Effect of Oleic Acid Level under Constant n-6/n-3 and P/S Ratios of Dietary Fats on Lipid Metabolism in Rats

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The effect of dietary oleate levels (18, 39, 57 and 74% of total fatty acids) on various lipid parameters was studied in rats given cholesterol-enriched diets containing fat with a constant P/S (3.1–3.2) and n-6/n-3 (5.4–6.2) ratio. High-oleic safflower oil was used as a source of oleic acid, and was replaced stepwise with a mixture of cotton seed and perilla seed oils. After three weeks of feeding, there were no significant differences in the concentrations of serum and liver cholesterol, although they tended to increase with an increasing dietary oleate level. A hypotriglyceridemic trend was observed toward an increasing proportion of oleic acid. The linoleate desaturation index, (dihomo-γ-linolenic acid+arachidonic acid)/linoleic acid, in tissue phosphatidylcholine tended to increase with an increasing proportion of oleate, whereas the production of prostacyclin by the aorta and thromboxane A2 by platelets was independent of the dietary oleate level. These results indicate that dietary oleate did not significantly modify the effect of polyunsaturated fatty acids on various lipid parameters under dietary conditions at which the P/S and n-6/n-3 ratios of the dietary fat were kept at an appropriate level to prevent ischemic heart disease.

Mono-unsaturated fatty acids (MUFAs), particularly oleic acid, have been considered neutral in their effect on the plasma cholesterol level. However, because of the relatively low mortality from ischemic heart disease in the Mediterranean countries where olive oil is commonly consumed,1,2 it is reasonable to consider that oleic acid has a specific function as a regulator of cholesterol metabolism. In fact, Grundy and his colleagues have recently observed in humans that, when substituted for saturated fat, oleic acid reduced the plasma cholesterol level without lowering the HDL-cholesterol level.2–5 However, this observation is not unanimous,6–11 although the effect of the oleate on experimental animals is not so much controversial as variable.12–18 The use of MUFAs as substitutes for polyunsaturated fatty acids (PUFAs), particularly linoleic acid, seems on one hand to be of benefit to evade the possible untoward effects of an excessive intake of linoleic acid.5,19 In addition, oleic acid also has an advantage over PUFAs in that it is less susceptible to autoxidation or rather inhibits lipid peroxidation.20

However, in the studies already cited, no attention has been paid to the effect of the difference in P/S and/or n-6/n-3 ratios of dietary fat, the experiments in general being merely a comparison between fats rich in oleic acid and those rich in linoleic or saturated fatty acids. In our study on the interrelated effects of n-6/n-3 and P/S ratios of dietary fats on lipid metabolism in rats, an n-6/n-3 ratio of approximately 5 and a P/S ratio of approximately 2 were shown to be desirable in respect of serum and liver lipid levels and of the balance of eicosanoid production.21 Thus, in the present study, in order to learn whether oleic acid can modify the influence of dietary fat on

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various lipid parameters, rats were given cholesterol-enriched diets containing different levels of oleic acid under constant P/S (3.1–3.2) and n-6/n-3 (5.4–6.2) ratios.

Materials and Methods

Animals and diets. Four-week-old specific pathogen-free male Sprague-Dawley rats (Seiwa Experimental Animals Co., Fukuoka) were used. They were divided into 4 groups (6 rats each) according to the level of dietary oleic acid. The dietary fats used were high-oleic safflower oil (Fuji Oil Co., Osaka), cotton seed oil (Ajinomoto Co., Tokyo) and perilla seed oil (presented by Lion Co., Tokyo). The diet composition was, by percentage weight: casein, 20; fat, 10; vitamin mixture (AIN-TM\(^7\)), 1.0; mineral mixture (AIN-TM\(^7\)), 3.5; choline bitartrate, 0.2; DL-methionine, 0.3; cellulose powder, 5; corn starch, 15, and sucrose to 100. Cholesterol (0.5%) and sodium cholate (0.125%) were added to the diet at the expense of sucrose. The fatty acid compositions of each dietary fat is shown in Table I. The animals were housed individually in an air-conditioned room (20–23°C, lights on from 08:00 to 20:00 hr) and were given free access to the experimental diets for 3 weeks. Food intake and body weight were recorded every other day. The animals were killed by withdrawing blood from the abdominal aorta under light diethyl ether anesthesia. A portion of the blood was clotted by incubating at 37°C for 30 min, and serum was obtained to measure the production of thromboxane A\(_2\) by platelets\(^{23,24}\). The remaining portion of the blood was clotted in ice, and the serum was harvested. The thoracic aorta was immediately excised to measure the prostacyclin production. The liver, heart and epididymal adipose tissue were also excised and stored at \(-20°C\).

Lipid analyses. Serum and liver lipids were measured as previously described\(^{25}\). Cholesterol in the aorta and adipose tissue was extracted and analyzed by gas-liquid chromatography (GLC) in a OV-17 column.\(^{26}\) Fatty acid compositions of tissue phosphatidylcholine and adipose tissue total lipids were analyzed by GLC in a SILAR 10C column.\(^{27}\)

Measurements of eicosanoids. Preincubated serum was diluted twenty times with 50 mm phosphate buffer at pH 7.3, containing 0.1% gelatin and 0.01% thimerosal, to measure thromboxane B\(_2\) by a radioimmunoassay (NEK-007, New England Nuclear, Boston, MA)\(^{21,28}\). 6-Keto-prostaglandin F\(_1\) \(\Delta_5\) produced during the incubation of the thoracic aorta at 25°C for 30 min was extracted\(^{29}\) and analyzed by radioimmunoassay (NEW-008, New England Nuclear)\(^{21,29}\).

Statistical analysis. Data were analyzed by one-way analysis of variance and a subsequent inspection of the difference between pairs of means by Duncan's multiple-range test\(^{30}\).

Results

Growth and liver weight. The rats initially weighed an average of 127 g, consumed 19.2–20.3 g of diet per day and gained 174–180 g of body weight in 3 weeks. There were no significant differences in these parameters among the groups. The relative liver weight was also comparable in general (average 6.72–7.32 g/100 g of body weight).

Tissue lipid concentration

As shown in Table II, there was an increasing trend in the concentration of serum and liver cholesterol with an accompanying increase in the level of dietary oleate, but the difference was not significant. The concentration of serum triglyceride tended to decrease with increasing dietary olate level, whereas that of liver triglyceride was higher in the rats fed with the

<table>
<thead>
<tr>
<th>Table 1. Fatty Acid Composition of Dietary Fats</th>
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<tbody>
<tr>
<td>Dietary fat</td>
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<tr>
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<tr>
<td></td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
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<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
</tbody>
</table>

The dietary fats were mixtures of high-oleic safflower oil, cotton seed oil and perilla seed oil. P/S = (18:2 + 18:3)/(14:0 + 16:0).
highest oleate level than in those fed with lower levels of the oleate. The concentration of phospholipid was not influenced by the type of dietary fat, while the concentrations of adipose tissue and aorta cholesterol remained unchanged regardless of the dietary oleate level (data not shown).

Table II. CONCENTRATION OF SERUM AND LIVER LIPIDS

<table>
<thead>
<tr>
<th>Tissue lipids</th>
<th>Group (oleate %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (18.1)</td>
</tr>
<tr>
<td>Serum (mg/dl)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>128 ± 9</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>329 ± 34</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>251 ± 20</td>
</tr>
<tr>
<td>Liver (mg/g)</td>
<td>52.8 ± 3.4</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>106 ± 6ab</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>34.4 ± 8.8</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 to 6 rats.

Table III. FATTY ACID COMPOSITION OF LIVER PHOSPHATIDYLCHOLINE

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Group (oleate %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (18.1)</td>
</tr>
<tr>
<td>14:0</td>
<td>1.0*</td>
</tr>
<tr>
<td>16:0</td>
<td>25.4</td>
</tr>
<tr>
<td>16:1</td>
<td>3.0*</td>
</tr>
<tr>
<td>18:0</td>
<td>13.6</td>
</tr>
<tr>
<td>18:1</td>
<td>10.3*ab</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>16.9*</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.3*</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>2.1*</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>17.8*ab</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.3</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.4</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>4.2*ab</td>
</tr>
</tbody>
</table>

Values are means for 6 rats.

Table IV. FATTY ACID COMPOSITION OF ADIPOSE TISSUE TOTAL LIPIDS

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Group (oleate %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (18.1)</td>
</tr>
<tr>
<td>14:0</td>
<td>1.3</td>
</tr>
<tr>
<td>16:0</td>
<td>23.4*ab</td>
</tr>
<tr>
<td>16:1</td>
<td>5.5</td>
</tr>
<tr>
<td>18:0</td>
<td>2.7*</td>
</tr>
<tr>
<td>18:1</td>
<td>25.9*</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>34.6*</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>5.2*</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>0.5*ab</td>
</tr>
</tbody>
</table>

Values are means for 6 rats.

Table II. CONCENTRATION OF SERUM AND LIVER LIPIDS

Fatty acid composition of the tissue lipids

The fatty acid composition of liver phosphatidylcholine is shown in Table III. The proportion of the oleate in phosphatidylcholine increased gradually, whereas that of the linoleate decreased in response to their dietary levels. In contrast, the proportion of arachidonate remained essentially unchanged irrespective of the dietary oleate and/or linoleate levels. Thus, the ratio of (20:3n-6 + 20:4n-6)/18:2n-6, a desaturation index for the linoleate in liver phosphatidylcholine, tended to increase when the dietary oleate level was relatively high. In general, similar response
trends could be seen in the fatty acid composition of heart and aortic phosphati-
dylcholine (data not shown), except for a significant increase in the linoleate desaturation in-
dex in the heart (2.06\(^a\), 2.01\(^b\), 2.53\(^ab\) and 3.65\(^b\) for groups A to D, respectively), and in the
aorta (2.03\(^ab\), 1.93\(^a\), 2.00\(^a\) and 2.91\(^b\), different
superscript letters denoting a significant difference at \( p < 0.05 \). The fatty acid composition
of adipose tissue total lipids reflected that of the dietary fats (Table IV). Thus,
the proportions of oleate, linoleate and \( \alpha \)-linolenate were dependent on their dietary
levels.

Eicosanoid production

There was no significant difference in the production by the aorta of prostacyclin and
by platelets of thromboxane A\(_2\) among the
groups, although the former tended to decrease
slightly with increasing level of dietary oleate,
and hence, a decreasing level of linoleate (Table
V).

<table>
<thead>
<tr>
<th>Group (oleate %)</th>
<th>Prostacyclin(^a) (ng/mg of aorta)</th>
<th>Thromboxane A(_2)(^b) (ng/ml of serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (18.1)</td>
<td>2.46 ± 0.50</td>
<td>544 ± 139</td>
</tr>
<tr>
<td>B (39.1)</td>
<td>2.48 ± 0.44</td>
<td>476 ± 124</td>
</tr>
<tr>
<td>C (56.5)</td>
<td>2.29 ± 0.50</td>
<td>605 ± 106</td>
</tr>
<tr>
<td>D (74.0)</td>
<td>1.94 ± 0.24</td>
<td>546 ± 114</td>
</tr>
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Values are means ± SE for 6 rats.
\(^a,b\) Measured as 6-keto-PGF\(_{1\alpha}\) and thromboxane B\(_2\),
respectively.

Discussion

In studies in which the effect of the dietary oleate level on lipid metabolism is being
examined, a change in the proportion of PUFAs in dietary fat is inevitable, insofar as
the P/S ratio and/or n-6/n-3 ratio are kept constant. If one is obliged to maintain the
proportion of PUFAs constant under the changing oleate levels, these ratios change very
widely. Since the P/S and n-6/n-3 ratios of
dietary fats, rather than the dietary level of
PUFAs, largely influence various lipid pa-
rameters,\(^21\) we chose the experimental con-
ditions for constant P/S and n-6/n-3 ratios in the
present study.

We used high-oleic safflower oil as a source of oleic acid instead of the more common olive
oil because the latter contains a relatively large amount of specific unsaponifiable matter,
squalene, which may influence cholesterol
metabolism.\(^31,32\) Consequently, the P/S ratio
adopted was to some extent higher than that
recommended for preventing hypercholester-
olemia, 1 to 2. However, in this situation, the
n-6/n-3 ratio could be maintained at an
appropriate level, approximately 6.\(^21\) In
addition, because of the specific character of
the animal species used, cholesterol and cholate
were added to the diet in order to magnify the
difference in the serum cholesterol level, and
hence, to facilitate an evaluation of the effect
of dietary fats. Thus, in the present study, we
could not discriminate the effect of cholesterol
from that of bile acid.

Under constant n-6/n-3 and P/S ratios for
dietary fat, oleic acid at different dietary levels
did not greatly increase the serum and liver
cholesterol levels when substituted for PUFAs.
Although Grundy and his co-workers have
reported that the plasma total- and LDL-
cholesterol levels were lower in humans given
fats high in oleate or linoleate than in those
given fats high in palmitate,\(^2-5,33\) the effect
of oleic acid in humans appears to be diverse
and depend on the diet used for comparison.\(^6-11\) The same is true for experimental
animals, including rats\(^13-16\) and rabbits.\(^17,18\)
Beynen\(^13\) observed a hypercholesterolemic
propensity of oleic acid in rats fed with
cholesterol-enriched, but not cholesterol-free
diets, whereas no difference in the concentra-
tion of serum cholesterol was found between
the oleate and linoleate,\(^15\) nor between olive
oil and a solid vegetable fat\(^14\) in rats fed with
diets free of cholesterol. We have also reported
the hypercholesterolemic nature of olive oil in
rats fed with cholesterol-free\(^12\) or cholesterol-
enriched\(^12,16\) diets compared to PUFA fats.

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**Table V.** Production of Aorta Prostacyclin and Platelet Thromboxane A\(_2\)

<table>
<thead>
<tr>
<th>Group (oleate %)</th>
<th>Prostacyclin(^a) (ng/mg of aorta)</th>
<th>Thromboxane A(_2)(^b) (ng/ml of serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (18.1)</td>
<td>2.46 ± 0.50</td>
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</tr>
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However, in these studies, the effect of the change in n-6/n-3 and P/S ratios on the serum cholesterol level was ignored. The present results at least support the view that oleic acid is not hypercholesterolemic, and that it is as effective as linoleic acid in the regulation of serum lipid levels in a cholesterol-loaded rat when the proportions of PUFAs are kept appropriate, although the serum cholesterol level tended to increase when most PUFAs were replaced by oleate. In this context, measurements of lipoprotein cholesterol will give more information regarding the effect of oleate. In some experiments with rats, dietary oleate compared to polyunsaturated fat tends to increase the concentration of serum cholesterol.\(^{13,16,17}\) However, in these studies, the effect of the difference in the P/S or n-6/n-3 ratio was ignored.

The concentration of serum triglyceride tended to decrease with increasing dietary oleate level in agreement with the observation in humans,\(^{41}\) suggesting the possible hypo-triglyceridemic effect of oleic acid. In contrast, liver triglyceride increased when rats were fed with dietary fat containing a high proportion of oleic acid in accordance with the reported data.\(^{13,15}\) Since the oleate does not appear to have stimulated the hepatic synthesis of triglyceride in fed rats,\(^{34}\) triglyceride accumulation may largely be related to an increased absorption of cholesterol as stimulated by oleic acid.\(^{35,36}\)

The increasing trend in linoleate desaturation in tissue phosphatidylcholine at a high dietary oleate levels seems to disagree with the observation that oleic acid competes with the desaturation of linoleic acid, although the magnitude of the interference appears to be moderate compared to \(\alpha\)-linolenic acid.\(^{37}\) Consumption of a large amount of linoleic acid has been shown to reduce its desaturation.\(^{38}\)

The production of prostacyclin by the aorta and of thromboxane \(A_2\) by platelets was apparently independent of the dietary oleate level. However, the prostacyclin production was slightly lower when the rats were fed with a high level of oleate accompanying a slight decrease in the proportion of arachidonate in aortic phosphatidylcholine. Thus, it seems unlikely that dietary oleic acid significantly influences eicosanoid synthesis under the dietary regimen employed.\(^{39}\)

In conclusion, with constant n-6/n-3 and P/S ratios, dietary oleate appeared to have no significant effect on the serum cholesterol level in the rats, except for a slight increase in liver triglyceride. There was no particular effect of dietary oleate on the production of eicosanoids which are involved in thrombosis. These results suggest that the effect exerted by the oleate on the various lipid parameters currently examined was comparable to that of the linoleate. Therefore, a portion of linoleic acid in the dietary fat could, at least in part, be replaced by oleic acid without significantly modifying the effect on lipid metabolism, insofar as the P/S and n-6/n-3 ratios are kept constant.

**References**