ELSEVIER

Journal of Nutritional Biochemistry 13 (2002) 289–295

THE JOURNAL OF Nutritional Biochemistry

Comparative effect of sesamin and episesamin on the activity and gene expression of enzymes in fatty acid oxidation and synthesis in rat liver

Masayo Kushiro[a,*], Takeshi Masaoka[b], Shinji Hageshita[b], Yoko Takahashi[a], Takashi Ide[a], Michihiro Sugano[c]

[a]*Laboratory of Nutrition Biochemistry, National Food Research Institute, 2–1-12 Kannondai, Tsukuba 305-8642 Japan*
[b]*Kadoya Sesame Mills Inc., 6188 Tonosho-cho, Shozu-gun, Kagawa 761-4101 Japan*
[c]*Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Kumamoto 862-8502 Japan*

Received 8 August 2001; received in revised form 10 October 2001; accepted 18 December 2001

Abstract

Sesamin is one of the most abundant lignans in sesame seed. Episesamin, a geometrical isomer of sesamin, is not a naturally occurring compound and is formed during the refining process of non-roasted sesame seed oil. We compared the physiological activities of these compounds in affecting hepatic fatty acid metabolism in rat liver. Rats were fed either a control diet free of lignan or diets containing 0.2% of sesamin or episesamin for 15 days. These lignans increased the mitochondrial and peroxisomal palmitoyl-CoA oxidation rates. However, the magnitude of the increases was greater with episesamin than with sesamin. Sesamin caused 1.7- and 1.6-fold increases in mitochondrial and peroxisomal activity, respectively, while episesamin increased these values 2.3- and 5.1-fold. These lignans also increased the activity and gene expression of various fatty acid oxidation enzymes. Again, the increase was much more exaggerated with episesamin (1.5- to 14-fold) than with sesamin (1.3- to 2.8-fold). Diets containing sesamin and episesamin lowered the activity and gene expression of hepatic lipogenic enzymes to one-half of those obtained in the animals fed a lignan-free diet. However, no significant differences in these parameters were seen between rats fed sesamin and episesamin. Responses to sesamin and episesamin of hepatic lipogenesis are, therefore, considerably different from those observed in fatty acid oxidation. These results show that the physiological activity of the commercial sesamin preparation containing equivalent amounts of both sesamin and episesamin in increasing hepatic fatty acid oxidation observed previously was mainly ascribable to that of episesamin but not to sesamin.

Keywords: Sesame lignan; Sesamin; Episesamin; β-oxidation; Rat liver

1. Introduction

Sesame seed contains considerable amounts of compounds collectively called as lignans. Sesamin is one of the most abundant lignan compounds and is epimerized during the acid-clay bleaching in the oil refining process to form episesamin [1] (Fig. 1). Therefore, commercial sesamin preparation contains both sesamin and episesamin at about 1:1 ratio. It has been demonstrated that this sesamin preparation exerts various physiological activities including being an anti-oxidant [2] and anti-carcinogen [3], blood pressure-lowering [4] and serum lipid-lowering [5–7] in experimental animals and humans, and it is generally considered that the physiological activity of this preparation is attributed to that of sesamin. However, there have been a lack of studies in which the physiological activities of sesamin and episesamin were compared. We demonstrated previously [7] that the sesamin preparation containing equivalent amounts of both sesamin and episesamin greatly increased the activity and gene expression of fatty acid oxidation enzymes at a dietary level of 0.1–0.5% in the rat liver. We also showed that the sesamin preparation caused a significant decrease in the activity and gene expression of hepatic fatty acid synthase and pyruvate kinase, the lipogenic enzymes. Therefore, reciprocal alterations in hepatic fatty acid oxidation and synthesis can account for the lipid-lowering effect of the sesamin preparation containing both sesamin and episesamin [7]. However, it is still uncertain which component of the sesamin preparation, sesamin or

Abbreviations: PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PPAR, peroxisome proliferator activated receptor; VLDL, very-low density lipoprotein.

* Corresponding author. Tel.: +81-298-38-8083; fax: +81-298-38-7996.

E-mail address: kushirom@nfri.affrc.go.jp (M. Kushiro).

0955-2863/02/$ – see front matter
PII: S0955-2863(01)00224-8

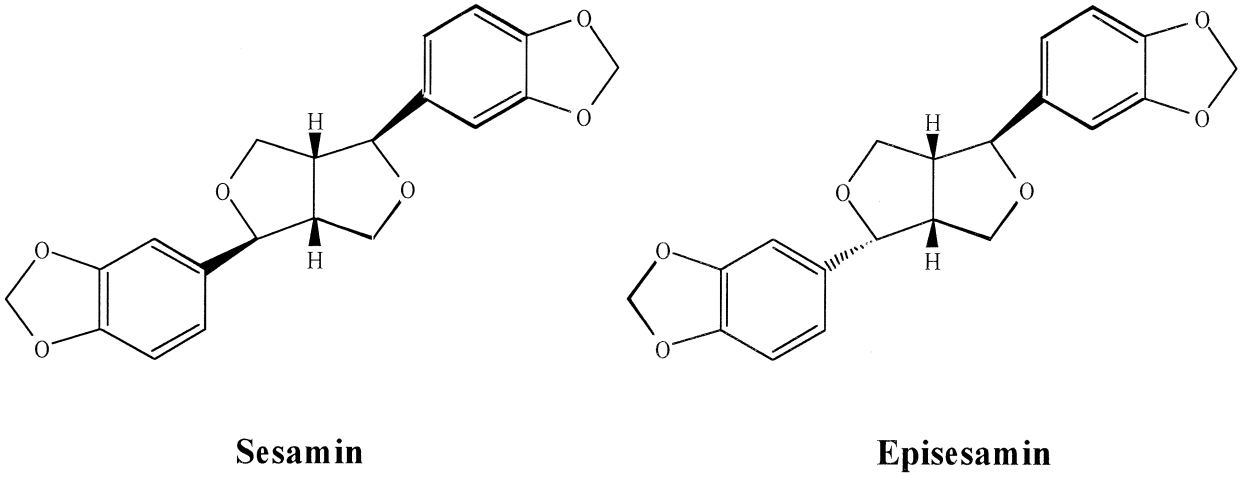

Fig. 1. Chemical structures of sesamin and episesamin.

episesamin, causes profound alteration in hepatic fatty acid metabolism. In this context, we prepared sesamin and episesamin preparations both free of their corresponding geometrical isomers, and examined their effects on the activity and gene expression of hepatic enzymes in fatty acid oxidation and synthesis in the rat.

2. Materials and methods

2.1. Materials

Malonyl-CoA was purchased from Sigma Chemical (St. Louis, MO). Acyl-CoA compounds used as substrates for enzyme activity assays were prepared as detailed previously [8,9]. Bovine serum albumin fraction V (essentially fatty acid-free) was a product of Boehringer Mannheim (Mannheim, Germany). [1-^{14}C]Palmitic acid, [α-^{32}P]dCTP and nylon filtrate (Hybond N^{+}) were purchased from Amersham International (Little Chalfont, Buckinghamshire, UK). Sesamin preparation with a purity of approximately 90% was prepared by molecular distillation of the scum obtained during the deodorizing process of sesame seed oil refinement. This preparation (200 g) was treated in ethanoic HCl solution to epimerize sesamin to form episesamin according to the procedures described by Beroza [10]. Reaction products were recrystallized in ethanol and subjected to silicic acid column chromatography using a solvent mixture of chloroform and methanol (9:1, v/v) to separate out sesamin and episesamin. Sesamin and episesamin obtained after this process were recrystallized in ethanol. Approximately 35 g each of sesamin and episesamin was obtained following these treatments. The sesamin and episesamin preparations were analyzed by high-performance liquid chromatography using a Capsel Pak AG 120 A C18 column (250 mm $\times$ 4.6 mm, Shiseido, Tokyo, Japan) with a mobile phase of methanol:water 7:3 v/v at a flow rate of 0.75 ml/min and monitoring at a wave length of 290 nm [11]. The analysis revealed that the sesamin and episesamin preparations contained negligible amounts of their corresponding geometrical isomers and purities were estimated to exceed 98%.

2.2. Animals and diets

Male Sprague-Dawley rats obtained from Charles River Japan, (Kanagawa, Japan) were housed individually in a room with controlled temperature (20–22°C), humidity (55–65%) and lighting (lights on from 7.00 to 19.00 hr), and fed on a commercial non-purified diet (type NMF, Oriental Yeast Co., Tokyo, Japan). After 7 days of acclimatization to the housing conditions, the rats were randomly divided into 3 groups consisted of 7–8 animals and fed either a purified control diet free of lignans or diets containing 0.2% of sesamin or episesamin for 15 days. The basal composition of the experimental diet was as follows (in weight %): casein, 20; palm oil, 10; cellulose, 2; mineral mixture [12], 3.5; vitamin mixture [12], 1.0; DL-methionine, 0.3; choline bitartrate, 0.2 and sucrose to 100. Experimental diets were served in the form of powder. We employed palm oil and sucrose as a dietary fat and carbohydrate sources, respectively, in the current study to compare the physiological activity of lignans to influence hepatic metabolism. Our preceding studies [8,9,13] demonstrated that hepatic fatty acid oxidation rate was considerably lower in rats fed palm oil than in the animals fed polyunsaturated fats. In addition, this saturated fat causes higher rate of hepatic lipogenesis [8,9,13]. Also, dietary sucrose compared to starch or dextrose increases hepatic lipogenesis. Thus, these dietary manipulations may facilitate the comparison of the physiological activity of lignans to enhance hepatic fatty acid oxidation and to decrease fatty acid synthesis. Lignans were added to the experimental diets in lieu of sucrose. We followed the guide of our institute in the care and use of laboratory animals.

2.3. Enzyme assays

After 15 days of the experimental period, rats were anesthetized using diethyl ether and killed by bleeding from the abdominal aorta. The livers were then quickly excised. About 2 g of each liver was homogenized with 15 ml of 0.25 mol/L sucrose containing 1 mmol/L EDTA and 3 mmol/L Tris-HCl (pH 7.2). The liver homogenates were centrifuged at 200,000 g for 30 min to obtain cytosols. Mitochondrial and peroxisomal palmitoyl-CoA oxidation rates as well as the activity of fatty acid oxidation enzymes were analyzed using whole liver homogenates as an enzyme source as described previously [7–9]. Cytosols were used as an enzyme source for the analysis of the activity of lipogenic enzymes [7–9]. The mitochondrial palmitoyl-CoA oxidation rate was measured on the day of slaughter using fresh enzyme preparations and other enzymes were analyzed using enzyme preparations stored at −30°C for up to 10 days.

2.4. RNA analysis

Hepatic RNA was extracted using the acid guanidium thiocyanate-phenol-chloroform method [14] and the mRNA levels of enzymes in fatty acid metabolism were analyzed by slot-blot and Northern-blot hybridization as detailed elsewhere [7,13]. Obtained values were corrected for those of a house-keeping gene (glyceraldehyde-3-phosphate dehydrogenase). Specific cDNA probes used to detect mRNAs of enzymes in fatty acid oxidation and synthesis except for acetyl-CoA carboxylase and ATP-citrate lyase were all the same as described elsewhere [7]. Reverse transcription and polymerase chain reaction was used to prepare cDNA probes for acetyl-CoA carboxylase and ATP-citrate lyase [7]. Specific up- and down-stream primers used were 5′-TGAGGTCCAGCATGTCCGG-3′ and 5′-CATGGCAA TCTGGAGCTGTG-3′ for acetyl-CoA carboxylase (length of PCR product was 1175 bp), and 5′-CTCAGCCATCCAGAAC CGG-3′ and 5′-AGGAAGTTGGCAGTGTGAGC-3′ for ATP-citrate lyase (1245 bp), respectively. As we confirmed the specificity of our cDNA probes by Northern-blot analysis, mRNA abundances were estimated using slot-blot hybridization.

2.5. Lipids analysis

Serum triacylglycerol, cholesterol, phospholipid, free fatty acid and glucose concentrations were assayed using commercial enzyme kits (Wako Pure Chemicals, Osaka, Japan). Liver lipids were extracted and purified [15]. Triacylglycerol, cholesterol and phospholipid contents in the lipid extract were analyzed as described previously [16].

2.6. Statistical analysis

Data were analyzed by the method of Snedecor and Cochran [17]. Thus, the Levene's test was adopted to inspect the constancy of variance of the observations. In cases where variances were found to be heterogeneous with this method, observations were transformed logarithmically. Subsequent Levene's test showed that the logarithmic transformations successfully rendered the variances of these data to be constant, and these transformed values were used for the subsequent statistical examinations. The data were then analyzed by one-way analysis of variance to establish the significance of the effect of dietary lignans before the examination of the significant differences of means. Examinations of significant differences of means were established using Tukey's test at the level of $P < 0.05$ [17]. All values were expressed as the mean ± SEM.

3. Results

3.1. Activity and gene expression of hepatic fatty acid oxidation enzymes

There were no significant differences in food intake (20–21 g/day) and growth (130–135 g/15 days) among the groups. Liver weight was significantly higher in the rats fed episesamin (6.58 ± 0.14 g/100 g body weight) than in those fed sesamin (5.55 ± 0.19 g/100 g body weight) or a lignan-free control diet (5.56 ± 0.18 g/100 g body weight).

Since the liver weights were significantly different among the groups, the enzyme activity was expressed as total activity (μmol/min per liver of 100 g body weight) (Tables 1 and 2). Diets containing sesame lignan compared to a control diet free of lignan significantly increased the mitochondrial palmitoyl-CoA oxidation rate (Table 1). The value was, however, significantly lower in rats fed sesamin than in those fed episesamin. Sesamin and episesamin also significantly increased the peroxisomal palmitoyl-CoA oxidation rate. The extent of increase was much more exaggerated with episesamin (5.1-fold) than with sesamin (1.6-fold).

We also analyzed the activities of various enzymes in the β-oxidation cycle and the auxiliary pathway. Although both sesamin and episesamin were effective in increasing all the enzyme activities in fatty acid oxidation, the magnitude of the responses was again more exaggerated with episesamin than with sesamin. Sesamin rather moderately increased the activities of fatty acid oxidation enzymes (1.3- to 2.1-fold), while episesamin more profoundly increased it (2.2- to 6.0-fold).

To confirm the result obtained with enzyme activity, we analyzed the mRNA levels of various fatty acid oxidation enzymes by slot-blot hybridization using specific cDNA probes. The values were corrected for those of a house-keeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The values for GAPDH were indistinguishable among the groups, and were 100 ± 7, 88.7 ± 6.3 and 101 ± 6% for rats fed lignan-free, sesamin and episesamin diets, respectively. Great diversity is characteristic of the β-oxi-

Table 1
Effects of sesame lignans on the activity and gene expression of fatty acid oxidation enzymes ([a,b,c]Values in a line not sharing a common superscript letter are significantly different at $P < 0.05$, n = 7–8)

	Groups		
	Lignan-free	Sesamin	Episesamin
Enzyme activity (μmol/min per liver of 100 g body weight)			
Palmitoyl-CoA oxidation			
Mitochondrial	1.67 ± 0.15[a]	2.89 ± 0.11[b]	3.83 ± 0.14[c]
Peroxisomal	1.69 ± 0.11[a]	2.76 ± 0.10[b]	8.56 ± 0.59[c]
Carnitine palmitoyltransferase	3.19 ± 0.30[a]	5.13 ± 0.29[b]	9.57 ± 0.55[c]
Acyl-CoA oxidase	1.21 ± 0.08[a]	1.78 ± 0.10[b]	7.20 ± 0.29[c]
3-Hydroxyacyl-CoA dehydrogenase	560 ± 24[a]	736 ± 52[b]	1493 ± 58[c]
3-Ketoacyl-CoA thiolase	176 ± 18[a]	253 ± 11[b]	390 ± 36[c]
Δ^3, Δ^2-enoyl-CoA isomerase	68.7 ± 2.1[a]	129 ± 6[b]	199 ± 6[c]
2,4-dienoyl-CoA reductase	2.37 ± 0.18[a]	5.06 ± 0.22[b]	10.5 ± 0.6[c]
mRNA level (%)			
Mitochondrial enzymes			
Carnitine palmitoyltransferase II	100 ± 0.2[a]	146 ± 9[b]	210 ± 9[c]
Long-chain acyl-CoA dehydrogenase	100 ± 12[a]	128 ± 8[b]	150 ± 6[b]
Trifunctional enzyme subunit α	100 ± 6[a]	180 ± 12[b]	282 ± 11[c]
Trifunctional enzyme subunit β	100 ± 12[a]	170 ± 19[b]	278 ± 28[c]
Mitochondrial 3-ketoacyl-CoA thiolase	100 ± 10[a]	184 ± 14[b]	278 ± 16[c]
Short-chain Δ^3, Δ^2-enoyl-CoA isomerase	100 ± 6[a]	222 ± 28[b]	661 ± 47[c]
2,4-dienoyl-CoA reductase	100 ± 9[a]	280 ± 22[b]	313 ± 32[c]
Peroxisomal enzymes			
Carnitine octanoyltransferase	100 ± 8[a]	131 ± 11[a]	173 ± 18[b]
Acyl-CoA oxidase	100 ± 5[a]	167 ± 15[a]	412 ± 32[c]
Peroxisomal bifunctional enzyme	100 ± 7[a]	256 ± 19[a]	1447 ± 130[b]
Peroxisomal 3-ketoacyl-CoA thiolase	100 ± 7[a]	217 ± 23[b]	491 ± 50[c]

All values are the mean ± SEM.

dation pathway [18]. The distinct enzyme species catalyzed the sequential enzyme reactions of mitochondrial and peroxisomal β-oxidation pathway. Moreover, several enzyme species differing in the substrate specificity are involved at each enzyme step of β-oxidation cycle and the auxiliary pathway at least in mitochondria. Acyl-CoA oxidase activity is present in peroxisomes but not in mitochondria. However, the activity of other fatty acid oxidation enzymes measured in the whole homogenate in the present study may represent sum of the activity of various enzymes located in both mitochondria and peroxisomes at a given condition of enzyme assay. Carnitine palmitoyltransferase activity is generally regarded to be specific in mitochondria. But there is still the possibility that this enzyme activity contains peroxisomal carnitine octanoyltransferase activity [19]. cDNA probe specific for each enzyme mRNA, however, can discriminate response of gene expression of individual enzymes. Both sesamin and episesamin increased the

Table 2
Effects of sesame lignans on the activity and gene expression of lipogenic enzymes ([a,b,c]Values in a line not sharing a common superscript letter are significantly different at $P < 0.05$, n = 7–8)

	Groups		
	Lignan-free	Sesamin	Episesamin
Enzyme activity (μmol/min per liver of 100 g body weight)			
Fatty acid synthase	28.6 ± 3.1[b]	11.7 ± 1.0[a]	13.7 ± 2.2[a]
ATP-citrate lyase	53.2 ± 4.4[b]	25.8 ± 3.0[a]	24.4 ± 2.7[a]
Glucose-6-phosphate dehydrogenase	77.9 ± 6.4[b]	43.3 ± 5.2[a]	37.1 ± 5.4[a]
Pyruvate kinase	250 ± 23[c]	157 ± 11[b]	96.8 ± 10.7[a]
mRNA level (%)			
Acetyl-CoA carboxylase	100 ± 7[b]	64.6 ± 3.9[a]	57.0 ± 3.0[a]
Fatty acid synthase	100 ± 14[b]	35.8 ± 4.8[a]	41.2 ± 8.7[a]
ATP-citrate lyase	100 ± 10[b]	53.4 ± 4.9[a]	59.1 ± 6.4[a]
Glucose-6-phosphate dehydrogenase	100 ± 6[c]	58.4 ± 3.5[b]	44.7 ± 2.8[a]
L-pyruvate kinase	100 ± 9[b]	50.8 ± 5.7[a]	35.4 ± 3.0[a]

All values are the mean ± SEM.

Table 3
Effects of sesame lignans on concentrations of serum components and liver lipids ([a,b,c]Values in a line not sharing a common superscript letter are significantly different at $P < 0.05$, n = 7–8)

	Groups		
	Lignan-free	Sesamin	Episesamin
Serum components (mmol/L)			
Triacylglycerol	3.83 ± 0.82[b]	1.77 ± 0.16[a]	1.79 ± 0.10[a]
Cholesterol	3.20 ± 0.22[b]	2.55 ± 0.14[a]	2.54 ± 0.18[a]
Phospholipid	3.36 ± 0.23[b]	2.60 ± 0.16[a]	2.64 ± 0.12[a]
Free fatty acid	1.35 ± 0.25	0.989 ± 0.096	0.98 ± 0.165
Glucose	4.94 ± 0.21	4.35 ± 0.60	4.31 ± 0.18
Liver lipids (μmol/liver of 100 g body weight)			
Triacylglycerol	298 ± 59	212 ± 26	291 ± 37
Cholesterol	41.1 ± 4.2	41.1 ± 2.1	43.8 ± 2.5
Phospholipid	242 ± 11[a]	253 ± 6[a]	364 ± 10[b]

All values are the mean ± SEM.

mRNA levels of mitochondrial and peroxisomal fatty acid oxidation enzymes. Again, the increases were considerably greater with episesamin than with sesamin. Sesamin increased the gene expression of fatty acid oxidation enzymes 1.3 to 2.8-fold, while episesamin increased these values 1.5 to 14-fold. All the values except for long-chain acyl-CoA dehydrogenase were significantly higher in rats fed episesamin than in those fed sesamin.

3.2. Activity and gene expression of lipogenic enzymes

The activity of hepatic lipogenic enzymes including fatty acid synthase, ATP-citrate lyase, glucose 6-phosphate dehydrogenase and pyruvate kinase in rats fed diets containing sesamin and episesamin became about one-half of those in the animals fed a control diet free of these lignans (Table 2). However, in contrast to the situation in fatty acid oxidation enzymes, the values were comparable between rats fed sesamin and episesamin except for those of pyruvate kinase. The pyruvate kinase activity was significantly lower in rats fed episesamin than in those fed sesamin.

We also analyzed the gene expression of enzymes involved in fatty acid synthesis by slot-blot hybridization. With respect to pyruvate kinase, there are four types named L, M_1, M_2 and R in mammals [20]. As L type is the major isozyme in the liver, we used cDNA for L type as a probe to detect hepatic pyruvate kinase mRNA. Diets containing sesamin and episesamin reduced mRNA levels of lipogenic enzymes including acetyl-CoA carboxyalse, fatty acid synthase, ATP-citrate lyase, glucose 6-phosphate dehydrogenase and pyruvate kinase to about one-half of those obtained with a control diet. Consistent with the results obtained with the enzyme activity, no significant differences in these parameters except for those of glucose 6-phosphate dehydrogenase were observed between the animals fed sesamin and episesamin. Glucose 6-phosphate dehydrogenase mRNA level was significantly lower in rats fed episesamin than in those fed sesamin.

3.3. Serum and liver lipid levels

Diets containing sesamin and episesamin, compared to a lignan-free diet, significantly decreased the serum concentrations of triacylglycerol, cholesterol and phospholipid (Table 3). No significant differences in these parameters were seen between the animals fed sesamin and episesamin. Dietary lignans did not affect the serum free fatty acid and glucose concentrations.

No significant differences in the liver triacylglycerol and cholesterol contents were seen among the groups. The hepatic phospholipid content became significantly higher in the episesamin group than in other groups.

4. Discussion

We previously demonstrated that the sesamin preparation containing equivalent amounts of sesamin and episesamin caused profound increases in the activity and gene expression of hepatic fatty acid oxidation enzymes [7]. However, the physiological activities of the individual components of the sesamin preparation, sesamin and episesamin, remained unclarified. In the present study, we showed that both of these compounds were effective in increasing enzyme activity in hepatic fatty acid oxidation. We also clearly demonstrated that episesamin compared to sesamin is much more effective in increasing hepatic β-oxidation activity. This observation was further sustained by the results obtained with the analysis of mRNA levels of enzymes involved in the fatty acid oxidation pathway. Therefore, episesamin rather than sesamin in the sesamin preparation containing both lignans may primarily be responsible for its physiological activity in increasing hepatic fatty acid oxidation as observed previously [7].

It is a general consensus that peroxisome proliferator activated receptor (PPAR), a member of the nuclear receptor superfamily, is involved in the regulation of the gene expression of fatty acid oxidation enzymes [21]. It has been

demonstrated that diverse xenobiotics called peroxisome proliferators are the ligands to activate PPAR and hence increase hepatic fatty acid oxidation [21]. Typical peroxisome proliferators such as the hypolipidemic drugs clofibric acid and Wy 14, 643, and the industrial plasticiser monoethylhexyl phtahlate possess a carboxylic acid group in their structure [22]. Although sesamin and episesamin do not contain a carboxylic acid group in their structure, the current and previous [7] observations support the consideration that the sesamin and episesamin are the ligands of PPAR to induce fatty acid oxidation enzyme gene expression in the liver. There is the possibility that the efficacy of sesamin and episesamin as the ligands to activate PPAR may be mutually different each other and hence cause divergent effects on gene expression and the activity of hepatic fatty acid oxidation enzymes. Studies to compare sesamin- and episesamin-dependent activation of PPAR using a cotransfection assay in cultured cells [21,23] or a ligand-binding assay [24] are required to confirm this possibility.

Umeda-Sawada et al. [11] studied the metabolic fate of sesamin and episesamin following the administration of the sesamin preparation containing equivalent amounts of these compounds. Sesamin and episesamin concentrations in various tissues reached a plateau at around 3–6 hrs after an administration of the sesamin preparation and rapidly decreased thereafter. Despite the fact that the sesamin preparation contained equivalent amounts of sesamin and episesamin, the concentrations of episesamin in the tissues were more than 2-fold higher than those of sesamin during the 1–9 hrs after an administration. They also showed that the rates of lymphatic transport of sesamin and episesamin were indistinguishable from each other. Therefore, there is the possibility that differences in metabolic fate rather than the efficacy to activate PPAR between sesamin and episesamin are responsible for the divergent effects of these compounds in increasing hepatic fatty acid oxidation.

We demonstrated in the previous study [7] that the sesamin preparation containing both sesamin and episesamin decreased hepatic activity and gene expression of fatty acid synthase and pyruvate kinase, the lipogenic enzymes. Therefore, we suggested that the sesamin preparation, unlike the other peroxisome proliferators [25,26], has a unique propensity in decreasing hepatic fatty acid synthesis. In the present study, we confirmed that both sesamin and episesamin lowered the activity and gene expression of these enzymes. Moreover, we demonstrated that these lignans also lowered the activity and gene expression of ATP-citrate lyase and glucose 6-phosphate dehydrogenase. Although we have not measured the enzyme activity, these compounds decreased the gene expression of acetyl-CoA carboxylase as well. Therefore, it is apparent that both sesamin and episesamin have a propensity in decreasing hepatic fatty acid synthesis. However, in contrast to the observation made in fatty acid oxidation enzyme activity and gene expression, these compounds were equally effective in decreasing the parameters for fatty acid synthesis. It is considered that the gene expression of hepatic lipogenic enzymes is controlled by the mechanism independent of PPAR [25–27]. Therefore, it is not surprising that sesamin and episesamin are equally effective in decreasing hepatic lipogenesis despite the fact that these compounds affect hepatic fatty acid oxidation differently. We showed in the previous study [7] that the sesamin preparation containing both sesamin and episesamin dose-dependently lowered the activity and gene expression of fatty acid synthase and pyurvate kinase. These values in rats fed a diet containing 0.2% of the sesamin-episesamin mixture reached one-half of those observed in the animals fed a lignan-free diet, but no further decreases occurred in the diets containing 0.5% of the preparation. In the present study, diets containing 0.2% of sesamin or episesamin lowered the activity and gene expression of lipogenic enzymes to about one-half of those observed in the animals fed a lignan-free diet. Therefore, it is expected that these lignans at this dietary level already exerted maximal physiological activity in decreasing hepatic lipogenesis. There is still the possibility, however, that sesamin and episesamin exert different effects on fatty acid synthesis at dietary levels lower than 0.2%. Detailed studies are still required to clarify this issue.

Alterations in hepatic fatty acid synthesis and oxidation are the crucial factors affecting the assembly and secretion of very-low density lipoproteins (VLDL) and hence modify serum lipid levels [28–30]. In fact, sesamin- and episesamin-dependent changes in hepatic fatty acid synthesis and oxidation accompanied the decreases in serum lipid levels. Despite the fact that episesamin increased hepatic fatty acid oxidation more than sesamin did, the serum lipid levels were comparable between the animals fed these compounds. Therefore, it is possible that decreases in the activity and gene expression of lipogenic enzymes rather than the increases in that of fatty acid oxidation enzymes may primarily be responsible for the serum lipid-lowering effects of these lignans. Also, there is the possibility that sesame lignans affect intestinal absorption of lipids [6] or the gene expression of proteins other than those involved in fatty acid metabolism that regulate VLDL synthesis and secretion [31] and hence reduce serum lipid levels. In this context, dietary lignans failed to affect hepatic triacylglycerol content in the present study in spite that they increased hepatic fatty acid oxidation and decreased fatty acid synthesis. The result may indicate that dietary lignans not only decrease triacylglycerol synthesis but also decrease secretion of this lipid molecule as VLDL resulting in unalteration of hepatic triacylglycerol content. Therefore, detailed comparative studies regarding the effects of sesamin and episesamin on these processes are still required.

In conclusion, we demonstrated that episesamin compared to sesamin at a 0.2% dietary level is more competent in increasing activity and gene expression of hepatic fatty acid oxidation enzymes of rats. Therefore, the physiological activity of the preparation containing equivalent amounts of both sesamin and episesamin in increasing hepatic fatty acid

oxidation as observed previously [7] is primarily ascribable to that of episesamin rather than sesamin. These compounds also lowered the activities and gene expression of lipogenic enzymes, but no differences in these parameters were seen between rats fed sesamin and episesamin. Therefore, responses to sesamin and episesamin of the enzymes in hepatic fatty acid oxidation and synthesis are mutually different to each other.

References

[1] Y. Fukuda, M. Nagata, T. Osawa, M. Namiki, Contribution of lignan analogues to antioxidative activity of refined unroasted sesame seed oil, J. Am. Oil Chem. Soc. 63 (1986) 1027–1031.

[2] K. Yamashita, M. Kagaya, N. Higuti, Y. Kiso, Sesamin and alpha-tocopherol synergistically suppress lipid-peroxide in rats fed a high docosahexaenoic acid diet, Biofactors 11 (2000) 11–13.

[3] N. Hirose, F. Doi, T. Ueki, K. Akazawa, K. Chijiiwa, M. Sugano, K. Akimoto, S. Shimizu, H. Yamada, Suppressive effect of sesamin against 7,12-dimethylbenz[a]-anthracene induced rat mammary carcinogenesis, Anticancer Res. 12 (1992) 1259–1265.

[4] Y. Matsumura, S. Kita, Y. Tanida, Y. Taguchi, S. Morimoto, K. Akimoto, T. Tanaka, Antihypertensive effect of sesamin. III. Protection against development and maintenance of hypertension in stroke-prone spontaneously hypertensive rats, Biol. Pharm. Bull. 21 (1998) 469–473.

[5] F. Hirata, K. Fujita, Y. Ishikura, K. Hosoda, T. Ishikawa, H. Nakamura, Hypocholesterolemic effect of sesame lignan in humans, Atherosclerosis 122 (1996) 135–136.

[6] N. Hirose, T. Inoue, K. Nishihara, M. Sugano, K. Akimoto, S. Shimizu, H. Yamada, Inhibition of cholesterol absorption and synthesis in rats by sesamin, J. Lipid Res. 32 (1991) 629–638.

[7] L. Ashakumary, I. Rouyer, Y. Takahashi, T. Ide, N. Fukuda, T. Aoyama, T. Hashimoto, M. Mizugaki, M. Sugano, Sesamin, a sesame lignan, is a potent inducer of hepatic fatty acid oxidation in the rat, Metabolism 1999;48:1303–1313.

[8] T. Ide, M. Murata, M. Sugano, Stimulation of the activities of hepatic fatty acid oxidation enzymes by dietary fat rich in α-linolenic acid in rats, J. Lipid Res. 37 (1996) 448–463.

[9] Y. Kabir, T. Ide, Activity of hepatic fatty acid oxidation enzymes in rats fed α-linolenic acid, Biochim. Biophys. Acta 1304 (1996) 105–119.

[10] M. Beroza, Epiasarinin, a diastereomer of sesamin and asarinin, Stereochemistry of, 2,6-diaryl-*cis*-3,7-dioxabicyclo [3.3.0]octane, J. Am. Chem. Soc. 78 (1956) 5082–5084.

[11] R. Umeda-Sawada, M. Ogawa, O. Igarashi, The metabolism and distribution of sesame lignans (sesamin and episesamin) in rats, Lipids 34 (1999) 633–637.

[12] American Institute of Nutrition, Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies, J. Nutr. 107 (1977) 1340–1348.

[13] T. Ide, H. Kobayashi, L. Ashakumary, I.A. Rouyer, Y. Takahashi, T. Aoyama, T. Hashimoto, M. Mizugaki, Comparative effects of perilla and fish oils on the activity and gene expression of fatty acid oxidation enzymes in rat liver, Biochim. Biophys. Acta 1485 (2000) 23–35.

[14] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction, Anal. Biochem. 162 (1987) 156–159.

[15] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226 (1957) 497–509.

[16] T. Ide, H. Okamatsu, M. Sugano, Regulation by dietary fats of 3-hydroxy-3-methylglutaryl-coenzyme A reductase in rat liver, J. Nutr. 108 (1978) 601–612.

[17] G.W. Snedecor, W.G. Cochran, Statistical methods. 8th ed. Ames, IA: Iowa State University Press, 1989.

[18] T. Hashimoto, Peroxisomal beta-oxidation enzymes, Cell. Biochem. Biophys. 32 (2000) 63–72.

[19] S.J. Choi, D.H. Oh, C.S. Song, A.K. Roy, B. Chatterjee, Molecular cloning and sequence analysis of the rat liver carnitine octanoyltransferase cDNA, its natural gene and the gene promoter, Biochim. Biophys. Acta 1264 (1995) 215–222.

[20] H. Inoue, T. Noguchi, T. Tanaka, Complete amino acid sequence of rat L-type pyruvate kinase deduced from the cDNA sequences, Eur. J. Biochem. 154 (1986) 465–469.

[21] K. Schoonjans, B. Staels, J. Auwerx, Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression, J. Lipid Res. 37 (1996) 907–925.

[22] C.D. Soontjens, J.J. Rafter, J.Å. Gustafsson, Ligands for orphan receptors?, J. Endocrinol. 150 (1996) S241–S257.

[23] S.A. Kliewer, B.M. Forman, B. Blumberg, E.S. Ong, U. Borgmeyer, D.J. Mangelsdorf, K. Umesono, R.M. Evans, Differential expression and activation of a family of murine peroxisome proliferator-activated receptors, Proc. Natl. Acad. Sci. USA 91 (1994) 7355–7359.

[24] G. Krey, O. Braissant, F. L'Horset, E. Kalkhoven, M. Perroud, M.G. Parker, W. Wahli, Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay, Mol. Endcrinol. 11 (1997) 779–791.

[25] T. Aoyama, J.M. Peters, N. Iritani, T. Nakajima, K. Furihata, T. Hashimoto, F.J. Gonzalez, Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor α (PPARα), J. Biol. Chem. 273 (1998) 5678–5684.

[26] D.B. Jump, S.D. Clarke, Regulation of gene expression by dietary fat, Annu. Rev. Nutr. 19 (1999) 63–90.

[27] B. Ren, A.P. Thelen, J.M. Peters, F.J. Gonzalez, D.B. Jump, Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor α, J. Biol. Chem. 272 (1997) 26827–26832.

[28] H.G. Windmueller, A.E. Spaeth, *De novo* synthesis of fatty acid in perfused rat liver as a determinant of plasma lipoprotein production, Arch. Biochem. Biophys. 122 (1967) 362–369.

[29] T. Ide, J.A. Ontko, Increased secretion of very low density lipoprotein-triglyceride following inhibition of long chain fatty acid oxidation in isolated rat liver, J. Biol. Chem. 256 (1981) 10247–10255.

[30] T. Ide, H. Oku, M. Sugano, Reciprocal response to clofibrate in ketogenesis and triglyceride and cholesterol secretion in isolated rat liver, Metabolism 31 (1982) 1065–1072.

[31] H. Jamil, C.-H. Chu, J.K. Dickson Jr., Y. Chen, M. Yan, S.A. Biller, R.E. Gregg, J.R. Wetterau, D.A. Gordon, Evidence that microsomal triglyceride transfer protein is limiting in the production of apolopoprotein B-containing lipoproteins in hepatic cells, J. Lipid Res. 39 (1998) 1448–1454.