Sesamin, A Sesame Lignan, Is a Potent Inducer of Hepatic Fatty Acid Oxidation in the Rat

Lakshmikuttyamma Ashakumary, Isabelle Rouyer, Yoko Takahashi, Takashi Ide, Nobuhiro Fukuda, Toshifumi Aoyama, Takashi Hashimoto, Michinao Mizugaki, and Michihiro Sugano

The effects of sesamin, one of the most abundant lignans in sesame seed, on hepatic fatty acid oxidation were examined in rats that were fed experimental diets containing various amounts (0%, 0.1%, 0.2%, and 0.5%) of sesamin (a 1:1 mixture of sesamin and episesamin) for 15 days. Dietary sesamin dose-dependently increased both mitochondrial and peroxisomal palmitoyl-coenzyme A (CoA) oxidation rates. Mitochondrial activity almost doubled in rats on the 0.5% sesamin diet. Peroxisomal activity increased more than 10-fold in rats fed a 0.5% sesamin diet in relation to rats on the sesamin-free diet. Dietary sesamin greatly increased the hepatic activity of fatty acid oxidation enzymes, including carnitine palmitoyltransferase, acyl-CoA dehydrogenase, acyl-CoA oxidase, 3-hydroxyacyl-CoA dehydrogenase, enoyl-CoA hydratase, and 3-ketoacyl-CoA thiolase. Dietary sesamin also increased the activity of 2,4-dienoyl-CoA reductase and Δ^3,Δ^2-enoyl-CoA isomerase, enzymes involved in the auxiliary pathway for β-oxidation of unsaturated fatty acids dose-dependently. Examination of hepatic mRNA levels using specific cDNA probes showed a sesamin-induced increase in the gene expression of mitochondrial and peroxisomal fatty acid oxidation enzymes. Among these various enzymes, peroxisomal acyl-CoA oxidase and bifunctional enzyme gene expression were affected most by dietary sesamin (15- and 50-fold increase by the 0.5% dietary level). Sesamin-induced alterations in the activity and gene expression of carnitine palmitoyltransferase I and acyl-CoA oxidase were in parallel with changes in the mitochondrial and peroxisomal palmitoyl-CoA oxidation rate, respectively. In contrast, dietary sesamin decreased the hepatic activity and mRNA abundance of fatty acid synthase and pyruvate kinase, the lipogenic enzymes. However, this lignan increased the activity and gene expression of malic enzyme, another lipogenic enzyme. An alteration in hepatic fatty acid metabolism may therefore account for the serum lipid–lowering effect of sesamin in the rat.

SESAMIN, one of the most abundant lignans in sesame seed and oil, profoundly influences lipid metabolism in experimental animals. Sesamin feeding is associated with reduced serum levels of triacylglycerol,[1-3] cholesterol,[2-5] and phospholipid[1] in rodents. This compound is also effective to prevent an increase in serum triacylglycerol following ethanol consumption in the rat.[6] The cholesterol-lowering effect of sesamin is also demonstrated in humans.[7] In addition, dietary sesamin reduces hepatic concentrations of triacylglycerol[2,4,8] and cholesterol[1,4,5,8] but increases phospholipid levels accompanying liver hypertrophy,[1,2,4,8] although temporally.[5] With regard to the mechanism underlying the hypocholesterolemic effect of dietary sesamin, Hirose et al[4] demonstrated in rats that it increased fecal cholesterol excretion and reduced the hepatic activity of 3-hydroxy-3-methylglutaryl-coenzyme A (CoA) reductase, a rate-limiting enzyme in cholesterol biosynthesis. It is therefore plausible that dietary sesamin decreases serum cholesterol through combined effects on the intestinal absorption and hepatic synthesis of cholesterol.

A plausible alternative factor modifying serum lipid concentrations is a change in the rate of fatty acid synthesis and oxidation in the liver. Alterations in fatty acid synthesis[9] and oxidation[10,11] modify the availability of fatty acids for triacylglycerol synthesis, in turn altering very–low-density lipoprotein production by the liver. Diverse chemicals called peroxisome proliferators, which include the hypolipidemic agent clofibrate and related compounds, have been demonstrated to enhance hepatic fatty acid oxidation and consequently reduce serum lipid levels.[11-13] These compounds, like sesamin, cause liver hypertrophy accompanying an increase in the hepatic phospholipid level. We therefore hypothesized that sesamin is an inducer of hepatic fatty acid oxidation which thus reduces serum lipid levels. We demonstrate in the present study that sesamin greatly increases the activity and gene expression of hepatic mitochondrial and peroxisomal fatty acid oxidation enzymes. Although confirmation is still required, the results also indicate that sesamin, similar to clofibrate and related compounds, induces hepatic fatty acid oxidation through a mechanism involving the peroxisome proliferator–activated receptor (PPAR), a member of the nuclear receptor superfamily.

MATERIALS AND METHODS

Materials

Malonyl-CoA was purchased from Sigma Chemical (St Louis, MO). 3-Keto-octanoyl-CoA was enzymatically prepared according to the method of Thorpe.[14] Other acyl-CoA compounds used as substrates for enzyme activity assays were prepared as detailed previously.[15,16] Bovine serum albumin fraction V (essentially fatty acid–free) was obtained from Boehringer (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 (a 1:1 mixture of sesamin and episesamin) was a gift from Suntry (Osaka, Japan).

From the Laboratory of Nutrition Biochemistry, National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Tsukuba; Department of Biological Resource Sciences, Faculty of Agriculture, Miyazaki University, Miyazaki; Department of Biochemistry, Shinshu University School of Medicine, Nagano; Department of Pharmaceutical Sciences, Tohoku University Hospital, Miyagi; and Department of Human Life Sciences, Prefectural University of Kumamoto, Kumamoto, Japan.

Submitted January 14, 1999; accepted April 27, 1999.

Supported in part by the Bio-Renaissance Program of the Ministry of Agriculture, Forestry and Fisheries (BRP-98-I-A-2).

Address reprint requests to Takashi Ide, PhD, Laboratory of Nutrition Biochemistry, National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, 2-1-2 Kannondai, Tsukuba Science City 305-8642, Japan.

0026-0495/99/4810-0024$10.00/0

Animals and Diets

Male Sprague-Dawley rats obtained from Charles River (Kanagawa, Japan) were housed individually in a room with controlled temperature (20° to 22°C), humidity (55% to 65%), and lighting (lights on from 7 AM to 7 PM) and fed a commercial nonpurified diet (type NMF; Oriental Yeast, Tokyo, Japan). After 7 days of acclimatization to the housing conditions, rats were randomly divided into four groups and fed purified experimental diets containing various amounts of sesamin (0% to 0.5%) for 15 days. The basal composition of purified experimental diets was as follows (in weight %): 20 casein, 15 corn starch, 15 palm oil, 2 cellulose, 3.5 mineral mixture,[17] 1.0 vitamin mixture,[17] 0.3 DL-methionine, 0.2 choline bitartrate, and sucrose to 100. Sesamin was added to the experimental diets in lieu of sucrose.

Enzyme Assays

Upon termination of the experimental period, the rats were anesthetized using diethyl ether and killed by bleeding from the abdominal aorta. The livers were then quickly excised. About 3 g of each liver was homogenized with 7 vol 0.25-mol/L sucrose containing 1 mmol/L EDTA and 3 mmol/L Tris hydrochloride (pH 7.0) and centrifuged at 500 × *g* for 10 minutes. The supernatant fraction was centrifuged at 9,000 × *g* for 10 minutes to isolate the mitochondria. The mitochondrial fraction was washed twice with 0.25 mol/L sucrose containing 1 mmol/L EDTA and 3 mmol/L Tris hydrochloride (pH 7.0) and finally suspended in the same medium. The rates of mitochondrial and peroxisomal oxidation of fatty acids were measured in the whole-liver homogenate using a [1-^{14}C]palmitoyl-CoA substrate as detailed elsewhere.[15,16] The activity of hepatic fatty acid oxidation enzymes was analyzed as detailed previously.[15,16] Carnitine palmitoyltransferase I activity was measured radiochemically using freshly isolated mitochondria. Carnitine palmitoyltransferase activity was also measured spectrophotometrically in a freeze-thawed preparation of whole homogenate. The activity of acyl-CoA dehydrogenase was analyzed using isolated mitochondria as an enzyme source. The activities of other fatty acid oxidation enzymes were measured in the whole-liver homogenate. Malic enzyme, pyruvate kinase, and fatty acid synthase activities were measured using the 9,000 × *g* supernatant fraction of liver homogenate.[15,16,18] The activity of glutamate dehydrogenase, a marker enzyme for mitochondria, was measured in both the whole homogenate and isolated mitochondria as described elsewhere.[19] The rate of mitochondrial oxidation of fatty acids and the activity of carnitine palmitoyltransferase I were measured on the day of slaughter using fresh enzyme preparations, and other enzymes were analyzed using enzyme preparations stored at −40°C for up to 10 days.

Preparation of cDNA Probes

cDNA probes to detect the respective mRNAs for acyl-CoA oxidase,[20] peroxisomal bifunctional enzyme,[21] mitochondrial trifunctional enzyme subunits α and β,[22] and 2,4-dienoyl-CoA reductase[23] were all the same as described previously. Reverse transcription–polymerase chain reaction (PCR) was used to prepare cDNA probes for the other fatty acid oxidation and synthesis enzymes using a kit (Gene Amp RNA PCR kit; Perkin Elmer, Foster City, CA) according to the procedures recommended by the manufacturer, with slight modifications. Specific sense and antisense primers used for PCR preparation of the respective cDNAs are shown in Table 1. The primers were chosen from cDNA sequences available in the GenBank data base (accession number: carnitine palmitoyltransferase II, J05470; long-chain acyl-CoA dehydrogenase, J05029; peroxisomal 3-ketoacyl-CoA thiolase, J02749; short-chain Δ^3,Δ^2-enoyl-CoA isomerase, M61112; fatty acid synthase, J03514; and L-type pyruvate kinase, M17685), except for carnitine palmitoyltransferase I, mitochondrial 3-ketoacyl-CoA thiolase, and malic enzyme. The primers for malic enzyme were chosen from the cDNA sequence reported by Magnuson et al,[24] and primers for mitochondrial 3-ketoacyl-CoA thiolase were from the sequence reported by Arakawa et al.[25] The primers used for amplification of carnitine palmitoyltransferase I cDNA were the same as those reported by Asins et al.[26] DNA sequences of the amplification products were partially determined by direct sequencing to confirm their identities with reported cDNA sequences of corresponding enzymes. The PCR products were purified by agarose gel electrophoresis and used as probes to detect mRNAs for the respective enzymes. The cDNA probe for β-actin was provided by Wako Pure Chemicals (Osaka, Japan).

RNA Analysis

Hepatic RNA was extracted by the acid guanidium thiocyanate-phenol-chloroform method.[27] RNA samples (40 μg) were denatured and applied to a nylon membrane using a slot-blot apparatus (Bio-Rad Laboratories, Hercules, CA) and fixed with UV irradiation. Northern blot analysis was performed by standard procedures. RNA samples (40 μg) were denatured and electrophoresed on a 1.1% agarose gel containing 0.66 mol/L formaldehyde and then transferred to a nylon membrane and fixed with UV irradiation. RNAs on nylon membranes were hybridized with specific cDNA probes labeled with [α-^{32}P]dCTP and quantified by an imaging analyzer (Bio-Rad Laboratories).

Lipid Analysis

Serum and liver triacylglycerol and phospholipid concentrations were measured as described previously.[28] Serum cholesterol, free fatty acid, and glucose concentrations were measured using commercial enzyme kits (Wako Pure Chemicals). Liver cholesterol was analyzed as described previously.[11]

Statistical Analysis

The data were analyzed by the method of Snedecor and Cochran[29] as detailed elsewhere,[30] and significant differences in mean values were determined at a P level less than .05.

Table 1. Primers for PCR Preparation of cDNA Probes

Enzyme	Sense Primer	Antisense Primer
Carnitine palmitoyltransferase I	5′-GCAGGGATACAGAGAGGAGG-3′	5′-GGAAAGGTGAGTCGACTGC-3′
Carnitine palmitoyltransferase II	5′-ACACCAGCTACATCTCAGGC-3′	5′-TCGGGAAGTCATCTAGGCAG-3′
Long-chain acyl-CoA dehydrogenase	5′-GGAGAAGTGAGTAGAGAGCT-3′	5′-TGTCTCACGTAGTTCCTGGT-3′
Mitochondrial 3-ketoacyl-CoA thiolase	5′-AGGTGTGTTTATCGTTGCTGC-3′	5′-GCCTTAACTCGTGAACCAGG-3′
Short-chain Δ^3,Δ^2-enoyl-CoA isomerase	5′-TGACAAGAGCATCCGAGGTG-3′	5′-AGTTCTGGATGTTAGCCTCTC-3′
Peroxisomal 3-ketoacyl-CoA thiolase	5′-GTGAAGCCAAAGCCTGAGTG-3′	5′-TTCCCAGCTTCTCCACACAG-3′
Fatty acid synthase	5′-CTGAATCTGAGTATCCTGCTG-3′	5′-TGTTGATGATAGACTCCAGGC-3′
Malic enzyme	5′-CTATTGTGGTGACTGATGGAG-3′	5′-TCTGACACTTGCTGGGATATG-3′
L-Pyruvate kinase	5′-AAGTTCCAGACAAGGGGTGAT-3′	5′-ACGGTAGAGCAAGGGGAAGA-3′

RESULTS

There were no significant differences in food intake (21 to 23 g/d) and growth (147 to 153 g/15 d) among the rat groups. Dietary sesamin increased the liver weight of rats dose-dependently (5.65 ± 0.20, 5.69 ± 0.22, 6.21 ± 0.21, and 7.61 ± 0.23 g/100 g body weight for control, 0.1% sesamin, 0.2% sesamin, and 0.5% sesamin groups, respectively). The difference between the control group and the 0.5% sesamin group was statistically significant ($P < .05$).

Activity of Hepatic Fatty Acid Oxidation Enzymes

Since dietary sesamin considerably increased the liver weight of the animals, the enzyme activity was calculated as total activity (micromoles per minute per liver per 100 g body weight) and the results are presented as a percentage of the values in rats fed a sesamin-free diet (Figs 1 and 2). Similar sesamin effects on fatty acid oxidation enzyme activities were confirmed even when expressed in terms of the specific activity (nanomoles per minute per milligram of protein).

Dietary sesamin increased mitochondrial and peroxisomal fatty acid oxidation rates dose-dependently. The diet containing 0.5% sesamin nearly doubled the mitochondrial activity and increased the peroxisomal activity more than 10-fold compared with the diet free of sesamin (Fig 1).

We also examined the effect of sesamin on the activity of various enzymes of fatty acid oxidation in rat liver (Fig 2). The activity was analyzed using the whole homogenate as an enzyme source, except for carnitine palmitoyltransferase I and acyl-CoA dehydrogenase, for which the activity was measured using isolated mitochondria. To express the activity of these enzymes as total activity, the activity of a marker enzyme for mitochondria (glutamate dehydrogenase) was measured in both the whole homogenate and the mitochondrial fractions, and the recovery of cell organelles from the whole homogenate was calculated by the activity of glutamate dehydrogenase found in isolated mitochondria. Dietary sesamin increased the total activity of glutamate dehydrogenase in the liver homogenate dose-dependently (1,518 ± 86, 1,713 ± 68, 1,974 ± 92, and 2,073 ± 34 μmol/min/liver/100 g body weight for rats fed diets containing 0%, 0.1%, 0.2%, and 0.5% sesamin, respectively). Dietary sesamin considerably increased the recovery of mitochondria (24.8% ± 1.2%, 33.6% ± 3.9%, 36.9% ± 2.1%, and 48.3% ± 6.1% for rats fed diets containing 0%, 0.1%, 0.2%, and 0.5% sesamin, respectively). The differences in glutamate dehydrogenase activity and mitochondrial recovery between the control group and the 0.2% and 0.5% sesamin groups were statistically significant ($P < .05$). Dietary sesamin also increased the hepatic mitochondrial protein content. It was 341 ± 24 mg/liver/100 g body weight in rats fed a diet free of sesamin and increased as the dietary level of sesamin increased (364 ± 23, 428 ± 38, and 583 ± 50 mg/liver/100 g body weight in rats fed diets containing 0.1%, 0.2%, and 0.5% sesamin, respectively). The difference in mitochondrial protein content between the control group and the 0.5% sesamin group was statistically significant ($P < .05$).

Sesamin increased hepatic fatty acid oxidation enzyme activity dose-dependently (Fig 2A to I). Even at a 0.1% dietary level, sesamin detectably increased hepatic fatty acid oxidation enzyme activity, and significant differences relative to a sesamin-free diet were generally observed with diets containing 0.2% and 0.5% sesamin. However, the magnitude of the response to dietary sesamin of the respective enzymes was considerably different. The activities of carnitine palmitoyltransferase I, acyl-CoA dehydrogenase, and enoyl-CoA hydratase measured with crotonyl-CoA and *trans*-2-octenoyl-CoA substrates were two to 2.5 times higher in rats fed a 0.5% sesamin diet versus those fed a sesamin-free diet (Fig 2A, C, and E). Dietary sesamin more profoundly increased the activities of other enzymes. Carnitine palmitoyltransferase activity (Fig 2B) measured spectrophotometrically in a freeze-thawed preparation of whole homogenate was fourfold higher in rats fed a 0.5% sesamin diet versus those fed a sesamin-free diet. The magnitude of the response was therefore greater than that observed with carnitine palmitoyltransferase activity measured radio-

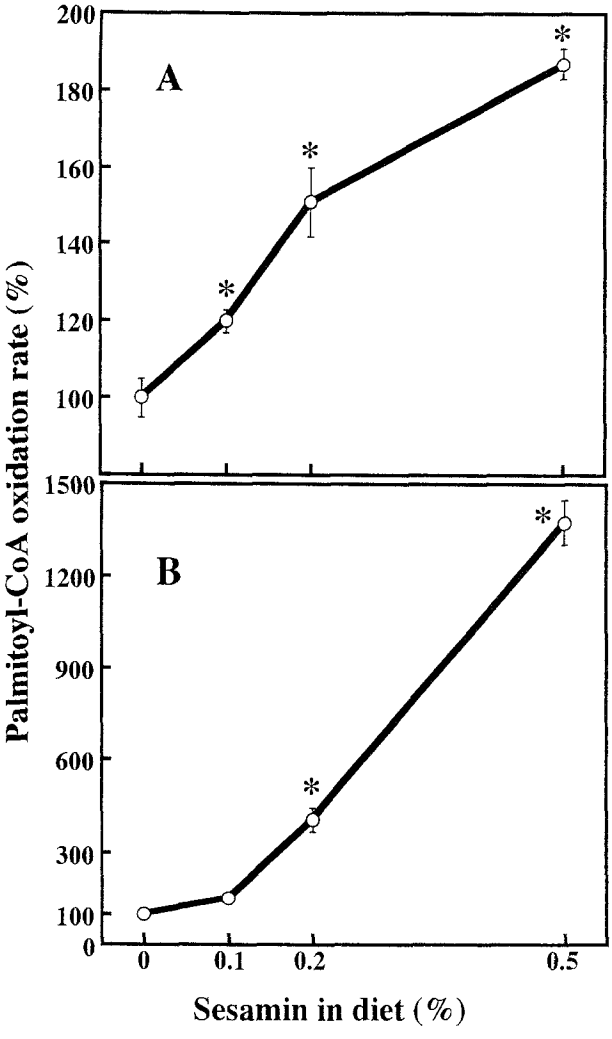

Fig 1. Effect of sesamin on mitochondrial (A) and peroxisomal (B) palmitoyl-CoA oxidation rate in rat liver. Rats were fed experimental diets containing various amounts of sesamin (0%-0.5%) for 15 days. Activities were calculated as total activity (μmol/min/liver/100 g body weight) and are presented as a percentage of the activity in rats fed a sesamin-free diet. Mitochondrial and peroxisomal palmitoyl-CoA oxidation rates in rats fed a sesamin-free diet were 2.17 ± 0.12 and 2.54 ± 0.11 μmol/min/liver/100 g body weight, respectively. Values are the mean ± SE of 7-8 rats. *$P < .05$ *v* sesamin-free group.

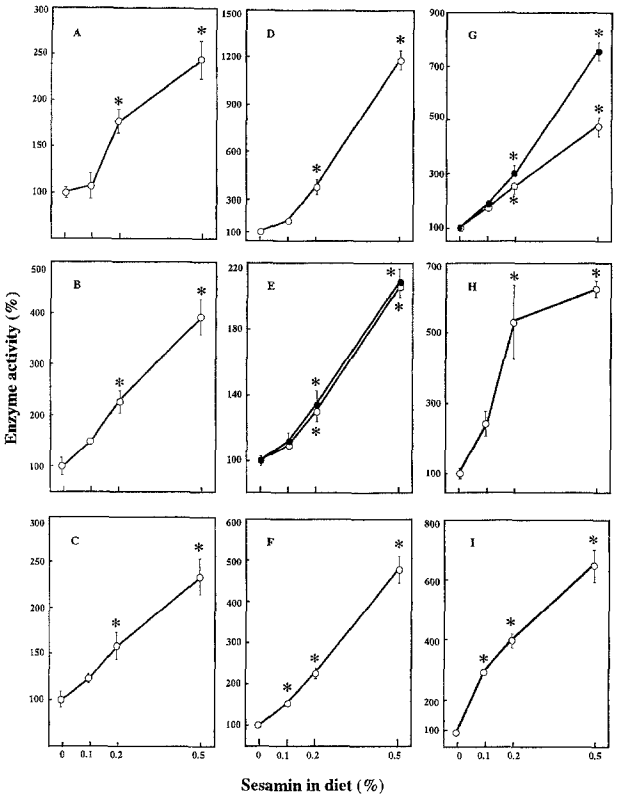

Fig 2. Effect of sesamin on the activity of fatty acid oxidation enzymes in rat liver. The experiment was the same as described in Fig 1. Activities were calculated as total activity and are expressed as a percentage of the activity in the sesamin-free group. Carnitine palmitoyltransferase I and acyl-CoA dehydrogenase activities were analyzed using the mitochondrial fraction as an enzyme source. Other enzyme activities were measured using the whole homogenate as an enzyme source. Carnitine palmitoyltransferase I activity was analyzed radiochemically in freshly isolated mitochondria (A). Carnitine palmitoyltransferase activity was also analyzed spectrophotometrically in the whole homogenates (B). Acyl-CoA dehydrogenase and acyl-CoA oxidase activity are shown in C and D, respectively. Palmitoyl-CoA was used as a substrate to assay these enzymes. Enoyl-CoA hydratase activity (E) was assayed using crotonyl-CoA (○) and *trans*-2-octenoyl-CoA (●) substrates. Acetoacetyl-CoA was used as a substrate for 3-hydroxyacyl-CoA dehydrogenase to assay the activity (F). 3-Ketoacyl-CoA thiolase activity (G) was analyzed using acetoacetyl-CoA (○) and 3-keto-octanoyl-CoA (●) substrates. Sorboyl-CoA was used as a substrate for 2,4-dienoyl-CoA reductase to assay the activity (H). *trans*-3-Hexenoyl-CoA was used as a substrate for Δ^3,Δ^2-enoyl-CoA isomerase to assay the activity (I). Enzyme activity in rats fed a sesamin-free diet was (in μmol/min/liver/100 g body weight) as follows: carnitine palmitoyltransferase I, 2.02 ± 0.12; carnitine palmitoyltransferase, 4.75 ± 0.80; acyl-CoA dehydrogenase, 18.2 ± 1.7; acyl-CoA oxidase, 2.14 ± 0.19; enoyl-CoA hydratase, 2,304 ± 117 using crotonyl-CoA and 2,088 ± 65 using *trans*-2-octenoyl-CoA substrates; 3-hydroxyacyl-CoA dehydrogenase, 380 ± 26; 3-ketoacyl-CoA thiolase, 75.3 ± 6.2 using acetoacetyl-CoA and 106 ± 8 using 3-keto-octanoyl-CoA substrates; 2,4-dienoyl-CoA reductase, 4.74 ± 0.70; and Δ^3,Δ^2-enoyl-CoA isomerase, 25.3 ± 2.3. Values are the mean ± SE of 7-8 rats. *$P < .05$ *v* sesamin-free group.

chemically in isolated mitochondria (carnitine palmitoyltransferase I activity). Also, the activity of 3-hydroxyacyl-CoA dehydrogenase was five times higher in the 0.5% sesamin group versus the control group. Dietary sesamin increased 3-ketoacyl-CoA thiolase activity measured with both acetoacetyl-CoA and 3-keto-octanoyl-CoA substrates (Fig 2F and G). The increase by dietary sesamin was more prominent for activity measured using the 3-keto-octanoyl-CoA substrate versus the acetoacetyl-CoA substrate. The activity of peroxisomal acyl-CoA oxidase (Fig 2D) progressively increased as the dietary level of sesamin increased, and the enzyme activity was 12-fold higher in rats fed a 0.5% sesamin diet versus those fed a sesamin-free diet.

In addition to the activity of carnitine palmitoyltransferases and enzymes in the β-oxidation cycle, we analyzed the activity of auxiliary enzymes (2,4-dienoyl-CoA reductase and Δ^3,Δ^2-enoyl-CoA isomerase) involved in the pathway for the oxidation of unsaturated fatty acids. Increasing the dietary level of sesamin progressively increased the activities of these enzymes, and the activities were more than sixfold higher in rats fed a 0.5% sesamin diet versus those fed the control diet free of sesamin (Fig 2H and I).

Gene Expression of Fatty Acid Oxidation Enzymes

We analyzed the mRNA abundance of mitochondrial and peroxisomal fatty acid oxidation enzymes by slot-blot hybridization using specific cDNA probes (Figs 3 and 4). The values are expressed as a percentage of the value in rats fed a sesamin-free diet, designated as 100. Specific cDNA probes were used to discriminate the gene expression of carnitine palmitoyltransferases located in outer (carnitine palmitoyltransferase I) and inner (carnitine palmitoyltransferase II) mitochondrial membranes. Dietary sesamin increased the mRNA levels of both carnitine palmitoyltransferases I and II (Fig 3A and B). However, the extent of the increase was more marked with carnitine palmitoyltransferase II than with the enzyme isoform I. There are various acyl-CoA dehydrogenase molecules differing in substrate specificity.[31-33] The mRNA abundance of long-chain specific acyl-CoA dehydrogenase[32] was analyzed using a specific cDNA probe. Sesamin administration significantly increased the gene expression of this mitochondrial enzyme dose-dependently (Fig 3C). The mitochondrial trifunctional enzyme possessing long-chain specific enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase activities is composed of two subunits (α and β) encoded by different genes.[22] The gene expression of the enzyme α and β subunits was coordinately induced by sesamin, and the diet containing 0.5% sesamin caused a threefold to fourfold increase in these values (Fig 3D and E). The hepatic mRNA level of mitochondrial 3-ketoacyl-CoA thiolase was increased by sesamin dose-dependently (Fig 3F). Compared with a sesamin-free diet, the diet containing 0.5% sesamin caused a 4.5-fold increment in the mRNA abundance of this mitochondrial enzyme.

Of the enzymes involved in auxiliary pathways, at least two 2,4-dienoyl-CoA reductases differing in molecular weight exist in mitochondria.[34,35] Since cDNA encoding a larger, but not a smaller, isoform has been cloned,[23] this cDNA was used to detect mRNA of 2,4-dienoyl-CoA reductase. Two different cDNAs for mitochondrial Δ^3,Δ^2-enoyl-CoA isomerase have been cloned and sequenced.[36,37] The cDNA probe used in this study was prepared according to the cDNA sequence reported by Pihlajaniemi and Hiltunen[36] for short-chain specific Δ^3,Δ^2-enoyl-CoA isomerase. Dietary sesamin even at a 0.1% level increased 2,4-dienoyl-CoA reductase and Δ^3,Δ^2-enoyl-CoA

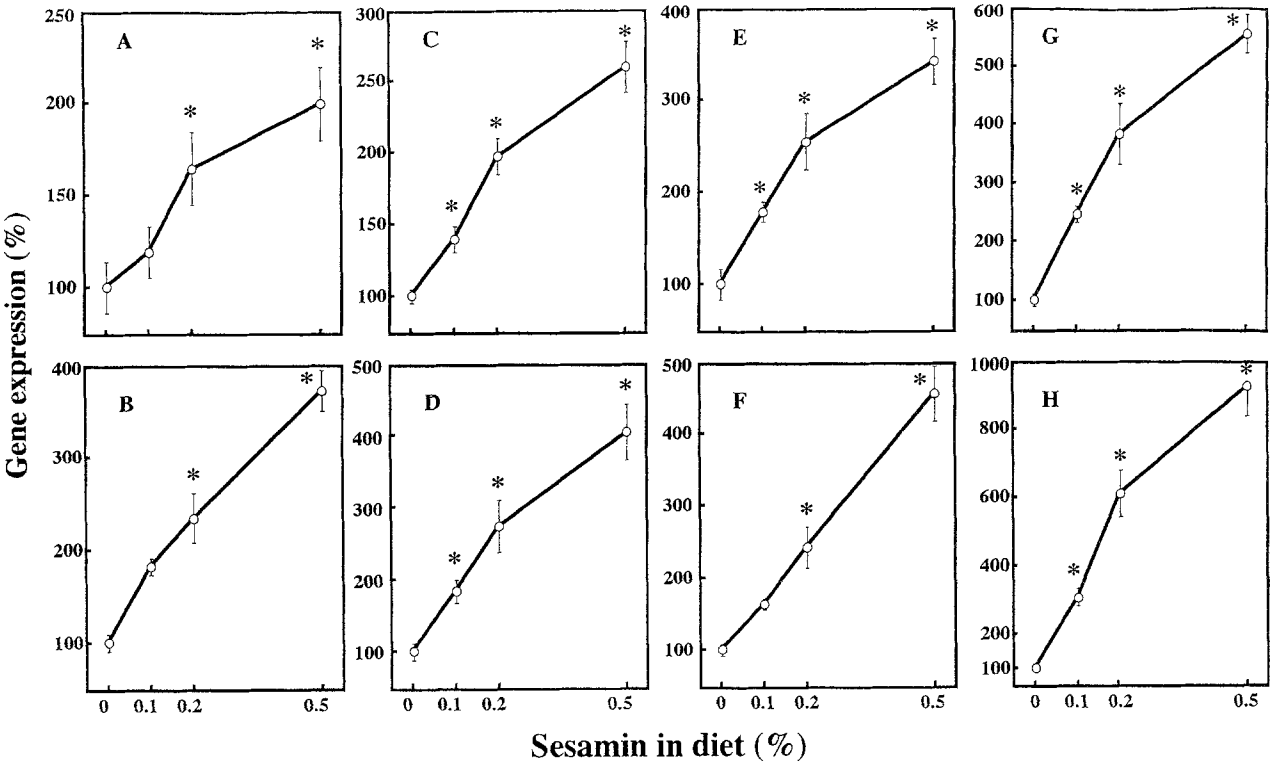

Fig 3. Effect of sesamin on the gene expression of mitochondrial fatty acid oxidation enzymes in rat liver. The experiment was the same as described in Fig 1. Values are expressed as a percentage of those for the sesamin-free group. cDNA probes specific for carnitine palmitoyltransferase I (A), carnitine palmitoyltransferase II (B), long-chain acyl-CoA dehydrogenase (C), mitochondrial trifunctional enzyme subunits α (D) and β (E), mitochondrial 3-ketoacyl-CoA thiolase (F), 2,4-dienoyl-CoA reductase (G), and short-chain Δ^3,Δ^2-enoyl-CoA isomerase (H) were used to assay hepatic mRNA levels by slot-blot hybridization. Values are the mean ± SE of 7-8 rats. *$P < .05$ *v* sesamin-free group.

isomerase gene expression more than twofold (Fig 3G and H), and a diet containing 0.5% sesamin caused a sixfold increase in the reductase gene expression and a 10-fold increase in the isomerase gene expression.

Dietary sesamin also increased the abundance of mRNA for peroxisomal enzymes (Fig 4A to C). A diet containing 0.1% sesamin doubled the acyl-CoA oxidase mRNA level (Fig 4A). mRNA abundance was more than fivefold and 15-fold higher with 0.2% and 0.5% sesamin, respectively, versus a sesamin-free diet. Among the peroxisomal enzymes, the gene expression of peroxisomal bifunctional enzyme possessing both enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities[21] was affected most by dietary sesamin (Fig 4B). Sesamin as low as 0.1% caused a fivefold increase in the peroxisomal bifunctional enzyme mRNA level. Increasing the dietary level of sesamin progressively increased the gene expression of this enzyme, and it was more than 15 times higher on a 0.2% sesamin diet and 50 times higher on a 0.5% sesamin diet versus the control diets free of sesamin. Among the peroxisomal enzymes, the gene expression of peroxisomal 3-ketoacyl-CoA thiolase was affected least by sesamin, but it was six times higher on a 0.5% sesamin diet than on a sesamin-free diet (Fig 4C). Dietary sesamin did not affect the expression of a housekeeping gene (β-actin). The values for β-actin gene

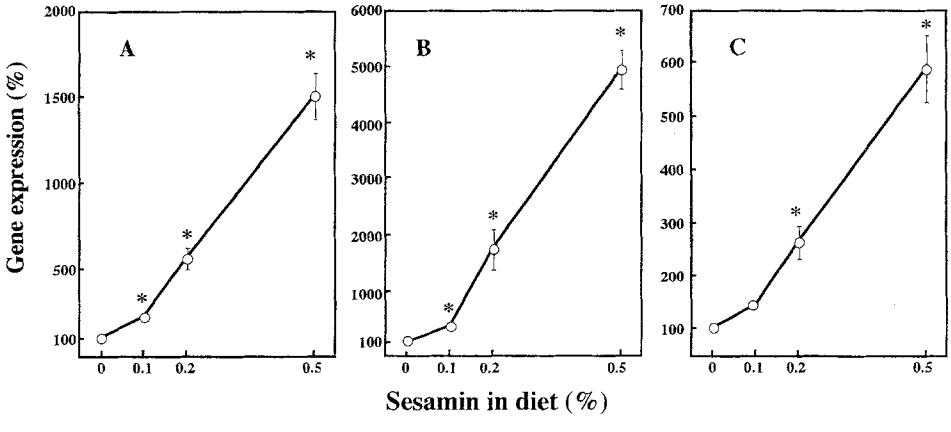

Fig 4. Effect of sesamin on the gene expression of peroxisomal fatty acid oxidation enzymes in rat liver. The experiment was the same as described in Fig 1. Values are expressed as a percentage of those for the sesamin-free group. cDNA probes specific for acyl-CoA oxidase (A), peroxisomal bifunctional enzyme (B), and peroxisomal 3-ketoacyl-CoA thiolase (C) were used to assay hepatic mRNA levels by slot-blot hybridization. Values are the mean ± SE of 7-8 rats. *$P < .05$ *v* sesamin-free group.

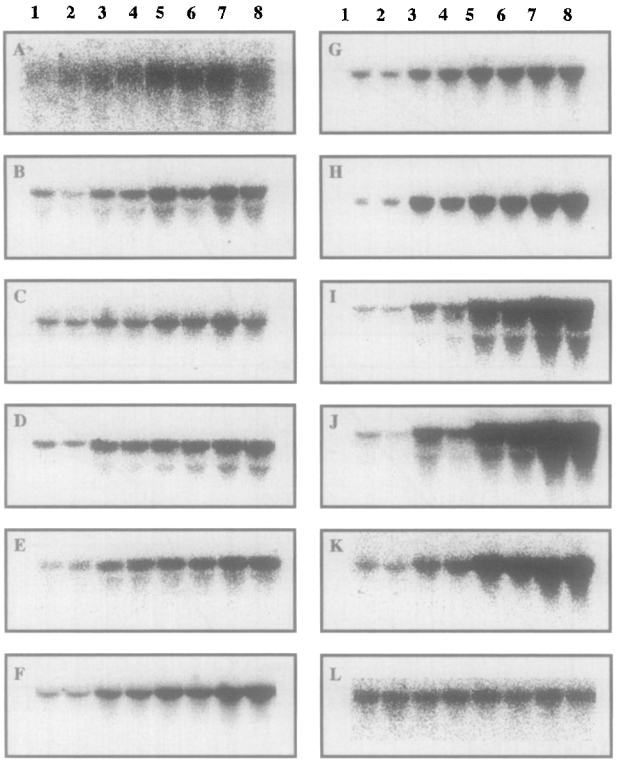

Fig 5. Northern blot analysis of mRNA for fatty acid oxidation enzymes in rat liver. The experiment was the same as described in Fig 1. RNA samples (40 μg) were denatured and subjected to electrophoresis on 1.1% agarose gel containing 0.66 mol/L formaldehyde, and then transferred to a nylon membrane and fixed with UV irradiation. RNA on nylon membranes was hybridized with radiolabeled cDNA probes specific for the mRNA of carnitine palmitoyltransferase I (A), carnitine palmitoyltransferase II (B), long-chain acyl-CoA dehydrogenase (C), mitochondrial trifunctional enzyme subunits α (D) and β (E), mitochondrial 3-ketoacyl-CoA thiolase (F), 2,4-dienoyl-CoA reductase (G), short-chain Δ^3,Δ^2-enoyl-CoA isomerase (H), acyl-CoA oxidase (I), peroxisomal bifunctional enzyme (J), peroxisomal 3-ketoacyl-CoA thiolase (K), and β-actin (L). Lanes 1 and 2, RNA from rats fed a sesamin-free diet; lanes 3 and 4, 0.1% sesamin diet; lanes 5 and 6, 0.2% sesamin diet; lanes 7 and 8, 0.5% sesamin diet.

expression were 100% ± 7%, 110% ± 8%, 110% ± 5%, and 106% ± 5% for 0%, 0.1%, 0.2%, and 0.5% sesamin diets, respectively. A sesamin-dependent increase in the gene expression of fatty acid oxidation enzymes as determined by slot-blot hybridization was further confirmed by RNA analysis by Northern-blot hybridization. Increasing dietary levels of sesamin progressively increased the mRNA signal intensities for various fatty acid oxidation enzymes, while those for β-actin remained constant (Fig 5A to L).

Activity and Gene Expression of Enzymes in Fatty Acid Synthesis

Figure 6 shows the activity and gene expression of enzymes involved in fatty acid synthesis. Mammals have four types of pyruvate kinase, namely L, M_1, M_2, and R.[38] The L type is the major isozyme in the liver. M_1 predominates in muscle, heart, and brain. M_2 is found only in fetal tissues, and R is specifically located in red blood cells. We therefore used cDNA for L isozyme as a probe to detect hepatic pyruvate kinase mRNA. Sesamin at a 0.1% dietary level reduced fatty acid synthase activity (Fig 6A) and gene expression (Fig 6D) to about half the levels obtained with a sesamin-free diet. However, increasing dietary levels of sesamin did not cause a further reduction of these indices. Pyruvate kinase activity (Fig 6B) and gene expression (Fig 6E) decreased as the dietary levels of sesamin increased. In contrast, increasing dietary levels of sesamin progressively increased both the activity (Fig 6C) and mRNA abundance (Fig 6F) of malic enzyme. They were approximately two times higher on a 0.5% sesamin diet versus a sesamin-free diet. Northern blot analysis of hepatic RNA in rats fed varying amounts of sesamin confirmed that dietary sesamin decreased

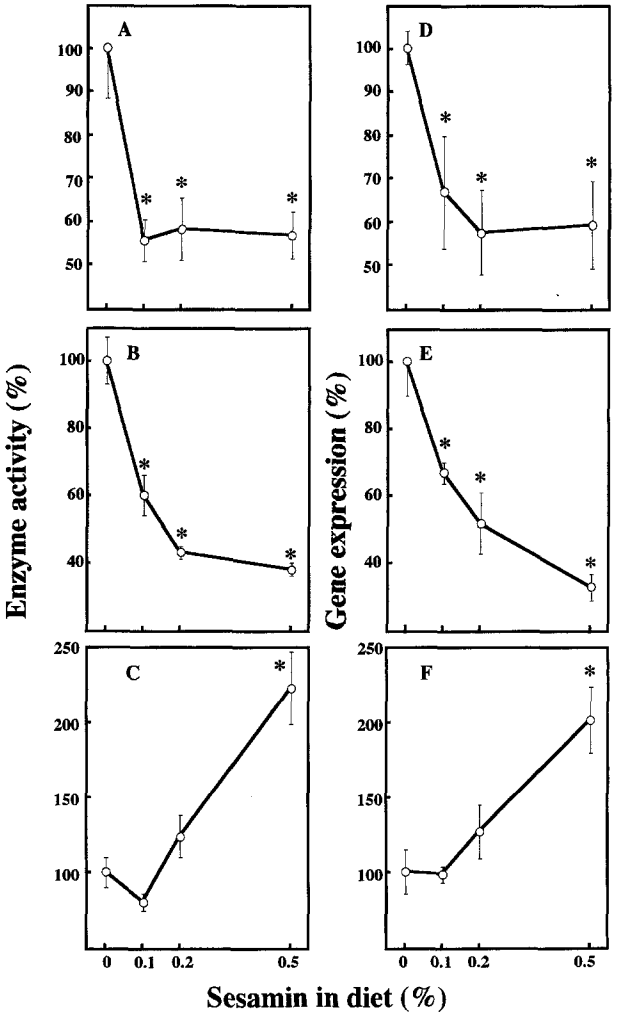

Fig 6. Effect of sesamin on the activity and gene expression of lipogenic enzymes in rat liver. The experiment was the same as described in Fig 1. Enzyme activities were calculated as total activity, and are presented as a percentage of that in the sesamin-free group. Gene expression is also presented as a percentage. The activities of fatty acid synthase (A), pyruvate kinase (B), and malic enzyme (C) were analyzed in the 9,000 × *g* supernatant fraction of liver homogenate, and mRNA levels of fatty acid synthase (D), L-pyruvate kinase (E), and malic enzyme (F) were assayed using specific cDNA probes. Enzyme activity in rats fed a sesamin-free diet was (μmol/min/liver/100 g body weight) as follows: fatty acid synthase, 14.8 ± 2.1; pyruvate kinase, 195 ± 14; and malic enzyme, 41.5 ± 4.3. Values are the mean ± SE of 7-8 rats. *$P < .05$ *v* sesamin-free group.

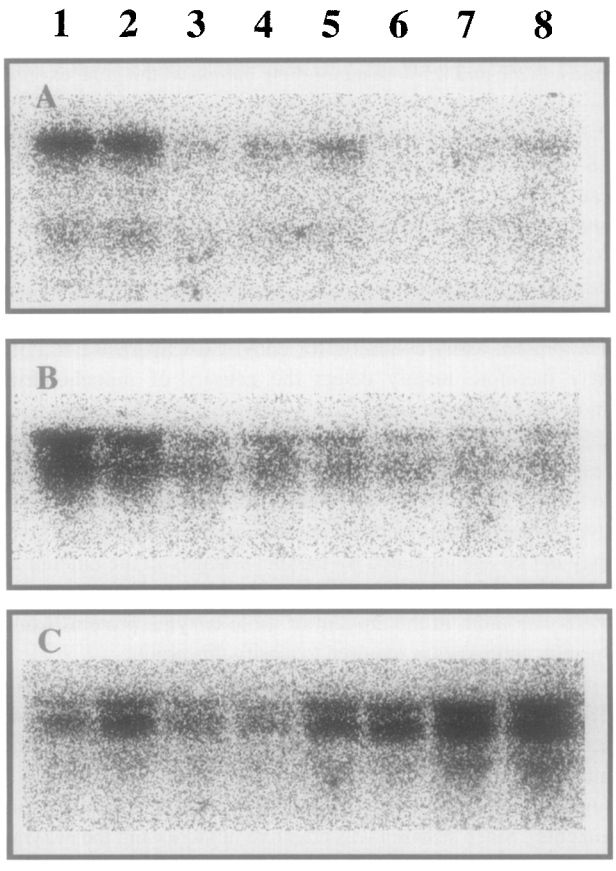

Fig 7. Northern blot analysis of mRNA of lipogenic enzymes in rat liver. The experiment was the same as described in Fig 1. RNA samples were treated as described in Fig 5. RNA on nylon membrane was hybridized with radiolabeled cDNA probes specific for mRNA of fatty acid synthase (A), L-pyruvate kinase (B), and malic enzyme (C). Lanes 1 and 2, RNA from rats fed a sesamin-free diet; lanes 3 and 4, 0.1% sesamin diet; lanes 5 and 6, 0.2% sesamin diet; lanes 7 and 8, 0.5% sesamin diet.

the mRNA level of fatty acid synthase (Fig 7A) and L-pyruvate kinase (Fig 7B), but progressively increased that of malic enzyme (Fig 7C).

Serum and Liver Lipid Levels

Dietary sesamin dose-dependently decreased serum triacylglycerol and free fatty acid concentrations (Table 2). Serum cholesterol was significantly lower in rats fed 0.1% and 0.2% sesamin diets versus those fed a sesamin-free diet. However, the value in rats fed a 0.5% sesamin diet was comparable to that in the animals fed a sesamin-free diet. Although no significant differences were found, similar changes were observed in the serum phospholipid concentration. Sesamin did not affect the serum glucose concentration. There were no significant differences in the hepatic triacylglycerol concentration and content among groups. Hepatic cholesterol decreased as dietary levels of sesamin increased, and the value was significantly lower in rats fed a 0.5% sesamin diet than in those fed a sesamin-free diet. However, no significant differences were found among groups when the value was expressed in terms of the content. Sesamin dose-dependently increased the hepatic phospholipid concentration and content. Hepatic phospholipid content was 75% higher in rats fed a 0.5% sesamin diet versus those fed a sesamin-free diet.

DISCUSSION

Sesamin Induces the Activity and Gene Expression of Hepatic Enzymes in Fatty Acid Oxidation

The present study unequivocally demonstrates that sesamin greatly induces the activity and gene expression of hepatic enzymes involved in fatty acid oxidation. The extent of the increase with sesamin was more marked in peroxisomal versus mitochondrial activity as observed with a variety of chemical compounds called peroxisome proliferators.[12,13] Although histological confirmation is still required, sesamin may therefore be considered a naturally occurring peroxisome proliferator. A sesamin-dependent increase in fatty acid oxidation activity was detectable at a dietary level as low as 0.1%. Polyunsaturated fatty acids of the n-3 series such as α-linolenic,[15,16] eicosapentaenoic, and docosahexaenoic,[39,40] have been demonstrated to induce hepatic fatty acid oxidation. But considerably higher amounts were required to obtain a detectable increase in hepatic fatty acid oxidation as compared with sesamin.[15,16,39,40] Thus, sesamin appears to be the most potent inducer of hepatic fatty acid oxidation among the various naturally occurring compounds reported thus far. Although previous observations[1,2,4,5,8] that sesamin decreased hepatic levels of triacylglycerol and cholesterol were not necessarily confirmed in the present study, it dose-dependently increased the hepatic phospholipid level accompanying liver hypertrophy as reported elsewhere.[1,2,4,5,8] This observation indicates that dietary sesamin, like xenobiotic peroxisomal proliferators, may proliferate liver organelles includ-

Table 2. Effects of Dietary Sesamin on Serum Lipid and Glucose Concentrations and Liver Lipid Levels (mean ± SE)

Parameter	Sesamin-Free	0.1% Sesamin	0.2% Sesamin	0.5% Sesamin
Serum components (μmol/dL)				
Triacylglycerol	285 ± 46	269 ± 42	183 ± 22*	97 ± 12*
Cholesterol	265 ± 20	221 ± 17*	214 ± 17*	285 ± 10
Phospholipid	349 ± 29	305 ± 18	311 ± 10	354 ± 8
Free fatty acid	145 ± 17	109 ± 16	99.6 ± 8.3*	73.7 ± 4.5*
Glucose	979 ± 39	906 ± 58	904 ± 37	978 ± 40
Liver lipids				
Concentration (μmol/g)				
Triacylglycerol	34.5 ± 5.6	34.4 ± 3.5	31.7 ± 3.0	29.8 ± 3.4
Cholesterol	7.25 ± 0.39	6.92 ± 0.24	6.86 ± 0.24	6.15 ± 0.23*
Phospholipid	36.8 ± 0.6	40.2 ± 0.6*	43.4 ± 0.8*	47.8 ± 1.0*
Content (μmol/liver/100 g body weight)				
Triacylglycerol	196 ± 35	199 ± 28	196 ± 19	227 ± 28
Cholesterol	41.1 ± 2.9	39.5 ± 2.4	42.6 ± 2.2	46.9 ± 2.8
Phospholipid	208 ± 9	228 ± 7	269 ± 8*	363 ± 6*

*$P < .05$ *v* sesamin-free group.

ing mitochondria and peroxisomes and thus cause liver hypertrophy accompanying an increase in the phospholipid level in this tissue. The increase in mitochondrial protein content and glutamate dehydrogenase activity in the liver of rats fed sesamin in the present study supports the proposal that sesamin at least causes a proliferation of mitochondria.

Great diversity is characteristic of the β-oxidation pathway. Several enzyme species differing in substrate specificity are involved at each enzyme step of the β-oxidation cycle. The fatty acid oxidation enzyme activity measured in cell-free enzyme preparations therefore represents the sum of the activity of various enzymes at a given condition of enzyme assay. However, a cDNA probe specific for each enzyme mRNA can discriminate the response for the gene expression of individual enzymes. In the present study, it was confirmed that dietary sesamin increased the gene expression of various enzymes in the mitochondrial and peroxisomal β-oxidation pathway.

In the present study, the increase with dietary sesamin was much higher for carnitine palmitoyltransferase activity measured spectrophotometrically in freeze-thawed whole homogenate versus the activity measured radiochemically in freshly isolated mitochondria. The latter activity represents carnitine palmitoyltransferase I activity.[41] Freezing-thawing mitochondria inactivates carnitine palmitoyltransferase I but not transferase II,[41] indicating that the transferase activity measured spectrophotometrically using a freeze-thawed whole homogenate may most likely represent transferase II activity. Consistent with this assumption, the analysis of mRNA levels using cDNA probes specific for carnitine palmitoyltransferase I and II showed that dietary sesamin increased the mRNA level of transferase II more than that of isozyme I. The mitochondrial palmitoyl-CoA oxidation rate paralleled the activity and gene expression of carnitine palmitoyltransferase I rather than the enzyme activity measured spectrophotometrically and carnitine palmitoyltransferase II gene expression, supporting the consideration that the transferase I is a rate-limiting enzyme in mitochondrial fatty acid oxidation.[41]

Four distinct acyl-CoA dehydrogenase species differing in substrate specificity catalyze the first step of the mitochondrial β-oxidation cycle of fatty acids with different chain length. These correspond to short-,[32] medium-,[31] long-,[32] and very–long-chain[33] acyl-CoA dehydrogenases. Dietary sesamin increased mitochondrial acyl-CoA dehydrogenase activity measured with palmitoyl-CoA as a substrate accompanying an increase in the long-chain acyl-CoA dehydrogenase gene expression analyzed with a specific cDNA probe. The responses to dietary sesamin of the enzyme activity and gene expression correlated well with each other, indicating that the enzyme activity measured using long-chain acyl-CoA substrate most likely represents long-chain acyl-CoA dehydrogenase activity. It is also possible that dietary sesamin increases the activity and gene expression of other enzyme species differing in substrate specificity.[31-33] The measurement of enzyme activity using different acyl-CoA substrates differing in chain length and gene expression with cDNA probes specific for each enzyme is required to clarify this issue.

At least three hepatic proteins, ie, mitochondrial trifunctional enzyme subunit α, mitochondrial monofunctional short-chain specific enoyl-CoA hydratase,[42] and peroxisomal bifunctional enzyme,[21] show enoyl-CoA hydratase activity. In the present study, 0.2% and 0.5% sesamin diets increased the gene expression of mitochondrial trifunctional enzyme subunit α by 2.7-fold and fourfold and peroxisomal bifunctional enzyme by 18- and 50-fold, respectively. However, these sesamin diets caused only 1.3-fold and twofold increases, respectively, in enoyl-CoA hydratase activity. We observed that sesamin at a 0.2% dietary level did not affect the mRNA level of mitochondrial monofunctional short-chain specific enoyl-CoA hydratase (Ashakumary and Ide, unpublished observation, June 1998). The present assay condition for enoyl-CoA hydratase activity may therefore mainly detect the activity of mitochondrial short-chain specific enoyl-CoA hydratase rather than mitochondrial and peroxisomal multifunctional enzymes, resulting in a response to sesamin that is lower than estimated from changes in the mRNA levels of these multifunctional enzymes. Alternatively, there is the possibility that alterations in the mRNA level may not be accompanied by parallel changes in the amount of enzyme protein. Examination of the alterations induced by dietary sesamin in the amount of these enzyme proteins using specific antibodies is required to clarify this point.

Dietary sesamin increased the activity of 3-hydroxyacyl-CoA dehydrogenase measured using the acetoacetyl-CoA substrate accompanying the increase in gene expression of enzyme molecules possessing this enzyme activity (mitochondrial trifunctional enzyme subunit α and peroxisomal bifunctional enzyme). Since sesamin was effective in increasing the enzyme activity measured with the short-chain substrate, it may also increase the gene expression of mitochondrial short-chain specific 3-hydroxyacyl-CoA dehydrogenase.[43]

Sesamin increased 3-ketoacyl-CoA thiolase activity measured with both acetoacetyl-CoA and 3-keto-octanoyl-CoA substrates. However, the extent of the increase was more marked with the 3-keto-octanoyl-CoA substrate versus the acetoacetyl-CoA substrate. It is plausible that the enzyme activity measured with the latter substrate represents the sum of the activities for mitochondrial and peroxisomal enzyme molecules possessing 3-ketoacyl-CoA thiolase, acetoacetyl-CoA thiolase in the mitochondrial ketogenic pathway, and cytosolic acetoacetyl-CoA thiolase in the cholesterogenic pathway, as well. Since it is expected that these enzymes are under different control mechanisms, the change induced by dietary sesamin in the enzyme activity obtained with 3-keto-octanoyl-CoA represents the more exact figure for the response of hepatic 3-ketoacyl-CoA thiolase. In fact, we previously showed[16] that dietary α-linolenic acid, an inducer of hepatic β-oxidation, increased the enzyme activity measured with the acetoacetyl-CoA substrate in the 500 × *g* supernatant fraction of the liver homogenate, but not in the cytosolic fraction. As expected, dietary sesamin increased the gene expression of all enzyme molecules possessing 3-ketoacyl-CoA thiolase, including mitochondrial trifunctional enzyme subunit β and mitochondrial and peroxisomal 3-ketoacyl-CoA thiolases.

Dietary sesamin greatly increased the gene expression of acyl-CoA oxidase. The changes observed paralleled those for the peroxisomal palmitoyl-CoA oxidation rate and acyl-CoA oxidase activity, supporting the concept that acyl-CoA oxidase

is an enzyme that regulates peroxisomal fatty oxidation.[13,20] The marked increase by sesamin in the gene expression of peroxisomal bifunctional enzyme supports its role as a potent inducer of peroxisomes. The extent of the increase in gene expression was considerably low in peroxisomal 3-ketoacyl-CoA thiolase among the peroxisomal enzymes, and was only slightly higher than that for mitochondrial 3-ketoacyl-CoA thiolase. It has been demonstrated that peroxisome proliferators such as clofibrate and di-(2-ethylhexyl)phthalate increase the mRNA levels of both mitochondrial[44] and peroxisomal[45] 3-ketoacyl-CoA thiolases. However, studies comparing the effects of peroxisome proliferators on the gene expression of these enzyme molecules are lacking.

2,4-Dienoyl-CoA reductase and Δ^3,Δ^2-enoyl-CoA isomerase are auxiliary enzymes required for degradation by the β-oxidation pathway of unsaturated fatty acids.[23,34-37] In the present study, dietary sesamin increased not only the activity and mRNA level of carnitine palmitoyltransferases and various enzymes involved in the β-oxidation cycle but also those of 2,4-dienoyl-CoA reductase and Δ^3,Δ^2-enoyl-CoA isomerase. Thus, these auxiliary enzymes are coordinately regulated with other β-oxidation enzymes. In this context, peroxisome proliferators like clofibrate induce the activity and gene expression of these auxiliary enzymes.[35]

Sesamin Effects on the Activity and Gene Expression of Hepatic Enzymes in Fatty Acid Synthesis

The present study demonstrates that sesamin affects not only hepatic fatty acid oxidation but also synthesis. Pyruvate kinase, an enzyme involved in the glycolytic pathway in the liver, is coordinately regulated with other enzymes involved in lipogenesis, and is considered to participate in the regulation of fatty acid synthesis.[38,46] Dietary sesamin decreased the activity and gene expression of fatty acid synthase and pyruvate kinase. Although sesamin increased the activity and gene expression of malic enzyme, the results possibly indicate that this compound increases fatty acid oxidation but reduces fatty acid synthesis in the liver. Sesamin behaves like a peroxisome proliferator in modulating fatty acid oxidation, but peroxisome proliferators such as clofibrate[47] and Wy-14,643[48] did not affect the activity of hepatic lipogenic enzymes including fatty acid synthase, acetyl-CoA carboxylase, glucose-6-phosphate dehydrogenase, and ATP-citrate lyase. Therefore, sesamin appears to be distinct from other peroxisome proliferators in its effect on hepatic fatty acid synthesis.

The potential role of the PPAR, a member of the nuclear receptor superfamily, in regulating lipid metabolism has been well demonstrated.[49,50] Various types of PPAR (α, γ_1, γ_2, and δ) have been identified in rodents and humans. PPARα is highly expressed in liver and may play a crucial role in regulating lipid metabolism in this tissue.[49,50] Peroxisome proliferators activate PPAR to induce gene expression of hepatic fatty acid oxidation enzymes. Aoyama et al[48] demonstrated that not only peroxisomal but also mitochondrial fatty acid oxidation enzyme gene expression is regulated through a PPARα-dependent mechanism. There is no evidence to support the involvement of PPAR in regulating the gene expression of enzymes in fatty acid synthesis, except for malic enzyme.[48-52] The available information[48-51] indicates that PPAR upregulates the expression of the gene encoding malic enzyme without influencing that of other enzymes involved in hepatic fatty acid synthesis. Despite the fact that sesamin decreased the activity and gene expression of fatty acid synthase and pyruvate kinase, it induced the activity and gene expression of malic enzyme in the present study. This observation supports the consideration that sesamin, like other peroxisome proliferators, induces the gene expression of hepatic fatty acid oxidation enzymes through a PPAR-dependent mechanism. A cotransfection assay[48,50] using cultured cells to demonstrate sesamin-dependent activation of PPAR is required to confirm this hypothesis. Sesamin may decrease the activity and gene expression of fatty acid synthase and pyruvate kinase through a mechanism independent of PPAR.

Sesamin and Serum Lipids

Examination of the enzyme activity in fatty acid oxidation and synthesis using liver homogenate and of the mRNA levels of corresponding enzyme proteins in the present study indicated that sesamin increases fatty acid oxidation but decreases fatty acid synthesis in the liver. Fukuda et al[53] recently demonstrated that dietary sesamin increases ketone body production in perfused rat liver. Therefore, the sesamin-dependent increase in hepatic fatty acid oxidation enzyme activity apparently associates with the enhancement of fatty acid oxidation in the intact tissue. A significant decrease with dietary sesamin in the serum free fatty acid concentration also supports the consideration that this compound increases hepatic fatty acid oxidation. An increase in hepatic fatty acid oxidation with a concomitant decrease in fatty acid synthesis may cause a decrease in the availability of fatty acid for triacylglycerol synthesis and consequently a decrease in the assembly and secretion of triacylglycerol-rich lipoproteins,[9-11,53] thus accounting for the serum lipid-lowering effect of sesamin.[1-8] In the present study, sesamin decreased serum levels of triacylglycerol dose-dependently. Sesamin at 0.1% and 0.2% dietary levels also significantly decreased the serum cholesterol level, but sesamin at a 0.5% dietary level failed to decrease it. Changes in the serum phospholipid level paralleled changes in cholesterol. This observation suggests that a high dietary level of sesamin increases hepatic production of high-density lipoprotein that is rich in cholesterol and phospholipid and thus fails to decrease the serum cholesterol level. However, this consideration cannot be supported by the observation that peroxisome proliferators downregulate the gene expression of apolipoprotein C-I and C-II,[49,50] protein components of high-density lipoprotein in rodents. Further study is needed to clarify this point.

In conclusion, the present study demonstrates that sesamin, a sesame seed lignan, greatly increases the activity and gene expression of hepatic fatty acid oxidation enzymes. The extent of the increase was more marked in peroxisomal than in mitochondrial enzymes. In contrast, dietary sesamin decreased the activity and gene expression of fatty acid synthase and pyruvate kinase. Hence, sesamin increases hepatic fatty acid oxidation while it concomitantly decreases fatty acid synthesis. These changes in hepatic fatty acid metabolism may be responsible for the serum lipid-lowering effect of dietary sesamin. Despite the observation that sesamin decreased the

activity and gene expression of fatty acid synthase and pyruvate kinase, this compound, like peroxisome proliferators, increased the activity of malic enzyme and the expression of the gene encoding this enzyme possessing a peroxisome proliferator response element in the promoter region.[45,51,52] Conceivably, therefore, sesamin may increase the gene expression of hepatic fatty acid oxidation enzymes through the PPAR signaling pathway.

REFERENCES

1. Sugano M, Inoue T, Koba K, et al: Influence of sesame lignans on various lipid parameters in rats. Agric Biol Chem 54:2669-2673, 1990

2. Hirose N, Doi F, Ueki T, et al: Suppressive effect of sesamin against 7,12-dimethylbenz[α]anthracene induced rat mammary carcinogenesis. Anticancer Res 12:1259-1266, 1992

3. Ogawa H, Sasagawa S, Murakami T, et al: Sesame lignans modulate cholesterol metabolism in the stroke-prone spontaneously hypertensive rat. Clin Exp Pharmacol Physiol 22:S310-S312, 1995 (suppl 1)

4. Hirose Y, Inoue T, Nishihara K, et al: Inhibition of cholesterol absorption and synthesis in rats by sesamin. J Lipid Res 32:629-638, 1991

5. Ogawa T, Makino T, Hirose N, et al: Lack of influence of low blood cholesterol levels on pancreatic carcinogenesis after initiation with *N*-nitrosobis(2-oxopropyl)amine in Syrian golden hamsters. Carcinogenesis 15:1663-1666, 1994

6. Akimoto K, Kigawa Y, Akamatsu T, et al: Protective effect of sesamin against liver damage caused by alcohol or carbon tetrachloride in rodents. Ann Nutr Metab 37:218-224, 1993

7. Hirata F, Fujita K, Ishikura Y, et al: Hypocholesterolemic effect of sesame lignan in humans. Atherosclerosis 122:135-136, 1996

8. Gu JY, Wakizono Y, Tsujita A, et al: Effects of sesamin and alpha-tocopherol, individually or in combination, on the polyunsaturated fatty acid metabolism, chemical mediator production, and immunoglobulin levels in Sprague-Dawley rats. Biosci Biotechnol Biochem 59:2198-2202, 1995

9. Windmueller HG, Spaeth AE: De novo synthesis of fatty acid in perfused rat liver as a determinant of plasma lipoprotein production. Arch Biochem Biophys 122:362-369, 1967

10. Ide T, Ontko JA: Increased secretion of very low density triglyceride following inhibition of long chain fatty acid oxidation in rat liver. J Biol Chem 256:10247-10255, 1981

11. Ide T, Oku H, Sugano M: Reciprocal response to clofibrate in ketogenesis and triglyceride and cholesterol secretion in isolated rat liver. Metabolism 31:1065-1072, 1982

12. Lazarow PB, De Duve C: A fatty acyl CoA oxidising system in rat liver peroxisomes enhanced by clofibrate, a hypolipidemic drug. Proc Natl Acad Sci USA 73:2043-2046, 1976

13. Reddy JK: Peroxisomal lipid metabolism. Annu Rev Nutr 14:343-370, 1994

14. Thorpe C: A method for the preparation of 3-ketoacyl-CoA derivatives. Anal Biochem 155:391-394, 1986

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

16. Kabir Y, Ide T: Activity of hepatic fatty acid oxidation enzymes in rats fed α-linolenic acid. Biochim Biophys Acta 1304:105-119, 1996

17. American Institute of Nutrition: Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 107:1340-1348, 1977

18. Ide T, Hirabayashi S, Kano S, et al: Soybean phospholipid dependent reductions in triacylglycerol concentration and synthesis in the liver of fasted-refed rats. Biochim Biophys Acta 1124:163-170, 1992

19. Schmidt E, Schmidt WS: Glutamate dehydrogenase, in Bergmeyer HU, Graßl M (eds): Methods of Enzymatic Analysis (English ed 3). Deerfield Beach, FL, VHC Publishers, 1985, pp 216-227

20. Miyazawa S, Hayashi H, Hijikata M, et al: Complete nucleotide sequence of cDNA and predicted amino acid sequence of rat acyl-CoA oxidase. J Biol Chem 256:8131-8137, 1987

21. Osumi T, Ishii N, Hijikata M, et al: Molecular cloning and nucleotide sequence of the cDNA for rat peroxisomal enoyl-CoA: hydratase-3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme. J Biol Chem 260:8905-8910, 1985

22. Kamijo T, Aoyama T, Miyazaki J, et al: Molecular cloning of the cDNAs for the subunits of rat mitochondrial fatty acid β-oxidation multienzyme complex. Structural and functional relationship to other mitochondrial and peroxisomal β-oxidation enzymes. J Biol Chem 268:26452-26460, 1993

23. Hirose A, Kamijo K, Osumi T, et al: cDNA cloning of rat liver 2,4-dienoyl-CoA reductase. Biochim Biophys Acta 1049:346-349, 1990

24. Magnuson MA, Morioka H, Tecce MF, et al: Coding nucleotide sequence of rat liver malic enzyme mRNA. J Biol Chem 261:1183-1186, 1986

25. Arakawa H, Takiguchi M, Amaya Y, et al: cDNA-derived amino acid sequence of rat mitochondrial 3-oxoacyl-CoA thiolase with no transient presequence: Structural relationship with peroxisomal isozyme. EMBO J 6:1361-1366, 1987

26. Asins G, Luís R, Serra D, et al: Gene expression of enzymes regulating ketogenesis and fatty acid metabolism in regenerating rat liver. Biochem J 299:65-69, 1994

27. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159, 1987

28. Ide T, Okamatsu H, Sugano M: Regulation by dietary fats of 3-hydroxy-3-methylglutaryl-coenzyme A reductase in rat liver. J Nutr 108:601-612, 1982

29. Snedecor GW, Cochran WG: Statistical Methods (ed 8). Ames, IA, Iowa University Press, 1989

30. Ide T, Kano S, Yanagita T, et al: Dietary modifications of the biliary bile acid glycine:taurine ratio and activity of hepatic bile acid-CoA:amino acid *N*-acyltransferase (EC 2.3.1) in the rat. Br J Nutr 72:93-100, 1994

31. Matsubara Y, Kraus JP, Ozasa H, et al: Molecular cloning and nucleotide sequence of cDNA encoding the entire precursor of rat liver medium chain acyl coenzyme A dehydrogenase. J Biol Chem 262:10104-10108, 1987

32. Matsubara Y, Indo Y, Naito E, et al: Molecular cloning and nucleotide sequence of cDNAs encoding the precursors of rat long-chain acyl-coenzyme A, short-chain acyl-coenzyme A, and isovaleryl-coenzyme A dehydrogenases. J Biol Chem 264:16321-16331, 1989

33. Aoyama T, Ueno I, Kamijo T, et al: Rat very–long-chain acyl-CoA dehydrogenase, a novel mitochondrial acyl-CoA dehydrogenase gene product, is a rate-limiting enzyme in long-chain fatty acid beta-oxidation system. cDNA and deduced amino acid sequence and distinct specificities of the cDNA-expressed protein. J Biol Chem 269:19088-19094, 1994

34. Hakkola EH, Hiltunen JK: The existence of two mitochondrial isoforms of 2,4-dienoyl-CoA reductase in the rat. Eur J Biochem 215:199-204, 1993

35. Hakkola EH, Hiltunen JK, Autio-Harmainen HI, et al: Mitochondrial 2,4-dienoyl-CoA reductases in the rat: Differential responses to clofibrate treatment. J Lipid Res 35:1820-1828, 1994

36. Pihlajaniemi T, Hiltunen JK: Amino acid sequence similarities of the mitochondrial short chain Δ^3,Δ^2-enoyl-CoA isomerase and peroxisomal multifunctional Δ^3,Δ^2-enoyl-CoA isomerase, 2-enoyl-CoA hydra-

tase, 3-hydroxyacyl-CoA dehydrogenase enzyme in rat liver. J Biol Chem 266:10750-10753, 1991

37. Tomioka Y, Hirose A, Moritani H, et al: cDNA cloning of mitochondrial Δ^3,Δ^2-enoyl-CoA isomerase of rat liver. Biochim Biophys Acta 28:109-112, 1992

38. Inoue H, Noguchi T, Tanaka T: Complete amino acid sequence of rat L-type pyruvate kinase deduced from the cDNA sequences. Eur J Biochem 154:465-469, 1986

39. Willumsen N, Skorve J, Hexeberg S, et al: The hypotriglyceridemic effect of eicosapentaenoic acid in rats is reflected in increased mitochondrial fatty acid oxidation followed by diminished lipogenesis. Lipids 28:683-690, 1993

40. Willumsen N, Hexeberg S, Skorve J, et al: Docosahexaenoic acid shows no triglyceride-lowering effects but increases the peroxisomal fatty oxidation in liver of rats. J Lipid Res 34:13-22, 1993

41. McGarry JD, Brown NF: The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem 244:1-14, 1997

42. Minami-Ishii N, Taketani S, Osumi T, et al: Molecular cloning and sequence analysis of the cDNA for rat mitochondrial enoyl-CoA hydratase. Structural and evolutionary relationships linked to the bifunctional enzyme of the peroxisomal-oxidation system. Eur J Biochem 185:73-78, 1989

43. El-Fakhri M, Middleton B: The existence of an inner-membrane-bound, long acyl-chain-specific 3-hydroxyacyl-CoA dehydrogenase in mammalian mitochondria. Biochim Biophys Acta 713:270-279, 1982

44. Mire S, Takiguchi M, Matsue H, et al: Molecular cloning of cDNA for rat mitochondrial 3-oxoacyl-CoA thiolase. Eur J Biochem 154:479-484, 1986

45. Fujiki Y, Rachubinski RA, Mortensen RM, et al: Synthesis of 3-ketoacyl-CoA thiolase of rat liver peroxisomes on free polyribosomes as a larger precursor. Induction of thiolase mRNA activity by clofibrate. Biochem J 226:697-704, 1985

46. Noguchi T, Iritani N, Tanaka T: Molecular mechanism of induction of key enzymes related to lipogenesis. Proc Soc Exp Biol Med 200:206-209, 1992

47. Kochan Z, Bukato G, Swierczynski J: Inhibition of lipogenesis in rat brown adipose tissue by clofibrate. Biochem Pharmacol 46:1501-1506, 1993

48. Aoyama T, Peters JM, Iritani N, et al: Altered constitutive expression of fatty acid–metabolizing enzymes in mice lacking the peroxisome proliferator–activated receptor α (PPARα). J Biol Chem 273:5678-5684, 1998

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

50. Latruffe N, Vamecq J: Peroxisome proliferators and peroxisomal proliferator activated receptors (PPARs) as regulators of lipid metabolism. Biochimie 79:81-94, 1997

51. Hertz R, Nikodem V, Ben-Ishai A, et al: Thyromimetic mode of action of peroxisome proliferators: Activation of 'malic' enzyme gene transcription. Biochem J 319:241-248, 1996

52. Ren B, Thelen AP, Peters JM, et al: Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator–activated receptor α. J Biol Chem 272:26827-26832, 1997

53. Fukuda N, Miyagi C, Zhang L, et al: Reciprocal effects of dietary sesamin on ketogenesis and triacylglycerol secretion by the rat liver. J Nutr Sci Vitaminol 44:715-722, 1998