Comparisons of the Effects of Dietary Fats on Serum and Liver Lipid Levels of Rats

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The ratio of polyunsaturated-to-saturated fatty acids (P/S) in dietary fat is crucial in regulating the serum levels of cholesterol (CHL) in experimental animals and in humans as well. 1,2,3 However, recent studies show that the hypocholesterolemic effect of polyunsaturated fatty acids (PUFAs) differs greatly among the individual PUFAs: the effect of γ-linolenic acid is markedly greater than that of the parent molecule, linoleic acid. 4,5 In addition, α-linolenic, arachidonic, or eicosapentaenoic acids exert a much greater CHOL-lowering activity than the linoleic acid. 6,7,8,9 However, since these effects were examined independently, it is difficult to compare the efficacy of these PUFAs directly.

This study was therefore undertaken to compare directly the hypocholesterolemic effects of linoleic, α-linolenic and γ-linolenic acid using safflower oil (SFO), perilla oil (PRO), and evening primrose oil (EPO), respectively. Since different saturated fatty acids differently influence the plasma CHOL level, 10,11,12 we also compared the effects of beef tallow (BT), lard (LA), and partially hydrogenated rapeseed oil (PHR) simultaneously.

Four-week-old Wistar male rats (Kyudo Co., Kumamoto) were used. The composition of the diet was by weight percent: vitamin-free casein, 20; fat, 10; vitamin mixture, 1.0; mineral mixture, 4; choline chloride, 0.15; cellulose, 4; cholesterol, 1.0; sodium cholate, 0.25; and sucrose to 100%. The vitamin and mineral mixtures were as described by Harper. The fatty acid compositions of dietary fats (donated by Nippon Oil & Fats Co., Ltd.) are shown in Table I. The animals were housed individually in an air-conditioned room (22–24°C), lights on from 7:00 to 19:00 and were given free access to the diets and water for 2 weeks. Food intake and body weight were recorded every other day. Feces were collected for 2 days before killing. The animals were killed by decapitation and serum and liver lipids were extracted by method of Folch et al. 13 Triglyceride (TG), CHOL and phospholipid (PL) in serum and liver were measured as described elsewhere. 14,15 Fatty acid compositions and fecal neutral steroids were analyzed by gas-liquid chromatography and acidic steroids were analyzed enzymatically. 16,17 Data were analyzed by one-way analysis of variance and the statistical significance of the difference of the means

Table I. Fatty Acid Composition of Dietary Fats

<table>
<thead>
<tr>
<th>Dietary fat</th>
<th>Fatty acids (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>16:0</td>
</tr>
<tr>
<td>BT</td>
<td>27.7</td>
</tr>
<tr>
<td>PHR</td>
<td>5.2</td>
</tr>
<tr>
<td>SFO</td>
<td>6.7</td>
</tr>
<tr>
<td>PRO</td>
<td>5.8</td>
</tr>
<tr>
<td>EPO</td>
<td>6.1</td>
</tr>
</tbody>
</table>

* LA, lard; BT, beef tallow; PHR, hydrogenated rapeseed oil; SFO, safflower oil; PRO, perilla oil; EPO, evening primrose oil.

Table II. Comparison of metabolic Responses to Dietary Fats

<table>
<thead>
<tr>
<th>Serum lipids (mg/dl)</th>
<th>Cholesterol</th>
<th>HDL-cholesterol</th>
<th>Atherogenic index</th>
<th>Triglyceride</th>
<th>Phospholipid</th>
<th>Liver lipids (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Free</td>
<td>Ester (%)</td>
<td>Total</td>
<td>Free</td>
<td>Ester (%)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>451 ± 32.8a</td>
<td>59.3 ± 8.1b</td>
<td>86.9 ± 0.9</td>
<td>39.1 ± 1.7a</td>
<td>10.5 ± 0.6a</td>
<td>133 ± 38.7</td>
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<tr>
<td></td>
<td>622 ± 64.3b</td>
<td>88.1 ± 13.9a</td>
<td>85.8 ± 0.9</td>
<td>34.1 ± 3.4a</td>
<td>17.3 ± 1.1a</td>
<td>116 ± 15.1</td>
</tr>
<tr>
<td></td>
<td>483 ± 77.5a</td>
<td>73.1 ± 17.6a</td>
<td>84.9 ± 0.9</td>
<td>33.8 ± 3.6a</td>
<td>13.3 ± 2.6a</td>
<td>93.5 ± 12.9</td>
</tr>
<tr>
<td></td>
<td>216 ± 18.9a</td>
<td>73.9 ± 4.3bc</td>
<td>82.4 ± 1.6</td>
<td>32.8 ± 0.6a</td>
<td>5.6 ± 0.6b</td>
<td>141 ± 19.3</td>
</tr>
<tr>
<td></td>
<td>163 ± 15.2a</td>
<td>25.3 ± 3.6bc</td>
<td>84.5 ± 1.1</td>
<td>35.4 ± 1.9a</td>
<td>3.6 ± 0.6d</td>
<td>115 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>137 ± 8.4a</td>
<td>18.4 ± 1.7c</td>
<td>86.6 ± 0.9</td>
<td>46.1 ± 5.5a</td>
<td>2.0 ± 0.4b</td>
<td>105 ± 5.9</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 rats.

* LA, lard; BT, beef tallow; PHR, hydrogenated rapeseed oil; SFO, safflower oil; PRO, perilla oil; EPO, evening primrose oil.
was evaluated by the method of Snedecor at the level of $p<0.05$.\textsuperscript{131}

The rats initially weighing an average of 149 g consumed 16.9--17.5 g
of diet per day and gained 89--97 g of body weight in 2 weeks. There
were no significant differences in these parameters among the groups.

The relative liver weight was also comparable in general (average 5.7--6.0 g/100 g of body weight).

Table II summarizes the serum and liver lipid concentrations. In
accordance with the P/S concept, dietary fats manifested differential effects on the serum total CHOL level; rats fed plant oils showed generally lower total CHOL values than those fed animal fats.\textsuperscript{59} Among the saturated fats, BT was hypercholesterolemic in relation to LA, probably due to the lower content of linoleic acid. On the other hand, the concentration of total CHOL in the P-HR group was comparable with that in the LA group, irrespective of the lack of linoleic acid and the presence of a large proportion of trans-octadecenoate; the less hyperlipidemic effect of PHR can be explained by the neutral behavior of trans-octadecenoate\textsuperscript{180} and a possible hypercholesterolemic potential of stearic acid.\textsuperscript{60} These results indicate that the linoleic acid content of dietary fats is a factor responsible for governing serum total CHOL levels, while trans-fatty acids may, at least, not be hypercholesterolemic. On the other hand, there was no significant difference in the concentration of serum total CHOL levels that among plant oils. However, when considered the content of individual PUFAs among these plant oils, it is apparent that $\gamma$-linolenic acid has a greater hypercholesterolemic effect in comparison with $\omega$-linolenic and/or linoleic acid. The result was consistent with the observations by Horrobin and his colleagues,\textsuperscript{61,62} Sugano et al.\textsuperscript{63} and Garg et al.\textsuperscript{64} The free CHOL level also responded similarly to that of the total CHOL level. As a result, the proportion of the estrone total CHOL remained unchanged. High Density Lipoprotein (HDL)-CHOL was also analyzed with an enzymatic Kit (HDL-CHOL-test Wako), but no significant difference was observed among the groups. However, the atherogenic index expressed as (Total-CHOL - HDL-CHOL)/HDL-CHOL was significantly lower in rats fed plant oils than in those fed animal fats; among plant oils, the value was lowest in rats fed EPO followed by PRO and SFO. The difference among these groups was statistically significant. The serum TG level was comparable among the groups, while the serum PL level was generally lower in rats fed plant oils than in those fed animal fats, the reduction being most prominent in rats fed PRO or EPO followed by SFO.

The concentration of hepatic total CHOL was significantly lower in rats fed SFO or EPO than in those fed animal fats or hydrogenated fat; $\gamma$-linolenic acid was most effective in this respect. SFO as compared to other plant oils has shown to increase hepatic total CHOL levels in cholesterol-free diets, but not in cholesterol-enriched diets,\textsuperscript{65} consistent with our results. In contrast, PRO did not reduce liver total CHOL. The decrease in total CHOL on feeding EPO was largely due to the decrease in the CHOL ester, since the free CHOL level remained unchanged. On the other hand, dietary SFO and EPO, but not PRO, caused a significant accumulation of hepatic TG levels as compared with fats rich in saturated fatty acids, BT and PHR, but not LA. The PL level was comparable among the groups.

Dry weight of the feces was comparable (average 2.0--2.1 g/2 days). The rate of microbial transformation of cholesterol to coprostanol as well as fecal excretion of neutral and acidic steroids (Table II) were not influenced by the type of dietary fats, suggesting that mechanism(s) independent of the change in fecal steroid excretion seems to be responsible for the hypocholesterolemic activity of dietary PUFAs.

The results of this study showed a hypocholesterolemic effects of plant oils. Among these oils, EPO may be more appropriate than PRO or SFO in terms of the treatment of hypercholesterolemia.

References