Effect of Dietary Fats and Sesamin on the Lipid Metabolism and Immune Function of Sprague-Dawley Rats

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We examined the effect of three dietary fats, safflower oil (SAF) rich in linoleic acid, borage oil (BOR) rich in γ-linolenic acid, and perilla oil (PER) rich in α-linolenic acid, on the lipid metabolism, and chemical mediator and immunoglobulin levels in Sprague-Dawley rats, as well as the dietary effect of sesame-seed antioxidative sesamin. The serum cholesterol, phospholipid, triglyceride, prostanandin E\textsubscript{2} level and splenic leukotreine B\textsubscript{2} level were lower in the rats fed on BOR or PER than in those fed on SAF. SES feeding suppressed the expression of the lipid-decreasing effect of BOR, but not in the rats fed on PER. In respect of the fatty acid composition of the liver and spleen, PER feeding gave a lower arachidonic acid level, and higher eicosapentaenoic and docosahexaenoic acid levels than SAF feeding did, while the effect of BOR feeding was marginal. The effect of SES feeding on fatty acid composition was much smaller than that of dietary fats. In respect of immunoglobulin production, PER + SES feeding gave the lowest IgE productivity in the mesenteric lymph node lymphocytes. These results suggest that PER feeding regulated lipid metabolism and exerted an anti-allergic effect by a different mechanism from that with BOR feeding.

Key words: sesamin; polyunsaturated fatty acid; chemical mediator; immunoglobulin

There has been a recent increasing trend in the incidence of allergic disorders, in particular hypersensitivity to food or airborne allergens. Among the 4 types of allergic reactions, the Type I allergy plays an important role in the general occurrence of allergies.\textsuperscript{11} With this type of allergy, the induction of allergen-specific IgE and chemical mediator release from basophils or mast cells are essential steps for expression of the allergy. On the other hand, allergen-specific IgA inhibits the process by inhibiting allergen absorption and IgG by competing with IgE.\textsuperscript{11} Thus, class-specific regulation of Ig production and inhibition of chemical mediator release may be useful for alleviating allergic symptoms.

It has been reported that these immune reactions were enhanced or suppressed by various food components. For example, enhanced IgE production and inhibited IgA and IgG production by rat lymphocytes have been reported in bile acids,\textsuperscript{2} hydrogen peroxide\textsuperscript{3,4} and polyunsaturated fatty acids (PUFAs).\textsuperscript{5,6} On the other hand, PUFAs exerted an anti-allergic effect by inhibiting the leukotriene (LT) release from mast cells,\textsuperscript{7} as well as various antioxidants such as tea polyphenols\textsuperscript{6,7} and flavonoids.\textsuperscript{8-10}

Among them, PUFAs most strongly regulated these immune reactions.\textsuperscript{11-19} In general, PUFAs of the n-3 family such as α-linolenic acid (ALA) suppressed hypersensitivity, and those of the n-6 family such as linoleic acid (LA) enhanced the allergic response.\textsuperscript{20,21} However, some n-6 PUFAs such as γ-linolenic acid (GLA) have been shown to reduce hypersensitivity.\textsuperscript{22-24} To clarify the immunoregulatory effect of these PUFAs, the dietary effect of safflower oil (SAF) rich in LA, borage oil (BOR) rich in GLA, and perilla oil (PER) rich in ALA was compared.

Sesamin is an antioxidative lignan from sesame oil and has been reported to exert diverse physiological effects. It specifically interfered with the metabolism of essential fatty acids by inhibiting Δ5-desaturase in microorganisms\textsuperscript{25} and in rats.\textsuperscript{26,27} Such inhibition of Δ5-desaturase leads to an accumulation of dihomo-γ-linolenic acid (DGLA) and the reduction of arachidonic acid (AA). The former is a starting material for 1-series prostanadins (PG) synthesis such as PGE\textsubscript{1}, which exerts favorable functions,\textsuperscript{28,29} and the latter is a starting fatty acid for 4-series LT synthesis which induces the type I allergy. This suggests that the Δ5-desaturase inhibitor could suppress the allergic reaction. In fact, it has been shown that an oral administration of sesamin and α-tocopheryl strongly suppressed LTC\textsubscript{4} production in rat lung tissue.\textsuperscript{30,31} In the present study, the combined effect of dietary fats and sesamin on rat immune functions and lipid metabolism is examined to clarify their immunoregulatory mechanism.

Material and Methods

Animals and diets. Four-week-old male Sprague-Dawley rats (Seiwa Experimental Animals, Fukuoka, Japan) housed individually in stainless steel mesh cages were acclimatized for 7 days after their arrival on a nonpurified diet (NMF, Oriental Yeast Co., Tokyo, Japan) in an air-conditioned room (20-23°C, lights on 0800-2000 hr). The rats weighing an average of 139 g were divided into 3 groups of 6 rats each and given free access to the diet and water. The diet, according to the recom-

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Table 1. Fatty Acid Compositions of the Dietary Fats

<table>
<thead>
<tr>
<th>Group</th>
<th>SAF</th>
<th>BOR</th>
<th>PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>15.6</td>
<td>11.9</td>
<td>8.0</td>
</tr>
<tr>
<td>16:1</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>18:0</td>
<td>2.7</td>
<td>4.9</td>
<td>26.5</td>
</tr>
<tr>
<td>18:1</td>
<td>20.2</td>
<td>18.0</td>
<td>0.0</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>60.6</td>
<td>33.2</td>
<td>15.9</td>
</tr>
<tr>
<td>18:3n-6</td>
<td>—</td>
<td>26.7</td>
<td>—</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>—</td>
<td>—</td>
<td>48.3</td>
</tr>
</tbody>
</table>

SAF, safflower oil; palm oil = 7:2 (w/w); BOR, borage oil; PER, perilla oil.

Mendations of American Institute of Nutrition, contained the following ingredients by weight percentage: casein, 20; dietary fat, 5; AIN76 mineral mixture, 3.5; AIN76 vitamin mixture, 1; choline bitartrate, 0.2; DL-methionine, 0.3; cellulose, 5; sesamin 0.1; corn starch, 15; and sucrose to 100. The dietary fat was PER (Ohta Oil Co., Okazaki, Japan), BOR (Bioriginal Food and Science Corp. Co., Saskatchewan, Canada) or safflower oil (Rinoru Oil Mill Co., Nagoya, Japan). The fatty acid composition of each of these oils is given in Table 1. To make the content of PUFA comparable in the three dietary fats, palm oil (Fuji Oil Co., Osaka, Japan) was added to SAF at a 2:7 (w/w) ratio. The mineral and vitamin mixtures were purchased from Oriental Yeast Co., Tokyo, Japan. Sesamin, 99.5% purity as total lignans, was presented by Suntory Ltd. (Osaka, Japan) and was a 1:1 mixture of sesamin and episesamin. The body weight and food intake of each animal were recorded every other day. After 4 weeks of feeding, each rat was killed by withdrawing blood from the abdominal aorta under diethyl ether anesthesia and the serum was harvested. The liver, spleen and epididymal adipose tissue were immediately excised into ice-cold saline, blotted, and weighed. The lymphocytes of the spleen and mesenteric lymph node (MLN) were isolated as described previously and cultured in an RPMI1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; Intergen Co., Purchase, NY, U.S.A.).

Lipid Analyses. The tissue and lymphocyte lipids were extracted by the method of Folch et al. Liver phosphatidylethanolamine (PC) and phosphatidylethanolamine (PE) were separated by thin-layer chromatography, and the fatty acid composition was analyzed by GLC (GC-8A, Shimadzu Co.) in a SILAR 10C column. The fatty acid composition of lymphocyte total lipids was analyzed by a GC-17A instrument (Shimadzu Co., Kyoto, Japan), in a CP-Sil 88 capillary silica column (50 m x 0.25 mm; Chrompack International, Middelburg, The Netherlands) as methyl esters that were prepared by esterification in 2 N HCl methanol. The GLC conditions were as follows: oven temperature, 240°C; injector temperature, 180°C; flow rate of helium, 1.5 ml/min; split ratio, 1/50. Blood lipoperoxides were measured as thiobarbituric acid reactive substances (TBARS). Serum total and HDL-cholesterol, triglyceride and phospholipid were enzymatically assayed with commercial kits (Cholesterol Test, HDL-Cholesterol Test, TG-G Test and PL-B Test, respectively, Wako Pure Chemicals, Osaka, Japan).

Measurements of eicosanoids. A sample of the spleen was homogenized in ice-cold phosphate-buffered saline (PBS, pH 7.4) and then incubated at 37°C for 20 min. Lekotriene B4 (LTB4) was extracted by the method of Moqbel et al. and measured by a radio immunoassay, using a commercial kit (NEK-030, DuPont NEN Research Products, Boston, MA, U.S.A.) under a linear relationship between the tissue weight and incubation time. Serum was diluted with 15% ethanol and acidified with 0.1 N HCl to pH 3.8. PGE2 was extracted by the method of Green et al. and measured by an enzymatic immunoassay kit (PGE2 Monoclonal EIA Kit, Cayman Chemical, Ann Arbor, MI, U.S.A.) under the condition of the production being linear with respect to the serum volume and incubation time at 25°C. Hista- mine was measured as reported elsewhere, after mixing the serum with 1 N HCl and centrifuging at 1,000 x g for 30 min.

Analysis of T-lymphocyte subsets. Lymphocytes of the spleen and MLN, at 1 x 106 cells/ml, were added to either R phycoerythrin (RPE)-labeled mouse monoclonal anti-rat CD4+ or fluorescein isothiocyanate (FITC)-labeled mouse monoclonal anti-rat CD8+ (Sero- tec, Kidlington, Oxford, U.K.). After incubating for 30 min at 4°C, the lymphocytes were collected by centrifugation at 160 x g for 5 min and rinsed three times with PBS containing 10% FBS. The washed lympho- cytes were fixed by 2% paraformaldehyde and then counted by a flow cytometer (Epics Profile II, Coulter Electronics, Beds, U.K.).

Measurements on the immunoglobulins. The lymphocytes of MLN and the spleen, at 2 x 106 cells/ml, were cultured for 4 hr to measure IgE, or for 24 hr to measure lgA, lgG and lgM at 37°C in a 10% FBS-RPMI 1640 medium. The concentrations of immunoglobulins in the culture supernatant were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) as described previously.

Statistical analysis. The data were analyzed by Dun- can's new multiple-range test to determine the exact nature of the differences among groups.

Results

Growth and serum lipid level

After 4 weeks of feeding, there was no difference in the food intake and weight gain among the 6 dietary groups (data not shown), and no difference in the tissue weight among them, except for the liver weight. The relative liver weight of the rats fed on BOR + SES was significantly higher than that of each other dietary group (data not shown).

Table 2 shows the effect of dietary fats and SES on the serum lipid levels in the Sprague-Dawley rats. The total cholesterol level was the lowest in the BOR group and
Table 2. Effect of Dietary Fats and Sesamin on Serum Lipid Concentrations

<table>
<thead>
<tr>
<th>Serum Lipid</th>
<th>SAF</th>
<th>BOR</th>
<th>PER</th>
<th>SAF + SES</th>
<th>BOR + SES</th>
<th>PER + SES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>146 ± 85</td>
<td>109 ± 106</td>
<td>124 ± 155</td>
<td>137 ± 65</td>
<td>128 ± 125</td>
<td>115 ± 85</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>97.8 ± 3.55</td>
<td>76.6 ± 4.95</td>
<td>78.5 ± 3.86</td>
<td>103 ± 65</td>
<td>98.8 ± 8.35</td>
<td>78.8 ± 4.35</td>
</tr>
<tr>
<td>(Total-HDL)/HDL</td>
<td>0.49 ± 0.05</td>
<td>0.42 ± 0.08</td>
<td>0.57 ± 0.07</td>
<td>0.34 ± 0.07</td>
<td>0.31 ± 0.05</td>
<td>0.43 ± 0.11</td>
</tr>
<tr>
<td>Phospholipid (mg/dl)</td>
<td>347 ± 365</td>
<td>266 ± 207</td>
<td>225 ± 268</td>
<td>325 ± 298</td>
<td>411 ± 178</td>
<td>207 ± 219</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>201 ± 255</td>
<td>138 ± 135</td>
<td>160 ± 105</td>
<td>129 ± 126</td>
<td>329 ± 555</td>
<td>110 ± 99</td>
</tr>
<tr>
<td>Blood TBARS (nmol MDA/ml)</td>
<td>5.87 ± 0.26</td>
<td>4.59 ± 0.28</td>
<td>5.05 ± 0.20</td>
<td>5.90 ± 0.53</td>
<td>6.77 ± 1.08</td>
<td>4.56 ± 0.34</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE of 6 rats. *Values without the same superscript letter are significantly different at p < 0.05. TBARS, thiobarbituric acid-reactive substances; MDA, malondialdehyde.

highest in the SAF group. SES feeding modified the effect of dietary fats, elevating the total cholesterol level in the BOR group, but lowering it in the SAF and PER groups. The HDL level was significantly lower in the BOR and PER groups than in the SAF group. SES feeding elevated the HDL level in the BOR group, but the effect was negligible in the other groups. The ratio of LDL (total-HDL) and HDL was lowest in the BOR group and highest in the PER group. In the SES-fed groups, this ratio was lower than that in each SES-free group. These results suggest that BOR feeding was the most effective for improving the serum cholesterol level among the dietary fats employed here and that SES feeding enhanced the effect of the dietary fats.

The serum phospholipid level was the lowest in the PER group and highest in the SAF group. SES feeding markedly increased the level in the BOR group, but the effect was negligible in the other groups. The serum triglyceride level was the lowest in the PER group and highest in the SAF group, as was the case with the phospholipid level. SES feeding markedly increased this level in the BOR group, but decreased it in the SAF group. The blood TBARS level was similar among the dietary fat groups. The effect of SES addition was small, although a significant increase of TBARS level was observed in the BOR group.

Chemical mediator levels in the serum and spleen

As shown in Fig. 1, dietary fats and SES affected the levels of the serum chemical mediators. The serum histamine level was higher in the BOR group than in the other dietary fat groups, although the difference was not significant. SES feeding slightly decreased this level in the SAF and BOR groups and enhanced it in the PER group, although the change was not significant.

On the contrary, the effect of dietary fats on the serum PGE₂ level was marked: the level was significantly lower in the BOR and PER groups than in the SAF group. SES feeding decreased the level in the SAF group, but the effect was small in the other groups. Dietary fats exerted a similar effect on the splenic LTB₄ level, but the effect was smaller than that on the serum PGE₂ level. The splenic LTB₄ level was lowest in the PER group and highest in the SAF group. SES feeding significantly enhanced the level in the BOR group, but the effect was small in the other groups.

Fatty acid composition

To clarify the mechanism which induced a change in the eicosanoid level, the effect was examined of dietary fats and SES on the fatty acid composition of various tissues. As shown in Table 3, the proportion of DGLA (20:3n-6) in liver PC was significantly higher in the BOR and PER groups than in the SAF group. SES feeding significantly increased the level in the BOR group, but not in the SAF and PER groups. The proportion of AA

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Fig. 1. Effects of Dietary Fats and Sesamin on Serum Histamine and Prostaglandin E₂ Levels, and on Splenic Leukotriene B₄ Level. Each value is the mean ± SE of 6 rats. *Values without the same superscript letter are significantly different at p < 0.05.
Table 3. Effect of Dietary Fats and Sesamin on the Polysaturated Fatty Acid Compositions of Liver Phosphatidylcholine and Phosphatidylethanolamine

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>SAF</th>
<th>BOR</th>
<th>PER</th>
<th>SAF + SES</th>
<th>BOR + SES</th>
<th>PER + SES</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2(n-6)</td>
<td>6.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.3 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.4 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.3 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.7 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.8 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.2 ± 0.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.5 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.5 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.1 ± 0.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.6 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>1.1 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.3 ± 0.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.5 ± 0.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.0 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.1 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.6 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>34.9 ± 0.5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>37.2 ± 0.8&lt;sup&gt;f&lt;/sup&gt;</td>
<td>14.4 ± 1.9&lt;sup&gt;f&lt;/sup&gt;</td>
<td>36.7 ± 0.5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>38.9 ± 0.2&lt;sup&gt;f&lt;/sup&gt;</td>
<td>13.4 ± 1.3&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>0.2 ± 0.0&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.3 ± 0.0&lt;sup&gt;g&lt;/sup&gt;</td>
<td>9.8 ± 0.5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.2 ± 0.0&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.3 ± 0.0&lt;sup&gt;g&lt;/sup&gt;</td>
<td>9.5 ± 1.3&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>2.4 ± 0.1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>2.1 ± 0.2&lt;sup&gt;h&lt;/sup&gt;</td>
<td>10.4 ± 0.9&lt;sup&gt;h&lt;/sup&gt;</td>
<td>2.4 ± 0.1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>2.4 ± 0.1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>9.4 ± 0.7&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Index
- Δ5 desaturation: 36.9 ± 4.3<sup>a</sup>
- Δ6 desaturation: 5.4 ± 0.5<sup>b</sup>

PE
- 18:2(n-6): 36.3 ± 0.5<sup>a</sup>
- 18:3(n-6): 0.1 ± 0.0<sup>b</sup>
- 20:3(n-3): 12.1 ± 1.8<sup>c</sup>
- 20:5(n-3): 0.2 ± 0.0<sup>d</sup>
- Δ5 desaturation: 5.6 ± 1.3<sup>e</sup>
- Δ6 desaturation: 0.4 ± 0.0<sup>f</sup>

Each value is the mean ±SE of 6 rats. *Values without the same superscript letter are significantly different at p < 0.05. Δ5 desaturation index = (20:4(n-6) + 20:3(n-6)) / 18:2(n-6); PC, phosphatidylcholine; PE, phosphatidylethanolamine.

Table 4. Effect of Dietary Fats and Sesamin on the Polysaturated Fatty Acid Compositions of Spleen Total Lipid

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>SAF</th>
<th>BOR</th>
<th>PER</th>
<th>SAF + SES</th>
<th>BOR + SES</th>
<th>PER + SES</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2(n-6)</td>
<td>11.2 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.4 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.5 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.7 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.0 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.8 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0 ± 0.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.9 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.0 ± 0.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0 ± 0.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.5 ± 0.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.6 ± 0.1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.8 ± 0.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.4 ± 0.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.4 ± 0.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.7 ± 0.1&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>1.3 ± 0.0&lt;sup&gt;g&lt;/sup&gt;</td>
<td>4.0 ± 0.2&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.4 ± 0.0&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.3 ± 0.1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.9 ± 0.1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.4 ± 0.1&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>23.1 ± 0.3&lt;sup&gt;i&lt;/sup&gt;</td>
<td>21.7 ± 0.8&lt;sup&gt;j&lt;/sup&gt;</td>
<td>11.8 ± 0.3&lt;sup&gt;j&lt;/sup&gt;</td>
<td>22.8 ± 0.9&lt;sup&gt;j&lt;/sup&gt;</td>
<td>25.0 ± 0.5&lt;sup&gt;j&lt;/sup&gt;</td>
<td>12.7 ± 0.5&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>0.0 ± 0.0&lt;sup&gt;k&lt;/sup&gt;</td>
<td>0.0 ± 0.0&lt;sup&gt;k&lt;/sup&gt;</td>
<td>3.9 ± 0.1&lt;sup&gt;k&lt;/sup&gt;</td>
<td>0.0 ± 0.0&lt;sup&gt;k&lt;/sup&gt;</td>
<td>0.0 ± 0.0&lt;sup&gt;k&lt;/sup&gt;</td>
<td>3.8 ± 0.2&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>0.4 ± 0.0&lt;sup&gt;l&lt;/sup&gt;</td>
<td>0.0 ± 0.0&lt;sup&gt;l&lt;/sup&gt;</td>
<td>1.4 ± 0.1&lt;sup&gt;l&lt;/sup&gt;</td>
<td>0.3 ± 0.1&lt;sup&gt;l&lt;/sup&gt;</td>
<td>0.4 ± 0.0&lt;sup&gt;l&lt;/sup&gt;</td>
<td>1.4 ± 0.0&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Index
- Δ5 desaturation: 17.7 ± 0.1<sup>a</sup>
- Δ6 desaturation: 1.0 ± 0.0<sup>b</sup>

Each value is the mean ±SE of 6 rats. *Values without the same superscript letter are significantly different at p < 0.05. Δ5 desaturation index = (20:4(n-6) + 20:3(n-6)) / 18:2(n-6); Δ6 desaturation index = (20:4(n-6) + 20:3(n-6)) / 18:2(n-6).

(20:4n-6) was significantly lower in the PER group than in the SAF and BOR groups. SES feeding did not affect the AA level in liver PC. The proportion of EPA (20:5n-3) was significantly higher in the PER group than in the SAF and BOR groups and was not affected by SES feeding.

In the case of liver PE, the proportion of DGLA (20:3n-6) was higher in the BOR group than in the SAF and PER groups. SES feeding slightly decreased the level in the SAF group, but increased it in the BOR and PER groups. The proportion of AA (20:4n-6) was much lower than in the case of PC and significantly low in the PER group as was the case with PC. In addition, the level was lower in the BOR group than in the SAF group, although the difference was not significant. No decrease in AA level was apparent in the BOR + SES group, while SES feeding further decreased the level in the PER group. The proportion of EPA (20:5n-3) was significantly higher in the PER group, but was not affected by SES feeding, as was seen in the case of PC.

A similar tendency but slightly different results were obtained for the PUFA levels of spleen total lipid (Table 4). The proportion of DGLA (20:3n-6) was higher in the BOR group than in the SAF and PER groups, as was the case with liver PE, but was not affected by SES feeding. A significant decrease in the proportion of AA (20:4n-6) was apparent in the PER and PER + SES groups, but not in the BOR or BOR + SES group, irrespective of a significant decrease in the LTB4-releasing activity of the spleen. EPA (20:5n-3) was only detected in the PER group, and the level was not affected by SES feeding.

*Serum immunoglobulin level and its productivity of lymphocytes*
### Table 5. Effects of Dietary Fats and Sesamin on Serum Immunoglobulin Levels

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Group</th>
<th>SAF</th>
<th>BOR</th>
<th>PER</th>
<th>SAF + SES</th>
<th>BOR + SES</th>
<th>PER + SES</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA (µg/ml)</td>
<td></td>
<td>39.7 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.7 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.5 ± 4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.2 ± 5.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.7 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.1 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgE (ng/ml)</td>
<td></td>
<td>4.0 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgG (mg/ml)</td>
<td></td>
<td>9.6 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.0 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.4 ± 7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.0 ± 15.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.9 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgM (µg/ml)</td>
<td></td>
<td>135.2 ± 5.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124.6 ± 9.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>142.9 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>136.2 ± 10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>132.4 ± 6.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>126.6 ± 5.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE of 6 rats. <sup>a</sup>Values without the same superscript letter are significantly different at p<0.05.

The effect of dietary fats on the serum Ig level was then examined. As shown in Table 5, there was no significant difference in the serum Ig level among the dietary groups. On the other hand, some differences were observed in the Ig productivity of the spleen and MLN lymphocytes. In the spleen lymphocytes, a tendency for decreased IgA productivity was observed in the PER group, although the difference was not significant (Fig. 2). IgE productivity was highest in the PER group and lowest in the BOR group. SES feeding enhanced IgE productivity in the SAF and BOR groups, but decreased it in the PER group. IgG productivity was significantly lower in the BOR group than in the other dietary fat groups. While SES feeding generally decreased IgG productivity, it enhanced it in the BOR and PER groups. Similar results were found for IgM productivity.

In the MLN lymphocytes, IgA productivity was not affected by dietary fats and SES feeding. IgE productivity was lower in the BOR and PER groups than in the SAF group, although the difference was not significant. SES feeding markedly decreased IgE productivity only in the PER group. IgG productivity was significantly lower in the BOR group than in the SAF and PER groups, as was the case in the spleen lymphocytes. However, SES feeding decreased IgG productivity in the

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**Fig. 2.** Effects of Dietary Fats and Sesamin on Immunoglobulin Productivity of the Rat Spleen and Mesenteric Lymph Node Lymphocytes. Each value is the mean ± SE of 6 rats. <sup>a</sup>Values without the same superscript letter are significantly different at p<0.05.
PER group, although it exerted enhancing activity in the splenocytes. IgM productivity was significantly lower in the PER group than in the SAF and BOR groups, and SES feeding decreased it to the level of the PER group.

To clarify the Ig production-regulating mechanism for these dietary components, the effect on the T cell population was examined. Dietary fats exerted a significant effect on the proportion of CD4+ cells, but not on the proportion of CD8+ cells (data not shown). In the SAF group, the proportion of CD4+ cells was 35.1% in the spleen and 53.2% in MLN. In the BOR group, this figure increased to 41.6% in the spleen and 63.9% in MLN. No increase in CD4+ cells was apparent in the BOR + SES group. The proportion of CD8+ cells was around 20% in the spleen and MLN lymphocytes, and the effect of dietary fats and SES was negligible.

**Discussion**

ALA is the starting fatty acid for n-3 PUFA synthesis and is metabolized to EPA and DHA. These n-3 PUFAs have been reported to be anti-allergic and to reduce inflammation in human patients. LA is the starting fatty acids for n-6 PUFAs and is metabolized to GLA, DGLA, and AA. Since 4-series LTs which induce the type I allergy are produced from last metabolite AA, an excessive intake of LA is believed to enhance this type of allergy. However, an oral administration of GLA has been reported to reduce inflammation in human patients as well as with n-3 PUFA. In the present study, we compared the dietary effects of LA, GLA, and ALA on such immune functions as the chemical mediator release and Ig production in Sprague-Dawley rats, using dietary fats rich in these PUFAs. Among them, SAF is rich in LA, BOR rich in GLA, and PER rich in ALA.

Although the serum histamine level was elevated in the rats fed on BOR, the serum PGE1 level was much lower in the BOR and PER groups than in the SAF group. In addition, the splenic LTB4 level was also lower in the BOR and PER groups than in the SAF group. Since a marked reduction in the proportion of AA was observed in the liver phospholipids and spleen total lipid of the rats fed on PER, such a decrease in the 2-series PG and 4-series LT levels may have been due to the reduction in AA level. In addition, significant increases in EPA and DHA were observed in the rats fed on PER, especially in the liver phospholipids. In vitro experiments have shown that EPA and DHA inhibited the 5-lipoxygenase pathway and LTB4 production in neutrophils and monocytes and in rat peritoneal exudate cells. These results suggest that n-3 PUFAs suppress the production of AA metabolites by at least two mechanisms, reducing the substrate level and inhibiting the enzymatic activity.

However, no decrease in AA level was apparent in the rats fed on BOR. Similarly, an oral administration of evening primrose oil rich in GLA decreased the LTB4 release activity of peritoneal exudate cells in Brown Norway rats without decreasing the AA level in liver phospholipids. These results suggest that a GLA-rich diet reduced the production of 2-series PG and 4-series LT by a different mechanism with PER. In the rats fed on BOR, the accumulation of DGLA was marked in both the liver phospholipids and spleen total lipid. DGLA is the substrate for 1-series PG and it has been reported that an oral administration of EPO elevated the tissue PGE1 level and suppressed chronic inflammation. Such an increase in PGE1 level may suppress LTB4 synthesis, as has been reported in human neutrophils. SES also affected PUFA metabolism by inhibiting Δ5-desaturase. Inhibition of this enzyme leads to an accumulation of DGLA and a reduction of AA in n-6 PUFAs. However, the effect of SES feeding on the levels of DGLA and AA in the liver phospholipids and spleen total lipid was much weaker than that of BOR feeding.

In addition to their effect on lipid metabolism and eicosanoid production, PUFA and antioxidants affected the immunoglobulin production of rat lymphocytes. PUFAs enhanced IgE production by the rat spleen and MLN lymphocytes, and this enhancing activity increased with the number of double bonds. Since the elevation of IgE level was inhibited in the presence of lipophilic antioxidative α-tocopherol, but not in the presence of hydrophilic ascorbic acid, the oxidation of PUFAs under lipophilic circumstances such as the cell membrane seems to be essential for enhanced IgE production. When dietary fats rich in n-6 and/or n-3 PUFAs were fed to Sprague-Dawley rats at the 5% level, there was no significant difference between the serum TBARS and IgE levels. This suggests that the effect of PUFA oxidation on Ig production was negligible at this low dietary fat level. However, a significant influence was observed in the Ig productivity of lymphocytes. In the rats fed on PER and SES, IgE productivity was significantly lower than that from PER and the other dietary groups. In addition, a marked increase in the IgG and IgM productivity of splenocytes was observed in the rats fed on PER and SES. These results suggest that PER and SES cooperatively regulated the Ig productivity of rat lymphocytes. Similarly, a combined administration of SES and α-tocopherol gave much higher IgG and IgM productivity of MLN lymphocytes and induced a lung tissue-specific decrease of LTC4 productivity. These results indicate that food components can synergistically regulate immune functions in a complex manner. To establish a diet for optimizing immune functions, clarification of the synergism between food components is essential.

In the case of serum lipid levels, a reciprocal effect was observed between BOR and SES. The total and HDL cholesterol levels were low in the rats fed on BOR, but these decreases were canceled in the rats fed on SES + BOR. A similar tendency was observed in the phospholipid and triglyceride levels. Although PER feeding gave lower levels of cholesterol, phospholipid, and triglyceride than in the rats fed on SAF, the addition of SES did not markedly change the levels. These results suggest that the effect of feeding SES could compete with the effect of BOR, but not with that of PER. This means that PER feeding regulates lipid metabolism by a different mechanism from that with BOR feeding. Since both GLA and ALA have been reported to be as anti-allergic as EPA and DHA, the balanced inges-
tion of dietary fats may be essential to normalize lipid metabolism and the immune function.

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