— **Regular Article** —

Unsaturated Fatty Acids in Fish Oil Play a Role in Adequate Fat Distribution to Plasma, Liver and White Adipose Tissue

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The components bringing the effects of fish oil on glucose and lipid metabolism are unclear. We used hydrogenated fish oil, which has saturated fatty acids with the same carbon chain lengths as the unsaturated fatty acids in fish oil, to clarify the functions of these unsaturated fatty acids on the improvements in lipid and glucose metabolism in mice. Mice divided into 3 groups were fed different diets: fish oil diet (FO), hydrogenated fish oil diet (HFO), and soybean oil diet (SBO) as a control. Body weight gain and white adipose tissue weight in the HFO and FO groups were significantly decreased compared with those in the SBO group. However, in the HFO group, the triglyceride (TG) levels in plasma were significantly decreased, while the lipids levels in the liver were remarkably increased compared with those in the FO group. Regarding the fatty acid composition in the liver and white adipose tissue in the HFO group, in parallel with the up-regulation of stearoyl-CoA desaturase 1 mRNA, relative amounts of C16 : 1 and C18 : 1 were significantly increased. By contrast, blood glucose levels in the oral glucose tolerance test had not deteriorated in the HFO group. Our results indicate that unsaturated fatty acids in the FO diet decrease lipid levels in the liver and maintain the balance of lipid levels in plasma, liver and white adipose tissue; in addition, in the HFO group, C16 : 1 and C18 : 1 synthesized in the liver and white adipose tissue may improve glucose tolerance and lipid metabolism.

Key words —— fish oil, unsaturated fatty acid, palmitoleic acid, oleic acid

INTRODUCTION

It has been reported that fish oil improves lipid and glucose metabolism. Fish oil decreases body weight[1)] and regulates lipid metabolism-related enzymatic activities,[2–5)] leading to a reduction in triglyceride (TG) and total cholesterol (TC) synthesis in the liver.[6)] Fish oil also affects glucose metabolism in mice.[7,8)] It has been considered that the main components in fish oil inducing these effects are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are mainly contained in fish oil. However, recent studies have indicated that the effects of fish oil on lipid and glucose metabolism are not limited to the effects of DHA and EPA. It has been observed that EPA and DHA do not mimic the physiological activity of fish oil in affecting hepatic fatty acid oxidation in rat[9)]; in addition, no reduction in plasma glucose and no plasma lipid changes were observed in mice fed DHA and EPA, in contrast to those fed fish oil.[10)] These reports indicate the presence of other factors besides DHA and EPA inducing the effects of fish oil.

The nature of oils is different by the length of carbon chain and the number of double bonds. Therefore, to investigate the components inducing the effects of fish oil, we noticed that hydrogenated fish oil (HFO) has saturated fatty acids with the same carbon chain lengths as the unsaturated fatty

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Table 1. Profiles of Dietary Oils

Fatty acid (%)		SBO	FO	HFO
14:0	(Myristic acid)	0.1	3.0	2.9
16:0	(Palmitic acid)	9.7	18.2	22.9
16:1	(Palmitoleic acid)	—	4.2	1.4
18:0	(Stearic acid)	3.7	4.9	26.4
18:1 n-9	(Oleic acid)	25.6	18.8	0.1
18:2 n-6	(Linoleic acid)	50.1	1.3	—
18:3 n-3	(α-Linolenic acid)	7.1	0.8	—
20:0	(Arachidic acid)	0.4	—	12.4
20:4 n-6	(Arachidonic acid)	0.1	2.0	—
20:5 n-3	(EPA)	—	6.8	—
22:0	(Behenic acid)	0.5	—	27.4
22:5 n-6	(Docosapentaenoic acid; DPA)	—	—	—
22:6 n-3	(DHA)	—	22.8	—
S : M : P		15 : 26 : 57	10 : 10 : 14	95 : 2 : 0
n-6/n-3 ratio		7.0	0.1	0
trans-fatty acid (%)		2	< 2	< 2

acids in fish oil.

Present study was designed by using HFO to clarify the functions of unsaturated fatty acids in fish oil responsible for the improvements in lipid and glucose metabolism in mice.

MATERIALS AND METHODS

Animals —— C57BL/6J female mice were obtained from CLEA Japan, Inc. (Tokyo, Japan) at 6 weeks of age; they were given free access to a standard diet pellet (MF; Oriental Yeast, Tokyo, Japan) and water for a week to stabilize the metabolic conditions before the start of the experiments. The mice were maintained at a constant temperature of 23 ± 3°C and humidity of 55 ± 10% with a fixed artificial light cycle (12 hr light and 12 hr dark). All procedures were approved by the Josai University Animal Care and Use Committee and complied with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Diets —— Three different experimental diets containing 40 fat energy percent were used: fish oil diet (FO); extensively hydrogenated fish oil diet (HFO), which has saturated fatty acids with the same carbon chain lengths as unsaturated fatty acids in fish oil; soybean oil diet (SBO) as a control. In the supplementary experiment, four experimental diets were used; the diet consisted of 10 soybean oil energy percent (LF-SBO), SBO, FO substituted 10 energy percent of lipid for soybean oil (FO+SBO), HFO substituted 10 energy percent of lipid for soybean oil (HFO+SBO) to supply essential fatty acids. The fatty acid compositions of dietary oils were measured by gas chromatography, and the resulting profiles are shown in Table 1. The composition of the diets was based on the AIN-93G[11] with modifications, as described previously.[12] AIN-93G is recommended as diets consisted of purified nutritional components for laboratory rodents during growth periods by the American Institute of Nutrition.[11] LF-SBO contained 69% carbohydrate, 10% fat, and 21% protein on a calorie basis. Other experimental diets contained 39% carbohydrate, 40% fat, and 21% protein on a calorie basis. Each serving was replaced with new servings once a day.

Purified nutritional components and soybean oil were purchased from Oriental Yeast and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and the fish oils (FO and HFO) were a gift from NOF CORPORATION (Tokyo, Japan).

Experimental Procedures —— The mice were given free access to FO, HFO, SBO and water for 11 wk. The body weight of the mice was measured once a week during the experimental period. The energy intake and fecal weight were measured at 7 wk. The oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were conducted at 8 and 9 wk, respectively. At 11 wk, fasting blood glucose levels were measured after a 4-hr fast, and mice were killed with an intraperitoneal injection of somnopentyl (Kyoritsu Seiyaku Corporation, Tokyo, Japan). Blood samples were collected from the postcaval vein, and plasma was stored at −30°C for analyses. The weights of the liver, parame-

trial white adipose tissue (WAT), and gastrocnemius were measured, and the tissue samples were then immediately frozen in liquid nitrogen and stored at −80°C.

In the supplementary experiment, control group was fed LF-SBO and other mice were fed SBO for 12 wk to induce obesity. Then, mice fed SBO were divided into two groups, and fed FO+SBO and HFO+SBO for an additional 3 wk. At 15 wk, mice were killed with an intraperitoneal injection of somnopentyl. Blood samples, liver and WAT were treated as mentioned previously. Mice were given free access to water in all periods.

Measurement of Food Intake and Absorbed Energy —— Food intake was measured as previously described.[12] The measurement of food intake was continued for 4 days, and the values from 4 days were averaged. The standard error of food intake was calculated from the variation of daily intake of each group.

The feces were collected for the same 4 days as the measurement of food intake. For measurement of energy intake, each diet was freeze-dried by a vacuum-freeze dryer (LABCONCO, Asahi Life Science Co., Ltd., Saitama, Japan) to evaporate the water. The freeze-dried diet and feces then became a powder, and the physical fuel values of the diet and feces were measured by the auto-calculating bomb calorimeter (CA4PJ, Shimadzu Corporation, Kyoto, Japan). Energy intake was calculated by food intake (g) × physiological fuel value of diet (kcal/g). The absorbed energy per day was then calculated by subtracting the fecal energy from the energy intake. Therefore, the absorbed energy was not the absolutely absorbed energy. The standard error of energy absorption was the variation of the daily absorbed energy of each group for 4 days.

OGTT and ITT —— In OGTT, D-glucose (1 mg/g body weight; Glucose Injection, Daiichi Sankyo Company, Ltd., Tokyo, Japan) was administered orally after a 6-hr fast. In ITT, insulin (0.75 mU/g body weight; Humulin, Eli Lilly Japan KK., Kobe, Japan) was injected intraperitoneally. Blood samples were obtained at the indicated times by cutting the tail end. Blood glucose levels were measured using a glucose analyzer ASCENSIA DEXTER ZII (Bayer Health Care, Osaka, Japan).

Measurement of Lipid Levels in Plasma, Liver, and Gastrocnemius —— TG and TC levels in plasma were measured by colorimetric slides using the FUJIDRICHEM analyzer (DRI-CHEM 3500, FUJIFILM Medica Co., Ltd., Tokyo, Japan). NEFA levels were measured using the Wako NEFA C test kit (Wako Pure Chemical Industries, Ltd.).

Each liver section (100–200 mg) and one side of gastrocnemius (100–200 mg) was homogenized in phosphate buffer solution (pH 7.4) by a polytron (PT 3100, Kinamatica, Inc., Littau/Lucerne, Switzerland), and total lipid extracts were obtained by the method described by Bligh and Dyer.[13] In the liver and gastrocnemius, total lipids were measured, and TG, TC, and NEFA levels in the total lipid samples were measured using the Wako TG E, TC E, and NEFA C test kits (Wako Pure Chemical Industries, Ltd.).

Analysis of Fatty Acid Compositions in the Feces, Liver, and WAT —— Lipids were extracted from the feces, liver, and WAT of mice by the methods of Folch *et al.*[14] Fifty mM pentadecanoic acid (C15 : 0) as an internal standard, which was dissolved in ethanol, was added for each lipid extract: 15 μl for feces, 15 μl for liver, and 20 μl for WAT extract. The method used for lipid extraction has been described previously.[15] The fatty acid methyl esters were measured by gas-liquid chromatography (Shimadzu GC-14A equipped with FID and Chromatopac C-R4A were used as data processors) with a Rascot Silliar 5CP capillary column (0.2 μm × 50 m; Nihon Chromato Works, Tokyo, Japan).[15] The absorbed fatty acids per day were calculated by subtracting the sum of measured fecal fatty acids from the sum of measured fatty acids in the experimental diets. Therefore, the absorbed fatty acids were not the absolutely absorbed fatty acids.

RNA Isolation and Measurement of mRNA Levels by Real-time Reverse Transcription (RT)-PCR —— Total RNA was extracted from the liver and WAT using TRIzol Reagent (Invitrogen, Renfrewshire, U.K.) and an RNeasy kit (QIAGEN, Hilden, Germany). Quantitative real-time RT-PCR analysis was performed on 0.08–1.5 μg of total RNA with iCycler iQ (Bio-Rad laboratory, Inc., Tokyo, Japan) and the ABI Prism 7000 thermal cycler (Applied Biosystems, Tokyo, Japan) using QuantiTect SYBR Green RT-PCR (QIAGEN) with gene-specific primers: fatty acid elongase 6 (Elovl6) and stearoyl-CoA desaturase 1 (SCD1). The mRNA levels in both the liver and WAT in all groups are represented as ratios to the mRNA levels in the liver in the SBO group.

Statistical Analysis —— Statistical comparisons of the groups were made by one-way analysis of variance (ANOVA), and each group was compared with the others by Fisher's protected least significant dif-

Table 2. Food Intake, Fecal Weight and Absorbed Energy, and Body Weight, Liver and WAT Weight

	SBO	FO	HFO
Food intake (g/5 mice per day)	$13.0 \pm 0.8^{a)}$	$12.2 \pm 0.4^{a)}$	$17.8 \pm 0.4^{b)}$
Fecal weight (g/5 mice per day)	$1.3 \pm 0.1^{a)}$	$1.3 \pm 0.1^{a)}$	$4.3 \pm 0.1^{b)}$
Absorbed energy (kcal/5 mice per day)	51.2 ± 3.5	50.8 ± 1.7	52.2 ± 1.8
Absorbed fatty acids (g/5 mice per day)	$2.0 \pm 0.1^{a)}$	$1.5 \pm 0.1^{b)}$	$1.3 \pm 0.1^{b)}$
Absorbed fatty acids (mmol/5 mice per day)	$8.5 \pm 0.5^{a)}$	$6.6 \pm 0.2^{b)}$	$11.0 \pm 0.2^{c)}$
Initial body weight (g)	17.9 ± 0.4	17.7 ± 0.5	17.9 ± 0.4
Final body weight (g)	$27.4 \pm 2.6^{a)}$	$21.6 \pm 0.3^{b)}$	$22.5 \pm 0.7^{b)}$
Body weight gain (g)	$9.5 \pm 2.3^{a)}$	$3.9 \pm 0.3^{b)}$	$4.6 \pm 0.4^{b)}$
WAT weight (g/mouse)	$0.7 \pm 0.2^{a)}$	$0.2 \pm 0.0^{b)}$	$0.2 \pm 0.0^{b)}$
Liver weight (g/mouse)	$0.9 \pm 0.1^{a)}$	$0.9 \pm 0.0^{a)}$	$1.4 \pm 0.1^{b)}$

Food intake and the amount of feces were simultaneously measured for 4 days around 7 wk. Each data point represents the mean ± S.E. of 5 mice for 4 days. *a*), *b*), *c*) Values not sharing the same superscript are significantly different at $p < 0.05$ by Fisher's PLSD test. Mice were sacrificed after a 4-hr fast, and WAT and liver weights were measured at 11 wk. Each data point represents the mean ± S.E. of 5 mice. *a*), *b*) Values not sharing the same superscript are significantly different at $p < 0.05$ by Fisher's PLSD test.

ference (PLSD) test (Statview 5.0; SAS Institute Inc., Cary, NC, U.S.A.). In supplementary experiment, statistical comparisons of the groups were made by Student's t test. p values less than 0.05 were considered to indicate statistical significance. Values are means ± S.E.

RESULTS

Food Intake, Fecal Weight, and Absorbed Energy

Food intake and the fecal weight were significantly increased in the HFO group among the three groups ($p < 0.01$, Table 2). Absorbed energy of the FO, HFO, and SBO groups did not change significantly. Although absorbed fatty acids based on the weight per day were significantly decreased in the FO and HFO groups, those based on the mole per day were significantly decreased in the FO groups and were significantly increased in the HFO groups compared with those in the SBO group. These results were explained by the greater amount of short chain saturated fatty acids in the HFO diet.

Body Weight and Organ Weight

Final body weight, body weight gain, and WAT weight were significantly decreased in the FO and HFO groups compared with the SBO group ($p < 0.05$, Table 2). However, liver weight in the HFO group was significantly increased among the three groups ($p < 0.05$, Table 2).

Excretion of Fatty Acids in Feces

We analyzed the profiles of fatty acids in feces. Excretion of C14 : 0, C20 : 0, C22 : 0, and C26 : 0 was significantly increased in the HFO groups among the three groups ($p < 0.05$, Fig. 1). However, excretion of C16 : 0 did not differ among the three groups, and C18 : 0 was significantly decreased in the HFO and the FO groups compared with the SBO group ($p < 0.05$, Fig. 1).

Blood Glucose Levels, Insulin Levels, and Homeostasis Model Assessment-Insulin Resistance (HOMA-IR)

Blood glucose levels after a 4-hr fast were significantly decreased in the FO and HFO groups compared with the SBO group ($p < 0.05$, Table 3). However, there was no significant difference in insulin levels or HOMA-IR among the three groups (Table 3).

Lipids Levels in Plasma, Liver, and Gastrocnemius

TG levels in plasma in the HFO group were significantly decreased compared with those in the other groups ($p < 0.05$, Table 3). TC levels in plasma were significantly decreased in the FO and HFO groups compared with those in the SBO group ($p < 0.01$, Table 3). NEFA levels did not differ among the three groups. In the liver, total lipids, TG, TC, and NEFA levels in the FO group did not differ from those in the SBO group, however these levels were significantly increased in the HFO group among the three groups ($p < 0.05$, Table 3). In gastrocnemius, total lipids tended to increase in the FO and HFO groups compared with the SBO group ($p = 0.1$, Table 3). Although there was no significant difference in TG and NEFA levels among the

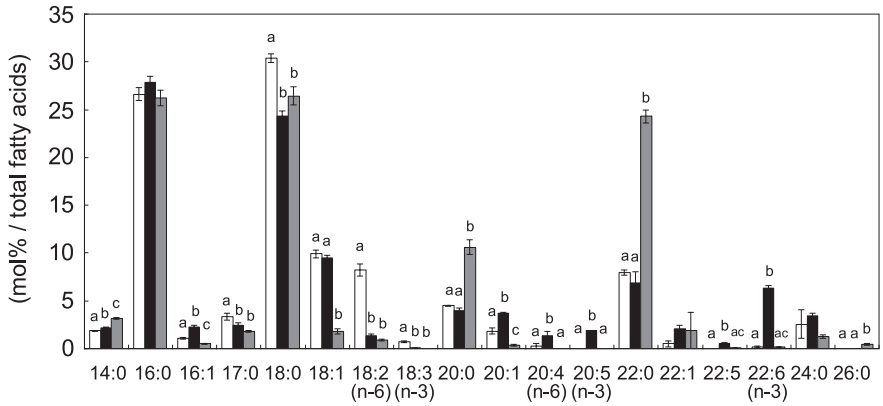

Fig. 1. Fatty Acids Composition in Feces

Fatty acids in feces were measured by gas chromatography. Each data point represents the mean ± S.E. of 5 mice. [a,b,c] Values not sharing the same superscript are significantly different at $p < 0.05$ by Fisher's PLSD test. □; SBO group, ■; FO group, ▒; HFO group.

Table 3. Plasma Parameters and Lipid Levels in Plasma, Liver and Gastrocnemius

	SBO	FO	HFO
Plasma			
Blood glucose (mmol/l)	7.06 ± 0.37[a)]	5.64 ± 0.42[b)]	5.80 ± 0.24[b)]
Insulin (pmol/l)	87.2 ± 17.0	80.1 ± 24.9	94.9 ± 22.9
HOMA-IR	4.6 ± 1.0	3.5 ± 1.3	4.1 ± 1.1
TG (mmol/l)	0.48 ± 0.04[a)]	0.40 ± 0.05[a)]	0.26 ± 0.05[b)]
TC (mmol/l)	1.64 ± 0.23[a)]	0.96 ± 0.07[b)]	1.14 ± 0.07[b)]
NEFA (mmol/l)	0.42 ± 0.06	0.35 ± 0.03	0.38 ± 0.04
Liver			
Total lipid (g/liver)	0.08 ± 0.01[a)]	0.07 ± 0.01[a)]	0.13 ± 0.02[b)]
TG (μmol/liver)	39.7 ± 7.2[a)]	26.3 ± 4.3[a)]	66.3 ± 8.8[b)]
TC (μmol/liver)	9.1 ± 0.9[a)]	7.2 ± 0.2[a)]	18.0 ± 2.6[b)]
NEFA (μmol/liver)	7.7 ± 0.7[a)]	6.5 ± 0.3[a)]	12.9 ± 1.3[b)]
Gastrocnemius			
Total lipid (mg/gastrocnemius)	1.91 ± 0.11	3.56 ± 0.66	3.10 ± 0.53
TG (μmol/gastrocnemius)	1.16 ± 0.33	0.84 ± 0.09	0.54 ± 0.05
TC (μmol/gastrocneimius)	0.41 ± 0.00[a)]	0.52 ± 0.02[b)]	0.42 ± 0.02[a)]
NEFA (μmol/gastrocnemius)	1.00 ± 0.03	1.08 ± 0.05	0.99 ± 0.03

Blood glucose levels were measured before sacrificed. Mice fed each diet for 11 wk were sacrificed after a 4-hr fast at 11 wk, and insulin levels in plasma, TG, TC, NEFA levels in plasma and the liver, total lipid levels in the liver and gastrocnemius were measured. Each data point represents the mean ± S.E. of 5 mice. *a*), *b*) Values not sharing the same superscript are significantly different at $p < 0.05$ by Fisher's PLSD test.

three groups, TC levels were significantly increased in the FO group among the three groups ($p < 0.05$, Table 3).

OGTT and ITT

Blood glucose levels in OGTT and ITT in all groups showed no significant difference at any time point (Fig. 2A and 2B).

Fatty Acid Composition in the Liver and WAT

Although lipid levels in the liver in the HFO group were significantly increased in all three groups, glucose tolerance in the group was not deteriorated (Fig. 2A and 2B). It was known that altered fatty acid compositions in the tissues also affect glucose tolerance. We therefore analyzed the fatty acid compositions in the liver and WAT.

Palmitoleic acid (C16 : 1), oleic acid (C18 : 1), and cerotic acid (C26 : 0) in the liver and C16 : 1 and C18 : 1 in the WAT were significantly higher in the HFO group compared to the other groups ($p < 0.05$, Fig. 3A and 3B).

mRNA Levels of Enzymes Involved in Fatty Acid Composition in the Liver and WAT

To clarify the causes of the characteristic alterations in fatty acid composition, especially in the HFO group, we measured the mRNA levels of

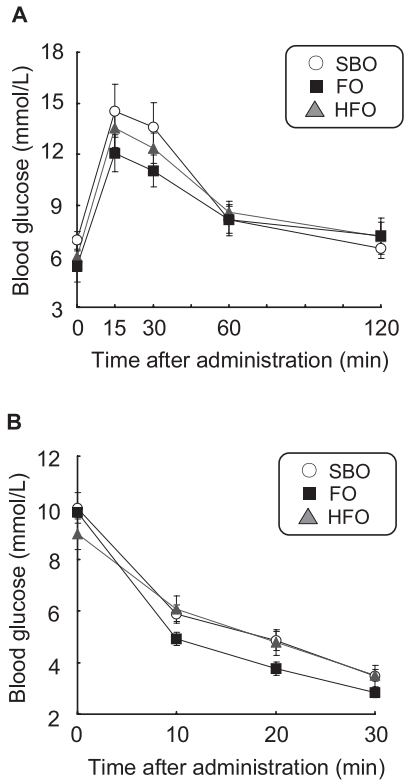

Fig. 2. OGTT and ITT

(A) OGTT. Mice after a 6-hr fast were administered D-glucose (1 mg/g body weight) orally at 8 wk. Blood glucose levels were measured at indicated times. Each data point represents the mean ± S.E. of 5 mice. (B) ITT. Mice were administered insulin (0.75 mU/g body weight) intraperitoneally at 9 wk. Blood glucose levels were measured at indicated times. Each data point represents the mean ± S.E. of 4–5 mice.

Table 4. Body Weight in the Obesity Inducing Period in the Supplementary Experiment

	LF-SBO	SBO
Initial body weight (g)	19.3 ± 0.4	19.3 ± 0.2
Final body weight (g)	27.2 ± 0.9	30.2 ± 1.0*
Body weight gain (g)	7.9 ± 0.8	10.9 ± 0.8*

Control group was fed LF-SBO and other mice were fed SBO for 12 wk to induce obesity. Data is shown as the mean ± S.E. of 4 mice. Astarisks indicate the significant difference at $p < 0.05$ by Student's t test between the LF+SBO and the SBO groups.

Table 5. Body Weight and Lipid Levels in Plasma and Liver in the Experimental Period in the Supplementary Experiment

	FO+SBO	HFO+SBO
Initial body weight (g)	30.2 ± 0.7	30.2 ± 1.7
Final body weight (g)	31.3 ± 0.9	26.8 ± 0.9*
Body weight gain (g)	1.1 ± 0.8	−3.5 ± 1.0*
WAT weight (g)	1.3 ± 0.1	0.7 ± 0.2*
Liver weight (g)	1.2 ± 0.2	1.2 ± 0.3
Plasma		
TG (mg/dL)	46.0 ± 4.5	23.0 ± 1.9**
TC (mg/dL)	52.7 ± 4.3	54.8 ± 9.2
Liver		
Total lipid (g/liver)	0.05 ± 0.0	0.08 ± 0.01*
TG (μmol/liver)	32.6 ± 2.8	48.9 ± 3.7*

Diet induced obese mice fed SBO for 12 wk were divided into two groups, and fed FO+SBO and HFO+SBO for an additional 3 wk. Mice were sacrificed after 4-hr fast at 15 wk. WAT and liver weights, TG, TC levels in plasma and total lipid, TG levels in liver were measured. Data is shown as the mean ± S.E. of 3–4 mice. Astarisks indicate the significant difference at $p < 0.05$ by Student's t test between the FO+SBO and the HFO+SBO groups.

Elovl6 and SCD1, which play key roles in the elongation and desaturation of fatty acids.

Elovl6 mRNA levels in the liver in the FO and the HFO groups were lower than those in the SBO group (Fig. 4A). In contrast, the mRNA levels in the WAT of the HFO group were higher than those in the SBO and FO groups. SCD1 mRNA levels in the liver and WAT in the HFO group were remarkably higher than those in the FO and SBO groups (Fig. 4B).

Supplementary Experiment

The HFO diet contained few or no essential fatty acids. Then we performed the supplementary experiment using diets substituted 10 energy percent of lipid for soybean oil. In the obesity inducing period, final body weight and body weight gain were significantly increased in the SBO group compared with those in the LF-SBO group (Table 4). In the experimental period, final body weight, body weight gain and WAT weight were significantly decreased in the HFO+SBO group compared with those in the FO+SBO group (Table 5). In the HFO+SBO group, plasma TG level was significantly decreased, and total lipid and TG levels in liver were significantly increased compared with those in the FO+SBO group (Table 5). These results indicated that added essential fatty acids did not contribute to lipid distribution in mice.

DISCUSSION

We used hydrogenated fish oil to investigate the role of unsaturated fatty acids in fish oil on lipid and glucose metabolism. The unsaturated fatty acids in the fish oil were found to be the main components maintaining the balance of TG levels in plasma, liver, and WAT.

In previous studies, fish oil has commonly been found to decrease TG levels in plasma and liver.[2, 8] Especially, fish oil has the powerful hypolipidemic

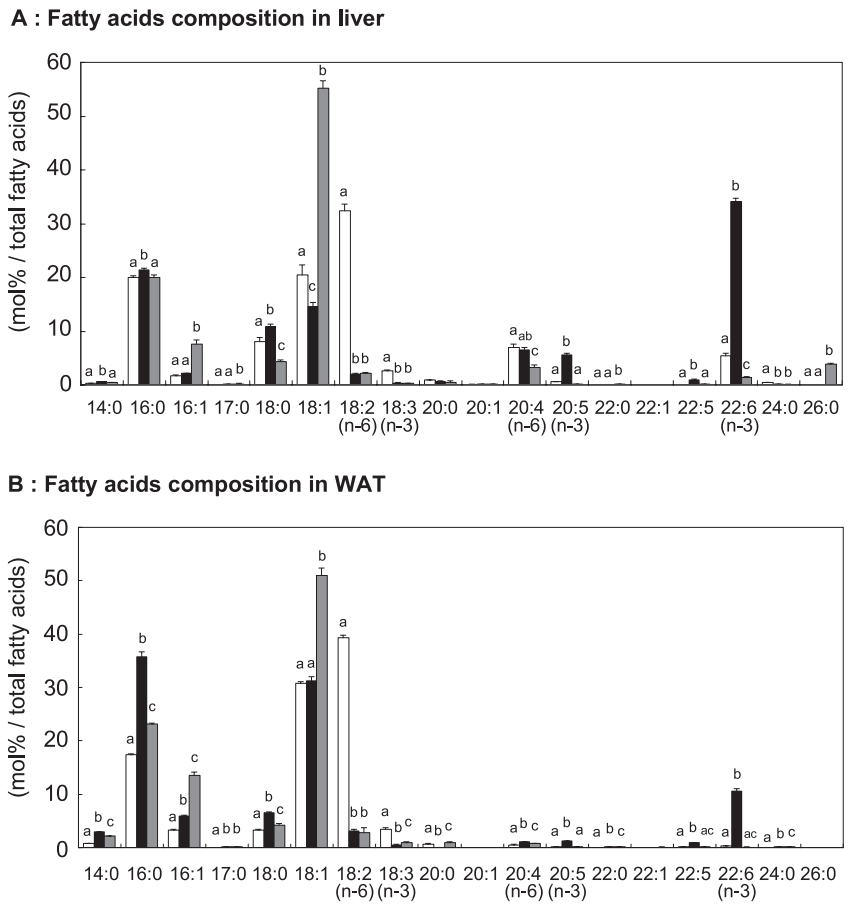

Fig. 3. Fatty Acid Compositions in Liver and WAT

Fatty acids in liver (A) and WAT (B) were measured by gas chromatography. Each data point represents the mean ± S.E. of 5 mice (A and B). [a,b,c]Values not sharing the same superscript are significantly different at $p < 0.05$ by Fisher's PLSD test. □; SBO group, ■; FO group, ▒; HFO group.

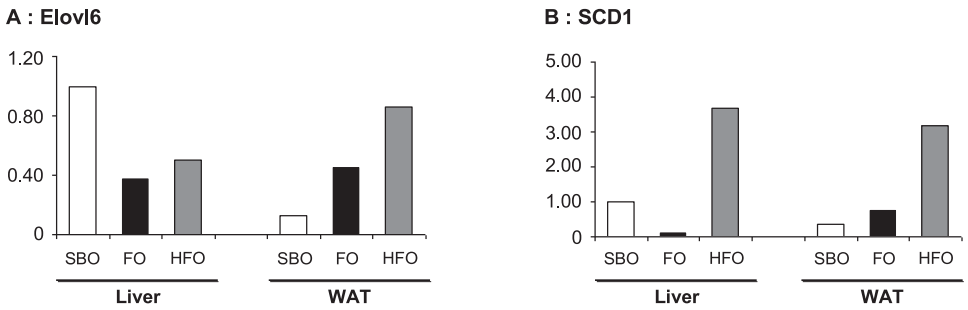

Fig. 4. Real-time RT-PCR of mRNA Involved Fatty Acid Metabolism in the Liver and WAT

Mice fed each diet for 11 wk were sacrificed after a 4-hr fast, and livers or WAT samples from five mice in each group were mixed, and total RNA was extracted. Quantitative real-time RT-PCR analysis was performed using total RNA extracted from the liver or WAT. Elovl6 (A) and SCD1 (B) mRNA levels in the liver and WAT of the SBO, FO and HFO groups. Levels of mRNA are represented as ratios to each liver mRNA level in the SBO group.

properties in liver, and it has been reported that fish oil and n-3 fatty acids improved nonalcoholic fatty liver disease.[16] In our study, the lipid levels (total, TG, TC, and NEFA) in the liver in the HFO group were remarkably increased, while the TG levels in plasma were significantly decreased compared with those in the FO group. It has been reported that TG levels were increased in both plasma and liver in mice fed beef diet containing saturated fatty acids similar to those in the HFO.[17] An incomplete HFO also decreased TG levels in plasma but did not change those in the liver in mice.[18] It has been re-

ported that C18 : 1-rich diet decreases TG levels in plasma and increases TG levels in liver by reduction of very-low density lipoprotein (VLDL) secretion from the liver.[12, 19] In our study, the amounts of C16 : 1 and C18 : 1 in the liver and WAT in the HFO group were remarkably increased. These findings suggest that C18 : 1 in the HFO group may contribute to low TG levels in plasma and high TG levels in liver. Then, it is speculated that unsaturated fatty acids in fish oil decrease hepatic TG levels and maintain the balance of TG levels between plasma and the liver.

On the other hand, body weight, WAT weight, and TC levels in plasma were decreased in the HFO group as well as in the FO group compared with those in the SBO group. The amounts of C16 : 1 and C18 : 1 in the liver and WAT in the HFO group were remarkably increased. These monounsaturated fatty acids might be synthesized by up-regulated SCD1 in the liver and WAT in the HFO group. The HFO diet excluded with polyunsaturated fatty acids increased SCD1 gene expression in the liver and WAT. Then, up-regulated SCD1 increased de novo synthesis of monounsaturated fatty acids from saturated fatty acids. A recent study in humans has indicated that body weight and body mass index decreased in a group fed macadamia nuts, which contain large amounts of C16 : 1.[20] In addition, synthesized C16 : 1 in adipose tissue leads to suppress lipogenesis and increase β-oxidation in the liver.[21] Therefore, a diet containing C16 : 1 or synthesized C16 : 1 may potentially lead to decrease in body weight and WAT weight depending on the increase in lipolysis and β-oxidation in the liver and WAT.

It was thought that TG accumulation in the liver in the HFO group would make glucose tolerance worse. However, there was no significant difference in blood glucose levels at any time point among the three groups in OGTT and ITT. It has been reported that polyunsaturated fatty acids and monounsaturated fatty acids (C16 : 1 and C18 : 1) improve glucose metabolism and glucose tolerance.[21–23] C16 : 1 increased the hepatic insulin signaling-mediated phosphorylation of Akt.[22] Furthermore, these results have suggested the possibility that C16 : 1 synthesized in adipose tissue is secreted to plasma and enhances insulin action in skeletal muscle in mice.[21] And in a human study, C18 : 1-rich diet decreased blood glucose levels.[23, 24] Therefore, synthesized C16 : 1 and C18 : 1 may improve glucose metabolism.

The intensively hydrogenated fish oil used in our experiments contained much amount of long-chain saturated fatty acids, especially C18 : 0 (stearic acid), C20 : 0 (arachidic acid), and C22 : 0 (behenic acid). These long chain saturated fatty acids are easily excreted into feces.[25] Therefore, the increase in food intake in the HFO group compensated for the loss of energy into feces and kept the energy absorption at levels similar to those in the other groups.

In summary, our results indicate that unsaturated fatty acids in fish oil decrease lipid levels in liver, and maintain the balance of lipid levels between plasma, liver, and WAT. In addition, it is possible that synthesized C16 : 1 and C18 : 1 in the HFO group improve glucose tolerance and affect lipid metabolism.

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