Long-Term Storage of Fish Oil Fatty Acids in the Liver, Not Adipose Tissue, in Humans

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Summary This study examined fish oil storage using samples from 7 autopsied patients. Four of these seven patients had been under intravenous alimentation for more than 2 months without supplementation of fish oils. Both subcutaneous and omental adipose tissues were rich in n-9 and n-6 fatty acids but poor in the n-3 fatty acids EPA and DHA. In contrast, fish oil fatty acids were abundant in liver lipids, especially in phospholipids. The absolute amounts of EPA and DHA in total lipids were calculated based on the actual weighed liver and on the estimated total adipose tissue mass. The mean liver EPA and DHA contents were 2.59 and 9.65 g, respectively, whereas mean adipose tissue EPA was 0.89 g and DHA 2.90 g. Thus the absolute fish oil fatty acid content in liver was about 3 times greater than that in the adipose tissue mass. Gas liquid chromatographic chemical-ionization mass spectrometry clearly identified the fish oil fatty acids from the autopsied patients as being the same as authentic standards purified from fish. These findings provide evidence that ingested fish oils are stored in the human liver for more than 2 months.

Key Words: fish oils, n-3 fatty acids, human

The potential role of fish oil fatty acids (n-3 fatty acids) like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the prevention of arteriosclerotic diseases [1] has attracted widespread attention for more than a decade. In general, dietary n-9 and n-6 fatty acids are known to be stored for a long period [2], usually in adipose tissue in humans [3, 4], and mobilized for utilization as free
fatty acids when needed [4]. Thus the habitual intake of certain oils like corn oil produces adipose tissue “corn oiliness” [4]. On the other hand, of interest are the reports of Wood et al. [5] and ourselves [6] who showed that human adipose tissue contains only trace amounts of EPA and DHA in spite of their constant presence in blood plasma. Plasma free fatty acids consist of those with chain lengths of less than C:18 [7], and neither EPA nor DHA is contained in the free fatty acid fraction. Therefore, it might be that ingested fish oil fatty acids are stored not in adipose tissue but in liver, which can secrete them as lipoprotein-lipids into the blood.

This study was conducted in order to investigate the possibility of the liver storage of fish oil fatty acid in humans.

SUBJECTS AND METHODS

To obtain the liver samples, we used as the source seven autopsied patients (4 males and 3 females) after permission had been given by their families. Clinical data on the subjects are given in Table 1. Cases 2, 4, and 7 had been eating regular meals until the period shortly before death, but the others had been under intravenous alimentation without fish oil supplementation for at least 2 months before death (in case 5, for nearly 4 months). At autopsy we obtained the portion of the liver with the minimum number of lesions and minimal amount of subcutaneous and omental adipose tissues and clotted blood. The tissues were stored at −40°C. In the analysis, a portion of each tissue specimen was homogenized after thawing and weighing. Tissue total lipids were extracted and purified [8]. Then, the lipid fractions from the total lipids in the liver and whole blood were separated in duplicate by thin-layer chromatography using silica gel G-60 plates (Merck, Darmstadt), with a solvent system of hexane/diethyl-ether/formic acid (40:10:1, v/v/v). In one series, lipid fractions scraped off from silica plates and extracted were dried, and then phospholipids (PL) [9], triglyceride (TG) [10], and cholesterol ester (CE) [11] were measured after dissolution in isopropanol. In the other series, PL, TG, and CE were hydrolyzed and their fatty acid methylated by BF₃ methanol. The fatty acid compositions of each lipid fraction were analyzed by gas
liquid chromatography with a flame ionization detector (Model G 300, Hitachi Co., Hitachi). In the analysis, a capillary column (CP-Sil 88 WCOT, Hitachi Co.) was used with He as a carrier gas. Prior to the identification process, the chemical-ionization method was performed with isobutane gas for both the sample EPA and the standard EPA (Cis 5, 8, 11, 14, 17; Sigma Co., St. Louis, MO). The standard EPA and its stated purity of greater than 99.0% were confirmed by gas liquid chromatography. Identification was carried out by a mass spectrometry apparatus (Model JMS-DX 303, Nippon-Denshi Co., Tokyo) after sequential application of ionized sample EPA and standard EPA.

Results are expressed as means±SEM. Student’s t-test was used to examine the significance of differences in paired as well as unpaired comparisons, and a p value of less than 0.05 was considered significant.

RESULTS

Lipid contents in the clotted blood and liver specimens are shown in Table 2. The fresh blood plasma from 15 control subjects who were suspected to be hepatitis B virus carriers [12, 13] contained 0.8±0.1 mmol/liter of TG, 4.7±0.1 mmol/liter of total cholesterol (TC), and 59.8±1.6 mmol/liter of PL. Thus, the slight decrease in TG and the relative maintenance of TC and PL in the whole blood are noteworthy. The effect of illness and therapies, including intravenous alimentation as well as the influence of membrane lipids from blood cells seem to have affected the blood lipid levels of the autopsied patients. Interestingly, the liver lipids had remained near the normal range in the autopsied patients in spite of their various different conditions. The control lipid values in the biopsied liver from the above 15 suspected hepatitis B carriers were 0.20±0.01 mmol/g for TG, 0.31±0.05 mmol/g for TC, and 10.0±1.0 mmol/g for PL. No differences in liver lipid fractions were thus found between the autopsied and biopsied cases. The main n-9, n-6, and n-3 series of fatty acids in TG, CE, and PL of whole blood and liver and in adipose tissue total lipids are shown in Table 3. Clear separation of adipose tissue TG, CE, and PL was difficult by thin-layer chromatography because
of tailing by the massive amount of TG. Therefore, only the data for fatty acids in total lipids are shown for subcutaneous and omental adipose tissues. Blood and liver fatty acids showed almost the same pattern for each lipid fraction; in the n-9 series C18:0 of PL and C18:1 of TG, in the n-6 series C18:2 of CE and C20:4 of PL, and in the n-3 series EPA (C20:5) and DHA (C22:6) of PL showed significant increases (p<0.05-0.0001). In adipose tissue total lipids, C18:1 and C18:2 were predominant, while fish oil fatty acids were found only scantily. The absolute amounts of EPA and DHA in whole liver and adipose tissue total lipids were calculated from the actual liver weight and from body adipose tissue mass estimated as 15% of body weight [14]. The amounts of EPA and DHA were 0.89±0.10 and 2.90±0.19 g, respectively, in the adipose tissue mass, while in the liver, the amount of EPA was 2.59±0.28 and that of DHA, 9.65±0.88 g. Both amounts were about three fold greater than those in adipose tissues. The gas liquid chromatographic mass spectrometry demonstrated similarity between liver EPA and highly purified standard EPA in both the base peak and the small fragmentation patterns around the base peak, as shown in Fig. 1.

**DISCUSSION**

The beneficial effects of fish oil fatty acids like EPA and DHA on hyperlipidemia and dyslipidemia continue to be emphasized in recent reports [15-18]. Whether or not “the gift from the sea” can also benefit familial hypercholesterolemia [19] and dyslipidemia in diabetes [20] remains to be elucidated.

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**Table 3. Percentage composition of main fatty acids in lipids of clotted blood, liver, and adipose tissue.**

<table>
<thead>
<tr>
<th>Tissue lipids</th>
<th>n-9 fatty acids</th>
<th>n-6 fatty acids</th>
<th>n-3 fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C18:0</td>
<td>C18:1</td>
<td>C18:2</td>
</tr>
<tr>
<td>TG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>5.9±0.2a</td>
<td>32.6±2.2b</td>
<td>10.9±0.8c</td>
</tr>
<tr>
<td>Liver</td>
<td>5.2±0.1a</td>
<td>27.5±1.2b</td>
<td>13.9±1.2ce</td>
</tr>
<tr>
<td>CE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1.9±0.2a</td>
<td>18.7±0.5bd</td>
<td>24.7±1.4de</td>
</tr>
<tr>
<td>Liver</td>
<td>4.8±0.1ae</td>
<td>14.2±3.2bd</td>
<td>18.3±0.5ce</td>
</tr>
<tr>
<td>PL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>10.9±0.8ae</td>
<td>19.3±0.2bd</td>
<td>11.6±0.4ce</td>
</tr>
<tr>
<td>Liver</td>
<td>16.2±0.6ae</td>
<td>12.0±0.3ed</td>
<td>15.8±0.1ce</td>
</tr>
<tr>
<td>Adipose tissue total lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>4.2±0.4</td>
<td>41.5±1.0</td>
<td>16.6±0.3</td>
</tr>
<tr>
<td>Omental</td>
<td>3.7±0.5</td>
<td>42.1±0.8</td>
<td>17.4±0.3</td>
</tr>
</tbody>
</table>

Values are means±SEM. C18:0 (%): 1a vs. 3a, p<0.01; 3a vs. 5a, p<0.001; 2a vs. 4a, n.s.; 4a vs. 6a, p<0.001. C18:1(%): 1b vs. 3b, p<0.001; 3b vs. 5b, n.s.; 2b vs. 4b, p<0.05; 4b vs. 6b, n.s. C18:2(%): 1c vs. 3c, p<0.001; 3c vs. 5c, p<0.001; 2c vs. 4c, p<0.05; 4c vs. 6c, n.s. C20:4(%): 1d vs. 3d, p<0.05; 3d vs. 5d, p<0.001; 2d vs. 4d, n.s.; 4d vs. 6d, p<0.001. C20:5(%): 1e vs. 3e, p<0.001; 3e vs. 5e, n.s.; 2e vs. 4e, p<0.02; 4e vs. 6e, p<0.05. C22:6(%): 1f vs. 3f, p<0.05; 3f vs. 5f, p<0.001; 2f vs. 4f, p<0.001; 4f vs. 6f, p<0.001.
the lipid-lowering and antithrombotic actions of fish oil fatty acids, attention is being given to their possible anti-neoplastic effect [21] and blood pressure-lowering action [22, 23]. These epidemiological and clinical data together with the finding that both EPA and DHA are hardly detected in adipose tissue despite their habitual intake [5, 6] prompted us to examine our hypothesis [6] of liver storage of fish oil fatty acids. As expected, we demonstrated a large amount of stored EPA and DHA in human livers. The amount stored in liver was estimated

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to be at least three-fold that in the total adipose tissue mass. The DHA/EPA ratio was about 3.5 for both liver and adipose tissue, and this ratio may have some significance since the administration of fish oils with different DHA/EPA ratios was shown recently to produce divergent plasma lipoprotein responses [24]. We are now analyzing EPA and DHA in the surgically resected liver from patients with various diseases. Study of 4 subjects (K. Sekine et al., unpublished) has shown that EPA and DHA are abundant in PL of high- and low-density lipoproteins and that their contents in resected liver are more than twice as high as those in the autopsied cases of this study. Thus the amount of fish oil fatty acids in human liver seems to decrease gradually with time. In 3 of the autopsied patients, fish oil fatty acids had not been ingested for at least 2 months, but their livers contained EPA and DHA in amounts only slightly below the mean value for the other autopsied patients. It is of interest to consider different patterns of storage of n-3 fatty acids between humans and animals of various species. In certain fishes such as cod [25, 26], EPA and DHA are known to be stored in both adipose tissue and liver; so further studies are needed in order to clarify the reason for this difference.

In humans, it might be that fish oil fatty acids ingested are incorporated into the chylomicron PL portion, which is then taken up in liver cells for storage as a fatty acid group of PL in chylomicron-remnants via the known liver remnant receptor [27]. EPA and DHA included in PL seem to be supplied constantly to circulating blood cells and peripheral tissues from the liver as a constituent of various lipoproteins.

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REFERENCES
