Dietary Sesame Seed Decreases Urinary Excretion of α- and γ-Tocopherol Metabolites in Rats

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Summary We previously showed that dietary sesame seed and its lignan inhibited γ-tocopherol metabolism to 2,7,8-trimethyl-2(′-carboxyethyl)-6-hydroxychroman (γ-CHEC), a γ-tocopherol metabolite, and markedly elevated tissue γ-tocopherol concentration in rats. The aim of this study was to clarify the effect of dietary sesame seed on α-tocopherol metabolism. Vitamin E-deficient rats fed a vitamin E-free diet for 4 wk were fed a diet containing α-tocopherol, α- and γ-tocopherol, or α-tocopherol with sesame seed for 7 d. Urinary excretion of 2,5,7,8-tetramethyl-2(′-carboxyethyl)-6-hydroxychroman (α-CHEC), a α-tocopherol metabolite, in rats fed α-tocopherol with sesame seed was inhibited (p<0.05) as compared with that in rats fed α-tocopherol alone, or α- and γ-tocopherol. The γ-CHEC excretion was also less (p<0.05) in rats fed α-tocopherol with sesame seed than that in rats fed α- and γ-tocopherol. The inhibition of α- and γ-CHEC excretion by sesame seed was accompanied by elevation (p<0.05) of the α- and γ-tocopherol concentration in the liver. These results suggest that dietary sesame seed inhibits not only γ-tocopherol metabolism to γ-CHEC but also α-tocopherol metabolism to α-CHEC in rats.

Key Words rats, sesame seed, tocopherol, vitamin E

Vitamin E is a potent fat-soluble antioxidant that inhibits lipid peroxidation in biological membranes. In nature, compounds with vitamin E activity are α-, β-, γ- or δ-tocopherols and α-, β-, γ- or δ-tocotrienols. Dietary sesame seed or its lignans, such as sesamin and sesaminol, markedly elevate γ-tocopherol concentration in rat plasma and tissues (1). The elevation of the γ-tocopherol concentration by sesame lignan is accompanied by inhibition of urinary excretion of 2,7,8-trimethyl-2(′-carboxyethyl)-6-hydroxychroman (γ-CHEC), a γ-tocopherol metabolite (2). In addition to the animal studies, several reports showed that the dietary sesame seed or sesame oil elevated plasma (serum) γ-tocopherol concentration in humans (3–5). Frank et al. (6) recently reported that consumption of sesame oil muffins significantly decreased the urinary excretion of γ-CHEC in humans. Therefore, sesame lignan elevates the γ-tocopherol concentration in the serum and tissues by inhibiting γ-tocopherol metabolism to γ-CHEC in humans and rats.

Despite our daily intake of relatively large amount of γ-tocopherol, the α-tocopherol concentration in the serum and tissues is much higher than the γ-tocoph-

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Abbreviations: α-CHEC, 2,5,7,8-tetramethyl-2(′-carboxyethyl)-6-hydroxychroman; γ-CHEC, 2,7,8-trimethyl-2(′-carboxyethyl)-6-hydroxychroman; CYP, cytochrome P450; α-TTP, α-tocopherol transfer protein.

Materials and Methods

Materials. RRR-α- and RRR-γ-tocopherol added to the diet and used as standards, and α- and γ-CHEC used as standards were generously donated by Eisai (Tokyo, Japan).

Animals and diets. Male Wistar rats (4-wk-old) were purchased from Japan SLC (Shizuoka, Japan) and maintained at 23°C with a 12-h light cycle (lights on from...
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0800 to 2000 h. Before the start of the experiment, rats were fed a vitamin E-free diet for 4 wk to deplete tissue vitamin E stores. Then, the rats were fed a diet containing 50 mg α-tocopherol/kg (the A group, n=6), 50 mg α-tocopherol/kg and 50 mg γ-tocopherol/kg (the AG group, n=6), or 50 mg α-tocopherol/kg and 200 g sesame seed/kg (the AS group, n=7) for 7 d. The α-tocopherol content in the diets of all groups and the γ-tocopherol content in the diets of the AG and AS groups were equal, because 200 g sesame seed contained a negligible amount of α-tocopherol and 50 mg γ-tocopherol. The diet composition is shown in Table 1. For the last 12 h, the urine was collected in a test tube kept cool with dry ice as soon as the rats urinated on a plastic tray under each wire screen-bottomed cage. The rats were deprived of food during urine collection. The urine was stored at −80°C under nitrogen until used for the determination of α- and γ-CEHC concentrations. The rats were killed by decapitation between 9000 and 1200 h, and the serum, and tissues were removed and stored at −80°C. 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.

α- and γ-CEHC excretion. Both conjugated and unconjugated α- and γ-CEHC in the urine were methylated and extracted by the method of Kiyose et al. (12), and the concentration was determined using HPLC with an electrochemical detector. Urine (0.5 mL) was added to 0.1 mL of 500 g/L ascorbic acid and 1 mL of 0.54 mmol/L EDTA. The urine sample was methylated in 3 mol/L methanolic hydrochloric acid at 60°C for 1 h under nitrogen, and the methylated tocopherol metabolite was extracted with hexane. The hexane was evaporated by nitrogen, and the residue was dissolved in 100 μL of 45% (v/v) acetonitrile containing 50 mmol/L sodium perchlorate; 10 μL of this solution was subjected to HPLC. Instrumentation used for HPLC was a Shimadzu LC-10AS (Shimadzu, Kyoto, Japan) with a Coulochem III electrochemical detector (MC Medical, Osaka, Japan) and an ODS-3 column (250x2.1 mm, GL Science, Tokyo, Japan). The mobile phase was 45% (v/v) acetonitrile containing 50 mmol/L sodium perchlorate, pH 3.6, and the flow rate was 0.2 mL/min. For coloumnetic detection, the analytical and guard cells were set to +0.4 V and +0.45 V, respectively.

Tocopherol concentration. Tissues were homogenized in distilled water. The tissue homogenate (0.5 mL) was put in a test tube, and 0.5 mL of ethanol containing 60 g/L pyrogallol and 0.45 μg of 2,2,5,7,8-pentamethyl-6-chroman as an internal standard were added. Then, 0.1 mL of 600 g/L potassium hydroxide was added and saponified at 70°C for 30 min. After the addition of 2.25 mL of 20 g/L sodium chloride, tocopherol was extracted with 0.5 mL of hexane containing 10% (v/v) ethylacetate. Serum (75 μL) was put in a test tube, and 90 ng of 2,2,5,7,8-pentamethyl-6-chroman as an internal standard was added. After the addition of 0.5 mL of distilled water and 1.0 mL of ethanol, tocopherol was extracted with 5 mL of hexane.

The tocopherol concentration was determined by HPLC (13). Instrumentation used for HPLC was a Shimadzu LC-10AS (Shimadzu) with a Shimadzu RF-10AXL fluorescence detector (excitation 298 nm, emission 325 nm). The analytical column used was a Develosil NH2-5 (4.6 × 150 mm, Nomura Chemical, Aichi, Japan). The mobile phase was hexane containing 1% (v/v) isopropylalcohol, and the flow rate was 1 mL/min.

Statistical analysis. Data are presented as means±SE. n=6 or 7. They were analyzed by 2-way ANOVA with Tukey’s multiple comparison test (Prism 4 for Windows, GraphPad Software, CA, USA). Because variances among groups were unequal, all data were logarithmically transformed before analysis by 2-way ANOVA. Differences were regarded as significant at p<0.05.

Results and Discussion

Tocopherol is known to undergo metabolism to phytyl chain-shortened metabolites, such as α-CEHC and γ-CEHC, excreted into urine in human (14–16) and rats (17–19). We previously reported that sesame seed or its lignan inhibited γ-tocopherol metabolism to γ-CEHC, and elevated the γ-tocopherol concentration in rat tissues (2). The present study examined whether dietary sesame seed inhibited not only γ-tocopherol but also α-tocopherol metabolism in vivo. Despite equal amounts of α-tocopherol in all diets, urinary excretion of α-CEHC in the AS group was 41% (p<0.05) and 38% (p<0.05) those in the A and AG groups, respectively (Fig. 1). The γ-CEHC excretion in the AS group was 41% (p<0.05) that in the AG group. Thus, dietary sesame seed inhibi-
ited \(\alpha\)- and \(\gamma\)-tocopherol metabolism in rats. In addition, the \(\alpha\)-tocopherol concentration in the liver of the AS group was 161\% (\(p<0.05\)) and 166\% (\(p<0.05\)) those of the A and AG groups, respectively, and the \(\gamma\)-tocopherol concentration of the AS group was 632\% (\(p<0.05\)) that of the AG group (Fig. 2). Thus, the inhibition of \(\alpha\)- and \(\gamma\)-CEHC excretion by sesame seed was accompanied by elevation of the \(\alpha\)- and \(\gamma\)-tocopherol concentrations in the liver. The \(\alpha\)-tocopherol concentration in the kidney, lung and serum of the AS group tended to be higher than those of the A and AG groups, and the \(\gamma\)-tocopherol concentration of the AS group was 632\% (\(p<0.05\)) that of the AG group (Fig. 2). Thus, the inhibition of \(\alpha\)- and \(\gamma\)-CEHC excretion by sesame seed was accompanied by elevation of the \(\alpha\)- and \(\gamma\)-tocopherol concentrations in the liver. The \(\alpha\)-tocopherol concentration in the kidney, lung and serum of the AS group tended to be higher than those of the A and AG groups, and the \(\gamma\)-tocopherol concentration was higher (\(p<0.05\)) in those tissues and serum of the AS group than that of the AG group. These data showed that the dietary sesame seed elevates the \(\alpha\)- and \(\gamma\)-tocopherol concentrations in some tissues by inhibiting their metabolism in rats.

Dietary tocopherol is absorbed in the intestine and secreted with triacylglycerol-rich chylomicron into lymph and blood. After lipolysis of chylomicron triacylglycerol by lipoprotein lipase, tocopherol is transported to the liver (20). \(\alpha\)-TTP in the liver catalyzes \(\alpha\)-tocopherol secretion by a non-Golgi-mediated pathway, and \(\alpha\)-tocopherol is incorporated into VLDL and subsequently transported to the various tissues by lipoproteins. In contrast, most of dietary \(\gamma\)-tocopherol is metabolized to \(\gamma\)-CEHC and excreted into urine because of its affinity for \(\alpha\)-TTP is only 9\% that of \(\alpha\)-tocopherol (21). Excess amount of \(\alpha\)-tocopherol is metabolized to \(\alpha\)-CEHC and excreted into urine. As a result, the \(\alpha\)-tocopherol concentration in the serum and tissues is much higher than the \(\gamma\)-tocopherol concentration, and the urinary excretion of \(\alpha\)-CEHC was much less than that of \(\gamma\)-CEHC. In fact, in the AG group fed same contents of both tocopherols, the \(\alpha\)-tocopherol concentration in the serum and tissues was much higher than the \(\gamma\)-tocopherol concentration (Fig. 2), while the \(\alpha\)-CEHC excretion was only 9\% that of the \(\gamma\)-CEHC excretion (Fig. 1). On the other hand, the pathway of the \(\alpha\)- and \(\gamma\)-tocopherol metabolism to \(\alpha\)- and \(\gamma\)-CEHC involves \(\omega\)-hydroxylation of phytol chains and the following \(\beta\)-oxidation, and the rate-limiting step is \(\omega\)-hydroxylation by cytochrome P450 (CYP) 4F (10, 22).

Sesame seed or its lignan may not affect the tocopherol absorption in the intestine because \(\alpha\)-tocopherol absorption into the lymph was not affected by sesaminol, a sesame lignan, in rats (23). Several reports showed that \(\alpha\)-TTP or its mRNA level in rat liver was changed by some conditions (24–26) but dietary ses-

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**Fig. 1.** Urinary excretion of tocopherol metabolites in rats fed a diet containing \(\alpha\)-tocopherol alone (A), \(\alpha\)- and \(\gamma\)-tocopherol (AG), or \(\alpha\)-tocopherol and sesame seed (AS) for 7 d. The insert shows \(\alpha\)-CEHC excretion at a different scale for clarity. Values are means±SE, \(n=6\) or 7. There were effects of CEHC (\(p<0.05\)), group (\(p<0.05\)) and their interaction (\(p<0.05\)) by 2-way ANOVA. Means not sharing a letter differ, \(p<0.05\). ND, not detected.

**Fig. 2.** Tocopherol concentration in tissues and serum of rats fed a diet containing \(\alpha\)-tocopherol alone (A), \(\alpha\)- and \(\gamma\)-tocopherol (AG), or \(\alpha\)-tocopherol and sesame seed (AS) for 7 d. Values are means±SE, \(n=6\) or 7. There were effects of tocopherol (\(p<0.05\)) and group (\(p<0.05\)) for each panel by 2-way ANOVA. Means not sharing a letter differ, \(p<0.05\). ND, not detected.
Sesame seed affected neither the α-TTP level in the liver of rats determined by immunoblot analysis nor its mRNA level determined by real-time PCR (unpublished data). Whether sesame lignan affects the α-TTP activity is not known at the present time. On the other hand, sesamin clearly inhibits the tocopherol and tocotrienol metabolism to their metabolites in vitro. Sontag and Parker (10) showed that sesamin inhibited α- and γ-tocopherol metabolism to α- and γ-CEHC in human and rat liver microsomes. You et al. (27) reported that sesamin dose-dependently inhibited δ-tocotrienol metabolism in a human lung epithelial cell line. These data suggest that sesamin inhibits α-hydroxylation of tocopherol and tocotrienol, the rate-limiting step of their metabolism. Therefore, the inhibition of CYP4F-dependent α-hydroxylation of α- and γ-tocopherol by sesame lignan may be a major point of the action of sesame seed on the tocopherol metabolism.

We previously reported that sesame seed or its lignan elevated the α-tocopherol concentrations in some tissues and plasma of rats (7–9, 23). However, in this study, the α-tocopherol concentration in the extrahepatic tissues tended to be elevated by sesame seed but the differences were not significant (Fig. 2). The reason why the significant elevation of the tissue α-tocopherol concentration was not detected may be due to a short feeding period, 7 d, of rats because the period of our previous studies was 4 or 8 wk.

In conclusion, the present study showed that the dietary sesame seed inhibited the urinary excretion of not only γ-CEHC but also α-CEHC in rats fed α- and γ-tocopherol. The inhibition by sesame seed was accompanied by elevation of α- and γ-tocopherol concentrations in the liver. These data suggest that sesame seed and its lignan enhance the biological activity of tocopherol in the tissues by inhibiting its metabolism.

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