

Human Nutrition and Metabolism Research Communication

Supplementation of Diets with α -Tocopherol Reduces Serum Concentrations of γ - and δ -Tocopherol in Humans^{1,2}

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ABSTRACT Despite promising evidence from *in vitro* experiments and observational studies, supplementation of diets with α -tocopherol has not reduced the risk of cardiovascular disease and cancer in most large-scale clinical trials. One plausible explanation is that the potential health benefits of α -tocopherol supplements are offset by deleterious changes in the bioavailability and/or bioactivity of other nutrients. We studied the effects of supplementing diets with *RRR*- α -tocopheryl acetate (400 IU/d) on serum concentrations of γ - and δ -tocopherol in a randomized, placebo-controlled trial in 184 adult nonsmokers. Outcomes were changes in serum concentrations of γ - and δ -tocopherol from baseline to the end of the 2-mo experimental period. Compared with placebo, supplementation with α -tocopherol reduced serum γ -tocopherol concentrations by a median change of 58% [95% CI = (51%, 66%), $P < 0.0001$], and reduced the number of individuals with detectable δ -tocopherol concentrations ($P < 0.0001$). Consistent with trial results were the results from baseline cross-sectional analyses, in which prior vitamin E supplement users had significantly lower serum γ -tocopherol than non-users. In view of the potential benefits of γ - and δ -tocopherol, the efficacy of α -tocopherol supplementation may be reduced due to decreases in serum γ - and δ -tocopherol levels. Additional research is clearly warranted. *J. Nutr.* 133: 3137–3140, 2003.

KEY WORDS: • tocopherol • vitamin E • randomized controlled trial

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Vitamin E is a collective term for eight naturally occurring compounds, four tocopherols (α -, β -, γ - and δ -) and four tocotrienols (α -, β -, γ - and δ -), that qualitatively exhibit the biological activities of α -tocopherol. The eight forms of vitamin E are not interconvertible in humans. Concentrations of α -tocopherol are higher in wheat germ oil, almond and sunflower oil; γ -tocopherol is the major form of vitamin E in corn oil and soybean oil; levels of tocotrienols are high in rice bran, barley, oats and palm oil (1).

To date, research has focused on the potential health effects of α -tocopherol because of its abundance in nature and its potent antioxidant effects. Although α -tocopherol is the major form of vitamin E in the blood, γ -tocopherol constitutes ~70% of the vitamin E in a typical American diet (2). Recent *in vitro* studies suggest that other tocopherols and tocotrienols have chemopreventive effects. γ -Tocopherol is more effective than α -tocopherol in inhibiting prostate cancer cell growth (3), reducing oxidative DNA damage (4), increasing superoxide dismutase activity (5) and scavenging mutagenic electrophiles such as peroxynitrite, a potent nitrating and oxidizing compound (6,7). In addition, γ -tocopherol and its major metabolite exhibit greater anti-inflammatory effects than α -tocopherol (8). The potential benefit of γ -tocopherol is further supported by some epidemiologic studies that documented inverse relationships between serum concentration of γ -tocopherol and coronary heart disease or the risk of developing prostate cancer (9–12). *In vitro* data suggest a health benefit of δ -tocopherol, which has stronger antiproliferative effects on preneoplastic and neoplastic mouse mammary epithelial cells than α - and γ -tocopherol (13).

Most toxicity studies of vitamin E have focused on α -tocopherol. In aggregate, these studies suggest that α -tocopherol is not mutagenic, teratogenic or carcinogenic (14). Doses of 10–720 mg α -tocopherol/d are considered to be a “range without side effect” (15). Toxicologically, daily doses of 10–150 mg were considered to be “absolutely safe” and those of 100–300 mg were believed to be harmless (16). Nonetheless, little is known about the effect of α -tocopherol supplementation on other forms of vitamin E that have potentially important biological effects. A few preliminary studies suggested that the use of α -tocopherol supplements reduced serum and tissue levels of γ -tocopherol (17–20), but no study in humans has investigated the effects on δ -tocopherol or other forms of vitamin E.

In this setting, we determined the effects of α -tocopherol supplementation on serum concentrations of γ - and δ -tocopherol in a double-masked, placebo-controlled, 2×2 factorial trial of vitamin E and vitamin C supplementation (21–23).

SUBJECTS AND METHODS

The institutional review boards of the Johns Hopkins Medical Institutions approved the trial protocol. All participants provided written informed consent.

Study population. The study population consisted of 184 adult nonsmokers recruited in the metropolitan area of Baltimore, MD. Eligibility criteria were a willingness to provide written informed consent and to take study pills, but no other vitamin supplements, for 2 mo. Major exclusion criteria were regular exposure to passive tobacco smoke for ≥ 1 h/d or consumption of ≥ 14 servings of alcohol/wk. Persons taking vitamin supplements were eligible after a 2-mo period of abstinence.

Conduct of trial. Participants had two in-person visits to ascertain eligibility and to provide baseline data, including a 12-h fasting blood sample. Eligible persons were randomly assigned to one of four supplementation groups: placebo (dicalcium phosphate, 380 mg/tablet, and soybean oil), vitamin C alone (500 mg ascorbate/tablet), vitamin E alone [400 IU (296 mg) RRR- α -tocopheryl acetate/capsule] and both vitamin C and vitamin E. The vitamin C supplements and corresponding placebo tablets were purchased from Consolidated Midland (Brewster, NY). The active vitamin E capsules and corresponding placebo capsules were donated by Henkel (LaGrange, IL). All participants, data collectors and laboratory technicians were unaware of group assignment. Participants were provided with study pills according to the group assignment, and were instructed to take two types of pills (vitamin C or placebo and vitamin E or placebo) each day and to avoid taking any vitamin supplements other than study pills during the study period. Two months after randomization, 12-h fasting blood samples were collected. Adherence with pill taking was assessed by pill counts and changes in serum concentration of α -tocopherol and ascorbic acid. The blood samples were allowed to clot for no > 15 min, and were then centrifuged at $2000 \times g$ for 15 min at room temperature. Serum specimens were portioned into polypropylene tubes and stored at -70°C until assayed.

Outcomes. The outcome variables were changes in serum concentrations of γ - and δ -tocopherol from baseline to the end of supplementation.

Laboratory assays. Serum α -, γ - and δ -tocopherol were measured by isocratic HPLC (24). The reproducibility (intra-assay CV) of each measure was assessed in 40 pairs of duplicate samples. The intra-assay CV were 3.3% for α -tocopherol and 2% for γ -tocopherol. The limit of detection for δ -tocopherol was $0.1 \mu\text{mol/L}$.

Statistical analysis. Because there were no changes in γ - or δ -tocopherol in the groups supplemented with vitamin C and there was no evidence of an interaction between vitamin C and vitamin E on the outcomes, trial results are presented by placebo vs. active α -tocopherol, across placebo and active vitamin C groups.

Baseline characteristics of participants were compared between placebo and active α -tocopherol groups. Median regression models were used to estimate the effects of α -tocopherol supplementation on changes in serum concentrations of α - and γ -tocopherol, with or without adjustment for baseline tocopherol concentration and other covariates. For δ -tocopherol, logistic regression was used to determine whether the presence of detectable levels at follow-up differed in the α -tocopherol and the placebo group, adjusted for baseline δ -tocopherol. Subgroup analyses were performed according to age (above vs. below median), gender, and chronic illness (hypertension, diabetes mellitus, or hypercholesterolemia vs. none of the above). For γ -tocopherol, additional subgroup analyses were performed according to baseline serum α - and γ -tocopherol concentrations (above vs. below the median, and above vs. below the 1st tertile). Analyses were repeated for the data with cholesterol standardization of α - and γ -tocopherol, and the pattern of the results was essentially the same. Hence, data without cholesterol standardization were presented. Statistical analyses were performed with the SAS system for Windows (version 8.1; SAS Institute, Cary, NC). Differences were considered significant at $P < 0.05$ (two-sided).

RESULTS

The characteristics of the participants in the placebo and active α -tocopherol supplementation groups were similar (Table 1). Follow-up rates and adherence to pill taking were high and did not differ by supplement group. Of the participants, 92% completed 2 mo of supplementation, and 93% took $\geq 90\%$ of study pills.

TABLE 1

Characteristics of trial participants given placebo or RRR- α -tocopheryl acetate

Characteristic ¹	Placebo (n = 93)	α -Tocopherol (n = 91)
Age, y	59.6 \pm 13.1	56.6 \pm 14.1
Women, %	52.7	58.2
Race, %		
African American	47.3	52.7
Caucasian	49.5	42.8
Other	3.2	5.5
Education		
>High school, %	83.9	74.7
Chronic illness, ² %	64.5	63.7
Prior vitamin E supplement use, %	11.8	16.5
Dietary intake ³		
Vitamin C, mg/d	136.0 [76.5, 178.0]	136.5 [88.0, 167.5]
Vitamin E, α -TE/d	6.8 [4.7, 10.0]	7.6 [5.4, 14.0]
Fruits/vegetables, servings/d	3.9 [2.2, 5.7]	3.5 [2.3, 5.3]
Serum ascorbic acid, $\mu\text{mol/L}$	61.3 [54.0, 71.0]	63.0 [50.0, 72.7]

¹ Continuous variables are presented as means \pm SD or medians [interquartile range].

² Hypertension, diabetes mellitus, or hypercholesterolemia.

³ Measured by the Block food-frequency questionnaire (47); α -TE, α -tocopherol equivalents.

After 2 mo of pill taking, α -tocopherol supplementation increased serum α -tocopherol concentration compared with placebo, but significantly reduced serum γ -tocopherol concentration (Table 2). Adjustment for baseline variables (cholesterol, age, gender, race, education, chronic illness, prior antioxidant use, and baseline serum α - and γ -tocopherol concentration) did not alter the pattern of these results. Reductions in γ -tocopherol due to α -tocopherol supplementation were significant in each subgroup that we examined, including the lowest third of baseline serum γ -tocopherol ($< 1.8 \mu\text{mol/L}$). In prior vitamin E supplement users ($n = 26$) who abstained from supplement use for 2 mo before enrollment, the baseline serum α -tocopherol concentrations were higher than those of nonusers [median (interquartile range) = 29.4 (26.0, 35.1) vs. 26.5 (22.3, 31.0) $\mu\text{mol/L}$, $P = 0.03$ by the Wilcoxon rank sum test], whereas the baseline serum γ -tocopherol concentrations were substantially lower [median (interquartile range) = 1.4 (1.1, 2.0) vs. 2.4 (1.9, 3.0) $\mu\text{mol/L}$, $P < 0.0001$].

At enrollment, serum δ -tocopherol concentrations were detectable in 50% of the participants in the placebo group and 46% of the participants in the α -tocopherol group (Table 2). At the end of supplementation, δ -tocopherol was detectable in 46% of the placebo group, but only 13% of the α -tocopherol group ($P < 0.0001$ for the difference, adjusted for baseline detectable levels). The same pattern of findings was evident in the subgroups defined by age, gender and history of chronic illness.

DISCUSSION

In this randomized controlled trial, supplementation of diets with RRR- α -tocopheryl acetate, 400 IU/d for 2 mo, significantly reduced serum concentration of γ -tocopherol by $\sim 60\%$, and significantly reduced the number of individuals with detectable serum concentrations of δ -tocopherol. The

TABLE 2

Serum levels of α-, γ- and δ-tocopherol in trial participants given placebo or RRR-α-tocopheryl acetate

	Placebo (n = 93)	α-Tocopherol (n = 91)
Serum α-tocopherol, μmol/L ¹		
Baseline	27.2 [23.2, 33.0]	26.4 [22.0, 29.5]
Follow-up	27.4 [22.1, 32.7]	38.5 [31.4, 55.0]
Median change (95% CI)	0.23 (-0.30, 0.77)	14.3 (10.8, 18.0)*
Difference in median change (95% CI)		14.2 (12.8, 15.5)*
Difference in median % change (95% CI)		57 (51, 63)*
Serum γ-tocopherol, μmol/L ¹		
Baseline	2.30 [1.70, 2.70]	2.20 [1.80, 3.00]
Follow-up	2.30 [1.80, 2.70]	1.00 [0.70, 1.40]
Median change (95% CI)	0.00 (-0.15, 0.15)	-1.30 (-1.51, -1.09)*
Difference in median change (95% CI)		-1.30 (-1.50, -1.10)*
Difference in median % change (95% CI)		-58 (-66, -51)*
Serum δ-tocopherol (% detectable)		
Baseline	50	46
Follow-up	46	13
OR (95% CI) ²		0.16 (0.07, 0.34)†

¹ Median [interquartile range] at baseline and at follow-up; change was defined as the change from baseline to the end of supplementation (follow-up). * Different from baseline, *P* < 0.0001. † *P* < 0.0001.

² Odds ratio of being detectable at follow-up, adjusted for baseline, in the active vitamin C group compared with the placebo group.

reductions occurred in each subgroup examined. Results from this trial corroborate findings from previous small-scale (n = 4–24), nonrandomized studies in which plasma or tissue levels of γ-tocopherol were reduced by daily supplementation of 30, 100 or 800 mg α-tocopherol for 4 wk or up to 1 y (17–20). Three of these four studies did not include a placebo group (17–19).

α-Tocopherol supplement use may reduce circulating γ- and δ-tocopherol concentrations due to competition for hepatic transfer. Intestinal absorption and delivery to the liver of tocopherol isomers and stereoisomers were similar (25–27), even after a high dose (1 g) of α-tocopherol and γ-tocopherol was ingested (28). However, a 30-kDa α-tocopherol transfer protein in the liver cytoplasm preferentially transfers RRR-α-tocopherol into nascent VLDL (27,29–33) and hence accounts for the higher concentration of RRR-α-tocopherol in serum. Compared with RRR-α-tocopherol, the relative affinity to α-tocopherol transfer protein is 9 and 2% for γ-tocopherol and δ-tocopherol, respectively (34). Although we did not determine the effects of α-tocopherol supplementation on serum levels of β-tocopherol and tocotrienols, these compounds might have also been reduced by α-tocopherol supplement use because of their weak affinity to α-tocopherol transfer protein; the affinity was estimated to be 38% for β-tocopherol and 12% for α-tocotrienol compared with α-tocopherol (28).

In our trial, 26 prior α-tocopherol supplement users abstained from supplement use for 2 mo before enrollment. At enrollment, the median concentrations of serum α-tocopherol in these individuals was only 3 μmol/L higher than that in nonusers, whereas serum γ-tocopherol concentrations of prior users was substantially lower than that of nonusers. A previous study estimated that the period required to reach a new steady-state distribution of tocopherols would be 2 y after 1 y of α-tocopherol supplementation (18). These findings suggest that the effects of long-term α-tocopherol supplement use on serum concentrations of γ- and δ-tocopherol are not only substantial but also prolonged.

The health effects of γ- and δ-tocopherol are unknown, but evidence from some epidemiologic studies has suggested potential benefits of γ-tocopherol. In a population-based, pro-

spective study, plasma γ-tocopherol concentrations were strongly and inversely associated with subsequent risk of prostate cancer [odds ratio = 0.21 (0.08, 0.54) in men in the highest quintile compared with men in the lowest quintile of serum γ-tocopherol concentrations, *P*-trend <0.001] (9). A protective association between plasma α-tocopherol concentrations and prostate cancer risk was observed only when γ-tocopherol concentrations were above the median (10). Consistent with these findings were results from the Alpha-Tocopherol, Beta-Carotene trial in which there was an inverse relationship between dietary intake of γ-tocopherol and the risk of developing prostate cancer in the α-tocopherol supplementation group (35), and an animal study that showed adding γ-tocopherol to the diet increased tissue levels of γ-tocopherol as well as α-tocopherol (36). Two case-control studies reported lower blood concentrations of γ-tocopherol in cases with coronary heart disease compared with controls (11,12). However, other studies did not find significant associations between adipose tissue levels of γ-tocopherol and myocardial infarction risk (37) or serum levels of γ-tocopherol and deaths from coronary heart disease (38).

In conclusion, use of α-tocopherol supplements significantly reduces serum γ-tocopherol and δ-tocopherol, both of which may have important biological effects. Potential health benefits of α-tocopherol supplements may be offset by deleterious changes in the bioavailability of other forms of tocopherols and tocotrienols, which might in part account for the null effects of α-tocopherol supplementation in most prevention trials of cardiovascular disease and cancer (39–46). Additional research is clearly warranted.

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