Abstract. Fatty liver disease is characterized by a wide spectrum of liver damage, i.e. simple steatosis may progress to advanced fibrosis and to cryptogenic cirrhosis via steatohepatitis, and ultimately to hepatocellular carcinoma. Eicosapentaenoic acid (EPA), a marine-derived n-3 fatty acid like docosahexaenoic acid (DHA), is an anti-thrombotic and hypolipidemic agent, and is an antagonist of platelet aggregation and an inhibitor of cholesterol and lipoprotein. In an attempt to confirm the hypolipidemic action of this agent, the effects of EPA on liver and plasma levels of lipids in mice fed a high-fat diet were investigated. EPA markedly reduced the fatty droplets in the liver cells and the liver weight, also lowering plasma levels of total cholesterol, free total cholesterol, phospholipids and triglyceride. It is suggested that n-3 fatty acid intake and fish consumption may be able to prevent the occurrence not only of metabolic syndrome, but also of fatty liver and non-alcoholic steatohepatitis.

Fatty liver disease is a new clinicopathological entity of emerging importance. It is characterized by a wide spectrum of liver damage, i.e. simple steatosis may progress to advanced fibrosis and to cryptogenic cirrhosis via steatohepatitis, and ultimately to hepatocellular carcinoma (1). It is suggested that chronic hepatocellular injury, necro-inflammation, stellate cell activation, progressive fibrosis and, ultimately, cirrhosis are initiated by the peroxidation of hepatic lipids and injury-related release of cytokines. Obesity is the single most significant risk factor for the development of fatty liver, i.e. obesity is also predictive of the presence of fibrosis, potentially progressing to advanced liver disease. From a histopathological point of view, insulin resistance plays a central role in the accumulation of triglyceride (TG) within the hepatocytes and in the initiation of the inflammatory cascade.

Low rates of cardiovascular disease in populations with a high intake of fish, such as Alaskan natives (2), Greenland Eskimos (3) and Japanese people (4), have suggested that fish consumption may prevent the occurrence of atherosclerosis and coronary heart disease (CHD). Eicosapentaenoic acid (EPA), a marine-derived n-3 polyunsaturated fatty acid, is an anti-thrombotic and hypolipidemic agent. This agent is an antagonist of platelet aggregation and an inhibitor of cholesterol and lipoprotein, and reduces serum levels of not only cholesterol but also TG in menopausal women (5). It has been suggested that the agent lowers hepatic cholesterol biosynthesis and enhances hepatic biliary secretion (6). Consumption of fish and n-3 fatty acid has been associated with a reduced risk of strokes, thrombotic infarction and CHD death, and has not been related to a risk of hemorrhagic stroke (7, 8).

In an attempt to confirm the hypolipidemic action of marine-derived n-3 fatty acids, in the present study, the effects of EPA on liver and plasma levels of lipids in mice fed a high-fat diet were investigated.

Materials and Methods

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Experimental animals. Male ICR mice (Charles River Japan, Yokohama, Japan), 8 weeks of age, were used. They were housed in plastic cages with wood shavings under controlled conditions (24±0.5˚C and 12 h of light from 06:00 to 18:00 h), in accordance with the principles outlined in the Guide for Animal Care and Use of the Committee of Tokyo Medical and Dental University, Japan.

Dietary treatment. All the mice had free access to a commercial normal diet (AIN-76A; Oriental Yeast Co. Ltd., Tokyo, Japan), which is a purified diet based on casein as the sole source of protein and tap water ad libitum. The daily food intake was approximately 5.0±0.5 g/mouse. The mice were divided into three groups of 15 each at the age of 10 weeks.

As previously reported (9), the animals in the high-fat diet group (HF-group) were each fed 5.0 g of a commercial high-fat diet (F2HFD2; Oriental Yeast Co., Ltd.), which consisted of 58% lard (w/w), 30% fish powder, 10% skim milk and a 2% vitamin and mineral mixture (equivalent to 7.5% carbohydrate, 24.5% protein and 60% fat), daily for 4 weeks.

The animals in the EPA-treated group (EP-group) each received 5.0 g of the high-fat diet (F2HFD2) supplemented with 18.9 mg of EPA, approximately 14-fold the human dose (2.7 g/day), when the body weights of human and mouse were 70 kg and 35 g, respectively.

The animals in the normal-control group (NC-group) were given the same normal diet (AIN-76A) alone. All the experimental procedures conformed to the regulations described in the Guide to the Care and Use of Laboratory Animals of the U.S. National Institute of Health (NIH).

Experimental procedure and histological evaluation. Five mice in each cage were daily given 25.0 g of the diet, and the body growth was checked weekly throughout the experiment.

All the mice were killed at 14 weeks of age by cervical dislocation after cardiac puncture under deep urethane anesthesia (1.5 g urethane/kg of body weight, Merck, Darmstadt, Germany) to measure the plasma levels of lipids and biochemical markers (SRL, Inc., Tokyo, Japan).

At autopsy, the removed testes, spleen, kidney and liver were weighed. Each liver was immediately fixed in a 10% formaldehyde buffer solution (pH 7.2), embedded in paraffin, prepared as 5-μm serial sections and stained with Mayer’s hematoxylin and eosin for histological examination.

Table I. Plasma levels of biochemical markers.

<table>
<thead>
<tr>
<th>Groups</th>
<th>HF (g/dl)</th>
<th>EP (g/dl)</th>
<th>NC (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>3.8±0.18</td>
<td>3.35±0.18</td>
<td>4.0±0.15</td>
</tr>
<tr>
<td>Gluc</td>
<td>165±8</td>
<td>175±15</td>
<td>163±8</td>
</tr>
<tr>
<td>BUN</td>
<td>20.4±1.3</td>
<td>17.3±1.0</td>
<td>22.8±0.9</td>
</tr>
<tr>
<td>Creat</td>
<td>0.0±0.01</td>
<td>0.0±0.01</td>
<td>0.0±0.01</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>40±4.4</td>
<td>50.6±4.6</td>
<td>50.6±4.6</td>
</tr>
<tr>
<td>LD (IU/l)</td>
<td>168±35</td>
<td>108±45</td>
<td>108±45</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>94±3.7</td>
<td>95.5±7.9</td>
<td>95.5±7.9</td>
</tr>
</tbody>
</table>

Data are the mean±S.E.M. HF: High-fat diet group; EP: EPA-treated group; NC: normal-control group; TP: total protein; Gluc: glucose; BUN: blood urea nitrogen; Creat: creatinine; AST: aspartate aminotransferase; LD: lactate dehydrogenase; ALP: alkaline phosphatase.

Figure 1. Liver weights (body weight/g). HF: High-fat diet group; EP: EPA-treated group; NC: normal-control group. Data are the means±SEM. **And *significantly different from HF-group at p<0.01 and 0.05, respectively.

Statistical analysis. All parameters were expressed as the mean±SEM. The significance of differences among groups was evaluated using the unpaired t-test and/or Wilcoxson’s rank test. A p-value less than 0.05 was considered statistically significant.

Results

Body growth and organ weights. There were no differences in the initial or final body weights or the body growth among the groups (data not shown). Differences in the wet weights of testis, spleen and kidney were not observed among the groups. However, liver weights in the EP-group were markedly lowered to 75.4% (p<0.01) of that of the HF-group, in which the liver weights increased to 107.8% of the NC-group (p<0.05) (Figure 1).

Histological examination of liver. As shown in Figure 2, hepatic lipid deposits appeared as vacuoles of small size within the cytoplasm of the liver cells in the mice fed the high-fat diet. However, the addition of EPA to the diet markedly reduced the number of fatty droplets in the cytoplasm of the liver cells in the mice of the EP-group.

Plasma lipids. Plasma levels of total cholesterol (TCh), free total cholesterol (FCh) and phospholipids (PhL) were enhanced by feeding a high-fat diet (p<0.01) (Figure 3A, B and C). However, supplementing with EPA markedly lowered the plasma levels of TCh, FCh and PhL to 46.1%, 47.9% and 49.5% of those in the HF-group.
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Figure 2. Histological structure of liver in each group (HE staining, original magnification ×288). A) Normal-control group (NC), B) high-fat diet group (HF) and C) EPA-treated group (EP).

Figure 3. Plasma levels of lipids in each group. A) total cholesterol (TCh), B) free total cholesterol (FCh), C) phospholipids (PhL) and D) triglyceride (TG). HF: High-fat diet group; EP: EPA-treated group; NC: normal-control group. Data are the means±SEM. **Significantly different from HF-group at p<0.01.
respectively ($p<0.01$). The plasma level of TG was remarkably reduced to 30.4% of that of the HF-group ($p<0.01$) (Figure 3D).

**Plasma biochemical markers.** There were few differences in the plasma levels of total protein, glucose, blood urea nitrogen and creatinine, and in the activities of aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase among the groups (Table I).

**Discussion**

The Adult Treatment Panel (ATP) III of the National Cholesterol Education Program (NCEP) has proposed a definition for “metabolic syndrome” to aid in the identification of individuals at risk for both CHD and type 2 diabetes (10). The definition incorporates thresholds for five easily measured variables linked to insulin resistance, i.e. waist circumference, TG, high density lipoprotein-cholesterol, fasting glucose concentration and blood pressure, and this classification for metabolic syndrome is confirmed when predefined limits of any three of the above five criteria are exceeded.

On the other hand, the World Health Organization (WHO) definition of metabolic syndrome is more complex and is focused on glucose dysregulation (11). Thus, the NCEP’s definition of metabolic syndrome rather than the WHO’s definition is expected to help identify individuals who may receive particular benefit from life-style measures to prevent CHD and diabetes. A higher plasma concentration of oxidized low density lipoprotein was associated with an increased incidence of metabolic syndrome overall, as well as its components of abdominal obesity, hyperglycemia, and hypertriglyceridemia (12).

Prospective cohort studies reported that n-3 fatty acid intake and fish consumption were associated (13) or not associated with a lower risk of CHD (14). EPA, in part converted from docosahexaenoic acid (DHA), is transformed into a nonaggregatory agent, thromboxane A3, which increases the synthesis of vasodilator, prostaglandin I3, leading to further reductions in platelet aggregation and increased vasodilation (15).

The Japan Eicosapentaenoic Acid Lipid Interventions study, a randomized trial of 18,645 Japanese that examined the effectiveness of 1.8 g of EPA per day plus a statin in reducing CHD rates reported that, after a follow-up of 4.5 years, the hazard ratio in the EPA versus control groups was 0.81 (95% CI: 0.68 to 0.96) for non-fatal coronary events (16). The Japan Public Health Center-based study, a 10-year prospective cohort study of 41,578 middle-aged Japanese, reported that dietary intake of marine-derived n-3 fatty acids had significant inverse associations with nonfatal coronary events. The multivariate-adjusted hazard ratio in the highest versus lowest quintiles of marine-derived n-3 fatty acid intake (median intake=2.1 versus 0.3 g/day, respectively) was 0.33 (95% CI: 0.16 to 0.63) for nonfatal coronary events (17).

In the present study, EPA markedly reduced fatty droplets in liver cells and liver weight, and lowered plasma levels of lipids. It is suggested that n-3 fatty acid intake and fish consumption may prevent the occurrence not only of metabolic syndrome but also of fatty liver and non-alcoholic steatohepatitis. Regular intake of blue fishes such as pompano, sardine, mackerel, herring and cod may be beneficial for the prevention of thrombotic infarction, and current dietary guidelines are recommending fish consumption at least twice or three times a week for the prevention of CHD.

**Competing Interests**

The Authors declare that they have no competing interests.

**Authors’ Contributions**

NEM made substantial contributions to the study’s conception and the analysis of the data, carried out the animal experiments under the guidance of SAS, and was involved in drafting the manuscript. SUZ carried out the pathological examination with NEM. KIK performed the statistical analysis. OKA participated in drafting the manuscript. SAK conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All Authors read and approved the final draft.

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**References**


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