Alpha and gamma tocopherol metabolism in healthy subjects and patients with end-stage renal disease

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Alpha and gamma tocopherol metabolism in healthy subjects and patients with end-stage renal disease.

Background. The metabolism of alpha and gamma tocopherol, the major components of vitamin E, have not been studied in uremic patients. The major pathway of tocopherol metabolism is via phytyl side chain oxidation, leaving carboxyethylhydroxycromans (CEHC) as metabolites. Alpha and gamma CEHC are water soluble, renally excreted, with known potent anti-inflammatory and antioxidative properties.

Methods. We examined serum alpha and gamma tocopherol and respective CEHC concentrations in 15 healthy subjects and 15 chronic hemodialysis patients.

Results. Serum alpha tocopherol levels were similar in hemodialysis patients (12.03 ± 1.34 μg/mL) and healthy subjects (11.21 ± 0.20 μg/mL), while gamma tocopherol levels were significantly greater in hemodialysis patients (3.17 ± 0.37 μg/mL) compared to healthy subjects (1.08 ± 0.06 μg/mL, P < 0.0001). Serum alpha and gamma CEHC levels were tenfold and sixfold higher in hemodialysis patients compared to healthy subjects, respectively (both P < 0.0001). Serum alpha and gamma tocopherol levels and CEHC metabolites were also measured after supplementation of alpha- or gamma-enriched mixed tocopherols in both hemodialysis patients and healthy subjects. Tocopherol administration resulted in modest or nonsignificant changes in serum tocopherol concentrations, while markedly increasing serum CEHC concentrations in both healthy subjects and hemodialysis patients. Hemodialysis resulted in no change in the serum alpha or gamma tocopherol concentrations while decreasing serum alpha CEHC and gamma CEHC levels by 63% and 53%, respectively (both P = 0.001 versus predialysis). Fourteen-day administration of gamma-enriched but not alpha tocopherols lowered median C-reactive protein (CRP) significantly in hemodialysis patients (4.4 to 2.1 mg/L, P < 0.02).

Conclusion. First, serum alpha and gamma CEHC accumulate in uremic patients compared to healthy subjects; second, supplementation with tocopherols dramatically increases serum CEHC levels in both healthy subjects and hemodialysis patients; and, finally, CEHC accumulation may mediate anti-inflammatory and antioxidative effects of tocopherol in hemodialysis patients.

Key words: tocopherol, ESRD, oxidative stress, CEHC, CRP.

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tially exported from hepatocytes and incorporated into lipoproteins, including low-density lipoproteins (LDL) [25, 26]. In vitro studies demonstrate superior antioxidant properties of alpha tocopherol in the prevention of LDL lipid peroxidation due to its lipid solubility and preferential incorporation into lipoproteins [27].

For many years, tocopherol metabolism was thought to proceed via a free radical attack on the chroman structure resulting in the formation of a tocopherylquinone. Degradation of the phytol side chain would then lead to the formation of alpha tocopheronic acid as well as the so-called Simon metabolites, which can be glucuronidated or sulfated and excreted in the urine [28, 29]. In the past decade, the discovery of urinary tocopherol metabolites with an intact chroman structure has been noted. These carboxyethyl-hydroxychromans (CEHC) are now known to be the major metabolites derived from each of the four parent tocopherol compounds [30–32]. This pathway of tocopherol metabolism derived from side chain degradation, leaves an intact chroman structure with potential antioxidant activity (Fig. 1). Of importance, the CEHC metabolites of tocopherols are water soluble and are known to be at least partially renally excreted.

To date, there are very few studies examining human tocopherol metabolism in vivo, and no published studies examining differential tocopherol metabolism in patients with ESRD. We therefore examined the metabolism of both alpha tocopherol and gamma tocopherol in patients with ESRD compared to age- and gender-matched healthy subjects.

**METHODS**

**Patient population**

Fifteen patients on chronic maintenance hemodialysis were recruited from hemodialysis units associated with the Division of Nephrology at Maine Medical Center and informed consent was obtained. Eligibility criteria required subjects to be between the ages of 30 and 60 years, and to have clinically acceptable hepatic function (transaminases <twice normal) and white blood cell counts between 4500 and 10,500. Individuals were excluded if they had chronic inflammatory diseases, evidence of recent bacterial infection, were pregnant or lactating, had clinically significant electrocardiogram (ECG) abnormalities, or a body mass index (BMI) >30 kg/m². Anti-inflammatory medications as well as alpha tocopherol >60 IU/day and vitamin C >500 mg/day supplements were also prohibited. Subjects continued with all prescribed medications as instructed by their physician for the duration of the study. The mean age of dialysis patients was 49.8 ± 1.9 years, with eight female and seven...
male subjects; seven of the patients were diabetic. Serum albumin was 3.65 ± 0.10 g/dL and Kt/Varea was 1.38 ± 0.06. Fifteen subjects who were age- and gender-matched healthy controls (mean age, 45.1 ± 1.8 years; eight females, seven males) were also recruited for the study. Informed consent was obtained from all study subjects.

Study protocols

Fifteen patients on chronic hemodialysis therapy and 15 healthy subjects were randomized to receive either an alpha tocopherol preparation or a preparation enriched in gamma tocopherol in a double-blinded fashion. The alpha and mixed tocopherol raw materials were purchased from Cargill (Minneapolis, MN, USA) and encapsulated by Soft Gel Technologies (Los Angeles, CA, USA). The identical white soft gel capsules contained either 99% RRR-alpha tocopherol or a mixture of 60% RRR-gamma tocopherol, 28% RRR-delta tocopherol, and 10% RRR-alpha tocopherol as measured by liquid chromatography–mass spectrometry. As there is relatively sparse published information on the metabolism of gamma tocopherol, subjects were allocated to this vitamin E isomer in a 2:1 ratio relative to the more comprehensively studied alpha tocopherol (i.e., ten dialysis patients and ten healthy subjects receiving gamma-enriched tocopherols versus five dialysis patients and five healthy subjects receiving alpha tocopherol).

Each subject underwent two separate protocols after randomization to a given type of tocopherol. In the first protocol, each subject received a single 600 mg dose of tocopherol preparation. Clinical laboratory measures and analysis of tocopherol levels were assayed 1 day before, and 1 and 6 days after the acute dose, respectively. For hemodialysis patients, tocopherol assays were also performed before and after dialysis on the day after tocopherol administration.

In the second protocol (performed after at least a 2-week washout from the first protocol), both hemodialysis patients and healthy subjects received a daily administration of 300 mg of the blinded tocopherol preparation for 14 consecutive days. Clinical parameters and laboratory assays were assessed at baseline, and after 7 and 14 days of tocopherol administration. Blood chemistries, complete blood count with differential, and vital signs were determined at each study visit. Serum alpha tocopherol, gamma tocopherol, alpha CEHC, and gamma CEHC were measured at all visits. Serum C-reactive protein (CRP), interleukin-6 (IL-6), and prealbumin levels were measured at the start and end of the 2-week dosing phase of the study. For hemodialysis patients, at the 14-day time point, blood samples were drawn pre- and postdialysis for measurements of alpha tocopherol, gamma tocopherol, alpha CEHC, and gamma CEHC. Patient safety was assessed by physical examination, vital signs, clinical laboratory evaluations, and reports of adverse events throughout the study.

Tocopherol and CEHC analysis

Reagents. Gamma tocopherol and alpha tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gamma and alpha tocopherol stock solutions were prepared individually in acetonitrile (4 mg/mL) and stored in amber vials at –80°C. Gamma CEHC was prepared at Galileo Laboratories, Inc. (Santa Clara, CA, USA), while alpha CEHC was purchased from Encore Pharmaceuticals (Riverside, CA, USA). Gamma and alpha CEHC stock solutions were prepared individually in acetonitrile (1 mg/mL) and stored in amber vials at –80°C. Acetonitrile and methanol used for extraction and high-performance liquid chromatography (HPLC) were purchased from Burdick and Jackson (Muskegon, MI, USA). Acetic acid, ethanol, and hexane were obtained from EM Science (Gibbstown, NJ, USA). Butylated hydroxy toluene (BHT), L-ascorbic acid, Escherichia coli β-glucoronidase, trifluoroacetic acid, and monobasic potassium phosphate were purchased from Sigma Chemical Co. Pooled, frozen human serum and serum with ethylenediaminetetraacetic acid (EDTA) used for background subtraction and standards was obtained from Valley Biomedical (Winchester, VA, USA). Centricon YM-30 and YM-50 membrane filtration devices were obtained from Millipore Corp. (Bedford, MA, USA).

Instruments. Tocopherol analyses were conducted by liquid chromatography–ultraviolet detection on an Agilent 1100 Series HPLC with diode array detector using an Alltima C18 HPLC column (5 μm, 150 x 2.1 mm) purchased from Alltech Associates, Inc. (Deerfield, IL, USA). CEHC analyses were conducted by liquid chromatography–mass spectrometry on an Agilent 1100 Series LC-MSD with diode array detector and electrospray ionization (ESI) source using the same type of HPLC column. Solvent removal was achieved using a Speedvac SC210A centrifugal evaporator (Savant Instruments, Holbrook, NY, USA).

Tocopherol standards. Standard mixtures of gamma and alpha tocopherol (1:1) in acetonitrile (10, 20, 50, 100, 500, 1000, and 2000 μg/mL each) were prepared from stock solutions on each day of analyses. Each standard mixture (10 μL) was added to 90 μL of pooled human serum to produce samples used to generate the standard curves (1, 2, 5, 10, 50, 100, and 200 μg/mL tocopherols).

Tocopherol extraction. Ethanol (150 μL) was added to each standard serum sample prepared as described above and to each study subject serum sample (100 μL) to precipitate proteins, and then 250 μL water was added to increase the volume for extraction. Tocopherols were extracted with 2 mL of hexane/ethyl acetate (5:1). After vortexing, centrifugation, and freezing the sample at –80°C, the upper, organic layer was removed and the solvents evaporated. The residue was resuspended in 100 μL of acetonitrile/methanol (1:1) and used for HPLC analysis.
**Tocopherol analysis.** Extracted tocopherols were separated by HPLC (25 µL injection) using an Alltima C_{18} reversed-phase HPLC column (5 µm, 150 × 2.1 mm) eluted with acetonitrile/methanol (80:20) with trifluoroacetic acid (0.1%) at a flow rate of 0.3 mL/min. Ultraviolet monitoring at 295 nm allowed detection of gamma and alpha tocopherol at 11.1 and 12.9 minutes, respectively.

**Tocopherol data analysis.** Data to create standard curves for gamma and alpha tocopherol was generated by duplicate or triplicate analysis of standards prepared as described above (1, 2, 5, 10, 50, 100, and 200 µg/mL tocopherols). For each sample, the integration of peaks was generated from the chromatogram at 295 nm and the background (extracted pooled human serum) was subtracted. Curve fitting was performed in Microsoft Excel. Linear or weighted (1/x or 1/x^2) standard curves of ultraviolet absorbance at 295 nm versus concentration in µg/mL were generated. Generally, the linear range for quantitation for gamma and alpha tocopherol was 1 to 100 µg/mL and the lower limit of quantitation (LLOQ) was 1 µg/mL. Tocopherol levels of samples from each study subject were calculated using the standard curve generated on the day of analysis.

**CEHC standards.** Standard mixtures of gamma and alpha CEHC (1:1) in water (50, 100, 250, 500, 1000, and 5000 ng/mL each) were prepared from stock solutions on each day of analysis. Each standard mixture (10 µL) was added to 90 µL of pooled human serum to produce samples used to generate the standard curves (5, 10, 25, 50, 100, 500 ng/mL CEHCs).

**CEHC extraction.** Ascorbic acid in water (10 µL, 5 mg/mL) was added to each standard serum sample prepared as described above and to each study subject serum sample (100 µL) to stabilize CEHCs, and then 100 µL β-glucuronidase solution [7500 units/mL in 10 mmol/L potassium phosphate buffer (pH 6.8)] was added to each sample to liberate conjugated CEHCs. Following the incubation period of 30 minutes at 37°C, 800 µL of methanol containing 10 µg/mL BHT was added to precipitate protein and extract the CEHCs. After vortexing and centrifugation, each supernatant was transferred to a Centricon YM-30 or YM-50 membrane filtration device, which had been prepared by addition of 1 mL of water. Extracts were then centrifuged at 3700 rpm at 15°C for 45 minutes to decontaminate the samples. Solvents were removed from the filtrate and the residue was resuspended in 100 µL 45% methanol–0.1% acetic acid containing 50 µg/mL ascorbic acid.

**CEHC analysis.** Extracted CEHCs were analyzed by liquid chromatography–mass spectometry (30 µL injection) using an Alltima C_{18} reversed-phase HPLC column (5 µm, 150 × 2.1 mm) eluted with a solvent gradient starting in water (0.1% HOAc)/methanol (55:45) for 1 minute, followed by a linear gradient to 80% methanol at 10 minutes at a flow rate of 0.25 mL/min. These conditions were maintained until 15 minutes when a quick (½ minute) gradient to 100% methanol was used to wash the column (held until 25 minutes). Monitoring the separation by mass spectrometry using an ESI source operating in the negative ion mode with selective ion monitoring (SIM) at 263.1 atomic mass unit (amu) (gamma CEHC) and 277.1 amu (alpha CEHC) allowed detection of gamma and alpha CEHC at 12.9 and 13.8 minutes, respectively.

**CEHC data analysis.** Data to create standard curves for gamma and alpha CEHC was generated by duplicate or triplicate analysis of standards prepared as described above (5, 10, 25, 50, 100, 500 ng/mL). For each sample, the integration of peaks was generated from the SIM chromatogram at 263.1 amu (gamma CEHC) and 277.1 amu (alpha CEHC), and the background (extracted pooled human serum) was subtracted. Curve fitting was performed in Microsoft Excel. Linear or weighted (1/x or 1/x^2) standard curves of SIM peak area versus concentration in ng/mL were generated. Generally, the linear range for quantitation was 10 to 500 ng/mL or 5 to 500 ng/mL and the LLOQ was 10 and 5 ng/mL for gamma and alpha CEHC, respectively. Samples measured above the linear range were diluted tenfold with pooled human serum and analyzed. CEHC levels of samples from each study subject were calculated using the standard curve generated on the day of analysis.

**Laboratory analysis**

Complete blood counts were determined using an EPEX Coulter Counter (Miami, FL, USA). Comprehensive metabolic profiles were measured using an autoanalyzer. High-resolution CRP was determined using nephelometry. Prealbumin was determined using an autoanalyzer. Serum IL-6 levels were determined by enzyme-linked immunosorbent assay (ELISA).

**Statistical analyses**

Wilcoxon signed rank tests were performed for within group comparisons, and Mann-Whitney U tests were performed for between group comparisons. The Spearman rank correlation test was used to measure the association between serum CRP and IL-6. Fisher’s exact test was used to compare the incidence of adverse experiences among treatment groups. These analyses were carried out using Intercooled Stata 6.0 for Windows 98/85/NT (Stata Corporation, College Station, TX, USA). Data are reported as means ± SEM unless otherwise noted.

**RESULTS**

**Baseline tocopherol and CEHC levels**

Serum alpha tocopherol, gamma tocopherol, alpha CEHC, and gamma CEHC levels were determined in all 15 healthy subjects and 15 hemodialysis patients. Serum alpha tocopherol levels were significantly higher than
gamma tocopherol levels in both healthy subjects (11.21 ± 0.20 μg/mL versus 1.08 ± 0.06 μg/mL, P < 0.0001) and hemodialysis patients (12.03 ± 1.34 μg/mL versus 3.17 ± 0.37 μg/mL, P < 0.0001) (Table 1). There were no significant differences in alpha tocopherol levels between healthy subjects and hemodialysis patients (P = 0.46). In contrast, serum gamma tocopherol levels were significantly greater in hemodialysis patients than healthy subjects (P < 0.0001).

Both serum alpha and gamma CEHC levels were markedly greater in hemodialysis patients than healthy subjects. Alpha CEHC levels were tenfold higher in the hemodialysis group compared to healthy subjects (75.65 ± 17.06 ng/mL versus 7.43 ± 1.31 ng/mL, P < 0.0001). Similarly, serum gamma CEHC levels were sixfold higher in the hemodialysis patients compared to healthy subjects (582.21 ± 120.35 ng/mL versus 95.30 ± 18.79 ng/mL, P < 0.0001). In both healthy subjects and hemodialysis patients, serum gamma CEHC was significantly higher than serum alpha CEHC levels (P < 0.0001).

**Table 1.** Baseline concentration of serum tocopherols and carboxylhydroxvchromans (CEHCs) [means ± SEM (median)]

<table>
<thead>
<tr>
<th>Serum level</th>
<th>Healthy subjects</th>
<th>Hemodialysis patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha tocopherol</td>
<td>11.21 ± 0.20 (11.09)*</td>
<td>12.03 ± 1.34 (12.53)*</td>
</tr>
<tr>
<td>Gamma tocopherol</td>
<td>1.08 ± 0.06 (1.08)*</td>
<td>3.17 ± 0.37 (3.41)</td>
</tr>
<tr>
<td>Alpha CEHC</td>
<td>7.43 ± 1.31 (6.63)*</td>
<td>3.75 ± 17.06 (52.87)*</td>
</tr>
<tr>
<td>Gamma CEHC</td>
<td>95.30 ± 18.79 (74.22)*</td>
<td>582.21 ± 120.35 (450.58)</td>
</tr>
</tbody>
</table>

*P < 0.0001 vs. gamma tocopherol (within column)  
*P < 0.0001 vs. hemodialysis patients (within row)  
*P < 0.0001 vs. gamma CEHC (within column)

Effect of a single dose of 600 mg of gamma-enriched tocopherols

A single 600 mg dose of a gamma tocopherol–enriched preparation was administered to 10 healthy subjects and 10 hemodialysis patients. Figure 3A demonstrates that in healthy subjects, gamma tocopherol administration was associated with a significant increase in serum gamma tocopherol levels on day 1 (1.09 ± 0.06 μg/mL versus 2.06 ± 0.15 μg/mL, P = 0.005) and day 6 (1.09 ± 0.06 μg/mL versus 1.74 ± 0.25 μg/mL, P = 0.04) after administration, with no significant changes observed in serum alpha tocopherol levels. In hemodialysis patients, gamma tocopherol administration resulted in a significant increase in serum gamma tocopherol levels at day 1 after administration (3.38 ± 0.52 μg/mL versus 5.17 ± 0.53 μg/mL, P = 0.04), which was no longer evident at day 6. No significant effects on serum alpha tocopherol levels were observed.

In healthy subjects, the administration of a gamma tocopherol–enriched preparation resulted in a marked increase in serum gamma CEHC levels day 1 after administration (67.16 ± 8.16 ng/mL versus 348.45 ± 79.00 ng/mL, P = 0.005) (Fig. 3B). Of interest, by day 6 after administration of a single dose, serum gamma CEHC levels had returned to baseline levels in healthy subjects (67.16 ± 8.16 ng/mL versus 69.96 ± 11.56 ng/mL, P = 0.72). In hemodialysis patients, the administration of a single 600 mg dose of gamma tocopherol–enriched preparation also resulted in a significant increase in serum gamma CEHC levels at day 1 after administration (730.82 ± 159.47 ng/mL versus 1,848.04 ± 349.91 ng/mL, P = 0.009). By day 6 after gamma tocopherol administration, serum gamma CEHC levels were no longer significantly different from baseline (730.82 ± 159.47 ng/mL versus 1036.45 ± 242.38 ng/mL, P = 0.07). Alpha CEHC levels did not vary in this patient population after administration of a single dose of gamma-enriched tocopherols.

Metabolism of a single dose of 600 mg alpha tocopherol administration

The effect of the administration of a single 600 mg dose of alpha tocopherol was examined in five healthy subjects and five hemodialysis patients. Figure 2A demonstrates there were no significant changes in either serum alpha tocopherol or serum gamma tocopherol levels on either day 1 or day 6 after alpha tocopherol administration in healthy subjects or hemodialysis patients.

Figure 2B demonstrates that in healthy subjects, there was a nonsignificant increase in alpha CEHC levels on day 1 after alpha tocopherol administration (9.66 ± 3.64 ng/mL versus 19.44 ± 6.62 ng/mL, P = 0.14), which diminished by day 6 (9.66 ± 3.64 ng/mL versus 15.10 ± 5.38 ng/mL, P = 0.35). Of interest, alpha tocopherol administration was also associated with a nonsignificant decrease in serum gamma CEHC levels on day 6 after administration to healthy subjects compared to prior to administration (151.58 ± 47.11 ng/mL versus 85.65 ± 30.86 ng/mL, P = 0.35). In hemodialysis patients, single-dose alpha tocopherol administration was not associated with a significant change in either serum alpha CEHC or gamma CEHC levels.
Fig. 2. Serum alpha and gamma tocopherol (A) and alpha and gamma carboxyethyl-hydroxychroman (CEHC) (B) levels in healthy controls and hemodialysis patients after a 600 mg dose of alpha tocopherol at day 0.
Fig. 3. Serum alpha and gamma tocopherol (A) and alpha and gamma carboxyethyl-hydroxychroman (CEHC) (B) levels in healthy controls and hemodialysis patients after a 600 mg dose of gamma-enriched tocopherols at day 0.
Fig. 4. Serum alpha and gamma tocopherol (A) and alpha and gamma carboxyethyl-hydroxychroman (CEHC) (B) levels in healthy controls and hemodialysis patients during a 14-day course of 300 mg alpha tocopherol/day.
tocopherol levels on either day 7 or day 14. However, after 7 and 14 days of alpha tocopherol administration, serum gamma tocopherol levels decreased significantly (1.36 ± 0.20 μg/mL versus 0.73 ± 0.14 μg/mL, P = 0.04; 1.36 ± 0.20 μg/mL versus 0.72 ± 0.10 μg/mL, P = 0.04, respectively).

In healthy subjects, alpha tocopherol administration did result in a significant increase in serum alpha CEHC levels at day 7 (10.63 ± 2.86 ng/mL versus 49.24 ± 22.08 ng/mL, P = 0.04) and day 14 (10.63 ± 2.86 ng/mL versus 35.93 ± 14.76 ng/mL, P = 0.04), while not affecting serum gamma CEHC levels (Fig. 4B). In hemodialysis patients, alpha tocopherol administration also significantly increased serum alpha CEHC levels at day 7 (56.43 ± 3.13 ng/mL versus 267.33 ± 35.06 ng/mL, P = 0.04) and day 14 (56.43 ± 3.13 ng/mL versus 354.42 ± 73.51 ng/mL, P = 0.04).

Serum gamma CEHC levels were significantly greater after 14 days of alpha tocopherol administration (393.21 ± 67.85 ng/mL versus 623.16 ± 204.25 ng/mL, P = 0.04). Thus, in both healthy subjects and hemodialysis patients, the major metabolic effect of alpha tocopherol administration was an increase in serum alpha CEHC levels without a change in serum alpha tocopherol levels, although minor effects on serum gamma tocopherol and gamma CEHC cannot be ruled out.

**Tocopherol metabolism during a 14-day course of 300 mg gamma-enriched tocopherols daily**

Figure 5A depicts serum alpha tocopherol, gamma tocopherol, alpha CEHC, and gamma CEHC levels after a 2-week administration of daily gamma-enriched tocopherol preparation. In healthy subjects, the administration of gamma-enriched tocopherols did not significantly change serum alpha tocopherol levels on day 7 or 14, but did result in a slight but significant increase in serum gamma tocopherol levels at day 7 (2.72 ± 0.39 μg/mL versus 5.50 ± 0.99 μg/mL, P = 0.03) and day 14 (2.72 ± 0.39 μg/mL versus 4.01 ± 0.43 μg/mL, P = 0.02). In hemodialysis patients, the administration of gamma tocopherol–enriched preparation for 14 days did not significantly affect alpha tocopherol levels or serum gamma tocopherol levels at day 7 (2.18 ± 0.40 μg/mL versus 3.89 ± 0.86 μg/mL, P = 0.13) or at day 14 (2.18 ± 0.40 μg/mL versus 3.30 ± 0.70 μg/mL, P = 0.20).

The administration of gamma tocopherol–enriched preparation resulted in a significant increase in serum gamma CEHC levels in healthy subjects at day 7 (66.95 ± 8.31 ng/mL versus 346.95 ± 69.80 ng/mL, P = 0.005) and at day 14 (66.95 ± 8.31 ng/mL versus 280.47 ± 82.25 ng/mL, P = 0.007). Gamma tocopherol administration was associated with a significant increase in serum alpha CEHC levels in healthy subjects at day 7 (5.16 ± 0.49 ng/mL versus 7.67 ± 1.43 ng/mL, P = 0.03) but not day 14 (P = 0.20). In hemodialysis patients, the administration of gamma tocopherol similarly resulted in a marked increase in serum gamma CEHC levels at day 7 (749.22 ± 175.82 ng/mL versus 3892.57 ± 987.59 ng/mL, P = 0.005) and at day 14 (749.22 ± 175.82 ng/mL versus 3395.44 ± 964.70 ng/mL, P = 0.005). Of interest, gamma tocopherol–enriched administration was also associated with significant elevations in serum alpha CEHC levels in hemodialysis patients at day 7 (41.54 ± 13.08 ng/mL versus 120.98 ± 35.49 ng/mL, P = 0.005) and day 14 (41.54 ± 13.08 ng/mL versus 109.12 ± 41.09 ng/mL, P = 0.005).

**Effects of the hemodialysis procedure on tocopherol metabolism**

The effects of the hemodialysis procedure on the clearance of serum alpha tocopherol, gamma tocopherol, alpha CEHC, and gamma CEHC was also examined both after the administration of a single 600 mg dose and after a 2-week course of 300 mg of the corresponding tocopherols. There was no significant effect of the dialysis procedure on serum gamma tocopherol levels (2.44 ± 0.56 μg/mL versus 2.37 ± 0.47 μg/mL, P = 0.64), nor serum alpha tocopherol (8.00 ± 1.30 μg/mL versus 8.55 ± 1.71 μg/mL, P = 0.58) (Table 2). Serum alpha CEHC levels decreased by 63% (190.88 ± 46.83 ng/mL versus 70.80 ± 18.34 ng/mL) and serum gamma CEHC levels decreased by 53% after the dialysis procedure (2471.35 ± 724.44 ng/mL versus 1163.83 ± 321.56 ng/mL, in both cases P = 0.001 versus predialysis). These data suggest that the water-soluble CEHC metabolites of tocopherols are readily dialyzable, while the more lipid-soluble parent compounds are not.

**Effects of tocopherol administration on the acute-phase inflammatory process**

It has recently been suggested that gamma tocopherol and its metabolite gamma CEHC may have potent anti-inflammatory effects, which are not shared by alpha tocopherol. We therefore compared the effects of a 2-week administration of alpha tocopherol and gamma-enriched tocopherols on serum CRP and serum IL-6 levels. For this analysis, a patient receiving gamma-enriched tocopherols who had an intercurrent hospitalization with surgery in the midst of the study was excluded from analysis. Figure 6B demonstrates that for hemodialysis patients taking daily gamma-enriched tocopherols for 2 weeks, median CRP declined from 4.4 to 2.1 mg/L (P < 0.02). Although there was a correlation between serum CRP and serum IL-6 at day 0 (rho = 0.98, P < 0.0001) and day 14 (rho = 0.89, P = 0.001), median serum IL-6 levels did not decline significantly (12.6 versus 10.9 pg/mL, P = 0.59) (Fig. 7B). In the five hemodialysis patients who received a 2-week course of alpha tocopherol therapy, there was no evidence of a difference in median serum CRP (12.2 versus 10.8 mg/L, P = 0.69) (Fig. 6A), while median serum IL-6 levels increased over the course of therapy (21.0 versus 32.9 pg/mL, P = 0.04) (Fig. 7A).
Fig. 5. Serum alpha and gamma tocopherol (A) and alpha and gamma carboxyethyl-hydroxychroman (CEHC) (B) levels in healthy controls and hemodialysis patients during a 14-day course of 300 mg gamma-enriched tocopherols/day.
The administration of alpha tocopherol either as a single high dose or as a 14-day course did not affect serum alpha tocopherol levels in either healthy subjects or hemodialysis patients. These data are in accordance with previous studies suggesting that serum alpha tocopherol concentration is saturable, resulting in an increase in metabolism to alpha CEHC. In fact, in healthy subjects, it has previously been suggested that measurements of urinary alpha CEHC can be used as a biomarker of adequate tissue alpha tocopherol stores [31]. Of potential importance, however, the administration of a 14-day course of alpha tocopherol resulted in a significant decrease in serum gamma tocopherol levels in healthy subjects and hemodialysis patients. These data are in accord with previous studies suggesting that alpha tocopherol can replace gamma tocopherol in lipid membranes and vice versa [52, 53]. Thus, biologic interactions between different tocopherol preparations would suggest that supplementation with mixed tocopherols (i.e., akin to “natural” or dietary tocopherols) may potentially avoid adverse biologic effects.

In contrast to alpha tocopherol, administration of a gamma tocopherol–enriched preparation resulted in significant increases in serum gamma tocopherol levels in healthy subjects. This suggests that serum gamma tocopherol levels are more modifiable by supplementation and may perhaps explain why observational studies have correlated serum gamma tocopherol levels with cardiovascular disease more consistently than serum alpha tocopherol levels [42–44]. Because the gamma tocopherol–enriched preparation also contained alpha tocopherol, it is impossible to tell from these studies whether administration of pure gamma tocopherol would affect serum alpha tocopherol levels.

A remarkable finding in this study is the dramatically elevated serum alpha and gamma CEHC levels in hemodialysis patients compared to healthy subjects. To our knowledge, there are only two previous studies in the literature measuring serum alpha and gamma CEHC levels in humans, and neither study examined patients with renal disease [54, 55]. The finding of six- to tenfold higher alpha and gamma CEHC levels in hemodialysis patients compared to healthy subjects confirms the important role of urinary excretion of the water-soluble metabolites of tocopherols in healthy subjects. Since the CEHC metabolites of tocopherols may have important biologic activities [23], these data strongly suggest that studies administering antioxidants to patients with renal failure must take into account antioxidant metabolism as part of study design. The finding that in both healthy subjects and hemodialysis patients, serum gamma CEHC concentrations are markedly higher than alpha CEHC concentrations, while conversely serum alpha tocopherol levels are markedly higher than serum gamma tocopherol levels, points to the crucial importance of the hepatic

### Table 2. Effect of hemodialysis on serum tocopherols and carboxyethyl-hydroxychromans (CEHCs) [means + SEM (median)]

<table>
<thead>
<tr>
<th>Serum level</th>
<th>Before dialysis</th>
<th>After dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha tocopherol ug/mL</td>
<td>8.00 ± 1.30</td>
<td>8.55 ± 1.71</td>
</tr>
<tr>
<td></td>
<td>(5.27)</td>
<td>(6.56)</td>
</tr>
<tr>
<td>Gamma tocopherol ug/mL</td>
<td>2.44 ± 0.56</td>
<td>2.37 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>(1.48)</td>
<td>(1.83)</td>
</tr>
<tr>
<td>Alpha CEHC ng/mL</td>
<td>190.88 ± 46.83</td>
<td>70.80 ± 18.34</td>
</tr>
<tr>
<td></td>
<td>(105.29)</td>
<td>(37.87)</td>
</tr>
<tr>
<td>Gamma CEHC ng/mL</td>
<td>2471.35 ± 724.44</td>
<td>1163.83 ± 521.56</td>
</tr>
<tr>
<td></td>
<td>(1428.15)</td>
<td>(501.02)</td>
</tr>
</tbody>
</table>

*P = 0.001 vs. after dialysis (within row)
tocopherol transport protein in differentiating the metabolic fate of tocopherols [25, 26].

In both healthy subjects and hemodialysis patients, the predominant effect of either alpha or gamma tocopherol administration is on serum levels of the corresponding CEHC metabolite rather than parent compounds. A provocative hypothesis stemming from this observation is that the major biologic effects associated with administration of high doses of tocopherols may also be mediated through the CEHC metabolites. Since the tissue distribution of the water-soluble CEHC metabolites differs markedly from the more lipid-soluble parent tocopherols, this would suggest that administration of high-dose tocopherols will affect extracellular and intracellular antioxidant status more than lipoproteins and cell membranes. In this context, it is tempting to speculate that the beneficial effects seen in the SPACE Trial, where high-dose alpha tocopherol was administered to hemodialysis patients for a prolonged time course, may have been due to the likely buildup of serum alpha CEHC levels rather than the effects of alpha tocopherol in this patient population. Since serum CEHC accumulates to a greater degree in renal failure, the relative potency of tocopherol administration would be enhanced in the presence of renal failure due to decreased renal clearance of CEHC metabolites. In this context, it is worth noting that, whereas in the general population studies of alpha tocopherol administration have generally had mixed results [56], studies in patients with ESRD have almost invariably reported some potent and beneficial biologic effects [20, 45, 46, 57–62].

A potentially important observation in this study is that the administration of the gamma tocopherol–enriched preparation, but not the alpha tocopherol preparation, significantly reduced CRP concentrations in hemodialysis patients. Although we did not observe a consistent reduction in CRP with alpha tocopherol, we cannot rule out a similar effect from this form of the vitamin due to the small number of subjects given alpha tocopherol. While alpha tocopherol is a potent lipoprotein and cell membrane–associated antioxidant, several recent studies have demonstrated that gamma tocopherol and gamma CEHC may additionally function as potent anti-inflammatory agents in vitro. Both gamma tocopherol and gamma CEHC at physiologically relevant concentrations inhibit the cyclooxygenase-2 (COX-2) enzyme in macrophages and epithelial cells to a much greater degree than alpha tocopherol [23]. Gamma tocopherol is also highly potent in quenching reactive nitrogen species [63, 64]. It is important, however, to recognize that the sample size in the present study, while sufficient to understand the metabolism of tocopherols in healthy subjects and patients with renal disease, may not be sufficient to allow definitive interpretation of their relative efficacy as in vivo anti-inflammatory agents. Similarly, the observation of a slight increase in serum IL-6 levels after alpha tocopherol administration is in contradistinction to other studies in diabetic patients and may reflect the relatively small sample size in the present study [65].

In this study, the hemodialysis procedure had no significant effect on either serum alpha tocopherol or gamma tocopherol levels. In contrast, hemodialysis resulted in a 63% reduction in serum alpha CEHC levels and a 53% reduction in gamma CEHC levels. Significant dialytic clearance of alpha and gamma CEHC is to be expected given their low molecular weight and water solubility, whereas both parent compounds are highly lipid-soluble and thus significant dialytic clearance is not expected.

Several limitations to the present study are acknowledged. The liquid chromatography–mass spectrometry
CONCLUSION

This is the first study to examine the metabolism of vitamin E, in both healthy subjects and hemodialysis patients. The tocopherol metabolites alpha and gamma CEHC accumulate in the serum of uremic patients compared to healthy subjects, and supplementation with tocopherols dramatically increases serum CEHC levels in both healthy subjects and hemodialysis patients. Administration of gamma- but not alpha-enriched tocopherols to maintenance hemodialysis patients significantly reduced median serum CRP. Further studies assessing the potential anti-inflammatory and antioxidative effects of tocopherol administration in uremic patients are required.

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