Sex differences in the inhibition of γ-tocopherol metabolism by a single dose of dietary sesame oil in healthy subjects

Jan Frank, Sangeun Lee, Scott W Leonard, Jeffrey K Atkinson, Afaf Kamal-Eldin, and Maret G Traber

ABSTRACT

Background: γ-Tocopherol has unique properties that may be beneficial in sustaining optimal human health, but hepatic vitamin E metabolism enhances γ-tocopherol turnover.

Objective: Our aim was to determine the extent to which dietary sesame lignans alter human α- and γ-tocopherol metabolism and elimination as carboxyethyl hydroxychromans (CEHCs).

Design: Healthy participants (n = 5 women and 5 men) in a randomized, crossover study (with 4-wk washout) consumed muffins prepared with either corn oil or unrefined sesame oil (sesamin, 94 mg; sesamolin, 42 mg), along with a capsule containing a 1:1 molar ratio of deuterium-labeled d_5-α- and d_5-γ-tocopherol acetates (≥50 mg each). Plasma and urine were collected up to 72 h; unlabeled and labeled tocopherol and CEHC concentrations were determined by liquid chromatography–mass spectrometry.

Results: Sesame oil muffin consumption in men, but not in women, decreased (P < 0.05) areas under plasma d_5-γ-CEHC concentration-time curves (area under the curve) and maximum concentrations. However, in both sexes urinary d_5-γ-CEHCs were decreased for 24 h following sesame oil muffin consumption.

Conclusions: In humans, γ-tocopherol metabolism can be inhibited by the simultaneous consumption of γ-tocopherol and sesame lignans. The observed differences between men and women with respect to vitamin E metabolism warrant further investigation. Am J Clin Nutr 2008;87:1723–9.

INTRODUCTION

γ-Tocopherol has unique properties that may be beneficial in sustaining optimal human health and preventing disease (1). For example, γ-tocopherol as a result of the unsubstituted 5-position on the chromanol ring can scavenge reactive nitrogen species (RNS) (2). RNS damage in humans has been substantiated by the detection of higher 5-nitro-γ-tocopherol concentrations in smokers (3) and Alzheimer’s disease patients (4). γ-Tocopherol reportedly inhibits thrombogenesis (5), decreases inflammation (6), as well as reduces cancer cell proliferation (7, 8). Moreover, γ-tocopherol, but not α-tocopherol, levels were reduced in coronary heart disease patients (9–11), suggesting that there may be benefits in raising plasma γ-tocopherol concentrations (5, 12).

Although the liver expresses the α-tocopherol transfer protein, which is responsible for salvaging α-tocopherol from the excretory pathway and returning it to the liver, hepatic metabolism appears to be a key factor in the discrimination between tocopherols (13). In the fruit fly Drosophila melanogaster, because the fly lacks α-tocopherol transfer protein, the selective accumulation of α-tocopherol has been attributed to metabolism of non-α-tocopherols (14). Vitamin E forms are metabolized to side chain–truncated, water-soluble carboxyethyl hydroxychromans (CEHCs) (15–18) and are excreted in urine (19–21) and bile (22, 23). Cytochrome P_500 (CYP) enzymes catalyze the initial α-hydroxylation of the side chain before its shortening by enzymes of the β-oxidation pathway (17, 18). Importantly, these processes have a higher catalytic activity toward the non-α-vitamins (15, 17).

Sesamin, an abundant lignan in sesame seeds and oils, when fed to rats, substantially increased plasma γ-tocopherol, but not α-tocopherol concentrations (24, 25). In human hepatocellular liver carcinoma (HepG2) cell line and primary rat hepatocytes, sesamin inhibited the degradation of non-α-tocopherols to their corresponding CEHC metabolites (18, 26). Consistently, dietary sesame seeds or sesame lignans (sesamin and sesaminol) reduced the urinary excretion of γ-CEHC in rats (21). Increased blood γ-tocopherol concentrations in response to sesame lignan intake have also been reported in 2 controlled human studies (27, 28). Thus, the lack of γ-tocopherol retention in blood is greatly influenced by hepatic metabolism.

Our previous studies using deuterium-labeled α- and γ-tocopherols demonstrated that not only does γ-tocopherol turn over faster than does α-tocopherol but that γ-CEHC and γ-tocopherol disappear from the plasma at the same rates (29). Moreover, women had a faster γ-tocopherol disappearance rate than did men. These data suggest that vitamin E metabolism is critical in regulating plasma γ-tocopherol concentrations in humans. To evaluate the hypothesis that the simultaneous consumption of vitamin E with sesame oil containing sesame lignans could decrease vitamin E metabolism and thus increase plasma...
γ-tocopherol concentrations, a randomized, crossover study was carried out in both women and men. The participants consumed deuterium-labeled α- and γ-tocopherol acetates along with a breakfast containing either sesame or corn oil muffins; after a 4-wk washout, the subjects repeated the study with the opposite muffin.

SUBJECTS AND METHODS

Materials

α-5,7-(C2H3)2 tocopheryl acetate (dα-α-TAc) was a gift from Dr. James Clark of Cognis Nutrition and Health (LaGrange, IL). γ-3,4-(3H) tocopheryl acetate (dγ-γ-TAc) was prepared from γ-tocopherol as described (30). The dα-α- and dγ-γ-TAc were diluted in tocopherol-stripped corn oil at a 1:1 molar mixture, and gelatin capsules containing ~50 mg of each α- and γ-TAc were prepared. The dα-α- to dγ-γ-tocopherol molar ratio was determined by liquid chromatography–mass spectrometry (LC-MS) to be 0.98. The internal standard, α-5,7,8-(C2H3)2 tocopheryl acetate (dα-α-TAc), was provided by Carolyn Good of The Bell Institute of Health and Nutrition (Minneapolis, MN) and was synthesized by Isotec, Inc (Miamisburg, OH). Cold-pressed, unrefined sesame oil (a gift from Henry Lamotte GmbH, Bremen, CA). Trolox, γ-tocopherol, ascorbic acid, butylated hydroxytoluene (BHT), potassium hydroxide (KOH), lithium perchlorate, and β-glucuronidase (type H-1, contains minimum 300,000 U/g β-glucuronidase activity and minimum 10 000 U/g sulfatase activity) were obtained from Sigma-Aldrich (St. Louis, MO). Diethyl ether was obtained from Mallinckrodt Baker, Inc (Phillipsburg, NJ), and high-pressure liquid chromatography–grade methanol, ethanol, hexane, and glacial acetic acid were from Fisher (Fair Lawn, NJ).

Subjects

Healthy participants (5 women, 5 men) were recruited, and written informed consent was obtained before inclusion in the trial. Participants were not smokers, did not take any dietary supplements at least 3 wk before and during the study, and restricted their physical activity to <5 h/wk. Routine blood serum chemistry assays were performed at Good Samaritan Hospital (Corvallis, OR) and were within the normal limits for all subjects. For subject characteristics, see Table 1. The study protocol was approved by the Institutional Review Board at Oregon State University.

Experimental diets and study design

Corn and sesame oils used for the preparation of the muffins were equalized with respect to concentrations of α-tocopherol (17.9 mg/100 g corn oil; 15.6 mg/100 g sesame oil) and γ-tocopherol (84,9 mg/100 g corn oil; 84.0 mg/100 g sesame oil). The 2 muffins made with either oil contained ~13.0 mg α-tocopherol and 2.5 mg γ-tocopherol. The 2 sesame oil muffins provided 135.4 mg sesame lignans (sesamin, 93.8 mg; sesamolin, 41.6 mg). Sesame oil or corn oil (with equalized vitamin E concentrations in each oil) was used to prepare muffins from 650 g wheat flour, 250 g sugar, 25 g baking powder, 10 g salt, 130 g whole egg, 440 g milk, and 275 g oil by mixing all ingredients and weighing 50 g of each oil and γ-TAc were prepared. The dα-α- to dγ-γ-tocopherol molar ratio was determined by liquid chromatography–mass spectrometry (LC-MS) to be 0.98. The internal standard, α-5,7,8-(C2H3)2 tocopheryl acetate (dα-α-TAc), was provided by Carolyn Good of The Bell Institute of Health and Nutrition (Minneapolis, MN) and was synthesized by Isotec, Inc (Miamisburg, OH). Cold-pressed, unrefined sesame oil (a gift from Henry Lamotte GmbH, Bremen, CA). Trolox, γ-tocopherol, ascorbic acid, butylated hydroxytoluene (BHT), potassium hydroxide (KOH), lithium perchlorate, and β-glucuronidase (type H-1, contains minimum 300,000 U/g β-glucuronidase activity and minimum 10 000 U/g sulfatase activity) were obtained from Sigma-Aldrich (St. Louis, MO). Diethyl ether was obtained from Mallinckrodt Baker, Inc (Phillipsburg, NJ), and high-pressure liquid chromatography–grade methanol, ethanol, hexane, and glacial acetic acid were from Fisher (Fair Lawn, NJ).

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Subject characteristics and blood lipids at screening

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 5)</th>
<th>Men (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.4 ± 1.8</td>
<td>30.6 ± 2.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 0.6</td>
<td>24.9 ± 1.0</td>
</tr>
<tr>
<td>Serum lipids (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.40 ± 0.22</td>
<td>4.97 ± 0.11</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>2.40 ± 0.17</td>
<td>3.14 ± 0.13</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.33 ± 0.06</td>
<td>0.52 ± 0.08</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>0.72 ± 0.15</td>
<td>1.12 ± 0.18</td>
</tr>
<tr>
<td>Total lipids</td>
<td>5.1 ± 0.2</td>
<td>6.1 ± 0.3</td>
</tr>
<tr>
<td>Plasma tocopherols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (µmol/L)</td>
<td>19.4 ± 3.4</td>
<td>18.3 ± 2.4</td>
</tr>
<tr>
<td>γ-Tocopherol (µmol/L)</td>
<td>1.8 ± 0.5</td>
<td>3.5 ± 1.5</td>
</tr>
<tr>
<td>α-Tocopherol per lipids (µmol/mol)</td>
<td>3.8 ± 0.3</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>γ-Tocopherol per lipids (µmol/mol)</td>
<td>0.34 ± 0.4</td>
<td>0.57 ± 0.10</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM. Shown are the baseline serum lipids, plasma tocopherols, and the tocopherol/lipid ratios. Lipids are defined as the sum of the serum total cholesterol and triglycerides, expressed as mmol/L. Statistical differences between values of women and men were calculated by an unpaired Student’s t test.

2 P < 0.05.

3 P < 0.01.

Quantification of labeled and unlabeled plasma tocopherols

Tocopherols were extracted from plasma according to the method of Podda et al (31). Briefly, plasma was saponified with
saturated alcoholic KOH and the tocopherols extracted with hexane. An appropriate aliquot was dried under N2-gas, the residue resuspended in MeOH:H2O (1:1, by vol) containing \( \text{d}_6\)-α-tocopherol as internal standard and injected into the LC system. For LC-MS analysis of labeled and unlabeled tocopherols, a Waters (Milford, MA) 2690 Separations Module equipped with a Symmetry LC-18 column (Waters; 4.6 × 75 mm, 3.5-μm particle size; mobile phase, 100% methanol; flow rate, 1 mL/min) and a negative atmospheric pressure chemical ionization probe was used as previously described (32).

Quantification of labeled and unlabeled plasma and urine CEHC metabolites

Plasma and urinary CEHCs were extracted using a modified method of Lodge et al (33). In brief, a known amount of trolox (internal standard) was added to either 0.5 mL plasma or 1 mL urine, before incubation for 30 min at 37 °C with 100 \( \mu \)L \( \beta \)-glucuronidase (10 mg/mL; dissolved in 0.01 mol/L potassium phosphate buffer, pH 6.8), and 20 \( \mu \)L H2O2 containing 1% ascorbic acid. After incubation, the samples were acidified by the addition of 10 \( \mu \)L 12 mol/L HCl. CEHCs were extracted with 5 mL of diethyl ether, dried under nitrogen gas, and resuspended in H2O:MeOH (1:1, by vol) containing 0.1% (by vol) acetic acid, with 10 \( \mu \)L injected into the LC system described above equipped with a SymmetryShield RP-18 column (Waters; 3.0 × 150 mm, 3.5-μm particle size), and the solvent was delivered by a modified gradient method of Himmelfarb et al (34). The system was first equilibrated with 50:50 H2O:MeOH (both containing 0.1% acetic acid) for 1 min, followed by a linear gradient to 80% MeOH at 6 min at a flow rate of 0.25 mL/min. These conditions were maintained for 15 min, followed by a 5-min wash period with 95% MeOH, at which time original conditions were returned to and run for 5 min before injection of the proceeding sample. Samples were detected using a Micromass (Manchester, UK) ZQ 2000 single-quadrupole mass spectrometer with an electrospray ionization source [capillary voltage, 2.5 V; sample cone voltage, ~30 V; desolvation temperature, 150 °C; desolvation gas (nitrogen) flow, 160 L/h; nebulizer gas (nitrogen) pressure, 80 psi; cone gas (nitrogen) flow, 50 L/h], dwell time per compound, 0.20 s. Single-ion recording mass-to-charge (m/z) ratios for molecular ions were as follows: \( \text{d}_6\)-α-CEHC, m/z 277; \( \text{d}_6\)-α-CEHC, m/z 283; \( \text{d}_6\)-γ-CEHC, m/z 263; \( \text{d}_2\)-γ-CEHC, m/z 265, and trolox, m/z 249. Sample CEHC concentrations were calculated from the ratio of the peak area of the corresponding ion to that of the internal standard trolox peak; deuterated CEHCs were calculated using the corresponding nondeuterated CEHC standards.

Quantification of plasma triacylglycerols and cholesterol and urinary creatinine

Plasma triacylglycerols and cholesterol were determined using the respective ThermoDMA Kits (Louisville, CO). Urinary creatinine was quantified spectrophotometrically at a wavelength of 500 nm after reaction with picric acid according to the Jaffé reaction (35).

Statistical analyses

The maximal concentrations (\( C_{\text{max}} \)) and the time to reach maximal concentrations (\( T_{\text{max}} \)) were identified by visual inspection of each individual’s plasma concentration data. Areas under the curve (AUC) were calculated using the trapezoidal rule. Fractional disappearance rates (FDRs) of \( \text{d}_6\)-α- and \( \text{d}_6\)-γ-tocopherols and \( \text{d}_2\)-α- and \( \text{d}_2\)-γ-CEHCs were calculated separately for each individual as described previously (36). Repeated measures multivariate analysis of variance was performed using JMP Statistical Discovery software (version 5.0.1a; SAS Institute, Cary, NC) to evaluate effects attributed to oil type and to sex. When significant interactions were found, then an unpaired Student’s \( t \) test for between-sex comparisons or a one-tailed, paired Student’s \( t \) test for comparisons of the 2 treatments within the same subjects was carried out. Differences were considered significant

FIGURE 1. Mean (±SEM) plasma \( \text{d}_6\)-α- and \( \text{d}_6\)-γ-tocopherol and \( \text{d}_2\)-γ-carboxyethyl hydrochromanols (CEHC) concentrations. Plasma \( \text{d}_6\)-α-tocopherol (A), \( \text{d}_6\)-γ-tocopherol (B), and \( \text{d}_2\)-γ-CEHC (C) concentrations (μmol/L) following ingestion of muffins prepared from unrefined sesame oil (squares, dotted lines) or corn oil (triangles, solid lines) simultaneously with a capsule containing 50 mg each \( \text{d}_6\)-α- and \( \text{d}_6\)-γ-tocopherol acetates in men (\( n = 5 \), filled symbols) and in women (\( n = 5 \), open symbols). Plasma samples were collected periodically for 72 h. By 72 h, the \( \text{d}_6\)-γ-tocopherol (B) concentrations were below levels of detection for many subjects, and thus the means are not shown.
at a \( P < 0.05 \) level. Data are expressed as means \( \pm \) SEM unless otherwise noted.

**RESULTS**

**Participant characteristics**

All participants had normal blood levels of analytes measured in routine chemistry panels (data not shown) and were within the normal range for body mass index and blood lipids (Table 1). Men, compared with women, had significantly higher plasma \( \gamma \)-tocopherol concentrations as well as serum total and LDL cholesterol at the time of screening; plasma \( \alpha \)-tocopherol concentrations were similar in all subjects (Table 1). Neither plasma \( \alpha \)-tocopherol nor \( \gamma \)-tocopherol concentrations were different between men and women when expressed per serum total lipids (sum of cholesterol and triacylglycerols) because total lipid concentrations were lower in women than in men (\( P < 0.05 \)).

No significant differences in the dietary intakes of macro- and micronutrients, including vitamin E, were observed between treatment groups or between men and women throughout the trials (data not shown).

**TABLE 2**

Kinetic parameters calculated from plasma \( d_\alpha \)-\( \alpha \)-tocopherol, \( d_\gamma \)-\( \gamma \)-tocopherol, and \( d_\gamma \)-\( \gamma \)-carboxyethyl hydrochromanol (CEHC) concentrations in subjects consuming \( d_\alpha \)-\( \alpha \)-tocophenyl acetate (TAc) and \( d_\gamma \)-\( \gamma \)-TAc together with sesame oil or corn oil muffins, respectively

<table>
<thead>
<tr>
<th></th>
<th>( d_\alpha )-( \alpha )-Tocopherol</th>
<th>( d_\gamma )-( \gamma )-Tocopherol</th>
<th>( d_\gamma )-CEHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (( \mu )mol/L \cdot h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>164 ( \pm ) 19</td>
<td>20 ( \pm ) 5</td>
<td>4.8 ( \pm ) 1.0</td>
</tr>
<tr>
<td>P for oil</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P for sex</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P for oil \x/\x sex</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (( \mu )mol/L)</td>
<td>4.2 ( \pm ) 0.5</td>
<td>1.3 ( \pm ) 0.3</td>
<td>0.18 ( \pm ) 0.05</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>162 ( \pm ) 27</td>
<td>21 ( \pm ) 3</td>
<td>0.29 ( \pm ) 0.08</td>
</tr>
<tr>
<td>P for oil</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P for sex</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P for oil \x/\x sex</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (h)</td>
<td>16 ( \pm ) 3</td>
<td>10 ( \pm ) 2</td>
<td>13 ( \pm ) 3</td>
</tr>
<tr>
<td>Corn oil</td>
<td>19 ( \pm ) 3</td>
<td>10 ( \pm ) 1</td>
<td>14 ( \pm ) 2</td>
</tr>
<tr>
<td>P for oil</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>P for sex</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P for oil \x/\x sex</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>FDR (pools/d)</td>
<td>0.57 ( \pm ) 0.01</td>
<td>2.15 ( \pm ) 0.14</td>
<td>1.38 ( \pm ) 0.09</td>
</tr>
<tr>
<td>Corn oil</td>
<td>0.45 ( \pm ) 0.01</td>
<td>1.49 ( \pm ) 0.04</td>
<td>1.19 ( \pm ) 0.02</td>
</tr>
<tr>
<td>P for oil</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P for sex</td>
<td>&lt;0.002</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P for oil \x/\x sex</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\( ^1 \) AUC, area under the curve; \( ^{21} \) \( C_{\text{max}} \), maximum concentration; \( ^{2} \) \( T_{\text{max}} \), time to reach maximal concentration; FDR, fractional disappearance rate. No \( d_\alpha \)-CEHC was detected in plasma. Repeated-measures MANOVA was performed using JMP Statistical Discovery software (SAS Institute, Cary, NC) to evaluate effects attributed to oil type and to sex. When there were significant interactions, post hoc \( t \) tests were performed.

\( ^2 \) \( \pm \) SEM (all such values).

\( ^3 \) \( P < 0.05 \) for paired comparisons of corn with sesame oil within each sex.

\( ^4 \) \( P < 0.05 \) for unpaired comparisons between men and women within each treatment group.

**Plasma tocopherols and CEHCs**

With respect to plasma \( d_\alpha \)-\( \alpha \)-tocopherol concentrations and calculated kinetic parameters, sesame oil compared with corn oil-containing muffins had no significant effects on any of these parameters (Figure 1A and Table 2). Specifically, plasma \( d_\alpha \)-\( \alpha \)-tocopherol AUC, \( C_{\text{max}} \), and \( T_{\text{max}} \) values did not differ between the treatments (Table 2). Participants' plasma unlabeled (\( d_0 \))-\( \alpha \)-tocopherol and \( d_\gamma \)-\( \gamma \)-tocopherol concentrations were unresponsive to the type of muffin consumed (data not shown).

In contrast, consumption of sesame oil compared with corn oil muffins delayed the time of the peak (\( T_{\text{max}} \)) in the plasma kinetics of concurrently ingested \( d_\gamma \)-\( \gamma \)-tocopherol in both men and women (Figure 1B and Table 2). The dietary treatments did not significantly alter any of the other parameters of \( d_\gamma \)-\( \gamma \)-tocopherol kinetics (Table 2).

Differences between plasma \( \alpha \)- and \( \gamma \)-tocopherol concentrations have been attributed in part to increased \( \gamma \)-tocopherol metabolism (13). Therefore, we sought to evaluate the efficacy with which sesame oil might alter vitamin E metabolism by measuring plasma CEHCs derived from the administered deuterated \( \alpha \)- and \( \gamma \)-tocopherols. Similar to our previous studies, no \( d_\alpha \)-CEHC was detected in plasma or urine (29), suggesting that the dose of...
**DISCUSSION**

This study was designed to test the hypothesis that humans consuming sesame lignans (sesamin and sesamolin) from unrefined sesame oil would have increased plasma \( \gamma \)-tocopherol concentrations likely as a result of inhibiting the metabolism of \( \gamma \)-tocopherol to \( \gamma \)-CEHC. These expectations are in accordance with previously published findings from human (27, 28) and rat (24, 25, 38, 39) studies that showed markedly increased plasma \( \gamma \)-tocopherol concentrations after dietary intervention with sesame seeds, sesame oil, or isolated sesame lignans. Although sesame oil consumption delayed the peak in \( d_2 \)-\( \gamma \)-tocopherol concentrations in both men and women (Table 2), none of the other \( d_2 \)-\( \gamma \)-tocopherol kinetic parameters were altered by sesame oil. However, we found that plasma \( d_2 \)-\( \gamma \)-CEHC AUCs and \( C_{\text{max}} \) were significantly lower in men after consuming sesame oil muffins compared with corn oil muffins (Figure 1C and Table 2), suggesting that sesame lignans interfered with \( d_2 \)-\( \gamma \)-tocopherol metabolism. Surprisingly, similar decreases in response to sesame oil consumption were not found for women’s plasma \( d_2 \)-\( \gamma \)-CEHC concentrations, although sesame delayed the peak in the \( d_2 \)-\( \gamma \)-CEHC concentrations in both men and women.

In conjunction with the significant decrease in the plasma \( d_2 \)-\( \gamma \)-CEHC AUCs and \( C_{\text{max}} \), a reduction in urinary \( d_2 \)-\( \gamma \)-CEHC excretion was observed in both sexes during the first 24 h of the sesame oil trial. It is likely that women’s vitamin E metabolism was affected by sesame lignans, as observed previously (27), but did not result in differences in their plasma \( d_2 \)-\( \gamma \)-CEHC concentrations. This speculation is supported by the findings that overall urinary excretion of unlabeled \( \alpha \)- and \( \gamma \)-CEHC and \( d_2 \)-\( \gamma \)-CEHC was higher in women than in men (Table 3).

The percentage of the \( d_2 \)-\( \gamma \)-tocopherol dose recovered over 72 h as urinary \( d_2 \)-\( \gamma \)-CEHC in men nearly doubled during the corn oil (2.1% ± 0.6%) intervention compared with the sesame intervention (1.2% ± 0.4%), but these differences did not reach
TABLE 3

Urinary excretion of \(d_6\)-\(\alpha\)-carboxyethyl hydrochromanol (CEHC), \(d_6\)-\(\gamma\)-CEHC and \(d_2\)-\(\gamma\)-CEHC (nmol/g creatinine) over 24 h in subjects consuming \(d_6\)-\(\alpha\)-tocophenyl acetate (TAc) and \(d_2\)-\(\gamma\)-tocophenyl acetate (TAc) together with sesame oil or corn oil muffins, respectively.\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Women ((n = 5))</th>
<th>Men ((n = 5))</th>
<th>Women ((n = 5))</th>
<th>Men ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>0.87 ± 0.15(^2)</td>
<td>0.23 ± 0.04</td>
<td>2.00 ± 0.26</td>
<td>1.00 ± 0.21</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>0.92 ± 0.44</td>
<td>0.11 ± 0.03</td>
<td>1.83 ± 0.60</td>
<td>0.59 ± 0.17</td>
</tr>
<tr>
<td>(P) for sex</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.008</td>
<td>&lt;0.008</td>
</tr>
<tr>
<td>(P) for oil × sex</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.055</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^1\) Repeated-measures MANOVA was performed using JMP Statistical Discovery software (SAS Institute, Cary, NC) to evaluate effects attributed to oil type and to sex.

\(^2\) \(x \pm \text{SEM (all such values).}\)

statistical significance; in women the overall percentage of dose excreted was 2.5% ± 0.7% and 2.3% ± 0.5% during the corn and sesame oil trials, respectively. Parker et al (26) previously demonstrated in cells in culture that the sesame lignan, sesamin, at very low concentrations (1 \(\mu\)mol/L) almost completely inhibited the formation of the side chain-truncated metabolite \(\gamma\)-CEHC from \(\gamma\)-tocopherol. They proposed the involvement of cytochrome P450 (CYP) enzymes in the initial \(\omega\)-hydroxylation of the terminal methyl group of the tocopherol side chain (18) and its inhibition by sesamin (26). These findings offer a ready explanation for the pronounced decrease in plasma \(\gamma\)-CEHCs concentrations because of sesame lignan consumption in our study. This mechanism is further supported by reports of a reduced excretion of \(\gamma\)-CEHC in the urine of rats fed sesame seeds, isolated sesame lignans (sesamin or sesamolin), or ketoconazole (21). The data presented herein, however, show in humans that \(\gamma\)-tocopherol metabolism can be inhibited by the simultaneous consumption of \(\gamma\)-tocopherol and sesame lignans. It is remarkable that this \(\gamma\)-tocopherol-sparing activity was observed after the consumption of only a single oral dose of 94 mg sesamin and 42 mg sesamolin. Repeated ingestion of sesame lignans results in an even more pronounced increase in \(\gamma\)-tocopherol concentrations, as observed by Cooney et al (28) in subjects consuming muffins prepared with ground sesame seeds on 3 consecutive days.

The potent inhibition of CYP-mediated vitamin E metabolism by sesame lignans is of particular importance with regard to clinical nutrition. Chemicals with a methylenedioxyphenyl function, such as sesamin and sesamolin, are known to form complexes with CYPs, thereby irreversibly inactivating the enzymes (40). CYPs are centrally involved in the detoxification of xenobiotics, including many pharmaceutical agents. CYP3A4, for example, metabolizes >50% of prescription drugs (41). Thus, simultaneously ingested sesame lignans may alter the bioavailability and biopotency of drugs by altering their in vivo conversion to the bioactive forms or by slowing down their elimination from the body. Furthermore, phase I enzymes, such as CYPs, are involved in the activation, as well as the elimination, of procarcinogens; thus, sesame lignans may hypothetically interfere with cancer development. In female rats, feeding a mixture of sesamin and episesamin at 0.2% (by weight) in the diet significantly reduced the formation of chemically induced mammary carcinomas (42).

Throughout the current investigation, we observed considerable differences in the handling, metabolism, and excretion of tocopherols and their water-soluble metabolites between men and women. The faster disappearance of both plasma \(d_6\)-\(\alpha\)-tocopherol and \(d_2\)-\(\gamma\)-tocopherol in women compared with men (Table 2) and increased urinary excretion of \(\gamma\)-CEHC relative to \(\alpha\)-CEHC is consistent with our previous results (29). In general, the production of \(\alpha\)-CEHC from \(\alpha\)-tocopherol is limited (17, 18). Our previous study examining the relative metabolism of \(\alpha\)-tocopherol and \(\gamma\)-tocopherol showed in normal individuals that plasma \(\alpha\)-CEHC concentrations are 1/10th of those of \(\gamma\)-CEHC (29). Additionally, Schultz et al (43) showed that \(\alpha\)-tocopherol intakes in humans had to be over 150 mg/d to detect plasma \(\alpha\)-CEHC. We therefore did not find it surprising that \(d_6\)-\(\alpha\)-CEHC was not detectable with a single dose of 50 mg/d.

These data suggest that further studies comparing vitamin E metabolism in men and women are warranted.

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