Vitamin E is a fat-soluble antioxidant that inhibits lipid peroxidation in biological membranes. In nature, compounds with vitamin E activity are α-, β-, γ- or δ-tocopherol and α-, β-, γ- or δ-tocotrienol. α- and γ-tocopherol are abundant in dietary vitamin E while tocotrienol is only present in some plant sources, such as palm oil and rice bran, while daily foods contain low levels of tocotrienol. The dietary vitamin E isoforms are absorbed in the small intestine, secreted with triacylglycerol-rich chylomicrons into the lymph and blood, and then transported to the liver (1, 2). The vitamin E isoform α-tocopherol is preferentially incorporated into VLDL and transported to tissues by lipoprotein (3, 4) because of its high affinity for α-tocopherol transfer protein (αTTP) (5). In contrast, the other vitamin E isoforms, including γ-tocopherol and tocotrienol, are catabolized and excreted. Therefore, α-tocopherol has the highest biological activity among vitamin E isoforms.

All vitamin E isoforms undergo catabolism to phytol short-chain carboxyethyl-hydroxychromans (CEHC) such as 2,5,7,8-tetramethyl-2′(2′-carboxyethyl)-6-hydroxychroman (αCEHC), a metabolite of α-tocopherol and α-tocotrienol, and 2,7,8-trimethyl-2′(2′-carboxyethyl)-6-hydroxychroman (γCEHC), a metabolite of γ-tocopherol and γ-tocotrienol (6–8). The catabolic pathway involves ω-hydroxylation of the phytol chain and subsequently β-oxidation (9, 10). The rate-limiting step in humans is ω-hydroxylation of vitamin E by cytochrome P450 (CYP) 4F2 (11, 12). CEHC is then conjugated with glucuronate in humans or with sulfate in rats and subsequently excreted into urine. Both ketoconazole and sesamin, inhibitors of CYP4F-dependent vitamin E catabolism, increase the vitamin E content in various tissues in rats (13, 14), suggesting that vitamin E catabolism is a critical determinant of tissue vitamin E content.

The liver is considered to be the most important tissue for vitamin E catabolism to CEHC. This is supported by the catabolic conversion of vitamin E in hepatic microsomes and HepG2 cells (11, 12), as well as CEHC accumulation in rat and mouse liver (15, 16). However, it was also reported by You et al. (17) that human lung epithelial A549 cells can catabolize vitamin E, suggesting that extrahepatic tissues can also catabolize vitamin E.

As described above, vitamin E absorbed via the small intestine and is secreted into the circulatory system. 

Key Words  carboxyethyl-hydroxychroman, tocopherol, tocotrienol, vitamin E
Tissue Distribution of Vitamin E Metabolites

The small intestine is secreted with triacylglycerol-rich chylomicrons into the lymph and blood. We previously reported that lipolysis of triacylglycerol-rich chylomicrons in the circulation is necessary for vitamin E transport to the liver and subsequent transport to other tissues (18). This is because treatment with Triton WR1339, an inhibitor of triacylglycerol-rich lipoprotein catabolism, completely inhibited the transport of absorbed vitamin E to the liver and caused its accumulation in the plasma of rats following vitamin E administration. This also indicates that the level of vitamin E that accumulates in the plasma is similar to the amount absorbed in vivo. In Triton WR1339-treated rats, the plasma tocotrienol concentration following tocotrienol administration was much lower than the α-tocopherol concentration following α-tocopherol administration, suggesting poor absorption of tocotrienols in these rats. Moreover, we found a significant amount of γ-CEHC in the small intestine of rats following the administration of γ-tocotrienol (14). Therefore, we hypothesized that vitamin E catabolism in the small intestine during absorption was responsible for the low circulating levels of tocotrienol.

Therefore, it is important to determine whether vitamin E undergoes catabolism in the small intestine, and whether its catabolism during absorption influences the bioavailability of dietary vitamin E. In the present study, we determined the CEHC content in various tissues, including the small intestine, in rats orally administered with vitamin E isoforms. We measured the total amounts of conjugated and unconjugated CEHC by methylation with methanolic HCl followed by HPLC analysis because the hydrolysis of sulfate-conjugated CEHC efficiently released free CEHC from rat samples (19). An analog of CEHC, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), was used as an internal standard, as previously reported (15).

MATERIALS AND METHODS

Materials. Purified RRR-α-tocopherol and RRR-γ-tocopherol were used for oral administration. A tocotrienol mixture extracted from natural sources comprised 339 mg/g α-tocotrienol, 40 mg/g β-tocotrienol, 471 mg/g γ-tocotrienol, 110 mg/g δ-tocotrienol, and 1 mg/g RRR-δ-tocopherol. α-, β-, and γ-tocopherol were undetectable in the mixture. RRR-α-Tocopherol, RRR-γ-tocopherol, the tocotrienol mixture, and CEHC were generously donated by Eisai Food & Chemical (Tokyo, Japan). Trolox and ketoconazole were purchased from Sigma-Aldrich Japan (Tokyo, Japan).

Animal study. Male Wistar rats (6 wk of age) were purchased from Japan SLC, Inc. (Shizuoka, Japan) and were maintained at 24˚C with a 12-h light cycle (lights on from 0800 to 2000 h). Before the start of the experiment, rats were fed a vitamin E-free diet (18), for 4 wk to deplete tissue stores of α-tocopherol and CEHC. This study was approved by the Laboratory Animal Care Committee of Nagoya University of Arts and Sciences, and all procedures were performed in accordance with the Animal Experimentation Guidelines of Nagoya University of Arts and Sciences.

In Expt. 1, the rats were orally administered (by gavage) with 1 mL of emulsion containing 10 mg of α-tocopherol (αT group, n=5), 10 mg of γ-tocopherol (γT group, n=6) or 29.5 mg of tocotrienol mixture (T3 group, n=6). All three emulsions contained 200 mg of sodium taurocholate, 200 mg of triolein, and 50 mg of albumin. The rats had free access to food until vitamin E administration and were deprived of food thereafter. Twenty-four hours after administration, the rats were killed by decapitation. The liver, kidney, heart, lung, adrenal grand, spleen and brain were removed and stored at −80˚C until the concentrations of CEHC and vitamin E were measured. The small intestine was divided into three equal sections. The middle section was washed three times with 10 mL of ice-cold saline using a glass syringe, and then stored at −80˚C.

In Expt. 2, the rats were orally administered (by gavage) with 1 mL of emulsion containing 10 mg of γ-tocopherol (γT group, n=6) or 10 mg of γ-tocopherol plus 10 mg of α-tocopherol (γT+αT group, n=6). Both emulsions contained 200 mg of sodium taurocholate, 200 mg of triolein, and 50 mg of albumin. The rats had free access to food until vitamin E administration and were deprived of food thereafter. Twenty-four hours after administration, the rats were killed by decapitation. For the last 12 h, urine was collected in a test tube cooled on dry ice as soon as the rats urinated on a plastic tray under each wire screen-bottomed cage. The small intestine and the other tissues were collected as described in Expt. 1.

In Expt. 3, the rats were orally administered (by gavage) with 1 mL of emulsion containing 10 mg of γ-tocopherol (γT group, n=5), or 10 mg of γ-tocopherol plus 50 mg ketoconazole/kg body weight (γT+KCZ group, n=6). Both emulsions contained 200 mg of sodium taurocholate and 200 mg of triolein. The rats were deprived of food for 12 h before oral administration of the emulsion. Six hours after administration, the rats were killed by decapitation. The small intestine and liver were collected as described in Expt. 1.

Measurement of vitamin E and metabolite concentrations. The vitamin E concentration was determined by HPLC with a fluorescence detector, as described previously (18). The total amounts of conjugated and unconjugated CEHC were determined by the method of Kiyose et al. (20) with some modifications. Briefly, 1 mL of sample solution was added to 0.5 mL of 0.5 mg/L trolox used as an internal standard, and 0.1 mL of 100 g/L ascorbic acid. The sample was methylated in 3 mol/L methanolic HCl at 60˚C for 1 h under nitrogen along with a standard solution comprising 1 mL of methanol plus 0.5 mg/L CEHC or 0.25 mg/L trolox. The methylated metabolite and trolox were then extracted with hexane. The hexane was evaporated by nitrogen, and the residue was dissolved in 200 µL of 36% (v/v) acetonitrile containing 50 mmol/L sodium perchlorate. Ten microliters of this solution was subjected to HPLC analysis.

HPLC was done using a Shimadzu LC-10AI with a Coulochem III electrochemical detector (MC Medical, Japan).
Osaka, Japan) and an ODS-3 column (250×2.1 mm, GL Science, Tokyo, Japan). The mobile phase was 36% (v/v) acetonitrile containing 50 mmol/L sodium perchlorate, pH 3.6, and the flow rate was 0.3 mL/min. For coulometric detection, the analytical and guard cells were set to +0.6 V and +0.65 V, respectively. The methylated compounds of αCEHC, γCEHC, and trolox in the standard and samples were clearly separated by HPLC (Fig. 1). There was a linear relationship between the standard concentration ratio (CEHC/trolox) and the peak area ratio (CEHC/trolox). Urinary creatinine excretion was determined using a commercial kit and was used to normalize the CEHC excretion in Expt. 2.

Statistical analysis. Data are presented as means±

![Fig. 1. Structures of αCEHC, γCEHC, trolox and their methylated compounds (A), HPLC chromatograms of methylated compounds extracted from standard and rat samples (B), and calibration curves for the standard compounds (C). The samples shown in (B) were prepared from rats 24 h after oral administration of α- and γ-tocopherol in Expt. 2. For the calibration curve in (C), 1 mL of 0.25 mg/L trolox containing 0.125, 0.25, 0.5, 1.0 or 1.5 mg/L CEHC was methylated. αCEHC-Me, methylated αCEHC; γCEHC-Me, methylated γCEHC; trolox-Me, methylated trolox.](image)
SE for 5–6 samples per group. Data were analyzed using Student’s t test for parameters with equal variance or by Welch’s test for parameters with unequal variance. In Expt. 1, data were analyzed by 1-way ANOVA followed by Tukey’s post-hoc test. Differences were regarded as significant at $p<0.05$. All data were analyzed using GraphPad Prism 5 for Windows (GraphPad Software, La Jolla, CA, USA).

**RESULTS**

In Expt. 1, at 24 h after the administration of $\alpha$-tocopherol ($\alpha$T), $\gamma$-tocopherol ($\gamma$T) or a tocotrienol mixture (T3) in Expt. 1. Values are means±SE, $n=5$ ($\alpha$T group) or 6 ($\gamma$T and T3 groups). Means not sharing a letter differ at $p<0.05$. ND, not detected; $\alpha$T3, $\alpha$-tocotrienol; $\gamma$T3, $\gamma$-tocotrienol.

over, in the kidney of the T3 group, the $\gamma$CEHC concentration was much higher than the $\gamma$-tocotrienol concentration. A small quantity of vitamin E metabolites was detected in the heart and lung, but neither $\alpha$CEHC nor $\gamma$CEHC was detected in the adrenal grand, spleen, or brain (data not shown).

In Expt. 2, the $\gamma$CEHC concentration in the small intestine was higher in the $\gamma$T+$\alpha$T group than in the $\gamma$T group (Fig. 3). In contrast, the urinary excretion of $\gamma$CEHC did not differ between the $\gamma$T+$\alpha$T and $\gamma$T groups ($p=0.11$). The $\gamma$-tocopherol concentration in the serum, heart, lung, and kidney was low in the $\gamma$T+$\alpha$T group, whereas the $\gamma$-tocopherol concentration in the liver and small intestine was not affected by simultaneous administration of $\alpha$-tocopherol. In Expt. 3, the $\gamma$CEHC concentration in the small intestine and liver of the $\gamma$T+KCZ group was much lower than that of the $\gamma$T group (Fig. 4), whereas the $\gamma$-tocopherol concentration was higher in the $\gamma$T+KCZ group than in the $\gamma$T group.

Fig. 2. Concentrations of vitamin E isoforms and their metabolites in various tissues and in serum 24 h after oral administration of $\alpha$-tocopherol ($\alpha$T), $\gamma$-tocopherol ($\gamma$T) or a tocotrienol mixture (T3) in Expt. 1. Values are means±SE, $n=5$ ($\alpha$T group) or 6 ($\gamma$T and T3 groups). Means not sharing a letter differ at $p<0.05$. ND, not detected; $\alpha$T3, $\alpha$-tocotrienol; $\gamma$T3, $\gamma$-tocotrienol.
**DISCUSSION**

It is important to determine whether vitamin E isoforms are catabolized in the small intestine because its catabolism during absorption influences the bioavailability of dietary vitamin E. In the present study, we measured the content of vitamin E metabolites in various tissues, including the small intestine, in rats following oral administration of vitamin E isoforms. We measured the total amounts of conjugated and unconjugated CEHC by methylation followed by HPLC with an electrochemical detector, using trolox as an internal standard. Freiser and Jiang (21) showed that about 80% of the vitamin E metabolites in rat plasma was CEHC, or its sulfate, and the plasma concentration of other long-chain intermediate metabolites (e.g., 9′-, 11′-, 13′-carboxychromanols) and their sulfates was extremely low. Similarly, there were no major peaks in the chromatograms of the small intestine, liver, or serum, except for the peaks corresponding to the methylated CEHC compounds and trolox (Fig. 1). We previously showed that γCEHC was present in the rat small intestine following the administration of γ-tocopherol or γ-tocotrienol (14). In addition, we found that αCEHC accumulated in the small intestine following the administration of α-tocopherol or α-tocotrienol (Fig. 2).

Dietary α-tocopherol decreases γ-tocopherol concen-
tration in various extrahepatic tissues and its excretion into bile in rats (22, 28), suggesting that \( \alpha \)-tocopherol enhances \( \gamma \)-tocopherol catabolism in the liver. Since Sontag and Parker (12) revealed that \( \alpha \)-tocopherol was a positive effector of \( \gamma \)-tocopherol \( \alpha \)-hydroxylation in rat liver microsomes, we determined the effects of \( \alpha \)-tocopherol on \( \gamma \)-tocopherol catabolism on tissue metabolite content in vivo. \( \alpha \)-Tocopherol administration decreased the \( \gamma \)-tocopherol concentration in the serum, heart, lung, and kidney (Fig. 3). We speculate that the extremely low serum \( \gamma \)-tocopherol level caused by \( \alpha \)-tocopherol administration was probably caused by the high affinity of \( \alpha \)-tocopherol to \( \alpha TTP \) in the liver. The \( \gamma \)CEHC concentration in the small intestine was increased by simultaneous administration of \( \alpha \)-tocopherol, while that in the serum and liver did not change (Fig. 3). The excretion of \( \gamma \)CEHC was not affected (\( p=0.11 \)) by \( \alpha \)-tocopherol, whereas Kiyose et al. (20) reported that \( \alpha \)-tocopherol administration increased urinary excretion of \( \gamma \)CEHC in rats. Therefore, we believe that \( \alpha \)-tocopherol enhances \( \gamma \)-tocopherol catabolism to \( \gamma \)CEHC in vivo.

Ketoconazole is an imidazole antifungal agent and is known as a potent inhibitor of some families of CYP because of the formation of a coordinate bond between its imidazole ring and CYP heme iron. We previously reported that ketoconazole increased the vitamin E isoform content in various tissues and in the serum of rats (14). In this study, ketoconazole completely inhibited \( \gamma \)-tocopherol catabolism to \( \gamma \)CEHC in the small intestine and in the liver (Fig. 4). Thus, we can conclude that CYP-dependent catabolism regulates the vitamin E concentration in the small intestine and in the liver. We previously found that sesame seed and its lignans, such as sesamin and sesaminol, inhibited vitamin E catabolism (13, 23). These data suggest that dietary sesame seed enhances vitamin E absorption in the small intestine.

After administration of \( \gamma \)-tocopherol or \( \gamma \)-tocotrienol, the \( \gamma \)CEHC concentration in the kidney was higher than that in the small intestine, despite the low level of \( \gamma \)-tocopherol or \( \gamma \)-tocotrienol in the kidney (Fig. 2). In addition, in rats treated with \( \gamma \)-tocotrienol, the metabolite concentration was higher than the \( \gamma \)-tocotrienol concentration in the kidney. The high level of \( \gamma \)CEHC in the kidney is very interesting because \( \gamma \)CEHC is an endogenous natriuretic factor that inhibits potassium channels in the thick ascending limb cells of the kidney (24). Moreover, the kidney highly expresses CYP4F2 in humans, suggesting that the kidney is capable of catalyzing vitamin E to CEHC.

Some studies have shown that A549 cells, a human lung epithelial cell line, catalyzed vitamin E isoforms to their metabolites (17, 21, 25), whereas H69AR cells, another human lung epithelial cell line, and rat primary pneumocytes did not exhibit such activity (17). The previously reported undetectable level of CYP4F mRNA in the rat lung (26, 27) and the extremely low levels of \( \alpha \)CEHC and \( \gamma \)CEHC in the lung, as reported in the present study, suggest that the lung is unable to catabolize vitamin E.

In this study, we found significant accumulation of \( \alpha \)CEHC and \( \gamma \)CEHC in the serum, liver, small intestine and kidney of rats. The metabolite concentration in the small intestine was influenced by \( \alpha \)-tocopherol and a CYP inhibitor. These data indicate that the small intestine catabolizes vitamin E isoforms via a CYP-dependent pathway. Therefore, we conclude that some dietary vitamin E is catabolized to CEHC in the small intestine and then secreted into the circulatory system. The catabolism of vitamin E isoforms in the small intestine during absorption probably influences their bioavailability.

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