Dietary Sesame Lignans Decrease Lipid Peroxidation in Rats Fed Docosahexaenoic Acid

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Summary We have previously reported that dietary sesamin and sesaminol, major lignans of sesame seed, elevate the α -tocopherol concentration and decrease the thiobarbituric acid reactive substance (TBARS) concentration in the plasma and liver of rats. In this study, the effects of dietary sesamin and sesaminol on the lipid peroxidation in the plasma and tissues of rats fed docosahexaenoic acid (DHA, 22:6 n-3) were examined. Male Wistar rats (4-wk-old) were divided into the following six experimental groups: control group, fed a basal diet; sesamin group, fed a diet with sesamin (2 g/kg); sesaminol group, fed a diet with sesaminol (2 g/kg); DHA group, fed a diet containing DHA (5 g/kg); DHA+sesamin group, fed a diet containing DHA with sesamin; and DHA+sesaminol group, fed a diet containing DHA with sesaminol. Each diet contained either 0.01 or 0.05 g D- α -tocopherol/kg, and the rats were fed the respective experimental diet for 5 wk. The dietary DHA elevated the TBARS concentration and also increased the red blood-cell hemolysis induced by the dialuric acid. The dietary sesamin and sesaminol lowered the TBARS concentrations and decreased the red blood hemolysis. The dietary sesamin and sesaminol elevated the α -tocopherol concentrations in the plasma, liver, and brain of the rats fed a diet with or without DHA. These results suggest that dietary sesame lignans decrease lipid peroxidation as a result of elevating the α -tocopherol concentration in rats fed DHA.

Key Words docosahexaenoic acid, lipid peroxidation, sesamin, sesaminol, α -tocopherol

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. The chemical properties of these vitamin E isoforms include antioxidative activities. We have previously shown that dietary sesame seed or its lignan, such as sesamin and sesaminol, elevates the tocopherol concentrations in rats fed tocopherol (1–6), and the tocotrienol concentrations in rats fed the tocotrienol-rich fraction extracted from palm oil (7–9). Lemcke-Norjrvi et al. (10) recently reported that dietary sesame oil elevated the γ -tocopherol concentration in the serum of Swedish women. Thus, sesame lignan elevates the vitamin E

concentration in humans and rats. We have also shown that the thiobarbituric acid-reactive substance (TBARS) concentrations in the tissues and plasma (or serum) of rats are decreased by dietary sesame seed or its lignan (1, 2, 4–6). Therefore, dietary sesame seed may decrease lipid peroxidation through its effect on vitamin E activity.

Docosahexaenoic acid (DHA, 22:3 n-6), a major polyunsaturated fatty acid in fish oil, is susceptible to peroxidation because of its high degree of unsaturation. DHA or DHA-rich fish oil intake elevates the TBARS concentrations in humans (11–14), rats, (15, 16) and mice (17, 18). Some reports have shown that DHA-rich fish oil intake lowers the α -tocopherol concentration in humans (11), rats (15, 16), and mice (18, 19). These reports suggest that α -tocopherol acts as a potent antioxidant against the lipid peroxidation induced by DHA. In this study, we determined whether or not dietary sesame lignan decreases lipid peroxidation in rats fed DHA.

MATERIALS AND METHODS

Materials. Sesamin and sesaminol were kindly provided by Takemoto Oil & Fat (Aichi, Japan). D- α -Tocopherol was kindly donated by Eisai (Tokyo, Japan). DHA was kindly provided by Suntory (Osaka, Japan).

Animals and diets. Male Wistar rats (3-wk-old) were purchased from Japan SLC (Shizuoka, Japan). They

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Abbreviations: CYP, cytochrome P₄₅₀; DHA, docosahexaenoic acid; HPLC, high-performance liquid chromatography; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substance; α -TTP, α -tocopherol transfer protein.

Table 1. Compositions of the experimental diets.

	Toc 0.01						Toc 0.05					
	Con- trol	Sesa- min	Sesa- minol	DHA	DHA+ sesamin	DHA+ sesaminol	Con- trol	Sesa- min	Sesa- minol	DHA	DHA+ sesamin	DHA+ sesaminol
	(g/kg diet)											
Casein	200	200	200	200	200	200	200	200	200	200	200	200
DL-Methionine	3	3	3	3	3	3	3	3	3	3	3	3
Starch	150	150	150	150	150	150	150	150	150	150	150	150
Sucrose	500	498	498	495	493	493	500	498	498	495	493	493
Cellulose powder	50	50	50	50	50	50	50	50	50	50	50	50
Lard	50	50	50	50	50	50	50	50	50	50	50	50
Mineral mixture ¹	35	35	35	35	35	35	35	35	35	35	35	35
Vitamin mixture ²	10	10	10	10	10	10	10	10	10	10	10	10
Choline bitartrate	2	2	2	2	2	2	2	2	2	2	2	2
D-α-Tocopherol	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.05	0.05	0.05	0.05	0.05
DHA				5	5	5				5	5	5
Sesamin		2			2			2			2	
Sesaminol			2			2			2			2

¹ AIN-76 (20), ² vitamin E-free AIN-76A (21). The rats were fed a diet with either 0.01 g/kg (Toc 0.01) or 0.05 g/kg (Toc 0.05) of α-tocopherol. DHA, docosahexaenoic acid.

were maintained at 24.5°C with a 12-h light cycle (lights on from 0800 to 2000 h) and allowed free access to water and food. The rats were fed a commercial diet (CE-2, Japan Clea, Tokyo, Japan) for 7 d before the start of the experiment and then fed the experimental diet for 5 wk. The composition of the experimental diet is shown in Table 1. The rats were sacrificed between 1000 and 1200 h, and all procedures were performed in accordance with the Animal Experimentation Guidelines of Nagoya University.

The rats were fed a basal diet (control group, *n*=6), a diet containing 2 g sesamin/kg (sesamin group, *n*=6), a diet containing 2 g sesaminol/kg (sesaminol group, *n*=6), a diet containing 5 g DHA/kg (DHA group, *n*=6), a diet containing 2 g sesamin and 5 g DHA/kg (sesamin+DHA group, *n*=6), or a diet containing 2 g sesaminol and 5 g DHA/kg (sesaminol+DHA group, *n*=6). Each diet contained either 0.01 or 0.05 g D-α-tocopherol/kg. The rats were fed the respective experimental diet for 5 wk. After 24 h of food deprivation, the rats were anesthetized with sodium pentobarbital and blood was drawn from the heart using a heparinized needle and syringe. The liver and brain were taken and stored at -80°C until use for the determination of TBARS and α-tocopherol concentrations.

Determination of TBARS concentrations. TBARS concentrations in the tissue and plasma were determined by the methods of Ohkawa et al. (22) and Yagi (23), respectively. Liver or brain (0.5 g each) was homogenized in 4.5 mL of 11.5 g/L potassium chloride. The tissue homogenate (0.3 mL) was put in a test tube, and 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 were added. After boiling for 1 h, TBARS was extracted with 5 mL of

butanol containing 62.5 g/L pyridine and the color developed was measured with a UV-1600 spectrophotometer (Shimadzu, Kyoto, Japan) at 532 nm. The TBARS concentration is presented as nmol malondialdehyde (MDA), using MDA as the external standard.

Determination of red blood cell hemolysis. Oxidative hemolysis of red blood cells was measured using dialuric acid (24). Heparinized blood was centrifuged at 500×*g* for 5 min, and blood cells were washed with 9 g/L sodium chloride three times. Then, the blood cells diluted with 9 g/L sodium chloride were incubated with dialuric acid at 37°C for 45 min and centrifuged at 500×*g* for 5 min. The color of the supernatant was measured with a UV-1600 spectrophotometer (Shimadzu) at 540 nm.

Determination of plasma pyruvate kinase activity. Plasma pyruvate kinase activity was determined by the method of Gutmann and Bernt (25) using a system coupled with lactate dehydrogenase and NADH. Plasma was added to 0.1 mol/L triethanolamine (pH 7.5) containing 0.15 mmol/L NADH, 1 mmol/L phosphoenolpyruvic acid, 4 mg/L lactate dehydrogenase, and 3 mmol/L ADP. The reduction of NADH was measured spectrophotometrically at 340 nm for 5 min at 37°C using the molar absorption coefficient for NADH. A unit of pyruvate kinase activity was equivalent to the activity needed to catalyze the reaction of 1 μmol of ADP to ATP for 1 min.

Determination of α-tocopherol concentration. Liver or brain (0.5 g each) was homogenized in 4.5 mL of distilled water. The liver or brain 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 the internal standard were added.

Fig. 1. Effects of dietary sesamin and sesaminol on thiobarbituric acid reactive substance (TBARS) concentrations in the plasma, liver, and brain of rats fed diets with or without docosahexaenoic acid (DHA). The rats were fed a basal diet (Control), a diet with sesamin (Sesamin), a diet with sesaminol (Sesaminol), a diet containing DHA (DHA), a diet containing DHA with sesamin (DHA+sesamin), or a diet containing DHA with sesaminol (DHA+sesaminol), for 5 wk. Each diet contained either 0.01 g/kg (A) or 0.05 g/kg (B) of α -tocopherol. Values are means $\pm$ SE, $n=6$. Means not sharing a letter are different, $p < 0.05$. MDA, malondialdehyde.

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. Plasma (75 μ L) was put in a test tube, and 90 ng of 2,2,5,7,8-pentamethyl-6-chroman as the 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 α -tocopherol concentration was determined by HPLC (26). Instrumentation used for HPLC was a LC-10AS (Shimadzu) with a RF-10AXL fluorescence detector (Shimadzu) at excitation 298 nm and emission 325 nm. The analytical column used was a Develosil NH₂-5 (4.6 $\times$ 150 mm, Nomura Chemical, Aichi, Japan). The mobile phase was hexane containing 1% (v/v) isopropylalcohol, and the flow rate was 1 mL/min.

Determination of docosahexaenoic acid content. Total lipids were extracted from the plasma and liver by the method of Bligh and Dyer (27). Triacylglycerol and phospholipids were separated by Sep-Pak Plus Silica Cartridges (Nihon Waters, Tokyo, Japan) and methylated in 3 mol/L methanolic hydrochloric acid at 50°C for 3 h. The fatty acid methyl esters were extracted with hexane and measured by gas-liquid chromatography using a GC-12APF with a flame ionization detector (Shimadzu). The analytical glass column was an Advance-DS 5% Chromosorb W80/100 glass column (3.2 mm $\times$ 2.1 m, Shinwa Chemical Industries, Kyoto, Japan). The column and injector temperatures were 190°C and 240°C, respectively.

Statistical analysis. Data are presented as means $\pm$ SE, $n=6$. They were analyzed by one-way ANOVA with Fisher's post-hoc test. Differences with a p -value < 0.05 were regarded as significant.

RESULTS

Dietary DHA or sesame lignan did not affect the food intake or growth of rats during the experimental period

Fig. 2. Effects of dietary sesamin and sesaminol on red blood-cell hemolysis and plasma pyruvate kinase activity of rats fed diets with or without docosahexaenoic acid (DHA). The rats were fed a basal diet (Control), a diet with sesamin (Sesamin), a diet with sesaminol (Sesaminol), a diet containing DHA (DHA), a diet containing DHA with sesamin (DHA+sesamin), or a diet containing DHA with sesaminol (DHA+sesaminol), for 5 wk. The diet contained 0.01 g α -tocopherol/kg. The hemolysis was determined using dialuric acid. Values are means $\pm$ SE, $n=6$. Means not sharing a letter are different, $p < 0.05$. U, a unit which catalyzes the reaction of 1 μ mol of ADP to ATP for 1 min.

(data not shown).

In the rats fed a diet with 0.01 g α -tocopherol/kg (Fig. 1A), the TBARS concentration in the liver and brain of the DHA group was higher ($p < 0.05$) than that of the control group. The dietary sesamin and sesaminol lowered ($p < 0.05$) the TBARS concentration in the plasma, liver, and brain of the rats fed diets with or without DHA. In the rats fed a diet with 0.05 g α -tocopherol/kg (Fig. 1B), the TBARS concentration in the plasma and liver of the DHA group was higher ($p < 0.05$) than that of the control group. The dietary

Fig. 3. Effects of dietary sesamin and sesaminol on α -tocopherol concentrations in the plasma, liver, and brain of rats fed diets with or without docosahexaenoic acid (DHA). The rats were fed a basal diet (Control), a diet with sesamin (Sesamin), a diet with sesaminol (Sesaminol), a diet containing DHA (DHA), a diet containing DHA with sesamin (DHA+sesamin), or a diet containing DHA with sesaminol (DHA+sesaminol), for 5 wk. Each diet contained either 0.01 g/kg (A) or 0.05 g/kg (B) of α -tocopherol. Values are means+SE, $n=6$. Means not sharing a letter are different, $p<0.05$.

sesamin or sesaminol lowered ($p<0.05$) the TBARS concentration in the plasma, liver, and brain of the rats fed diets with or without DHA.

The red blood-cell hemolysis induced by dialuric acid in the rats fed the diet with 0.01 g α -tocopherol/kg was measured (Fig. 2). The red blood-cell hemolysis of the DHA group was much greater ($p<0.05$) than that of the control group. The hemolysis of the DHA+sesamin and DHA+sesaminol groups were much lower ($p<0.05$) than that of the DHA group. The hemolysis of the DHA+sesaminol group was not different ($p\geq 0.05$) from those of the control, sesamin and sesaminol groups fed diets without DHA. The pyruvate kinase activity in the plasma of the rats fed the diet with 0.01 g α -tocopherol/kg was also measured (Fig. 2). The pyruvate kinase activity of the DHA+sesamin group was lower ($p<0.05$) than that of the DHA group. The dietary sesaminol lowered ($p<0.05$) the pyruvate kinase activity in the plasma of the rats fed diets with or without DHA.

The α -tocopherol concentrations in the plasma, liver, and brain are shown in Fig. 3. In the rats fed the diet with 0.01 g α -tocopherol/kg (Fig. 3A), the α -tocopherol concentration in the plasma and liver of the DHA group was lower ($p<0.05$) than that of the control group. The dietary sesamin or sesaminol elevated ($p<0.05$) the α -tocopherol concentrations in the plasma, liver, and brain. The α -tocopherol concentrations in the plasma and liver of the DHA+sesamin and DHA+sesaminol groups were not different ($p\geq 0.05$) from that of each control group. The dietary DHA also lowered ($p<0.05$) the α -tocopherol concentrations in the plasma and liver of the rats fed the diet with 0.05 g α -tocopherol/kg, although it did not affect ($p\geq 0.05$) the α -tocopherol concentration in the brain (Fig. 3B). The dietary sesamin and sesaminol elevated ($p<0.05$) the α -tocopherol concentration in the plasma, liver, and brain of the rats fed diets with or without DHA.

The dietary sesamin did not affect the triacylglycerol concentrations in the plasma and liver in this study

Fig. 4. Effect of dietary sesamin on the percentage of docosahexaenoic acid (DHA) in the fatty acid composition of the plasma and liver of rats fed diets with or without DHA. The rats were fed a basal diet (Control), a diet with sesamin (Sesamin), a diet containing DHA (DHA), or a diet containing DHA with sesamin (DHA+sesamin), for 5 wk. The diet contained 0.01 g α -tocopherol/kg. Values are means+SE, $n=6$. Means not sharing a letter are different, $p<0.05$.

(data not shown). The dietary DHA elevated the percentage of DHA in the fatty acid composition of both triacylglycerol and phospholipids in the plasma and liver of the rats fed the diet with 0.01 g α -tocopherol/kg (Fig. 4). The DHA percentage of the plasma triacylglycerol of the DHA+sesamin group was higher ($p<0.05$) than that of the DHA group, although the effect of sesamin was not detected ($p\geq 0.05$) in the plasma phospholipids. However, the dietary sesamin did not affect the DHA percentage of the triacylglycerol or phospholipids in the liver.

DISCUSSION

Several reports have shown that DHA or DHA-rich fish oil intake elevates the TBARS concentrations in

humans (11-14), rats (15, 16), and mice (17, 18). In this study, dietary DHA elevated the TBARS concentrations in the liver and brain of rats fed a diet with 0.01 g α -tocopherol/kg (Fig. 1A) and the plasma and liver of rats fed a diet with 0.05 g α -tocopherol/kg (Fig. 1B). Dietary DHA also increased the red blood-cell hemolysis induced by dialuric acid, and tended to elevate the plasma pyruvate kinase activity in rats fed the diet with 0.01 g α -tocopherol/kg (Fig. 2). Thus, dietary DHA induces lipid peroxidation and weakness of the biological membranes because of its high degree of unsaturation. We have previously shown that dietary sesamin and sesaminol decrease the TBARS concentrations in the liver of rats fed a diet containing 0.01 g α -tocopherol/kg and sesamin or sesaminol (2). The dietary lignans also prevented red blood-cell hemolysis and lowered the plasma pyruvate kinase activity (2). In this study, the dietary sesame lignans significantly lowered the TBARS concentrations in the plasma, liver, and brain of the rats fed diets with or without DHA (Fig. 1). It also prevented red blood-cell hemolysis and lowered the plasma pyruvate kinase activity in the rats fed DHA (Fig. 2). Thus, sesame lignans decrease lipid peroxidation and improve the weakness of biological membranes in rats fed diets with DHA.

DHA-rich fish oil intake lowers the α -tocopherol concentration in humans (11), rats (15, 16), and mice (18, 19). These data suggest that α -tocopherol acts as an antioxidant for the inhibition of lipid peroxidation in DHA-rich biological membranes. The dietary sesame lignans elevated the α -tocopherol concentrations in the plasma, liver, and brain of the rats fed diets with or without DHA (Fig. 3). Moreover, dietary sesame seed elevated the α -tocopherol concentration in the red blood cell of rats fed diets with 0.01 or 0.05 g α -tocopherol/kg (2). These results suggest that dietary sesame lignans inhibit the lipid peroxidation induced by DHA intake by elevating the α -tocopherol concentration. Dietary sesamin also elevated the DHA content in the plasma triacylglycerol (Fig. 4). This result suggests the possibility that elevation of the α -tocopherol concentration utilizing dietary sesamin prevents the degradation of DHA in plasma triacylglycerol.

On the other hand, we have studied the effect of dietary sesame seed or its lignans on the vitamin E metabolism in rats. Dietary α -tocopherol is absorbed in the intestine and carried to the liver as a result of the uptake of chylomicron remnants (28). Sesame lignan does not affect the absorption of α -tocopherol nor chylomicron secretion by the intestine (9). α -Tocopherol transfer protein (α -TTP), tocopherol-associated protein, and tocopherol-binding protein have been reported as the tocopherol-regulatory proteins that determine the tissue tocopherol levels (29). It is thought that α -TTP plays an important role in the discrimination of vitamin E isoforms (30). α -TTP catalyzes α -tocopherol secretion by a novel non-Golgi-mediated pathway in liver cells, and α -tocopherol is incorporated into very-low-density lipoprotein and subsequently transported to the various tissues by lipoproteins (31). Some reports show that the

α -TTP concentration in the liver of rats changed during α -tocopherol deficiency (32) and in the developmental period after birth (33). However, the dietary sesame seed did not affect the α -TTP concentration in the liver of rats fed α -tocopherol with sesame seed (data not shown). The excess amount of α -tocopherol is metabolized to carboxychroman (34) and excreted into urine. It has recently been suggested that the ω -oxidation of tocopherol, the initiating and rate-limiting step of tocopherol degradation to carboxychroman, is catalyzed by some cytochrome P₄₅₀ (CYP) isoforms (5, 35-37). Sesamin inhibits the CYP-dependent oxidation of γ -tocopherol in HepG2 (35) and microsomes expressing recombinant human CYP4F (37), and the dietary sesamin and sesaminol decrease the urinary excretion of carboxychroman in rats fed γ -tocopherol (5). These data suggest that dietary sesame lignans elevate the tocopherol concentration by inhibiting the vitamin E metabolism. Therefore, the sesame lignans may inhibit the α -tocopherol metabolism to carboxychroman in rats fed DHA.

Both sesamin and sesaminol elevated the α -tocopherol concentration in the plasma, liver, and brain of rats (Fig. 3). The α -tocopherol concentration in the liver of the rats fed the diets with sesaminol for 5 wk was higher than that of the rats fed the diets with sesamin. We previously showed a high α -tocopherol concentration in the plasma and liver of rats fed a diet with sesaminol for 8 wk (2). However, the γ -tocopherol concentrations in the liver, kidney, brain, and serum, and the urinary excretion of carboxychroman of the rats fed diets containing α -tocopherol with sesamin or sesaminol for a short period (3 d) did not differ (5). These data suggest that the inhibitory effects of sesamin and sesaminol on the tocopherol metabolism were almost the same. The reason why the α -tocopherol concentration in the liver of the rats fed diets with sesaminol for a long period (5 or 8 wk) was higher than that of the rats fed diets with sesamin is unknown. In contrast to sesamin, sesaminol has a hydroxyl group (1) and shows antioxidative activity. We previously suggested that sesaminol was a potent antioxidant against lipid peroxidation in vivo (9). Sesaminol may act as an antioxidant against the oxidative degradation of α -tocopherol and lipid peroxidation in biological membranes. Moreover, Nakai et al. (38) recently identified some metabolites of sesamin in rat bile after the oral administration of sesamin, and suggested that the metabolites had radical-scavenging activity and decreased lipid peroxidation in vitro. Therefore, sesame lignans may decrease lipid peroxidation not only elevating the α -tocopherol concentration, but also by their antioxidative activity or that of their metabolites directly.

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