} \pm$ SD.

³ $P = 0.03$.

⁴Activity sufficient to work up a sweat at least once a week.

⁵Defined on the basis of a systolic blood pressure ≥ 140 mm Hg, a diastolic blood pressure ≥ 90 mm Hg, a physician's diagnosis, or the use of hypertension medication.

⁶Defined on the basis of a fasting glucose concentration ≥ 126 mg/dL, a physician's diagnosis, or the use of diabetes medication.

⁷Defined on the basis of a total cholesterol concentration ≥ 240 mg/dL or the use of hypercholesterolemia medication.

⁸Measured by the Block food-frequency questionnaire.

⁹ $P = 0.02$ (Wilcoxon's rank-sum test).

¹⁰Trolox (Hoffmann-La Roche, Nutley, NJ).

¹¹Measured by enzyme immunoassay.

¹²Measured by gas chromatography-mass spectrometry ($n = 11$ per group).

TABLE 2

Changes in concentrations of serum vitamins, urinary F₂-isoprostanes, urinary malondialdehyde (MDA) + 4-hydroxyalkenals, and serum oxygen-radical absorbance capacity (ORAC)¹

Measure	Within-group change				Main effect ²		Interactive effect ³
	Placebo	Vitamin C	Vitamin E	Vitamins C + E	Vitamin C	Vitamin E	
Ascorbic acid (μmol/L)	-0.1 ± 2.1	20.3 ± 3.8	2.6 ± 2.7	22.3 ± 5.4	20.1 ± 3.7 (<i>P</i> = 0.0001)	2.3 ± 3.7 (<i>P</i> = 0.53)	-0.8 ± 7.4 (<i>P</i> = 0.92)
α-Tocopherol (μmol/L)	-0.3 ± 0.5	0.1 ± 0.7	15.0 ± 2.2	17.5 ± 1.7	1.5 ± 1.4 (<i>P</i> = 0.30)	16.3 ± 1.4 (<i>P</i> = 0.0001)	2.0 ± 2.9 (<i>P</i> = 0.49)
F ₂ -isoprostanes (pg/mg creatinine) ⁴	38.7 ± 137.0	-175.6 ± 190.1	-462.7 ± 427.2	-88.7 ± 137.9	79.9 ± 242.2 (<i>P</i> = 0.74)	-207.3 ± 242.2 (<i>P</i> = 0.40)	588.2 ± 484.4 (<i>P</i> = 0.23)
MDA + 4-hydroxyalkenals (nmol/mmol creatinine)	13.3 ± 68.5	29.1 ± 73.1	-23.0 ± 70.7	98.3 ± 70.7	68.5 ± 70.8 (<i>P</i> = 0.33)	16.5 ± 70.8 (<i>P</i> = 0.82)	105.5 ± 141.5 (<i>P</i> = 0.46)
ORAC (μmol Trolox/L) ⁵	-167.0 ± 76.7	139.5 ± 60.4	-102.8 ± 85.6	-21.9 ± 73.3	194.0 ± 75.0 (<i>P</i> = 0.01)	-48.6 ± 75.0 (<i>P</i> = 0.52)	-225.6 ± 150.0 (<i>P</i> = 0.13)

¹ $\bar{x} \pm SE$.

²The difference in measures between the active vitamin C (or vitamin E) and placebo vitamin C (or vitamin E) groups, averaged over the 2 groups that were or were not assigned to take active vitamin E or vitamin C.

³The difference in the effect of vitamin C on the measures between active vitamin E and placebo vitamin E groups.

⁴Measured by gas chromatography–mass spectrometry (*n* = 11 per group).

⁵Trolox (Hoffmann-La Roche, Nutley, NJ).

additional benefit beyond that of each vitamin alone. Adjustment for unbalanced baseline variables (ie, serum ascorbic acid concentrations, race, and other variables (age, sex, education, chronic illness, alcohol consumption, and prior antioxidant use) did not alter the pattern of these results. The same results persisted after exclusion of data from subjects who had diabetes, hypercholesterolemia, or both and of data from persons who changed their medication use during the trial (2 began taking premarin, 1 discontinued the use of premarin, and 1 discontinued cholesterol-lowering therapy).

Urinary 8-iso-PGF_{2α} measured by enzyme immunoassay was significantly correlated with urinary F₂-isoprostanes measured by GC-MS: Pearson's *r* = 0.68 (*P* < 0.0001) and 0.52 (*P* = 0.0004) at baseline and at the end of supplementation, respectively. Neither the main effect nor the interactive effect of supplementation on F₂-isoprostanes was significant, probably because of the small sample size (*n* = 11 per group) (Table 2). Neither vitamin had a main or interactive effect on urinary MDA + 4-hydroxyalkenals. Vitamin C, but not vitamin E, significantly increased serum ORAC (Table 2).

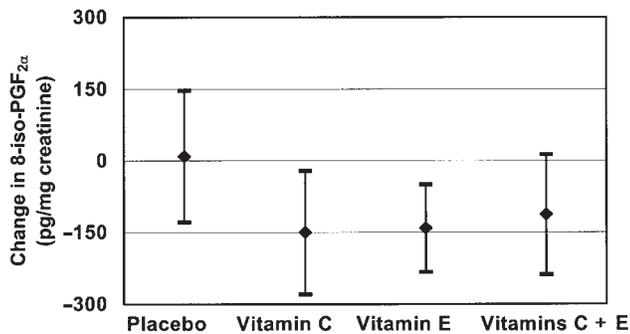


FIGURE 1. Mean changes (and 95% CIs) in urinary 8-iso-prostaglandin F_{2α} (PGF_{2α}): 9.0 (-125.1, 143.1) in the placebo group, -150.0 (-275.4, -24.6) in the vitamin C group, -141.3 (-230.5, -52.1) in the vitamin E group, and -112.5 (-234.8, 9.8) in the vitamins C + E group. There was no synergistic interactive effect of vitamins C and E (*P* = 0.12).

Results of the analyses of the subgroups with serum α-tocopherol concentrations less than the lowest tertile at baseline were similar to those of the total group, with the following notable findings. In the subgroup with baseline serum α-tocopherol concentrations less than the lowest tertile (24.4 μmol/L), supplementation with a combination of vitamins C and E increased serum ascorbic acid and α-tocopherol to concentrations that were nearly twice those by each vitamin alone (Table 3). However, the sparing effect was not significant and did not result in an additional reduction in urinary 8-iso-PGF_{2α}. No sparing effect of vitamin E supplements on serum ascorbic acid was observed in the subgroup that had lower serum ascorbic acid concentrations at baseline.

DISCUSSION

In this placebo-controlled trial of nonsmoking adults, vitamin C, vitamin E, and a combination of both vitamins reduced the in vivo lipid peroxidation as measured by urinary 8-iso-PGF_{2α}. No synergistic effects of these 2 vitamins on lipid peroxidation were observed. Neither vitamin had a main effect or an interactive effect on urinary MDA + 4-hydroxyalkenals. Vitamin C, but not vitamin E, increased serum ORAC.

The core design of this trial permitted a direct assessment of the interactive effects of vitamin C and vitamin E supplements on in vivo lipid peroxidation. Although some in vitro experiments and animal studies suggest that vitamin C potentiates the antioxidant effects of vitamin E by reducing tocopheroxyl radicals (17–20), we detected no such benefit on in vivo lipid peroxidation. One explanation is that there is a floor or threshold effect, ie, as a result of endogenous processes such as normal aerobic metabolisms and phagocytosis, a certain level of oxidative damage is inevitable. Alternatively, other antioxidants might be more effective than is exogenous vitamin C in reducing tocopheroxyl radicals.

This trial is by far the largest that assessed, in a rigorous fashion, the main and interactive effects of vitamin C and vitamin E on in vivo lipid peroxidation. Previous studies suggest a beneficial effect of antioxidant vitamins on 8-iso-PGF_{2α}, but most of these studies were conducted in selected samples and did not have a placebo-control group. In an uncontrolled study of 22 hypercho-

TABLE 3

Changes in concentrations of serum vitamins and urinary 8-iso prostaglandin F_{2α} (8-iso-PGF_{2α}) in the subgroup with serum α-tocopherol concentrations less than the lowest tertile (24.4 μmol/L) at baseline¹

Measure	Within-group change				Main effect ²		Interactive effect ³
	Placebo (n = 14)	Vitamin C (n = 13)	Vitamin E (n = 16)	Vitamins C + E (n = 19)	Vitamin C	Vitamin E	
Ascorbic acid (μmol/L)	-3.6 ± 2.7	15.8 ± 7.6	2.1 ± 3.8	30.0 ± 7.5	23.6 ± 6.2 (P = 0.0004)	10.0 ± 6.3 (P = 0.11)	8.5 ± 12.5 (P = 0.50)
α-Tocopherol (μmol/L)	0.12 ± 0.8	0.5 ± 0.5	9.6 ± 2.1	15.1 ± 2.0	2.9 ± 1.7 (P = 0.08)	12.0 ± 1.7 (P = 0.0001)	5.0 ± 3.4 (P = 0.14)
8-Iso-PGF _{2α} (pg/mg creatinine)	-55.8 ± 78.4	-216.2 ± 117.1	-96.6 ± 71.6	-101.6 ± 96.8	-82.7 ± 95.1 (P = 0.39)	36.9 ± 94.8 (P = 0.70)	155.5 ± 189.7 (P = 0.42)

¹ $\bar{x} \pm SE$.

²The difference in measures between the active vitamin C (or vitamin E) and placebo vitamin C (or vitamin E) groups, averaged over the 2 groups that were or were not assigned to take active vitamin E or vitamin C.

³The difference in the effect of vitamin C on the measures between active vitamin E and placebo vitamin E groups.

⁴Measured by gas chromatography–mass spectrometry (n = 11 per group).

lesterolemic patients, supplementation with 100 or 600 mg vitamin E/d for 2 wk significantly reduced urinary 8-iso-PGF_{2α} by 34% and 58%, respectively (13). In another uncontrolled study (9), in which 5 heavy smokers took vitamin C (2 g/d) and 4 heavy smokers took a combination of vitamin C (2 g/d) and vitamin E (800 IU/d) for 5 d, reductions in urinary 8-iso-PGF_{2α} (29% and 23%, respectively) were documented. Vitamin E alone (either 100 IU/d in 5 moderate smokers or 800 IU/d in 7 heavy smokers) did not suppress urinary 8-iso-PGF_{2α}.

In contrast with our findings, one study in apparently healthy persons showed no benefits on lipid peroxidation of α-tocopherol supplements at dosages of 200, 400, 800, 1200, or 2000 IU/d for 8 wk (29). The characteristics of the study participants in that study ($\bar{x} (\pm SD)$ age of 38 ± 12.5 and 120% of ideal weight) were different from those of the participants in our trial [58 ± 13.7 y and a body mass index (in kg/m²) of 28.8 ± 5.3]. The sample size was just 5 in each dosage group of that study.

In the subgroup with lower serum α-tocopherol concentrations at baseline, the increases in serum ascorbic acid and α-tocopherol concentrations resulting from supplementation with both vitamins C and E were approximately twice those by each vitamin alone. However, the sparing effect was not significant and did not result in additional reduction in urinary 8-iso-PGF_{2α}. These findings further support the notion that a floor or threshold effect on lipid peroxidation might have been achieved by use of either vitamin alone. No sparing effect of either vitamin was observed in the subgroup with baseline serum vitamin C concentrations in the lowest tertile, presumably because baseline vitamin C concentrations were not low enough or because oxidized ascorbic acid concentrations were reduced by other antioxidants, such as glutathione and the NADPH system.

The finding that vitamin C supplementation significantly increased serum ORAC is consistent with data from in vitro studies that showed vitamin C to be more effective than other antioxidants in plasma in inhibiting lipid peroxidation initiated by peroxy radicals in the aqueous phase (15, 16). Supplementation with vitamin E did not increase serum ORAC, possibly because the site of action of vitamin E is nonaqueous (ie, the lipophilic component of lipoproteins). Vitamin C and vitamin E, either alone or combined, did not affect urinary MDA + 4-hydroxyalkenals. The limited reproducibility of the colorimetric assay for MDA + 4-hydroxyalkenals hinders its use and may have accounted for the absence of treatment effects.

Urinary 8-iso-PGF_{2α} measured with an enzyme immunoassay was significantly correlated with F₂-isoprostanes measured by GC-MS. Both methods also had similar intraassay reproducibility. Because the GC-MS method is labor-intensive, the enzyme immunoassay may be a satisfactory alternative in studies with large sample sizes.

The effect of antioxidant vitamin supplements on clinical outcomes remains uncertain. Prospective cohort studies observed a lower risk of coronary heart disease in men and women after the consumption of vitamin E supplements (≥ 100 IU/d) for > 2 y (30, 31) and a lower risk of cardiovascular death in a sample of the US population after the consumption of vitamin C supplements for a median of 10 y (32). Large-scale secondary prevention trials, however, have reported inconsistent results (33–37). These discrepancies may have been attributed to differences in dosages and durations of supplementation, host factors, medication use, and genetic disposition (38). Most importantly, lipid peroxidation occurs in the early stage of atherosclerosis (39, 40), and protections against damage may be more effective in early rather than late stages of atherosclerotic cardiovascular disease.

The results of this trial have several implications pertaining to the use of antioxidant vitamins as a means to prevent atherosclerotic cardiovascular diseases, particularly with respect to primary prevention. First, supplementation of the usual diet with vitamin C (500 mg/d) or vitamin E (400 IU/d) resulted in a reduction in vivo lipid peroxidation. Second, the extent of the reduction in lipid peroxidation by each vitamin or the combination thereof was similar. A daily vitamin C dose of 500 mg is attainable through the diet, whereas it is virtually impossible to consume 400 IU vitamin E/d through food alone. Third, the lack of a synergistic interactive effect of these 2 vitamins suggests that, for the large number of Americans who consume antioxidant vitamins, supplementation with both vitamins may not confer additional benefit on lipid peroxidation beyond that by either vitamin alone.

Among the strengths of the present study are its core design (a placebo-controlled 2 × 2 factorial trial) and large sample size. High rates of follow-up and adherence with pill-taking, along with effective masking, enhance its internal validity. We enrolled a demographically heterogeneous group of participants (55% women, 50% African Americans), many of whom had risk factors for atherosclerotic cardiovascular disease. Hence, the results of this trial should be generalizable to individuals who choose to take antioxidant supplements, including persons who are at risk

of atherosclerotic cardiovascular diseases (eg, hypertensive, diabetic, or hypercholesterolemic patients). The potential limitations of this study include the brief duration of pill-taking (2 mo), which, however, exceeded the duration of other studies (9, 13). In the present study, the magnitude of the mean increase in serum α -tocopherol concentration in the vitamin E and the vitamins C + E groups was similar to that of the group treated with 400 IU vitamin E/d in the Cambridge Heart Antioxidant Study (33), in which the median follow-up time was 510 d.

The results of the present trial provide evidence of the effects of 2 widely used antioxidant vitamins and, for the first time, their interactive effects on *in vivo* lipid peroxidation in humans. Specifically, supplementation with 500 mg vitamin C/d or 400 IU vitamin E/d for 2 mo resulted in a reduction in lipid peroxidation of $\approx 10\%$ on the basis of the measured urinary excretion of 8-iso-PGF_{2 α} , but supplementation with a combination of both vitamin C and vitamin E conferred no additional benefit. 

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