Comparative Effects of Sesame Seeds Differing in Lignan Contents and Composition on Fatty Acid Oxidation in Rat Liver

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Abstract: We compared the physiological activities of sesame seeds rich in lignans from three varieties (Gomazou, Maruhime and Maruemon), and those from a conventional cultivar (Masekin) in rats. The sum of the values of fat-soluble lignans (sesamin and sesamolin) in seeds of Gomazou, Maruhime and Maruemon varieties was approximately double the value in Masekin. Seeds from Maruemon contained fat-soluble lignan most exclusively as sesamin while other varieties contained sesamin and sesamolin at about a 2:1 ratio. After a 16 d experiment, sesame seeds, added at 200 g/kg to the experimental diets, increased the activity and mRNA levels of fatty acid oxidation enzymes. Increases were stronger with seeds rich in lignans than with seeds from Masekin. In contrast, sesame seeds lowered the activity and mRNA levels of lipogenic enzymes. However, sesame seeds from all the varieties were comparable in affecting these parameters. Serum triacylglycerol concentrations were lower in rats fed diets containing sesame seeds rich in lignans than in those fed a diet free of sesame seeds or a diet containing seeds from the Masekin variety. Serum malondialdehde (a marker of lipid peroxidation) was lower in rats fed diets containing sesame seeds rich in lignans than in those fed a sesame seed-free diet or Masekin diet. It is apparent that sesame seeds rich in lignans, irrespective of lignan composition, more profoundly affect hepatic fatty acid oxidation and serum triacylglycerol levels and possibly attenuate oxidative stress. Therefore, consumption of sesame seeds rich in lignans hopefully results in physiological activity to promote health.

Key words: sesame lignans, hepatic fatty acid oxidation, hepatic lipogenesis, serum lipids, rat

1 INTRODUCTION

Sesame seeds contain compounds collectively known as lignans. Sesamin and sesamolin are extractable in oil, and sesame seed and its unrefined oil contain these two lignans at a ratio of about 2:1. Another major sesame lignan is sesaminol, which can exist as a mono-, di-, or tri-glucoside and is not extractable in oil. During the refining of sesame oil, sesamin is epimerized during acid-clay bleaching to form episesamin, while most of the sesamolin is degraded, with some converted to sesaminol. Fat-soluble sesame lignans, including sesamin, episesamin and sesamolin, have physiological effects, including anti-oxidant, anti-carcinogenic, blood pressure-lowering and serum lipid-lowering in experimental animals and humans. In relation to the serum lipid-lowering propensity of sesame lignans, we previously found that sesame lignans markedly increased the activity and gene expression of fatty acid oxidation enzymes in rat liver. Also, sesame lignans decreased hepatic activity and mRNA levels of enzymes involved in fatty acid synthesis. All these findings suggested that the consumption of sesame seeds has health benefits, and enrichment of the lignans would potentiate the characteristics of sesame in improving human health. We therefore bred and established a sesame variety named Gomazou (GZ) with seeds containing sesamin and sesamolin at twice the rate of a conventional variety. We demonstrated in rats that consumption of sesame seeds from GZ compared to those from a conventional variety effectively increased hepatic activity of enzymes involved in fatty acid oxidation, although seeds
form these varieties were equally effective in decreasing the values for fatty acid synthesis in rats. These changes are associated with profound decreases in serum triacylglycerol levels in rats fed seeds from GZ. However, it is not still clear if these changes are solely ascribable to the differences in the concentration of fat-soluble lignans (sesamin and sesamolin) in seeds. There is a possibility that compounds other than lignans are also involved in the physiological activities of seeds from GZ variety.

We recently completed the breeding of two novel sesame varieties with seeds rich in lignans, named Maruhime (MH) and Maruemon (ME)\(^{20}\). The sum of the values for the concentrations in seeds of fat-soluble lignans (sesamin and sesamolin) was twice as high as in a conventional variety and was approximately the same between the seeds from MH and ME. However, lignan compositions were markedly different. MH contained sesamin and sesamolin at about a 2:1 ratio, and this was also the case for ME (a conventional variety) and GZ (a variety with seeds rich in lignans). However, ME contained fat-soluble lignans almost exclusively as sesamin, and sesamolin contents were less than one tenth of sesamin\(^{20}\). It has been demonstrated that the physiological activities of various fat-soluble lignans (sesamin, episesamin and sesamolin) affecting hepatic fatty acid metabolism are considerably different\(^{15-17}\). Therefore, there is a possibility that the propensity to affect hepatic fatty acid metabolism is considerably different among the seeds from these sesame varieties. Taking this into consideration, in the present study, we compared the physiological activities of seeds from various sesame varieties differing in lignan contents and compositions affecting hepatic fatty acid metabolism in rats.

2 EXPERIMENTAL

2.1 Animals and diets

Male Sprague-Dawley rats obtained from Charles River Kanagawa, Japan were housed individually in a room with controlled temperature (20-22\(^\circ\)C), humidity (55-65\%) and lighting (lights on from 07:00 to 19:00 h), and fed on a commercial non-purified diet (type NMF; Oriental Yeast Co., Tokyo, Japan) containing 1 mM EDTA and 3 mM Tris-HCl. After 7 d of acclimatization to the housing conditions, rats were randomly divided into groups (6-7 rats per group) with the same mean body weights and fed experimental diets for 16 d. The compositions of the experimental diets are shown in Table 1. Sesame seed powders were added at a rate of 200 g/kg. We used sesame seed powder from a conventional variety that is popular in Japan (Masekin (MK)) and seeds rich in fat-soluble lignans (sesamin and sesamolin) from Gomazou (GZ)\(^{19}\), Maruhime (MH)\(^{20}\) and Maruemon (ME)\(^{20}\). The sesamin and sesamolin contents of sesame seeds were determined as described previously\(^{25}\), and were 4.1 and 2.4 g/kg for MK, 8.7 and 3.4 g/kg for GZ, 8.7 and 4.1 g/kg for MH and 12.6 and 0.4 g/kg for ME, respectively. Therefore, the sum of the values of sesamin and sesamolin was about two times higher in GZ, MH and ME than in MK. MK, GZ and MH contained sesamin and sesamolin at about a 2:1 ratio, but ME contained sesamin but almost no sesamolin. The sesame seeds differed in seed color: brown for MK and GZ, white for MH and black for ME, respectively. Analyses revealed that sesame seeds from MK, GZ, MH and ME varieties contained 272, 246, 243 and 263 g protein/kg and 539, 542, 562 and 503 g lipid/kg, respectively. Based on this information, the amounts of casein and oil mixture composed of corn and safflower oils (65:35, wt/wt) were reduced in diets containing sesame seeds to adjust protein and fat contents to be indistinguishable among the diets. Casein contained 942 g protein/kg. Sesame is reported to contain 108 g dietary fiber per kg\(^{21}\). Therefore, the cellulose contents of the diets containing sesame were reduced accordingly. Thus, the various experimental diets contained similar amounts of protein, fat and dietary fiber. Fatty acid compositions determined by GLC were similar among the experimental diets and are shown in Table 1. The compositions of mineral and vitamin mixtures were the same as those recommended by the American Institute of Nutrition\(^{22}\). Animals had free access to the diets and water during the experimental period. This study was approved by the review board of the animal ethics of our university and we followed the university’s guidelines in the care and use of laboratory animals.

2.2 Enzyme assays

At the termination of the experiment, rats were killed by bleeding from the abdominal aorta under isoflurane anesthesia. The livers were then quickly excised. About 2 g of each liver was homogenized with 15 mL of 0.25 M sucrose containing 1 mM EDTA and 3 mM Tris-HCl (pH 7.0) and centrifuged at 12,000 xg for 20 min. The activity of hepatic fatty acid oxidation enzymes was analyzed using whole liver homogenate as an enzyme source\(^{23}\). Peroxisomal palmitoyl-CoA oxidation rate (KCN-insensitive acyl-CoA-dependent NAD reduction rate) and acyl-CoA oxidase activity were measured using palmitoyl-CoA as a substrate. Carnitine acyltransferase and enoyl-CoA hydratase activities were measured using substrates differing in carbon chain length: acetyl-CoA (C2), octanoyl-CoA (C8) and palmitoyl-CoA (C16) for the former and crotonoyl-CoA (C4) and trans-2-ocenoyl-CoA (C8) for the latter. 3-Hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase activities were measured using acetoacetyl-CoA and 2,4-dienoyl-CoA reductase activity was analyzed using sorboyl-CoA as substrates, respectively. The activity of enzymes in fatty acid synthesis was measured using a 12,000 xg supernatant fraction as detailed previously\(^{23}\).
Table 1  Compositions of experimental diets and their fatty acid composition and lignan content.

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Control</th>
<th>Sesame varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MK</td>
</tr>
<tr>
<td>Sesame seed powder</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>136</td>
</tr>
<tr>
<td>Oil Mixture</td>
<td>120</td>
<td>12.2</td>
</tr>
<tr>
<td>Cellulose</td>
<td>30</td>
<td>8.4</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Sucrose</td>
<td>450</td>
<td>443</td>
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</table>

Fatty acid composition (g/100 g oil)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>MK</th>
<th>GZ</th>
<th>MH</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>16:0</td>
<td>6.6</td>
<td>7.0</td>
<td>6.4</td>
<td>6.2</td>
<td>6.6</td>
</tr>
<tr>
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<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>18:0</td>
<td>1.1</td>
<td>3.5</td>
<td>3.0</td>
<td>3.5</td>
<td>3.4</td>
</tr>
<tr>
<td>18:1</td>
<td>47.3</td>
<td>48.1</td>
<td>44.8</td>
<td>47.7</td>
<td>47.1</td>
</tr>
<tr>
<td>18:2</td>
<td>44.5</td>
<td>40.9</td>
<td>45.4</td>
<td>42.0</td>
<td>42.3</td>
</tr>
<tr>
<td>18:3</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>20:0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>22:0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Lignans (g/kg)

<table>
<thead>
<tr>
<th>Lignan</th>
<th>Control</th>
<th>MK</th>
<th>GZ</th>
<th>MH</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sesamin</td>
<td>0</td>
<td>0.83</td>
<td>1.75</td>
<td>1.75</td>
<td>2.52</td>
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<tr>
<td>Sesamolin</td>
<td>0</td>
<td>0.48</td>
<td>0.68</td>
<td>0.82</td>
<td>0.07</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>1.31</td>
<td>2.43</td>
<td>2.57</td>
<td>2.59</td>
</tr>
</tbody>
</table>

Oil mixture represents a mixture of corn and safflower oil (65:35, wt/wt). Protein and fat contents of sesame were determined, and amounts of casein and oil mixture were reduced in diets containing sesame to adjust protein and fat contents to be indistinguishable among diets. Also, amounts of cellulose contents were reduced in diets containing sesame according to the information on dietary fiber content of sesame provided by Resources Council, Science and Technology Agency of Japan (21). Fatty acid compositions of diets were determined in quadruplicate and average values are shown. MK, Masekin; GZ, Gomazou; MH, Maruhi; ME, Maruemon.

2.3 mRNA analysis

RNA in the liver was extracted and mRNA abundance was analyzed by quantitative real-time PCR, as detailed elsewhere. mRNA abundance was calculated as a ratio to the β-actin level in each cDNA sample and expressed as a fold-change, assigning a value of 1 for rats fed a diet free of sesame seeds.

2.4 Analyses of serum and liver components

Liver triacylglycerol and phospholipid concentrations were determined as described previously. The liver cholesterol concentration was analyzed as detailed elsewhere. Serum triacylglycerol, cholesterol, and phospholipid concentrations were assayed using commercial enzyme kits (Wako Pure Chemical, Osaka, Japan). Malondialdehyde in serum was analyzed by HPLC as thiobarbituric acid adducts. Hepatic carnitine was analyzed by the method of Pearson et al.
2.5 Statistical Analysis

Microsoft Excel add-in software (Excel Statistics 2010, Social Survey Research Information Co., Tokyo, Japan) was used for statistical analysis. The constancy of the variance and normality of the distribution of the observations were evaluated by Levene’s test and the Kolmogorov-Smirnov test, respectively. If variances were heterogeneous and/or the distributions were not normal, they were transformed logarithmically. The transformations were successful in rendering the variance of the observation constant and the distribution of data normal, and hence the transformed values were used for subsequent statistical evaluations. The data were analyzed with one-way ANOVA to establish whether the effect of sesame seeds was significant, and significant differences of the means at a level of \( p < 0.05 \) were evaluated with the two-sided Tukey’s test.

3 RESULTS

3.1 Growth parameters and liver weights

Average body weight at the start of the experiments was 144 ± 1 g. There were no significant differences in food intake (average values were 19.1-20.7 g/day for the various groups), body weight at the time of killing (293-312 g) and growth (138-155 g/16 d). Liver weights were significantly higher in rats fed a diet containing sesame seeds from Gomazou (GZ) (5.56 ± 0.15 g/100 g body weight) than in animals fed a diet without sesame seeds (5.07 ± 0.09 g/100 g body weight). However, no other significant differences in this parameter were seen among groups.

3.2 Activity and mRNA levels of hepatic fatty acid oxidation enzymes

Diets containing sesame seeds increased various hepatic enzymes involved in fatty acid oxidation (Fig. 1). The peroxisomal palmitoyl-CoA oxidation rate and activities of various enzymes involved in fatty acid oxidation were significantly higher in rats fed diets containing sesame seeds from various varieties than in the animals fed a control diet free of sesame seeds. Among the groups of animals fed diets containing sesame seeds, the values except for the activities of carnitine acyltransferase measured using octanoyl-CoA and palmitoyl-CoA substrates were significantly higher in rats fed diets containing sesame seeds from Gomazou (GZ), Maruemon (ME) and Maruhime (MH) varie-

![Fig. 1](image_url)

Effect of dietary sesame seeds on the activity of enzymes involved in fatty acid oxidation in rat liver. Values are the means with their standard errors (n = 6-7). Rats were fed a control diet free of sesame seeds (control) or a diet containing sesame powder from a conventional variety (Masekin (MK)) or diets containing sesame seeds rich in lignans from Gomazou (GZ), Maruime (MH) and Maruemon (ME) varieties. Means without a common letter differ, \( p < 0.05 \).
ties rich in lignan than in the animals fed a diet containing sesame seeds from a conventional variety Masekin (MK). The activities of carnitine acyltransferase measured using octanoyl-CoA and palmitoyl-CoA were the same between rats fed sesame seeds from MK and ME, respectively. The activities of various enzymes were comparable among rats fed sesame seeds rich in lignans from GZ, MH and ME varieties, except in a few cases (peroxisomal palmitoyl-CoA oxidation rate, activity of carnitine acyltransferase measured with palmitoyl-CoA substrate, activity of 3-hydroxyacyl-CoA dehydrogenase).

All the diets containing sesame seeds compared to a diet free of sesame seeds significantly increased mRNA levels of various peroxisomal enzymes involved in fatty acid oxidation and a peroxisomal membrane protein (peroxin 11α) (Fig. 2). Sesame seeds rich in lignans from GZ, ME and MH varieties were much more competent than the seeds from a conventional cultivar MK in increasing these parameters, except for carnitine octanoyltransferase where there was no significant difference in the mRNA levels between rats fed sesame seeds from MK and GZ varieties.

Sesame seeds also effectively increased mRNA levels of various hepatic mitochondrial enzymes involved in fatty acid oxidation except carnitine palmitoyltransferase 1a (Fig. 3). Sesame seeds from GZ and ME but not MK and MH varieties significantly increased the mRNA level of this enzyme. The mRNA levels of carnitine O-acetyltransferase, carnitine palmitoyltransferase 1b, carnitine palmitoyltransferase 2, 3-ketoacyl-CoA thiolase and 2,4-dienoyl-CoA reductase were significantly higher in rats fed sesame seeds rich in lignans from GZ, MH and ME varieties than in the animals fed the seeds from MK variety. Also, seeds from MH and ME but not GZ varieties significantly increased mRNA levels of carnitine/acetyl carnitine translocase and enoyl-CoA delta isomerase 1 compared to seeds from MK.

3.3 Activity and mRNA levels of enzymes involved in fatty acid synthesis

All the diets containing sesame seeds significantly decreased fatty acid synthase, ATP-citrate lyase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and pyruvate kinase activities relative to the control diet without sesame seeds (Table 2). The activities of these enzymes except for pyruvate kinase were comparable among rats fed sesame seeds from various varieties. Pyruvate kinase activities were significantly lower in rats fed seeds from GZ, MH and ME varieties than in the animals fed seeds from MK variety. The diet containing sesame

![Fig. 2](image-url) Effect of dietary sesame seeds on mRNA levels of peroxisomal fatty acid oxidation enzymes and a peroxisomal membrane protein (peroxin 11α) in rat liver. Values are the means with their standard errors (n = 6-7). Rats were fed a control diet free of sesame seeds (control) or a diet containing sesame powder from a conventional variety (Masekin (MK)) or the diets containing sesame seeds rich in lignans from Gomazou (GZ), Maruhime (MH) and Maruemon (ME) varieties. Means without a common letter differ, p <0.05.
seeds from MK did not affect malic enzyme activity, but those containing seeds from GZ, MH and ME varieties significantly increased it.

We also measured mRNA levels of proteins involved in hepatic lipogenesis. There are two types of acetyl-CoA carboxylase, i.e., alpha and beta. The alpha but not beta form appears to be involved in fatty acid synthesis in cytosol. Mammalian tissues contain 3 distinct isoforms of malic enzyme (malic enzyme 1, 2 and 3). Malic enzyme 1 appears to be involved in the regulation of lipogenesis. There are four isoforms of pyruvate kinase in mammals. L-Pyruvate kinase is an enzyme expressed in the liver. Adiponutrin is a protein presumed to be involved in the regulation of lipogenesis. Consistent with the observations made on enzyme activity, sesame seeds from various varieties significantly reduced mRNA levels of acetyl-CoA carboxylase, fatty acid synthase, ATP-citrate lyase, glucose 6-phosphate dehydrogenase, and L-pyruvate kinase. Seeds from various varieties were equally effective in decreasing the values of these enzymes except for L-pyruvate kinase. The reduction was weaker in rats fed seeds from MK variety than in the animals fed seeds rich in lignans from GZ, MH and ME varieties. The values were comparable among rats fed sesame seeds rich in lignans from three different varieties.

3.4 Serum and liver lipid levels

Serum triacylglycerol concentrations were significantly lower in groups of rats fed diets containing sesame seeds than in the animals fed a control diet without sesame seeds (Table 3). Among rats fed diets containing sesame seeds, the values were significantly lower in the animals fed seeds.
### Table 2  Effects of dietary sesame seeds on the activity of lipogenic enzymes and mRNA levels of genes related to lipogenesis in rat liver.

<table>
<thead>
<tr>
<th>Enzyme activity (nmol/min per mg protein)</th>
<th>Control</th>
<th>MK</th>
<th>GZ</th>
<th>MH</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid synthase</td>
<td>31.7 ± 2.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.3 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.79 ± 1.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.41 ± 1.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.17 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ATP-citrate lyase</td>
<td>71.2 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.3 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.8 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.2 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.4 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose 6-phosphate dehydrogenase</td>
<td>72.8 ± 6.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.6 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.6 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.7 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.42 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>6-Phosphogluconate dehydrogenase</td>
<td>143 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.5 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.6 ± 5.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.4 ± 5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.5 ± 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Malic enzyme</td>
<td>69.0 ± 5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.5 ± 4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.5 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Pyruvate kinase</td>
<td>892 ± 45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>300 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>176 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>189 ± 17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>161 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
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<table>
<thead>
<tr>
<th>mRNA level (fold change)</th>
<th>Acetyl-CoA carboxylase</th>
<th>Fatty acid synthase</th>
<th>ATP-citrate lyase</th>
<th>Glucose 6-phosphate dehydrogenase</th>
<th>6-Phosphogluconate dehydrogenase</th>
<th>Malic enzyme</th>
<th>L-Pyruvate kinase</th>
<th>Adiponutrin</th>
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<tbody>
<tr>
<td></td>
<td>1.00 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.530 ± 0.035&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.448 ± 0.030&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.529 ± 0.040&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.03 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.61 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.53 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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</table>

Values are the means ± SEM, n = 6-7. Means in a row with superscripts without a common letter differ, *p*<0.05. MK, Masekin; GZ, Gomazou; MH, Maruhime; ME, Maruemon.

**Fig. 4**  Effect of dietary sesame seeds on mRNA levels of Cd36 and carnitine transporter and carnitine concentration in rat liver. Values are the means with their standard errors (n = 6-7). Rats were fed a control diet free of sesame seed (control) or a diet containing sesame powder from a conventional variety (Masekin (MK)) or diets containing sesame seeds rich in lignans from Gomazou (GZ), Maruhime (MH) and Maruemon (ME) varieties. Means without a common letter differ, *p*<0.05.
concentration was higher in rats fed seeds from MK variety than in the other groups. Dietary sesame seeds significantly reduced serum concentrations of malondialdehyde. The decreases were much more effective in increasing the activity of enzymes involved in fatty acid oxidation compared to that of seeds from the conventional variety. The values in rats fed diets containing 0.1, 0.2 and 0.5% of the lignan preparations were about one-half that observed in rats fed a lignan-free diet, but were almost comparable to that of seeds from the conventional variety.

Our previous studies showed that fat-soluble sesame lignans (sesamin, episesamin and sesamolin) increased the activity and mRNA levels of various enzymes involved in fatty acid oxidation, presumably through a peroxisome activated-receptor activated receptor α (PPARα) dependent mechanism, while decreasing the parameters of lipogenic enzymes through a sterol regulatory element binding protein-1 (SREBP-1) dependent mechanism. Therefore, it is conceivable that seed-dependent changes in hepatic fatty acid oxidation and lipogenesis could be ascribable to fat-soluble lignans. The observations in the previous and current studies that sesame seeds rich in lignans from GZ, MH and ME varieties were more effective than the conventional MK variety in increasing hepatic fatty acid oxidation support this consideration. However, the impact of seeds rich in lignan on hepatic lipogenic enzymes except for malic enzyme and pyruvate kinase was comparable to that of seeds from the conventional variety. In relation to this, we previously observed that the dose-dependent effects of a lignan preparation containing equivalent amounts of sesamin and episesamin on the activity and mRNA levels of hepatic fatty acid synthase were saturable. The values in rats fed diets containing 0.1, 0.2 and 0.5% of the lignan preparations were about one-half that observed in rats fed a lignan-free diet, but were almost comparable among rats fed diets containing different seeds from various varieties except for one instance where the phospholipid level was significantly lower in rats fed seeds from ME variety than in the animals fed seeds from MH variety. Sesame seeds significantly reduced serum concentrations of malondialdehyde. The decreases were stronger with diets containing seeds rich in lignans from GZ, MH and ME varieties than in seeds from MK variety.

No significant differences were seen in hepatic triacylglycerol concentration among groups. Hepatic cholesterol concentration was higher in rats fed seeds from MK variety than in the other groups. Dietary sesame seeds significantly increased hepatic phospholipid concentrations. Among the groups of rats fed sesame seeds, the value was the highest in the animals fed seeds from GZ variety.

4 DISCUSSION

We previously showed that the consumption of sesame seeds decreased hepatic activity and mRNA levels of lipogenic enzymes while increasing these parameters of fatty acid oxidation. We also observed that seeds from Gomazou (GZ) sesame variety, called 0732 line in our previous report, contained twice as much sesamin and sesamolin as seeds from a conventional Masekin (MK) variety and were much more effective in increasing the activity of enzymes involved in fatty acid oxidation. These findings were confirmed in the present study. In addition, we observed that our recently established sesame varieties (MH and ME) rich in lignans were as effective as GZ in affecting hepatic fatty acid oxidation.
amounts of the lignan. Sesame seeds also decreased the activity and mRNA levels of L-pyruvate kinase, an enzyme involved in the glycolytic pathway, and decreases were much stronger with seeds rich in lignans. Therefore, the situation was somewhat different from those observed with fatty acid synthase, ATP-citrate lyase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Studies indicated that lipogenic enzymes and pyruvate kinase are coordinately regulated under various nutritional and physiological conditions\(^{35,36}\) and are under the control of SREBP-1. The present observation, however, implies that different mechanisms exist in the regulation of pyruvate kinase and lipogenic enzymes. The response to dietary sesame seeds of malic enzyme was completely different from those of other enzymes. Actually, seeds from MK did not affect the activity and mRNA levels of this enzyme while seeds rich in lignans from GZ, MH and ME significantly increased these parameters, except in rats fed a diet containing seeds from ME where malic enzyme activity was not significantly different from that observed in rats fed a sesame seed-free diet and a diet containing seeds from a conventional MK variety. It has been reported that the expression of the genes of malic enzyme 1 is dually regulated by SREBP-1 and PPAR\(\alpha\). Lignans that are contained in sesame seeds appear to be agonists of PPAR\(\alpha\)\(^{14}\) and hence are expected to stimulate gene expressions of malic enzyme 1 and, at the same time, the ligans should conversely down-regulate their mRNA expression through a SREBP-1-dependent mechanism\(^{40}\). It is conceivable that the PPAR\(\alpha\)-dependent mechanism overcame the SREBP-1-dependent mechanism in rats fed sesame seeds rich in lignans and hence increased the activity and mRNA levels of malic enzyme.

Sesame seeds increased mRNA expressions of Cd36 and carnitine transporter. We also observed that up-regulation of carnitine transporter mRNA expression by sesame seeds was associated with increased carnitine concentration in the liver. The responses were much greater with seeds rich in lignans than with seeds from a conventional variety. It has been indicated that Cd36\(^{38}\) and carnitine transporter genes\(^{39}\) are under the control of PPAR\(\alpha\). We previously observed that fat-soluble lignans increased the mRNA expression of Cd36, a transporter involved in fatty acid uptake, and carnitine transporter\(^{15}\). Therefore, it is conceivable that these changes represent the consequence of PPAR\(\alpha\) activation by the lignans contained in sesame seeds.

We previously observed that the physiological activities of various fat-soluble lignans are considerably different\(^{15-17}\). Compared to sesamin, sesamolin and episesamin had a much stronger effect on fatty acid oxidation and miscellaneous genes involved in fatty acid transport and metabolism, except for those involved in lipogenesis\(^{15}\). However, the present observation showed that seeds from ME variety, which contained fat-soluble lignan predominantly as sesamin and was almost devoid of sesamolin, was as effective as seeds from GZ and MH varieties, which contained sesamin and sesamolin at about a 2:1 ratio, in affecting the parameter of hepatic fatty acid oxidation. The reason for this is not clear at present. Seeds from GZ and MH varieties contained considerable amounts of sesamolin, but the amounts were still considerably low relative to those of sesamin. Therefore, these levels may not be high enough to cause differential effects on hepatic fatty acid oxidation among rats fed seeds from GZ, MH and ME varieties. There is the possibility that the lignan levels in experimental diets containing sesame seeds rich in lignans (about 2.5 g/kg) were high enough to cause saturable effects to enhance hepatic fatty acid oxidation. This may account for the failure to observe differential effects of seeds from GZ, MH and ME varieties. The observation\(^{44}\) that at least a lignan preparation containing equivalent amount of sesamin and episesamin linearly increased hepatic fatty acid oxidation up to the dietary levels of 5g/kg may not support this consideration. Alternatively, there is a possibility that a compound(s) other than sesamin and sesamolin also contributes to the physiological activity of sesame seeds affecting fatty acid oxidation. In this situation, seed contents of sesamin and sesamolin may not be the only determinant in affecting hepatic fatty acid oxidation. Sesaminol, one of the lignans contained in sesame seeds as mono-, di- and triglucoside, is an alternative candidate affecting hepatic fatty acid oxidation. A previous study\(^{4}\) showed that sesame seeds contained considerable amounts of sesaminol (1.6-8.2 g of the aglycon in kg of seeds) as di- and triglucoside. Kang et al.\(^{4}\) showed that defatted sesame flour, containing 8.7 g sesaminol glucosides, 0.7 g sesamin and 0.3 g sesamolin in 1 kg, reduced hepatic but not serum concentrations of triacylglycerol and cholesterol in rabbits. As defatted sesame flour was almost devoid of sesamin and sesamolin, the reductions in hepatic lipids may be ascribed to sesaminol. Therefore, this study raised the possibility that sesaminol, similar to fat-soluble lignans, has the propensity to affect hepatic lipid metabolism. As complicated methods are required to analyze sesaminol\(^{4}\), we were not able to measure the contents of this lignan in the sesame seeds employed in the present study. Elucidation of the physiological activity of sesaminol in affecting hepatic fatty acid metabolism and determination of the sesaminol contents in sesame seeds are required to clarify this point.

It has been demonstrated that dietary sesamin and sesamolin reduced oxidative stress in experimental animals, although these lignans do not act as antioxidants \textit{in vitro}\(^{4,40}\). Studies indicated that these fat-soluble lignans are metabolized to compounds having antioxidant propensities and hence ameliorate oxidative stress\(^{4,41}\). Consistent with these observations, ingestion of sesame seeds has been demonstrated to reduce oxidative stress in rats\(^{42}\) and humans\(^{43,44}\). In the present study, we confirmed the results.
of a previous study\textsuperscript{42} that sesame seeds reduced serum concentrations of malondialdehyde in rats. Moreover, sesame seeds rich in lignans from GZ, MH and ME varieties were much more competent than those from a conventional variety, MK. The results therefore support the consideration that lignans are responsible for the sesame seed-dependent reduction in oxidative stress.

Alterations in fatty acid synthesis\textsuperscript{45} and oxidation\textsuperscript{37, 46} modify the availability of fatty acids for triacylglycerol synthesis, and in turn alter the very-low density lipoprotein production by the liver. Therefore, the change in the rate of fatty acid synthesis and oxidation in the liver is a factor modifying serum lipid concentrations. We found in the present study that seeds rich in lignans from GZ, MH and ME varieties lowered serum triacylglycerol more than the MK variety. The activity and mRNA levels of enzymes in fatty acid synthesis were lower in the groups of rats fed sesame seeds differing in lignan content than in those fed a control diet free of lignans, but they were the same among the four groups of rats fed sesame seeds. Activities of enzymes in fatty acid oxidation were, however, higher in the three groups of animals fed seeds from GZ, MH and ME varieties than in those fed MK variety. Therefore, an alteration in very-low-density lipoprotein production through the change in hepatic fatty acid oxidation may primarily be responsible for the serum triacylglycerol-lowering effect of sesame rich in lignans relative to a conventional variety.

Alterations in the rate of synthesis and uptake from circulation of fatty acid as well as the rate of its degradation by fatty acid oxidation pathway could be responsible mechanisms to affect hepatic triacylglycerol levels. Despite the fact that sesame seeds decreased hepatic lipogenesis but increased fatty acid oxidation, they failed to affect hepatic triacylglycerol concentration in the present study. This may be due to the up-regulation of Cd36 expression. This study was supported by a grant-in-aid for scientific research (Scientific Research C, no. 25450177) from the Japan Society for the Promotion of Science.

5 CONCLUSION

Sesame seeds rich in sesamin and sesamolin from our three established sesame varieties compared to those from a conventional variety significantly increased the activity and mRNA levels of enzymes involved in hepatic fatty acid oxidation accompanying the lowering of serum triacylglycerol. In addition, sesame seeds rich in lignans compared to seeds from a conventional variety significantly reduced serum malondialdehyde, indicating the superior health-promoting propensities of seeds rich in lignans from the three sesame varieties that we established. Among the seeds rich in lignans from three varieties, the potency of the variety containing fat-soluble lignan almost exclusively as sesamin to affect hepatic fatty acid oxidation was the same as that of seeds from the two varieties which contained sesamin and sesamolin at about a 2:1 ratio. This did not agree with the previous observations\textsuperscript{15–17} that sesamin was less effective than sesamolin in affecting hepatic fatty acid oxidation. In this context, the possibility that a compound(s) other than sesamin and sesamolin is involved in the sesame seed-dependent increase in hepatic fatty acid oxidation should be taken into consideration.

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