Effects of Dietary Supplementation of α-Tocopherol on Plasma Glutathione and DNA Repair Activities

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Abstract

In a randomized double-blind trial of α-tocopherol (vitamin E), we investigated the effects of α-tocopherol supplementation on lipid- and water-soluble antioxidants in plasma and DNA repair activities in peripheral mononuclear leukocytes. Baseline levels of antioxidants and DNA repair activities were assessed twice before α-tocopherol intervention: on day 1 (visit 1) and day 3 (visit 2). During the second visit, participants were randomized to receive one of three dosages of α-tocopherol, 15, 60, or 200 mg/day for 4 weeks. The same biochemical measurements as at baseline were repeated twice after intervention: on day 17 (visit 3) and day 31 (visit 4). A total of 31 healthy volunteers were eligible for the study, completed all four visits and were included in the final data analysis. At baseline, no appreciable differences of dietary intake of vitamin E and plasma α-tocopherol were observed among the three dosage groups. In general, supplementation of α-tocopherol for 2–4 weeks resulted in a dose-dependent increase of plasma level of α-tocopherol (compared to baseline); significant increases of plasma α-tocopherol at visits 3 and 4 were observed in the two higher dosage groups, 60 and 200 mg, but not in the lowest dosage group, 15 mg. At visit 4 (but not visit 3), plasma glutathione levels were significantly elevated (compared to baseline) in the two higher dosage groups, 60 and 200 mg, but not in the lowest dosage group, 15 mg. In addition, there was an increase in the lipid protection ratio by supplementation of α-tocopherol for 2–4 weeks in the two higher dosage groups, 60 and 200 mg, but not in the lowest dosage group, 15 mg. In general, there were no consistent effects of α-tocopherol on DNA repair activities in peripheral mononuclear leukocytes after being adjusted for baseline DNA repair activities. Results from this study demonstrate the interrelationship between α-tocopherol and other antioxidants in plasma; total plasma antioxidants can be modulated by short-term dietary supplementation of α-tocopherol.

Introduction

Data from both animal and epidemiological studies suggest that dietary antioxidants may have protective effects against certain types of cancer. Specifically, α-tocopherol (vitamin E), a well-studied lipid-soluble antioxidant, was associated with a reduced risk of cancer in two longitudinal epidemiologic studies (1, 2) but not in two other studies (3, 4). The potential use of α-tocopherol in human cancer chemoprevention has been evaluated in lung cancer (5), oral leukoplasia (6), and colorectal polyps (7, 8). The antimutagenic and antiproliferative effects of α-tocopherol may contribute to its chemopreventive property (9). Intracellularly, α-tocopherol is localized in the lipid-rich membrane and may serve to protect the membrane against LPO by reacting with lipid peroxyl and alkoyl radicals (10). Antioxidants do not function alone; both lipid- and water-soluble antioxidants may interact with each other to achieve overall protective action against oxidant-induced damage. For example, upon oxidation, the regeneration of α-tocopherol requires further coupling in the reducing systems, such as GSH/glutathione disulfide and ascorbate (vitamin C; Ref. 11). In a study of GSH in the red blood cells of humans, rabbits, and rats, oral administration of vitamin E can increase the levels of GSH (12). Furthermore, in GSH-depleted hepatocytes, α-tocopherol has protective effects against chemical-induced cellular injury, suggesting an interrelationship between α-tocopherol and GSH in maintaining the cellular antioxidant defense system (13).

DNA damage and defective DNA repair are associated with the development of human cancer (14–16). Although genetic factors can contribute to genomic instability and defective DNA repair, environmental exposure and dietary factors may also play a role in regulating DNA damage and repair by modulating the antioxidant/oxidant balance (17). Antioxidants can protect cells from DNA damage by directly removing reactive free radicals, thus reducing the amount of DNA damage, which may lead to tumorigenesis. Accumulation of DNA damage and/or cellular reduction/oxidation imbalance may contribute to decreased DNA repair capacity (18, 19). Furthermore, different studies suggested that α-tocopherol can protect cells against DNA damage and improve DNA repair activity.
Study Design. During the first visit, subjects completed a baseline questionnaire that included demographic information, medical history, family history of cancer, and (for women) reproductive history. Dietary intake was assessed by the NCI Food Frequency Questionnaire (23). Height, weight, and blood pressure were obtained at the baseline interview. BMI was calculated as weight (kg)/height (m)². All biochemical measurements were performed twice at baseline (days 1 and 3) and twice after intervention (days 17 and 31). After blood was drawn on day 3 (visit 2), the subjects were randomized to receive 15, 60 or 200 mg/day of α-tocopherol. The three types of capsules were assigned three different codes by the statistician (N. D.), who recorded the code in a sealed envelope and retained the envelope unopened until the data analysis was completed. Neither the study participants nor the laboratory technicians had knowledge of the dosage level assigned. Compliance was measured at visits 3 and 4 by calendar and pill count.

Study Dosage. The average daily intake of α-tocopherol for male and female adults is 9.8 and 7.1 mg, respectively (24). This is close to the RDA of 10 and 8 mg for male and female adults, respectively. To determine the dosage for this trial, we sought the optimal dosage for modulations of overall antioxidant defense system and DNA repair. We chose to test three dosages of α-tocopherol in this pilot study, 15 mg (1.5 × average intake), 60 mg (6 × average intake) and 200 mg (20 × average intake). The purpose of this study was to test a range of dosages that might have an effect on plasma antioxidants and DNA repair activities.

Initial Sample Handling. At each visit, a total of 40 ml of venous blood were obtained from each subject by a trained phlebotomist. Heparinized whole blood samples were collected in a nonfasting state between 9:00 and 11:00 a.m., stored in a dark container, transported to the laboratory within 2 hours of phlebotomy, and processed for PML isolation immediately. After the plasma was isolated, the PML fraction was separated by lymphocyte separation medium (LSM), a formulation based on Ficoll-sodium diatrizoate as described previously (25). Freshly isolated PMLs were washed once with Dulbecco’s PBS, resuspended in Dulbecco’s PBS, and used for DNA repair measurements (ADPRT and UDS). The plasma for micronutrient analyses was frozen immediately and stored in liquid nitrogen until assay.

Profile of Plasma Antioxidants. Frozen plasma samples with equal storage time (1 year) were batched and shipped to Panton Laboratories (San Diego, CA) on dry ice for measurement of different antioxidants. The lipid-soluble antioxidants were determined by a high-performance liquid chromatography method as described previously (26, 27) with improvements made by Panton Laboratories. Different antioxidants were separated with a methanol-based mobile phase on a C-18 column with large pore size and uniform particle size. These were monitored by a programmable wavelength monochromometer followed by an electrochemical detector. In a single run, data were obtained on antioxidants including retinol, α-tocopherol, lycopene, α-carotene, β-carotene, and ubiquinol-10. Other analyses (i.e., uric acid, total cholesterol, apolipoprotein B, and triglycerides) included in the Panton Profile were performed on a Beckman CX5 Autoanalyzer. The lipid protection ratio was calculated as the sum of the μm concentrations of the lipid-soluble antioxidants in plasma, which are embedded within the lipoprotein particles, divided by the μm concentration of cholesterol. This ratio is considered proportional to the number of antioxidant molecules protecting the low density lipoprotein and lipoprotein α particles. Because the lipid soluble antioxidant molecules are inserted into the lipoprotein particles, the lipid protection ratio may serve as a measure of the “concentration” of these antioxidants in that solution. It was suggested that lipid protection ratio may be a better measure of the level of antioxidants in other compartments than the crude plasma level. Levels of plasma α-tocopherol were adjusted for plasma triglycerides and cholesterol, as suggested by Horwitt (28).

Determination of Plasma GSH. The plasma GSH levels were measured by an enzymatic recycling method described by Griffith (29) and modified by Hu et al. (30). The assay was based on the oxidation of GSH by DTNB and the reduction by NAPDH catalyzed by glutathione reductase. The assay was initiated by mixing 0.7 ml of 0.3 mM NAPDH, 0.1 ml of 6 mM DTNB, and 0.1 ml of phosphate buffer [all solutions were prepared in sodium phosphate buffer (10 mM, pH 7.4)]. The reaction was initiated by mixing 0.7 ml of 0.3 mM NAPDH, 0.1 ml of 6 mM DTNB, and 0.1 ml of phosphate buffer [all solutions were prepared in sodium phosphate buffer (10 mM, pH 7.4)]. The reaction was initiated by mixing 0.7 ml of 0.3 mM NAPDH, 0.1 ml of 6 mM DTNB, and 0.1 ml of phosphate buffer [all solutions were prepared in sodium phosphate buffer (10 mM, pH 7.4)]. 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Quantitation of MNNG- and UV-induced UDS. Freshly isol-
solutions. The absorbance was continuously measured in a 0.5-
mL incubation mixture. For UV-induced UDS, cells in the experimental group were irradiated with UV (60 mJ/cm²). The DNA repair reaction was initiated by the addition of 5 μCi of [³H]thymidine and hydroxyurea (final concentration, 10 M). After incubation at 37°C for 22 h, the reaction was terminated by the addition of 0.5 μl ice-cold 20% TCA/10% sodium pyrophosphate and washed once with 0.5 ml ice-cold 10% TCA/5% sodium pyrophosphate. RNA was hydrolyzed with 0.5 ml KOH (0.33 n) at 37°C for 60 min, then 0.5 ml 20% TCA/10% sodium pyrophosphate was added, and the tubes were stored at −20°C overnight. After three more washes with 1 ml ice-cold 10% TCA/5% sodium pyrophosphate, DNA was extracted with 0.8 ml 5% TCA at 90°C for 20 min. The radioactivity (dpm) in the samples was determined by using a Wallac 1410 liquid scintillation counter (Pharmacia, Gaithersburg, MD).

ADPRT Enzyme Activity Assay. Two forms of ADPRT activity were experimentally determined. The physiological or constitutive ADPRT activity is associated with cellular growth, expression, and differentiation. The activated ADPRT activity involves induction via the introduction of DNA strand breaks with deoxyribonuclease, radiation, or carcinogens/mutagens. After a standardized exposure to 100 μM H₂O₂, both the constitutive and the activated levels of ADPRT were measured. The procedure was adapted from the permeabilized cell technique of Berger (31) with modifications as described (17, 32). Duplicate samples of 0.5 × 10⁶ PMLs were cultured in PBS with different concentrations of autologous plasma (0, 1, and 20%) for 30 min at 37°C in the presence or absence of 100 μM of H₂O₂. The cells were harvested by centrifugation at 500 × g for 10 min and then permeabilized at 4°C by suspension in ice-cold permeabilization buffer (10 mM Tris-HCl (pH 7.8)-1 mM EDTA-30 mM 2-mercaptoethanol-4 mM MgCl₂). The cytoskeletons were recovered by centrifugation and then re-suspended in 91 μl reaction mixture: 47 μl permeabilization buffer, 28 μl 100 mM Tris-HCl (pH 7.8), 120 mM MgCl₂, 10.8 μl of nicotinamide adenine dinucleotide (NAD) (2 mM), and 5 μl of [³H]adenine-labeled NAD⁺ (final concentration, 220 μM). Incubation was conducted at 30°C for 15 min. The reaction was terminated by addition of 0.5 ml 3 M NaCl at 60°C for 10 min. The ADP-ribose-protein complexes were precipitated with ice-cold 7% TCA and then collected onto nitrocellulose filters (Millipore HAWP 02500). The data were recorded as dpm TCA precipitable (ppt) [³H]adenine-labeled NAD⁺/0.5 × 10⁶ cells in the presence (activated) or absence (constitutive) of 100 μM H₂O₂.

Statistical Analysis. All the assays for antioxidants and DNA repair were carried out four times: day 1 (visit 1), day 3 (visit 2), 2 weeks after supplementation (visit 3), and 4 weeks after supplementation (visit 4). Assay values of visits 1 and 2 were averaged to provide a baseline value. Fixed-effect ANOVA was used to assess differences in plasma α-tocopherol and GSH at baseline among the three dosage groups. Paired t tests and paired Mann-Whitney-Wilcoxon tests were used to assess augmentation in plasma α-tocopherol or GSH at either visit 3 or 4 compared to baseline, for each of the dosage groups separately. In addition, repeated-measures ANOVA was used simultaneously to compare assay values at visits 3 and 4 to baseline for each of the dosage groups separately. Repeated-measures ANOVA was also used to compare changes in assay levels over time among the three dosage groups. In the ANOVA models, covariate adjustment for baseline levels of plasma-adjusted α-tocopherol was always included. All statistical analyses were carried out by using the Statistical Analysis System (SAS Institute, Cary, NC) on a personal computer. Results were considered significant at the two-sided P < 0.05 level.

### Table 1 Distribution of study participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>A (15 mg)</th>
<th>B (60 mg)</th>
<th>C (200 mg)</th>
<th>P value</th>
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<tr>
<td>Age (mean)</td>
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<td>35.5</td>
<td>34.9</td>
<td>0.51&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>Sex</td>
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<tr>
<td>Male</td>
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<td>2</td>
<td>4</td>
<td>0.59&lt;sup&gt;+&lt;/sup&gt;</td>
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<tr>
<td>White</td>
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<td>8</td>
<td>8</td>
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<tr>
<td>Non-white</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0.42&lt;sup&gt;+&lt;/sup&gt;</td>
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<tr>
<td>α-Tocopherol (μM)</td>
<td>0.097 ± 0.020</td>
<td>0.103 ± 0.014</td>
<td>0.106 ± 0.015</td>
<td>0.41&lt;sup&gt;+&lt;/sup&gt;</td>
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<tr>
<td>Vitamin E intake (mg)</td>
<td>7.33 ± 2.89</td>
<td>7.27 ± 3.28</td>
<td>11.05 ± 6.63</td>
<td>0.118&lt;sup&gt;+&lt;/sup&gt;</td>
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</table>

<sup>a</sup> P value from ANOVA.
<sup>b</sup> P value from Fisher’s exact test.
<sup>c</sup> Data are presented as mean ± SD of baseline adjusted plasma α-tocopherol levels.
<sup>d</sup> Total of 10 participants were randomized to group C; plasma sample for α-tocopherol measurement was not available from one subject.
<sup>e</sup> Data are presented as mean ± SD of baseline daily dietary vitamin E intake, estimated by the NCI Food Frequency Questionnaire.
Effects of α-Tocopherol on Glutathione and DNA Repair

Table 2: Effects of α-tocopherol supplements on plasma-adjusted α-tocopherol levels

<table>
<thead>
<tr>
<th>α-tocopherol dose (mg/day)</th>
<th>n</th>
<th>Baseline, μM</th>
<th>Visit 3, μM</th>
<th>% of baseline</th>
<th>Visit 4, μM</th>
<th>% of baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10</td>
<td>0.097 ± 0.020 (0.091)</td>
<td>0.091 ± 0.023 (0.090)</td>
<td>101*</td>
<td>0.110 ± 0.027 (0.107)</td>
<td>118</td>
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<tr>
<td>60</td>
<td>11</td>
<td>0.103 ± 0.014 (0.103)</td>
<td>0.132 ± 0.027 (0.136)*</td>
<td>131</td>
<td>0.129 ± 0.031 (0.124)*</td>
<td>127</td>
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<td>200</td>
<td>9</td>
<td>0.106 ± 0.015 (0.114)</td>
<td>0.161 ± 0.031 (0.151)*</td>
<td>146</td>
<td>0.146 ± 0.035 (0.141)*</td>
<td>138</td>
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</table>

*Repeated measures ANOVA, P = 0.02, for testing dose effects over time.

Table 3: The effects of α-tocopherol supplements on plasma glutathione levels

<table>
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<th>α-tocopherol dose (mg/day)</th>
<th>n</th>
<th>Baseline, μM</th>
<th>Visit 3, μM</th>
<th>% of baseline</th>
<th>Visit 4, μM</th>
<th>% of baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10</td>
<td>31.7 ± 13.4 (32.4)*</td>
<td>29.2 ± 16.3 (33.6)</td>
<td>89*</td>
<td>29.5 ± 14.9 (34.1)</td>
<td>106</td>
</tr>
<tr>
<td>60</td>
<td>11</td>
<td>33.7 ± 7.1 (34.6)</td>
<td>35.5 ± 11.5 (36.3)</td>
<td>110</td>
<td>40.4 ± 12.0 (39.9)*</td>
<td>126</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
<td>33.1 ± 13.5 (38.2)</td>
<td>37.3 ± 6.6 (39.5)</td>
<td>141</td>
<td>42.2 ± 6.6 (39.9)*</td>
<td>167</td>
</tr>
</tbody>
</table>

*Repeated measures ANOVA, P = 0.024, for testing dose effects over time.

Results

Quality control procedures were applied to all laboratory analyses. Pantox Laboratories measured a "high" and "low" control every day before or after running samples. These controls were recorded, and if they moved out of range, corrective action was undertaken. The CV for repeated determinations on subsequent days range from 2% (ascorbate) to 10% (β-carotene). For DNA repair measurements, repeated determinations could not be performed because freshly isolated PMLs were used; however, a human lymphoblastoid cell line, CCRF, was used as external quality control sample. The week-to-week variation of ADPRT activity was determined over a 35-week period; the CV ranged from 31% (constitutive) to 37% (activated). The CV for duplicate samples ranged from 7% (UV-UDS) to 22% (ADPRT).

A total of 31 (23 females and 8 males) healthy volunteers completed the study and were included in the final data analysis. Characteristics of study subjects by dosage group, including age, sex, race, baseline plasma α-tocopherol levels, and baseline dietary intake of vitamin E are summarized in Table 1. There were no significant differences in sex, race, age, and baseline dietary intake of vitamin E among dosage groups at baseline. The mean adjusted plasma α-tocopherol level was 0.102 μM for all participants in the study, and there was no evidence of appreciable differences among the three dosage groups at baseline (ANOVA, P = 0.41). The average age for females was slightly older than for males (38 versus 33 years, respectively); however, the difference was not significant (t test, P = 0.34). There was no difference in BMI between males and females (t test, P = 0.91). Most of the plasma micronutrients at baseline were similar by sex; however, mean uric acid levels were significantly higher (P = 0.0072) in males (336.8 μM) than females (250.7 μM). Most measures of baseline DNA repair capacity were similar in males and females (induced ADPRT with 0, 1, and 20% plasma and UV-induced UDS); however, mean MNNG-induced UDS was significantly higher in males (3341 dpm/10^6 cells) than females (2018 dpm/10^6 cells). All of the ANOVAs were adjusted for age, sex, and BMI. Because this did not alter the results, only the results from unadjusted ANOVAs were reported.

For none of the numeric variables were there significant deviations from normality. However, because of the small sample size, all of the ANOVAs were repeated with variables log-transformed and confirmed the results of the untransformed analyses. The effects of α-tocopherol supplements on adjusted plasma α-tocopherol levels are summarized in Table 2. The overall test for differences in adjusted plasma α-tocopherol among dosage groups over time was statistically significant (repeated measures ANOVA, P = 0.022). At visits 3 and 4, adjusted plasma α-tocopherol levels were significantly increased compared to baseline among subjects in the two higher dosage groups, 60 and 200 mg, but not in the lowest dosage group, 15 mg. Adjusted plasma α-tocopherol was significantly increased by 27 and 38% at visit 4 in the 60- and 200-mg dosage groups, respectively. The level of increase was similar at visits 3 and 4, which suggested that the increases may stay relatively constant during the period of 2 weeks between visits 3 and 4.

The effects of α-tocopherol supplements on plasma GSH levels are summarized in Table 3. The mean overall baseline GSH was 32.9 μg/ml for all the participants in the study. The plasma GSH levels were similar in the three dosage groups at baseline (ANOVA, P = 0.92). The overall test for differences in GSH among dosage groups over time was statistically significant (repeated measures ANOVA, P = 0.024). At visit 4 (but not visit 3), GSH was significantly increased (compared to baseline) in the two higher dosage groups, 60 and 200 mg, but not in the lowest dosage group, 15 mg. For example, there was a 67% increase of plasma GSH (mean of the paired difference within each individual) from 33.1 to 42.2 μg/ml in the 200-mg dosage group (paired Wilcoxon test, P < 0.05).

To demonstrate the variations in the response of each individual to α-tocopherol, Figs. 1 and 2 graphically show the within-subject augmentation of plasma α-tocopherol and GSH by dosage group. The mean lipid- and water-soluble antioxidant levels for each dosage group at baseline, visit 3, and visit 4 are summarized in Table 4. In addition to adjusted α-tocopherol and GSH, significant dosage effects were also seen for α-carotene and β-carotene, although the changes in these two vari-

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Fig. 1. Within-subject augmentation of plasma α-tocopherol. Data points for each subject are presented as before (baseline) and after (visits 3 and 4) α-tocopherol intervention. A, 15-mg/day dosage group; B, 60-mg/day dosage group; C, 200-mg/day dosage group.

Fig. 2. Within-subject augmentation of plasma GSH. Data points for each subject are presented as before (baseline) and after (visits 3 and 4) α-tocopherol intervention. A, 15-mg/day dosage group; B, 60-mg/day dosage group; C, 200-mg/day dosage group.

ables were not consistent over time. Increases in the lipid protection ratio were consistent, but of only borderline statistical significance ($P = 0.058$).

Data on DNA repair activities are summarized in Table 5 separately for each dosage group and time of visit. There were no dose-dependent effects over time on any of the DNA repair measurements by α-tocopherol supplements under our experimental condition. To help elucidate the findings in Tables 4 and 5, and for a selection of variables, the mean within-dosage augmentation (compared to baseline) was calculated in the three dosage groups at visits 3 and 4. For example, at visit 4, subjects in the 15-mg dosage group experienced a 0.014-μM mean increase in plasma...
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Table 4 The effects of α-tocopherol supplements on plasma antioxidant defense system and lipid status

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
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<th>Visit 4</th>
<th>Repeated measures ANOVA P value&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>Lipid-soluble antioxidants</td>
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<tr>
<td>Unadjusted α-tocopherol (μM)</td>
<td>24.2</td>
<td>28.9</td>
<td>22.9</td>
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<tr>
<td>Adjusted α-tocopherol&lt;sup&gt;&lt;sup&gt;a&lt;/sup&gt;&lt;/sup&gt;</td>
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<td>0.10</td>
<td>0.11</td>
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<td>α-carotene (μM)</td>
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<td>0.33</td>
<td>0.13</td>
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<td>β-carotene (μM)</td>
<td>0.46</td>
<td>0.76</td>
<td>0.36</td>
<td>0.50</td>
</tr>
<tr>
<td>Lycopene (μM)</td>
<td>0.66</td>
<td>1.10</td>
<td>0.84</td>
<td>0.76</td>
</tr>
<tr>
<td>Lipid protection ratio&lt;sup&gt;&lt;sup&gt;d&lt;/sup&gt;&lt;/sup&gt;</td>
<td>5.79</td>
<td>6.39</td>
<td>5.23</td>
<td>5.47</td>
</tr>
<tr>
<td>Water-soluble antioxidants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C (μM)</td>
<td>44.9</td>
<td>42.0</td>
<td>47.6</td>
<td>53.1</td>
</tr>
<tr>
<td>Uric acid (μM)</td>
<td>270</td>
<td>282</td>
<td>267</td>
<td>285</td>
</tr>
<tr>
<td>GSH (μg/ml)</td>
<td>31.7</td>
<td>33.7</td>
<td>33.1</td>
<td>29.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Dosage groups: A, 15 mg/day; B, 60 mg/day; C, 200 mg/day.

<sup>b</sup>For testing dose effects over time.

<sup>d</sup>For testing dose effects over time.

<sup>2</sup>α-tocopherol concentration divided by plasma triglycerides and cholesterol.

<sup>d</sup>Ratio of total micromolar concentrations of all lipid antioxidants divided by the concentration of cholesterol (μM).

Table 5 Effects of α-tocopherol supplements on DNA repair measurements

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Repeated measures ANOVA P value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADPRT (dpm/0.5 × 10&lt;sup&gt;6&lt;/sup&gt; cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% plasma</td>
<td>20342</td>
<td>20532</td>
<td>18421</td>
<td>24865</td>
</tr>
<tr>
<td>1% plasma</td>
<td>20080</td>
<td>19317</td>
<td>15053</td>
<td>21410</td>
</tr>
<tr>
<td>20% plasma</td>
<td>3613</td>
<td>4133</td>
<td>2671</td>
<td>4416</td>
</tr>
<tr>
<td>UDS (dpm/1 × 10&lt;sup&gt;6&lt;/sup&gt; cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNNG induced</td>
<td>2034</td>
<td>2611</td>
<td>2411</td>
<td>2007</td>
</tr>
<tr>
<td>UVC induced</td>
<td>2363</td>
<td>2020</td>
<td>2460</td>
<td>2014</td>
</tr>
</tbody>
</table>

<sup>a</sup>Dosage groups: A, 15 mg/day; B, 60 mg/day; C, 200 mg/day.

<sup>b</sup>For testing dose effects over time.

<sup>c</sup>P < 0.05, compared to baseline, one-sample Wilcoxon test.

Discussion

Reactive oxygen species, which can damage DNA, proteins, carbohydrates, and lipids, are constantly generated in cells; α-tocopherol can eliminate pro-oxidants, decrease DNA damage, and function as part of an antioxidant defense system. The potential role of micronutrients and related compounds in the cellular antioxidant defense system and their interrelationship were previously proposed (33). In this study, we demonstrated a dose-dependent increase of plasma α-tocopherol and GSH after short-term supplementation of α-tocopherol. Two mechanisms are proposed: a) α-tocopherol has a “sparing effect” on GSH levels as well as other antioxidants; and b) α-tocopherol may affect the regulation of enzymes that are involved in the synthesis and/or metabolism of GSH. From this study, we suggest that different components of the antioxidant defense system interact with each other and provide an overall protection against reactive oxygen species.

Several studies have suggested that the chemopreventive properties of α-tocopherol include reducing micronuclei frequencies (6), preventing peroxidative damage to DNA by dietary polyunsaturated fat (34), and inhibiting mammary tumors induced by 9,10-dimethyl-1,2-benzanthracene (35). In this study, we evaluated an additional potential mechanism of α-tocopherol in cancer prevention: modulation of DNA repair activities. Our earlier studies have shown that DNA repair activities can be modulated by oxidant/antioxidant balance; pretreatment of PMLs with H<sub>2</sub>O<sub>2</sub> or buthionine sulfoximine (depletion of GSH) resulted in a decrease of DNA repair activity (17, 36). Furthermore, our preliminary results from a colon cancer case-control study suggested a positive correlation between plasma GSH level and UV-induced UDS in PMLs. In this study, however, we did not detect any consistent effects of α-tocopherol on three DNA repair measurements, ADPRT and UV- and MNNG-induced UDS.

Data from this study should be interpreted with caution because several factors may affect the study results. It is obvious that a sample size of 10 in each dosage group with a trial period of 4 weeks allows only preliminary conclusions and must be considered as a pilot project for a larger confirmatory study in the future. Furthermore, under our experimental conditions, assay for DNA repair activities by UDS may be influenced by the initial DNA damage levels. Two confounding factors may affect our ability to detect the effects of α-tocopherol on DNA repair: a) supplementation of α-tocopherol may protect cells from DNA damage and result in lower DNA repair synthesis, which may even out the effects of α-tocopherol on DNA repair; and b) if there is a delayed response to α-tocopherol.

Unpublished observation.
erol, a longer trial period may be required to evaluate its effects on DNA repair. However, it is also possible that the protective effect of α-tocopherol against carcinogenesis may support a mechanism for the protective effect of α-tocopherol against DNA damage, without affecting DNA repair activities. Wei et al. (37) reported that the use of vitamin supplements was not correlated with DNA repair capacity in T lymphocytes transfectected with a damaged plasmid. However, plasma antioxidants were not measured in their study; the relationship between antioxidants and DNA repair activity remains to be explored.

In this study, short-term supplements of α-tocopherol increase both plasma α-tocopherol and GSH in a time- and dosage-dependent fashion, but do not have consistent effects on DNA repair activities. Handelman et al. (38) raised important issues concerning the steady state after a change of α-tocopherol intake, which should be addressed in future trials. It was estimated that more than 2 years are required for the α-tocopherol/γ-tocopherol ratio to reach a new steady state after a change in α-tocopherol intake. Also, in a cross-sectional measurement in five subjects who reported long-term use of α-tocopherol supplements (≥250 mg/day), and in five other subjects who reported no supplement use, the adipose α-tocopherol/γ-tocopherol ratio clearly discriminated between the two groups. In the future, a longer trial period of α-tocopherol may be required. In addition, the adipose α/γ-tocopherol ratio may be valuable in ranking individuals according to long-term α-tocopherol intake (38).

The normal intake of vitamin E in United States diets ranges between 4 and 22 IU/day in adults who are not taking vitamin E supplements (average values, 11–13 IU/day). This intake maintains an average blood level of approximately 23 μM, very similar to the mean baseline plasma α-tocopherol level of 27.9 μM among our subjects. Concerning the selection of appropriate cohort for dietary chemoprevention studies, one important concept is that supplementation of α-tocopherol to subjects on a nutritionally adequate diet does not always provide additional benefit or protection (39); therefore, supplementation of micronutrients to subjects on a nutrient-deficient diet may provide maximal benefit or protection against cancer. In summary, short-term supplements of α-tocopherol can modulate the overall antioxidant defense system but without consistent effects on DNA repair activities. On the basis of the results from this small pilot study, future confirmatory studies should address import issues related to the selection of study cohort, the optimal dose of α-tocopherol, the duration of trial, and the specificity of DNA repair assays.

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References

Effects of dietary supplementation of alpha-tocopherol on plasma glutathione and DNA repair activities.

J J Hu, G C Roush, M Berwick, et al.