

Fish Oil Prevents Excessive Hepatic Lipid Accumulation without Inducing Oxidative Stress

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ABSTRACT

We examined the effects of fish oil (FO) on high-cholesterol diet-induced hepatic lipid accumulation and oxidative stress. Female C57BL/6J mice were fed diets consisting of safflower oil (SO), 1 en% FO (1FO), 2 en% FO (2FO), or 20 en% FO (20FO) with or without 2 weight% (wt%) cholesterol (SO/CH, 1FO/CH, 2FO/CH, and 20FO/CH groups, respectively) for 8 weeks. The hepatic triacylglyceride levels were significantly lower in the 2FO/CH and 20FO/CH groups than in the SO/CH group. The hepatic mRNAs of fatty acid oxidation-related genes were upregulated and the fatty acid synthesis-related genes were downregulated by the FO feeding. Adverse effects were not observed in the plasma levels of indicators of oxidative stress in response to the consumption of FO up to 20 en%. These results suggest that FO consumption in the range of 2–20 en% prevents hepatic lipid accumulation, thus improving lipid metabolism without causing oxidative stress.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a simple form of hepatic steatosis, the additional presence of oxidative stress and stimulating inflammatory cytokines results in non-alcoholic steatohepatitis. NAFLD is recognized as one of the most common causes of chronic liver disease, and it is closely associated with cardiovascular diseases and diabetes [1-3]. Thus, preventing lipid accumulation in the liver may be a promising method for improving insulin resistance, diabetes, and other metabolic syndromes.

It has been demonstrated that eicosapentaenoic acid (EPA) consumption improves hepatic steatosis [4]. A 12-month supplementation of n-3 polyunsaturated fatty acids (PUFAs; 1-g capsule daily) improved liver steatosis in NAFLD patients [5], and fish oil (FO) consumption reduces the risk of developing cardiovascular disease by decreasing the plasma triglyceride density [6]. Furthermore, many findings have indicated that the effects of n-3 PUFAs and FO are observed due to the inhibition of fatty acid synthesis via suppression of the expression of lipogenic genes such as sterol regulatory element-binding protein (SREBP), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and stearoyl-CoA desaturase (SCD) 1 and the induction of fatty acid oxidation by activating peroxisome proliferator-activated receptor (PPAR) α , which is a nuclear receptor that regulates acyl-CoA oxidase (AOX) and uncoupling protein (UCP) 2 in the liver [7-15]. Many previous studies including ours used high dosages of FO [20–50 energy% (en%)] to investigate the physiological effects of FO [7-9, 12-16]. However, our latest research demonstrated that low doses of FO (2 or 5 en%) improved hepatic lipid accumulation induced by high-cholesterol diet consumption [17]. As described above, it has been demonstrated that PUFAs in FO improve lipid metabolism. However, EPA and docosahexaenoic acid (DHA), the major PUFAs of FO, are very susceptible to oxidation, which tends to generate free radicals and lipid peroxides that can

have adverse health effects. The oxidative stress caused by free radicals and lipid peroxides induces diabetes and other diseases [18]. In previous studies using FO, EPA and DHA were reported to increase the lipid peroxides levels in plasma and the liver [19, 20]. Conversely, other reports indicated that lipid peroxide levels were not increased by PUFA feeding [21, 22]. To study the effects of FO consumption, it is very important to assess both its efficacy and toxicity.

In this study, to evaluate the minimum effective dose and toxicity of FO, we examined the influence of 1, 2, and 20 en% FO with or without 2 weight% (wt%) cholesterol on lipid metabolism and oxidative stress.

2. Material and methods

2.1. 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 (MF, Oriental Yeast Co, Tokyo, Japan) for 1 week to acclimatize the animals to their new conditions. Animals were reared in a room with controlled temperature ($23 \pm 2^{\circ}\text{C}$), humidity ($55 \pm 10\%$), and a 12-h day cycle (7:00 AM-7:00 PM) at the Josai University Life Science Center. The mice were divided into six groups ($n = 5$ in each group). All groups were fed a diet containing 60 en% carbohydrates, 20 en% fat, and 20 en% protein with or without 2 wt% cholesterol (CH). The details of the diets and abbreviated expressions (experimental groups) are shown in Table 1. β -Corn starch, casein, sucrose, cellulose powder, AIN-93G mineral mix, and AIN-93 vitamin mix were obtained from Oriental Yeast Co., Ltd (Tokyo, Japan). L-cysteine, *t*-butylhydroquinone, and cholesterol were obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). In this

study, dietary fats consisted of a mixture of safflower oil (SO) and FO to maintain the total fat energy level at 20 en% (20 en% SO/0 en% FO, 19 en% SO/1 en% FO, 18 en% SO/2 en% FO, or 0 en% SO/20 en% FO). SO (Benibana Foods, Tokyo, Japan) contained 78 wt% oleic acid (18:1n-9). FO (NOF Co., Tokyo, Japan) contained 7 wt% EPA (20:5n-3), 24 wt% DHA (22:6n-3), and 1 wt% α -tocopherol. Feed and water were freely provided. The feed was changed at 10:00 AM every day, and the residual quantity was measured daily. All animal studies were conducted in accordance with the “Standards Relating to the Care and Management of Experimental Animals” (Notice No. 6 of the Office of Prime Minister dated March 27, 1980) and the guidelines of Institutional Animal Care and Use Committee of Josai University.

2.2. Computed tomography (CT)

After 8 weeks of feeding, the mice were starved for 3 h and intraperitoneally anesthetized with pentobarbital sodium (Dainippon Sumitomo Pharma, Osaka, Japan). The abdominal composition of the mice was examined by X-ray CT (LaTheta LCT-100, Aloka, Tokyo, Japan). Contiguous 2-mm slice images between the second lumbar (L2) and fourth lumbar (L4) were used for the quantitative assessment using LaTheta software (version 2.10). Fat was divided into visceral and subcutaneous fat and evaluated quantitatively.

2.3. Collection of blood and tissue samples

After CT, the animals were dissected. Blood samples were drawn from the inferior vena cava and treated with EDTA-2Na. The liver and white adipose tissues (WATs) around the uterus were removed immediately and weighed. Photographs of the liver were taken using a

digital camera. A piece of liver tissue was excised from the median lobe of the liver. Five liver samples were individually collected for each group and fixed with 10% neutral buffered formalin (Wako Pure Chemical Industries, Ltd). The samples were embedded in paraffin, cut into sections, and stained with hematoxylin-eosin (H&E) for histopathological examination by Kotobiken Medical Laboratories, Inc. (Tokyo, Japan). Plasma was obtained by centrifugation (900×g, 4°C, 10 min) and frozen at −80°C for storage until analysis. The organs removed were frozen in liquid nitrogen and stored at −80°C.

2.4. Quantification of hepatic and plasma lipids

Hepatic lipids were extracted from approximately 100 mg of liver tissue for each mouse in accordance with the method of Folch et al [23]. Triacylglyceride and total cholesterol in the liver were measured using Triglyceride E-Test and Cholesterol E-Test kits (Wako Pure Chemical Industries, Ltd), respectively. Quantification of plasma triacylglyceride and total cholesterol levels was performed using the same test kits. To measure plasma high-density lipoprotein (HDL) cholesterol levels, HDL-Cholesterol E-Test kit (Wako Pure Chemical Industries, Ltd) was used.

2.5. Quantification of mRNA expression

Total RNA was extracted from the liver and intestinal tissue of each mouse using Trizol (Invitrogen) in accordance with the manufacturer's protocol. Quantification of mRNA expression levels by real-time RT-PCR was performed using the ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). mRNA was amplified using QuantiTect SYBP Green and QuantiTect RT Mix (Qiagen, Hilden, Germany). The thermal cycling conditions were as follows: reverse transcription at 50°C for 30 min, initial activation

at 95°C for 15 min, 40 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. A housekeeping transcript, glyceraldehyde-3-phosphate dehydrogenase, was used as an endogenous control gene. The primers used for real-time PCR analysis are listed in supplementary Table S1.

2.6. Assessment of oxidative stress

The diacron of reactive oxygen metabolites (d-ROMs) test was used to assess lipid peroxidation and was performed according to a commercially available method (d-ROMs test, Free Radical Analytical System, Diacron, Grosseto, Italy). Plasma was obtained from the tail vein 0, 1, 4, and 8 weeks after experimental diet feeding. The observed data were expressed in conventional units (Carratelli Units, U.CARR), and 1 U.CARR corresponds to 0.8 mg/L hydrogen peroxide (H₂O₂).

2.7. Statistical analysis

Data were represented as the mean \pm SD. A one-way analysis of variance (ANOVA) was performed on the data. Differences among groups were compared using Tukey-Kramer test. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Food intake and body and tissue weight

Food intake (kcal/5 mice/day) during the study was comparable among the diet groups. Although final body weight increased in all groups, body weight gain, i.e., final body weight – initial body weight, was not significantly different among the groups. Liver weights were significantly increased by the intake of diet containing 2 wt% cholesterol with SO or 1 en%

FO. No large differences were observed in liver weight among the other groups. Compared with the SO group, WAT weight tended to be lower in the 20FO/CH group (Table 2).

3.2. Assessment of abdominal fat tissues using X-ray CT

Visceral fat levels were not changed by low-dose FO feeding. However, the 20FO group exhibited a tendency of decreased visceral fat compared with the SO group (Fig. 1B). Subcutaneous fat levels were also similar (Fig. 1C).

3.3. Liver morphology, histology, and lipid levels

The morphology and histology of the liver are shown in Fig. 2. The livers of the SO/CH group mice were pale (Fig. 2A), and many lipid droplets were detected in tissue specimens (Fig. 2B). Compared with the SO/CH group, the 1FO/CH group displayed slightly less liver discoloration. In the 2FO/CH group, the discoloration was further suppressed and the amount of lipid droplets decreased. The livers of the 20FO and 20FO/CH group mice had a normal color and displayed no lipid droplets.

As shown in Fig. 3A, hepatic triacylglyceride levels were significantly increased by the intake of diet containing 2 wt% cholesterol with SO or low-dose FO (1 and 2 en%). Compared with the SO/CH group, the 1FO/CH group exhibited no change in triacylglyceride levels; however, the triacylglyceride levels were tended to decreased in the 2FO/CH group. Hepatic total cholesterol levels in the SO/CH, 1FO/CH, and 2FO/CH groups were increased significantly by the supplementation of dietary cholesterol (Fig. 3B). However, the 1FO/CH and 2FO/CH groups exhibited significantly decreased total cholesterol levels compared with the SO/CH group. Hepatic total cholesterol and triacylglyceride levels were similar between the 20FO and 20FO/CH groups.

3.4. Plasma lipid levels

Plasma triglyceride levels were not significantly different among the groups (Fig. 4B). Plasma total cholesterol levels were increased by the supplementation of dietary cholesterol in all diet groups. Total cholesterol levels were similar among the SO, 1FO, and 2FO groups, but the 20FO group displayed significantly decreased total cholesterol levels compared with the SO group (Fig. 4C). HDL cholesterol levels were decreased significantly in the 20FO group compared with the SO group, and the decreased levels were markedly increased by cholesterol feeding (Fig. 4D).

3.5. Liver mRNA expression levels of genes involved in lipid metabolism

To examine the effects of FO and cholesterol consumption on lipid metabolism in the liver, the mRNA expression of lipid metabolism-related genes was measured (Table 3). SREBP-1c, FAS, and SCD1 mRNA levels were not different between the SO and low-dose (1 and 2 en%) FO groups. FAS mRNA levels were reduced by cholesterol supplementation, but SCD-1 mRNA levels were not affected. Conversely, in the 20 en% FO groups, FAS and SCD-1 mRNA levels were significantly lower than those in the SO group, irrespective of cholesterol supplementation. The mRNA levels of AOX, a PPAR α target gene involved in fatty acid oxidation, were significantly higher in the 20 en% FO groups than in the SO groups, irrespective of cholesterol supplementation. And, the mRNA levels of UCP-2, which is involved in heat production, were also significantly higher in the 20 en% FO groups. The expression of these genes did not increase significantly in low-dose (1 and 2 en%) FO groups. SREBP-2 mRNA levels were significantly lower in the cholesterol supplementation groups than in the no cholesterol supplementation groups. The mRNA levels of HMG-CoA reductase,

a rate-limiting enzyme of cholesterol synthesis, did not change in response to low-dose FO consumption alone, but they were reduced by cholesterol supplementation. In contrast, the 20 en% high-dose FO groups displayed significantly lower HMG-CoA reductase mRNA levels than the SO group. The mRNA levels of ABCG5 and G8, two cholesterol transporters, were increased in all cholesterol-supplemented groups, except the 20FO/CH groups. The mRNA levels of cholesterol 7 α -hydroxylase, which is involved in bile acid synthesis, tended to increase with cholesterol supplementation. Catalase (CAT) mRNA levels were not significantly different among the groups. The mRNA levels of superoxide dismutase (SOD), which are antioxidant enzymes, were significantly increased in the 20FO group compared with the SO group. Cholesterol loading did not affect the expression of these genes.

3.6. Oxidative stress

Oxidative stress indices are shown in Fig. 5. Regarding plasma d-ROM levels after experimental diet feeding for 1, 4, and 8 weeks, significant changes were not observed in response to FO and/or cholesterol feeding at any point. In all groups, d-ROM levels were high after 4 weeks of experimental diet feeding, but thereafter, further increases were not observed. Plasma thiobarbituric acid reactive substances (TBARS) levels tended to be lower in the 20 en% FO groups. However, major changes were not observed in response to FO and cholesterol feeding.

4. Discussion

In this study, to determine the minimum effective dose of FO for improving lipid metabolism and to examine the effect of FO on oxidative stress, female C57BL/6J mice were fed diets containing 1, 2, or 20 en% FO with or without 2 wt% cholesterol for 8 weeks.

Body weight and parametrial WAT weight were not different among the 1FO, 2FO, and SO groups, but a decreasing tendency was observed in the 20FO group compared with the SO group. Previous studies described that high-dose FO feeding decreased body and WAT weight [16, 24, 25]; however, low-dose FO consumption did not affect these parameters [8, 17]. The present results are consistent with those of these reports.

Excessive accumulation of lipids in the liver, which is one of the causes of NAFLD, is involved in the development of insulin resistance and cardiovascular disease. Consistent with our previous reports [16, 17], it is suggested that in addition to high-dose FO intake, low-dose 2 en% FO intake, which is a common intake level in Japanese, can prevent fatty liver provoked by high-cholesterol diets. However, it is suggested that the suppressive effect is not sufficient at a dose of 1 en% FO. These results indicate that the 2 en% FO intake in daily meals is effective for preventing fatty liver.

We examined the effects of low-dose FO and cholesterol feeding on the expression of lipid metabolism-related genes in the liver. HMG-CoA reductase and SREBP-2 mRNA levels tended to decrease in response to cholesterol loading. Conversely, the mRNA levels of ABCG5 and ABCG8, which are induced by LXR and involved in biliary sterol excretion, were increased by cholesterol supplementation. It is considered that cholesterol is reduced in the liver to maintain cholesterol homeostasis in the body. In the 1 and 2 en% FO groups, FAS and SCD-1 mRNA levels were not significantly different from those in the SO group. Hepatic PPAR α and AOX mRNA levels were tended to increase in the 2 en% FO groups. And, in the 20 en% FO group, in line with a previous report [16], the expression of fatty acid synthesis-related genes decreased and that of fatty acid oxidation-related gene increased significantly compared with the expression of these genes in the SO group. These results suggested that the inhibition of lipid accumulation in the liver by the 2 and 20 en% FO intake

was the result of changes in the expression of genes involved in lipid metabolism in the liver.

EPA and DHA, the major PUFAs of FO, are very susceptible to peroxidation because of their high degree of unsaturation. Some previous studies reported that EPA or DHA consumption increased lipid peroxide levels in plasma and the liver [19, 20]. Tsuduki et al. [26] reported that FO intake increases oxidative stress, decreases cellular function, and causes organ dysfunction in senescence-accelerated (SAMP8) mice, thereby promoting aging and shortening the lifespan of mice. However, in this study, the plasma TBARS levels were not increased by FO intake up to 20 en%. Moreover, the plasma TBARS levels in the 20FO group tended to be lower compared with those in the SO group. The plasma levels of d-ROMs, a marker of oxidative stress, were not significantly different among the groups at any stage. Some reports described that FO intake aggravated oxidation stress [19, 20]. However, Frenoux et al. [22] reported that PUFA supplementation enhances the total anti-oxidant status and resistance to lipid peroxidation. In addition, Calviello et al. [21] reported that low doses of dietary EPA and DHA did not increase the susceptibility to oxidative stress. In this study, the mRNA expression of SOD and CAT, which are involved in enzymatic antioxidant, was observed. SOD was significantly increased and CAT tended to increase in the 20FO groups compared with the SO groups. These results suggest the possibility that oxidation stress was not increased because antioxidant enzyme activities were elevated. However, further examination is needed regarding this issue in the future.

In conclusion, consumption of FO containing 2–20 en% fat prevents hepatic lipid accumulation, thus improving lipid metabolism without inducing oxidative stress.

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Disclosure Summary

The authors have nothing to declare.

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Legends for figures and tables

Fig. 1. CT-based fat tissues composition analysis of 16-week-old female mice.

Representative X-ray CT images of mice fed SO, SO/CH, 1FO, 1FO/CH, 2FO, 2FO/CH, 20FO, and 20FO/CH for 8 weeks, at the L3 level (A). The areas indicated with pink, yellow, and light-blue are visceral fat, subcutaneous fat, and muscle, respectively. CT-estimated amounts of visceral fat (B), and subcutaneous fat (C) in the abdominal area of L2–L4. Values represent means \pm S.D. (n=4-5). Means with different letters are different at the $p<0.05$ level by Tukey-Kramer test.

Fig. 2. Liver morphology and tissue histology of 16-week-old female mice.

Liver morphology (A) , H&E-stained liver sections (B) in mice fed SO, SO/CH, 1FO, 1FO/CH, 2FO, 2FO/CH, 20FO, and 20FO/CH for 8 weeks.

Fig. 3. Hepatic lipid levels of 16-week-old female mice.

Hepatic triacylglyceride (TAG) (A) and total cholesterol (TC) (B) in mice fed SO, SO/CH, 1FO, 1FO/CH, 2FO, 2FO/CH, 20FO, and 20FO/CH for 8 weeks. Values represent means \pm S.D. (n=4-5). Means with different letters are different at the $p<0.05$ level by Tukey-Kramer test.

Fig. 4. Blood glucose and plasma lipid levels of 16-week-old female mice.

Blood glucose (A), triacylglyceride (TAG) (B), Total cholesterol (TC) (C), and high-density lipoprotein cholesterol (HDL-C) (D) in mice fed SO, SO/CH, 1FO, 1FO/CH, 2FO, 2FO/CH, 20FO, and 20FO/CH for 8 weeks. Values represent means \pm S.D. (n=4-5). Means with different letters are different at the $p<0.05$ level by Tukey-Kramer test.

Fig. 5. Plasma d-ROMs and TBARS levels of 16-week-old female mice.

Plasma d-ROMs for 0,1,4,8 weeks of experimental diets fed (A). Plasma d-ROMs for 8 weeks of experimental diets fed (B) and plasma TBARS for 8 weeks of experimental diets fed (C). Values represent means \pm S.D. (n=4-5). Statistical significance is indicated for the \dagger -2FO compared to the SO. Means with different letters are different at the $p<0.05$ level by Tukey-Kramer test.

Fig.1
A

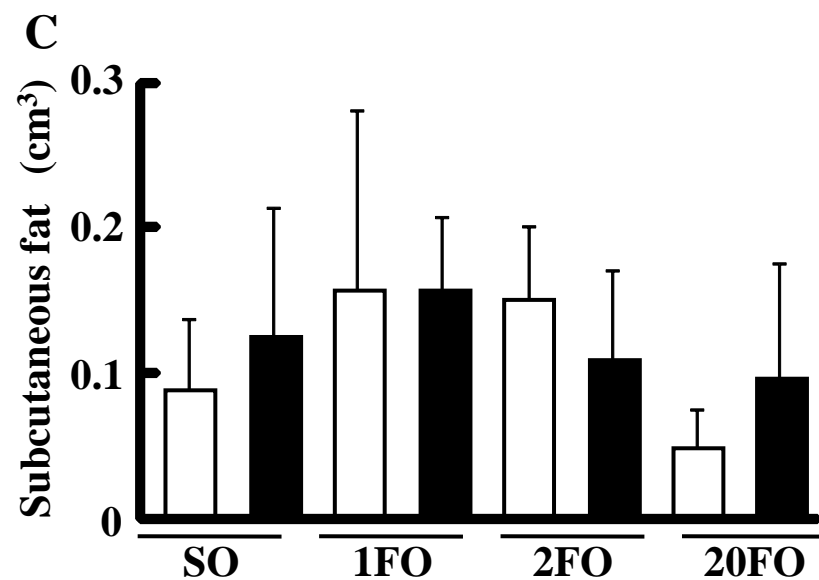
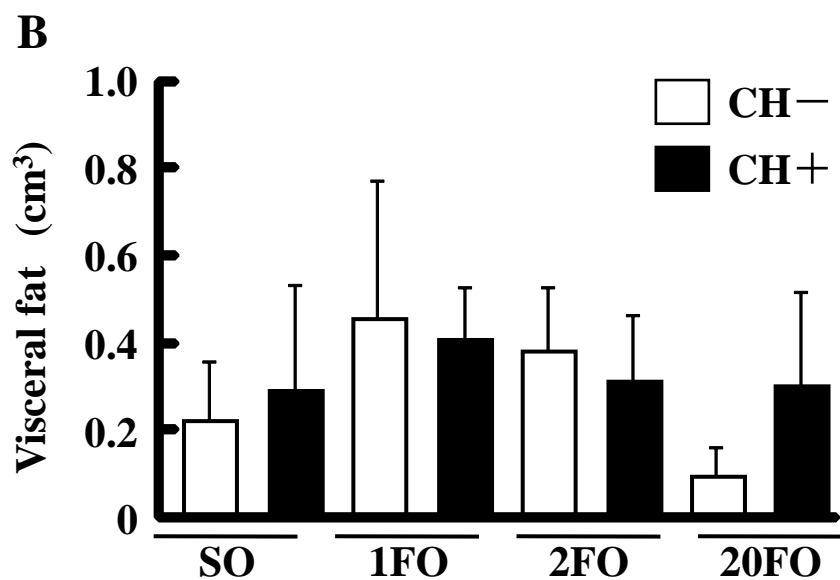
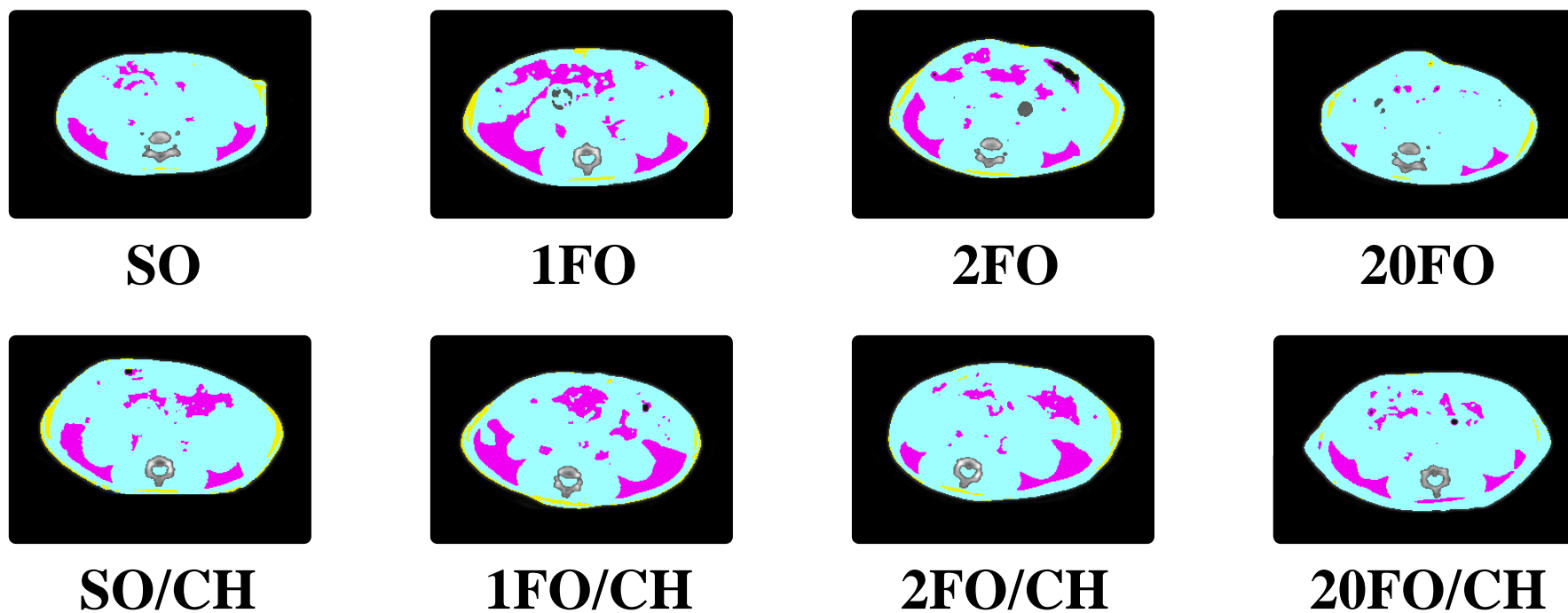
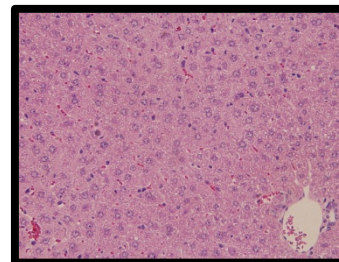
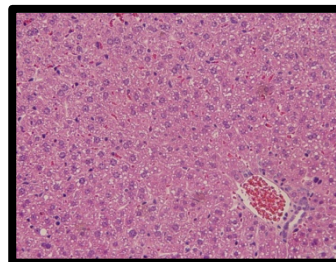
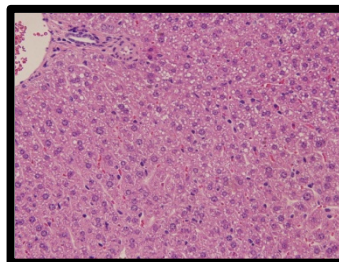
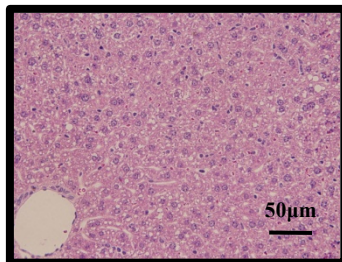


Fig.2

A



B



SO

1FO

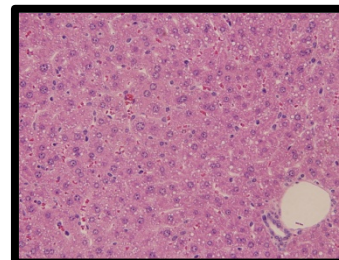
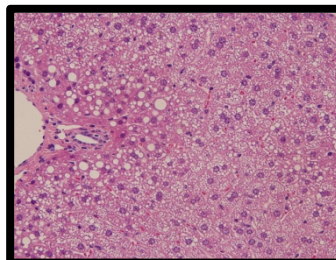
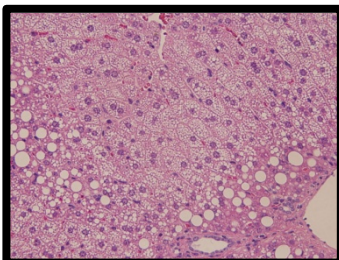
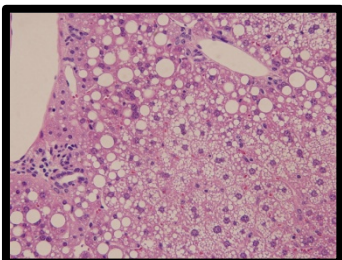
2FO

20FO

A



B



SO/CH

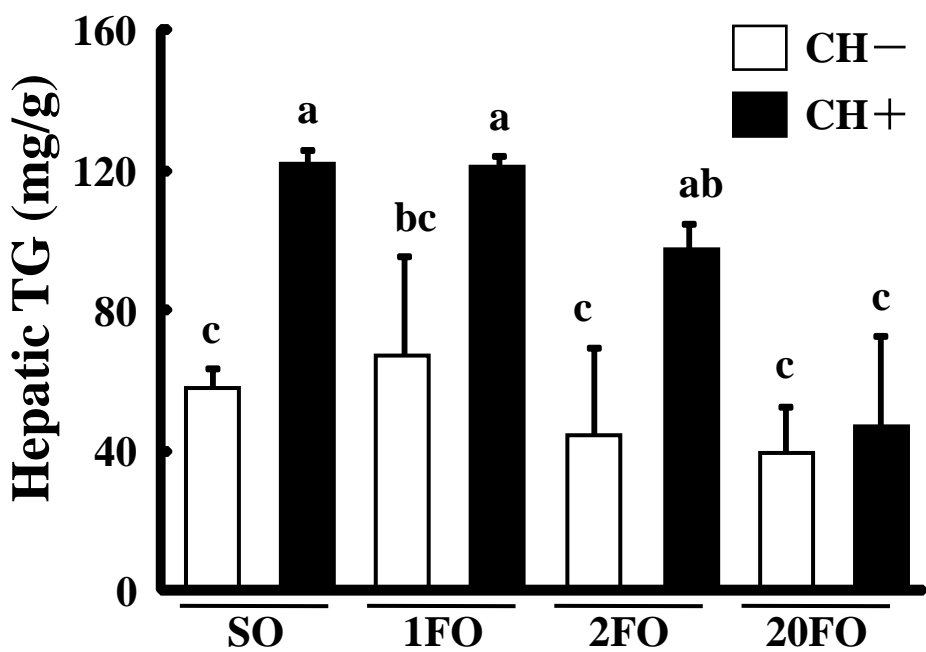
1FO/CH

2FO/CH

20FO/CH

Fig.3

A



B

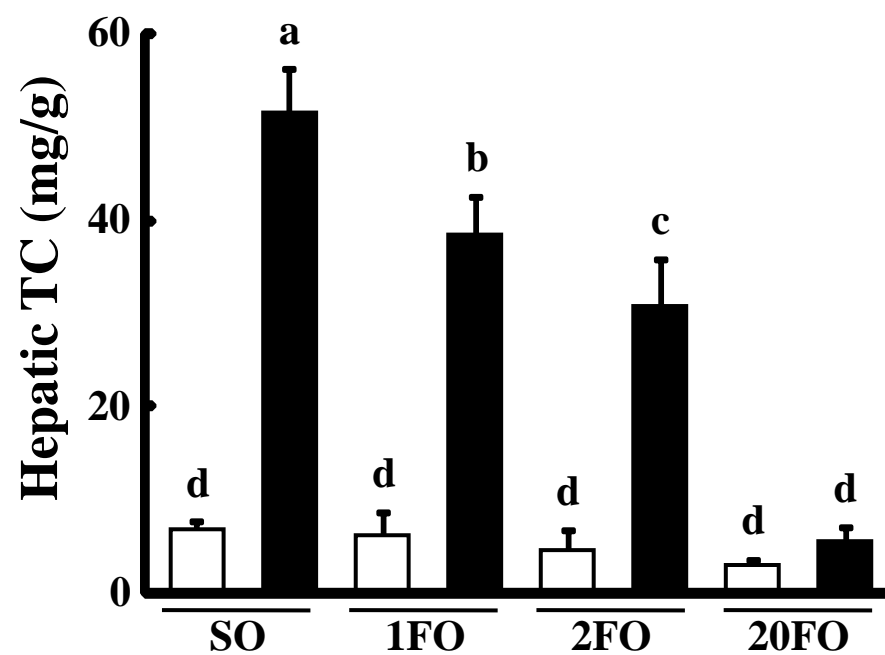


Fig.4

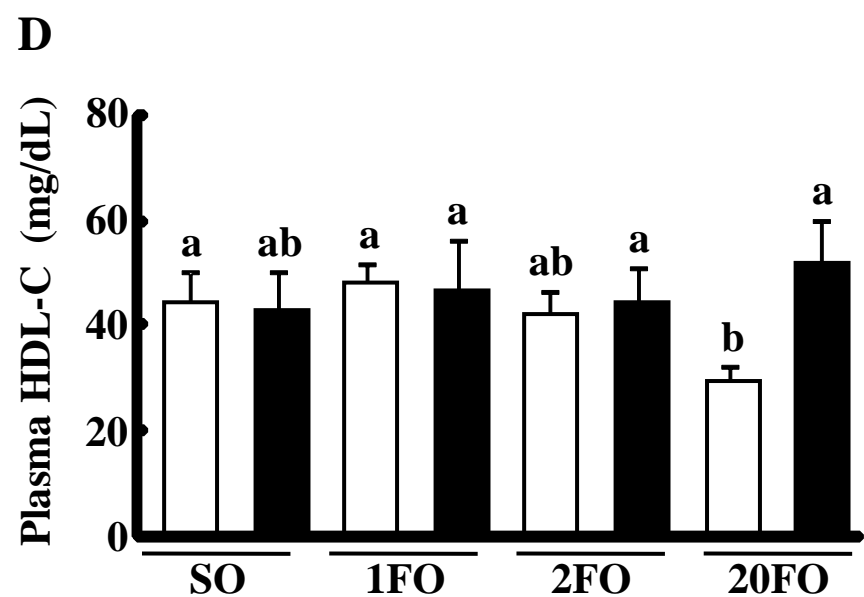
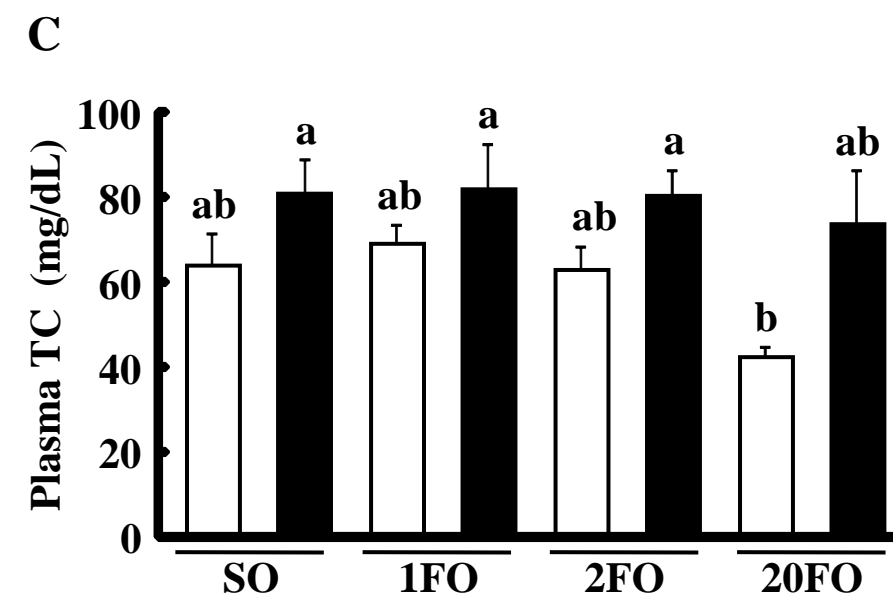
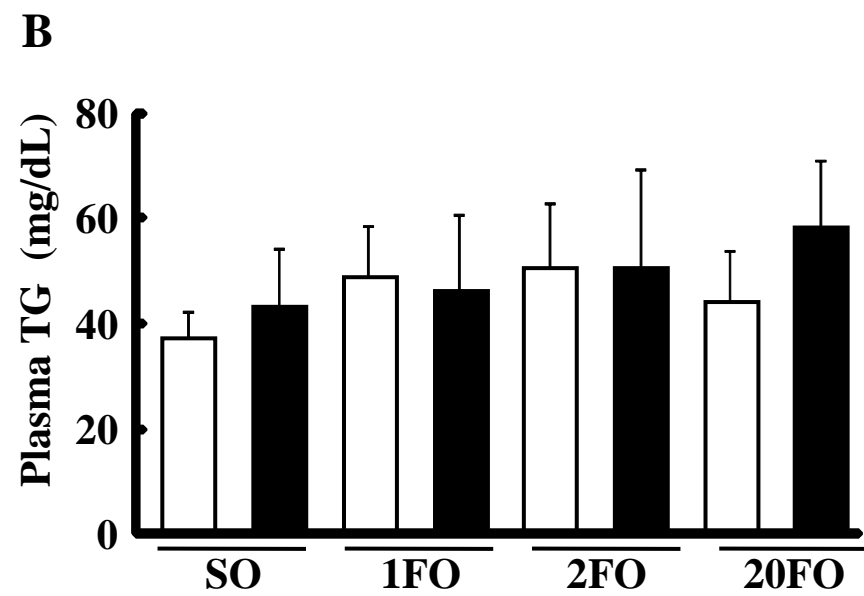
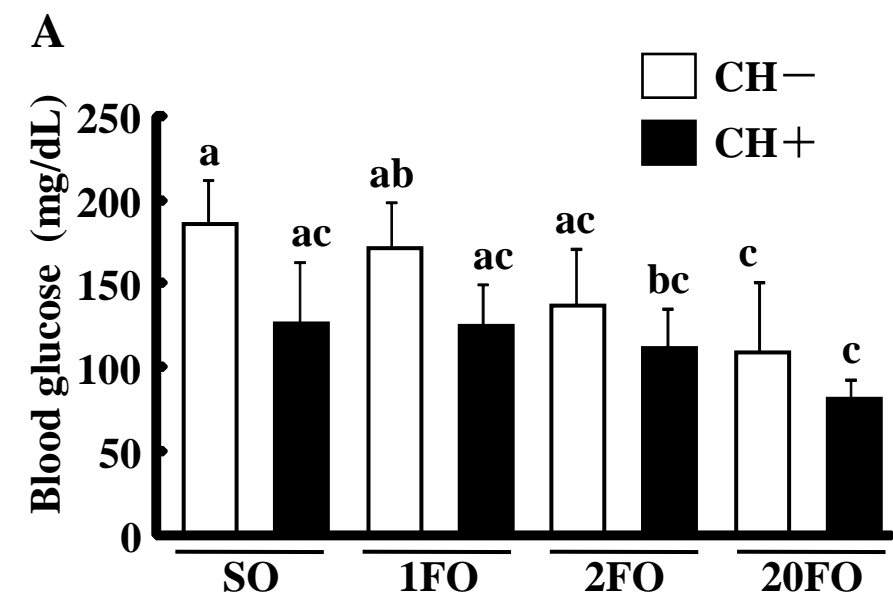


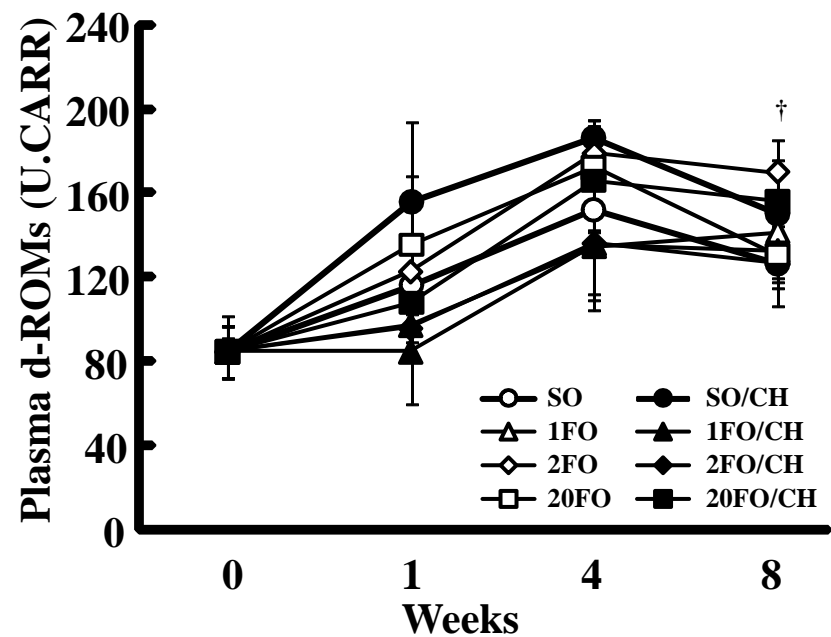
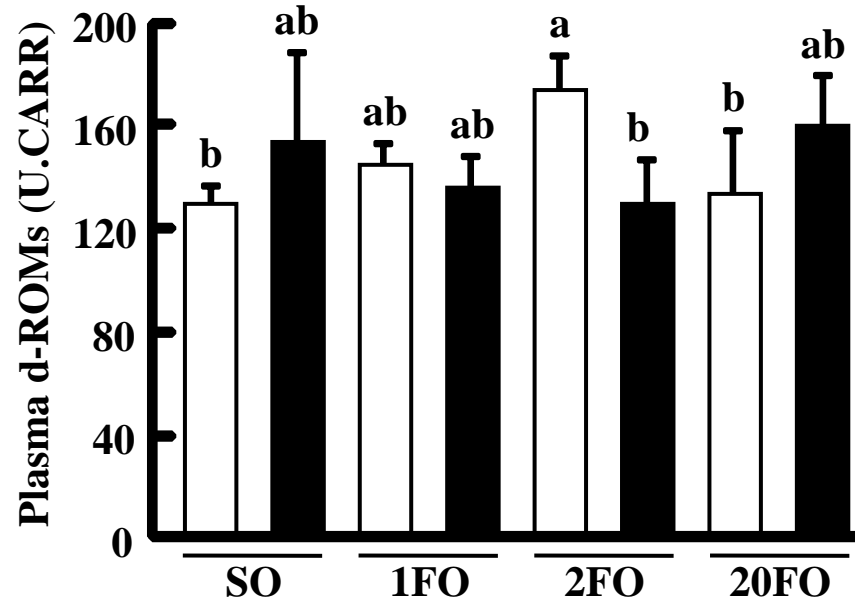
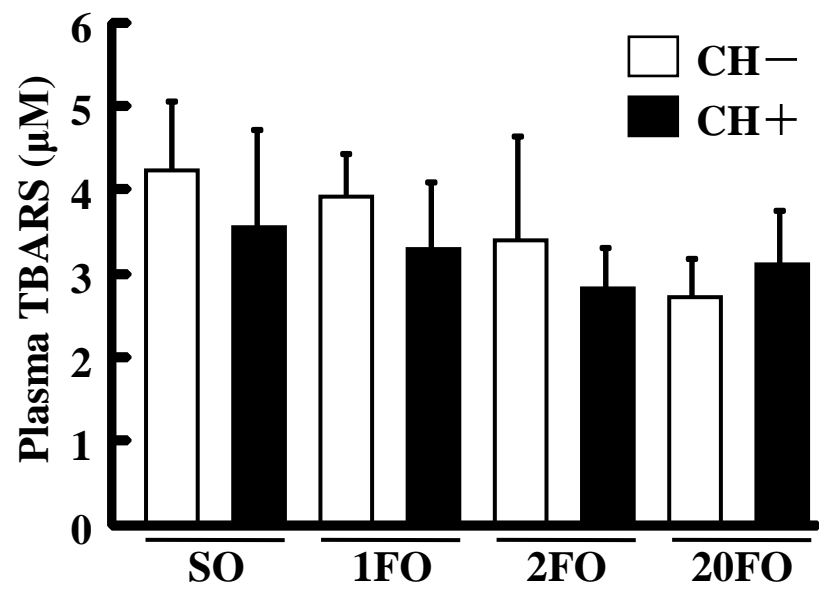
Fig.5**A****B****C**

Table 1. Composition of the experimental diets

Diet	SO	SO/CH	1FO	1FO/CH	2FO	2FO/CH	20FO	20FO/CH
Ingredients(g)								
Safflower oil	8	8	7.2	7.2	6	6	-	-
Fish oil	-	-	0.4	0.4	0.8	0.8	8	8
Casein	20	20	20	20	20	20	20	20
Sucrose	10.37	10.37	10.37	10.37	10.37	10.37	10.37	10.37
β -starch	51.83	51.83	51.83	51.83	51.83	51.83	51.83	51.83
Vitamin mix *	1	1	1	1	1	1	1	1
Mineral mix	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Cellulose powder	5	5	5	5	5	5	5	5
L-cysteine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Tert-butyl hydroquinone	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016
cholesterol	-	2	-	2	-	2	2	2
Total(g)	100	102	100	102	100	102	102	102
Energy (kcal/100g)	374.02	366.68	374.02	366.68	374.02	366.68	366.68	366.68
Fat Energy ratio (%)	19.7	19.7	19.7	19.7	19.7	19.7	19.7	19.7

*Vitamin mix and mineral mix were based on the AIN-93G formation. Vitamin mix substituted 0.25% sucrose for choline bitartrate.

Table 2. Body and tissue weight of 16-week-old female mice

Parameter	SO	SO/CH	1FO	1FO/CH	2FO	2FO/CH	20FO	20FO/CH
Initial body weight (g)	16.70 ± 1.11	16.68 ± 0.93	16.66 ± 0.82	16.66 ± 0.78	16.68 ± 0.70	16.66 ± 0.70	16.68 ± 0.65	16.76 ± 0.66
final body weight (g)	21.05 ± 1.65	22.14 ± 2.22	22.06 ± 2.05	22.52 ± 1.19	22.60 ± 2.06	22.35 ± 1.30	20.51 ± 1.12	22.20 ± 0.52
Liver weight (g)	0.92 ± 0.12 ^{bc}	1.28 ± 0.13 ^a	0.91 ± 0.09 ^c	1.09 ± 0.09 ^b	0.90 ± 0.13 ^c	0.96 ± 0.10 ^{bc}	0.85 ± 0.06 ^c	1.00 ± 0.06 ^{bc}
Liver weight/body weight (%)	4.38 ± 0.42 ^{bc}	5.64 ± 0.32 ^a	4.96 ± 1.91 ^{bc}	4.83 ± 0.19 ^{ab}	3.98 ± 0.28 ^{bc}	4.29 ± 0.19 ^{bc}	4.13 ± 0.31 ^c	4.51 ± 0.20 ^{bc}
White adipose tissue weight (g)	0.32 ± 0.17	0.46 ± 0.22	0.52 ± 0.29	0.51 ± 0.11	0.49 ± 0.09	0.45 ± 0.13	0.20 ± 0.10	0.41 ± 0.16

Values represent means ± S.D. (n=4-5). Means with different letters are different at the p<0.05 level by Tukey–Kramer test.

Table 3. Expression of genes associated with lipid metabolism in the liver

	SO	SO/CH	1FO	1FO/CH	2FO	2FO/CH	20FO	20FO/CH
SREBPs								
SREBP-1c	1.00 ± 0.26 ^{ab}	1.29 ± 0.38 ^a	0.97 ± 0.29 ^{ab}	1.68 ± 0.45 ^a	0.93 ± 0.04 ^{ab}	1.28 ± 0.66 ^a	0.41 ± 0.45 ^b	0.40 ± 0.13 ^b
SREBP-2	1.00 ± 0.12 ^a	0.48 ± 0.05 ^b	0.96 ± 0.18 ^a	0.48 ± 0.05 ^b	0.80 ± 0.2 ^a	0.44 ± 0.03 ^b	0.79 ± 0.15 ^a	0.40 ± 0.05 ^b
Fatty acid biosynthesis								
FAS	1.00 ± 0.47 ^a	0.46 ± 0.07 ^{ac}	0.61 ± 0.3 ^{ac}	0.41 ± 0.17 ^{ac}	0.90 ± 0.58 ^{ab}	0.35 ± 0.06 ^{bc}	0.14 ± 0.02 ^c	0.17 ± 0.11 ^c
SCD1	1.00 ± 0.40 ^a	1.21 ± 0.34 ^a	0.97 ± 0.35 ^a	1.20 ± 0.18 ^a	0.97 ± 0.41 ^a	1.14 ± 0.16 ^a	0.11 ± 0.06 ^b	0.35 ± 0.17 ^b
Cholesterol homeostasis								
HMG-CoA Reductase	1.00 ± 0.43 ^a	0.37 ± 0.08 ^{ab}	1.00 ± 0.44 ^a	0.45 ± 0.11 ^{ab}	0.93 ± 0.47 ^a	0.45 ± 0.07 ^{ab}	0.40 ± 0.11 ^{ab}	0.30 ± 0.08 ^b
ABCG5	1.00 ± 0.30 ^c	1.74 ± 0.60 ^{ab}	1.07 ± 0.24 ^c	2.09 ± 0.50 ^a	1.10 ± 0.15 ^{bc}	1.94 ± 0.65 ^{ac}	1.10 ± 0.23 ^{bc}	1.16 ± 0.29 ^{bc}
ABCG8	1.00 ± 0.27 ^{bc}	1.43 ± 0.28 ^{ac}	1.07 ± 0.40 ^c	1.67 ± 0.24 ^a	1.01 ± 0.22 ^{bc}	1.66 ± 0.34 ^{ab}	0.98 ± 0.22 ^{bc}	1.00 ± 0.28 ^{bc}
Fatty acid β-oxidation								
PPARα	1.00 ± 0.19 ^b	1.10 ± 0.33 ^{ab}	1.17 ± 0.19 ^{ab}	1.46 ± 0.31 ^{ab}	1.44 ± 0.41 ^{ab}	1.64 ± 0.06 ^a	1.58 ± 0.45 ^b	1.64 ± 0.13 ^{ab}
AOX	1.00 ± 0.21 ^d	1.20 ± 0.19 ^{cd}	1.07 ± 0.19 ^{cd}	1.40 ± 0.25 ^{bd}	1.34 ± 0.19 ^{cd}	1.49 ± 0.09 ^{bc}	1.86 ± 0.37 ^{ab}	2.07 ± 0.26 ^a
UCP2	1.00 ± 0.16 ^{cd}	0.86 ± 0.14 ^{cd}	1.20 ± 0.34 ^{bc}	0.70 ± 0.08 ^d	1.21 ± 0.22 ^{bc}	0.80 ± 0.17 ^{cd}	1.71 ± 0.11 ^a	1.57 ± 0.29 ^{ab}
Bile acid bioynthesis								
CYP7A1	1.00 ± 0.95	1.71 ± 0.82	1.28 ± 1.03	1.29 ± 1.03	0.80 ± 0.42	2.30 ± 1.20	0.35 ± 0.20	1.07 ± 0.18
CYP8B1	1.00 ± 0.38 ^b	1.78 ± 0.45 ^b	1.63 ± 0.51 ^b	1.10 ± 0.26 ^b	1.22 ± 0.53 ^b	1.57 ± 0.12 ^b	3.20 ± 0.48 ^a	3.33 ± 0.37 ^a
Antioxidant enzyme								
CAT	1.00 ± 0.10	0.97 ± 0.13	0.96 ± 0.14	1.12 ± 0.16	1.07 ± 0.06	1.08 ± 0.09	1.20 ± 0.12	1.15 ± 0.19
SOD	1.00 ± 0.01 ^c	1.03 ± 0.13 ^c	1.07 ± 0.11 ^{bc}	1.18 ± 0.13 ^{ac}	1.10 ± 0.11 ^{ac}	1.22 ± 0.17 ^{ac}	1.29 ± 0.07 ^{ab}	1.32 ± 0.07 ^a

The mRNA expression levels in liver of mice fed SO, SO/CH, 1FO, 1FO/CH, 2FO, and 2FO/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 Tukey–Kramer test.