



Original Article

Effects of fish oil-enriched Sasa-kamaboko diet in decreasing hepatic lipid levels in a mouse fatty liver disease model

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ABSTRACT — The influence of fish oil-enriched Sasa-kamaboko (a Japanese processed seafood) diet on lipid metabolism was investigated using mice lacking farnesoid X receptor (FXR) as a fatty liver disease model. Sasa-kamaboko (SK) was made from Alaska pollock surimi (fish paste), enriched with fish oil (0%, 2.5%, or 5.0%) and then freeze-dried. *Fxr*-null mice were fed the dried SK, mixed with AIN-93M chow in the ratio 1:1 (50% SK diet) or 1:3 (25% SK diet), for 4 weeks. Hepatic triglyceride levels, and total cholesterol levels were significantly decreased, which were dependent on the amount of added fish oil in 25% and 50% SK diets. Hepatic fatty acid synthase (Fas), acetyl-CoA carboxylase 1 (Acc1), and stearoyl CoA desaturase 1 (Scd1) mRNA levels and Fas protein levels were decreased in groups fed fish oil-enriched 50% SK diets. Brain and serum non-esterified fatty acid levels were significantly decreased in the groups fed fish oil-enriched 50% SK diet, whereas brain and serum, (but not hepatic) phospholipid levels were significantly increased in the groups. Hepatic, serum and brain n-3 polyunsaturated fatty acid, docosahexaenoic acid (DHA; 22:6 n-3), and eicosapentaenoic acid (EPA; 20:5 n-3) levels (except for brain EPA levels) were significantly increased in groups fed fish oil-enriched 50% SK diet, whereas hepatic and serum mono-unsaturated fatty acid, oleic acid (18:1 n-9), and palmitoleic acid (16:1 n-7) levels were significantly decreased in the groups. These results suggest that a diet containing fish oil-enriched SK enhances hepatic lipid-lowering through the alteration of hepatic fatty acid composition in a fatty liver disease mouse model.

Key words: Fatty liver disease, Fish oil, *Fxr*-null mice, Triglyceride, Functional food, Accumulation

INTRODUCTION

Kamaboko is a type of processed seafood product common in Japanese surimi (fish paste) product (Hall and Ahmad, 1997). Sasa-kamaboko (SK), a variety of kamaboko evolved in Sendai city more than a century ago, consists of a minced fillet of white fish (Alaska pollock) formed in the shape of a bamboo leaf and then baked into a fish cake. Fish oil contains an abundance of n-3 poly-

unsaturated fatty acids (n-3 PUFAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). As mammals cannot synthesize n-3 PUFAs, fatty acids must be derived from exogenous sources. These fatty acids perform different physiological functions, which include a regulation of lipid metabolism and inflammation through the activation of nuclear receptors and transcriptional factors. Several studies have reported the beneficial effects of n-3 PUFA supplementation on triglyceridemia, blood

pressure, inflammation, and insulin sensitivity (Sekiya *et al.*, 2003; Delarue *et al.*, 2006; Calder, 2012; Di Minno *et al.*, 2012). Moreover, recent studies reported that n-3 PUFAs could prevent non-alcoholic fatty liver disease (NAFLD) (Monteiro *et al.*, 2014; Jump *et al.*, 2015; Delarue and Lalles, 2016) as they possessed the lowering effect because of the upregulation of lipid oxidation through an activation of peroxisome proliferator-activated receptor alpha (PPAR α). Moreover, n-3 PUFAs have the ability to prevent the NAFLD using a downregulation of lipid synthesis through a suppression of the nuclear abundance of activated sterol regulatory element-binding protein 1c (SREBP-1c) (Worgall *et al.*, 1998).

Fish oil-enriched SK has been developed from high-grade Alaska pollock surimi to produce high-quality functional food, taking advantage of beneficial fish oil components, such as DHA and EPA. In addition, dietary fish proteins, such as Alaska pollock, have a lipid-lowering effect (Venugopal and Shahidi, 1995; Wergedahl *et al.*, 2004). Thus, SK alone is likely to have a lipid-lowering effect without the addition of fish oil.

We compared lipid lower effects between oil-enriched fish SK and SK alone using farnesoid X receptor (FXR)-null mice as a fatty liver disease model. *Fxr*-null mice developed hepatic steatosis and chronic inflammation. They displayed significant elevated levels of hepatic bile acids in livers, developing triglycerides, non-esterified fatty acids (NEFAs), and cholesterol (Sinal *et al.*, 2000). Furthermore, hepatomegaly and elevated hepatic damage-associated diagnostic markers, such as serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP), are observed in *Fxr*-null mice (Sinal *et al.*, 2000). Hence, *Fxr* deficient mice are the suitable model for NAFLD. On the other hand, (Miyata *et al.*, 2017), *Fxr*-null mice fed an AIN-93M fish oil-substituted diet demonstrate a decrease in hepatic damage diagnostic markers, hepatic and serum triglyceride and total cholesterol levels.

In the present study, we aimed to evaluate the influence of fish oil-enriched SK feeding in a mouse fatty liver disease model (*Fxr*-null mice). To mimic the dietary intake, fish oil-enriched SK was freeze-dried and mixed with AIN-93M diet in the ratios 1:1 (50% SK diet) and 1:3 (25% SK diet). *Fxr*-null mice fed these diets for 4 weeks displayed a decrease in hepatic triglyceride and total cholesterol levels. Their tissue fatty acid composition was altered.

MATERIALS AND METHODS

Materials

Fxr-null mice were provided by Dr Frank J. Gonzalez (National Institute of Health, Bethesda, MD) (Sinal *et al.*, 2000). Fish oil (DHA-22k) which was prepared from tuna and bonito, was provided by Maruha Nichiro Corp. (Tokyo, Japan) (Miyata *et al.*, 2016). DHA and EPA contents were 26.7% and 6.7%, respectively. SK and fish oil-enriched SK from Alaska pollock surimi was provided by Maruyo Suzuki Company (Siogama, Japan). Fish oil was mixed with Alaska pollock surimi in the ratio 1:40 (2.5% fish oil-enriched SK) or ratio 1:20 (5% fish oil-enriched SK). The SK nutritional composition was: protein 12.0%, lipid 0.1%, hydrocarbon 12.8%, water 73.1%, and ash 2.0%. The nutritional composition of 5% fish oil-enriched SK was: protein 12.6%, lipid 1.7%, hydrocarbon 13.6%, water 69.9%, and ash 2.2%. The 3 varieties of SK (0%, 2.5% and 5%) were freeze-dried, and then each was mixed with AIN-93M diet (Oriental Yeast Co. Ltd., Tokyo, Japan) in the ratio 1:1 (50% SK diet) or ratio 1:3 (25% SK diet) by weight, producing 6 different diets.

Animal treatment, sample collection, and histological analysis

Fxr-null mice were housed under a standard 12-hr light–dark cycle (9 a.m.–9 p.m.). Before performing the experiments, the mice were fed standard rodent chow (MF; Oriental Yeast Co. Ltd., Tokyo, Japan) and water *ad libitum*. Age-matched groups female mice of 10–12-week-old were used for the experiments. Our previous study demonstrated that female *Fxr*-null mice were more sensitive to changes in lipid metabolism than males. After 4 weeks of feeding with the SK diets, the mice were euthanized between 9 a.m. and 11 a.m. All the experiments were performed in accordance with the National Fisheries University (Shimonoseki, Japan) animal experiments guidelines. The protocol was approved by the Institutional Animal Care and Use Committee of National Fisheries University (Permission No. 2017-17-2).

Determination of hepatic damage-associated diagnostic markers and hepatic lipid levels

ALT and ALP activities were measured using the commercial kits, Transaminase CII-B-test Wako for ALT and Alkali-phospha B-test Wako for ALP (Wako Pure Chemicals, Osaka, Japan). Hepatic samples were prepared as described previously (Miyata *et al.*, 2010). Hepatic triglyceride, NEFA, total cholesterol and phospholipids were determined using the Triglyceride E-test

Wako, NEFA E-test Wako, Cholesterol E-test Wako and Phospholipid C-test Wako (Wako Pure Chemicals), respectively.

Determination of mRNA levels

Hepatic total RNA was isolated using the acid guanidine–phenol–chloroform method. Single-strand cDNA was synthesized using an oligo (dT) primer and a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The cDNA templates were used for real-time quantitative polymerase chain reaction (qPCR) using SYBR Premix Ex Taq™ II (Tli RNaseH Plus) (Takara Bio, Otsu, Japan) with the TP870 Thermal Cycler Dice Real Time System (Takara Bio). The relative mRNA levels were calculated by the comparative threshold cycle method. The following specific forward and reverse primers were used for real-time qPCR: *β-actin*, sense, 5'-ACCCTGTGCTGCTCACCGA-3' and antisense, 5'-CTGGATGGCTACGTAGACACC-3'; *stearoyl CoA desaturase 1 (Scd1)*, sense, 5'-AGTTAACCCTGAATGCGAGG-3' and antisense, 5'-GAGGTCCATTAGCACTTGCC-3'; *hydroxymethylglutaryl-CoA reductase (Hmgcr)*, sense, 5'-CTGCACCATGCCATCTATAG-3' and antisense, 5'-GACAATTCCTCCAGCCATTAC-3'; *hydroxymethylglutaryl-CoA synthase (hmgcs)*, sense, 5'-GACATTGCTATATATGCCACAGGA-3' and antisense, 5'-CAGGGCCACAGCTC-CAACT-3'; *acetyl-CoA carboxylase 1 (Acc1)*, sense, 5'-ACAGTGAAGGCTTACGTCTG-3' and antisense, 5'-TGTGGACGATGGAGTCCATG-3'; *fatty acid synthase (Fas)*, sense, 5'-CGGTCTGGAAAGCTGAAGGATC-3' and antisense, 5'-CGGAGTGAGGCTGGGTTGA-3'.

Western blot analysis

The hepatic S9 fractions were prepared as described previously (Miyata *et al.*, 2011). Rabbit anti-Fas antibody (H300: Santa Cruz Biotechnology) and monoclonal anti- β -actin antibody (AC-15: Sigma-Aldrich, St. Louis, MO) were used as primary antibodies. Goat anti-mouse IgG (H + L), horseradish peroxidase-conjugated (Thermo Scientific, Waltham, MA), goat anti-rabbit IgG (whole molecule), horseradish peroxidase-conjugated (Sigma-Aldrich) were used as secondary antibodies. Detection of immunoblot signaling was performed using Clarity Western ECL Substrate (Bio-Rad, Hercules, CA) and ChemiDoc XRS Plus (Bio-Rad).

Fatty acid analysis

Hepatic, brain, serum and SK diet lipids were extracted by the Folch method. Fatty acid methyl esters were

prepared using BF₃-methanol alkylation reagent (Merck, Darmstadt, Germany). The fatty acid composition was analyzed using gas chromatography (GC-2014: Shimadzu Corporation, Kyoto, Japan) with a TC-FFAP capillary column (30 m × 0.25 mm) (GL Sciences Inc., Tokyo, Japan). Methyl esters were identified by comparison with the retention times of standard fatty acids (Miyata *et al.*, 2016).

Statistical analysis

Data are presented as means ± S.D. or mean ± S.E. In animal experiments, the statistical significance was analyzed using one-way ANOVA followed by the Dunnett's method using the Excel software (Social Survey Research Information Co. Ltd., Tokyo, Japan) for evaluating differences between the mean values of each group. Statistical significance was established at $p < 0.05$.

RESULTS

SK diet and SK diet-fed mice

SK weight decreased to less than 33% by freezing dry. Each dried SK was mixed with AIN-93M diet by the ratio of 1 to 1 (50% SK diet) or 1 to 3 (25% SK diet). The DHA concentration of the SK diets was measured by gas chromatography. The 50% control SK diet (0% fish oil) contained 1.56 mg DHA g⁻¹ (Fig. 1). Significant 2-fold and 3-fold increases in DHA concentration were observed in the 2.5% and 5% fish oil-enriched SK diets, respectively. At the end of 4 weeks, no significant differences in body weight gain and food intake were observed between any of the diet groups of the *Fxr*-null mice. The activities

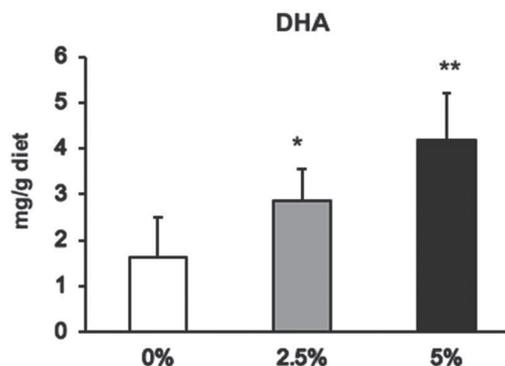


Fig. 1. DHA concentration in SK diets. DHA concentrations in the 50% SK diets were measured by gas chromatography. Values are presented as mean ± S.E. (n = 3). Significant differences (* $p < 0.05$; ** $p < 0.01$) were assessed by Dunnett's test. 0%, 2.5% and 5% indicate added ratios of fish oil to Alaska pollock surimi.

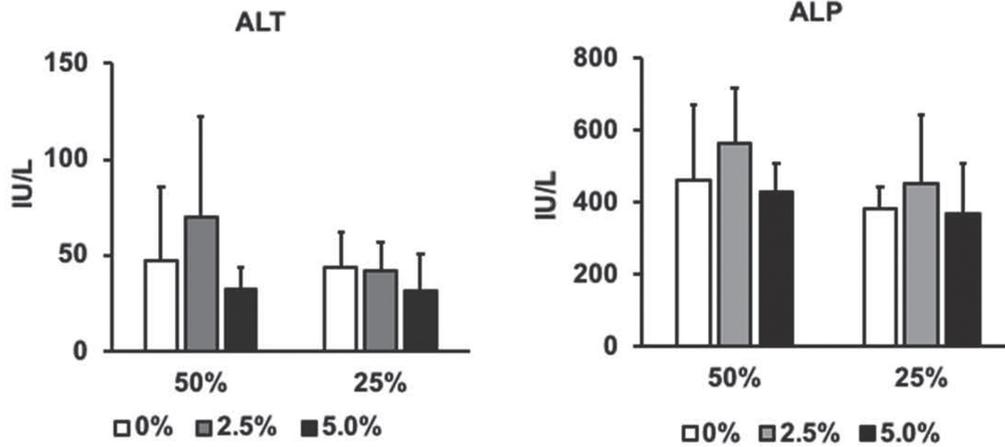


Fig. 2. Hepatic diagnostic damage markers in *Fxr*-null mice fed SK diets. *Fxr*-null mice were fed 50% or 25% SK diets for 4 weeks. Values are presented as mean \pm S.D. (n = 6). 0%, 2.5% and 5% indicate added ratios of fish oil to Alaska pollock surimi.

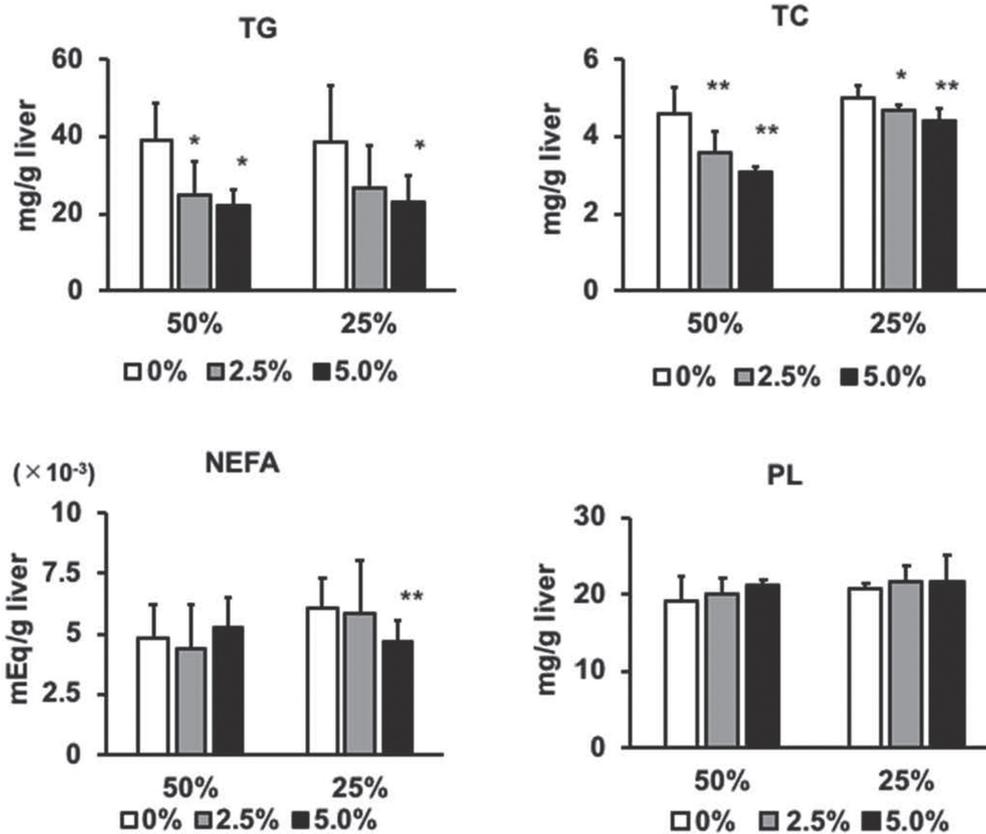


Fig. 3. Hepatic lipid levels in *Fxr*-null mice fed SK diets. *Fxr*-null mice were fed 50% or 25% SK diets for 4 weeks. Values are presented as mean \pm S.D. (n = 6). Significant differences (* p < 0.05; ** p < 0.01) were assessed by Dunnett's test. 0%, 2.5% and 5% indicate added ratios of fish oil to Alaska pollock surimi.

Effects of fish oil addition food

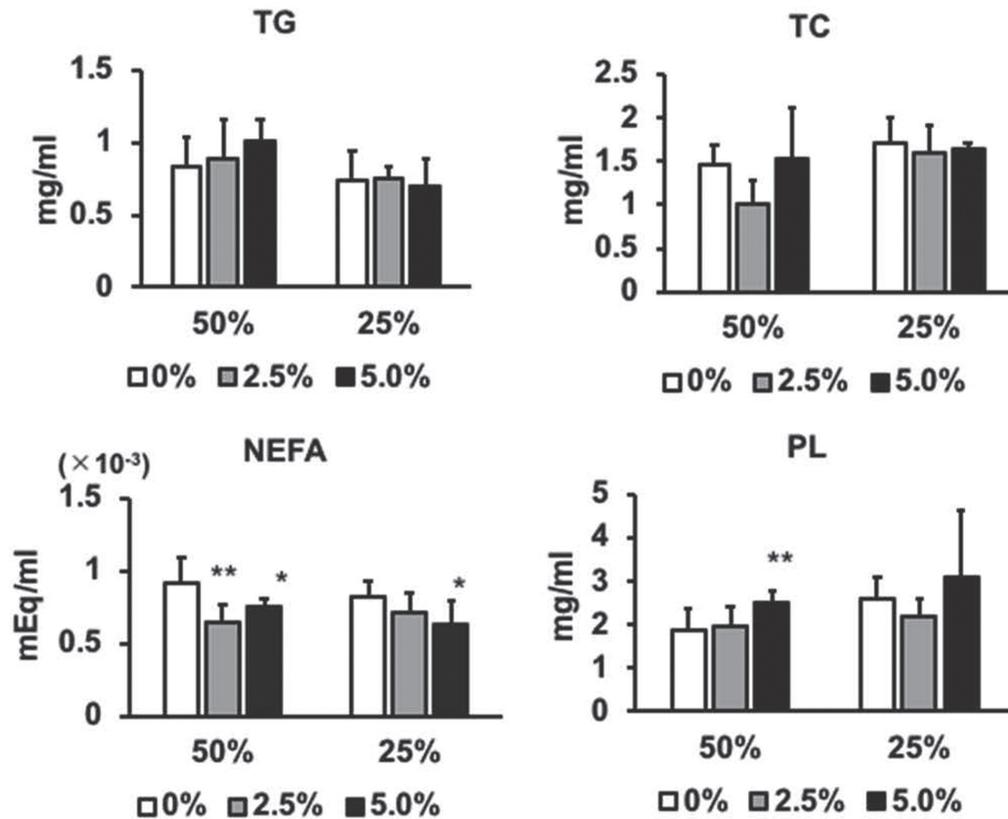


Fig. 4. Serum lipid levels in *Fxr*-null mice fed SK diets. *Fxr*-null mice were fed 50% or 25% SK diets for 4 weeks. Values are presented as mean \pm S.D. (n = 6). Significant differences (* p < 0.05; ** p < 0.01) were assessed by Dunnett's test. 0%, 2.5% and 5% indicate added ratios of fish oil to Alaska pollock surimi.

of ALT and ALP (hepatic damage-associated diagnostic markers) were not decreased in groups receiving the fish oil-enriched SK diets (2.5% and 5.0%) (Fig. 2).

Changes in lipid levels in liver, serum and brain

We measured lipid levels in liver, brain and serum of *Fxr*-null mice fed SK diets. Significant decreases in hepatic triglyceride and total cholesterol levels were observed in *Fxr*-null mice fed the fish oil-enriched SK diets (Fig. 3). Decreases in hepatic total cholesterol levels for fish oil-enriched diets were more clearly observed in *Fxr*-null mice fed the 50% SK diet than in those fed the 25% SK diet, whereas the decreases in hepatic triglyceride levels were similar in both diets. Significant decreases in hepatic NEFA levels were observed in *Fxr*-null mice fed the 25% SK diet enriched with fish oil (5%), whereas no significant changes in hepatic levels were observed in fish oil groups. No significant decreases in serum triglyceride and total cholesterol levels were observed in fish oil groups, whereas a significant decrease in serum NEFA

levels and a significant increase in serum phospholipid levels were observed (Fig. 4). The alteration of brain lipid levels due to the addition of fish oil was similar to that of serum lipid levels (Fig. 5). Brain NEFA and phospholipid levels, (but not triglyceride and total cholesterol levels) varied significantly in the mice fed the fish oil-enriched 50% SK diet.

Expression levels of hepatic lipid synthesis enzymes

We measured the expression levels of hepatic fatty acid and cholesterol synthesis enzymes to identify the mechanism for the reduction in hepatic triglyceride and total cholesterol levels in the fish oil groups. The hepatic mRNA levels of fatty acid synthase (Fas, Acc1, and Scd1) were clearly decreased in fish oil-enriched diet groups, whereas the hepatic mRNA levels of cholesterol synthase (Hmgcr and Hmgcs) were not significantly decreased (Table 1). Consistent with the change in the mRNA levels, hepatic Fas protein levels were significant-

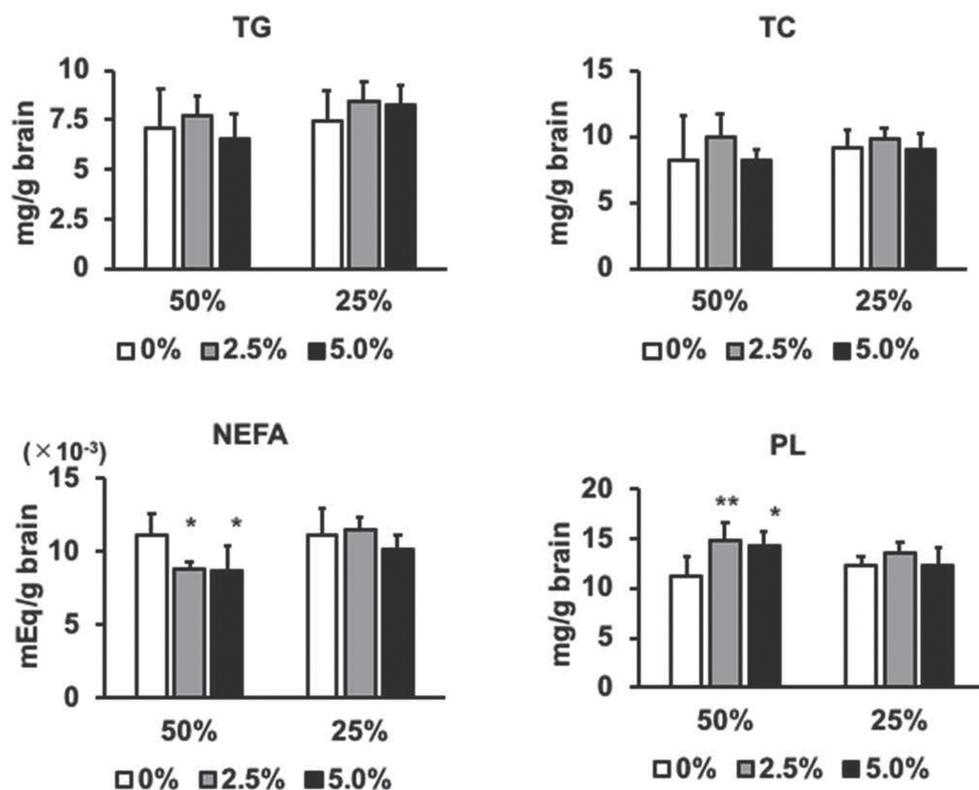


Fig. 5. Brain lipid levels in *Fxr*-null mice fed SK diets. *Fxr*-null mice were fed 50% or 25% SK diets for 4 weeks. Values are presented as mean \pm S.D. ($n = 6$). Significant differences ($*p < 0.05$; $**p < 0.01$) were assessed by Dunnett's test. 0%, 2.5% and 5% indicate added ratios of fish oil to Alaska pollock surimi.

Table 1. Hepatic mRNA levels of lipid synthetic genes.

	0%	2.5%	5.0%
Fas	1.00 \pm 0.85	0.34 \pm 0.31*	0.30 \pm 0.27*
Scd1	1.00 \pm 0.65	0.17 \pm 0.15*	0.13 \pm 0.10*
Acc1	1.00 \pm 0.33	0.52 \pm 0.35*	0.40 \pm 0.21*
Hmgcr	1.00 \pm 1.25	0.95 \pm 1.06	0.51 \pm 0.74
Hmgcs	1.00 \pm 1.22	1.10 \pm 1.01	0.83 \pm 1.25

Fxr-null mice were fed 50% SK diets for 4 weeks. The mRNA levels were normalized to that of β -actin, and the mRNA levels of *Fxr*-null mice of 0% group were set at 1. 0%, 2.5% and 5% indicate added ratios of fish oil to Alaska pollock surimi. Values are presented as mean \pm S.D. ($n = 6$). Significant differences were assessed by the Dunnett's test ($*p < 0.05$ vs. 0% group). Fas, fatty acid synthase; Scd1, stearoyl CoA desaturase 1; Acc1, acetyl-CoA carboxylase 1; Hmgcr, hydroxymethylglutaryl-CoA reductase; Hmgcs, hydroxymethylglutaryl-CoA synthase.

ly decreased in fish oil-enriched diet groups (Fig. 6).

Changes in fatty acid composition among liver, brain and serum

We explored the alteration of liver, serum and brain fatty acid composition due to the addition of fish oil. DHA (22:6 n-3) levels were significantly increased in liver, serum, and brain of fish oil-enriched diet groups

(Fig. 7), whereas EPA (20:5 n-3) levels were significantly increased in liver and serum, but not in the brain. Brain EPA levels were extremely low compared to DHA levels. On the other hand, levels of the n-6 PUFA arachidonic acid (20:4 n-6) were not changed in any of the tissues (Table 2). Levels of the mono-unsaturated fatty acids oleic acid (18:1n-9) and palmitoleic acid (16:1n-7) were significantly decreased in liver and serum, but not in the

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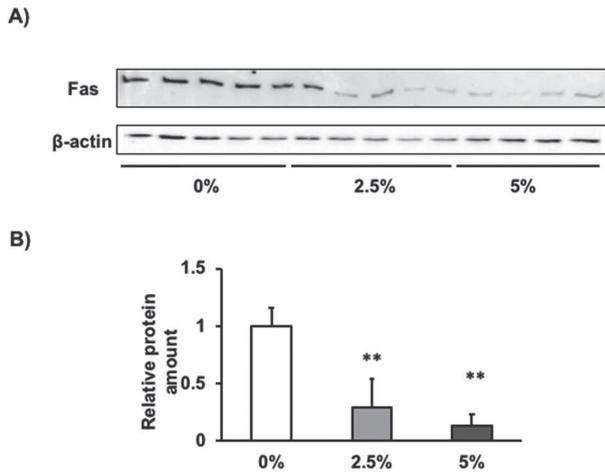


Fig. 6. Hepatic expression levels of Fas protein in *Fxr*-null mice fed SK diets. *Fxr*-null mice were fed 50% SK diets for 4 weeks. Values are presented as mean \pm S.D. (n = 4 or 5). Significant differences ($*p < 0.05$; $**p < 0.01$) were assessed by Dunnett's test. 0%, 2.5% and 5% indicate added ratios of fish oil to Alaska pollock surimi.

brain. Levels of the saturated fatty acids palmitic acid (16:0) and stearic acid (18:0) were significantly increased in the brain, but not in liver and serum. The alteration in each fatty acid level was similar in liver and serum, while alterations of levels in the brain were somewhat different.

DISCUSSION

The present study demonstrated that the addition of fish oil to SK enhanced a reduction of hepatic triglycerides and total cholesterol and altered tissue fatty acid composition. The lowering effect of hepatic lipids due to the addition of fish oil to SK was observed in the 25% SK diet. These results indicate that the addition of fish oil to surimi-based products is useful for the development of high-quality functional foods.

The fish oil-enriched SK diets contained higher levels of triglyceride than the control SK diet, yet hepatic triglyceride levels decreased in *Fxr*-null mice fed fish oil-enriched SK diets. A fish oil-enriched SK diet likely decreases hepatic fatty acid synthesis. The present study demonstrated that hepatic protein levels of fatty

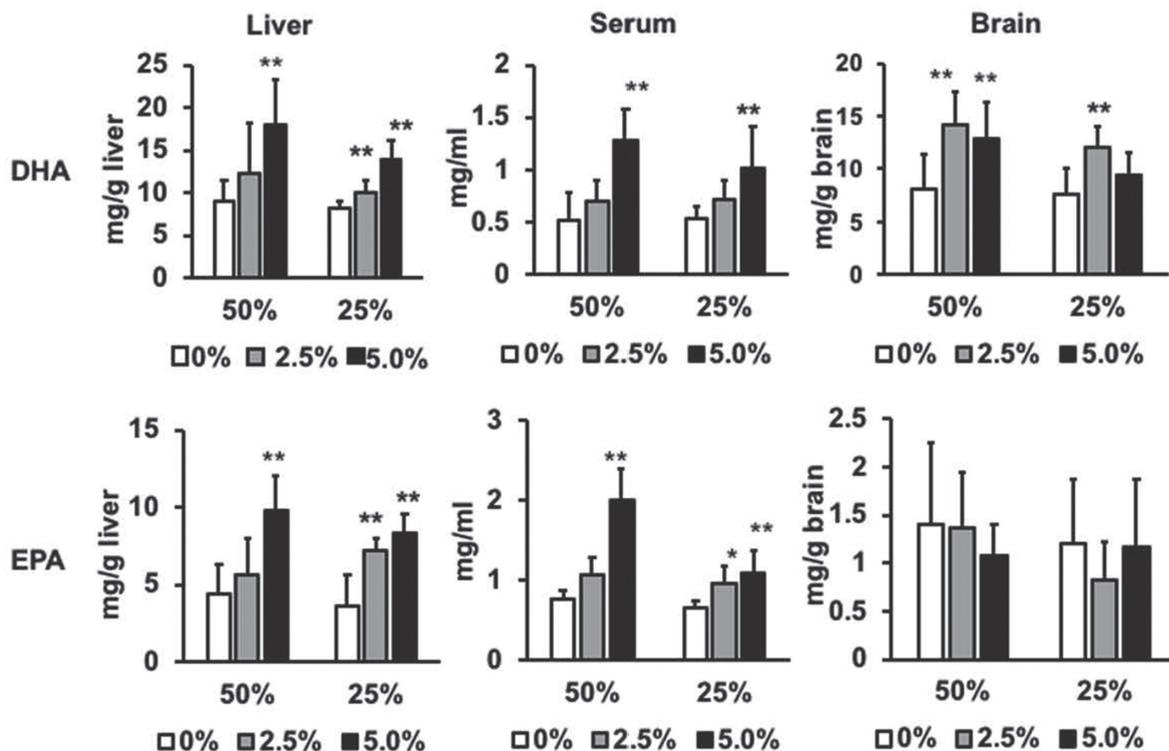


Fig. 7. Hepatic, serum and brain DHA and EPA concentrations in *Fxr*-null mice fed SK diets. *Fxr*-null mice were fed 50% or 25% SK diets for 4 weeks. Values are presented as mean \pm S.D. (n = 6). Significant differences ($*p < 0.05$; $**p < 0.01$) were assessed by Dunnett's test. 0%, 2.5% and 5% indicate added ratios of fish oil to Alaska pollock surimi.

Table 2. Relative contents of each fatty acid in liver, serum and brain.

Liver	0%	2.5%	5%
Palmitic acid	1.00 ± 0.54	0.89 ± 0.34	1.02 ± 0.16
Stearic acid	1.00 ± 0.23	0.88 ± 0.37	1.17 ± 0.24
Palmitoleic acid	1.00 ± 0.82	0.20 ± 0.09*	0.24 ± 0.12*
Oleic acid	1.00 ± 0.35	0.39 ± 0.26**	0.41 ± 0.26**
Arachidonic acid	1.00 ± 0.27	0.61 ± 0.23*	0.86 ± 0.17
Eicosapentaenoic acid	1.00 ± 0.45	1.29 ± 0.53	2.24 ± 0.50**
Docosahexaenoic acid	1.00 ± 0.28	1.37 ± 0.57	2.02 ± 0.58**
Serum			
Palmitic acid	1.00 ± 0.60	0.87 ± 0.14	0.96 ± 0.06
Stearic acid	1.00 ± 0.57	0.74 ± 0.14	0.95 ± 0.12
Palmitoleic acid	1.00 ± 0.36	0.65 ± 0.19	0.59 ± 0.08*
Oleic acid	1.00 ± 0.61	0.57 ± 0.18*	0.53 ± 0.03*
Arachidonic acid	1.00 ± 0.41	0.72 ± 0.20	1.42 ± 0.33
Eicosapentaenoic acid	1.00 ± 0.07	0.66 ± 0.14	1.25 ± 0.24**
Docosahexaenoic acid	1.00 ± 0.54	1.37 ± 0.40	2.52 ± 0.59**
Brain			
Palmitic acid	1.00 ± 0.23	1.30 ± 0.13*	1.24 ± 0.29*
Stearic acid	1.00 ± 0.26	1.43 ± 0.35*	1.44 ± 0.28*
Palmitoleic acid	1.00 ± 0.13	0.59 ± 0.38	0.51 ± 0.39
Oleic acid	1.00 ± 0.21	1.53 ± 0.11**	1.20 ± 0.41
Arachidonic acid	1.00 ± 0.34	1.48 ± 0.25*	1.34 ± 0.43
Eicosapentaenoic acid	1.00 ± 0.60	0.96 ± 0.42	0.77 ± 0.22
Docosahexaenoic acid	1.00 ± 0.40	1.75 ± 0.38**	1.58 ± 0.43*

Fxr-null mice were fed 50% SK diets for 4 weeks. The fatty acid levels of *Fxr*-null mice of 0% group were set at 1. Values are presented as mean ± S.D. (n = 6). Significant differences were assessed by Dunnett's test (* $p < 0.05$; ** $p < 0.01$ vs. 0% group). 0%, 2.5% and 5% indicate added ratios of fish oil to Alaska pollock surimi.

acid synthase (Fas) were decreased in fish oil-enriched diet groups. DHA and EPA are the principal natural ligands for PPAR α , and increases in hepatic DHA and EPA levels could activate hepatic PPAR α signaling, resulting in the stimulation of hepatic β -oxidation of fatty acids (Di Minno *et al.*, 2012; Jump *et al.*, 2005; Nakamura *et al.*, 2014).

The addition of fish oil to the diet decreased hepatic triglycerides and total cholesterol levels in *Fxr*-null mice, whereas this elevated brain and serum (but not hepatic) phospholipid levels. Overall, a fish oil-enriched diet is likely to reduce the triglyceride levels and elevates phospholipid levels in the body. Fatty acids in triglycerides derived from fish oil might be incorporated into brain and serum phospholipids. Phospholipids are the main components of cell membranes and are involved in the transport of several lipids, such as cholesterol and triglycerides (Yang *et al.*, 2018). Increases in brain phospholipid levels might lead to the alteration of brain function.

The addition of fish oil to the diet elevated brain DHA levels in *Fxr*-null mice fed SK diets. Because brain DHA is involved in the activation of neural cells (Echeverria *et al.*, 2017; Ghasemi Fard *et al.*, 2018), these data raise the

possibility that a diet containing fish oil-enriched SK activates brain function. On the other hand, the fish oil-enriched SK diets elevated hepatic and serum EPA (but not brain EPA) levels. Increases in serum EPA levels were not reflected in brain EPA levels. Furthermore, brain EPA levels were much lower than brain DHA levels. Rodent studies have suggested that the brain maintains the EPA low levels through extremely rapid rates of EPA β -oxidation and elongation and desaturation to docosapentaenoic acid and DHA (Igarashi *et al.*, 2013; Chen and Bazinet, 2015). EPA does not seem to be heavily recycled within brain phospholipids and triglycerides. In the liver, the addition of fish oil did not elevate levels of the saturated fatty acids, palmitic acid (16:0) and stearic acid (18:0), which caused a reduction in the levels of the mono-unsaturated fatty acids oleic acid and palmitoleic acid although palmitic acid (27.6%) and stearic acid (27.1%) are the main fatty acids in the used fish oil (Miyata *et al.*, 2016). These results raise the possibility that saturated and mono-unsaturated fatty acids are preferential β -oxidized compared to PUFAs. Hepatic and serum fatty acid compositions similarly altered the addition of fish oil, while brain and serum fatty acid compositions responded differently.

Fatty acid metabolism might be largely different between liver and brain (Bazinet and Laye, 2014).

In conclusion, the addition of fish oil to a SK diet lowered the liver lipid levels in a mouse fatty liver disease model. The result demonstrates that a fish oil-enriched SK diet might both prevent and improve fatty liver disease. The present study is expected to contribute to the development of high-quality functional food through the addition of fish oil components.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

- Bazinet, R.P. and Laye, S. (2014): Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat. Rev. Neurosci.*, **15**, 771-785.
- Calder, P.C. (2012): Mechanisms of action of (n-3) fatty acids. *J. Nutr.*, **142**, 592S-599S.
- Cardoso, C., Afonso, C. and Bandarra, N.M. (2016): Dietary DHA and health: cognitive function ageing. *Nutr. Res. Rev.*, **29**, 281-294.
- Chen, C.T. and Bazinet, R.P. (2015): beta-oxidation and rapid metabolism, but not uptake regulate brain eicosapentaenoic acid levels. *Prostaglandins Leukot. Essent. Fatty Acids*, **92**, 33-40.
- Delarue, J. and Lalles, J.P. (2016): Nonalcoholic fatty liver disease: roles of the gut and the liver and metabolic modulation by some dietary factors and especially long-chain n-3 PUFA. *Mol. Nutr. Food Res.*, **60**, 147-159.
- Delarue, J., Li, C.H., Cohen, R., Corporeau, C. and Simon, B. (2006): Interaction of fish oil and a glucocorticoid on metabolic responses to an oral glucose load in healthy human subjects. *Br. J. Nutr.*, **95**, 267-272.
- Di Minno, M.N., Russolillo, A., Lupoli, R., Ambrosino, P., Di Minno, A. and Tarantino, G. (2012): Omega-3 fatty acids for the treatment of non-alcoholic fatty liver disease. *World J. Gastroenterol.*, **18**, 5839-5847.
- Echeverria, F., Valenzuela, R., Catalina Hernandez-Rodas, M. and Valenzuela, A. (2017): Docosahexaenoic acid (DHA), a fundamental fatty acid for the brain: new dietary sources. *Prostaglandins Leukot. Essent. Fatty Acids*, **124**, 1-10.
- Ghasemi Fard, S., Wang, F., Sinclair, A.J., Elliott, G. and Turchini, G.M. (2018): How does high DHA fish oil affect health? A systematic review of evidence. *Crit. Rev. Food Sci. Nutr.*, 1-44.
- Hall G.M. and Ahmad N.H. (1997): Surimi and fish-mince products. *Fish Processing Technology*, 74-92.
- Igarashi, M., Chang, L., Ma, K. and Rapoport, S.I. (2013): Kinetics of eicosapentaenoic acid in brain, heart and liver of conscious rats fed a high n-3 PUFA containing diet. *Prostaglandins Leukot. Essent. Fatty Acids*, **89**, 403-412.
- Jump, D.B., Botolin, D., Wang, Y., Xu, J., Christian, B. and Demeure, O. (2005): Fatty acid regulation of hepatic gene transcription. *J. Nutr.*, **135**, 2503-2506.
- Jump, D.B., Depner, C.M., Tripathy, S. and Lytle, K.A. (2015): Potential for dietary omega-3 fatty acids to prevent nonalcoholic fatty liver disease and reduce the risk of primary liver cancer. *Adv. Nutr.*, **6**, 694-702.
- Miyata, M., Kinoshita, Y., Shinno, K., Sugiura, Y. and Harada, K. (2016): Hepatic n-3/n-6 polyunsaturated fatty acid shift improves hepatic steatosis in farnesoid X receptor-null mice. *Fish. Sci.*, **82**, 529-536.
- Miyata, M., Nomoto, M., Sotodate, F., Mizuki, T., Hori, W., Nagayasu, M., Yokokawa, S., Ninomiya, S. and Yamazoe, Y. (2010): Possible protective role of pregnenolone-16 alpha-carbonitrile in lithocholic acid-induced hepatotoxicity through enhanced hepatic lipogenesis. *Eur. J. Pharmacol.*, **636**, 145-154.
- Miyata, M., Sakaida, Y., Matsuzawa, H., Yoshinari, K. and Yamazoe, Y. (2011): Fibroblast growth factor 19 treatment ameliorates disruption of hepatic lipid metabolism in farnesoid X receptor (Fxr)-null mice. *Biol. Pharm. Bull.*, **34**, 1885-1889.
- Miyata, M., Shinno, K., Kinoshita, T., Kinoshita, Y. and Sugiura, Y. (2017): Fish oil feeding reverses hepatomegaly and disrupted hepatic function due to the lack of FXR signaling. *J. Toxicol. Sci.*, **42**, 671-681.
- Monteiro, J., Leslie, M., Moghadasian, M.H., Arendt, B.M., Allard, J.P. and Ma, D.W. (2014): The role of n - 6 and n - 3 polyunsaturated fatty acids in the manifestation of the metabolic syndrome in cardiovascular disease and non-alcoholic fatty liver disease. *Food Funct.*, **5**, 426-435.
- Nakamura, M.T., Yudell, B.E. and Loor, J.J. (2014): Regulation of energy metabolism by long-chain fatty acids. *Prog. Lipid Res.*, **53**, 124-144.
- Sekiya, M., Yahagi, N., Matsuzaka, T., Najima, Y., Nakakuki, M., Nagai, R., Ishibashi, S., Osuga, J., Yamada, N. and Shimano, H. (2003): Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. *Hepatology*, **38**, 1529-1539.
- Sinal, C.J., Tohkin, M., Miyata, M., Ward, J.M., Lambert, G. and Gonzalez, F.J. (2000): Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell*, **102**, 731-744.
- Venugopal, V. and Shahidi, F. (1995): Value-added products from underutilized fish species. *Crit. Rev. Food Sci. Nutr.*, **35**, 431-453.
- Wergedahl, H., Liaset, B., Gudbrandsen, O.A., Lied, E., Espe, M., Muna, Z., Mork, S. and Berge, R.K. (2004): Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion of HDL cholesterol, and lowers acyl-CoA:cholesterol acyltransferase activity in liver of Zucker rats. *J. Nutr.*, **134**, 1320-1327.
- Worgall, T.S., Sturley, S.L., Seo, T., Osborne, T.F. and Deckelbaum, R.J. (1998): Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. *J. Biol. Chem.*, **273**, 25537-25540.
- Yang, Y., Lee, M. and Fairn, G.D. (2018): Phospholipid subcellular localization and dynamics. *J. Biol. Chem.*, **293**, 6230-6240.