Inhibition of cholesterol absorption and synthesis in rats by sesamin

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Abstract The effects of sesamin, a lignan from sesame oil, on various aspects of cholesterol metabolism were examined in rats maintained on various dietary regimens. When given at a dietary level of 0.5% for 4 weeks, sesamin reduced the concentration of serum and liver cholesterol significantly irrespective of the presence or absence of cholesterol in the diet, except for one experiment in which the purified diet free of cholesterol was given. On feeding sesamin, there was a decrease in lymphatic absorption of cholesterol accompanying an increase in fecal excretion of neutral, but not acidic, steroids, particularly when the cholesterol-enriched diet was given. Sesamin inhibited micellar solubility of cholesterol, but not bile acids, whereas it neither bound taurocholate nor affected the absorption of fatty acids. Only a marginal proportion (ca. 0.15%) of sesamin administered intragastrically was recovered in the lymph. There was a significant reduction in the activity of liver microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase after feeding sesamin, although the activity of hepatic cholesterol 7α-hydroxylase, drug metabolizing enzymes, and alcohol dehydrogenase remained uninfluenced. Although the weight and phospholipid concentration of the liver increased unequivocally on feeding sesamin, the histological examination by microscopy showed no abnormality, and the activity of serum GOT and GPT remained unchanged. Since sesamin lowered both serum and liver cholesterol levels by inhibiting absorption and synthesis of cholesterol simultaneously, it deserves further study as a possible hypocholesterolemic agent of natural origin. —**Hirose, N., T. Inoue, K. Nishihara, M. Sugano, K. Akimoto, S. Shimizu, and H. Yamada.** Inhibition of cholesterol absorption and synthesis in rats by sesamin. *J. Lipid Res.* 1991. **32:** 629–638.

Supplementary key words sesamin • HMG-CoA reductase • cholesterol 7α-hydroxylase • micellar solubility • bile salts • steroid excretion

Sesame has long been used extensively as a traditional health food in the Orient for various purposes. However, no attention has been directed to the physiologically active components in this seed. Sesamin, one of the lignans existing exclusively and abundantly in sesame, is a most intriguing component since it exerts an efficient antioxidant activity (1–5), and lipid peroxidation is considered to be one of the causes of various deteriorative disorders (6–8). Our recent preliminary study on the physiological effect of the mixture of sesame lignans in rats suggested a possible function of sesamin in the regulation of lipid metabolism (9) as observed in the microorganism *Mortierella alpina* 1S-4 (10).

Thus it is important to elucidate the mechanism of action of sesame lignans, in particular sesamin, on lipid metabolism. Since sesame oil has been shown to be slightly more hypocholesterolemic than corn oil regardless of the comparable fatty acid composition (11), the present study was focused on the effect of sesamin on various aspects of cholesterol metabolism in rats.

METHODS

Animals and diets

Male Wistar rats (Seiwa Experimental Animals, Fukuoka) initially weighing ca. 140 g were used in the feeding studies. The animals were housed individually in an air-conditioned room (22–24°C with a 12-h light cycle) and were given experimental diets and water ad libitum. Three sets of feeding experiments were performed. In experiment I rats were fed purified diets under the reversed light and dark cycle. The diets were prepared according to the formula recommended by the American Institute of Nutrition (12) and contained (weight percent): casein, 20; corn oil, 10; vitamin mixture (AIN-TM[76]), 1; mineral mixture (AIN-TM[76]), 3.5; choline bitartrate, 0.2; DL-methionine, 0.3; cellulose, 5; corn starch, 15; and sucrose to 100. Cholesterol (0.5%) and sesamin (0.5%)

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; GLC, gas-liquid chromatography; HPLC, high performance liquid chromatography.

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were added at the expense of sucrose. Sesamin was a gift from Suntory Ltd. (Osaka) and was 99.5% pure as analzyed by HPLC (5). Feces were collected at the end of the 3rd week for a 2-day period and lyophilized prior to measurement of fecal steroids. After 4 weeks, rats were killed by decapitation between 0900 and 1000 h for the measurement of liver enzyme activities and serum and liver lipids. In experiments II and III rats were fed a powdered commercial stock diet (Type NMF, Oriental Yeast Co., Tokyo) with and without sesamin (0.5%) under the ordinary light and dark cycle. In Exp. II, rats were decapitated at night (0100 h) for the measurement of liver enzyme activities and serum and liver lipids. In Exp. III, rats were decapitated between 0900 and 1000 h for the measurement of the activities of serum GOT and GPT and liver microsomal drug metabolizing enzymes.

Assays of hepatic enzyme activities

The activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) (13) and cholesterol 7α-hydroxylase activity (EC 1.14.13.17) (14) in the liver microsomes was measured as described elsewhere. The activity of aminopyrine N-demethylase (15), aniline hydroxylase (EC 1.14.14.1) (16), and alcohol dehydrogenase (EC 1.1.1.1) (17) was assayed in the 10,000 *g* supernatant of liver homogenate. The homogenate was prepared in 7 volumes of 10 mM Na/K-phosphate buffer (pH 7.4) containing 1.15% KCl. Protein was measured by the method of Lowry et al. (18). DL-3-Hydroxy-3-methyl[3-^{14}C]glutaryl coenzyme A (1.92 GBq/mmol), DL-[2-^{14}C]mevalonic acid lactone (2.0 GBq/mmol), and [4-^{14}C]cholesterol (2.04 GBq/mmol) were obtained from Amersham International plc, Buckinghamshire.

Lipid analyses

Serum and liver lipids were extracted and purified according to the method of Folch, Lees, and Sloane Stanley (19). Cholesterol, triglyceride, and phospholipid were measured as described elsewhere (20). Serum high density lipoprotein (HDL)-cholesterol was analyzed using a commercial kit (HDL-C·2[DAIICHII], Daiichi Pure Chemicals Co., Ltd., Tokyo) on the supernatant obtained after precipitation of very low density and low density lipoproteins with dextran sulfate-$MgCl_2$. Fecal steroids were analyzed by gas–liquid chromatography (GLC) on an OV-17 column for neutral steroids and an AN-600 column for acidic steroids (21, 22).

Lymphatic absorption of cholesterol and fatty acid

Rats weighing about 300 g maintained on commercial rat chow were subjected to cannulation of the left thoracic lymph vessel cephalad to the cisterna chyli by the method of Vahouny et al. (23). Lipid emulsions for intragastric administration contained 50 mg bovine serum albumin (fatty acid-free, Sigma Chemical Co., St. Louis, MO), 200 mg corn oil, 200 mg sodium taurocholate, and 10 mg cholesterol (containing 1 μCi [4-^{14}C]cholesterol) and 50 mg of sesamin per 3 ml of distilled water. The emulsion not containing sesamin served as the control. The mixture was emulsified by sonication just prior to use. Lymph was collected periodically for a total of 24 h and analyzed for cholesterol radioactivity by liquid scintillation counting (Aloka LSC-1000, Tokyo) (24). The fatty acid composition in the lymph was analyzed by GLC on a SILAR 10C column (25), and the concentration was calculated using pentadecanoic acid as an internal calibration standard.

Analyses of sesamin in the serum, liver, lymph, and stool

Lipid extracted from serum, liver, and lymph were saponified with ethanolic KOH and unsaponifiable material was extracted with petroleum ether (26). For determination of sesamin, the unsaponifiable fraction was

TABLE 1. Effects of sesamin on food intake, growth, and liver weight

Exp. No. and Group	n	Food Intake	Body Weight Gain	Relative Liver Weight
		g/day	*g/4 weeks*	*g/100 g body weight*
Exp. I				
Purified diet	6	19.7 ± 0.5	197 ± 8	4.17 ± 0.33^a
Diet + sesamin	6	20.4 ± 0.6	199 ± 7	5.37 ± 0.19^b
Diet + cholesterol	6	20.9 ± 0.5	199 ± 8	5.48 ± 0.29^b
Diet + cholesterol and sesamin	7	21.6 ± 0.4	204 ± 8	6.76 ± 0.25^c
Exp. II				
Commercial chow	7	27.5 ± 0.3	205 ± 3	4.38 ± 0.11^a
Chow + sesamin	8	28.6 ± 0.4	198 ± 4	6.48 ± 0.14^b
Exp. III				
Commercial chow	6	25.3 ± 0.4	220 ± 10	4.85 ± 0.12^a
Chow + sesamin	7	23.8 ± 0.6	197 ± 10	6.03 ± 0.23^b

Values are means ± SEM. Rats initially weighing an average of 139 g were fed experimental diets for 4 weeks. Values with different letters are significantly different in each experiment ($P < 0.05$). Both sesamin and cholesterol were added to the diet at a 0.5% level.

TABLE 2. Effects of sesamin on concentrations of serum cholesterol

Exp. No. and Group	n	Cholesterol	HDL-Cholesterol
		mg/100 ml serum	
Exp. I			
Purified diet	6	108 ± 4[a]	65.4 ± 2.1[a]
Diet + sesamin	6	110 ± 5[a]	65.1 ± 5.7[a]
Diet + cholesterol	6	136 ± 8[b]	52.1 ± 3.3[a]
Diet + cholesterol and sesamin	7	102 ± 5[a]	36.9 ± 2.8[b]
Exp. II			
Commercial chow	7	69.1 ± 5.2[a]	
Chow + sesamin	8	55.5 ± 3.0[b]	

Values are means ± SEM. Values with different letters are significantly different in each experiment ($P < 0.05$). See footnote to Table 1.

transformed to trimethylsilyl derivatives and analyzed by GLC on a 3% OV-17 column coated on Gas Chrome Q, 60–80 mesh (Gasukuro Kogyo Inc., Tokyo) (26). 5α-Cholestane (Nacalai Tesque Inc., Kyoto) was used as an internal standard. Fecal sesamin was measured by the method used for the fecal neutral steroid analysis. Sesamin in serum and liver was also analyzed by HPLC (Waters 600E as an injector and Waters 490E as a detector, Millipore Corporation, Milford, MA) using a TSKgel ODS-120T column (Tosoh Ltd., Tokyo) and 2,2,5,7,8-pentamethyl-6-hydroxy coumarin as an internal standard (5).

Micellar solubility of cholesterol

Micellar solubility of cholesterol or its binary mixture with sesamin in vitro was measured by the method of Ikeda, Tanabe, and Sugano (26). Micellar solutions (5 ml) containing 0.6 mM egg yolk lecithin, 1 mM oleic acid, and 0.5 mM monoolein and different levels (1 and 2 mM) of cholesterol with or without sesamin (0.5–10 mM) were prepared. The solution was emulsified by sonication (2 min at 37°C, Ultrasonic Disrupture Model UR-200P, Tomy Seiko Co., Ltd., Tokyo) and ultracentrifuged at 100,000 *g* for 60 min at 37°C. The supernatant was collected for the determination of cholesterol and sesamin by GLC as described above, and bile acid by the method of Eaton and Klassen (27).

Binding ability to taurocholate

Binding ability of cholestyramine and sesamin with taurocholate was measured by the method of Krichevsky and Story (28). The mixtures containing 100 μmol tauro [carbonyl-^{14}C]cholic acid (sodium salt) (2.07 GBq/mmol, Amersham International plc, Buckinghamshire) in 5 ml of 0.1 M Tris-HCl buffer, pH 7.4, and 1–500 mg binding substances were incubated at 37°C for 2 h, and the radioactivity in the supernatant (15,000 *g* for 15 min) was measured by liquid scintillation counting.

Histological examination

In Exp. I, liver was stained with hematoxylin-eosin, sudan-III and oil red, and examined by light microscopy.

Statistical analysis

Data were analyzed by a one-way analysis of variance followed by inspection of all differences by Duncan's multiple range test (29).

RESULTS

Growth parameters and liver weight

Table 1 shows the growth parameters and liver weight. Food intake and body weight gain were comparable among the groups in each experiment. Sesamin significantly increased relative liver weight in all experiments. In a separate experiment, we observed that both food intake and body weight gain of rats were comparable between the control and sesamin (0.2%) groups for a period of 45 days.

Serum and liver cholesterol concentrations

Concentrations of serum cholesterol are summarized in **Table 2**. With purified diets (Exp. I), sesamin significant-

TABLE 3. Effects of sesamin on concentrations of liver lipids

Exp. No. and Group	n	Liver Lipids			Liver Microsomal Lipids	
		Cholesterol	Triglyceride	Phospholipid	Cholesterol	Phospholipid
		mg/g liver			*nmol/mg protein*	
Exp. I						
Purified diet	6	2.54 ± 0.13[a]	7.11 ± 0.78[a]	32.2 ± 0.7[a]	85.3 ± 8.8	734 ± 39
Diet + sesamin	6	1.95 ± 0.06[b]	7.57 ± 0.40[a]	39.4 ± 0.2[b]	81.2 ± 7.8	752 ± 48
Diet + cholesterol	6	20.8 ± 2.2[c]	23.5 ± 2.7[b]	29.0 ± 0.6[c]	82.4 ± 10.4	831 ± 67
Diet + cholesterol and sesamin	7	9.13 ± 1.02[d]	15.1 ± 1.9[c]	39.3 ± 1.1[b]	75.5 ± 9.6	785 ± 73
Exp. II						
Commercial chow	7	2.86 ± 0.19[a]	4.50 ± 0.81	29.7 ± 0.7[a]		
Chow + sesamin	8	1.82 ± 0.04[b]	3.81 ± 0.47	34.0 ± 0.5[b]		

Values are means ± SEM. Values with different letters are significantly different in each experiment ($P < 0.05$). See footnote to Table 1.

TABLE 4. Effects of sesamin on activities of key enzymes in the synthesis of cholesterol and bile acid in liver microsomes

Exp. No. and Group	n	HMG-CoA Reductase	Cholesterol 7α-Hydroxylase
		pmol/min per mg protein	
Exp. I			
Purified diet	6	203 ± 12[a]	20.7 ± 4.3
Diet + sesamin	6	151 ± 11[b]	26.1 ± 6.1
Diet + cholesterol	6	51.6 ± 2.0[c]	21.0 ± 3.7
Diet + cholesterol and sesamin	6	29.0 ± 2.4[d]	23.5 ± 4.2
Exp. II			
Commercial chow	7	269 ± 27[a]	
Chow + sesamin	8	172 ± 13[b]	

Values are means ± SEM. Values with different letters are significantly different in each experiment ($P < 0.05$). See footnote to Table 1.

ly reduced the concentration of serum cholesterol in rats fed a cholesterol-enriched diet. When rats were fed a stock diet essentially free of cholesterol (Exp. II), sesamin lowered the serum cholesterol concentration sigificantly. The concentration of HDL-cholesterol was also significantly lower in rats fed sesamin. However, there were no differences in the concentrations of serum triglyceride and phospholipid among the groups in each experiment and they were in the normal ranges (data not shown). In preliminary experiments, the effect of sesamin on plasma cholesterol level was dose-dependent (0.1-0.5%).

As shown in **Table 3**, sesamin significantly lowered the concentration of liver cholesterol in each set of experiments, but it did not lower microsomal cholesterol. The concentration of liver triglyceride was significantly low when rats were fed sesamin with cholesterol (Exp. I). Sesamin unequivocally increased the concentration of liver phospholipid significantly, whereas there was no increase in the microsomal phospholipid level.

Serum GOT and GPT activity

In Exp. III, no significant change in serum GOT and GPT activities was observed due to sesamin (79.5 ± 1.7 and 72.3 ± 4.8 IU/l for GOT, and 17.3 ± 1.0 and 17.0 ± 0.5 IU/l for GPT in the group with and without sesamin, respectively).

Hepatic enzyme activities

As **Table 4** shows, the activity of liver microsomal HMG-CoA reductase was significantly lower in rats fed

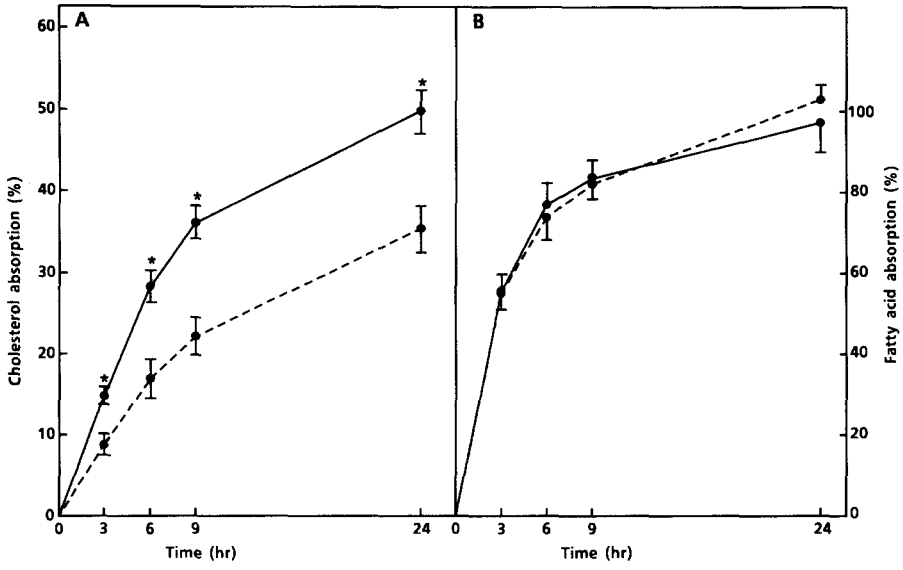

Fig. 1. Effects of sesamin on the lymphatic absorption of cholesterol (A) and fatty acids (B). Lymph fistula rats were administered intragastrically an emulsion containing 10 mg [^{3}H]cholesterol with or without 50 mg sesamin. Lymph was collected for 24 h and analyzed for cholesterol radioactivity and fatty acid concentration. Each point represents the mean ± SEM for seven rats. *Significantly different from the sesamin group ($P < 0.05$); (———) control; (-----) sesamin.

TABLE 5. Effects of sesamin on fecal steroid excretion in rats fed purified diets (Exp. I)

Fecal Steroid	Group (No. of Rats) Purified Diet (6)	Purified Diet + Sesamin (6)	Purified Diet + Cholesterol (6)	Purified Diet + Cholesterol & Sesamin (7)
Feces (g/day, dry wt)	1.40 ± 0.10^{a}	1.72 ± 0.13^{ab}	1.53 ± 0.11^{a}	2.08 ± 0.12^{b}
Daily excretion (mg/day)				
Neutral steroids				
Coprostanol	4.15 ± 0.60^{a}	0.69 ± 0.18^{b}	13.3 ± 1.3^{c}	0.43 ± 0.05^{b}
Cholesterol	3.84 ± 0.36^{a}	8.44 ± 0.38^{b}	35.0 ± 4.6^{c}	71.0 ± 4.8^{d}
Total	7.99 ± 0.96^{a}	9.13 ± 0.40^{a}	48.3 ± 5.6^{b}	71.4 ± 4.8^{c}
Acidic steroids				
Lithocholic	1.08 ± 0.41^{a}	4.97 ± 0.70^{b}	1.29 ± 0.16^{a}	3.92 ± 0.39^{b}
Deoxycholic	1.57 ± 0.47	n.d.	1.95 ± 0.25	n.d.
Chenodeoxycholic	0.04 ± 0.02^{a}	0.23 ± 0.06^{b}	0.07 ± 0.03^{ab}	0.14 ± 0.06^{ab}
Hyodeoxycholic + ursodeoxycholic	3.78 ± 1.71^{a}	n.d.	0.88 ± 0.41^{ab}	0.10 ± 0.06^{b}
Cholic	0.21 ± 0.09^{a}	0.59 ± 0.17^{a}	0.18 ± 0.13^{a}	1.90 ± 0.26^{b}
12-Ketolithocholic	0.02 ± 0.01^{a}	0.20 ± 0.04^{a}	0.18 ± 0.04^{a}	1.10 ± 0.20^{b}
α-Muricholic	0.98 ± 0.39^{a}	0.25 ± 0.13^{a}	6.17 ± 1.21^{b}	0.61 ± 0.21^{a}
β-Muricholic	0.59 ± 0.23^{a}	0.58 ± 0.17^{a}	9.69 ± 2.37^{b}	7.81 ± 1.31^{b}
Total	8.30 ± 2.22^{ab}	6.86 ± 1.02^{a}	20.8 ± 2.6^{c}	15.9 ± 1.9^{bc}
Steroid concentration (mg/g feces)				
Neutral steroids				
Coprostanol	2.93 ± 0.28^{a}	0.43 ± 0.13^{b}	8.75 ± 0.71^{c}	0.21 ± 0.02^{b}
Cholesterol	2.74 ± 0.13^{a}	5.03 ± 0.37^{b}	22.4 ± 1.5^{c}	34.4 ± 1.0^{d}
Total	5.67 ± 0.40^{a}	5.46 ± 0.45^{a}	31.1 ± 1.4^{b}	34.6 ± 1.0^{c}
Acidic steroids				
Lithocholic	0.78 ± 0.30^{a}	2.88 ± 0.23^{b}	0.85 ± 0.09^{a}	1.91 ± 0.16^{c}
Deoxycholic	1.07 ± 0.27	n.d.	1.30 ± 0.17	n.d.
Chenodeoxycholic	0.03 ± 0.02^{a}	0.13 ± 0.02^{b}	0.05 ± 0.02^{ab}	0.07 ± 0.03^{ab}
Hyodeoxycholic + ursodeoxycholic	2.76 ± 1.17^{a}	n.d.	0.57 ± 0.25^{ab}	0.045 ± 0.03^{b}
Cholic	0.16 ± 0.06^{a}	0.32 ± 0.07^{a}	0.11 ± 0.08^{a}	0.93 ± 0.11^{b}
12-Ketolithocholic	0.017 ± 0.01^{a}	0.11 ± 0.02^{a}	0.11 ± 0.02^{a}	0.54 ± 0.09^{b}
α-Muricholic	0.67 ± 0.25^{a}	0.17 ± 0.09^{a}	4.15 ± 0.88^{b}	0.30 ± 0.04^{a}
β-Muricholic	0.40 ± 0.15^{a}	0.33 ± 0.07^{a}	6.19 ± 1.19^{b}	3.82 ± 0.61^{b}
Total	5.88 ± 1.39^{a}	3.94 ± 0.33^{a}	13.6 ± 1.2^{b}	7.75 ± 0.80^{a}

Values are means ± SEM. Values with different letters are significantly different ($P < 0.05$). Feces were collected at the end of the 3rd week for a 2-day period. See footnote to Table 1.

sesamin than in the corresponding control rats irrespective of the presence or absence of cholesterol in the diet, whereas the activity of cholesterol 7α-hydroxylase was comparable among the groups. The activities of aminopyrine N-demethylase (3.05 ± 0.14 and 2.77 ± 0.10 μmol of formaldehyde formed per g of liver per 30 min for the control and sesamin groups, respectively) and aniline hydroxylase (34.9 ± 2.9 and 31.0 ± 1.1 μg of *p*-aminophenol formed per g of liver per 20 min), both the drug metabolizing enzymes, and alcohol dehydrogenase (39.8 ± 4.6 and 38.5 ± 4.4 IU/g liver) in the 10,000 *g* supernatant fraction of the liver homogenate were all comparable in rats fed with and without sesamin (Exp. III).

TABLE 6. Concentrations of sesamin in serum, liver, and feces

Exp. No. and Group	n	Serum	Liver	Feces	Feces	Daily Intake	Fecal Excretion
				mg/day	*mg/g feces*	*mg/day*	*%*
Exp. I							
Purified diet + sesamin	6	n.d.	n.d.	16.3 ± 2.0	9.5 ± 0.8	109 ± 4	14.9 ± 1.7
Diet + cholesterol and sesamin	7	n.d.	n.d.	22.3 ± 2.2	10.8 ± 0.8	118 ± 4	18.8 ± 1.7
Exp. II							
Commercial chow + sesamin	7	n.d.	n.d.	54.5 ± 2.8	5.8 ± 0.2	159 ± 4	34.2 ± 1.6

Values are means ± SEM; n.d., not detectable. Feces were collected at the end of the 3rd and 4th weeks for a 2-day period in Exps. I and II, respectively. Weight of dried feces excreted in Exp. II was 9.38 ± 0.28 and 8.07 ± 0.24 g/day for rats fed diets with and without sesamin, respectively.

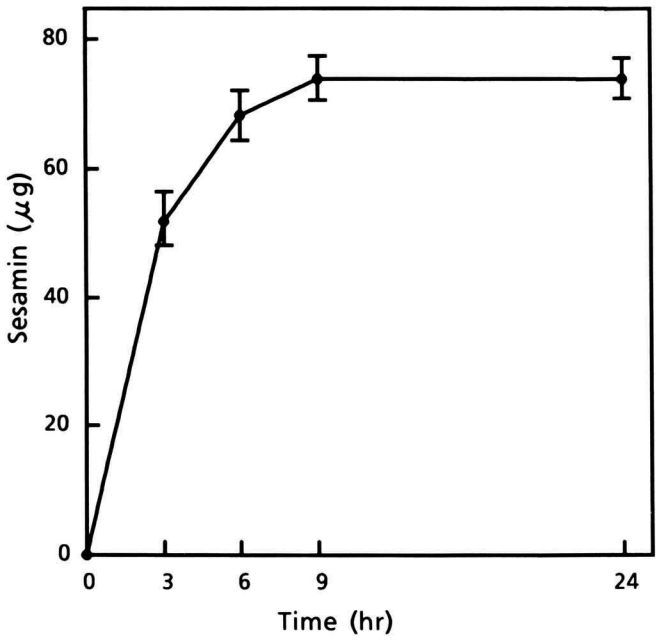

Fig. 2. Lymphatic absorption of sesamin. Lymph fistula rats were administered intragastrically a fat emulsion containing 10 mg cholesterol and 50 mg sesamin. Lymph was collected for 24 h and analyzed for sesamin. The total amount of sesamin absorbed in the lymph for 24 h was 73.7 ± 3.2 μg (0.15 ± 0.01% of the dose). Each point represents mean ± SEM for three rats.

Inhibition of cholesterol absorption by sesamin

The effect of sesamin on the lymphatic absorption of cholesterol was followed for 24 h after intragastric administration of a fat emulsion. As shown in **Fig. 1A**, sesamin decreased the absorption of exogenous cholesterol significantly ($P < 0.05$) at all time points. The lignan inhibited cholesterol absorption approximately 30% as compared to the control over a 24-h period. The lymph flow was comparable in both groups (93.3 ± 8.4 and 92.1 ± 4.4 ml/24 h). Fig. 1B shows a time course of the absorption of fatty acids in the lymph. Sesamin did not influence fatty acid absorption.

Fecal steroid excretion

The data for fecal steroid excretion in Exp. I are summarized in **Table 5**. The weight of dried feces was significantly higher in rats fed sesamin irrespective of the presence or absence of cholesterol in the diet. Neutral steroid excretion (coprostanol + cholesterol), both in terms of the concentration and the daily fecal excretion, also increased significantly by sesamin when the diet was enriched with cholesterol. In contrast, there was a decreasing trend of daily excretion of bile acids in rats fed sesamin compared to the corresponding paired rats, and the concentration of bile acids was significantly lower in the sesamin group when rats were fed cholesterol. The microbial transformation of cholesterol to coprostanol was significantly depressed by sesamin. The ratio of coprostanol/cholesterol in the two control groups with and without cholesterol was 1.06 ± 0.05 and 0.41 ± 0.06 compared to the corresponding sesamin supplemented groups, 0.08 ± 0.02 and 0.01 ± 0.00, respectively; the difference was statistically significant ($P < 0.01$). The composition of fecal bile acids also changed. Deoxycholic acid was not detectable in the two sesamin groups. Sesamin reduced the fraction of hyodeoxycholic + ursodeoxycholic acids markedly and it was not detected in rats fed a diet free of cholesterol, whereas the amount of chenodeoxycholic and lithocholic acids was significantly higher in sesamin-fed rats than in the corresponding control rats. The concentration of α-muricholic acid was also low in rats fed sesamin.

Concentrations of sesamin in serum, liver, lymph, and feces

The concentration of sesamin in serum, liver, and feces is summarized in **Table 6**. Since no sesamin was detected in the control groups, only the data for rats fed sesamin were shown. By gas–liquid chromatography, sesamin was

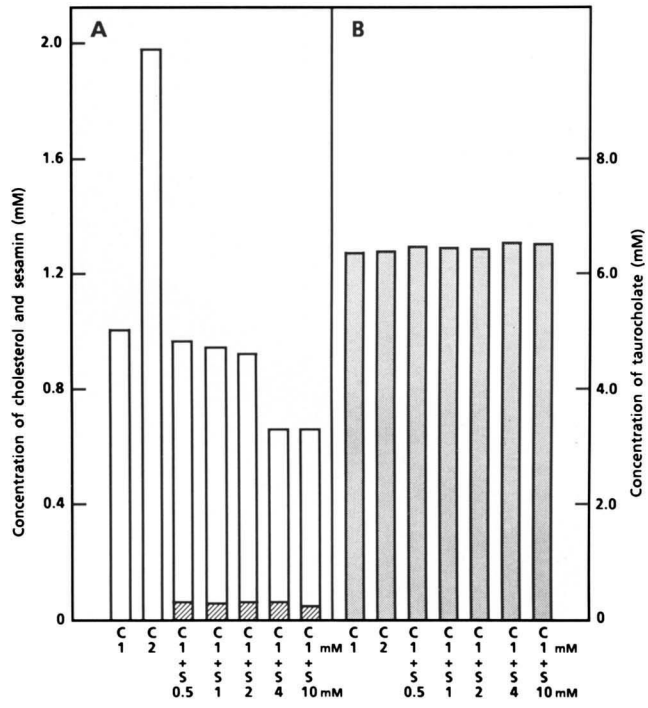

Fig. 3. Effects of sesamin on micellar solubility of cholesterol (A) and taurocholate (B). Cholesterol (1 and 2 mM) or mixtures of cholesterol (1 mM) and sesamin (0.5, 1, 2, 4, and 10 mM) were sonicated for 2 min at 37°C in 15 mM sodium phosphate buffer, pH 7.4, containing 6.6 mM sodium taurocholate, 0.6 mM egg yolk lecithin, 1 mM oleic acid, 0.5 mM monoolein, and 132 mM sodium chloride. After the mixture was incubated at 37°C overnight and centrifuged at 100,000 *g* for 60 min at 37°C, cholesterol (C) and sesamin (S) and taurocholate in the supernatant were analyzed. Results are means of two incubations. Experimental error was within 5%; □ cholesterol; ▨ sesamin; ▩ taurocholate.

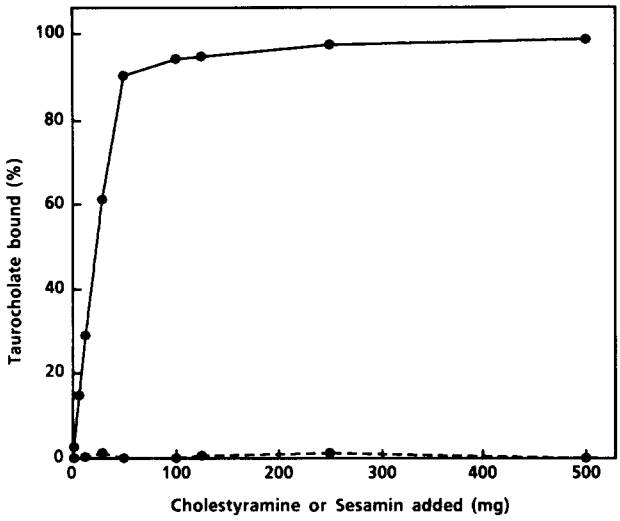

Fig. 4. Binding of cholestyramine and sesamin with taurocholate. The mixture containing 100 μmol [^{14}C]taurocholate in 5 ml of 0.1 M Tris-HCl buffer, pH 7.4, and various amounts of cholestyramine or sesamin was incubated for 2 h at 37°C. The supernatant was analyzed for radioactivity. Results are means of two incubations. Experimental error was within 2%; (——) cholestyramine; (-----) sesamin.

essentially not detected in the serum and liver (< 1.4 μg/ml serum and < 14 μg/g liver). However, it was detected by HPLC at concentrations of 0.17 ± 0.03 μg/ml serum and 1.32 ± 0.13 μg/g liver. Around 15 to 19% of ingested sesamin was excreted in the feces in rats fed a purified diet and approximately 34% in rats fed a non-purified diet. As shown in **Fig. 2**, only a limited amount of sesamin was absorbed and found in the lymph, and the proportion of sesamin recovered during 24 h was 0.15% out of 50 mg introduced to the stomach.

Effects of sesamin on micellar solubility of cholesterol in vitro

As shown in **Fig. 3A**, sesamin lowered micellar solubility of cholesterol to approximately 65% when the concentration of sesamin was more than four times that of cholesterol. In contrast, no effect of sesamin on the solubility of bile acids was observed (Fig. 3B). The concentration of sesamin in the micellar solution was apparently uninfluenced under the experimental conditions used, approximately 20 μg/ml.

Binding of sesamin with taurocholate

As shown in **Fig. 4**, sesamin did not bind taurocholate even at extremely high concentrations, in the range where the bile acid-binding resin, cholestyramine, bound bile acid quantitatively.

Histological examination

As shown in **Fig. 5**, the light microscopic examination showed no significant abnormalities in the livers of rats fed a purified diet with sesamin. Cholesterol feeding resulted in the marked fatty degeneration, but sesamin showed a significant protective effect.

DISCUSSION

Sesame is a traditional health food and has been considered to elicit medicinal value for the prevention of various regressive diseases such as atherosclerosis, hypertension, and aging in the Oriental countries for thousands of years. However, essentially no scientific information is available regarding the beneficial effect of sesame or sesame oil. Although Koh (11) observed that sesame oil tended to reduce the serum cholesterol level of rats compared to corn oil, in spite of the comparable fatty acid composition of the two oils, no reasonable explanation for such an effect was given and no attention was paid to the lignan components in sesame oil.

Most of the studies on lignan, a specific constituent abundant in sesame and sesame oil, has been focused on its potential as an antioxidant in vitro (1–5) and, as far as we know, there appear to be no in vivo studies. Our preliminary study on the lignan mixture extracted from sesame oil focused on the effect on the desaturation of linoleate to arachidonate and showed that lignans interfere with this reaction (9) as in the case of the microorganisms (10). In order to extend this observation, in the present study we fed rats with sesamin (0.5%) which is a main constituent of lignan in sesame (1–1.5%) and sesame oil (0.5–1%), and examined its effect on lipid metabolism, and, in particular cholesterol.

Sesamin affected neither the body weight gain nor food intake at the dietary level of 0.5%, but increased relative liver weight and the concentration of liver phospholipid (Table 1). However, no specific histological changes were observed under light microscopic examination of the liver. Activities of GOT and GPT in serum and drug metabolizing enzymes in the liver all remained unchanged. However, the pathological significance of this observation needs to be confirmed.

Sesamin reduced the serum and liver cholesterol levels both in rats fed cholesterol-free or cholesterol-enriched diets except for one occasion in which the purified diet free of cholesterol was given. In addition, the concentration of liver triglyceride was also reduced in rats fed a cholesterol-enriched diet, showing the prevention of fatty degeneration due to dietary cholesterol (Table 3 and Fig. 5). The hypocholesterolemic activity of sesamin can at least in part be explained by the inhibition of the intestinal absorption of cholesterol as reflected by the significant reduction in cholesterol in the thoracic lymph (Fig. 1A), accompanied by an increase in the fecal excretion of neutral steroids (Table 5).

In addition, sesamin reduced the activity of HMG-CoA reductase, the rate-limiting enzyme of cholesterol

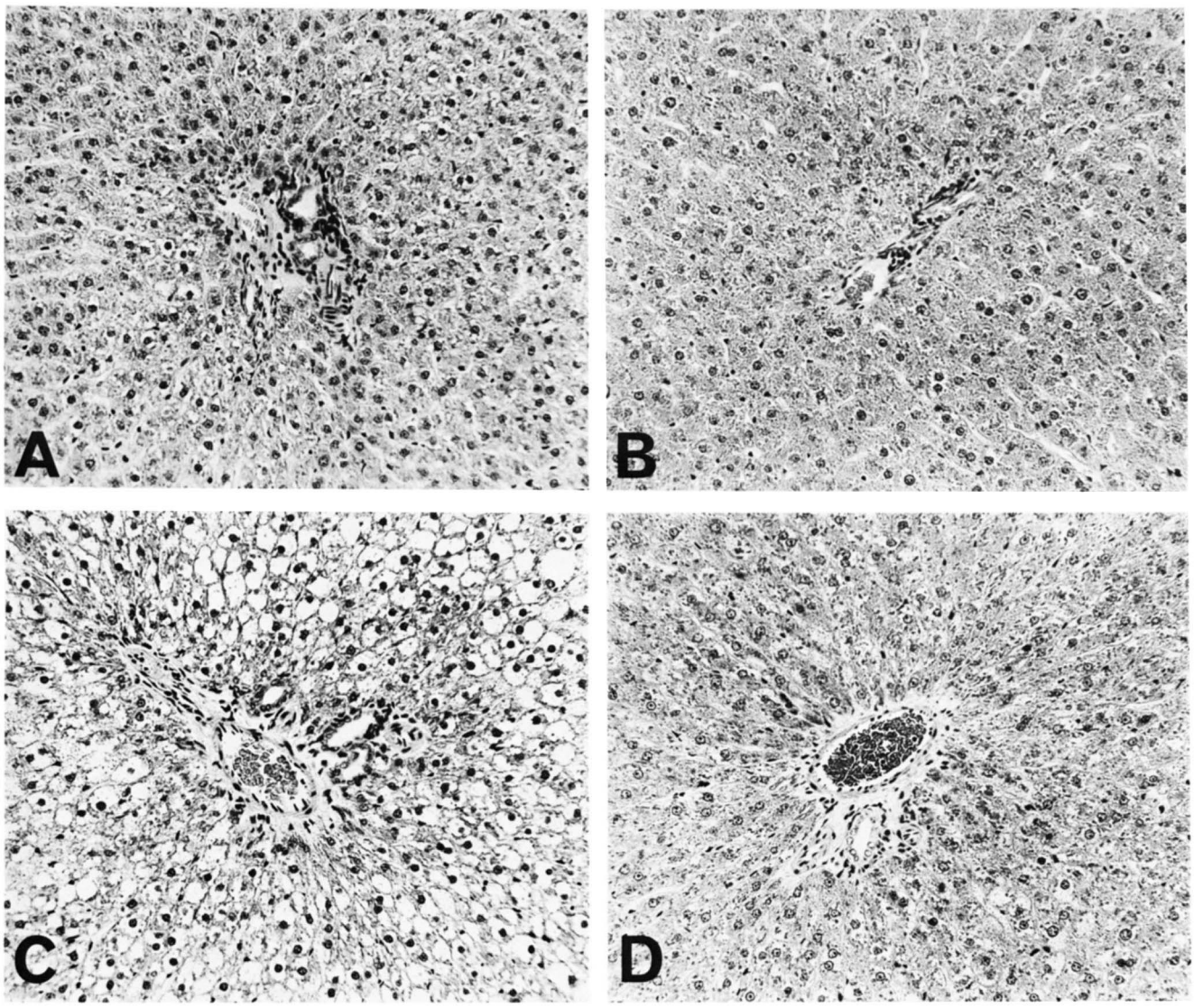

Fig. 5. Light microscopic examination of the liver with hematoxylin-eosin staining. (× 170). A, rat given a purified diet; B, rat given a purified diet with sesamin; C, rat given a purified diet supplemented with cholesterol; D, rat given a purified diet supplemented with cholesterol and sesamin. A and B showed no abnormality. C showed a marked fatty degeneration, whereas D showed a protection against the fatty change.

synthesis in liver microsomes, not only in rats fed a cholesterol-free diet but also in those fed a cholesterol-enriched diet (Table 4). Although the activity of cholesterol 7α-hydroxylase was not influenced significantly, it was, on the basis of the total activity, significantly higher ($P < 0.05$) in rats fed sesamin (1.80 ± 0.31 nmol/min per liver per 100 g body weight) than in the corresponding control rats (1.02 ± 0.16), suggesting an increased catabolism of cholesterol. Although it is known that HMG-CoA reductase undergoes feed-back regulation (30), the concentration of cholesterol in the liver was lower in rats fed sesamin. Sabine and James (31) proposed the hypothesis that the fluidity of microsomes is a determinant of the activity of HMG-CoA reductase. In the present study, however, the concentration of microsomal cholesterol and the ratio of cholesterol to phospholipid were not affected by sesamin (see Table 3). Our data are in agreement with the report by Ide, Okumatsu, and Sugano (13) who observed no consistent correlation between the HMG-CoA reductase activity and microsomal cholesterol concentration. Thus, the decrease in HMG-CoA reductase activity may be attributed to the direct inhibition of the enzyme by sesamin.

Cholestyramine exerts a hypocholesterolemic effect through sequestration of bile acids (32). Sesamin did not show any binding ability to taurocholate in vitro (Fig. 4),

and the concentration of bile acid in the miceller solution was not influenced by a wide range of added sesamin (Fig. 3B), suggesting that the hypocholesterolemic effect of sesamin is not attributed to the interference with bile acid availability in the intestine. In fact, the lignan did not influence the fecal excretion of bile acids.

There exists a correlation between the micellar solubility of cholesterol and the extent of cholesterol absorption (26). An approximate 35% reduction of the cholesterol concentration in the micellar solution when the molar concentration of sesamin was more than four times that of cholesterol (Fig. 3A) explains, in part, the mode of the inhibition of intestinal absorption of cholesterol by sesamin. The increase in fecal neutral steroid excretion is additional evidence to support the reduction of cholesterol absorption. In addition to interference with cholesterol absorption, sesamin appears to modulate the intestinal bacterial flora, since the rate of transformation of cholesterol and bile acids in the intestine was reduced markedly (Table 5).

Since only a marginal proportion of sesamin was solubilized in the micelles, it seems at least likely that sesamin directly interacts with cholesterol and subsequently reduces the micellar solubilization of cholesterol. In contrast, sesamin did not affect the fatty acid recovery in the lymph (Fig. 1B), suggesting no inhibitory effect on fat absorption but rather a specific interference with cholesterol absorption.

Very low concentration of sesamin was detected in serum and liver lipids. Micellar solubility of sesamin was in the range of 0.5–12% depending on the amount added to the system, and sesamin recovered in the lymph was only 0.15% of that administered intragastrically (Fig. 2). These results indicate that only limited amounts of sesamin are absorbed in the body. However, the fecal excretion of sesamin accounted for 15–34% of the amounts ingested (Table 6), suggesting a possible transformation of sesamin by the enteral bacterial flora. Kaku and Ri (33) were able to convert sesamin to pinoresinol dimethyl ether, and l-asarinin to eudesimin. Pinoresinol is a water-soluble antioxidant known to have a hypotensive activity (34). Thus, there is a possibility that sesamin itself may have an extremely strong activity at a low level and/or unknown metabolite(s) of sesamin formed in the gut may exert the physiological activity after being absorbed. The possibility of in vivo transformation of sesamin also cannot be ruled out.

Since no hypocholesterolemic substance has been reported so far that inhibits both cholesterol absorption from the intestine and cholesterol synthesis in the liver simultaneously, the clinical application of sesamin as a new and efficient hypocholesterolemic agent deserves consideration.

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