Interaction of Fenofibrate and Fish Oil in Relation to Lipid Metabolism in Mice

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Aim: The aim of our study is to elucidate the interactive effects on lipid metabolism of fenofibrate and two fish oils with EPA and DHA contents in mice.

Methods: Female C57BL/6J mice were fed purified experimental diets containing safflower oil (SO), EPA-rich menhaden oil (MO) or DHA-rich tuna oil (TO) with or without 0.1% fenofibrate for 8 weeks. At the end of the experiments, we measured plasma lipids and hepatic triglycerides and cholesterol, and the hepatic mRNA expression of lipogenic and lipidolytic genes.

Results: Plasma TG levels fell in the group fed MO or TO alone and fell significantly in all fenofibrate-treated groups. Although plasma total cholesterol levels fell significantly in fish oil-fed groups, fenofibrate treatments increased significantly plasma total cholesterol levels in these fish oil groups, but not in the group fed SO alone; however, hepatic triglyceride and total cholesterol levels markedly decreased in MO- or TO-fed mice. In lipid synthesis, the hepatic mRNA level of SREBP-1c was not reduced in either fish oil group; however, Insig-1 mRNA decreased in MO and TO feeding groups by about half and FAS or SCD-1 mRNA decreased significantly in MO and TO feeding groups, compared with the SO feeding group. In both fish oil groups, SREBP-2 mRNA decreased significantly and HMG-CoA reductase mRNA also decreased with/without fenofibrate. On the other hand, fenofibrate supplementation significantly induced the mRNA expression of AOX and UCP-2, which play a role in lipid catabolism, in all diets. CYP7A1 mRNA increased markedly in mice fed MO diet with fenofibrate, compared with TO diet with fenofibrate.

Conclusion: These data suggest that differences in dietary contents of EPA and DHA do not influence the inhibition of lipogenesis, and that fenofibrate supplementation stimulates fatty acid oxidation, regardless of the oil type; however, cholesterol catabolism was induced by a combination of EPA-rich fish oil and fenofibrate, which suggests that EPA has a greater synergistic ability for cholesterol catabolism induction by fenofibrate than DHA.


Key words; Eicosapentaenoic acid, Docosahexaenoic acid, Lipogenesis, Fatty acid oxidation

Introduction

Fish oil reduces plasma triglyceride levels in patients with hyperlipidemia and is useful for improving cardiovascular disease. The effects of fish oil are attributed to n-3 PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The lipid-lowering actions of n-3 PUFA mainly result from the inhibition of lipogenesis and stimulation of fatty acid oxidation in the liver. Several studies in vivo and in vitro have demonstrated that n-3 PUFA decreases the expression of genes coding for lipogenesis enzymes, such as fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD), and S14 protein, by lowering sterol regulatory element binding protein-1 (SREBP-1). In addition, n-3 PUFA also increases the expression of genes involved in fatty acid oxidation, such as acyl-CoA oxidase (AOX), medium-chain acyl-CoA dehydrogenase (MCAD), lipoprotein lipase (LPL), fatty acid binding protein (FABP),...
uncoupling protein-2 (UCP-2), and carnitine palmitoyltransferase-1 (CPT-1), through the activation of peroxisome proliferator-activated receptor alpha (PPARα)⁹⁻¹³.

Fenofibrate, a hypolipidemic drug in the fibrate class, is known as an agonist for PPARα, which is a ligand-activated transcription factor. PPARα regulates the expression of genes involved in triglyceride hydrolysis and fatty acid oxidation. A number of reports have shown that fenofibrate decreases fatty acid synthesis and increases hepatic fatty acid oxidation, thus reducing the amount of fatty acid available to triglyceride synthesis.⁰¹⁻¹⁷ Fenofibrate lowers plasma triglycerides and raises HDL-cholesterol.

Previous studies have reported that the lipid-lowering effects of EPA and DHA are different. Botolin et al. using rat primary hepatocytes incubated with EPA or DHA, demonstrated that DHA strongly suppressed nuclear SREBP-1 production compared to EPA. Conversely, EPA consistently induced PPARα in vitro while DHA did not. EPA, not DHA, has a hypotriglyceridemic effect. Freyland et al. also suggested that EPA possesses potent triglyceride-lowering properties in vivo, and stimulates the oxidation of fatty acids more than DHA. In addition, the combination of EPA and DHA resulted in a strongly hypolipidemic effect compared to the effect of each alone.

The aim of our study was to elucidate the interaction between the drug efficacy of fenofibrate and two kinds of fish oils with different degrees of EPA and DHA contents in mice.

Materials and Methods

Animals and Diets
Female C57BL/6J mice were obtained from Tokyo Laboratory Animals Science Co. (Tokyo, Japan) at 7 weeks of age and fed a normal laboratory diet (CE2; Clea Japan Inc., Tokyo, Japan) for 1 week to stabilize the metabolic conditions. Mice were housed in a 12-h light/12-h dark cycle at a constant temperature of 22 ± 2°C and humidity of 55 ± 10%. Mice were divided into six groups (five mice in each group), fed purified experimental diets containing safflower oil (SO), EPA-rich menhaden oil (MO) and DHA-rich tuna oil (TO) with or without 0.1% fenofibrate (Sigma, St Louis, MO, USA) for 8 weeks. The purified experimental diets contained, on an energy basis, 63% carbohydrate, 20% fat and 26% protein. Safflower oil contained 46% oleic acid (18:1n-9) and 45% linoleic acid (18:2n-6) as the main fatty acids; menhaden oil (Sigma, St Louis, MO, USA) contained 10–15% EPA (20:5n-3) and 8–15% DHA (22:6n-3); and tuna oil contained 7% EPA (20:5n-3) and 24% DHA (22:6n-3). The animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Josai Life Science Center.

Collection of Blood and Tissue Samples
Animals were anesthetized at about 10:00 a.m. by intraperitoneal injection of pentobarbital sodium (0.08 mg/g of body weight; Nembutal, Abbott, North Chicago, IL, USA) at the end of the experiment. Blood samples were obtained from the inferior vena cava after 3h fasting. Plasma triglyceride, total cholesterol and HDL-cholesterol concentrations were measured by the enzyme assays in the individual E-Test Wako kit (Wako Pure Chemical Industries, Osaka, Japan). Plasma adiponectin and leptin concentrations were measured by ELISA assays according to the manufacturer’s instructions (Morinaga Institute of Biological Science, Japan and Otsuka Pharmaceutical, Japan). Livers were rapidly isolated and weighed. Aliquots of liver were immediately homogenized to extract RNA, and the rest of the liver was immediately frozen in liquid nitrogen and stored at −80°C until analysis. Brown adipose tissue (BAT) and white adipose tissue (WAT) were handled in the same way as the liver.

RNA Extraction and Real-Time PCR
Total RNA was prepared from liver tissues for each mouse using Trizol reagents (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. RNA expression levels in the liver were analyzed by real-time PCR quantification. Real-time PCR was performed on an ABI Prism 7000 Sequence Detection Systems (Applied Biosystems Inc., CA, USA) using a QuantiTect SYBR Green Real-time PCR kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. The PCR primers used were as follows: SREBP-1c, 5‘ primer, 5‘-GGAGCCATGGAGTGCCCACATT-3’ and 3’ primer, 5‘-GGCCCGGGAGTAGCATTG-3’; SREBP-2, 5’ primer, 5‘-GCGTTCGAGAAGTTGGAGACC-3’ and 3’ primer, 5‘-TTGCTCTGAAAAACAAAT-3’; HMG-CoA reductase, 5’ primer, 5‘-TCACAGTGACTGAGCTTCAGCA-3’ and 3’ primer, 5‘-TCATCTTCTACACCCAGAGG-3’; FAS, 5’ primer, 5‘-TCACACCTGTGGCCTCGTGCAGAGAAGCGAG-3’ and 3’ primer, 5‘-TGTATTGCAGGCTTTAGCA-3’ and 3’ primer, 5‘-ACAAAGTTGCTCTGAAAAAACAATAC-3’; Insig-1, 5’ primer, 5‘-TGTCATTCTCGAGGTCATCT-3’ and 3’ primer, 5‘-ACAAAGTTGCTCTGAAAAAACAATAC-3’; SCD-1, 5’ primer, 5‘-CCGGAGACCCCTTAGATCGA-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’ primer, 5‘-TGCGGTCCATAGG-3’ and 3’
5’-TTTGTGGGATGCCTTTGTGATG-3’ and 3’ primer, 5’-AGCCGAAGCAGCAGCATGAT-3’; CYP7A1, 5’ primer, 5’-CTCTGATTTTTCTCTCAAGCTGGTCA-3’ and 3’ primer, 5’-CCCAGGCATTGCTCTTTGAT-3’; PPAR γ, 5’ primer, 5’-GTGGCTGCTATAATTTGCTGTG-3’ and 3’ primer, 5’-GGCCTTGAAACCAACCA-3’; UCP-2, 5’ primer, 5’-CCTGCTTCACCACCTTCTTGAT-3’, was used as an endogenous control gene. PCR products were evaluated by inspecting their melting curves.

Statistical Analysis
Data from multiple groups were compared by one-way ANOVA. When significant, each group was compared with the others by Fisher’s protected least significant difference (PLSD) test (Statview 4.0, Abacus Concepts). Values are shown as the means ± SD. Statistical significance was defined as p < 0.05.

Results

Body and Tissue Weight Change
In the absence of fenofibrate, final body weights showed no significant differences among mice fed different diets; however, the addition of 0.1% fenofibrate significantly reduced the body weight in SO- and MO-fed groups. The ratios of liver weight increased about 2-fold in fenofibrate-treated groups compared to untreated groups fed the respective diets. While WAT and BAT weights did not differ for three lipid sources, these weights were reduced significantly by fenofibrate treatment in the respective groups by 40–60% (Table 1).

Plasma and Hepatic Lipid Parameters
Plasma triglyceride levels were significantly lower 19% in TO-fed mice than in SO-fed mice in the absence of fenofibrate. Furthermore, they were reduced significantly in all fenofibrate-treated groups relative to the respective non-fenofibrate-treated groups. Although plasma cholesterol levels were 30% lower in MO-fed mice and 37% lower in TO-fed mice than in SO-fed mice, fenofibrate treatment increased those levels 2-fold in MO- and TO-fed mice. Additionally, HDL-cholesterol levels increased in all fenofibrate-treated groups (Table 2).

In the absence of fenofibrate, liver triglyceride levels fell significantly, by about 90%, in MO- and TO-fed mice, respectively, compared to SO-fed mice. Fenofibrate treatment decreased the lipid levels by about 70% in the liver from mice fed the SO diet, but no additive effect of fenofibrate was shown in the lipid levels in the liver of fish oil-fed mice. Similar to liver triglyceride levels, liver cholesterol levels also fell markedly, by about 70% in MO- and TO-fed mice in the absence of fenofibrate (Table 2).

Levels of plasma adiponectin and leptin, adipocyte-released hormones, are shown in Table 3. In the absence of fenofibrate, plasma adiponectin levels increased significantly in MO and TO groups compared to SO group; however, the increment was suppressed by fenofibrate treatment. Plasma leptin levels were markedly reduced in all fenofibrate treatment groups.

Plasma AST and ALT Levels
To investigate the effect of fenofibrate on liver
function, plasma AST and ALT were measured (Table 3). Plasma AST increased by about 1.5-fold and plasma ALT increased by about 3-fold with fish oil and fenofibrate treatment, compared with fish oil alone.

Expression Levels of Hepatic Genes Involved in Lipid Metabolism

To clarify the interactive effect of fenofibrate and two different fish oils on lipid metabolism, various mRNA levels of hepatic genes involved in cholesterol and fatty acid metabolism were analyzed. As shown in Fig. 1, SREBP-1c mRNA did not change with either fish oil diet while fenofibrate treatment slightly increased these mRNA expressions in the SO-or TO-fed group; however, SREBP-1-controlled genes, such as FAS and SCD-1 mRNA, decreased significantly in MO and TO groups by 80-90%, compared with the SO group. The Insig-1 mRNA level also decreased significantly in MO and TO groups by about 70%. On the other hand, fenofibrate did not influence these gene expressions in any group.

Various mRNA expressions for cholesterol metabolism are shown in Fig. 2. In the absence of fenofibrate, SREBP-2 mRNA decreased by 30-40% in fish oil-fed groups compared with the SO-fed group, and these changes were not affected by fenofibrate treatment. MO- and TO-fed mice without fenofibrate also showed markedly decreased levels of HMG-CoA reductase mRNA by about 80%; however, fenofibrate treatments did not affect mRNA expressions in fish oil groups. LDL-receptor mRNA expression decreased significantly in TO-fed mice, and increased by fenofibrate administration. CYP7A1 mRNA levels did not change in mice fed fish oil alone, but increased in mice fed the MO or TO diet with fenofibrate, by about 5.0-fold or 3.4-fold respectively, compared to mice fed each fish oil alone. The increment of CYP7A1 mRNA expression with fenofibrate treatment was greater in MO-fed mice than TO-fed mice. A similar expression pattern was shown in CYP8B1 mRNA levels (Fig. 3).

Various mRNA expressions of fatty acid oxidation are shown in Fig. 4. PPARα mRNA levels showed minor differences in all groups with/without fenofibrate; however, AOX mRNA expression slightly increased in MO or TO groups by 1.9-fold or 1.6-fold, respectively, compared with the SO group. Fenofibrate treatments markedly increased these expressions 5.7-

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Table 2. Plasma triglyceride, total cholesterol and HDL-cholesterol concentration and liver triglyceride and total cholesterol concentration in C57BL/6J mice fed safflower oil, menhaden oil and tuna oil with/without 0.1% fenofibrate diet for 8 weeks

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<tr>
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<th>FF</th>
<th>Plasma triglyceride (mg/dL)</th>
<th>Plasma total cholesterol (mg/dL)</th>
<th>Plasma HDL-cholesterol (mg/dL)</th>
<th>Liver triglyceride (mg/total liver)</th>
<th>Liver total cholesterol (mg/total liver)</th>
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<tr>
<td>SO</td>
<td>−</td>
<td>65 ± 13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79 ± 18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.82 ± 39.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.68 ± 2.21&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>MO</td>
<td>−</td>
<td>58 ± 3&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>55 ± 8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>33 ± 7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.16 ± 4.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.15 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>TO</td>
<td>−</td>
<td>53 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49 ± 11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28 ± 5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.47 ± 2.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>SO</td>
<td>+</td>
<td>40 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66 ± 24&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>58 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.95 ± 14.09&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>MO</td>
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<td>108 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TO</td>
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<td>109 ± 13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.72 ± 1.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.98 ± 0.13&lt;sup&gt;b,c&lt;/sup&gt;</td>
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Values are the mean ± SD (n = 5). Groups sharing different letters are significantly different, ANOVA with Fisher’s protected least significant difference (PLSD) test, p < 0.05. SO: safflower oil-fed group, MO: menhaden oil-fed group, TO: tuna oil-fed group. FF: fenofibrate

Table 3. Plasma adiponectin, leptin, AST and ALT levels in C57BL/6J mice fed safflower oil, menhaden oil and tuna oil with/without 0.1% fenofibrate diet for 8 weeks

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<tr>
<th></th>
<th>FF</th>
<th>Plasma adiponectin (μg/mL)</th>
<th>Plasma leptin (ng/mL)</th>
<th>Plasma AST (IU/L)</th>
<th>Plasma ALT (IU/L)</th>
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<tr>
<td>SO</td>
<td>−</td>
<td>36.5 ± 3.7&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>20.2 ± 11.0&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>8 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>MO</td>
<td>−</td>
<td>67.3 ± 5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.8 ± 11.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33 ± 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>TO</td>
<td>−</td>
<td>70.5 ± 12.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.4 ± 11.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38 ± 7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>SO</td>
<td>+</td>
<td>32.7 ± 4.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.3 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MO</td>
<td>+</td>
<td>39.5 ± 8.4&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<td>TO</td>
<td>+</td>
<td>43.5 ± 5.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>54 ± 11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
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Values are the mean ± SD (n = 5). Groups sharing different letters are significantly different, ANOVA with Fisher’s protected least significant difference (PLSD) test, p < 0.05. AST: aspartate aminotransferase, ALT: alanine aminotransferase. SO: safflower oil-fed group, MO: menhaden oil-fed group, TO: tuna oil-fed group. FF: fenofibrate
fold, 4.3-fold and 4.5-fold in SO, MO and TO groups, respectively, compared with each lipid source. While UCP-2 mRNA levels did not differ according to dietary lipid sources, fenofibrate supplementation caused the mRNA expression to increase 3.4-fold in the SO group and 2.4-fold or 2.8-fold in MO or TO groups, compared to the respective non-fenofibrate groups.

**Discussion**

The major findings of this study are that 1) body weight and adipose tissue weight decreased by fenofibrate treatment, regardless of difference in lipid sources; 2) mRNA expressions of lipogenic genes, such as FAS, SCD-1 and HMG-CoA reductase, were markedly decreased in MO and TO diets, but did not change with fenofibrate treatment; 3) mRNA expressions of fatty acid oxidation- and thermogenesis-related genes, such as AOX and UCP-2, were not affected by differences in lipid sources, but were markedly increased by fenofibrate treatment; and 4) the combination of MO and fenofibrate more than TO and fenofibrate markedly increased CYP7A1 mRNA expression.

In this study, fenofibrate treatment decreased body weight, adipose tissue weight, and consequently decreased plasma leptin levels markedly, regardless of differences in lipid sources. The increment of the plasma adiponectin level by fish oil feeding was also inhibited with fenofibrate treatments. Tsuchida et al. reported that PPARα activation by its agonist, Wy-14,643 treatment, up-regulated the expression of the adiponectin receptor (AdipoR) 1 and AdipoR2 in WAT, but not increased serum adiponectin concentrations. In our study, fenofibrate also decreased plasma adiponectin levels accompanied by WAT weight declination. We considered that adiponectin sensitivity increased with fenofibrate treatment. Although fenofibrate was effective to improve lipid metabolism regulation, when fenofibrate was used with fish oil, this combination resulted in increased liver tissue and
plasma ALT levels.

In rodent studies, diets containing 25% menhaden fish oil, 60% tuna fish oil, and 25% fish oil decreased SREBP-1c mRNA by 60, 86, and 80%, respectively, compared with their respective controls, while 40% sardine or tuna fish oil decreased...
mature SREBP-1 protein but not its mRNA\(^6\). Our finding showed that ingestion of a 20% menhaden or tuna oil diet did not decrease SREBP-1c mRNA expression levels, but markedly reduced the expressions of SREBP-targeting genes such as FAS and SCD-1. As shown in Fig. 1, ingestion of these fish oils also significantly decreased mRNA levels of Insig-1, which are regulated coordinately with SREBP-1 mature protein levels\(^{27}\). Our previous investigation reported that fish oil decreased the ratio of mature SREBP-1 protein to its precursor\(^6\), and also suggested that fish oil inhibited the maturation process of the SREBP-1 precursor.

PPAR\(\alpha\) are ligand-activated transcriptional factors and members of the nuclear hormone receptor superfamily\(^{20, 28}\). Fibrates\(^{29}\) and n-3 PUFA\(^{30}\) are ligands of the PPAR\(\alpha\). Evidence indicates that PPAR\(\alpha\) mediates the action of fibrates on lipid homeostasis\(^{31}\), leading to alteration of the expression of genes involved in lipid and lipoprotein metabolism\(^{32}\). Fenofibrate supplementation did not enhance the effect of fish oil on SREBPs or cholesterologenic or lipogenic enzyme mRNAs (Fig. 1, 2). This study suggests that the hypolipidemic effect of fish oil is principally produced by SREBP-1c mRNA down-regulation, not by PPAR\(\alpha\) activation in the liver.

Fatty acid oxidation genes AOX and UCP-2 are stimulated by PPAR\(\alpha\) activity, and these inductions are markedly induced by fenofibrate\(^{33, 34}\). It is reported that fish oil also induces AOX and UCP-2 mRNA expression\(^{29, 34}\). In our experiment, fenofibrate supplementation also markedly increased AOX and UCP-2 mRNA levels, but fish oil ingestion did not.

In this study, SREBP-2 mRNA expression decreased in both fish oil groups. SREBP-2 is a relatively selective activator of cholesterol synthesis\(^{35}\), which helps to explain our results that plasma and liver total cholesterol concentrations decreased markedly in MO- and TO-fed mice. In humans, treatment with fibrates is consistently associated with an increase in plasma HDL-cholesterol\(^{18}\). Our result is also consistent with the previous observation that fenofibrate treatment markedly increased plasma HDL-cholesterol in all groups. The data presented here indicate that the
increment of the plasma total cholesterol level in fenofibrate-supplemented groups is due to the increase of the plasma HDL-cholesterol level (Table 2).

Fish oil up-regulates CYP7A1 as well as increasing bile acid and cholesterol excretion. Furthermore, fenofibrate induces CYP7A1 expression through PPARα. Our data showed that CYP7A1 mRNA was not affected by fish oil alone, whereas its expression in the MO or TO diet with fenofibrate increased significantly compared with the SO diet with fenofibrate, and the induced expression with the MO diet with fenofibrate was higher than with the TO diet with fenofibrate. CYP7A1 is induced by PPAR/RXR or LXR/RXR, but is induced by PPAR/LXR. Fish oil stimulates RXRα expression markedly more than LXR. This observation suggests that EPA induces RXRα mRNA more than DHA and that the marked increase of CYP7A1 mRNA expression in the MO diet with fenofibrate group in this study was induced by formation of a PPAR/RXR or LXR/RXR combination from the induction of RXRα and PPARα by EPA and fenofibrate, respectively.

To verify this hypothesis, further investigations of both precursor or mature SREBP-1 protein activity and cholesterol catabolism regulation mechanisms are required.

Acknowledgments

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