J Nutr Sci Vitaminol, **53**, 383–392, 2007

Dietary Sesame Seed and Its Lignan Increase Both Ascorbic Acid Concentration in Some Tissues and Urinary Excretion by Stimulating Biosynthesis in Rats

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(Received January 23, 2007)

Summary We previously showed that the intake of sesamin, a major lignan in sesame seed, decreased lipid peroxidation and elevated tocopherol concentration in rat tissues. In this study, we examined the effect of dietary sesame seed and sesamin on the ascorbic acid concentration in rat tissues. Rats (4-wk-old) were fed either a vitamin E-free diet, or a diet containing 50 mg γ-tocopherol/kg, one containing 2 g sesamin/kg, one containing 50 mg γ-tocopherol/kg and 2 g sesamin/kg, or one containing 200 g sesame seed/kg for 28 d. The dietary sesamin and sesame seed elevated ascorbic acid concentrations in the liver and kidney, and increased urinary excretion in those Wistar rats. The dietary sesamin also elevated the hepatic mRNA levels of cytochrome P450 (CYP) 2B, and UDP-glucuronosyltransferase (UGT) 1A and 2B. In contrast, neither the sesamin nor the sesame seed affected the liver concentration of ascorbic acid in ODS rats with a hereditary defect in ascorbic acid synthesis, though the dietary sesame seed elevated the UGT1A and 2B mRNA levels in the liver. In addition, the sesame seed elevated the γ-tocopherol concentration in the various ODS rat tissues and the ascorbic acid concentrations in the kidney, heart and lung, while reducing the thiobarbituric acid reactive substance concentration in the heart and kidney. These results suggest that dietary sesame seed and its lignan stimulate ascorbic acid synthesis as a result of the induction of UGT1A and the 2B-mediated metabolism of sesame lignan in rats. The data of ODS rat studies also suggest that dietary sesame seed enhances antioxidative activity in the tissues by elevating the levels of two antioxidative vitamins, vitamin C and E.
Key Words ascorbic acid, sesamin, sesame seed, UDP-glucuronosyltransferase, vitamin C

Dietary sesame seed or its lignan, such as sesamin and sesaminol, elevates tocopherol concentration in the serum and tissues of rats fed tocopherol (*1*–*5*). Dietary sesame lignan elevates the tocopherol concentration in tissues by inhibiting tocopherol metabolism to its metabolite because the dietary sesamin or sesaminol markedly decreases the urinary excretion of 2,7,8-trimethyl-2(2′-carboxyethyl)-6-hydroxychroman (γ-CEHC), a major metabolite of γ-tocopherol, in rats fed a diet containing γ-tocopherol (*4*). We also showed that the concentration of thiobarbituric acid reactive substance (TBARS) in the tissues and plasma (or serum) was decreased by dietary sesame seed or its lignan (*1*, *2*, *4*, *5*). It was recently reported that dietary sesame seed or sesame oil consumption reduced the urinary excretion of γ-CEHC (*6*), elevated the plasma (or serum) γ-tocopherol concentration (*7*–*9*), and decreased serum TBARS concentration (*9*) in humans. These data suggest that dietary sesame lignan elevates tocopherol concentration in the serum and tissues by inhibiting its metabolism, and decreases lipid peroxidation by inducing a high tocopherol concentration in humans and rats.

Ascorbic acid is a potent water-soluble antioxidant that prevents biological compounds from oxidation with tocopherol. In this study, we examined the effect of dietary sesamin and sesame seed on the ascorbic acid concentration in the tissues of rats. Ascorbic acid is biosynthesized from glucose in rats, although guinea pigs and primates including humans cannot synthesize it because of their lack of L-gulono-γ-lactone oxidase which catalyzes the terminal step of the ascorbic acid biosynthesis pathway (Fig. 1). It is well-known that ascorbic acid biosynthesis is stimulated in rats by some exogenous small lipophilic compounds such as drugs and xenobiotics (*10*). Metabolism of these compounds is accompanied by the formation of glucuronate, a precursor of ascorbic acid, from UDP-glucuronate. Therefore, we also examined whether dietary sesamin and sesame seed stimulated ascorbic acid biosynthesis in

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Abbreviations: γ-CEHC, 2,7,8-trimethyl-2(2′-carboxyethyl)-6-hydroxychroman; CYP, cytochrome P_{450}; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substance; UDP, uridine diphosphate; UGT, UDP-glucuronosyltransferase.

rats by determining the urinary excretion of ascorbic acid and the hepatic mRNA levels of two major drug-metabolizing enzymes, UDP-glucuronosyltransferase (UGT) and cytochrome P450 (CYP). Furthermore, determining the ascorbic acid concentration in ODS rats fed sesamin and sesame seed allowed us to clarify the effect of dietary sesame lignan on ascorbic acid biosynthesis. The ODS rat (genotype *od*/*od*) is a mutant Wistar rat with a hereditary defect in ascorbic acid biosynthesis because of its lack of L-gulono-γ-lactone oxidase (*11*) (Fig. 1).

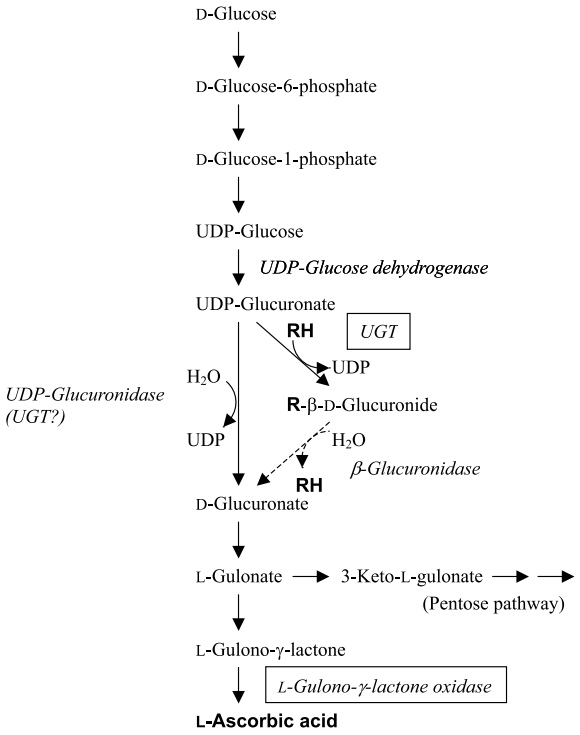

Fig. 1. Ascorbic acid synthesis pathway in rats. UDP, uridine diphosphate; UGT, UDP-glucuronosyltransferase.

MATERIALS AND METHODS

Materials. Roasted white sesame seed and sesamin extracted from sesame oil were donated by Shinsei (Aichi, Japan) and Takemoto Oil & Fat (Aichi, Japan), respectively. *RRR*-γ-Tocopherol added to diets and used as a standard for HPLC was donated by Eisai (Tokyo, Japan).

Animals and diets. Wistar and ODS rats (genotype *od*/*od*) were purchased from Japan SLC, Inc. (Shizuoka, Japan) and CLEA Japan, Inc. (Tokyo, Japan), respectively. They were maintained at 24°C with a 12-h light cycle (lights on from 0800 to 2000), and allowed free access to water and food. The rats were fed an experimental diet for 28 d and killed between 900 and 1200 h. The composition of the experimental diet is shown in Table 1. This study was approved by the Laboratory Animal Care Committee of the Nagoya University of Arts and Sciences, and all procedures were performed in accordance with the Animal Experimentation Guidelines of that institution.

Experiment 1. Wistar rats (4-wk-old) were fed a vitamin E-free diet (control group, $n=6$), and diets containing 50 mg γ-tocopherol/kg (γ-Toc group, $n=6$), a 2-g sesamin/kg diet (sesamin group, $n=6$), a 50-mg γ-tocopherol and 2-g sesamin/kg diet (γ-Toc+sesamin group, $n=6$), or a 200-g sesame seed/kg diet (sesame seed group, $n=6$). Two hundred grams of sesame seed contains 50 mg γ-tocopherol and 2 g sesame lignans. For their final 24 h, the rats were deprived of food, and urine samples were collected in 250 g/L metaphospho-

Table 1. Composition of the experimental diets.

Component	Control	γ-Toc	Sesamin	γ-Toc+sesamin	Sesame seed
			g/kg diet		
Casein[1]	200.0	200.0	200.0	200.0	160.0[2]
L-Cystine	3.0	3.0	3.0	3.0	3.0
Mineral mixture[3]	35.0	35.0	35.0	35.0	35.0
Vitamin mixture[4]	10.0	10.0	10.0	10.0	10.0
Choline	2.5	2.5	2.5	2.5	2.5
Corn oil[5]	100.0	100.0	100.0	100.0	—[2]
Cellulose powder	50.0	50.0	50.0	50.0	50.0
Sucrose	100.0	100.0	100.0	100.0	100.0
Cornstarch	499.5	499.5	497.5	497.5	439.5
Crushed sesame seed[6]	—	—	—	—	200.0[2]
Sesamin	—	—	2.0	2.0	—
			mg/kg diet		
γ-Tocopherol	—	50	—	50	—[2]
Ascorbic acid[7]	300	300	300	300	300

[1] Vitamin free-casein (Wako Pure Chemical Industries, Ltd., Osaka, Japan). [2] Sesame seed (200 g) contained 40 g protein, 100 g oil and 50 mg γ-tocopherol. [3] AIN93-MX (*12*). [4] Vitamin E-free AIN93-VX (*12*). [5] Vitamin E-stripped corn oil (Funabashi Noujou, Chiba, Japan). [6] Roasted white sesame seed (Shinsei, Aichi, Japan). [7] Ascorbic acid was added in each diet for ODS rats to prevent scurvy (*13*).

ric acid and stored at −25°C until the ascorbic acid determination. They were then killed by decapitation, and the liver, kidney, adrenal gland, spleen, thymus, jejunum, heart, lung and brain were taken and stored at −80°C until use.

Experiment 2. ODS rats (4-wk-old) were fed a vitamin E-free diet (control group, $n=4$), and diets containing 50 mg γ-tocopherol/kg (γ-Toc group, $n=4$), a 50-mg γ-tocopherol and 2-g sesamin/kg diet (γ-Toc+sesamin group, $n=4$), or a 200-g sesame seed/kg diet (sesame seed group, $n=4$). Tissues and urine were sampled and handled as described for Experiment 1.

Experiment 3. ODS rats (4-wk-old) were fed a diet containing 50 mg γ-tocopherol/kg (γ-Toc group, $n=4$) or one containing 200 g sesame seed/kg diet (sesame seed group, $n=4$). Liver was excised and stored at −80°C until use.

Vitamin E concentration. After putting a tissue homogenate (0.5 mL) in a test tube, we added 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, after which 0.1 mL of 600 g/L potassium hydroxide was then 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 was added as an internal standard. 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 (*14*). Instrumentation used for HPLC was a Shimadzu LC-10AS (Shimadzu, Kyoto, Japan) with a Shimadzu RF-10AXL fluorescence detector (excitation 298 nm, emission 325 nm). The analytical column used was a Develosil NH_2–5 (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.

Ascorbic acid concentrations. Tissue was homogenized in 50 g/L of ice-cold metaphosphoric acid and centrifuged for 15 min at 1,600×*g*. Ascorbic acid concentration in the supernatant or urine was measured by the dinitrophenylhydrazine method (*15*) with a modification by which the oxidation of ascorbic acid was accomplished with 2,6-dichlorophenol-indophenol.

mRNA levels in Experiment 1. Total RNA was extracted from liver by the method of Chomczynski and Sacchi (*16*) and subjected to Northern blot analysis. Twenty micrograms of the extracted RNA were separated by electrophoresis on 10 g/L agarose gel containing 66 g/L formaldehyde, 40 mmol/L 3-(*N*-morpholino) propanesulfonic acid buffer (pH 7.0), 10 mmol/L sodium acetate, and 1 mmol/L EDTA. RNA was denatured by heating at 55°C for 15 min in 3-(*N*-morpholino)propanesulfonic acid buffer containing 500 g/L formamide and 66 g/L formaldehyde. The electrophoresis buffer was 40 mmol/L 3-(*N*-morpholino)propanesulfonic acid (pH 7.0) containing 10 mmol/L sodium acetate and 1 mmol/L EDTA. After electrophoresis, RNA was transferred directly onto a nitrocellulose membrane (Hybond-N+, Amersham Biosciences, Tokyo, Japan) in 10×SSC (1×SSC is 150 mmol/L NaCl and 15 mmol/L sodium citrate, pH 7.0). The membrane was then baked at 80°C for 2 h.

cDNA probes were labeled with [^{32}P] dCTP using a labeling kit (Megaprime DNA Labelling System, Amersham Biosciences). Hybridization with a probe (0.9 MBq/L of ^{32}P-labeled cDNA) was carried out overnight at 42°C in a solution containing 500 g/L formamide, 5×SSC, 5×Denhardt's solution, 10 g/L SDS, 50 mmol/L sodium phosphate (pH 6.5), and 0.5 g/L denatured salmon sperm DNA. The membrane was washed twice with 2×SSC containing 1 g/L SDS for 15 min at room temperature, and twice with 0.1×SSC containing 1 g/L SDS for 15 min at 50°C. The washed membrane was subjected to autoradiography, and the radioactivities on the bands were quantified with the Bioimage Analyzer System (BAS 2000II, Fuji Photographic Film, Kanagawa, Japan). Each value was normalized to the apolipoprotein E mRNA level.

The isolation of rat UGT2B1 cDNA (*17*) was described previously (*18*). cDNA clones for rat UGT1A6 (*19*), rat L-gulono-γ-lactone oxidase (*20*) and rat apolipoprotein E (*21*) were kindly provided, respectively, by T. Iyanagi of Himeji Institute of Technology (Hyogo, Japan), M. Nishikimi of Wakayama Medical University (Wakayama, Japan), and J. M. Taylor of Gladstone Foundation Laboratories (San Francisco, CA). A cDNA clone for rat CYP1A2 (*22*) was the generous gift of K. Kawajiri of Saitama Cancer Research Institute (Saitama, Japan). A *Pst*I-*Taq*I fragment (330 bp) of a plasmid pcP-450mc-3 (*23*) was used as a probe. This fragment hybridizes with both CYP1A2 and 1A1 mRNA as separate bands in Northern blot analysis. A cDNA clone for rat CYP2B1 (*24*) was kindly provided by Y. Fujii-Kuriyama of Tohoku University (Miyagi, Japan). A *Pst*I fragment (1.6 kbp) of a plasmid pcP-450pb-4 (*24*) was used as a probe. This fragment hybridizes with both CYP2B1 and 2B2 mRNA as a single band in Northern blot analysis.

mRNA levels in Experiment 3. RNA was extracted from liver by the method of Chomczynski and Sacchi (*16*). Total RNA (5 μg) was reverse transcribed by M-MLV reverse transcriptase using an oligo dT primer. The cDNA encoding UGT1A, UGT2B, CYP1A, CYP2B or glyceraldehyde-3-phosphate dehydrogenase was amplified by real-time PCR (ABI Prism 7300 System, Applied Biosystems Japan, Tokyo, Japan) using specific primers and probes (TaqMan Gene Expression Assays, Applied Biosystems Japan). The primers and probes were as follows; UGT1A6, Rn00756113_mH; UGT2B3, Rn01790037_g1; CYP1A1, Rn00487218_m1; CYP2B15, Rn00755182_g1. The cDNA was quantified using ABI Prism 7000 Sequence Detection Systems (Applied Biosystems Japan) from the cycle number for threshold signal detection. We used the glyceraldehyde-3-phosphate dehydrogenase mRNA level quantified with TaqMan Rodent GAPDH Control Regents (Applied Biosystems Japan) to normalize the UGT and CYP mRNA levels.

TBARS concentrations. TBARS concentration in the tissue was determined by the method of Ohkawa et al. (25). Tissue was homogenized in 11.5 g/L potassium chloride. The tissue homogenate (0.3 mL) was put in a test tube to which were added 0.2 mL of 80 g/L SDS, 1.5 mL of 20% (v/v) acetic acid, and 1.5 mL of 8 g/L thiobarbituric acid. After boiling for 1 h, TBARS was extracted with 5 mL of butanol containing 62.5 g/L pyridine, and the color that developed was measured with a spectrophotometer UV-1600 (Shimadzu) at 532 nm. The TBARS concentration is presented as nmol malondialdehyde (MDA), using MDA as an external standard.

Statistical analysis. Data are presented as means± SE, $n=6$ (Experiment 1) or 4 (Experiments 2 and 3). They were analyzed by one-way ANOVA with Tukey's multiple comparison test (Prism 4 for Windows, GraphPad Software, CA, USA) in Experiments 1 and 2. When variances among groups were unequal, the data were logarithmically transformed before analysis by one-way ANOVA. In Experiment 3, the data were analyzed by Student's t test when the variances were equal. When the variances were unequal, the significance of differences was analyzed by Welch's test. Differences were regarded as significant at $p<0.05$.

RESULTS

Experiment 1

Dietary sesame seed and sesamin did not affect ($p\geq 0.05$) the food intake, growth or tissue weights of Wistar rats (data not shown). γ-Tocopherol concentration in the serum and various tissues of the control group which was not fed γ-tocopherol was either extremely low or not detected. The γ-tocopherol concentrations in the tissues and serum of the γ-Toc+sesamin and sesame seed groups were much higher ($p<0.05$) than that of the γ-Toc group (Table 2); those concentrations in the tissues (except liver) and serum of the sesame seed group were higher ($p<0.05$) than those of the γ-Toc+sesamin group, and in the tissues and serum of the sesamin group were extremely low because that group was fed a diet without γ-tocopherol.

Ascorbic acid concentrations in the liver and kidney of the sesamin, γ-Toc+sesamin, and sesame seed groups were higher ($p<0.05$) than those of the control and γ-Toc groups (Fig. 2). Those concentrations in the lung of the sesamin, γ-Toc+sesamin, and sesame seed groups tended to be higher than those of the control and γ-Toc groups. Neither sesamin nor sesame seed intake affected ($p\geq 0.05$) the ascorbic acid concentration in the adrenal gland, spleen, jejunum, heart, thymus, or brain (data not shown). The concentrations in the serum of the sesamin and γ-Toc+sesamin groups were higher ($p<0.05$) than those of the control and γ-Toc groups, respectively. The urinary excretion of ascorbic acid of the sesamin, γ-Toc+sesamin, and sesame seed groups was increased ($p<0.05$).

UGT1A6 and 2B1 mRNA levels in the liver of the sesamin, γ-Toc+sesamin, and sesame seed groups were higher ($p<0.05$) than that of the control group (Fig. 3). The UGT2B1 mRNA level of the sesamin group was 353% ($p<0.05$) that of the control group, and that of the γ-Toc+sesamin group was 315% ($p<0.05$) that of the γ-Toc group. The CYP2B1/2 mRNA level was also higher ($p<0.05$) in the sesamin and γ-Toc+sesamin groups than that in the control and γ-Toc groups. In contrast, dietary sesamin had no effect ($p\geq 0.05$) on the CYP1A1, 1A2 or L-gulono-γ-lactone oxidase mRNA levels.

Experiment 2

Dietary sesame seed and sesamin did not affect ($p\geq 0.05$) the food intake, growth or tissue weight of ODS rats (data not shown). No signs of scurvy were observed in any ODS rats during the experiment. The γ-tocoph-

Table 2. γ-Tocopherol (γ-Toc) concentrations in tissues and serum of Wistar (Experiment 1) and ODS (Experiment 2) rats fed a vitamin E-free diet (Control), a diet containing γ-Toc, a diet containing sesamin alone (Sesamin), or with γ-tocopherol (γ-Toc+sesamin), or a diet containing sesame seed, for 28 d.

	Experiment 1				Experiment 2			
	γ-Toc	Sesamin	γ-Toc+Sesamin	Sesame seed	Control	γ-Toc	γ-Toc+Sesamin	Sesame seed
				nmol/g				
Liver	1.61±0.17[a]	0.86±0.07[a]	18.26±2.98[b]	23.09±2.42[b]	0.38±0.22[a]	5.81±0.31[b]	22.06±0.94[c]	54.55±1.68[d]
Kidney	1.70±0.38[a]	0.46±0.05[a]	17.54±1.20[b]	28.25±3.12[c]	0.48±0.19[a]	5.33±0.72[a]	21.43±2.95[b]	72.26±5.90[c]
Adrenal gland	10.51±0.62[a]	nd	101.21±9.31[b]	165.70±7.82[c]	5.64±1.85[a]	88.30±6.48[a]	303.22±54.62[b]	511.66±46.82[c]
Spleen	3.50±0.17[a]	1.44±0.10[a]	21.38±1.20[b]	45.41±3.43[c]	0.79±0.34[a]	16.22±0.94[b]	65.40±3.98[c]	127.10±5.40[d]
Jejunum	2.64±0.34[a]	0.67±0.02[a]	13.06±1.42[b]	23.02±1.46[c]	0.24±0.06[a]	9.67±0.74[b]	30.86±2.76[c]	75.10±5.11[d]
Heart	6.48±0.14[a]	2.16±0.19[a]	44.69±1.58[b]	89.52±5.98[c]	1.73±0.48[a]	13.37±0.86[b]	46.70±6.53[c]	137.02±3.38[d]
Lung	4.18±0.43[a]	1.68±0.17[a]	40.92±4.61[b]	70.73±3.41[c]	0.82±0.19[a]	12.96±1.46[a]	55.34±7.87[b]	137.76±13.06[c]
Thymus	1.44±0.02[a]	0.46±0.05[a]	10.70±0.53[b]	18.34±0.70[c]	0.53±0.19[a]	7.97±2.40[a]	34.37±11.64[b]	113.62±26.90[c]
Brain	0.38±0.07[a]	0.29±0.05[a]	4.66±1.42[b]	24.98±0.67[c]	0.14±0.10[a]	1.18±0.24[b]	4.80±0.62[c]	34.44±0.19[d]
				μmol/L				
Serum	0.17±0.02[a]	0.14±0.07[a]	1.58±0.50[b]	5.76±0.84[c]	0.43±0.14[a]	2.81±0.24[b]	11.52±0.65[c]	22.39±1.06[d]

Values are means±SE, $n=6$ (Experiment 1) and 4 (Experiment 2). Values in a row in each experiment with different letters differ, $p<0.05$. nd, not determined.

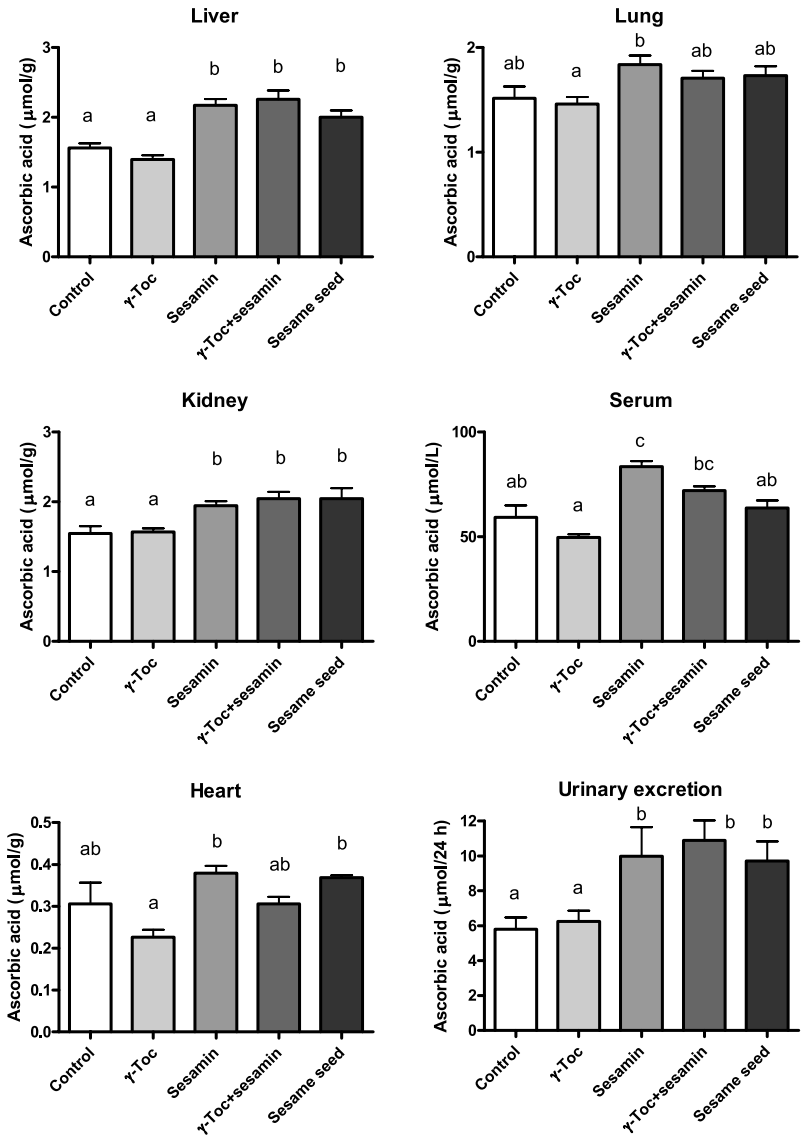

Fig. 2. Ascorbic acid concentration in tissues and serum, and its urinary excretion in Wistar rats fed a vitamin E-free diet (control), or diets containing γ-tocopherol (γ-Toc), sesamin, γ-tocopherol with sesamin (γ-Toc+sesamin), or sesame seed for 28 d (Experiment 1). Values are means+SE, $n=6$. Means not sharing a letter differ, $p<0.05$.

erol concentrations in the tissues and serum of the sesame seed and γ-Toc+sesamin groups were much higher ($p<0.05$) than those of the control and γ-Toc groups, and the concentration in the sesame seed group was higher ($p<0.05$) than that of the γ-Toc+sesamin group (Table 2).

Neither the ascorbic acid concentration in the tissues nor its urinary excretion was affected ($p\geq0.05$) by dietary sesamin, although the ascorbic acid concentrations in the kidney, heart and lung of the sesame seed group were higher ($p<0.05$) than that of the γ-Toc group (Fig. 4).

The TBARS concentration in the kidney of the sesame seed group was lower ($p<0.05$) than that of the control and γ-Toc groups (Fig. 5), and was also lower in the heart of the γ-Toc+sesamin and sesame seed groups ($p<0.05$) than that of the control or γ-Toc group. However, neither dietary sesamin nor sesame seed lowered ($p\geq0.05$) the TBARS concentration in the liver, spleen, lung, brain or serum (data not shown).

Experiment 3

The UGT1A6, 2B3 and CYP2B15 mRNA levels in the liver of the sesame seed group of ODS rats were higher ($p<0.05$) than those of the γ-Toc group (Fig. 6). The CYP1A1 mRNA level also tended to be elevated ($p=0.083$) by dietary sesame seed.

DISCUSSION

We have studied the effect of dietary sesame lignan on the tocopherol metabolism in rats (*1*–5). Ascorbic acid is a potent water-soluble antioxidant that has a protective effect against some oxidative stress associated with tocopherol. In this study, the effect of dietary sesamin and sesame seed on the ascorbic acid metabolism in rats was examined and was found to elevate the ascorbic acid concentration in some tissues and to increase its urinary excretion in Wistar rats (Fig. 2). Dietary sesamin and sesame seed also elevated the γ-

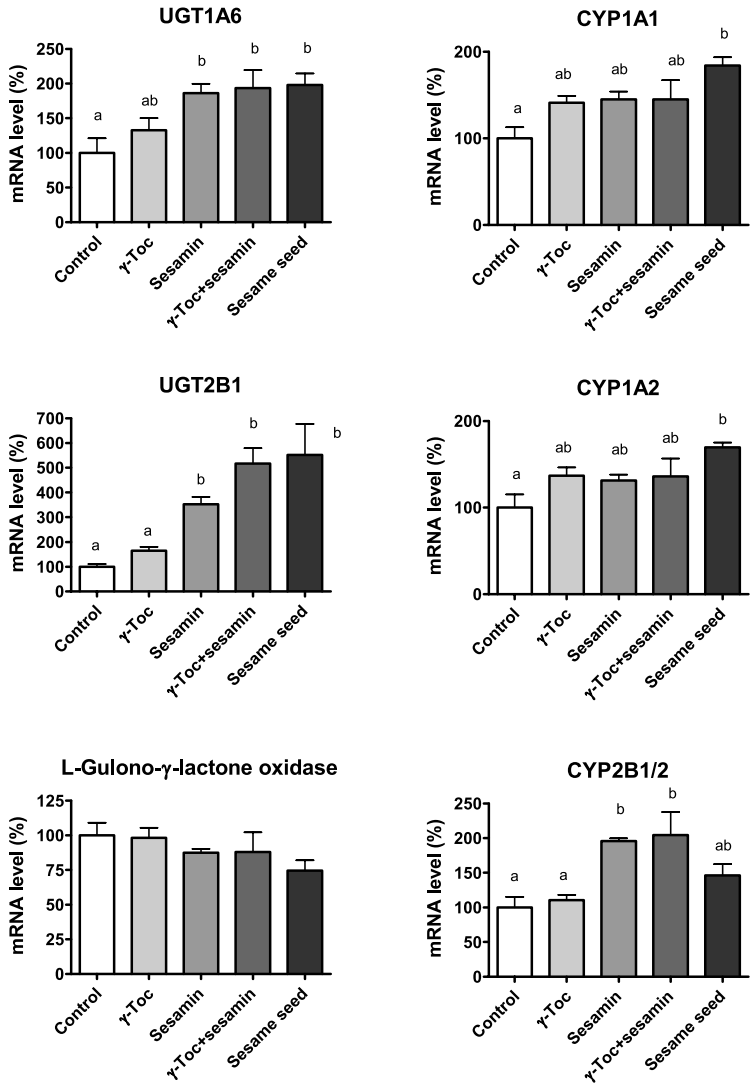

Fig. 3. UDP-glucuronosyltransferase (UGT), cytochrome P450 (CYP), and L-gulono-γ-lactone oxidase mRNA levels in liver of Wistar rats fed a vitamin E-free diet (control), or diets containing γ-tocopherol (γ-Toc), sesamin, γ-tocopherol with sesamin (γ-Toc+sesamin), or sesame seed for 28 d (Experiment 1). Values are means+SE, $n=6$, and are presented as a percentage of the mean of each control group. Means not sharing a letter differ, $p<0.05$. UDP, uridine diphosphate.

tocopherol concentrations in various tissues of rats fed γ-tocopherol (Table 2), as reported previously (*1*, *4*). Thus, sesame lignan elevates the tissue levels of two major antioxidants, vitamin C and E, in rats. In addition to their effect on vitamin levels, it was recently reported that some sesamin metabolites containing catechol moieties exhibited antioxidative activity in vitro (*26*). These results suggest that sesamin has an antioxidative effect on rat tissues by elevating the levels of antioxidative vitamins and sesamin metabolites, although sesamin in itself does not possess antioxidative properties.

Sesamin in liver is metabolized to mono- and di-catechol metabolites that are conjugated with glucuronate and excreted in bile (*26*, *27*). UGT transfers glucuronate to numerous substrates such as steroids, bile acids, bilirubin, hormones, dietary constituents, and xenobiotics (*28*, *29*). Several catechols were glucuronidated by rat recombinant UGT1A and 2B (*30*). We determined the effect of dietary sesamin and sesame seed on the UGT mRNA level in this study because UGT activity is regulated by its gene expression after treatment with some xenobiotics (*17–19*). As shown in Fig. 3, dietary sesamin and sesame seed elevated the UGT1A and 2B mRNA levels in the liver, while dietary sesamin alone also elevated the CYP2B mRNA level. Tsuruoka et al. (*31*), using DNA microarray analysis, also reported that CYP2B1 and 2B2 gene expression was up-regulated by an oral administration of sesamin in rats. These results suggest that sesamin metabolism is mediated by CYP2B, UGT1A and 2B.

Ascorbic acid is synthesized from glucose in rats (Fig. 1) (*32*, *33*), that synthesis is stimulated by some xenobiotics such as phenobarbital, 3-methylcholanthrene, aminopyrine, PCB or DDT, which is conjugated by glucuronate and excreted as a glucuronide (*18*, *34*). Administration of these xenobiotics increases liver ascorbic acid concentration and its urinary excretion in rats. The formation of D-glucuronate from UDP-glucose is rate-limiting in ascorbic acid synthesis because the activities of UDP-glucose dehydrogenase, which cata-

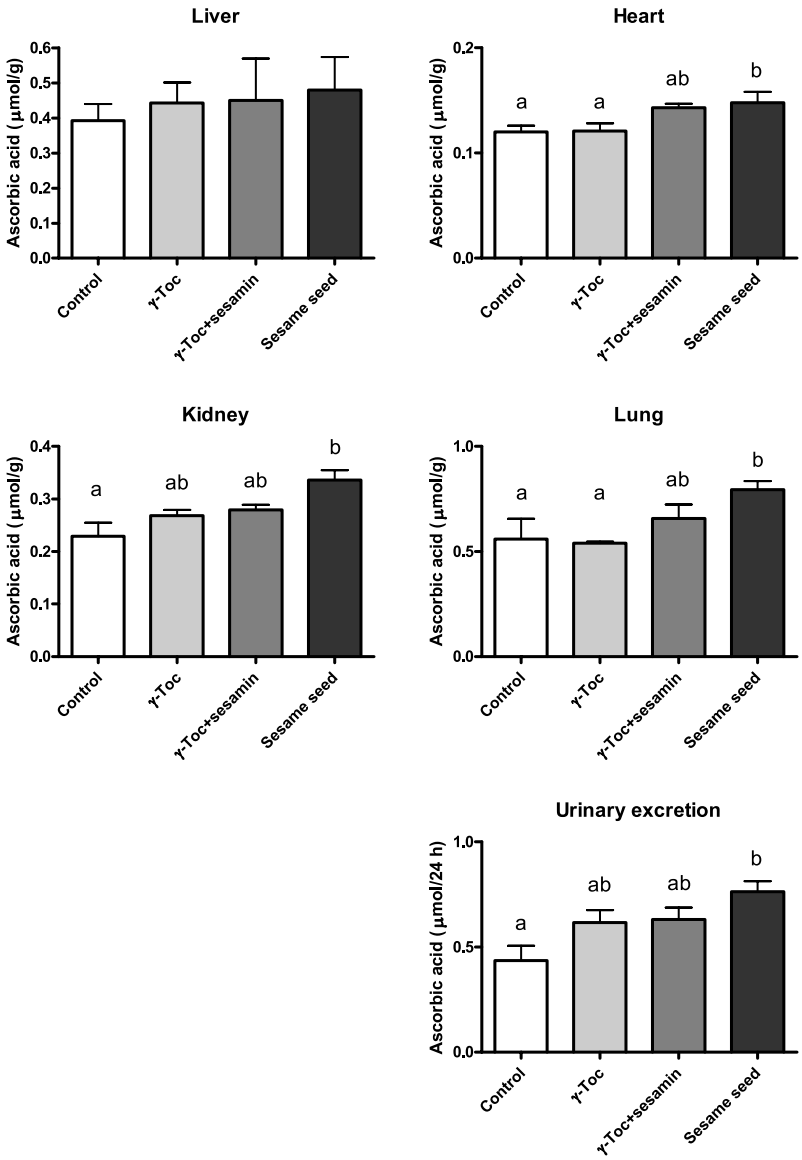

Fig. 4. Ascorbic acid concentration in tissues and its urinary excretion in ODS rats fed a vitamin E-free diet (control), or diets containing γ-tocopherol (γ-Toc), γ-tocopherol with sesamin (γ-Toc+sesamin), or sesame seed for 28 d (Experiment 2). Values are means+SE, $n=4$. Means not sharing a letter differ, $p<0.05$.

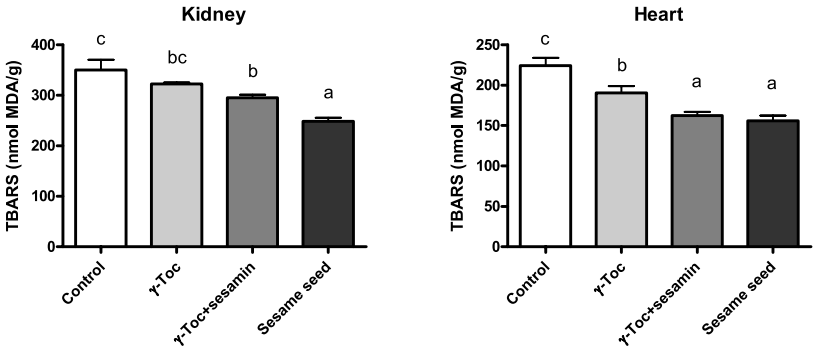

Fig. 5. Concentration of thiobarbituric acid reactive substance (TBARS) in kidney and heart of ODS rats fed a vitamin E-free diet (control), or diets containing γ-tocopherol (γ-Toc), γ-tocopherol with sesamin (γ-Toc+sesamin), or sesame seed for 28 d (Experiment 2). Values are means+SE, $n=4$. Means not sharing a letter differ, $p<0.05$. MDA, malondialdehyde.

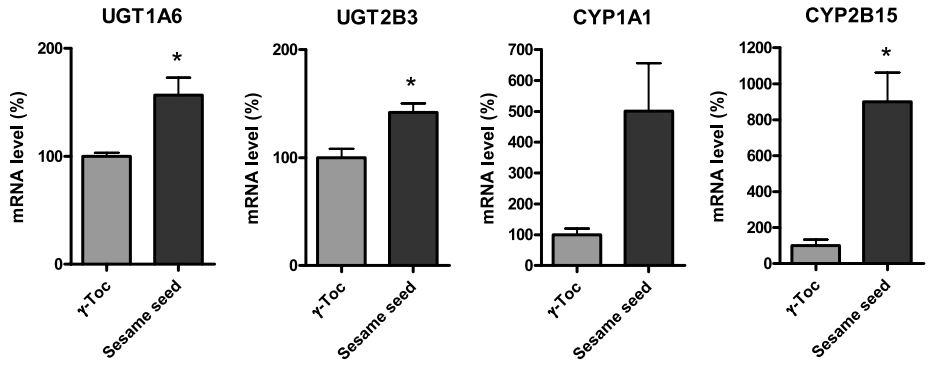

Fig. 6. UDP-glucuronosyltransferase (UGT) and cytochrome P450 (CYP) mRNA levels in liver of ODS rats fed a diet of either γ-tocopherol (γ-Toc) or sesame seed for 28 d (Experiment 3). Values are means+SE, $n=4$, and are presented as a percentage of the mean of the γ-Toc group. * Significantly different ($p<0.05$) from the γ-Toc group by t test. UDP, uridine diphosphate.

lyzes UDP-glucuronate formation from UDP-glucose, and UGT but not L-gulono-γ-lactone oxidase, are elevated by some xenobiotics such as PCB and DDT in rats (*34*, *35*). Because β-glucuronidase activity is elevated by PCB, DDT, chloretone or aminopyrine (*34*), some xenobiotics may stimulate ascorbic acid synthesis by the induction of a UGT/β-glucuronidase-mediated pathway. Administration of 3-methylcholanthrene metabolized by UGT1A increases the hepatic ascorbic acid concentration and its urinary excretion in Wistar rats but does not increase them in Gunn rats (genotype *j*/*j*) (*18*), a mutant strain of Wistar rat deficient in UGT1A activity because of a frameshift mutation of UGT1A gene (*36*), while the liver ascorbic acid concentration and its urinary excretion are markedly increased by phenobarbital metabolized by UGT2B in Gunn rats (*18*). These data also demonstrate the importance of UGT for the stimulation of ascorbic acid synthesis. Furthermore, Linster and Van Schaftingen (*37*, *38*) recently reported the direct formation of D-glucuronate from UDP-glucuronate by non-glucuronidable xenobiotics in rat hepatocytes and cell-free systems, suggesting that the UDP-glucuronidase activity is a side activity of UGT. Some UGT isoforms catalyze not only the transfer of a glucuronosyl group to an acceptor but also the hydrolysis of the glycosidic linkage in UDP-glucuronate, although the UDP-glucuronidase activity is much lower than the transfer activity (*38*). These data suggest that, when elevated by some xenobiotics, UGT activity directly stimulates ascorbic acid synthesis.

In Wistar rats in Experiment 1, dietary sesamin and sesame seed elevated both the hepatic UGT1A and 2B mRNA levels and the ascorbic acid concentration in the liver, enhancing its urinary excretion in the latter. The hepatic L-gulono-γ-lactone oxidase mRNA level was not changed by dietary sesamin and sesame seed as shown in rats administered some xenobiotics (*39*). Moreover, the intake of neither sesamin nor sesame seed affected the hepatic ascorbic acid concentration in ODS rats in Experiment 2, though the hepatic UGT1A and 2B mRNA levels were elevated by dietary sesame seed in Experiment 3. These results suggest that the increases in the hepatic ascorbic acid concentration and its urinary excretion by dietary sesame seed in Wistar rats are due to the stimulation of ascorbic acid biosynthesis by the induction of a UGT1A/2B-mediated metabolism of sesame lignan or its metabolite. Sugano et al. (*40–42*) have shown that dietary sesamin stimulates the expression of several genes related to fatty acid metabolism, and have suggested that sesamin is a ligand of peroxisome proliferator-activated receptor α, which induces hepatic UGT2B gene expression (*43*). Therefore, dietary sesamin may stimulate UGT2B gene expression by activating peroxisome proliferator-activated receptor α.

Ascorbic acid concentration in the heart and lung of ODS rats fed sesame seed was higher than that in rats fed γ-tocopherol, although its concentration in the liver did not differ (Fig. 4). These data confirmed that the elevation of ascorbic acid concentrations by dietary sesame seed in some extrahepatic tissues such as kidney, heart and lung was not only due to the stimulation of ascorbic acid synthesis. The dietary sesame seed lowered the TBARS concentration in the kidney and heart of ODS rats (Fig. 5) and markedly elevated the γ-tocopherol concentration in several tissues (Table 2). Therefore, dietary sesame seed may arrest ascorbic acid degradation by elevating the tocopherol concentration in the tissues. Sesame lignan metabolites may contribute to the antioxidative activity of sesame seed in vivo.

In conclusion, the present study demonstrated that dietary sesamin and sesame seed elevated the ascorbic acid concentration in some rat tissues, and that ascorbic acid biosynthesis may be stimulated as a result of the induction of a UGT1A/2B-mediated metabolism of sesame lignan. Dietary sesame seed enhances the antioxidative activity in tissues by elevating vitamin C and E levels.

Acknowledgments

This work was supported in part by a Grant-in-Aid (No. 15680018) for Scientific Research from the Japan Society for the Promotion of Science. The authors are grateful to Kaori Kawai (Nagoya University) for her technical assistance.

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