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REGULAR PAPER

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Fish oil suppresses obesity more potently in lean mice than in diet-induced obese mice but ameliorates steatosis in such obese mice

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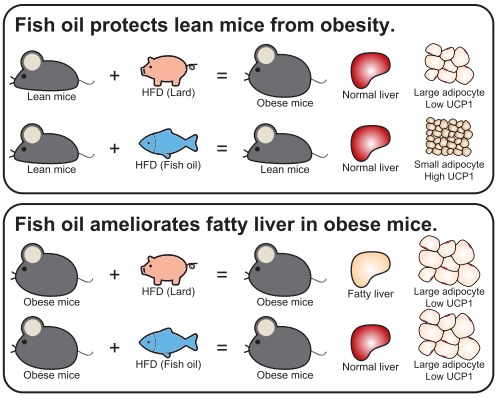
ABSTRACT

This study sought to clarify the antiobesity effects of fish oil (FO) in terms of prevention and amelioration. An isocaloric diet composed of lard or FO was given to lean C57BL/6J mice for the study of prevention and high-fat diet-induced obese (DIO) mice for the study of amelioration for 4 weeks. Body weight gain and food efficiency were potently suppressed by FO in lean mice compared to lard diet-fed mice. Uncoupling protein-1 (UCP-1) expression in inguinal white adipose tissue (WAT) was also significantly induced by FO in lean mice. FO also suppressed body weight gain and food efficiency in DIO mice but did not reduce body weight. FO ameliorated liver steatosis in DIO mice by mildly inducing UCP-1 in inguinal WAT. FO suppressed obesity more potently in lean mice than in DIO mice but ameliorated steatosis in the DIO mice.

Graphical Abstract



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Fish oil suppresses obesity more potently in lean mice than in diet-induced obese mice but ameliorates steatosis in such obese mice.

Keywords: obesity, fish oil, lard, EPA, DHA

Abbreviations: Cho: cholesterol; TG: triglyceride; DHA: docosahexaenoic acid; DIO: diet-induced obesity; EPA: eicosapentaenoic acid; FO: fish oil; HE: hematoxylin and eosin; PUFA: polyunsaturated fatty acids; PBS: phosphate-buffered saline; UCP-1: uncoupling protein-1; TNFα: tumor necrosis factor α; MCP-1: monocyte chemoattractant protein-1; WAT: white adipose tissue

The number of obese patients is increasing across the globe. Approximately 2.2 billion people are overweight; this corresponds to about one-third of the world's population. Moreover, approximately 712 million people are obese, which corresponds to about 10% of the global population (Afshin *et al.* 2017). Obesity is linked to lifestyle-related diseases such as diabetes, hypertension, dyslipidemia, and liver steatosis (Bluher 2019). Obese subjects may show hypertrophy of adipocytes, upregulation of adipocytokines such as tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1), and inflammation in white adipose tissue (WAT) (Ouchi *et al.* 2011). Inflammation of WAT impairs insulin signaling and glucose tolerance, which can cause diabetes (Smith and Kahn 2016; Czech 2020).

Excessive lipid intake causes hyperlipidemia and insulin resistance, which promotes lipid synthesis in the liver. These changes promote liver steatosis and dyslipidemia (Smith *et al.* 2020). Therefore, the prevention and amelioration of obesity are thought to be an important strategy for the prevention of a wide variety of lifestyle-related diseases.

Obesity is observed when one's energy intake exceeds energy consumption. Fatty acids possess more energy per gram than proteins and carbohydrates, and dietary lipids can activate the reward system (Fushiki 2014); thus, excess lipid intake further increases energy intake. The most typical lipid ingested with the diet is triglycerides (TGs) in which glycerol is bound to three fatty acid molecules, and the physicochemical properties of the lipid differ depending on the fatty acids.

Fatty acids are categorized as saturated and unsaturated. Palmitic acid and stearic acid, which are typical saturated fatty acids found in lard and palm oil, are mainly used as an energy source by the body. Thus, excessive intake of these fatty acids causes adipocyte hypertrophy. On the other hand, *n*-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are abundant in fish oil (FO) and their metabolites, show potent physiological activities such as antihyperlipidemic, antiinflammatory, and antithrombotic activities (Pirillo and Catapano 2013; Schunck *et al.* 2018; Preston Mason 2019). It has also been reported that FO enhances uncoupling protein (UCP)-1 expression, which is related to thermogenesis in WAT (Kim *et al.* 2015). UCP-1, expressed in the inner mitochondrial membrane, uncouples oxidative phosphorylation and releases the energy as heat (Garcia *et al.* 2018). Several food factors, including FO, show antiobesity effects by increasing UCP-1 expression in WAT and enhancing energy expenditure (Ohyama *et al.* 2016; Baskaran *et al.* 2016; Lee *et al.* 2017; Kagawa *et al.* 2020).

Previous studies describing the antiobesity effects of FO have investigated whether obesity can be prevented by feeding lean mice with a high-fat diet containing no FO and a high-fat diet containing FO (Kim *et al.* 2015; Caesar *et al.* 2015; Oliveira *et al.* 2019). However, it is unknown whether feeding a diet containing FO to obese mice improves obesity.

Table 1. Lard and fish oil-based high-fat diets composition

	LD	FD
	g/kg	
Casein	233.1	233.1
L-Cystine	3.5	3.5
Corn Starch	84.8	84.8
Maltodextrin	116.5	116.5
Sucrose	201.4	201.4
Cellulose	58.3	58.3
Soybean Oil	29.1	29.1
Lard	206.9	0
Menhaden Oil	0	206.9
Mineral Mix	11.7	11.7
Dicalcium Phosphate	15.1	15.1
Calcium Carbonate	6.4	6.4
Potassium Citrate, H_2O	19.2	19.2
Vitamin Mix	11.7	11.7
Choline Bitartrate	2.3	2.3
	kcal (%)	
Protein	20	20
Carbohydrate	35	35
Fat	45	45

The present study sought to clarify whether FO ameliorates established obesity and to confirm the preventive effects of FO on the onset of obesity. For this purpose, we fed lean mice and diet-induced obesity (DIO) mice a high-fat diet composed of lard and a high-fat diet composed of FO as an energy source.

Materials and methods

Animal experiment 1

All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Nihon University Animal Care and Use Committee (approval numbers: AP17B009 and AP19BRS085).

In the first experiment, C57BL/6J mice (8 weeks old, male; Charles River Laboratories Japan, Inc., Yokohama, Japan) were housed in a temperature-controlled room (22-23 °C) under a 12 h light/dark cycle. The mice were provided a normal chow diet (CRF-1; Oriental Yeast Co., Tokyo, Japan) and water *ad libitum*. After 2 weeks of acclimation, the mice were divided into two groups matched by body weight: the lard-diet (LD) group was given a high-fat diet containing mainly lard as a fat source (D12451; Research Diets Inc., New Brunswick, NJ, USA), and the FO-diet (FD) group was given a high-fat diet containing mainly FO as a fat source (D05122102; Research Diets Inc.) for 1 or 4 weeks. The compositions of the diets are shown in Table 1. In order to prevent the oxidation of fat in the diet, the amount of

diet to be fed at one time was stored in a container containing an oxygen absorber at –20 °C. Diets were replaced with fresh one daily during the experiment.

Following 1 or 4 weeks of LD or FD feeding, the mice were sacrificed by CO_2 euthanasia. After blood withdrawal by heart puncture with an EDTA-containing syringe, epididymal, perirenal, mesenteric, and subcutaneous WATs, and interscapular brown adipose tissue (BAT) were collected and then frozen immediately in liquid nitrogen. The samples were stored at 80 °C until analyses.

Animal experiment 2

In the second experiment, C57BL/6J mice (8 weeks old, male; Charles River Laboratories Japan, Inc.) were provided a normal chow diet (CRF-1; Oriental Yeast Co.) and water *ad libitum*. After 2 weeks of acclimation, the mice were given an LD for 8 weeks to induce obesity. These DIO mice were divided into 2 groups matched by body weight: the LD group was LD-fed for another 4 weeks, and the FD group was FD-fed for another 4 weeks. Blood and tissue samples were harvested as described above.

Histology and immunostaining

The harvested liver and inguinal adipose tissue samples were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) and embedded in Paraffin Wax II60 (Sakura Finetek Japan Co., Ltd., Tokyo, Japan). For hematoxylin and eosin (HE) staining of the liver sections, Mayer's Hematoxylin Solution (Fujifilm Wako Pure Chemical Corp., Osaka, Japan), and 1% Eosin Y Solution (Fujifilm Wako Pure Chemical Corp.) were used for the 5 µm sections. For immunostaining of the inguinal adipose tissue, 10 µm sections were subjected to Decloaking Chamber NxGen (Biocare Medial, Pacheco, CA, USA) for heat-induced epitope retrieval according to the manufacturer's instructions. After retrieval of the UCP-1 epitope, the sections were blocked with 5% bovine serum albumin in PBS, and incubated with anti-UCP-1 antibodies (1:200; Abcam, Cambridge, UK) at 4 °C overnight. Peroxidase-conjugated Affinipure Goat AntiRabbit IgG (H + L) (1:1000; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) was used as the secondary antibody; it was applied to the sections for 2 h and the horseradish peroxidase substrate was developed using an ImmPACT DAB Peroxidase Substrate Kit (Vector Laboratories, Inc., Burlingame, CA, USA) followed by counterstaining with hematoxylin. The localization of UCP-1 was observed with an Axio Imager A2 (Carl Zeiss, Oberkochen, Germany). ImageJ (Schneider *et al*. 2012) was employed to measure adipocyte sizes (µm) of 300 or more, and the cell size and cell number were summarized in a frequency distribution table.

Measurement of the plasma and liver concentrations of TGs and cholesterol (Cho)

The plasma concentrations of TGs and Cho and the contents of the lipids, which were extracted from the liver using the Bligh and Dyer method (Bligh and Dyer 1959), were determined using a Determiner TG Assay Kit (Hitachi Chemical Diagnostics Systems Co., Ltd., Tokyo, Japan) or Determiner TC Assay Kit (Hitachi Chemical Diagnostics Systems Co., Ltd.).

Reverse transcription and real-time PCR

Total RNA was extracted from liver and inguinal adipose tissue samples using RNAiso Plus (Takara Bio, Shiga, Japan). cDNA was synthesized using a PrimeScript RT Reagent Kit (Takara Bio) and real-time reverse transcription-PCR was performed using the fluorescent dye SYBR Green I with SYBR Premix Ex Taq and Perfect Real Time (Takara Bio) on the StepOne PCR system (Thermo Fisher Scientific, Waltham, MA, USA). The mouse UCP-1- and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)-specific primers used in the assay were designed based on GenBank information as listed in Table 2. The mRNA levels were quantified using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) and normalized to those of GAPDH.

Table 2. Primers used in qPCR

Gene	Forward (5′-3′)	Reverse (3′-5′)
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Gapdh	TGTGTCCGTCGTGGATCTG	GAGACAACCTGGTCCTCAGTG

Statistical analyses

All results are expressed as the mean ± standard error of the mean (SE) and were analyzed using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA). Statistical comparisons between groups were carried out using Student's *t*-tests. Differences were considered significant at $P < .05$.

Results

Effect of FO on lean mice

During the 4-week experimental period, body weight increased in a time-dependent manner in the LD-fed mice. On the other hand, the body weight was significantly lower in FD-fed mice than in LD-fed mice (Figure 1a). Body weight gain was also significantly lower in FD-fed mice than in LD-fed mice (Figure 1b); however, there were no differences in the daily food intake of the mice (Figure 1c). Food efficiency was remarkably lower in the FD-fed mice than in the LD-fed mice (Figure 1d). Consistent with these results, the weights of the epididymal, perirenal, mesenteric, and inguinal WATs and BATs were lower in FD-fed mice, but liver weight was not influenced by the FD diet (Figure 1e-j). Interestingly, the significant suppression of body weight and adipose tissue weight gain by FD was observed as early as 1 week after the start of feeding (Figure 1b, e-i).

Consuming a high-fat diet causes hyperlipidemia and elevated lipid levels in the liver (Nam *et al*. 2015). The plasma TG and Cho concentrations of the FD-fed mice at week 1 or 4 were significantly lower than those of the LD-fed mice (Figure 1k, l). Similarly, the liver TG content was significantly suppressed by FD feeding at week 4 (Figure 1m); however, no obvious differences were observed in lipid accumulation between these groups in HE-stained images of the liver tissues at 4 weeks after feeding (Figure 1n). These results indicate that FD did not cause obesity, hyperlipidemia, and hepatic lipid accumulation compared to LD.

Next, to clarify the antiobesity mechanism of FO, we focused on thermogenesis. Higher expression levels of thermogenesis-related factors were observed in mice fed a high-fat diet containing FO or n-3 PUFA, DHA, or EPA than in LD-fed mice (Kim *et al*. 2015; Oliveira *et al*. 2019). These data suggest that FO promotes energy expenditure by enhancing thermogenesis leading to its antiobesity effect. Therefore, we measured UCP-1 mRNA expression in inguinal WAT samples from each group. UCP-1 gene expression was significantly higher in FD-fed mice at week 1 than in LD-fed mice, and the same tendency was observed at week 4 (Figure 2a). Upregulation of UCP-1 protein expression was also

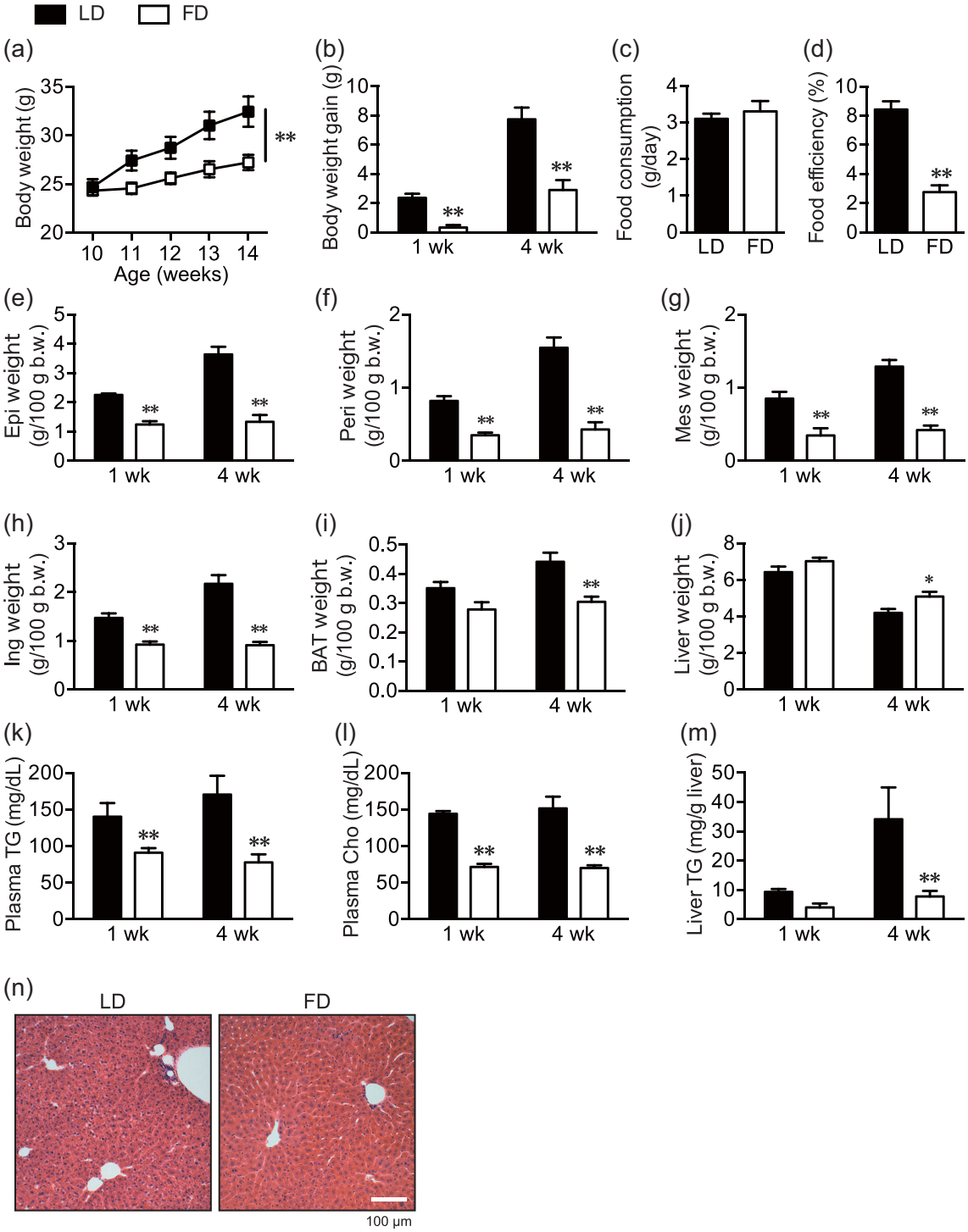

Figure 1. A high-fat diet composed of fish oil (FD) does not cause obesity compared to a high-fat diet composed of lard (LD). Ten-week-old C57BL/6J male mice were given FD or LD for 1-4 weeks. (a) Body weight; (b) body weight gain; (c) food consumption; (d) food efficiency; (e) epididymal adipose tissue (Epi) mass; (f) perirenal adipose tissue (Peri) mass; (g) mesenteric adipose tissue (Mes) mass; (h) inguinal adipose tissue (Ing) mass; (i) brown adipose tissue (BAT) mass; (j) liver mass; (k) plasma triglyceride concentration; (l) plasma cholesterol concentration; (m) liver triglyceride content; (n) representative HE-stained liver histology of FD and LD mice. Mean ± SE, $n = 6$, $^{**}P < .01$ versus the LD group.

observed by immunohistochemical staining of the inguinal WAT in FD-fed mice at both weeks 1 and 4 (Figure 2b).

We further confirmed the antiobesity effects of FO by measuring the size of adipocytes; more than 300 adipocytes in the inguinal WAT were measured. The adipocyte size of FD-fed mice tended to be smaller than that of LD-fed mice at week 1, and the tendency became remarkable at week 4 (Figure 2c). The median size of the adipocytes in LD-fed mice at week 4 was 2084 μm^2 while that in FD-fed mice was 473 μm^2. These results suggest that FO did not cause adipocyte hypertrophy and subsequent

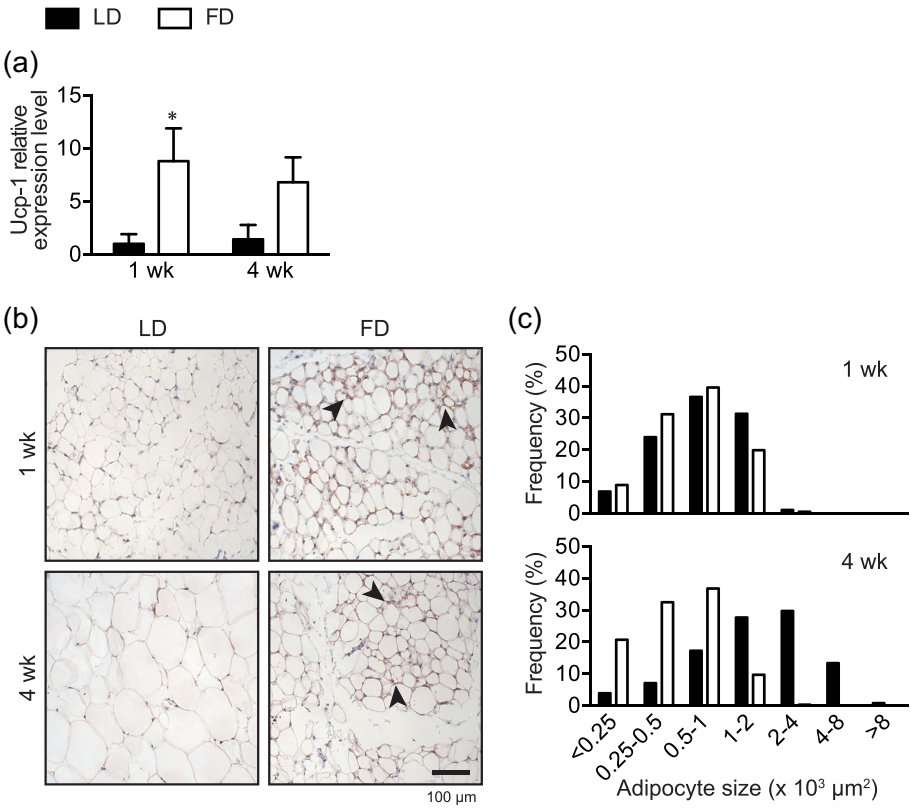

Figure 2. The formation of BAT was promoted in FD-fed mice compared to LD-fed mice. Ten-week-old C57BL/6J male mice were given FD or LD for 1 or 4 weeks. (a) UCP-1 mRNA expression level in inguinal adipose tissue. The level was expressed as ratio against LD-1wk; (b) representative immunohistochemical staining for UCP-1 in inguinal adipose tissues. A typical adipocyte expressing UCP-1 is indicated by arrowheads; (c) mean frequency of adipocyte diameter. Mean ± SE, $n = 6$, *$P < .05$ versus the LD group.

obesity by increasing thermogenesis via upregulated UCP-1 expression.

Effect of FO on DIO mice

Next, we examined whether FO can ameliorate established obesity. For the experiment, we produced DIO mice by feeding LD to C57BL/6J mice (10 weeks old) for 8 weeks. The DIO mice were employed to demonstrate amelioration of obesity by FO; the DIO mice were given FD for another 4 weeks (DIO-FD). As a control, DIO mice were further fed LD for 4 weeks (DIO-LD). The body weight increased in a time-dependent manner in DIO-LD, whereas in the DIO-FD mice the body weight slightly decreased up to 20 weeks from 18 weeks and then slightly increased again up to 22 weeks (Figure 3a). The body weight gain during the 4 weeks was almost completely suppressed in DIO-FD mice whereas a 5 g gain was observed in DIO-LD mice (Figure 3b). There were no differences in daily food intake between the DIO-LD and DIO-FD mice during the experimental period (Figure 3c); thus, the food efficiency was also significantly lower in DIO-FD mice (Figure 3d). On the other hand, the tissue weights (particularly WAT) tended to decrease at 4 weeks after FD feeding (Figure 3e). Taken together, these results indicate that FO suppressed the body weight gain of DIO mice but did not decrease the body weight of mice with established obesity.

Regarding the blood lipid level, the plasma Cho concentration was significantly lower in DIO-FD mice than in DIO-LD mice (Figure 3g), but no differences were observed in TG concentration (Figure 3f). A higher TG content and typical liver steatosis were more obvious in DIO-LD mice (Figure 3i, DIO-LD) than in lean mice (Figure 1n, LD). The typical steatosis was ameliorated in DIO-FD mice (Figure 3h, i).

Although FO suppressed body weight gain in DIO mice, both UCP-1 mRNA and protein expression in inguinal WAT were not significantly influenced by FD feeding for 4 weeks (Figure 4a, b). The adipocyte size of DIO-FD-fed mice tended to be smaller than that of DIO-LD-fed mice (Figure 4c). The median adipocyte size in DIO-LD-fed mice at week 4 was 2768 μm^2 while that in DIO-FD-fed mice was 1595 μm^2. These results suggest that FO suppressed body weight gain in DIO mice by inhibiting hypertrophy of adipocytes, but thermogenesis might not be potently involved in this mechanism.

Discussion

FO has various physiological functions in the body (Pirillo and Catapano 2013; Schunck *et al.* 2018; Preston Mason 2019). In this study, we examined whether FO shows not only a preventive but also an ameliorative effect on obesity by feeding a high-fat diet containing FO to mice before and after the onset of obesity.

In the first experiment, 10-week-old lean C57BL/6J mice were fed LD or FD. The LD-fed mice showed an increase in body weight (Figure 1a, b) and adipose tissue weight (Figure 1e-h). The liver TG content was also increased in LD-fed mice (Figure 1m). On the other hand, the increases in body weight and WAT weight were significantly lower in FD-fed mice compared to LD-fed mice (Figure 1a, b, e-h). These results indicate that a FO-containing

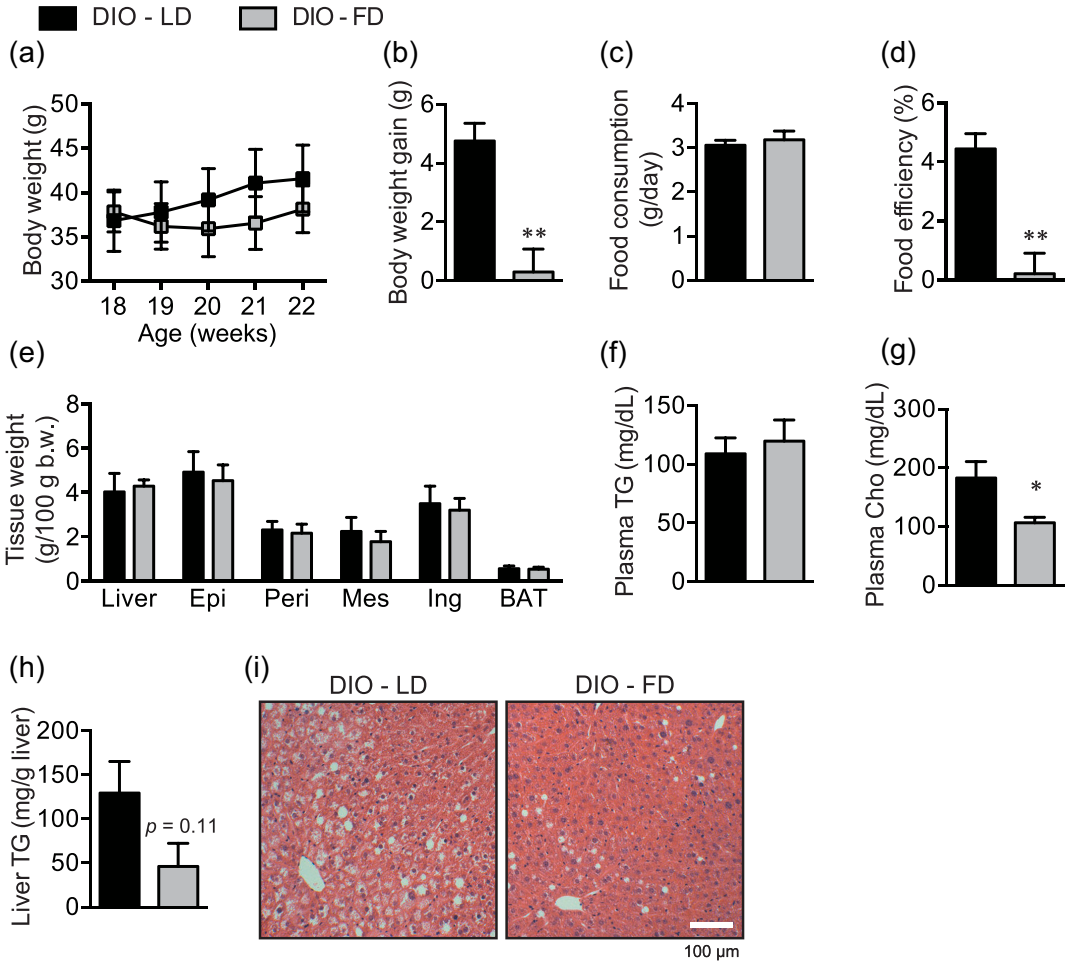

Figure 3. Feeding with FD did not worsen the obesity of DIO mice, but it could not decrease the body weight. Ten-week-old C57BL/6J male mice were given LD for 8 weeks to induce obesity. Then DIO mice were fed FD (DIO-FD) or LD (DIO-LD) for another 4 weeks. (a) Body weight; (b) body weight gain; (c) food consumption; (d) food efficiency; (e) tissue weights: liver, epididymal adipose tissue (Epi), perirenal adipose tissue (Peri), mesenteric adipose tissue (Mes), inguinal adipose tissue (Ing), and brown adipose tissue (BAT); (f) plasma triglyceride concentration; (g) plasma cholesterol concentration; (h) liver triglyceride content; (i) representative HE-stained liver histology of FD and LD mice. Mean ± SE, $n = 4$, $^{*}P < .05$ and $^{**}P < .01$ versus the DIO-LD group.

high-fat diet does not cause obesity compared to an isocaloric diet containing lard. Complications of obesity including diabetes are closely related to the mass of visceral WATs (Smith and Kahn 2016; Czech 2020). The weights of perirenal and mesenteric WATs of FD-fed mice were significantly lower than that of LD-fed mice (Figure 1f, g). These results indicate that FO may prevent the onset of diabetes induced by high fat diet.

In the second experiment employing DIO mice, the body weight gain of the DIO mice was suppressed by FD, but FO did not further decrease body weight (Figure 3a). Interestingly, the liver lipid content was reduced by FO in FD-fed mice (Figure 1m). These results suggest that FO can prevent the onset of obesity (Figure 1a), but there is little effect on weight loss once obesity is established (i.e., FO is not potent enough to ameliorate established obesity; Figure 3a). On the other hand, FO ameliorated liver steatosis (Figure 3h, i). In previous studies, mice fed a diet rich in PUFAs such as DHA and EPA showed significantly lower body weight gain than mice fed a high-fat diet containing lard (Ruzickova *et al.* 2004; Kim *et al.* 2015; Caesar *et al.* 2015; Oliveira *et al.* 2019); our results are consistent with these findings. As one of the possible mechanisms of action, *n*-3 PUFA present in FO activates transient receptor potential cation channel subfamily V member 1 (TRPV1), which is a receptor found in gastric and intestinal sensory nerves to enhance noradrenaline production. Noradrenaline induces UCP-1 production in brown adipocytes and beige cells via $\beta 3$ adrenergic receptors to upregulate thermogenesis and energy consumption, leading to antiobesity effects (Kim *et al.* 2015). In this study, UCP-1 gene and protein expression in WAT from FD-fed mice was higher than that in LD-fed mice, but the degree of browning (i.e., formation of beige cells) was weaker in DIO mice than in lean mice (Figures 2 and 4). Therefore, FO is less effective against established obesity; it does not possess enough activity to ameliorate obesity (Figures 2 and 4).

Next, we focused on the enhancement of energy consumption, which is independent of UCP-1. FO prevents diet-induced obesity even in UCP-1-deficient mice (Oliveira *et al.* 2019). It has also been reported that FO stimulates energy expenditure, independent of thermogenesis by UCP-1 and calcium uptake by sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) in both BAT and WAT (Oliveira *et al.* 2019). Regarding the UCP-1-independent enhancement of energy expenditure by FO, several lines of evidence have been published. EPA suppressed the expression of lipid synthesis-related enzymes in the liver and EPA enhanced energy consumption (Sato *et al.* 2010). Furthermore, PUFA and

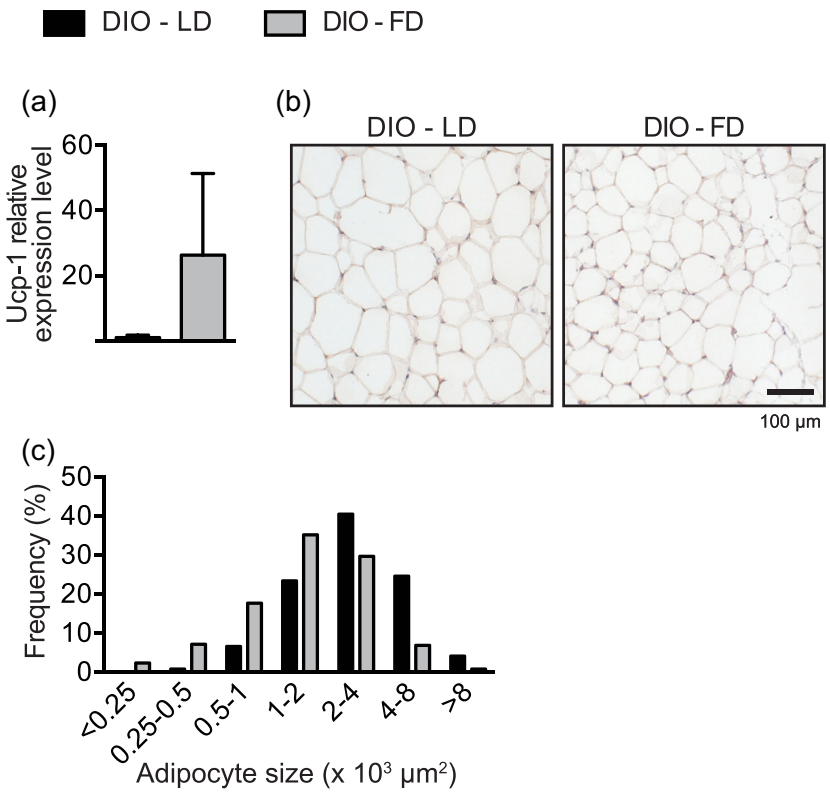

Figure 4. FD did not potently promote the formation of BAT in the DIO mice. (a) UCP-1 mRNA expression in inguinal adipose tissue. The level was expressed as ratio against LD-1wk; (b) representative inguinal adipose tissue immunohistochemically stained with UCP-1 (indicated by arrowheads); (c) representative immunohistochemical staining for UCP-1 in inguinal adipose tissues. Mean ± SE, $n = 4$.

FO inhibited lipid synthesis in the liver by suppressing the expression of SREBP1 (Yahagi *et al.* 1999; Xu *et al.* 1999; Kim *et al.* 1999). FO also promoted hepatic β-oxidation (Mori *et al.* 2007). In patients with obesity and type 2 diabetes, expression of hepatic lipid synthesis-related genes is upregulated by hypomethylation, and lipid synthesis in the liver is upregulated accordingly (Kirchner *et al.* 2016). In this study, we also observed lower levels of liver TGs, plasma TGs, and Cho in FD-fed mice than in LD-fed mice. These data suggest that FO suppressed lipid synthesis and upregulated β-oxidation leading to amelioration of a fatty liver (Figures 1k-m and 3f-i); however, the detailed relationship between suppression of lipid synthesis and enhancement of energy consumption remains unclear. The ratio of energy consumption by the liver against the whole-body energy metabolism accounts for about 20% (Wang *et al.* 2012); thus, enhancing lipolysis in the liver may lead to the prevention and amelioration of obesity. It has been reported that inflammation of WAT induced by high fat diet in obese diabetic mice was prevented by *n*-3 PUFA (Todoric *et al.* 2006). TNF-α protein level in WAT was also decreased by FO (Muurling *et al.* 2003). Since the lipid metabolism was influenced by inflammation (Minxuan *et al.* 2019), antiinflammatory activity of FO may also contribute the amelioration of liver steatosis induced by high fat diet. Further studies focusing on the antiinflammatory activity of FO are needed to clarify the relationship between the suppression of lipid synthesis in the liver and the upregulation of energy expenditure by FO.

FO-fed mice had the same food intake as LD-fed mice, but their body weight gain was suppressed. Thus, their food efficiency was significantly lower than that of LD-fed mice (Figures 1a-d and 3a-d). These data suggest that FO can be characterized as a lipid that is hard to store and easily metabolized. Regarding the chemical structure of lipids and their metabolism, medium-chain fatty acids are directly transported from the small intestine via the portal vein to the liver, where they are taken up by mitochondria in a carnitine-independent manner and rapidly metabolized (Papamandjaris *et al.* 1998). By contrast, long-chain fatty acids are absorbed from the small intestine via lymphatic vessels, and then enter the blood vessels via the subclavian vein for transportation throughout the body, suggesting that it takes longer for the metabolism of long-chain fatty acids after food intake (Papamandjaris *et al.* 1998). In a study that used stable isotopes, the intake of *n*-3 PUFAs inhibited the absorption of saturated fatty acids, including palmitic acid and stearic acid, while *n*-6 PUFAs did not show such effects (Yang *et al.* 2017). Taken together, these findings suggest that FO also affects the absorption of saturated fatty acids. As a next step, it will be necessary to evaluate the contribution of hepatic lipid metabolism and lipid absorption and excretion into the feces of FO-fed mice.

All studies on the antiobesity effects of FO reported to date have demonstrated the preventive effects of FO on obesity using comparisons with control mice fed a high-fat diet (Ruzickova *et al.* 2004; Kim *et al.* 2015; Caesar *et al.* 2015; Oliveira *et al.* 2019). Thus, it is unclear whether FO can actually ameliorate obesity. In this study, we confirmed that FO prevents obesity, and also that FO does not worsen the established obesity of DIO mice. Further, it ameliorated the typical liver steatosis observed in obesity by decreasing lipid accumulation in the liver. FO is expected to be a promising material for the

prevention of lifestyle-related diseases such as steatosis related to obesity.

Supplementary material

Supplementary material is available at *Bioscience, Biotechnology, and Biochemistry* online.

Author contribution

S.O., T.H., and T.S. designed the research; S.O., E.I., R.N., T.O., T.I., M.S., and A.M. conducted the research; and S.O., A.M., Y.O.M., T.H., and T.S. analyzed the data and wrote the paper. All authors read and approved the final manuscript.

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Disclosure statement

The authors declared no conflicts of interest.

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