13

Hepatic Lipid Accumulation by High Cholesterol Diet is Inhibited by the Low Dose Fish Oil in Male C57BL/6J Mice

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1. Introduction

Atherosclerotic diseases such as ischemic heart disease and cerebral infarction account for the major causes of death in many countries. It is well known that hyperlipidemia is closely associated with these diseases (Kodama, 1990). Fish oil consumption reduces lipogenesis and induces fatty acid oxidation in liver(Rustan, 1990; Halminski, 1991), and ameliorates plasma and hepatic lipid levels (Nestel, 1990). These effects of fish are greatly attributed to the action of n-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA). Several studies have reported that n-3 PUFA decreases the expression of genes coding for lipogenesis enzymes such as fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and stearoyl-CoA desaturase (SCD) through the lowering of sterol regulatory element binding protein-1 (SREBP-1) (Kim, 1999; Shimano, 1999; Nakatani, 2002). In addition, n-3 PUFA activates the peroxisome proliferator-activated receptor α (PPARα) (Krey, 1997). PPARα is a ligand-activated transcription factor that regulates the expression of genes involved in triglyceride hydrolysis and fatty acid oxidation (Latruffe, 1997; Nakatani, 2002; Schoonjans, 1996), such as acyl-CoA oxidase (AOX), lipoprotein lipase (LPL), medium-chain acyl-CoA dehydrogenase (MCAD), acyl-CoA synthetase (ACS), and uncoupling protein-2 (UCP-2).

Cholesterol is crucial for the components of animal cell membranes, and for the synthesis of bile acid and steroid hormone. Cholesterol in the inner cavity of the small intestine are entered in to epithelial cells by Niemann-Pick C1 like-1 (NPC1L1) protein on the brush border of epithelial cells of the small intestine (Davis, 2004), and cholesterol is released into the lymph duct. It is well known that cholesterols, including 22- and 24-hydroxy cholesterol, act as ligands for liver X receptor (LXR) α (Schultz, 2000). LXRα, a nuclear receptor, forms a heterodimer together with the retinoid X receptor (RXR) and combines with LXR element (LXRE) at the promoter of target genes to control transcription. LXR target genes include cholesterol 7α-hydroxylase (CYP7A1), ATP-binding cassette transporter (ABC)A1, ABCG1, and SREBPs, which are highly involved in cholesterol metabolism (Repa, 2000; Peet, 1998).

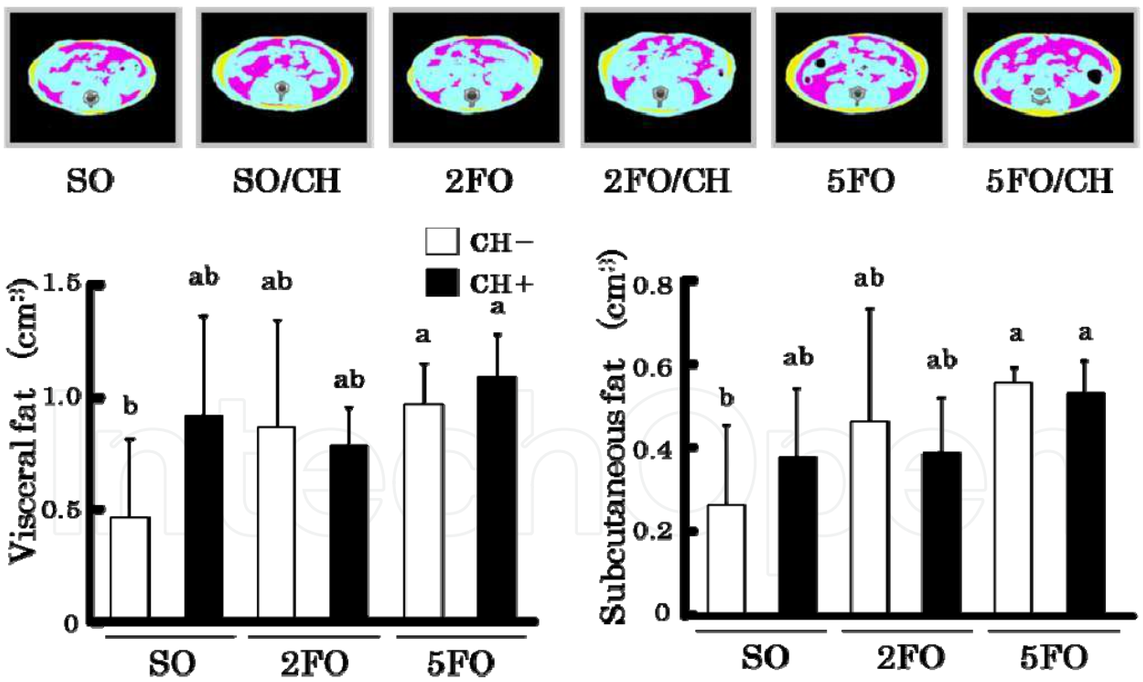
We previously indicated that 20en% fish oil diet dramatically inhibited hepatic lipids accumulation in female C57BL/6J mice fed 2% cholesterol (Hirako, 2010). In this study, we

determined whether 2 or 5en% fish oil, eqivalent to 10 or 25% of the total fat energy, improves lipid metabolism in high cholesterol diet fed male C57BL/6J mice. Mice were given SO diet consisted of 20en% safflower oil, 2FO diet consisted of 2en% fish oil plus 18en% safflower oil, 5FO diet consisted of 5en% fish oil plus 15en% safflower oil, and SO/CH, 2FO/CH, 5FO/CH are consisted of SO, 2FO, 5FO with 2%cholesterol. The body fat composition of mice was examined radiographically using an X-ray CT for experimental animals. Blood parameter and hepatic lipids were measured using enzymatic methods. Hepatic mRNA expression levels were measured by real-time RT-PCR.

2. Results and discussion

2.1 Body weight and tissue weight did not change with the low dose fish oil feeding in male C57BL/6J mice

Final body weights were not significantly different among the groups. No large difference in weights of the white adipose tissue around the uterus and brown adipose tissue from the interscapular region between the groups was observed. In previous study, we reported that body weight gain was significantly decreased in female C57BL/6J mice fed 20 en% fish oil (Hirako, 2010). However, our study observed that 5 en% fish oil feeding in female C57BL/6J mice did not modify body weights (data not shown). These results revealed that 2 and 5 en% fish oil feeding in male C57BL/6J mice did not modify body weight and adipose tissue weight



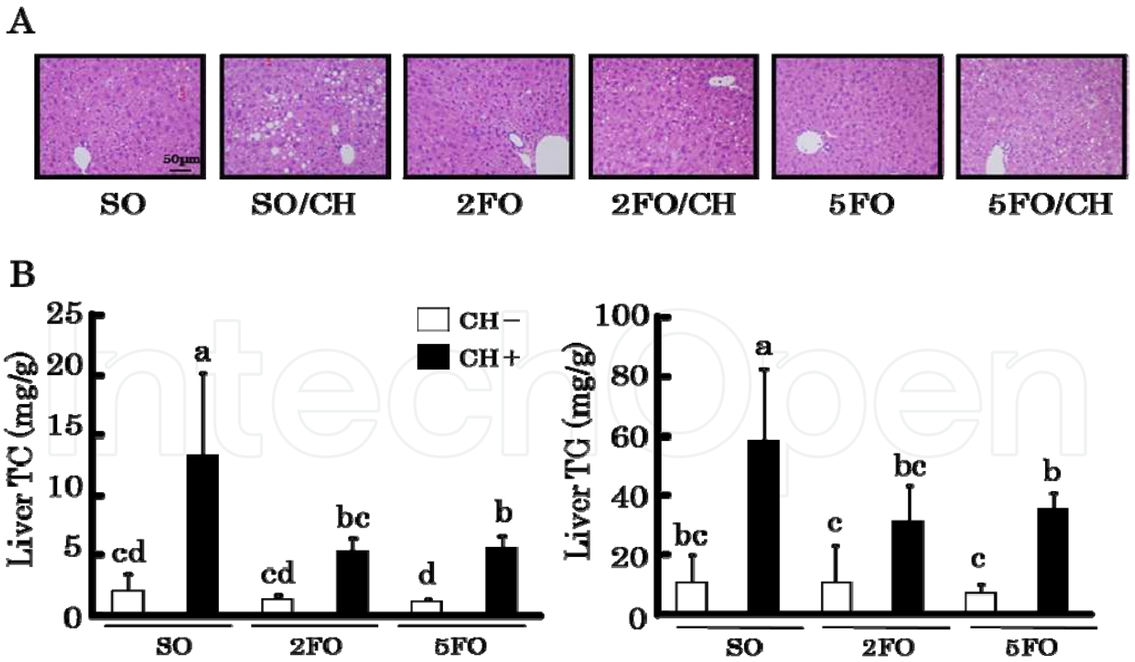
Representative X-ray CT images of mice fed SO, SO/CH, 2FO, 2FO/CH, 5FO, and 5FO/CH for 8 weeks, at the L3 level (upper). The areas indicated with pink, yellow, and light-blue are visceral fat, subcutaneous fat, and muscle, respectively. CT-estimated amounts of visceral fat and subcutaneous fat in the abdominal area of L2–L4(bottom). Values represent means ± S.D. (n=5). Means with different letters are different at the $p<0.05$ level by Fisher's protected least significant difference (PLSD) test.

Fig. 1. CT-based fat tissues composition analysis of 16-week-old male C57BL/6J mice.

as well as female C57BL/6J mice. CT scan analysis showed that visceral and subcutaneous fat mass were significantly increased in the 5FO and 5FO/CH groups, but not changed by cholesterol feeding (Figure 1). Meanwhile, these levels were high in all group compared with the fat mass in the female C57BL/6J mice (0.45±0.28cm^3 visceral fat in male C57BL/6J mice fed the SO diet and 0.24±0.13 cm^3 visceral fat in female C57BL/6J mice fed the SO diet).

2.2 Hepatic tissue histology and lipid levels modified with the low dose fish oil feeding in male C57BL/6J mice

Although the liver weight significantly increased in the SO/CH group compared with the SO group, there was no difference in the fish oil groups compared with the SO group (SO: 0.96 ± 0.10, SO/CH: 1.31 ± 1.2, 2FO: 1.00 ± 0.13, 2FO/CH: 1.11 ± 0.12, 5FO: 0.92 ± 0.08, 5FO/CH: 1.12 ± 0.12, $p<0.05$). The liver size in the SO/CH group had enlarged, and the entire surface had a pale color, suggestive of increased lipid storage. In contrast, the livers of the 2FO/CH and 5FO/CH groups were less pale and had a normal reddish appearance (data not shown). The hepatic tissues showed numerous lipid droplets in the livers of SO/CH group. However, these lipid droplets were markedly decreased in the 2FO/CH and 5FO/CH groups (Figure 2A). These results in the microscopic images of hepatic tissue were confirmed in hepatic lipid content reductions. Hepatic TG levels in both 2FO/CH and 5FO/CH group significantly decreased to about 50% of that in the SO/CH group. Hepatic TC levels also decreased by about 40% in both 2FO/CH and 5FO/CH group compared with the SO/CH group(Figure 2B). Fabbrini suggested that hepatic lipid accumulation is considered a risk factor for fatty liver and steatohepatitis, which promote the development



H&E-stained liver sections (A), total cholesterol (TC) and triglycerides (TG) (B) in mice fed SO, SO/CH, 2FO, 2FO/CH, 5FO, and 5FO/CH for 8. Values represent means ± S.D. (n=5). Means with different letters are different at the $p<0.05$ level by Fisher's protected least significant difference (PLSD) test.

Fig. 2. Liver tissue histology and lipid levels of 16-week-old male C57BL/6J mice.

of insulin resistance, dyslipidemia, and cardiovascular disease (Fabbrini, 2010). In this study, lipid deposition caused by high cholesterol feeding significantly decreased in the 2FO/CH and 5FO/CH groups, in which low dose fish oil was used as the lipid source. We confirmed that the 2 en% fish oil exerts ameliorating effects on hepatic lipid accumulation due to dietary cholesterol consumption in male C57BL/6J mice.

2.3 The hepatic mRNA levels of lipid metabolism-regulating genes modfied with the fish oil or cholesterol feeding in male C57BL/6J mice

The hepatic mRNA levels of lipid metabolism-regulating genes are shown in Table 1. The mRNA levels of SREBP-1c, which is transcription factor of genes related to lipogenesis, were not significantly changed in all groups. Low dose fish oil or cholesterol feeding did not particularly affect in FAS mRNA. However, SCD1 mRNA levels significantly decreased in the fish oil groups regardless of the addition the addition of cholesterol, compared with the SO/CH group. Previous studies showed that fish oil feeding decreases SREBP1c mRNA expression and/or mature protein production and results in the inhibition of SREBP1 target genes, such as ACC, FAS, and SCD-1 (Kim, 1999; Nakatani, 2002). In this study, different results were observed, suggesting that the inhibitory effect of fish oil on fatty acid biosynthesis is more clear at high dose fish oil.

	SO	SO/CH	2FO	2FO/CH	5FO	5FO/CH
SREBPs						
SREBP-1c	1.00 ± 0.31	1.27 ± 0.45	0.96 ± 0.56	1.21 ± 0.99	0.88 ± 0.29	0.81 ± 0.55
SREBP-2	1.00 ± 0.51^{c}	0.95 ± 0.06^{c}	1.88 ± 0.19^{a}	0.93 ± 0.14^{c}	1.41 ± 0.32^{b}	0.71 ± 0.12^{c}
Insig-1	1.00 ± 0.76^{bc}	1.44 ± 0.75^{abc}	2.42 ± 1.50^{a}	0.89 ± 0.52^{bc}	1.87 ± 0.57^{ab}	0.61 ± 0.20^{c}
Fatty acid biosynthesis						
FAS	1.00 ± 0.84^{a}	0.81 ± 0.58^{ab}	0.98 ± 0.39^{a}	0.58 ± 0.24^{ab}	0.85 ± 0.23^{ab}	0.32 ± 0.19^{b}
SCD1	1.00 ± 0.33^{ab}	1.46 ± 0.73^{a}	0.43 ± 0.32^{c}	0.82 ± 0.38^{bc}	0.35 ± 0.08^{c}	0.63 ± 0.19^{bc}
Cholesterol homeostasis						
HMGCo(A) Reductase	1.00 ± 0.30^{a}	0.67 ± 0.15^{bc}	0.85 ± 0.37^{ab}	0.66 ± 0.03^{bc}	1.07 ± 0.23^{a}	0.54 ± 0.10^{c}
ABCG5	1.00 ± 0.57^{c}	2.61 ± 0.29^{a}	0.94 ± 0.15^{c}	1.68 ± 0.25^{b}	0.81 ± 0.13^{c}	1.41 ± 0.18^{b}
ABCG8	1.00 ± 0.32^{d}	1.54 ± 0.32^{a}	1.25 ± 0.29^{cd}	2.18 ± 0.72^{ab}	0.95 ± 0.15^{d}	1.95 ± 0.43^{bc}
Fatty acid β-oxidation						
PPARα	1.00 ± 0.30^{b}	1.74 ± 0.74^{ab}	2.51 ± 1.03^{a}	2.32 ± 0.68^{a}	1.93 ± 0.49^{a}	1.97 ± 0.77^{a}
AOX	1.00 ± 0.49^{c}	1.54 ± 0.61^{bc}	1.66 ± 0.39^{b}	1.79 ± 0.43^{abc}	1.82 ± 0.33^{ab}	2.42 ± 0.66^{a}
UCP2	1.00 ± 0.32^{ab}	1.54 ± 0.32^{a}	0.99 ± 0.74^{ab}	1.19 ± 0.68a^{b}	0.70 ± 0.13^{b}	1.05 ± 0.38^{ab}
Bile acid bioynthesis						
CYP7A1	1.00 ± 0.57^{c}	3.12 ± 0.79^{ab}	1.27 ± 1.17^{c}	1.96 ± 0.80^{bc}	1.43 ± 0.81^{c}	3.63 ± 1.01^{a}
CYP8B1	1.00 ± 0.45^{b}	1.26 ± 0.29^{ab}	1.12 ± 0.47^{ab}	1.33 ± 0.32^{ab}	1.01 ± 0.19^{b}	1.55 ± 0.58^{a}

The mRNA expression levels in liver of male C57BL/6J mice fed SO, SO/CH, 2FO, 2FO/CH, 5FO, and 5FO/CH for 8 weeks. Values represent means ± S.D. (n=4-5). Means with different letters are different at the $p<0.05$ level by Fisher's protected least significant difference (PLSD) test.

Table 1. Expression of genes associated with lipid metabolism in the liver

The mRNA levels of PPARα and AOX, genes involved in fatty acid oxidation, were significantly higher in the fish oil groups regardless of the addition of cholesterol. However, the mRNA levels of UCP-2, which is involved in heat production, were unaffected by low dose fish oil or cholesterol feeding. The mRNA levels of ABCG5 and ABCG8, genes involved in cholesterol transport into the bile, were significantly induced with the cholesterol addition . Biliary cholesterol excretion increases due to the upregulation of these genes (Repa, 2002). These increases in ABCG5 and ABCG8 mRNA by cholesterol feeding are

crucial for the maintenance of cholesterol homeostasis. Indeed, the mRNA levels of CYP7A1, the rate-limiting gene in bile acid synthesis, were significantly increased in all cholesterol-supplemented groups. However, no such increases were observed in the expression of CYP8B1 mRNA. The synthesis of bile acid in the liver is important in the catabolic pathway of cholesterol. The bile acid systhesis is controlled by CYP7A1 (Berge, 2000). In this study, the mRNA levels of CYP7A1 were not significantly affected by fish oil feeding, but its levels significantly increased when cholesterol was added. This is consistent with previous observation that CYP7A1 mRNA level is increased on the addition of cholesterol to maintain the homeostasis of cholesterol.

3. Conclusion

Body weight gains and adipose tissue weights did not change with the low dose fish oil feeding in male C57BL/6J mice. Hepatic triglyceride and total cholesterol levels of the SO/CH group were dramatically increased compared to the SO group. However, in 2FO/CH and 5FO/CH groups, the hepatic lipids were significantly decreased compared to the SO/CH group. Low dose fish oil or cholesterol feeding did not particularly affect in SREBP-1C and FAS mRNA levels. But, PPARα and AOX mRNA levels were significantly higher in the fish oil groups regardless of the cholesterol addition. The present study indicates that low dose fish oil obviously improved the hepatic lipid accumulation by high cholesterol diet. And, this improving effect is partly due to the fatty acid degradation, which was facilitated by increased expression of fatty acid oxidation-related genes, such as AOX.

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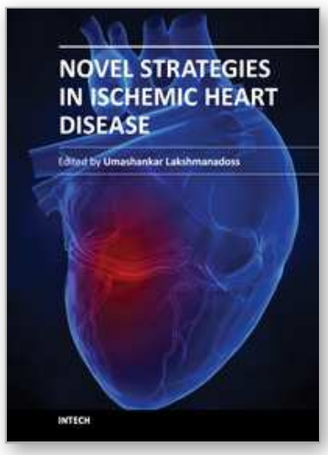

Novel Strategies in Ischemic Heart Disease

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