Note

Effect of Docosahexaenoic Acid and Sardine Oil Diets on the Ultrastructure of Hepatocytes in Adult Mice

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Summary The influence of docosahexaenoic acid (DHA) on the ultrastructure of hepatocytes was studied. Adult male mice of Crj:CD-1 (ICR) strain were fed a fat-free purified diet supplemented with 5% (by weight) of purified DHA, refined sardine oil, and palm oil. The mice were fed the DHA diet or the palm oil diet for 7 days, and the sardine oil diet or the palm oil diet for one month. There were significant ultrastructural changes in the hepatocytes between the mice fed palm oil diet and the animals fed DHA and sardine oil diets. Many lipid droplets in the tissues of mice fed the palm oil diet were observed. Few lipid droplets were contained in the hepatocytes from the mice fed DHA and sardine oil diets, but electron-dense bodies were found in their tissues. These electron-dense bodies were mainly found near the region of the nucleus, blood sinusoids and bile canaliculi. These results suggest that the dense bodies found in the DHA and sardine oil diet groups may appear as a result of acceleration of lipid metabolism in the liver of mice.

Key Words docosahexaenoic acid, palm oil, sardine oil, hepatocytes, ultrastructure, mice

Dietary fish oil influences various processes of lipid metabolism, including eicosanoid production in experimental animals and humans (1). These effects of fish oil have been exclusively attributed to n-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA). Plasma cholesterol-and triglyceride-lowering effects of fish oil have been found in animals (2) and humans (3). It has been shown that DHA has a more powerful suppressant effect on endogenous plasma cholesterol than EPA in mice (4). Thus, DHA seems to play an important role in reducing plasma lipids. However, there is little information on the ultrastructural changes in the liver cells of animals fed DHA and fish oil diets.

The present study was undertaken to clarify the ultrastructural changes of hepatocytes in adult mice fed DHA and sardine oil diets which have the effect of generally reducing plasma lipids.

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Materials and methods

Animals and materials. Male mice of Crj:CD-1 (ICR) strain (3 weeks old) were obtained from Charles River Japan Inc. (Atsugi, Kanagawa, Japan). The palm oil, refined sardine oil and purified DHA were supplied from NOF Corporation, Japan. The fatty acid composition is shown in Table 1. n-3 Polyunsaturated fatty acids were not detected in the palm oil. DHA accounted for about 90% of oil.

Experiment. Twenty-eight mice were randomly divided into four groups of seven animals each, and housed in suspended stainless-steel cages with wire mesh bottoms. The room was kept at 24.0±0.5°C and relative humidity of 65%. Room lighting consisted of alternating 12-h periods of light and dark. Animals were fed MF diet (Oriental Yeast Co., Ltd.) for 22 weeks in two groups (1 and 2), and for 20 weeks in the other groups (3 and 4). Then, MF was replaced with purified DHA diet (group 1), palm oil diet (groups 2 and 4) or refined sardine oil diet (group 3). The diets and water were provided ad libitum with groups 1 and 2 for 7 days, and with groups 3 and 4 for one month. Each diet contained 5% oil or fatty acid, and the remaining components were as follows: corn starch, 48.8%; casein, 25.0%; granulated sugar, 10.0%; cellulose powder, 5.0%; mineral mixtures, 4.0%; vitamin mixtures, 2.0%; L-methionine, 0.2%. Mineral and vitamin mixtures were purchased from Oriental Yeast Co., Ltd., Japan. The composition of mineral and vitamin mixtures has been described by Kohashi et al. (5). In order to prevent the changes in fatty acid composition during storage, each experimental diet was stored at −30°C (6). Body weight gain and food consumption were measured. At the end of the feeding trial, all mice were anesthetized with diethyl ether, and the liver was immediately removed.

Electron microscopy. Small tissue slices, approximately 1 mm in thickness, were prefixed for 2 h in cold 2.5% glutaraldehyde, washed in phosphate buffer (pH 7.3) for 1 h and postfixed in cold 1% osmium tetroxide in phosphate buffer (pH

Table 1. Fatty acid composition (%) of palm oil, refined sardine oil and purified DHA (22:6).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Palm oil</th>
<th>Sardine oil</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.1</td>
<td>8.3</td>
<td>—</td>
</tr>
<tr>
<td>16:0</td>
<td>46.6</td>
<td>18.0</td>
<td>—</td>
</tr>
<tr>
<td>16:1</td>
<td>—</td>
<td>10.1</td>
<td>1.2</td>
</tr>
<tr>
<td>18:0</td>
<td>3.8</td>
<td>1.9</td>
<td>—</td>
</tr>
<tr>
<td>18:1</td>
<td>37.5</td>
<td>14.5</td>
<td>0.3</td>
</tr>
<tr>
<td>18:2</td>
<td>9.8</td>
<td>1.6</td>
<td>0.3</td>
</tr>
<tr>
<td>18:3</td>
<td>—</td>
<td>0.9</td>
<td>—</td>
</tr>
<tr>
<td>20:0</td>
<td>0.2</td>
<td>3.6</td>
<td>—</td>
</tr>
<tr>
<td>20:1</td>
<td>—</td>
<td>6.0</td>
<td>—</td>
</tr>
<tr>
<td>20:4</td>
<td>—</td>
<td>0.6</td>
<td>1.8</td>
</tr>
<tr>
<td>20:5</td>
<td>—</td>
<td>15.9</td>
<td>1.4</td>
</tr>
<tr>
<td>22:6</td>
<td>—</td>
<td>8.0</td>
<td>88.3</td>
</tr>
</tbody>
</table>

EFFECT OF DHA ON LIVER ULTRASTRUCTURE

7.3) for 2 h, dehydrated in graded ethanols and propylene oxide, and embedded in Epon 812. Thin sections were prepared using an ultramicrotome, stained with lead citrate and uranyl acetate, and photographed on a JOEL 1200EX electron microscope.

Light microscopy. Thick (0.5 \( \mu \text{m} \)) sections were cut with glass knives, mounted on glass microscope slides, and stained with toluidine blue, and examined for histologic changes.

Results

There was a slight difference in food consumption (g/day) between the DHA (group 1; 4.5±0.3) and palm oil diet (group 2; 5.3±0.7) groups. However, no significant difference in food consumption (g/day) was noted between the refined sardine oil diet (group 3) and palm oil diet (group 4) groups. There was no significant difference in body weight between the DHA and palm oil diet groups, or between the refined sardine oil and palm oil diet groups. In the light microscopic observation, there were slight differences in the hepatocytes between the mice fed the palm oil diet and the animals fed the DHA diet. However, it was difficult to

Fig. 1. Electron micrograph of a liver cell from a mouse fed palm oil diet for 7 days. Lipid droplets are seen in the liver cell (magnification, \( \times 2,500 \); bar=2 \( \mu \text{m} \)).

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distinguish the lipid-like vesicles observed in mice fed palm oil diet from the
toluidine blue-stained vesicles in animals fed the DHA and sardine oil diets.

**Electron microscopic observation**

There were few ultrastructural differences in the hepatocytes of mice fed the
palm oil diet for 7 days and one month. Lipid droplets in the hepatocytes from the
animals fed the palm oil diet were also observed. Most lipid droplets were found
near the region of the nucleus and blood sinusoids. Some lipid droplets were
scattered throughout the cytoplasm of hepatocytes of mice fed the palm oil diet.
Electron-dense bodies were rarely found in the liver cells (Fig. 1).

The ultrastructure of the liver cells from animals fed the DHA diet is shown in
Fig. 2. Electron-dense bodies were found in the hepatocytes. They were mainly
found near the nucleus, blood sinusoids and bile canaliculi in hepatocytes.

The ultrastructure of liver cells in the mice fed the sardine oil diet for one
month (Fig. 3) was similar to that of the mice fed the DHA diet.

Dense bodies of various sizes were observed. Some electron-dense bodies
which were found in the liver cells from the mice fed the DHA and sardine oil diets

![Fig. 2. Electron micrograph of liver cells from a mouse fed DHA diet for 7 days.
Dense bodies are seen near the nucleus (magnification, ×2,500; bar=2μm).
Dense bodies (arrows).](image)
Different dietary oils results in distinctive morphological alterations in the hepatocytes of mice. Under electron microscopic observation, many lipid droplets were found in the hepatocytes of mice fed the palm oil diet. However, there were few lipid droplets in the liver cells of mice fed DHA and sardine oil diets.

Electron-dense bodies of various sizes were found in the hepatocytes of the mice fed the DHA diet and sardine oil diet. The existence of electron-dense bodies has been confirmed in the liver cells of pigs fed herring oil (7). However, there were very few of these dense bodies in the hepatocytes of mice fed the palm oil diet. These dense bodies may be a type of lysosome. Rats fed sea-lion oil exhibited a distribution of lipid vesicles oriented towards the canalicular region of hepatocytes and showed greater variation in vesicle sizes in hepatocytes (8), but it is not clear whether these dense bodies are associated with lipid vesicles. The plasma cholesterol-
ol- and triglyceride-reducing effects of fish oil have been observed in animals (2) and humans (3). Kobatake et al. suggested that fish oil rich in DHA has a more potent reducing effect on exogenous plasma cholesterol than oil rich in EPA (9). Compared to the effect of a coconut oil diet, the effect of a fish oil diet is associated with a higher binding affinity of LDL to liver plasma membranes (10). Furthermore, it has been reported that dietary fish oil increased biliary excretion of cholesterol compared to a corn oil diet (11). The mouse physiological function increased uptake of the lipoprotein from serum to the liver and increased biliary excretion by taking omega-3 fatty acid may be related to the dense bodies observed in our experiment. Our results suggest that the dense bodies found in the hepatocytes from mice fed the DHA and sardine oil diets may have appeared as a result of acceleration of cholesterol excretion in bile. Further studies are needed to clarify the relationship between the induction of the lysosome-like dense bodies and the action of DHA in hepatocytes.

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REFERENCES


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