

# The Effects of Vitamin C and Vitamin E on Oxidative DNA Damage: Results from a Randomized Controlled Trial<sup>1</sup>

Han-Yao Huang,<sup>2</sup> Kathy J. Helzlsouer, and Lawrence J. Appel

Department of Epidemiology, School of Hygiene and Public Health [H-Y. H., K. J. H.], and Department of Medicine, School of Medicine [L. J. A.], Johns Hopkins University, Baltimore, Maryland 21205-2223

## Abstract

Oxidative DNA damage may be important in mutagenic, carcinogenic, and aging processes. Although it is plausible that antioxidant vitamins may reduce oxidative DNA damage, evidence from human studies has been sparse and inconsistent. We determined the short-term effects of vitamin C (500 mg/day) and vitamin E (400 IU *d*- $\alpha$ -tocopheryl acetate/day) supplements on oxidative DNA damage in a double-masked, placebo-controlled, 2×2 factorial trial in 184 nonsmoking adults. Mean duration of supplementation was 2 months. Oxidative DNA damage was measured by 24-h urinary excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG). At baseline, urinary 8-OHdG (mean  $\pm$  SE; ng/mg creatinine) was associated with race (15.6  $\pm$  0.8 in African Americans *versus* 20.3  $\pm$  1.2 in Caucasians,  $P = 0.001$ ), prior antioxidant supplement use (18.6  $\pm$  0.8 in users *versus* 13.8  $\pm$  1.5 in non-users,  $P = 0.007$ ), and regular exercise (19.2  $\pm$  1.1 in exercisers *versus* 16.6  $\pm$  0.9 in non-exercisers,  $P = 0.04$ ). Fruit and vegetable intake and serum ascorbic acid were inversely associated with urinary 8-OHdG ( $P$ -trend = 0.02 and 0.016, respectively). The benefits of fruit and vegetable intake became evident with the consumption being at least three servings/day. At the end of supplementation, change from baseline in urinary 8-OHdG (mean  $\pm$  SE; ng/mg creatinine) was  $-0.6 \pm 1.4$  ( $P = 0.61$ ),  $0.6 \pm 1.1$  ( $P = 0.59$ ),  $0.5 \pm 1.0$  ( $P = 0.61$ ), and  $1.6 \pm 1.4$  ( $P = 0.27$ ) in the placebo, vitamin C alone, vitamin E alone, and combined vitamins C and E groups, respectively. In overall and subgroup analyses, there was no significant main effect or interaction effect of the supplements on urinary 8-OHdG. In conclusion, supplementation of diet with vitamin C (500 mg/day) and vitamin E (400 IU *d*- $\alpha$ -tocopheryl acetate/day) had no significant main effect or interaction effect on oxidative DNA damage as measured by urinary

8-OHdG in nonsmoking adults. However, several aspects of a healthy lifestyle were associated with lower oxidative DNA damage.

## Introduction

Oxidative damage to DNA by ROS<sup>3</sup> and RNS, such as hydrogen peroxide, hydroxyl radicals, singlet oxygen, and peroxytrite, may be important in mutagenic, carcinogenic, and aging processes (1–3). Sources of ROS and RNS are multiple, of which some are inevitable, such as normal aerobic metabolism and phagocytosis. During mitochondrial respiration, oxygen may undergo single electron transfer and generate superoxide anion radicals, which are normally converted to hydrogen peroxides by superoxide dismutase. Hydrogen peroxides can be transported across nuclear membrane and, in the presence of metal ions, produce hydroxyl radicals that cause oxidative modification of DNA. In addition, ROS and RNS may initiate and propagate lipid peroxidation, of which the major end products, malondialdehyde and 4-hydroxynonenal, possess genotoxic and mutagenic properties (4).

Oxidative damage to DNA may result in base modification, sugar damage, strand break, and DNA-protein cross-links. Of these, modification of guanine by hydroxyl radicals at the C-8 site, frequently estimated as 8-OHdG, is the most commonly studied lesion (5). Urinary excretion of 8-OHdG, the repair product from oxidative DNA modification by excision enzymes, is an *in vivo* measure of overall oxidative DNA damage (6). In *in vitro* studies, exposure of DNA to oxidants, such as singlet oxygen, UV light, radiation, and Fenton reaction, results in production of 8-OHdG (7–11). Also, high oxidative stress, such as smoking or extreme exercise, is associated with high 8-OHdG production (12–14). Although direct evidence that links 8-OHdG with cancer risk is lacking, increased 8-OHdG has been found in cancerous tissues (15). Urinary 8-OHdG was higher in small cell lung carcinoma patients compared with normal controls and increased in non-small cell lung carcinoma patients during the course of radiotherapy (16).

Evidence from observational epidemiological studies suggests that antioxidant vitamins may reduce the risk of developing cancer (17). Vitamin C and vitamin E are considered the most important water-soluble and lipid-soluble micronutrient antioxidants, respectively. *In vitro* studies suggest that vitamin C and vitamin E may protect against oxidative damage to DNA as measured by 8-OHdG (9, 18, 19), and that vitamin C may regenerate vitamin E by reducing tocopherol radicals (20, 21). However, in an animal feeding study, combinations of three dietary levels of vitamin C and vitamin E resulted in no protective effect on oxidative damage in the liver DNA of guinea

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<sup>2</sup> To whom requests for reprints should be addressed, at Department of Epidemiology, Johns Hopkins School of Hygiene and Public Health, 615 North Wolfe Street, E-6006, Baltimore, MD 21205-2223. Phone: (410) 502-9094; Fax: (410) 614-2632.

<sup>3</sup> The abbreviations used are: ROS, reactive oxygen species; RNS, reactive nitrogen species; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; CV, coefficient of variation.

pigs (22). In human studies (one cross-sectional study and four clinical trials), the effects of these antioxidant vitamins on 8-OHdG have been inconsistent (23–27).

We determined the effects of vitamin C and vitamin E, alone and combined, on oxidative DNA damage as measured by urinary excretion of 8-OHdG in a double-masked, placebo-controlled, 2×2 factorial trial.

## Materials and Methods

**Study Population.** The study population consisted of 184 community-dwelling nonsmokers recruited between February 1996 and June 1997 in Baltimore, Maryland. Eligibility criteria were willingness to provide written informed consent and to take study pills, but no other vitamin supplements, for 2 months. Exclusion criteria were current tobacco use, regular exposure to passive tobacco smoke for  $\geq 1$  h/day, and consumption of  $\geq 14$  alcohol beverages/week. Individuals who took vitamin supplements were eligible after a 2-month period of abstinence.

**Conduct of Trial.** Institutional review boards of the Johns Hopkins Medical Institutions approved the protocol of this trial. All participants provided written informed consent.

An in-person screening visit was conducted to ascertain eligibility and to obtain baseline data, including demographic characteristics, weight, height, body mass index (weight/height<sup>2</sup>, kg/m<sup>2</sup>), medical history, medication use, prior supplement use, regular exercise (regularly engage in any activity long enough to work up a sweat at least once a week), alcohol consumption, and habitual diet assessed by the Health Habits and History Questionnaire (National Cancer Institute version HHHQ FULL.JAN92; Ref. 28). The dietary questionnaire listed three serving sizes (small, medium, and large) and stated medium portions for food items, including fruits and vegetables. The DIETSYS 4.01 software was used to link the questionnaire with NHANES II nutrient content database, which was based on United States Department of Agriculture food composition data tapes and other sources.

Eligible and interested participants then attended one randomization visit and two follow-up visits (1 and 2 months after randomization) in which 12-h fasting blood samples and 24-h urine samples were obtained. Participants were randomized to one of four supplementation groups (placebo, vitamin C alone, vitamin E alone, and combined vitamin C and vitamin E), using a fixed randomization scheme generated by the Moses-Oakford algorithm (29) with a block size of 8 and an allocation ratio of 1:1:1:1. Study participants, data collectors, and laboratory technicians were masked to group assignment.

Blood samples were drawn from an antecubital vein into a Vacutainer, allowed to clot at room temperature for not more than 15 min, and then centrifuged at  $2000 \times g$  for 15 min at room temperature. Serum specimens were aliquoted into polypropylene storage tubes. Participants collected 24-h urine samples before the randomization visit and each follow-up visit. To prevent auto-oxidation, the 24-h urine collections were aliquoted into polypropylene storage tubes that contained butylated hydroxytoluene/ethanol solution at a final concentration of 100  $\mu\text{g}/\text{ml}$ . All biological specimens were stored at  $-70^\circ\text{C}$  until analysis.

Pills used in this study were: active vitamin C tablets (500 mg ascorbate/tablet) and corresponding placebo tablets (dicalcium phosphate, 380 mg/tablet), both purchased from Consolidated Midland Co., and active vitamin E capsules (400 IU *d*- $\alpha$ -tocopheryl acetate/capsule) and corresponding placebo capsules (soybean oil), both donated from Henkel Co. Participants were instructed to take two types of pills (vitamin C/

placebo and vitamin E/placebo) each day and to not change their diets or take other vitamin supplements for 2 months. Adherence with pill-taking was assessed by the average of pill counts (observed/expected number of pills consumed  $\times 100\%$ ) at each follow-up visit, changes in serum levels of ascorbic acid and  $\alpha$ -tocopherol from baseline, and self-reports (30).

**Outcome Measures.** The outcome measure in this study was change in urinary excretion of creatinine-adjusted 8-OHdG. Change was the difference between measurements obtained at baseline and the end of pill-taking. Reproducibility (intra-assay CV) of the laboratory measurements 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-OHdG was measured by an ELISA (16). Urine samples were centrifuged at  $300 \times g$  for 10 min to remove any particulate material. To each well of the ELISA kit, a 50- $\mu\text{l}$  urine sample and 50  $\mu\text{l}$  of reconstituted primary antibody were added. The plate was covered, incubated at  $37^\circ\text{C}$ , and mixed continuously for 1 h. The antibodies bound to the 8-OHdG in the sample were washed with 0.05% Tween 20/phosphoric acid buffer. An enzyme-labeled secondary antibody was added to the plate, which was then incubated at  $37^\circ\text{C}$  and mixed continuously for 1 h, and the unbound enzyme-labeled secondary antibody was washed away. The amount of antibody bound to the plate was determined by the color developed from the addition of a chromatic substrate (*o*-phenylenediamine) and read at 492 nm. Quantification of the 8-OHdG was achieved by comparing the optical densities of the chromogenic signal of each sample to that of an internal standard of known 8-OHdG concentrations. The intra-assay CV of this assay was 8.8%.

Serum ascorbic acid levels were measured based on the reduction of Fe(III) to Fe(II) by ascorbic acid, followed by chromogenic chelation of Fe(II) with ferrozine (31). Intra-assay CV of this assay was 3.2%. Serum  $\alpha$ -tocopherol levels were measured by isocratic high performance liquid chromatography (32). Intra-assay CV of this assay was 3.3%. Urinary creatinine was measured by a modified Jaffé reaction (33). Intra-assay CV of this assay was 2.7%.

**Statistical Methods.** Statistical analyses were performed on an “intention to treat” basis. The sample size of 184 individuals was estimated to provide statistical power of 80% to detect a 20% reduction in urinary excretion of 8-OHdG by either vitamin alone.

The associations between baseline characteristics and urinary 8-OHdG were assessed, using a *t* test or Wilcoxon Rank Sum test for discrete baseline variables and a linear regression model for continuous baseline variables. Multiple linear regression models were used to estimate the main effects and interaction effect of vitamin C and vitamin E supplementation on creatinine-adjusted urinary 8-OHdG, with or without adjustment for potential confounders or baseline variables that were unbalanced across groups. Post-hoc subgroup analyses were performed by ethnicity (African American *versus* Caucasians), regular exercise (yes *versus* no), fruit/vegetable intake ( $\leq 2$ , 3–4,  $\geq 5$  servings/day), chronic illness (hypertension, hypercholesterolemia, or diabetes mellitus *versus* none), serum vitamin level (lowest *versus* highest tertile), and urinary 8-OHdG excretion (lowest *versus* highest tertile) at baseline. Statistical tests were two-sided with an  $\alpha$  level of 0.05.

## Results

Of 318 persons screened, 184 individuals were randomized. The main reason for non-enrollment was loss of interest. Overall, the mean (SD) age of the trial participants was 58 (14) years

Table 1 Baseline characteristics by randomized group

Characteristic <sup>a</sup>	Randomized group			
	Placebo (n = 47)	Vitamin C (n = 46)	Vitamin E (n = 45)	Combined (n = 46)
Age	58.1 (13.8)	61.2 (12.3)	55.5 (14.5)	57.7 (13.8)
Women (%)	48.9	56.5	64.4	52.2
Race <sup>b</sup> (%)				
African American	48.9	45.6	71.1	34.8
Caucasian	46.8	52.2	24.4	58.7
Others	4.3	2.2	4.4	6.5
Education (%)				
>High school	83.0	84.8	68.9	80.4
Body mass index <sup>c</sup>	28.9 (4.8)	28.7 (5.4)	28.6 (5.1)	28.9 (5.9)
Regular exercise <sup>d</sup> (%)	53.2	52.2	62.2	56.5
Alcohol consumption (%)				
None	63.0	56.5	51.1	52.2
<2 drinks/day	37.0	43.5	48.9	47.8
Hypertension <sup>e</sup> (%)	45.6	34.8	53.3	52.2
Diabetes mellitus <sup>f</sup> (%)	17.0	13.0	8.9	8.7
Hypercholesterolemia <sup>g</sup> (%)	29.8	39.1	29.5	34.8
Prior supplement use (%)				
Any vitamin	31.9	32.6	26.7	30.4
Any antioxidant	19.1	17.4	22.2	15.2
Dietary intakes <sup>h</sup>				
Vitamin C (mg/day)	149.8 (96.3)	127.6 (69.5)	129.6 (70.6)	143.4 (80.0)
Vitamin E ( $\alpha$ -TE <sup>i</sup> /day)	8.8 (7.1)	7.4 (4.2)	8.5 (5.7)	11.1 (7.7)
Fruits/vegetables (servings/day)	3.8 (2.1)	4.1 (1.9)	3.9 (2.0)	3.9 (2.2)
Serum level				
Ascorbic acid <sup>j</sup> ( $\mu$ mol/l)	62.6 (15.3)	62.0 (14.7)	57.0 (17.8)	66.8 (13.2)
$\alpha$ -Tocopherol ( $\mu$ mol/L)	28.3 (8.1)	29.3 (7.7)	26.6 (7.0)	26.8 (7.0)
Urinary excretion <sup>k</sup>				
Creatinine (mg/dl)	94.6 (50.7)	84.8 (42.7)	96.6 (46.8)	90.9 (46.6)
8-OHdG (ng/mg creatinine)	17.6 (10.4)	19.3 (9.3)	16.5 (8.4)	17.7 (9.5)

<sup>a</sup> Continuous data presented as mean (SD).

<sup>b</sup>  $P = 0.03$  from Pearson  $\chi^2$  test.

<sup>c</sup> Weight/height<sup>2</sup> (kg/m<sup>2</sup>).

<sup>d</sup> Activity sufficient to work up a sweat, on average, at least once a week.

<sup>e</sup> Systolic blood pressure  $\geq 140$  mm Hg, diastolic blood pressure  $\geq 90$  mm Hg, physician-diagnosed hypertension, or on hypertension medication.

<sup>f</sup> Diabetes mellitus (fasting glucose level  $\geq 126$  mg/dl, physician-diagnosed diabetes, or on medication for diabetes).

<sup>g</sup> Low-density cholesterol  $\geq 160$  mg/dl, or on medication for hypercholesterolemia.

<sup>h</sup> Measured by Food Frequency Questionnaire.

<sup>i</sup>  $\alpha$ -Tocopherol equivalents.

<sup>j</sup>  $P = 0.02$  from Wilcoxon Rank Sum test.

<sup>k</sup> Sample size = 47, 43, 45, and 45 in the placebo, vitamin C alone, vitamin E alone, and combined vitamins C and E groups, because of missing data.

(range, 24–86); 55% were women, and 50% were African Americans. Baseline characteristics were similar across the four supplementation groups, except for race ( $P = 0.03$ ) and serum ascorbic acid levels ( $P = 0.02$ ; Table 1).

The association between baseline urinary 8-OHdG and participant characteristics is displayed in Table 2. Caucasian Americans had higher urinary 8-OHdG than African Americans ( $P = 0.001$ ). Individuals who regularly exercised had lower urinary 8-OHdG than non-exercisers ( $P = 0.04$ ). Individuals who had prior antioxidant supplement use had lower urinary 8-OHdG than non-users ( $P = 0.007$ ). Fruit and vegetable intake and serum ascorbic acid were significantly inversely associated with urinary 8-OHdG ( $P$ -trend = 0.02 and 0.016, respectively). The benefit of fruit and vegetable intake became evident with the consumption being at least three servings/day. In a multiple linear regression model in which race, serum vitamin C, prior antioxidant supplement use, body mass index, and fruits/vegetables intake were the independent variables, race (Caucasian versus African American) and serum vitamin C levels remained significant ( $P = 0.0001$  and 0.03, respectively), whereas prior antioxidant supplement use, body mass index, and fruits/vegetables intake approached statistical significance

( $P = 0.07$ , 0.06, and 0.09, respectively) after adjustment for other variables. When stratified by race, fruits/vegetables intake and prior antioxidant supplement use were significantly associated with baseline 8-OHdG in Caucasian Americans ( $P = 0.01$  and 0.02, respectively), whereas serum vitamin C levels were marginally associated with baseline 8-OHdG in African Americans ( $P = 0.07$ ).

Dietary intake of vitamin C,  $\alpha$ -carotene,  $\beta$ -carotene, cryptoxanthin, or lutein was inversely associated with urinary excretion of 8-OHdG at baseline after adjustment for total caloric intake. However, the trend was only statistically significant for lutein ( $P = 0.03$ ). Other examined nutrients, such as vitamin E, lycopene, retinol, and folate, were not associated with baseline 8-OHdG (data not shown).

The percentage of participants who completed the first follow-up visit in the placebo, vitamin C alone, vitamin E alone, and combined vitamins C and E groups was 100, 91.3, 100, and 97.8%, respectively. The percentage of participants who completed the second follow-up visit was 91.5, 89.1, 93.3, and 95.6%, respectively. Ninety-three % of participants took 90% or more of study pills. Serum vitamin C and vitamin E levels significantly increased in the active vitamin groups (Ta-

Table 2 Association between urinary 8-OHdG and participant characteristics at baseline<sup>a</sup>

Characteristics	n (%)	Urinary 8-OHdG (ng/mg creatinine)	<i>P</i> <sup>b</sup>
Age (yr)			
<40	21 (11.4)	17.0 ± 2.2	0.41
40–49	27 (15.8)	16.8 ± 1.9	
50–59	29 (15.8)	16.1 ± 1.7	
60–69	63 (35.5)	19.2 ± 1.2	
70+	40 (21.7)	17.9 ± 1.3	
Gender			
Women	100 (55.4)	17.0 ± 1.00	0.22
Men	80 (44.6)	18.8 ± 0.9	
Race <sup>c</sup>			
African American	89 (50.0)	15.6 ± 0.8	0.001
Caucasian	83 (45.7)	20.3 ± 1.2	
Chronic medical illness <sup>d</sup>			
No	62 (35.3)	18.1 ± 1.4	0.75
Yes	118 (64.7)	17.6 ± 0.8	
BMI (kg/m <sup>2</sup> )			
<30	110 (61.4)	17.4 ± 0.9	0.46
≥30	70 (38.6)	18.3 ± 1.0	
Exercise			
No	79 (44.0)	19.2 ± 1.1	0.04
Yes	101 (56.0)	16.6 ± 0.9	
Alcohol consumption			
None	101 (56.0)	17.8 ± 0.9	0.99
<2 drinks/day	79 (44.0)	17.8 ± 1.0	
Prior antioxidant supplement use			
No	148 (81.5)	18.6 ± 0.8	0.007
Yes	32 (18.5)	13.8 ± 1.5	
Fruit/vegetable intake		Median (range)	
≤2 servings/day	35 (20.8)	20.5 (4.9–43.3)	0.02 <sup>e</sup>
3–4 servings/day	80 (47.6)	16.7 (9.7–41.4)	0.05 <sup>f</sup>
≥5 servings/day	53 (31.5)	15.6 (0.8–39.0)	0.02 <sup>g</sup>
		Regression coefficient ± SE	
Serum ascorbic acid (μmol/l)		−0.11 ± 0.04	0.016
Serum α-tocopherol (μmol/l)		−0.09 ± 0.09	0.32

<sup>a</sup> Four participants were not included in the analysis because of missing data.

<sup>b</sup> Test for the mean difference between groups (for categorized variables) or for the slope in a linear regression model (for continuous variables).

<sup>c</sup> Eight participants were neither African American nor Caucasian.

<sup>d</sup> Hypertension, diabetes, or hypercholesterolemia.

<sup>e</sup> Test for trend, using median values of fruit/vegetable intake (servings/day) in each category.

<sup>f</sup> Comparison between groups of ≤2 and 3–4 servings/day.

<sup>g</sup> Comparison between groups of ≤2 and ≥5 servings/day.

ble 3). The difference in the changes in serum vitamin levels between the active and placebo supplement groups was statistically significant ( $P = 0.0001$  for both types of vitamins). Seventy-five % of participants reported perfect adherence with pill-taking with no difference between the active and placebo supplement groups.

Approximately 53% of participants correctly guessed the type of pills (*i.e.*, active or placebo) they took. There was no difference in the percentage correct between active and placebo groups. These results are consistent with chance alone, without any evidence of unmasking.

At the end of supplementation, mean ± SE change from baseline in urinary 8-OHdG (ng/mg creatinine) was  $-0.6 \pm 1.4$  (within group  $P = 0.27$ ),  $0.6 \pm 1.1$  ( $P = 0.59$ ),  $0.5 \pm 1.0$  ( $P = 0.61$ ), and  $1.6 \pm 1.4$  ( $P = 0.27$ ) in the placebo, vitamin C alone, vitamin E alone, and combined vitamins C and E groups, respectively (Table 3; Fig. 1). The main effects of vitamin C and vitamin E supplements on urinary 8-OHdG (ng/mg creatinine) were  $1.2 \pm 1.2$  ( $P = 0.34$ ) and  $1.1 \pm 1.2$  ( $P = 0.39$ ),

respectively, without evidence of an interaction effect ( $P = 0.93$ ). Adjustment for unbalanced baseline variables, *i.e.*, serum ascorbic acid levels and race, and for other variables (age, gender, exercise, fruit and vegetable intake, and prior antioxidant use) did not alter the pattern of the results. The lack of an effect from vitamin C and vitamin E supplements on 8-OHdG persisted in subgroups defined by ethnicity, exercise habit, fruit and vegetable intake, chronic illness, serum ascorbic acid, serum α-tocopherol, and urinary 8-OHdG levels at baseline.

## Discussion

In this placebo-controlled trial of nonsmoking adults, supplementation of diet with vitamin C and vitamin E for 2 months had no significant main effect or interaction effect on oxidative DNA damage, as measured by urinary excretion of 8-OHdG. The absence of a significant effect was evident in each subgroup that we examined. In cross-sectional analyses of baseline data, factors associated with a healthy lifestyle (*e.g.*, prior antioxidant supplement use, regular exercise, higher serum vitamin C levels, and a diet rich in fruits and vegetables) were significantly associated with lower urinary 8-OHdG excretion. Intriguingly, African Americans had lower urinary excretion of 8-OHdG than Caucasians at baseline.

The finding that vitamin C and/or vitamin E had no significant effects on urinary 8-OHdG suggests that systems other than these two vitamins may be more important in preventing or mitigating *in vivo* generation of 8-OHdG. It has been shown that formation of 8-OHdG is caused by hydroxyl radicals or singlet oxygen. Hydrogen peroxide, a source of hydroxyl radicals, is detoxified primarily through glutathione activity (34). Singlet oxygen is mostly quenched by β-carotene and lycopene *in vitro* (35). In addition, intracellular enzymatic antioxidants, such as superoxide dismutase, catalase, and peroxidases, might be more important defense systems against oxidative damage to DNA than vitamin C (active in extracellular space) and vitamin E (active in biomembranes). Despite the fact that vitamin C and vitamin E had no apparent effect on oxidative DNA damage as measured by urinary 8-OHdG, they may still protect other molecules, such as lipids and proteins, from oxidative damage.

In the context of our nonsignificant effect of vitamin supplementation on 8-OHdG, the significant inverse associations of baseline 8-OHdG with serum vitamin C levels, fruit and vegetable intake, exercise, and prior antioxidant supplement use are intriguing. This pattern of results is consistent with observational epidemiological studies that demonstrate lower risk for certain types of cancer among individuals who consumed more fruits and vegetables, had higher dietary intake of vitamin C or higher serum vitamin levels (17, 36), and exercised on a regular basis (37). It is also consistent with previous studies that demonstrate regular moderate exercise may up-regulate antioxidant systems and reduce oxidative damage (38). Several reasons could have explained our findings: (a) it is plausible that effects, if any, of vitamin C and/or vitamin E supplementation on urinary 8-OHdG levels cannot be observed over 2 months; and (b) vitamin C is a surrogate marker for other factors and aspects of a healthy lifestyle, particularly dietary factors other than vitamin C and vitamin E, may have beneficial effects on 8-OHdG. To date, few human studies have assessed the effects of nutrients other than vitamin C and vitamin E on 8-OHdG formation. In one small trial, a 28% reduction ( $P = 0.039$ ) in urinary 8-oxodG was observed in five nonsmokers who ate brussel sprouts 300 g/day for 3 weeks, in comparison to a 5% reduction ( $P = 0.72$ ) in five individuals whose diets were free of cruciferous vegetables (39). Equivocal effects of



Table 3 Change in serum ascorbic acid, serum  $\alpha$ -tocopherol, and urinary excretion of 8-OHdG

Measure	Within-group change (mean $\pm$ SE)				Main effect <sup>a</sup> ( $\pm$ SE)		Interaction effect <sup>b</sup> ( $\pm$ SE)
	Placebo	Vitamin C	Vitamin E	Combined	Vitamin C	Vitamin E	Vitamin C $\times$ Vitamin E
Ascorbic acid ( $\mu$ mol/l)	-0.1 $\pm$ 2.1 ( <i>P</i> = 0.96)	20.3 $\pm$ 3.8 ( <i>P</i> = 0.0001)	2.6 $\pm$ 2.7 ( <i>P</i> = 0.34)	22.3 $\pm$ 5.4 ( <i>P</i> = 0.0002)	20.1 $\pm$ 3.7 ( <i>P</i> = 0.0001)	2.3 $\pm$ 3.7 ( <i>P</i> = 0.53)	-0.8 $\pm$ 7.4 ( <i>P</i> = 0.92)
$\alpha$ -Tocopherol ( $\mu$ mol/l)	-0.3 $\pm$ 0.5 ( <i>P</i> = 0.48)	0.1 $\pm$ 0.7 ( <i>P</i> = 0.83)	15.0 $\pm$ 2.2 ( <i>P</i> = 0.0001)	17.5 $\pm$ 1.7 ( <i>P</i> = 0.0001)	1.5 $\pm$ 1.4 ( <i>P</i> = 0.30)	16.3 $\pm$ 1.4 ( <i>P</i> = 0.0001)	2.0 $\pm$ 2.9 ( <i>P</i> = 0.49)
8-OHdG (ng/mg creatinine)	-0.6 $\pm$ 1.3 (0.61)	0.6 $\pm$ 1.1 ( <i>P</i> = 0.59)	0.5 $\pm$ 1.0 ( <i>P</i> = 0.61)	1.6 $\pm$ 1.4 ( <i>P</i> = 0.27)	1.1 $\pm$ 1.2 ( <i>P</i> = 0.34)	1.1 $\pm$ 1.2 ( <i>P</i> = 0.39)	0.2 $\pm$ 2.4 ( <i>P</i> = 0.93)

<sup>a</sup> Main effect of vitamin C was calculated as the difference in measures between active vitamin C and placebo vitamin C groups, averaged over the two groups that were not assigned to take active vitamin E, and vice versa for the main effect of vitamin E.

<sup>b</sup> Interaction effect of vitamin C and vitamin E was estimated as the difference in the effect of vitamin C on the measures between active vitamin E and placebo vitamin E groups. Both main effects and interaction effect were estimated from a full model with two indicator variables and an interaction term (a cross-product of the two indicator variables).

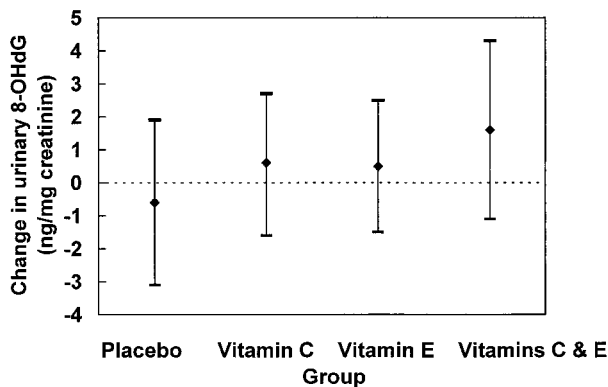


Fig. 1. Mean change (95% confidence interval) in urinary 8-OHdG by randomized group: -0.6 (-3.1-1.9), 0.6 (-1.6-2.7), 0.5 (-1.5-2.5), and 1.6 (-1.1-4.3) in the placebo, vitamin C alone, vitamin E alone, and combined vitamin C and vitamin E groups, respectively.

$\beta$ -carotene supplementation on oxidative DNA damage have been reported from two trials (40, 41).

The significant difference in urinary 8-OHdG between African Americans and Caucasian Americans is intriguing as well. Multiple regression analyses suggest that the difference was not confounded by the lifestyle factors that we examined. The exclusion of tobacco smokers and regular passive tobacco smokers from this trial also precludes smoking as a possible explanation. Hence, the observed difference may be attributable to differential genetic susceptibility, efficiency in detoxification or DNA repair, environmental factors not examined in this trial, or a combination of the above.

In this trial, participants' diets were unlikely to have changed during the supplementation period because the serum vitamin levels did not change from baseline to the end of supplementation in the placebo group. Furthermore, seasonal variations in the diets were unlikely to have confounded group comparisons because: (a) the majority (70%) of participants enrolled into and completed the trial in the Fall or the Spring; and (b) participants were evenly recruited into each supplement group over seasons via the blocking procedures of randomization.

Results from previous trials that assessed the impact of antioxidant vitamins on urinary 8-OHdG have been inconsistent. In a cross-over trial conducted in 30 healthy volunteers, supplementation of diet with vitamin C (500 mg/day) for 6 weeks reduced 8-oxoguanine but increased 8-oxoadenine, both measured in lymphocyte DNA by gas chromatography-mass

spectrometry. The authors interpreted these findings to be consistent with a pro-oxidant effect of vitamin C (23). Another trial of 30 healthy individuals demonstrated a significant decrease in 8-OHdG in mononuclear cell DNA but significant increases in serum and urinary 8-OHdG with vitamin C supplementation of 500 mg/day for 6 weeks (24). In contrast, a placebo-controlled trial in male smokers reported no benefits on urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine from supplementation for 2 months with 250 mg of slow release or regular vitamin C twice a day (*n* = 21 in each group), 100 mg *d*- $\alpha$ -tocopheryl acetate twice a day (*n* = 20), or a combination of 250 mg slow-released ascorbic acid and 100 mg *d*- $\alpha$ -tocopheryl acetate twice a day (*n* = 20; Ref. 25). In a cross-over exercise study of 11 men, vitamin C (1 g/day), vitamin E (533 mg/day), and  $\beta$ -carotene (10 mg/day) supplementation had no effects on urinary 8-OHdG (26). A cross-sectional study also showed no association between dietary intake of vitamin C and urinary 8-oxodG excretion (27). Inconsistent results from these studies might have been partly because of the uncertain validity of the 8-OHdG assays performed on DNA and design limitations such as small sample sizes. In this regard, the present trial is by far the largest to assess the individual effects as well as joint effects of vitamin C and vitamin E supplementation on oxidative DNA damage.

There have been concerns about the validity of methods used to measure 8-OHdG (42). Artfactual 8-OHdG may be formed in the isolation of DNA, particularly phenol-based DNA purification procedures, in the hydrolysis process of a high-performance liquid chromatography, or in the heating step of a gas chromatography/mass spectrometry. For the ELISA assay, other compounds such as oligonucleotides and 8-oxoguanosine may cross-react with antibody to 8-OHdG. However, these compounds may also be relevant markers of oxidative damage (24). Despite the potential limitation of ELISA assay, the fact that we detected several biologically plausible associations (*e.g.*, significant associations of urinary 8-OHdG with prior supplement use, serum ascorbic acid levels, regular exercise, and a diet rich in fruits and vegetables) suggests that cross-reactivity is not likely to corrupt the validity of the assay. The intra-assay coefficient of variation, 8.8%, also indicates satisfactory repeatability of the ELISA assay.

Among the strengths of the present trial are the large sample size and the core study design (*i.e.*, a 2 $\times$ 2 factorial trial with double masks and placebo-controls). High rates of follow-up and adherence with pill-taking, along with effective masking, enhance its internal validity. Results from this trial should be generalizable to nonsmoking adults who choose to take antioxidant vitamin C or vitamin E supplements.

Potential limitations of this study include the brief duration of pill-taking (2 months). We did not determine the effects of other doses of vitamin C and vitamin E in this trial. Depending on the concentration, redox potential, and inorganic chemistry of the cells, these vitamins may exert pro-oxidant effects that increase oxidative DNA damage and promote tumor cell growth *in vitro* (43, 44). Because of possible pro-oxidant effects, the effects of multiple dosages and long-term use of these vitamins deserves further investigation.

In conclusion, vitamin C and vitamin E had no significant main effect or interaction effect on oxidative DNA damage as measured by urinary 8-OHdG in nonsmoking adults. However, several aspects of a healthy lifestyle were associated with lower oxidative DNA damage.

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