Compounds with vitamin E activity are α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. The isoforms ingested through dietary intake are mainly α- and γ-tocopherol. Dietary tocopherol is absorbed in the small intestine and transported to the liver via chylomicron (1). α-Tocopherol has a high affinity for α-tocopherol transfer protein (α-TTP) and is transported from the liver to various tissues via very low-density lipoprotein even at low concentration. However, because γ-tocopherol has low affinity for α-TTP, it is difficult to transport from the liver to other tissues (2, 3). Excess α- and γ-tocopherol are metabolized to 2,5,7,8-tetramethyl-2(2′-carboxyethyl)-6-hydroxychroman (α-CEHC) and 2,7,8-trimethyl-2(2′-carboxyethyl)-6-hydroxychroman (γ-CEHC), respectively, by ω-hydroxylation followed by β-oxidation in the liver. α- and γ-CEHC are conjugated and excreted mainly into the urine (4, 5).

Two groups have researched carboxyethyl-hydroxychroman (CEHC) excretion in Japanese adults. Morinobu et al. (6) reported that excess intake of α-tocopherol in adult males (800 mg/d α-tocopherol for 28 d) increased the excretion of α- and γ-CEHC. They also reported that, in adult males, ingesting 186 mg/d γ-tocopherol for 28 d increased the excretion of γ-CEHC and sodium (7). Imai et al. (8) showed that α-tocopherol intake correlated with α-CEHC excretion in young adults. However, there are no reports comparing α- and γ-tocopherol metabolism. In addition to dietary intake, γ-tocopherol is also commercially available as a supplement to adjust water balance. Therefore, it is important to clarify the characteristics of γ-tocopherol metabolism. In this study, we tried to clarify the metabolic characteristics of γ-tocopherol by a crossover study in which healthy Japanese women ingested α- or γ-tocopherol.

**MATERIALS AND METHODS**

**Study design.** Participants (age 22.7 ± 1.7 y old, BMI 21.4 ± 0.9) provided written informed consent before participating in this study. This study was approved by the Ethical Committee of Aichi Gakusen University and Aichi Gakusen College (approval number 130008) and was conducted in accordance with the Declaration of Helsinki.

Six healthy women took a capsule (DHC Corporation, Tokyo, Japan) containing 134 mg of either d-α-tocopherol (dT group) or d-γ-tocopherol (γT group) at 0800 h after breakfast. Peripheral blood (300 μL) was collected from the fingertips at 0, 3, 6, 9, 12, 24, 36, 48, and 72 h after ingestion of α- or γ-tocopherol. Blood at 0 h was taken just before breakfast. Urine was collected...
from 12 h before to 72 h after tocopherol ingestion. Urine collection bottles were exchanged at 0, 3, 6, 9, 12, 24, 36, 48, and 72 h, and urine volume was recorded before storage. Except for blood and urine collection, participant behavior, including eating and drinking, during the experimental period was not particularly limited. Dietary content was recorded for 72 h and nutrient intake was calculated by the Standard Tables of Food Composition in Japan 2010. The serum and urine were stored at −30°C and −80°C, respectively, until determination of tocopherol and CEHC. After a 4-wk washout period, the ingestion of capsules (α- or γ-tocopherol) was switched and the above procedures repeated.

**Measurement of tocopherol, CEHC, sodium, and potassium.** Serum concentrations of α- and γ-tocopherol

<table>
<thead>
<tr>
<th>Dietary nutrient intake</th>
<th>αT group (mg/d)</th>
<th>γT group (mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1,350±8</td>
<td>1,360±22</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>49±2</td>
<td>52±1</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>37±2</td>
<td>42±2</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>199±3</td>
<td>189±2*</td>
</tr>
<tr>
<td>α-Tocopherol (mg/d)</td>
<td>2.9±0.2</td>
<td>2.8±0.05</td>
</tr>
<tr>
<td>γ-Tocopherol (mg/d)</td>
<td>3.0±0.6</td>
<td>1.9±0.3</td>
</tr>
<tr>
<td>Sodium (mg/d)</td>
<td>1,980±59</td>
<td>2,010±128</td>
</tr>
<tr>
<td>Potassium (mg/d)</td>
<td>1,560±129</td>
<td>1,740±38</td>
</tr>
</tbody>
</table>

Values are means±SE, n=6. *Significantly different from the value of the αT group (p<0.05).
Table 2. Kinetic parameters calculated from serum concentration of α- and γ-tocopherol, urinary excretion of α- and γ-CEHC, and their apparent excretion ratio.

<table>
<thead>
<tr>
<th></th>
<th>α-Tocopherol</th>
<th>γ-Tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0–72 h (μmol/L·h)</td>
<td>800±112</td>
<td>215±27*</td>
</tr>
<tr>
<td>Cmax (μmol/L)</td>
<td>53.5±4.4</td>
<td>16.4±1.0*</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>12.0</td>
<td>8.5±1.2*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>α-CEHC</th>
<th>γ-CEHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary excretion of CEHC (μmol/72 h)¹</td>
<td>8.94±1.05</td>
<td>24.8±4.9*</td>
</tr>
<tr>
<td>Apparent excretion ratio (%)²</td>
<td>2.87±0.34</td>
<td>7.71±1.52*</td>
</tr>
</tbody>
</table>

Values are means±SE, n=6. *Significantly different from the value of α-tocopherol or α-CEHC (p<0.05).

¹ Urinary excretion of CEHC (μmol/72 h)=[CEHC level after tocopherol intake (μmol/72 h)]−[CEHC level before tocopherol intake (μmol/12 h)]×6.

² Apparent excretion ratio (%)=[Urinary excretion of CEHC (μmol/72 h)]/[tocopherol intake via capsule (μmol)×100].

AUC0–72 h, area under the curve from 0 to 72 h; Cmax, serum maximal concentration; Tmax, time to maximal concentration.

RESULTS AND DISCUSSION

Dietary nutrient intake during the experiment (Table 1) was lower than the average intake for women in their 20s in National Health and Nutrition Survey in Japan 2016. There was no difference in nutrient intake, including tocopherol and fat, between the αT and γT groups, except that the intake of carbohydrates in the γT group was slightly lower.

In the αT group, the serum concentration of α-tocopherol tended to be higher at 9 h (p=0.0906) and was significantly higher at 12 and 24 h than at 0 h (Fig. 1). In the γT group, serum concentration of γ-tocopherol tended to be higher at 3 h (p=0.0761) and was higher at 6, 9, and 12 h than at 0 h. As shown in Table 2, the Tmax of γ-tocopherol was faster than that of α-tocopherol (8.5 vs 12.0 h). These results indicate that serum γ-tocopherol concentration after γ-tocopherol intake increased faster than the serum α-tocopherol concentration after α-tocopherol intake. The AUC0–72 h and Cmax of serum γ-tocopherol were as low as 27% and 31% of those of α-tocopherol, respectively. In addition, the slope of the approximate lines of the serum concentrations of α- and γ-tocopherol after 12 to 48 h was −0.0190 for γ-tocopherol and −0.00523 for α-tocopherol.

Urinary α-CEHC level from 3 h to 12 h was 0.45% of those of α- and γ-tocopherol. These results indicate that the urinary excretion of α- and γ-CEHC in the αT group was significantly lower than in the γT group. In addition, the slope of the approximate lines of the urinary excretion of α- and γ-CEHC in the αT group was significantly lower than in the γT group.

These results indicate that serum γ-tocopherol concentration after γ-tocopherol intake increased faster than the serum α-tocopherol concentration after α-tocopherol intake. The AUC0–72 h and Cmax of serum γ-tocopherol were as low as 27% and 31% of those of α-tocopherol, respectively. In addition, the slope of the approximate lines of the serum concentrations of α- and γ-tocopherol after 12 to 48 h was −0.0190 for γ-tocopherol and −0.00523 for α-tocopherol.

Urinary α-CEHC level from 12 h to 36 h after α-tocopherol intake was higher than that before α-tocopherol intake (−12–0 h) in αT group (Fig. 1). After γ-tocopherol intake, urinary γ-CEHC level from 3 h to 12 h was significantly higher, and that from 12 to 24 h tended to be higher (p=0.0790) than that before γ-tocopherol intake in the γT group. Urinary excretion of γ-CEHC for 72 h in the γT group was higher than urinary excretion of α-CEHC in the αT group (24.8 vs 8.9 μmol) (Table 2). Apparent excretion ratio of γ-CEHC was faster than that of α-CEHC.
for γ-CEHC (Table 2). Therefore, if there is not much difference in absorption rates of α- and γ-tocopherol (13), about 2.6 times more γ-tocopherol than α-tocopherol is metabolized and excreted. These data indicate that γ-CEHC excretion was high and the excretion was fast compared with that of α-CEHC. The results of serum tocopherol concentration and urinary CEHC excretion in this study showed that ingested γ-tocopherol is metabolized faster than α-tocopherol in healthy young women.

This is the first report showing that the increase in serum γ-tocopherol and γ-CEHC excretion after γ-tocopherol intake is faster than the increase in serum α-tocopherol and α-CEHC excretion after α-tocopherol intake. Although the rapid increase in serum γ-tocopherol suggests a rapid absorption of γ-tocopherol in the intestine, the precise absorption rate of α- and γ-tocopherol and the mechanism of their absorption are incompletely understood. It was thought for a long time that tocopherol is absorbed by passive diffusion, and that tocopherol is protected from its side-chain degradation in the liver (21). The excretion of α-tocopherol in the urine is due to the side-chain degradation in the liver (21). Therefore, γ-tocopherol may be more easily metabolized to γ-CEHC than α-tocopherol is to α-CEHC.

While serum α-tocopherol concentration was not changed by γ-tocopherol intake, serum γ-tocopherol concentration was reduced by α-tocopherol intake after 9–72 h (Fig. 1). Decrease of γ-tocopherol concentration by α-tocopherol intake was also reported by Morinobu et al. (6) and Huang and Appel (22). Because α-tocopherol has high affinity with α-TTP (2), serum γ-tocopherol concentration may decrease as a result of preferential transport of α-tocopherol from the liver to other tissues. γ-CEHC was first described by Wechter et al. (23) as a natriuretic factor that inhibited the K+ channel in the apical membrane of the thick ascending limb of the kidney. Oral administration of 20 mg γ-tocopherol enhanced the urinary excretion of sodium in rats fed a high-sodium diet but not in rats fed a normal diet (24). In humans, daily intake of 186 mg/d γ-tocopherol for 28 d enhanced urinary excretion of sodium 7 d after γ-tocopherol intake (7). However, in the present study, sodium excretion did not increase with ingestion of 134 mg γ-tocopherol (Fig. 1). From the results of this study, γ-CEHC derived from γ-tocopherol was excreted into urine after about 2 d. Therefore, when investigating physiological activity as a natriuretic factor of γ-CEHC, continuous intake of γ-tocopherol may be necessary.

Tocopherol metabolism is affected by plasma lipids (25) and metabolic syndrome (26). Therefore, in this study, young adults were recruited as participants in order to eliminate the influence of sickness such as metabolic disorder. Leonard et al. (19) showed that γ-tocopherol catabolism to γ-CEHC was faster for women than men. Traber et al. (23) recently reported that the disappearance rate of plasma α-tocopherol did not differ by age. The participants in this study were healthy women with an average age of 22.7 y. It is unknown whether the results obtained in this study also apply to men or other age groups. This is a future research topic.

In conclusion, we found that γ-tocopherol was metabolized and excreted faster than α-tocopherol in healthy young women. In addition, γ-tocopherol was excreted as its metabolite about 2.6 times more than α-tocopherol. γ-Tocopherol acts as a fat-soluble antioxidant and NO radical scavenger (27–29). Plasma concentration of γ-tocopherol but not α-tocopherol is inversely correlated with the incidence of coronary heart disease (30, 31). γ-CEHC is expected as a natriuretic factor as mentioned above. Therefore, to exert these beneficial physiological effects in vivo, continuous ingestion of γ-tocopherol may be necessary.

Acknowledgments

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REFERENCES

6) Morinobu T, Yoshikawa S, Hamamura K, Tamai H.
γ-Tocopherol Is Metabolized Faster than α-Tocopherol in Young Japanese Women


