

# $\alpha$ -Tocopherol disappearance is faster in cigarette smokers and is inversely related to their ascorbic acid status<sup>1-3</sup>

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## ABSTRACT

**Background:** Cigarette smokers have enhanced oxidative stress from cigarette smoke exposure and from their increased inflammatory responses.

**Objective:** The objective of this study was to determine whether cigarette smoking increases plasma  $\alpha$ -tocopherol disappearance in otherwise healthy humans.

**Design:** Smokers and nonsmokers ( $n = 10$ /group) were supplemented with deuterium-labeled  $\alpha$ -tocopheryl acetates (75 mg each of  $d_3$ -*RRR*- $\alpha$ -tocopheryl acetate and  $d_6$ -*all-rac*- $\alpha$ -tocopherols acetate) for 6 evenings (days -6 to -1). Plasma  $\alpha$ -tocopherols, ascorbic acid, uric acid, and  $F_{2\alpha}$ -isoprostanes were measured in blood samples collected on days -6 through 17. The urinary  $\alpha$ -tocopherol metabolite,  $\alpha$ -carboxy-ethyl-hydroxy-chroman ( $\alpha$ -CEHC), was measured on days -6, 0, and 17 in 24-h urine samples.

**Results:**  $F_{2\alpha}$ -isoprostanes were, on average,  $\approx 40\%$  higher in smokers than in nonsmokers. On day 0, plasma labeled and unlabeled  $\alpha$ -tocopherol concentrations were not significantly different between groups. Smoking resulted in faster fractional disappearance of plasma  $\alpha$ -tocopherol ( $0.215 \pm 0.011$  compared with  $0.191 \pm 0.009$  pools/d;  $P < 0.05$ ). Fractional disappearance rates of  $\alpha$ -tocopherol correlated with plasma ascorbic acid concentrations in smokers ( $P = 0.021$ ) but not in nonsmokers despite plasma ascorbic acid concentrations that were not significantly different between groups. By day 17, cigarette smoking resulted in lower plasma  $\alpha$ -tocopherol concentrations and urinary excretion of labeled and unlabeled  $\alpha$ -CEHC ( $P < 0.05$ ).

**Conclusions:** Cigarette smoking increased  $\alpha$ -tocopherol disappearance. Greater rates of  $\alpha$ -tocopherol disappearance in smokers appear to be related to increased oxidative stress accompanied by lower plasma ascorbic acid concentrations. Thus, smokers have an increased requirement for both  $\alpha$ -tocopherol and ascorbic acid. *Am J Clin Nutr* 2005;81:95-103.

**KEY WORDS** Oxidative stress, vitamin E, vitamin C, cigarette smoke, antioxidants, dietary requirements

## INTRODUCTION

Nearly 50 million Americans smoke cigarettes (1). The adverse health consequences of smoking have been largely attributed to the abundance of reactive oxygen species and reactive nitrogen species that readily react with various biomolecules. In fact, a single puff of cigarette smoke contains  $1 \times 10^{14-15}$  reactive oxygen species,  $\approx 500$  ppm nitric oxide, and other reactive nitrogen oxides (2). In addition to the stress of cigarette smoke,

cigarette smokers also have increased inflammatory responses that further enhance their oxidative stress (3, 4).

Increased oxidative stress from cigarette smoke results in higher dietary ascorbic acid requirements for cigarette smokers (5). However, research to date has been unable to accurately define vitamin E dietary requirements or determine whether cigarette smoking or other oxidative stresses increase these requirements. As a result, the 2000 recommended dietary allowance for vitamin E is based largely on the *in vitro* assessment of hemolysis in erythrocytes obtained from experimentally caused vitamin E deficiency in men after treatment with peroxide (5-7).

Clearly, *in vitro* studies have indicated that cigarette smoke exposure depletes plasma  $\alpha$ -tocopherol ( $\alpha$ -T) concentrations (8-10). However, plasma  $\alpha$ -T concentrations in humans trials (11-15) are often reported to not be significantly different between smokers and nonsmokers. Deuterium-labeled tocopherols have been used to determine the biokinetics, bioavailability, and metabolism of vitamin E in healthy humans and those with genetic abnormalities in lipoprotein metabolism and the  $\alpha$ -T transfer protein (16-20). Unfortunately, attempts to characterize plasma  $\alpha$ -T biokinetics in cigarette smokers with the use of deuterated tocopherols have not been entirely successful. Munro et al (21) supplemented smokers and nonsmokers on a single occasion with deuterated  $\alpha$ -T and then collected blood samples 6, 12, and 27 h after the supplement. Although smokers had lower plasma deuterated  $\alpha$ -T concentrations at each of the time points, it could not be elucidated whether these differences were due to reduced  $\alpha$ -T absorption or its faster plasma clearance. Traber et al (22)

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attempted to further clarify these differences by supplementing smokers and nonsmokers with deuterated  $\alpha$ -T for 7 d and collecting fasting blood samples on select days up to day 21 after supplementation. Although smokers had a faster deuterated  $\alpha$ -T disappearance, the findings were not statistically significant, likely because the investigation was underpowered.

Measurements of plasma and tissue  $\alpha$ -T concentrations have been used to assess human vitamin E status. Plasma or urinary  $\alpha$ -T metabolite ( $\alpha$ -carboxy-ethyl-hydroxy-chroman;  $\alpha$ -CEHC) concentrations may be another useful biomarker to assess vitamin E status. A nonoxidation product,  $\alpha$ -CEHC is synthesized by hepatocytes via a cytochrome P450-dependent pathway before urinary excretion (23, 24). In humans, urinary  $\alpha$ -CEHC is undetectable unless subjects are supplemented with sufficient  $\alpha$ -T to surpass a 30–40  $\mu\text{mol/L}$  plasma  $\alpha$ -T threshold (25), which suggests that metabolism occurs when adequate or excessive hepatic  $\alpha$ -T concentrations have been achieved.

In this investigation, we hypothesized that the higher magnitude of oxidative stress experienced by cigarette smokers than by nonsmokers would lead to more rapid plasma depletion of plasma  $\alpha$ -T and to decreased urinary  $\alpha$ -CEHC excretion. To test this hypothesis, we supplemented cigarette smokers and nonsmokers with deuterium-labeled  $\alpha$ -T and measured the disappearance of plasma  $\alpha$ -T and appearance of urinary  $\alpha$ -CEHC using liquid chromatography–mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GC-MS), respectively.

## SUBJECTS AND METHODS

### Study participants

The protocol for this study was approved by the Institutional Review Board at Oregon State University, and all participants provided written consent before enrollment. Healthy, normolipidemic volunteers ( $n = 10$  nonsmokers and 10 smokers) were selected for this study on the basis of age (18–35 y), nonnutritional supplement use ( $>6$  mo), and exercise status ( $<5$  h aerobic activity/wk). Participant characteristics are shown in **Table 1**. Nonsmokers ( $n = 6$  men and 4 women) were selected on the basis that they had never smoked and did not reside with a smoker. Smokers ( $n = 6$  men and 4 women) were selected if they smoked  $>10$  cigarettes/d. Cotinine, the metabolite of nicotine, was measured by using a radioimmunoassay (Diagnostics Products Corp, Los Angeles). A urinary cotinine concentration of  $>500$  ng/mL, as suggested by the manufacturer, was used as a cutoff to confirm smoking status.

To verify the participants' health status before enrollment in the study, a serum chemistry profile (**Table 2**) was performed at Good Samaritan Regional Medical Center (Corvallis, OR). Additionally, hemoglobin was measured in whole blood with a kit, according to manufacturer's instructions (Sigma Diagnostics, St Louis; procedure no. 525). Blood hematocrit was measured after 5 min of centrifugation (Statspin, Norwood, MA).

### Dietary analysis

To control for potential confounders with respect to differences in dietary nutrient consumption, all participants completed a 3-d food record (2 weekdays and 1 weekend day) during the investigation. Dietary intakes were analyzed by using FOOD PROCESSOR (version 7.9; ESHA Research, Salem, OR).

**TABLE 1**  
Participant characteristics at baseline

Characteristic	Nonsmokers ( $n = 10$ )	Smokers ( $n = 10$ )
Age (y)	19.5 $\pm$ 1.5 <sup>1</sup>	21.0 $\pm$ 3.1
Height (m)	1.69 $\pm$ 0.15	1.76 $\pm$ 0.13
Weight (kg)	63.6 $\pm$ 14.4	68.0 $\pm$ 9.0
BMI (kg/m <sup>2</sup> )	22.2 $\pm$ 3.2	22.0 $\pm$ 2.2
Nutrition supplements	None	None
Cigarettes smoked (no./d)	0	10–20
Urinary cotinine (ng/mL)	34 $\pm$ 13	2345 $\pm$ 1555
$\alpha$ -Tocopherol ( $\mu\text{mol/L}$ )	15.3 $\pm$ 2.8	14.6 $\pm$ 3.8
$\gamma$ -Tocopherol ( $\mu\text{mol/L}$ )	1.63 $\pm$ 0.6	1.56 $\pm$ 0.7
Ascorbic acid ( $\mu\text{mol/L}$ )	60 $\pm$ 22	55 $\pm$ 19
Uric acid ( $\mu\text{mol/L}$ )	273 $\pm$ 57	293 $\pm$ 47
F <sub>2</sub> $\alpha$ -Isoprostanes (pg/mL)	30.6 $\pm$ 6.7	46.7 $\pm$ 17.2 <sup>2</sup>
FRAP ( $\mu\text{mol/L}$ ) <sup>3</sup>	660 $\pm$ 106	575 $\pm$ 81 <sup>4</sup>
Total cholesterol (mmol/L)	4.34 $\pm$ 0.44	3.87 $\pm$ 0.96
HDL (mmol/L)	1.37 $\pm$ 0.17	1.45 $\pm$ 0.35
LDL (mmol/L)	2.42 $\pm$ 0.40	1.91 $\pm$ 0.64
Triacylglycerol (mmol/L)	1.19 $\pm$ 0.55	1.11 $\pm$ 0.43

<sup>1</sup>  $\bar{x} \pm$  SD (all such values).

<sup>2,4</sup> Significantly different from nonsmokers (unpaired Student's *t* test):

<sup>2</sup>*P* = 0.007, <sup>4</sup>*P* = 0.030.

<sup>3</sup> Ferritin reducing ability of plasma.

### Deuterated $\alpha$ -tocopherol

Capsules containing *RRR*- $\alpha$ -5-(CD<sub>3</sub>)- and *all-rac*- $\alpha$ -5,7-(CD<sub>3</sub>)<sub>2</sub> tocopheryl acetates (d<sub>3</sub>-*RRR*- $\alpha$ -TAc and d<sub>6</sub>-*all-rac*- $\alpha$ -TAc,

**TABLE 2**  
Blood chemistry and hematologic values of the subjects at screening<sup>1</sup>

Index	Normal range	Nonsmokers <sup>2</sup> ( $n = 10$ )	Smokers <sup>2</sup> ( $n = 10$ )
Sodium (mEq/L)	135–145	142 $\pm$ 2	142 $\pm$ 1
Potassium (mEq/L)	3.5–5.1	4.1 $\pm$ 0.2	4.6 $\pm$ 0.6
Chloride (mEq/L)	100–111	104 $\pm$ 1	103 $\pm$ 2
Bicarbonate (mEq/L)	22–30	25 $\pm$ 2	26 $\pm$ 3
Glucose (mg/dL)	70–105	83 $\pm$ 6	81 $\pm$ 11
BUN (mg/dL)	6–19	13 $\pm$ 3	13 $\pm$ 4
Creatinine (mg/dL)	0.4–1.1	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1
BUN:creatinine	6–30	16 $\pm$ 3	17 $\pm$ 4
Calcium (mg/dL)	8.4–10.2	9.8 $\pm$ 0.5	10.1 $\pm$ 0.4
Phosphorus (mg/dL)	2.7–4.5	3.8 $\pm$ 0.5	4.3 $\pm$ 0.7
Total protein (g/dL)	6.4–8.3	7.5 $\pm$ 0.2	7.3 $\pm$ 0.5
Albumin (g/dL)	3.4–5.0	4.4 $\pm$ 0.4	4.5 $\pm$ 0.3
SGOT (U/L)	0–31	20 $\pm$ 3	25 $\pm$ 19.2
LDH (U/L)	94–250	157 $\pm$ 11	201 $\pm$ 121
SGPT (U/L)	0–31	16 $\pm$ 6	18 $\pm$ 6
Alkaline phosphatase (U/L)	39–117	73 $\pm$ 24	80 $\pm$ 16
$\gamma$ -GT (U/L)	7–33	17 $\pm$ 9	14 $\pm$ 5
Total bilirubin (mg/dL)	0.0–1.0	0.6 $\pm$ 0.3	0.6 $\pm$ 0.2
Globulin (g/dL)	2.3–3.5	3.1 $\pm$ 0.4	2.8 $\pm$ 0.3
Hemoglobin (g/dL)	13.0–16.8	16.0 $\pm$ 1.4	15.4 $\pm$ 1.2
Hematocrit (%)	38–49	45.5 $\pm$ 4.1	45.9 $\pm$ 3.4

<sup>1</sup> Data from Good Samaritan Hospital, Corvallis, OR. BUN, blood urea nitrogen; SGOT, serum glutamic-oxaloacetate transaminase; LDH, lactate dehydrogenase; SGPT, serum glutamic-pyruvate transaminase; GT, glutamyl transferase. No significant differences (*P* > 0.05) were observed between the nonsmokers and the smokers (unpaired Student's *t* test), and all participants had blood chemistry values that were within the normal reference range.

<sup>2</sup> Values are  $\bar{x} \pm$  SD.

respectively) were a gift from the Natural Source Vitamin E Association and were synthesized by Eastman Kodak (Rochester, NY). The  $d_3$ -*RRR*- and  $d_6$ -*all-rac*- $\alpha$ -TAc were encapsulated in a gelatin capsule as nominal 1:1 mixtures in 150-mg quantities. The molar ratio of  $d_3$ -*RRR*- to  $d_6$ -*all-rac*- $\alpha$ -T was determined to be 0.98 (26).

### Study protocol

On 6 consecutive evenings, participants ingested the deuterated  $\alpha$ -T supplement immediately after a standard meal. On average, this meal contained 1143 kcal (43% carbohydrate, 17% protein, and 41% fat), 35 mg ascorbic acid, and 2.7 mg  $\alpha$ -T.

Blood samples were obtained after the subjects fasted overnight (10–12 h) on days  $-6$ ,  $-5$ ,  $-4$ ,  $-3$ ,  $-2$ ,  $-1$ ,  $0$ ,  $1$ ,  $2$ ,  $3$ ,  $4$ ,  $5$ ,  $6$ ,  $8$ ,  $10$ ,  $13$ ,  $15$ , and  $17$  (negative days denote the supplementation period). Blood was collected from the antecubital vein into blood collection tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) containing 0.05 mL 15%  $K_3EDTA$  or sodium heparin. Smokers were asked to refrain from smoking for 1 h before blood collection to alleviate the transient oxidative stress effects. Urine was collected for 24 h on 3 occasions: before supplementation ( $d - 6$ ) and on days  $0$  and  $17$ . After the total urine volume was determined, aliquots were collected and stored at  $-40^\circ C$  until analysis.

### Blood sample handling

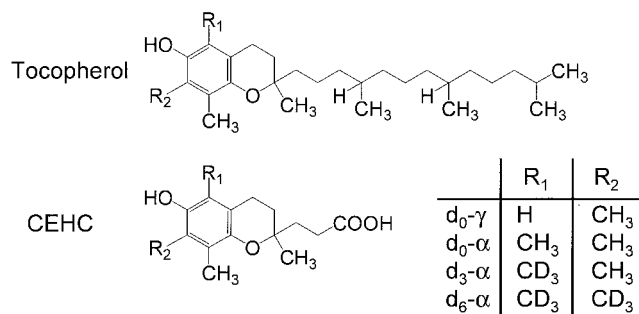
Blood tubes with additives were kept on ice for  $<30$  min before centrifugation. Plasma was separated by centrifugation ( $500 \times g$ , 15 min,  $4^\circ C$ ; model TJ-6; Beckman, Palo Alto, CA), separated into cryovials, snap frozen in liquid nitrogen, and then stored at  $-80^\circ C$  until analyzed. After plasma was separated, an aliquot was acidified (1:1) with 10% perchloric acid containing diethylenetriaminepentaacetic acid (1 mmol/L). This sample was then centrifuged ( $15\,000 \times g$ , 5 min,  $4^\circ C$ , Centrifuge 5415R; Eppendorf, Hamburg, Germany), the supernatant fraction was removed, snap frozen, and stored at  $-80^\circ C$  for future analysis of ascorbic and uric acid.

### Materials

Perchloric acid and HPLC-grade methanol were obtained from Fisher (Fair Lawn, NJ). The following were obtained from Sigma-Aldrich (St Louis): ascorbic acid, butylated hydroxytoluene, DTPA,  $FeCl_3$ , phosphate-buffered saline, potassium hydroxide, potassium phosphate trihydrate, TPTZ [2, 4, 6-tri(2-pyridyl)-*s*-triazine], and trolox. Chromatography pairing reagent, Q12 (1-dodecyltriethyl-ammonium phosphate), was purchased from Regis (Morton Grove, IL). The isotopic purity of  $d_9$ -*all-rac*- $\alpha$ -T was found to be 88.4%  $d_9$  and the remainder  $d_8$ .

### Tocopherol analysis

Labeled and unlabeled tocopherols (Figure 1) were extracted according to procedures previously described (27) and were analyzed with an LC-MS that consisted of a Waters 2690 Separations Module (Milford, MA) and a ZQ 2000 instrument single-quadrupole mass spectrometer (Micromass, Manchester, United Kingdom), as previously described (11). The LC-MS was equipped with an atmospheric pressure chemical ionization probe set to the negative ionization mode. The mass-to-charge ( $m/z$ ) ratios were as follows:  $d_0$ - $\alpha$ -T,  $m/z$  429.4;  $d_3$ - $\alpha$ -T,  $m/z$  432.4;  $d_6$ - $\alpha$ -T,  $m/z$  435.4;  $d_9$ - $\alpha$ -T,  $m/z$  438.4; and  $d_0$ - $\gamma$ -T,  $m/z$



**FIGURE 1.** Structures of unlabeled and labeled tocopherols and  $\alpha$ -carboxy-ethyl-hydroxy-chroman (CEHC). After supplementation with 75 mg each of  $d_3$ -*RRR*- $\alpha$ -tocopheryl acetate and  $d_6$ -*all rac*- $\alpha$ -tocopheryl acetate, plasma was analyzed by liquid chromatography–mass spectrometry for unlabeled  $\alpha$ - and  $\gamma$ -tocopherols ( $d_0$ - $\alpha$ -T and  $d_0$ - $\gamma$ -T) and deuterium-labeled  $\alpha$ -tocopherols ( $d_3$ - $\alpha$ -T and  $d_6$ - $\alpha$ -T). Urine was analyzed by gas chromatography–mass spectrometry for the metabolites of  $\alpha$ -T and  $\gamma$ -T as unlabeled  $\alpha$ - and  $\gamma$ -CEHC ( $d_0$ - $\alpha$ - and  $d_0$ - $\gamma$ -CEHC) and deuterium-labeled  $\alpha$ -CEHCs ( $d_3$ - $\alpha$ - and  $d_6$ - $\alpha$ -CEHC).

415.4. Calibration curves were prepared with the use of authentic standards, and *all-rac*- $\alpha$ -5,7,8-(CD<sub>3</sub>)<sub>3</sub>-T ( $d_9$ - $\alpha$ -T) was used as the internal standard. Concentrations of the tocopherol standards were determined spectrophotometrically (DU Series 600; Beckman, Fullerton, CA):  $\epsilon_{292\text{ nm}}^{\text{EtOH}} = 3270\text{ M}^{-1} \cdot \text{cm}^{-1}$  for  $\alpha$ -T and  $\epsilon_{298\text{ nm}}^{\text{EtOH}} = 3810\text{ M}^{-1} \cdot \text{cm}^{-1}$  for  $\gamma$ -T.

### Analysis of CEHCs

Urinary deuterium labeled and unlabeled  $\alpha$ - and  $\gamma$ -T metabolites ( $d_0$ ,  $d_3$ - and  $d_6$ - $\alpha$ -CEHC and  $d_0$ - $\gamma$ -CEHC; Figure 1) were measured by GC-MS according to a previously described method (28). The GC-MS consisted of an Agilent 6890 GC coupled with an Agilent 5973N MSD (Palo Alto, CA), and analyte concentrations were determined by electron impact ionization. In short, the urinary metabolites were extracted from 5 mL urine, derivatized with *N,O*-bis(trimethylsilyl) trifluoroacetamide and 1% trimethylchlorosilane, dried under nitrogen, and resuspended in hexane before injection. Sample volumes of  $1\ \mu\text{L}$  were injected onto the column with an HP 7683 auto-injector. Separations were performed on a DB-5MS column (30 m  $\times$  2.5 mm internal diameter 2.5  $\mu\text{m}$  film thickness; J & W Scientific, Folsom, CA) with helium as carrier gas. Quantification was performed by comparison with known amounts of an added internal standard (trolox).

### Ascorbic acid and uric acid analysis

Ascorbic and uric acids were measured by HPLC-electrochemical detection as previously described (29). Standards of ascorbic acid were prepared fresh daily and verified spectrophotometrically with the  $\epsilon_{265\text{ nm}} = 14\,500\text{ M}^{-1} \cdot \text{cm}^{-1}$ . Prepared uric acid standard was purchased from Pointe Scientific, Inc (Lincoln Park, MI).

### Ferritin reducing ability of plasma determination

To assess plasma antioxidant capacity, the ferritin reducing ability of plasma (FRAP) was measured according to the methods of Benzie and Strain (30). In brief, 40  $\mu\text{L}$  plasma (diluted 1:4) was mixed on a 96-well plate with 300  $\mu\text{L}$  freshly prepared FRAP reagent [25 mL sodium acetate buffer (300 mmol/L), 2.5

mL TPTZ (10 mmol/L), and 2.5 mL FeCl<sub>3</sub> (20 mmol/L)]. Samples were incubated for 15 min at 37 °C before reading at 550 nm on a Spectramax 190 microplate reader (Molecular Devices, Sunnyvale, CA). FRAP values were calculated by using trolox as a standard.

### Lipid analysis

Plasma F<sub>2α</sub>-isoprostanes were measured by GC-MS as previously described (31). Total cholesterol and triacylglycerol were measured with the use of kits obtained from Sigma Diagnostics (procedure no. 343 and 401, respectively) in accordance with the manufacturer's instructions.

### Mathematical analysis of tocopherol disappearance kinetics

Mathematical modeling was performed with the use of POOLFIT computer software, as was used in another investigation (32). The percentage of d<sub>3</sub>-was calculated from the plasma α-T concentrations for each subject, at each time point, and was fitted by a two-compartment model as previously described (22).

$$\%d_3\text{-}\alpha\text{-T} = [d_3\text{-}\alpha\text{-T}/(d_0\text{-}\alpha\text{-T} + d_3\text{-}\alpha\text{-T} + d_6\text{-}\alpha\text{-T})] \times 100 \quad (1)$$

The 2 compartments were assumed to have reached the same concentration at the end of 6 d of deuterated TAc supplementation. Fitting was performed by nonlinear least squares, assuming measurement error to have a constant CV. Fitting resulted in a fractional disappearance rate of α-T for each participant for which statistical comparisons could be made between nonsmokers and smokers. α-T half-lives were calculated as ln(2)/disappearance rate constant.

### Statistical analysis

Statistical analysis was performed with the use of GRAPH-PAD PRISM (version 4.0; GraphPad Software, San Diego). An unpaired Student's *t* test was used for all comparisons between smokers and nonsmokers. All correlations were calculated by linear regression. To examine the effects of ascorbic acid concentrations and smoking on the fractional disappearance rates of α-T, we conducted multiple linear regression analysis. All parameter estimates and *P* values were obtained by using PROC GLM (version 8; SAS Institute Inc, Cary, NC). Data were considered statistically significant at *P* < 0.05. All data are reported as means ± SEs unless otherwise noted.

## RESULTS

### Participant characteristics and analysis of dietary intakes

There were no significant differences in age, height, weight, body mass index, plasma lipids, α-T, γ-T, uric acid, or ascorbic acid at baseline between the smokers and nonsmokers (Table 1). However, smokers had a greater degree of oxidative stress, as marked by higher F<sub>2α</sub>-isoprostanes and lower FRAP. Participating cigarette smokers self-reported smoking between 10 and 20 cigarettes/d. Urinary cotinine values were correlated with the self-reported number of cigarettes smoked per day (*P* = 0.010, *R*<sup>2</sup> = 0.582) in the smokers. Nonsmokers had low or no measurable urinary cotinine concentrations (Table 1).

**TABLE 3**  
Dietary intakes of the nonsmokers and the smokers<sup>1</sup>

Nutrient	Nonsmokers ( <i>n</i> = 10)	Smokers ( <i>n</i> = 10)
Energy (kcal)	2703 ± 498	2807 ± 773
Carbohydrate (% of energy)	50.5 ± 4.9	52.5 ± 2.2
Protein (% of energy)	15.4 ± 1.5	14.1 ± 2.3
Fat (% of energy)	35.0 ± 3.9	31.9 ± 5.3
Ascorbic acid (mg) <sup>2</sup>	77.6 ± 25.3	73.4 ± 36.1
Vitamin E (mg α-T)	5.5 ± 1.1	5.3 ± 2.2

<sup>1</sup> All values are  $\bar{x} \pm$  SD. Dietary intakes were estimated from 3-d food records. α-T, α-tocopherol. There were no significant differences between groups (unpaired Student's *t* test).

<sup>2</sup> Correlation with plasma concentrations: *P* = 0.011, *R*<sup>2</sup> = 0.324.

Surprisingly, no significant differences in baseline ascorbic acid concentrations were observed between nonsmokers and smokers (Table 1). Because it is often reported that cigarette smokers have lower plasma ascorbic acid concentrations than do nonsmokers (3, 33–35), these results could be due to a seasonal effect (36–38) because this study was conducted in early autumn. However, although these results for ascorbic acid status were unplanned, this may help isolate the effects of cigarette smoking on α-T disappearance kinetics. None of the participants took nutritional supplements for ≥6 mo before our study. Dietary intakes between smokers and nonsmokers were not significantly different for any of the nutrients analyzed (Table 3).

### Plasma ascorbic and uric acids

After supplementation with deuterated α-TAcS, plasma ascorbic acid concentrations averaged from days 0 to 17 were not significantly different between nonsmokers and smokers (Table 4). Plasma ascorbic acid concentrations were correlated with 3-d dietary ascorbic acid intakes (Table 3). The uric acid concentration, another major plasma antioxidant, was also not significantly different between the groups when averaged from days 0 to 17 (Table 4).

### Ferritin reducing ability of plasma

FRAP is a measure of total antioxidant protection in plasma and is generally correlated with uric acid concentrations and to a

**TABLE 4**  
Plasma concentrations of lipids and antioxidant status markers after supplementation in the nonsmokers and the smokers<sup>1</sup>

	Nonsmokers ( <i>n</i> = 10)	Smokers ( <i>n</i> = 10)
Cholesterol (mmol/L)	4.36 ± 0.61	3.78 ± 0.94
Triacylglycerol (mmol/L)	1.00 ± 0.36	0.78 ± 0.29
Ascorbic acid (μmol/L)	52.1 ± 13.4	46.5 ± 17.8
Uric acid (μmol/L)	283.3 ± 41.3	278.2 ± 53.1
FRAP (μmol/L)	630 ± 56	554 ± 93 <sup>2</sup>
F <sub>2α</sub> -Isoprostanes (pg/mL)	32.4 ± 6.3	45.5 ± 16.0 <sup>3</sup>

<sup>1</sup> All values are  $\bar{x} \pm$  SD from days 0 to 17. FRAP, ferritin reducing ability of plasma.

<sup>2,3</sup> Significantly different from nonsmokers (unpaired Student's *t* test): <sup>2</sup>*P* = 0.021, <sup>3</sup>*P* = 0.014.

lesser extent with ascorbic acid concentrations (30). After supplementation, smokers had lower FRAP values than did nonsmokers (Table 4), which suggests that smokers had a lower plasma antioxidant status. When values were averaged from days 0 to 17 for each individual, FRAP correlated positively with uric acid concentrations ( $P < 0.0001$ ,  $R^2 = 0.663$ ) but not with plasma ascorbic acid concentrations ( $P = 0.53$ ,  $R^2 = 0.023$ ).

### Plasma isoprostanes

To assess oxidative stress,  $F_{2\alpha}$ -isoprostanes were measured on selected days throughout the study. Cigarette smokers, on average, had  $F_{2\alpha}$ -isoprostanes that were  $\approx 40\%$  higher than those of nonsmokers throughout the postsupplementation period (Table 4). During the supplementation period, plasma  $F_{2\alpha}$ -isoprostanes decreased slightly in the smokers ( $\approx 13\%$ ) from days  $-6$  ( $47 \pm 5$  pg/mL) to day 0 ( $41 \pm 4$  pg/mL), but these changes were not significantly different ( $P = 0.132$ ).

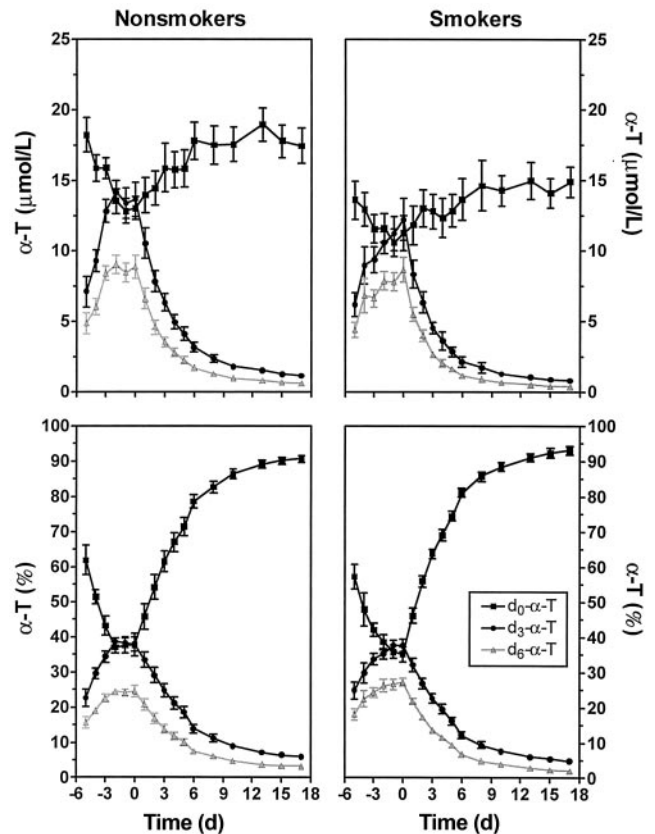
### Tocopherols

Before supplementation, plasma unlabeled  $\alpha$ -T and  $\gamma$ -T concentrations were not significantly different between smokers and nonsmokers (Table 1). After 6 d of supplementation with 75 mg each of  $d_3$ -*RRR*- $\alpha$ -TAc and  $d_6$ -*all-rac*- $\alpha$ -TAc, plasma total  $\alpha$ -Ts (sum of  $d_0$ -,  $d_3$ -, and  $d_6$ - $\alpha$ -T) more than doubled ( $P < 0.0001$ ) in nonsmokers (from  $15.3 \pm 2.8$   $\mu$ mol/L at baseline to  $35.5 \pm 5.8$   $\mu$ mol/L on day 0) and in smokers (from  $14.6 \pm 3.8$   $\mu$ mol/L at baseline to  $32.1 \pm 10.2$   $\mu$ mol/L on day 0). Day 0 plasma concentrations of  $d_0$ -,  $d_3$ -, and  $d_6$ - $\alpha$ -T and total  $\alpha$ -T were not significantly different between groups (Figure 2).

In both groups, plasma  $d_0$ - $\alpha$ -T concentrations decreased ( $P < 0.0001$ ) by  $\approx 19\%$  in response to deuterated  $\alpha$ -T supplementation, from  $14.9 \pm 0.7$   $\mu$ mol/L at baseline to  $12.1 \pm 0.8$   $\mu$ mol/L on day 0. Likewise, plasma  $\gamma$ -T concentrations decreased ( $P = 0.0011$ ) by  $\approx 38\%$  in both groups, from  $1.6 \pm 0.1$   $\mu$ mol/L at baseline to  $1.0 \pm 0.1$   $\mu$ mol/L on day 0.

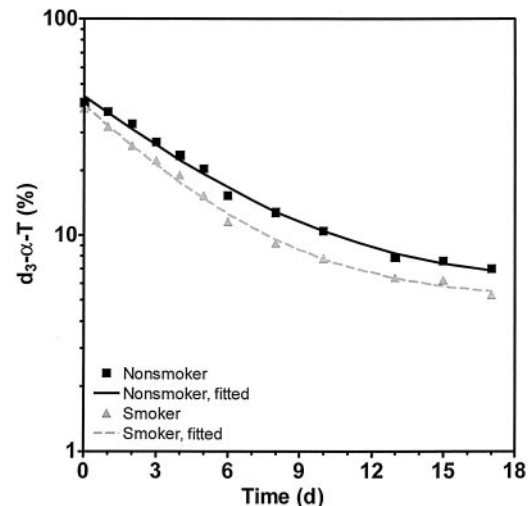
On day 0, plasma  $d_3$ - $\alpha$ -T concentrations in the nonsmokers ( $13.6 \pm 1.2$   $\mu$ mol/L) and smokers ( $12.2 \pm 1.5$   $\mu$ mol/L) were not significantly different, which suggested that both groups responded similarly to the 6 d of deuterium-labeled vitamin E supplementation (Figure 2). Likewise, plasma  $d_6$ - $\alpha$ -T concentrations were not significantly different between the nonsmokers ( $8.8 \pm 0.8$   $\mu$ mol/L) and the smokers ( $8.6 \pm 0.9$   $\mu$ mol/L). The ratio of  $d_3$ - $\alpha$ -T to  $d_6$ - $\alpha$ -T in the plasma was  $1.5 \pm 0.1$  during the supplementation period and subsequently increased to  $1.9 \pm 0.1$  after supplementation (days 0–17). These findings are similar to those from other human trials (16, 17, 39) that used deuterated *RRR*- and *all-rac*-tocopherols and, therefore, the subsequent results and discussion of  $\alpha$ -T disappearance kinetics will be limited to  $d_3$ - $\alpha$ -T for simplicity.

After 6 d of deuterated  $\alpha$ -T supplementation, the % $d_3$ - $\alpha$ -T on day 0 did not differ significantly between groups (Figure 2), and  $d_3$ - $\alpha$ -T represented  $\approx 40\%$  of total plasma  $\alpha$ -T. Mathematical modeling of the disappearance kinetics was performed on the % $d_3$ - $\alpha$ -T data from days 0 to 17, and fitted data from a representative smoker and nonsmoker are shown in Figure 3. The fractional disappearance rates of  $\alpha$ -T in cigarette smokers ( $0.215 \pm 0.011$ ) were  $\approx 13\%$  greater ( $P < 0.05$ ) than those in nonsmokers ( $0.191 \pm 0.009$  pools/d; Figure 4). Likewise, calculated  $\alpha$ -T half-lives were  $\approx 10$  h shorter in cigarette smokers ( $79.3 \pm 4.1$ ) than in nonsmokers ( $88.8 \pm 3.8$  h;  $P < 0.05$ ).

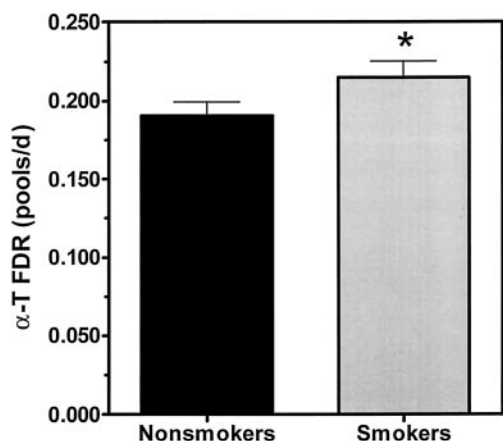


**FIGURE 2.** Time course of plasma  $d_0$ -,  $d_3$ -, and  $d_6$ - $\alpha$ -tocopherol ( $d_0$ -,  $d_3$ -, and  $d_6$ - $\alpha$ -T) in the smokers ( $n = 10$ ) and the nonsmokers ( $n = 10$ ) after supplementation for 6 d (days  $-6$  to day 0) with 75 mg each of  $d_3$ -*RRR*- $\alpha$ -tocopheryl acetate and  $d_6$ -*all-rac*- $\alpha$ -tocopheryl acetate after a standard meal. Values are means  $\pm$  SEs. All blood samples were collected in the morning after the subjects had fasted for  $\approx 12$  h.

Consistent with these results, the plasma  $d_3$ - $\alpha$ -T concentrations of the smokers on day 17 ( $0.9 \pm 0.3$   $\mu$ mol/L) were significantly lower than those of the nonsmokers ( $1.3 \pm 0.4$   $\mu$ mol/L;  $P < 0.05$ ). In addition, the  $d_0$ - $\alpha$ -T concentrations ( $14.1 \pm 1.0$



**FIGURE 3.** Modeled curve fit and actual percentages of  $d_3$ - $\alpha$ -tocopherol ( $d_3$ - $\alpha$ -T) from days 0 to 17 in a representative smoker and nonsmoker.  $\%d_3$ - $\alpha$ -T = [ $d_3$ - $\alpha$ -T]/( $d_0$ - $\alpha$ -T +  $d_3$ - $\alpha$ -T +  $d_6$ - $\alpha$ -T)  $\times$  100.



**FIGURE 4.** Mean ( $\pm$ SE) plasma fractional disappearance rates (FDRs) of the percentage of  $d_3$ - $\alpha$ -tocopherol ( $\alpha$ -T) in the smokers ( $n = 10$ ) and the nonsmokers ( $n = 10$ ). \*Significantly different ( $\approx 13\%$  greater) from the nonsmokers,  $P < 0.05$  (unpaired Student's  $t$  test).  $d_3$ - $\alpha$ -T half-lives were  $\approx 10$  h shorter in the smokers than in the nonsmokers.

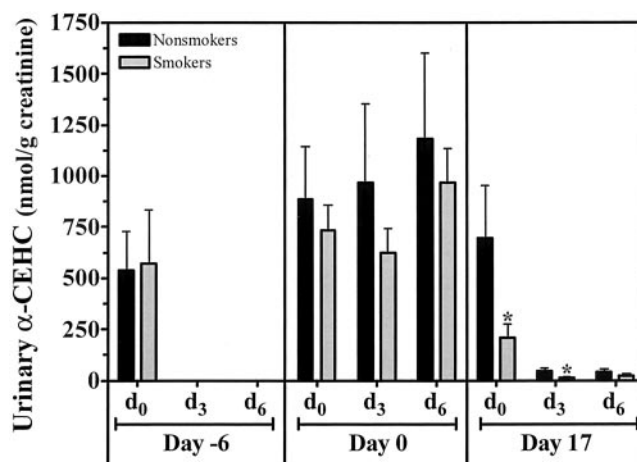
$\mu\text{mol/L}$ ) of the smokers were lower ( $P < 0.05$ ) than those of the nonsmokers ( $17.4 \pm 1.2 \mu\text{mol/L}$ ) by the end of the study. Despite these findings, there were no observable differences in  $d_0$ - $\alpha$ -T concentrations between smokers and nonsmokers before supplementation; moreover, dietary  $\alpha$ -T intakes (as assessed with dietary food records during the study) were not significantly different between the groups.

It has been suggested that plasma tocopherol concentrations should be adjusted for circulating lipid concentrations (40). However, in this investigation, no significant differences in plasma total cholesterol or triacylglycerol were observed between groups on any of the study days (Table 4). Therefore, labeled and unlabeled tocopherol concentrations were not adjusted for total lipids.

#### Urinary tocopherol metabolites

The metabolites of  $\alpha$ - and  $\gamma$ -T and  $\alpha$ - and  $\gamma$ -CEHC, respectively, were measured on 3 occasions during the investigation in 24-h urine samples: at baseline (day -6), after 6 d of supplementation (day 0), and on the last day of the study (day 17). Given the limited data on  $\alpha$ -T metabolism, we hypothesized that metabolite concentrations might serve as a marker for  $\alpha$ -T status. As shown in **Figure 5**, there were no significant differences in unlabeled and labeled  $\alpha$ -CEHC concentrations between the smokers and the nonsmokers at baseline or on day 0. Furthermore, the ratio of  $d_6$ - $\alpha$ -CEHC to  $d_3$ - $\alpha$ -CEHC on day 0 was  $1.7 \pm 0.1$  in both groups, which indicated a greater conversion of  $d_6$ -*all-rac*- $\alpha$ -T to  $\alpha$ -CEHC than did  $d_3$ -*RRR*-T. On day 17, 7 of 10 nonsmokers, but only 5 of 10 smokers, had detectable urinary deuterated  $\alpha$ -CEHC concentrations. Therefore, for the undetectable values, a value of half the detectable limit was substituted for statistical analyses. We observed that smokers excreted less urinary  $d_0$ - ( $P < 0.05$ ),  $d_3$ - ( $P < 0.05$ ), and  $d_6$ - $\alpha$ -CEHCs ( $P = 0.09$ ) than did nonsmokers. In addition, on day 17, plasma total  $\alpha$ -Ts or urinary total  $\alpha$ -CEHCs were lower among the smokers (**Table 5**). These data collectively support the notion that smokers had a lower  $\alpha$ -T status at the end of the study.

No significant differences in  $\gamma$ -CEHC excretion were observed at baseline between the smokers ( $3.13 \pm 0.54 \mu\text{mol/g}$  creatinine) and the nonsmokers ( $3.87 \pm 0.86 \mu\text{mol/g}$  creatinine),



**FIGURE 5.** Urinary mean ( $\pm$ SE) labeled ( $d_3$  and  $d_6$ ) and unlabeled ( $d_0$ )  $\alpha$ -carboxy-ethyl-hydroxy-chroman ( $\alpha$ -CEHC) excretion in the nonsmokers ( $n = 10$ ) and the smokers ( $n = 10$ ). Unlabeled urinary  $\alpha$ -CEHC was not significantly different between the smokers and the nonsmokers before supplementation with  $\alpha$ -tocopheryl acetate or on day 0. \*Significantly different from the nonsmokers,  $P < 0.05$  (unpaired Student's  $t$  test).

on day 0 between the smokers ( $3.18 \pm 0.70 \mu\text{mol/g}$  creatinine) and the nonsmokers ( $4.07 \pm 0.89 \mu\text{mol/g}$  creatinine), or on day 17 between the smokers ( $2.05 \pm 0.60 \mu\text{mol/g}$  creatinine) and the nonsmokers ( $3.44 \pm 0.85 \mu\text{mol/g}$  creatinine).

#### Interaction between $\alpha$ -T disappearance and ascorbic acid and uric acid

Ascorbic acid is reported to regenerate  $\alpha$ -tocopheroxyl radicals to  $\alpha$ -T in vitro (41), but this relation has not been shown in humans. Therefore, correlations were calculated between the  $\alpha$ -T fractional disappearance rate and mean plasma ascorbic acid concentrations for each group. As shown in **Figure 6**, the fractional disappearance rates of  $\alpha$ -T were correlated with plasma ascorbic acid in the cigarette smokers ( $P = 0.021$ ,  $R^2 = 0.509$ ) but not in the nonsmokers ( $P = 0.503$ ,  $R^2 = 0.058$ ). This observation suggests that the faster fractional disappearance rates of  $\alpha$ -T were related to lower ascorbic acid concentrations only in the presence of apparent oxidative stress, as observed in the smokers.

**TABLE 5**

Comparison between total plasma  $\alpha$ -tocopherol ( $\alpha$ -T) and total urinary  $\alpha$ -carboxy-ethyl-hydroxy-chroman ( $\alpha$ -CEHC)<sup>1</sup>

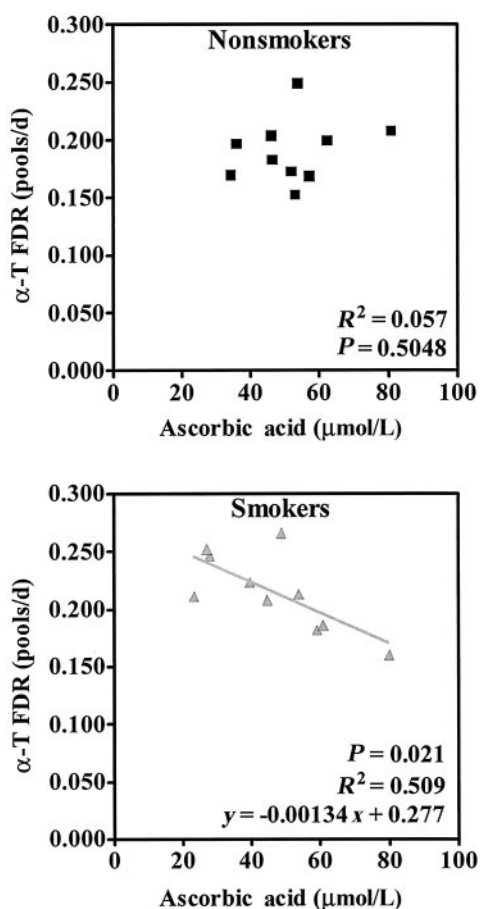
	Total $\alpha$ -T <sup>2</sup>		Total $\alpha$ -CEHC <sup>3</sup>	
	Nonsmokers ( $n = 10$ )	Smokers ( $n = 10$ )	Nonsmokers ( $n = 10$ )	Smokers ( $n = 10$ )
	$\mu\text{mol/L}$		$\text{nmol/g creatinine}$	
Day -6	$15.3 \pm 2.8$	$14.6 \pm 3.8$	$540 \pm 560$	$570 \pm 834$
Day 0	$35.5 \pm 5.6$	$32.1 \pm 10.2$	$3039 \pm 2889$	$2383 \pm 1068$
Day 17	$19.8 \pm 4.0$	$15.3 \pm 3.9^4$	$780 \pm 842$	$240 \pm 79^5$

<sup>1</sup> All values are  $\bar{x} \pm$  SD. Supplementation with deuterated (d)  $\alpha$ -T resulted in a doubling of total  $\alpha$ -T in both the nonsmokers and the smokers ( $P < 0.0001$ ) and in a 5.6- and 4.3-fold increase in total urinary  $\alpha$ -CEHC excretion in the nonsmokers and the smokers, respectively.

<sup>2</sup>  $d_0$ - +  $d_3$ - +  $d_6$ - $\alpha$ -T.

<sup>3</sup>  $d_0$ - +  $d_3$ - +  $d_6$ - $\alpha$ -CEHC.

<sup>4,5</sup> Significantly different from nonsmokers (unpaired Student's  $t$  test): <sup>4</sup> $P = 0.011$ , <sup>5</sup> $P = 0.039$ .



**FIGURE 6.** Relation between the fractional disappearance rates (FDRs) of  $\alpha$ -tocopherol ( $\alpha$ -T) and plasma ascorbic acid concentrations in the nonsmokers ( $n = 10$ ) and the smokers ( $n = 10$ ) by linear regression analysis. No significant relation was found in the nonsmokers by linear regression analysis, but a significant relation was found in the smokers. These data suggest that with increasing plasma ascorbic acid concentrations, the FDRs of plasma  $d_3$ - $\alpha$ -T decrease. Analysis with multiple linear regression showed that the slope of the smokers' regression was significantly different from that of the nonsmokers' regression ( $P = 0.0417$ ).

Because there was a significant correlation in the smokers, but not in the nonsmokers, we used multiple linear regression to assess whether the slopes of these regression lines differed between the nonsmokers and smokers and whether this relation remained constant at different plasma ascorbic acid concentrations. This analysis showed that the slope of the correlation in the nonsmokers was significantly different from that in the smokers ( $P = 0.0417$ ). When comparisons of predicted values were tested at low (34  $\mu\text{mol/L}$ ), intermediate (50  $\mu\text{mol/L}$ ), and high (80  $\mu\text{mol/L}$ ) ascorbic acid concentrations between groups, a significant difference between the smokers and the nonsmokers was observed only at the low ( $P = 0.014$ ) ascorbic acid concentration and not at the intermediate ( $P = 0.109$ ) or high ( $P = 0.229$ ) concentration. Therefore, the data suggest that when plasma ascorbic acid is low,  $\alpha$ -T is utilized at a greater rate in smokers than in nonsmokers.

A similar analysis was performed using uric acid because this water-soluble antioxidant is present in the plasma in significantly higher concentrations than is ascorbic acid. No significant correlations between the fractional disappearance rates of  $\alpha$ -T and

mean uric acid concentrations were observed in either the nonsmokers ( $P = 0.076$ ,  $R^2 = 0.343$ ) or the smokers ( $P = 0.446$ ,  $R^2 = 0.074$ ).

## DISCUSSION

Oxidative stress caused by cigarette smoking resulted in increased fractional disappearance rates of  $\alpha$ -T (Figure 4), consistent with an *in vivo* antioxidant function of  $\alpha$ -T. Smokers and nonsmokers were supplemented with deuterium-labeled  $\alpha$ -T (75 mg each of  $d_3$ -RRR- $\alpha$ -TAc and  $d_6$ -all-*rac*- $\alpha$ -TAc) for 6 d (Figure 2). Once supplementation ceased, the fractional disappearance rates and half-lives of  $\alpha$ -T in the smokers were  $\approx 13\%$  greater and  $\approx 10$  h shorter, respectively (Figure 4). As would be expected from these data, the plasma  $d_3$ - $\alpha$ -T concentrations of the smokers on day 17 were lower than those of the nonsmokers.

In cigarette smokers, but not in nonsmokers, fractional disappearance rates of  $\alpha$ -T were significantly correlated with plasma ascorbic acid concentrations (Figure 6). By multiple linear regression, it was determined that the greatest  $\alpha$ -T utilization occurred in smokers with low plasma ascorbic acid concentration. On the basis of the linear regression analysis of  $\alpha$ -T disappearance rates and mean plasma ascorbic acid concentrations, we estimated that cigarette smokers would require a plasma ascorbic acid concentration of 64.2  $\mu\text{mol/L}$  to have  $\alpha$ -T fractional disappearance kinetics similar to those of the nonsmokers (0.191 pools/d). This calculated estimate is  $\approx 38\%$  higher than the observed mean plasma ascorbic acid concentration of 46.5  $\mu\text{mol/L}$  in the smokers. Because dietary ascorbic acid was linearly correlated with plasma ascorbic acid in the smokers ( $P = 0.005$ ,  $R^2 = 0.697$ ), we further calculated that the smokers would require a total dietary ascorbic acid intake of 116 mg to achieve a plasma concentration of 64.2  $\mu\text{mol/L}$ . On average, this estimate would require that smokers consume 43 mg ascorbic acid/d above their reported dietary consumption (Table 3). These calculations are consistent with current dietary ascorbic acid recommendations, which suggest that smokers consume an additional 35 mg ascorbic acid/d above the recommendations for nonsmokers (90 and 75 mg/d for men and women, respectively) (5, 42).

Although fractional disappearance rates of  $\alpha$ -T in smokers were correlated with ascorbic acid concentrations (Figure 6) and smokers and nonsmokers had average plasma ascorbic acid concentrations that were not significantly different (Table 4), it was apparent that the range of ascorbic acid concentrations were greater in the smokers and that low ascorbic acid concentrations were observed in those smokers with the fastest  $\alpha$ -T disappearance rates. These data suggest that both low plasma ascorbic acid concentrations and oxidative stress are necessary to negatively influence the  $\alpha$ -T disappearance kinetics. To our knowledge, this is the first time that this interaction between ascorbic acid and  $\alpha$ -T has been shown in humans. Previously, guinea pigs fed 2 concentrations of deuterium-labeled  $\alpha$ -T and 3 concentrations of ascorbic acid did not show an interaction between these nutrients (43); however, no oxidative stress was applied to the guinea pigs in that investigation. Alternatively, *in vitro* studies conducted with H4IIE liver cells (44) or erythrocytes (45) subjected to oxidative stress sufficient enough to cause lipid peroxidation showed a sparing effect of  $\alpha$ -T when cells were treated with ascorbic acid. In addition, in another *in vitro* investigation it was

determined that the  $\alpha$ -tocopheroxyl radical formed within micellar and bilayer membrane systems could be effectively recycled by ascorbic acid found within the aqueous phase (41).


The results of our investigation showed that smokers have modestly higher requirements of  $\alpha$ -T than do nonsmokers. However, it should not be overlooked that the cohort of participants in this investigation was young (18–25 y) and had a limited history (2–5 y) and frequency (10–20 cigarettes/d) of smoking. Although it cannot be ascertained from the available data in this investigation, we can only speculate that these differences would be greater in older persons with a greater history and frequency of smoking because it is believed that aging in itself is an oxidative stress (46) and, thus, older smokers may require more vitamin E.

We measured plasma isoprostane concentrations as an index of oxidative stress. The smokers had higher plasma isoprostane concentrations than did the nonsmokers throughout the entire study. Although there was a modest reduction in circulating isoprostanes during deuterated  $\alpha$ -TAc supplementation (days –6 to day 0), these changes in isoprostanes were not statistically significant. In our investigation, we did not expect to observe changes in plasma isoprostanes because it has been reported that smokers supplemented with various doses of vitamin E for 5 d (47) or for 3 wk (48) had no significant effect in reducing urinary isoprostane excretion. However, in smokers and in nonsmokers ( $n = 100$ ) with elevated isoprostanes who were supplemented with 200 mg vitamin E for 1 y, there was a significant reduction in plasma isoprostanes (49). Thus, it is apparent that a longer intervention and possibly a larger sample size might be required to observe a significant reduction in isoprostanes with vitamin E supplementation.

In addition to plasma isoprostanes, we measured plasma FRAP as an index of antioxidant potential. We observed that the smokers, because of their increased oxidative stress, had 12% lower FRAP than did the nonsmokers. These data suggest that smokers have less plasma antioxidant potential, which would be consistent with their greater plasma isoprostane concentrations. Plasma uric acid is the greatest predictor of FRAP and accounts for  $\approx 60\%$  of the total predicted FRAP, whereas ascorbic acid contributes to  $\approx 15\%$  of the value (30). Uric acid has been reported to protect against peroxynitrite-mediated damage in experimental models of multiple sclerosis and central nervous system inflammation (50–52). However, excessive plasma uric acid concentrations are associated with the pathogenesis of gout (53). Both epidemiologic and experimental evidence suggest that uric acid is an independent risk factor for cardiovascular and renal disease (54). Note that all participants in this investigation had uric acid concentrations within normal limits (Table 4), and there were no significant differences in plasma uric acid concentrations between groups throughout the investigation. Therefore, we speculated that the smokers had significantly lower FRAP due to the subtle but nonsignificant differences among some or all of the other predictors of the assay, such as ascorbic acid—which was  $\approx 11\%$  lower ( $P > 0.05$ ) in the smokers than in the nonsmokers on average—throughout the investigation.

Last, the urinary metabolite of  $\alpha$ -T ( $\alpha$ -CEHC) was lower in the smokers than in the nonsmokers on day 17, which suggests that less  $\alpha$ -T metabolism occurred because there was less excess  $\alpha$ -T available for degradation. In addition, it was calculated that  $< 1\%$  of the labeled  $\alpha$ -T dose was excreted as  $\alpha$ -CEHC in the urine in both the smokers and the nonsmokers. These findings are similar

to those of another investigation that analyzed urinary deuterium-labeled  $\alpha$ -CEHC excretion from deuterium-labeled  $\alpha$ -T supplementation (26). Thus, other biological fluids, such as serum (55) or bile and fecal matter (56), may need to be analyzed to estimate the fate of metabolized  $\alpha$ -T.

Collectively, these data suggest that cigarette smokers, because of their elevated oxidative stress, require higher dietary  $\alpha$ -T intakes to maintain plasma  $\alpha$ -T concentrations that are similar to those of nonsmokers. The relation in smokers between faster disappearance rates of  $\alpha$ -T and lower plasma ascorbic acid concentrations warrants further assessment to determine whether intervention with ascorbic acid supplementation can modify  $\alpha$ -T disappearance kinetics in cigarette smokers. In addition, future investigations should attempt to characterize  $\alpha$ -T disappearance kinetics in a larger cohort of smokers with diverse ages and smoking habits, because this investigation had a limited number of young participants who had a relatively short smoking history and a low frequency of smoking. 

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RSB, TMB, and MGT participated in the study design and data collection and analyses and wrote the initial draft of the manuscript. RR conducted the mathematical analysis of  $\alpha$ -T disappearance and participated in the editing and review of the manuscript. TJM participated in the sample analysis for isoprostanes and contributed to the editing and review of the manuscript. None of the authors had a known conflict of interest.

## REFERENCES

1. Karnath B. Smoking cessation. *Am J Med* 2002;112:399–405.
2. Pryor WA. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. *Environ Health Perspect* 1997;105(suppl): 875–82.
3. Alberg A. The influence of cigarette smoking on circulating concentrations of antioxidant micronutrients. *Toxicology* 2002;180:121–37.
4. Brown KM, Morrice PC, Duthie GG. Erythrocyte vitamin E and plasma ascorbate concentrations in relation to erythrocyte peroxidation in smokers and nonsmokers: dose response to vitamin E supplementation. *Am J Clin Nutr* 1997;65:496–502.
5. US Institute of Medicine, Panel on Dietary Antioxidants and Related Compounds. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington, DC: National Academy Press, 2000.
6. Horwitt MK, Harvey CC, Duncan GD, Wilson WC. Effects of limited tocopherol intake in man with relationships to erythrocyte hemolysis and lipid oxidations. *Am J Clin Nutr* 1956;4:408–19.
7. Horwitt MK, Century B, Zeman AA. Erythrocyte survival time and reticulocyte levels after tocopherol depletion in man. *Am J Clin Nutr* 1963;12:99–106.
8. Handelman GJ, Packer L, Cross CE. Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. *Am J Clin Nutr* 1996;63:559–65.
9. Frei B, Forte TM, Ames BN, Cross CE. Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. *Biochem J* 1991;277:133–8.
10. Eiserich JP, van der Vliet A, Handelman GJ, Halliwell B, Cross CE. Dietary antioxidants and cigarette smoke-induced biomolecular damage: a complex interaction. *Am J Clin Nutr* 1995;62(suppl):1490S–500S.
11. Leonard SW, Bruno RS, Paterson E, et al. 5-nitro-gamma-tocopherol increases in human plasma exposed to cigarette smoke in vitro and in vivo. *Free Radic Biol Med* 2003;35:1560–7.
12. Dietrich M, Block G, Norkus EP, et al. Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase gamma-tocopherol in vivo after adjustment for dietary antioxidant intakes. *Am J Clin Nutr* 2003;77:160–6.

13. Malila N, Virtamo J, Virtanen M, Pietinen P, Albanes D, Teppo L. Dietary and serum alpha-tocopherol, beta-carotene and retinol, and risk for colorectal cancer in male smokers. *Eur J Clin Nutr* 2002;56:615–21.
14. Rust P, Lehner P, Elmadafa I. Relationship between dietary intake, antioxidant status and smoking habits in female Austrian smokers. *Eur J Nutr* 2001;40:78–83.
15. al Senaidy AM, al Zahrary YA, al Faqeeh MB. Effects of smoking on serum levels of lipid peroxides and essential fat-soluble antioxidants. *Nutr Health* 1997;12:55–65.
16. Acuff RV, Thedford SS, Hidirolou NN, Papas AM, Odom TA Jr. Relative bioavailability of *RRR*- and *all-rac*- $\alpha$ -tocopheryl acetate in humans: studies using deuterated compounds. *Am J Clin Nutr* 1994;60:397–402.
17. Burton GW, Traber MG, Acuff RV, et al. Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E. *Am J Clin Nutr* 1998;67:669–84.
18. Traber MG, Ramakrishnan R, Kayden HJ. Human plasma vitamin E kinetics demonstrate rapid recycling of plasma *RRR*-alpha-tocopherol. *Proc Natl Acad Sci U S A* 1994;91:10005–8.
19. Traber MG, Burton GW, Ingold KU, Kayden HJ. *RRR*- and *SRR*-alpha-tocopherols are secreted without discrimination in human chylomicrons, but *RRR*-alpha-tocopherol is preferentially secreted in very low density lipoproteins. *J Lipid Res* 1990;31:675–85.
20. Burton GW, Ingold KU, Cheeseman KH, Slater TF. Application of deuterated alpha-tocopherols to the biokinetics and bioavailability of vitamin E. *Free Radic Res Commun* 1990;11:99–107.
21. Munro LH, Burton G, Kelly FJ. Plasma *RRR*-alpha-tocopherol concentrations are lower in smokers than in non-smokers after ingestion of a similar oral load of this antioxidant vitamin. *Clin Sci (Lond)* 1997;92:87–93.
22. Traber MG, Winkhofer-Roob BM, Roob JM, et al. Vitamin E kinetics in smokers and nonsmokers. *Free Radic Biol Med* 2001;31:1368–74.
23. Birringer M, Drozan D, Brigelius-Flohe R. Tocopherols are metabolized in HepG2 cells by side chain omega-oxidation and consecutive beta-oxidation. *Free Radic Biol Med* 2001;31:226–32.
24. Sontag TJ, Parker RS. Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism. Novel mechanism of regulation of vitamin E status. *J Biol Chem* 2002;277:25290–6.
25. Schuelke M, Elsner A, Finckh B, Kohlschutter A, Hubner C, Brigelius-Flohe R. Urinary alpha-tocopherol metabolites in alpha-tocopherol transfer protein-deficient patients. *J Lipid Res* 2000;41:1543–51.
26. Traber MG, Elsner A, Brigelius-Flohe R. Synthetic as compared with natural vitamin E is preferentially excreted as alpha-CEHC in human urine: studies using deuterated alpha-tocopheryl acetates. *FEBS Lett* 1998;437:145–8.
27. Podda M, Weber C, Traber MG, Packer L. Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinols, and ubiquinones. *J Lipid Res* 1996;37:893–901.
28. Galli F, Lee R, Dunster C, Kelly FJ. Gas chromatography mass spectrometry analysis of carboxyethyl-hydroxychroman metabolites of alpha- and gamma-tocopherol in human plasma. *Free Radic Biol Med* 2002;32:333–40.
29. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci U S A* 1989;86:6377–81.
30. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal Biochem* 1996;239:70–6.
31. Morrow JD, Roberts LJ II. Mass spectrometry of prostanoids: F<sub>2</sub>-isoprostanes produced by non-cyclooxygenase free radical-catalyzed mechanism. *Methods Enzymol* 1994;233:163–74.
32. Berglund L, Witztum JL, Galeano NF, Khouw AS, Ginsberg HN, Ramakrishnan R. Three-fold effect of lovastatin treatment on low density lipoprotein metabolism in subjects with hyperlipidemia: increase in receptor activity, decrease in apoB production, and decrease in particle affinity for the receptor. Results from a novel triple-tracer approach. *J Lipid Res* 1998;39:913–24.
33. Faruque MO, Khan MR, Rahman MM, Ahmed F. Relationship between smoking and antioxidant nutrient status. *Br J Nutr* 1995;73:625–32.
34. Marangon K, Herbeth B, Lecomte E, et al. Diet, antioxidant status, and smoking habits in French men. *Am J Clin Nutr* 1998;67:231–9.
35. Mezzetti A, Lapenna D, Pierdomenico SD, et al. Vitamins E, C and lipid peroxidation in plasma and arterial tissue of smokers and non-smokers. *Atherosclerosis* 1995;112:91–9.
36. Cooney RV, Franke AA, Hankin JH, et al. Seasonal variations in plasma micronutrients and antioxidants. *Cancer Epidemiol Biomarkers Prev* 1995;4:207–15.
37. Woodhouse PR, Khaw KT. Seasonal variation of risk factors for cardiovascular disease and diet in older adults. *Int J Circumpolar Health* 2000;59:204–9.
38. Lenton KJ, Theriault H, Cantin AM, Fulop T, Payette H, Wagner JR. Direct correlation of glutathione and ascorbate and their dependence on age and season in human lymphocytes. *Am J Clin Nutr* 2000;71:1194–200.
39. Traber MG, Rader D, Acuff RV, Brewer HB Jr, Kayden HJ. Discrimination between *RRR*- and *all-racemic*-alpha-tocopherols labeled with deuterium by patients with abetalipoproteinemia. *Atherosclerosis* 1994;108:27–37.
40. Gross M, Yu X, Hannan P, Prouty C, Jacobs DR Jr. Lipid standardization of serum fat-soluble antioxidant concentrations: the YALTA study. *Am J Clin Nutr* 2003;77:458–66.
41. Bisby RH, Parker AW. Reaction of ascorbate with the alpha-tocopheroxyl radical in micellar and bilayer membrane systems. *Arch Biochem Biophys* 1995;317:170–8.
42. Kallner AB, Hartmann D, Hornig DH. On the requirements of ascorbic acid in man: steady-state turnover and body pool in smokers. *Am J Clin Nutr* 1981;34:1347–55.
43. Burton GW, Wronska U, Stone L, Foster DO, Ingold KU. Biokinetics of dietary *RRR*-alpha-tocopherol in the male guinea pig at three dietary levels of vitamin C and two levels of vitamin E. Evidence that vitamin C does not “spare” vitamin E in vivo. *Lipids* 1990;25:199–210.
44. Huang J, May JM. Ascorbic acid spares alpha-tocopherol and prevents lipid peroxidation in cultured H4IIE liver cells. *Mol Cell Biochem* 2003;247:171–6.
45. May JM, Qu ZC, Mendiratta S. Protection and recycling of alpha-tocopherol in human erythrocytes by intracellular ascorbic acid. *Arch Biochem Biophys* 1998;349:281–9.
46. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A* 1993;90:7915–22.
47. Reilly M, Delanty N, Lawson JA, FitzGerald GA. Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation* 1996;94:19–25.
48. Patrignani P, Panara MR, Tacconelli S, et al. Effects of vitamin E supplementation on F(2)-isoprostane and thromboxane biosynthesis in healthy cigarette smokers. *Circulation* 2000;102:539–45.
49. Kaikkonen J, Porkkala-Sarataho E, Morrow JD, et al. Supplementation with vitamin E but not with vitamin C lowers lipid peroxidation in vivo in mildly hypercholesterolemic men. *Free Radic Res* 2001;35:967–78.
50. Hooper DC, Scott GS, Zborek A, et al. Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood-CNS barrier permeability changes, and tissue damage in a mouse model of multiple sclerosis. *FASEB J* 2000;14:691–8.
51. Hooper DC, Spitsin S, Kean RB, et al. Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis. *Proc Natl Acad Sci U S A* 1998;95:675–80.
52. Scott GS, Hooper DC. The role of uric acid in protection against peroxynitrite-mediated pathology. *Med Hypotheses* 2001;56:95–100.
53. Gentili A. Advanced imaging of gout. *Semin Musculoskelet Radiol* 2003;7:165–74.
54. Alderman M, Aiyer KJ. Uric acid: role in cardiovascular disease and effects of losartan. *Curr Med Res Opin* 2004;20:369–79.
55. Morinobu T, Yoshikawa S, Hamamura K, Tamai H. Measurement of vitamin E metabolites by high-performance liquid chromatography during high-dose administration of alpha-tocopherol. *Eur J Clin Nutr* 2003;57:410–4.
56. Kiyose C, Saito H, Kaneko K, et al. Alpha-tocopherol affects the urinary and biliary excretion of 2,7,8-trimethyl-2 (2'-carboxyethyl)-6-hydroxychroman, gamma-tocopherol metabolite, in rats. *Lipids* 2001;36:467–72.