

Article

Fish Oil Increases Diet-Induced Thermogenesis in Mice

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Abstract: Increasing energy expenditure (EE) is beneficial for preventing obesity. Diet-induced thermogenesis (DIT) is one of the components of total EE. Therefore, increasing DIT is effective against obesity. We examined how much fish oil (FO) increased DIT by measuring absolute values of DIT in mice. C57BL/6J male mice were given diets of 30 energy% fat consisting of FO or safflower oil plus butter as control oil (Con). After administration for 9 days, respiration in mice was monitored, and then the data were used to calculate DIT and EE. DIT increased significantly by 1.2-fold in the FO-fed mice compared with the Con-fed mice. Body weight gain was significantly lower in the FO-fed mice. FO increased the levels of uncoupling protein 1 (*Ucp1*) mRNA and UCP1 protein in brown adipose tissue (BAT) by 1.5- and 1.2-fold, respectively. In subcutaneous white adipose tissue (subWAT), the levels of *Ucp1* mRNA and UCP1 protein were increased by 6.3- and 2.7-fold, respectively, by FO administration. FO also significantly increased the expression of markers of browning in subWAT such as fibroblast growth factor 21 and cell death-inducing DNA fragmentation factor α -like effector a. Thus, dietary FO seems to increase DIT in mice via the increased expressions of *Ucp1* in BAT and induced browning of subWAT. FO might be a promising dietary fat in the prevention of obesity by upregulation of energy metabolism.

Keywords: brown adipose tissue; browning; energy expenditure; n-3 fatty acid; uncoupling protein; white adipose tissue



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1. Introduction

Obesity results when energy intake continuously exceeds energy expenditure (EE). Total daily energy expenditure (TEE) is comprised of multiple components such as basal metabolic rate, diet-induced thermogenesis (DIT) and physical activity-related EE [1]. DIT is defined as an increase in EE above that of the fasting state and is related to digestion, intestinal absorption of nutrients and storage of these nutrients [2]. One of the methods to prevent overweight and obesity is to increase energy consumption by upregulation of DIT [3].

Brown adipose tissue (BAT) is the main site for the induction of DIT and cold-induced thermogenesis, which significantly contributes to controlling body temperature and EE [4]. Although BAT is considered to be abundant in small rodents and human infants and decreases with aging in human [5], recent studies showed that functional BAT was identified in adult human [6,7]. The thermogenic ability of BAT is principally dependent on uncoupling protein 1 (UCP1) [8,9]. UCP1 facilitates uncoupling of mitochondrial substrate oxidation from ATP production, which leads to energy release as heat from free fatty acid oxidation [4].

UCP1-ablated mice consumed less oxygen than wild-type mice during the eating period, that is, DIT was UCP1-dependent [10]. UCP1-deficient mice maintained in a room at 23 °C developed obesity with age; therefore, UCP1 may play an important role against

obesity [11]. UCP1 gene polymorphism (−3826 A/G) showed lowered capacity of thermic effect in response to dietary intake in healthy boys aged 8–11 years [12]. Thus, the function of UCP1 and activity promoting the activation of BAT greatly contribute to the increase of DIT.

BAT is strongly activated by exposure to cold and by pharmacological effects, such as that of β 3-adrenergic receptor agonist [6,13,14]. Moreover, it has been reported that BAT is activated by food ingredients such as capsinoids, thereby contributing to a reduction in body fat [15,16]. Fish oil (FO) also has anti-obesity effects in humans [17–19]. FO contains a high content of n-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which must be obtained from the diet or synthesized from alpha-linolenic acid in the body [20–22]. DHA and EPA bind to peroxisome proliferator-activated receptor (PPAR) α and thereby activate PPAR α [23,24], which is highly expressed in BAT [25]. PPAR α binds to the PPAR response element of the *Ucp1* gene to increase mRNA expression of *Ucp1* [26].

Recently, beige adipose tissue, which is produced by the browning of white adipose tissue (WAT), has been reported as a third type of adipose tissue in addition to WAT and BAT [27–29]. Beige adipocytes are strongly induced by some environmental conditions and external cues such as exposure to cold and some pharmacological treatments, and they have potent thermogenic ability similar to that of classical brown adipocytes [30]. FO treatment leads to the browning of WAT, increases thermogenic genes such as *Ucp1* [31–33], stimulates thermogenesis, as measured by rectal temperature [34,35], and increases EE without changes in food intake [36]. These studies only suggest the possibility that FO influences DIT, however, and how much FO actually increases DIT is still unknown.

We recently developed a new technique to measure absolute DIT values in mice by applying a methodology used in the measurement of DIT in human to mice using a respiratory chamber [37]. In the present study, we showed how much FO increased DIT through activation of BAT and browning of WAT. An increase in DIT may have potential impact on anti-obesity and therapy for diabetes [6,7,38], and the evidence shown in this study indicates that FO might be a promising dietary fat.

2. Results

2.1. Effects of Fish Oil Supplementation on DIT, EE, Activity and RER

Energy metabolism of mice was measured after 9 days of feeding of each experimental diet. The measurements of O₂ consumption, CO₂ production and activity (defined as the count per minute of any movement made by mouse) of the mice were carried out over a 22-h period. The DIT of the control fat (Con)-fed mice began to increase as soon as they started eating, was maintained at a high level during the dark period, and then decreased toward the end of the dark period (Figure 1a). However, DIT increased again after the start of the light period. Similar changes were observed in the FO-fed mice (Figure 1a). When comparing the DIT in the Con- and FO-fed mice every hour, DIT in the FO-fed mice was significantly higher at 0000 and 0300. EE in the dark period and light period was not different in the Con- and FO-fed mice (Dark: Con, 7615 ± 76 cal/h/kg^{0.75}; FO, 7582 ± 87 cal/h/kg^{0.75}; Light: Con, 6712 ± 83 cal/h/kg^{0.75}; FO, 6641 ± 92 cal/h/kg^{0.75}). However, DIT in the dark period was higher in the FO-fed mice than that in the Con-fed mice (Dark: Con, 1275 ± 22 cal/h/kg^{0.75}; FO, 1541 ± 32 cal/h/kg^{0.75}, $p < 0.01$; Light: Con, 1509 ± 47 cal/h/kg^{0.75}; FO, 1674 ± 46 cal/h/kg^{0.75}). There was no difference in activity every hour between the two groups (Figure 1b). Activity in the dark period and light period was not different in the Con- and FO-fed mice (Dark: Con, 238.2 ± 13.2 count/min; FO, 221.2 ± 23.4 count/min; Light: Con, 95.4 ± 5.8 count/min; FO, 96.5 ± 11.7 count/min). The respiratory exchange ratio (RER) in the FO-fed mice was higher at 0600 and 1300 than that in the Con-fed mice, but there was no significant difference at other times (Figure 1c). RER in the dark period and light period was not different in the Con- and FO-fed mice (Dark: Con, 0.881 ± 0.010; FO, 0.901 ± 0.006; Light: Con, 0.860 ± 0.005; FO, 0.893 ± 0.009). The total energy intake during DIT measurement was almost the same in the Con- and

the FO-fed mice (Figure 2a). Total DIT over 22 h was calculated from the area under each curve. Total DIT in the FO-fed mice was 1.2-fold higher than that in the Con-fed mice (Figure 2b). TEE over 22 h was also calculated from the area under each curve. The values of activity and TEE between the two groups were not different (Figure 2c,d). DIT (%) versus calorie intake was calculated by dividing total DIT by total calorie intake and is indicated as $DIT_{/intake}$ in Figure 2e. $DIT_{/intake}$ was 11.2% for the FO-fed mice, which was 1.2-fold higher than that for the Con-fed mice. DIT (%) versus TEE was calculated by dividing total DIT by TEE and is indicated as $DIT_{/TEE}$ in Figure 2f. $DIT_{/TEE}$ for the FO-fed mice was 22.3%, which was also 1.2-fold higher than that for the Con-fed mice.

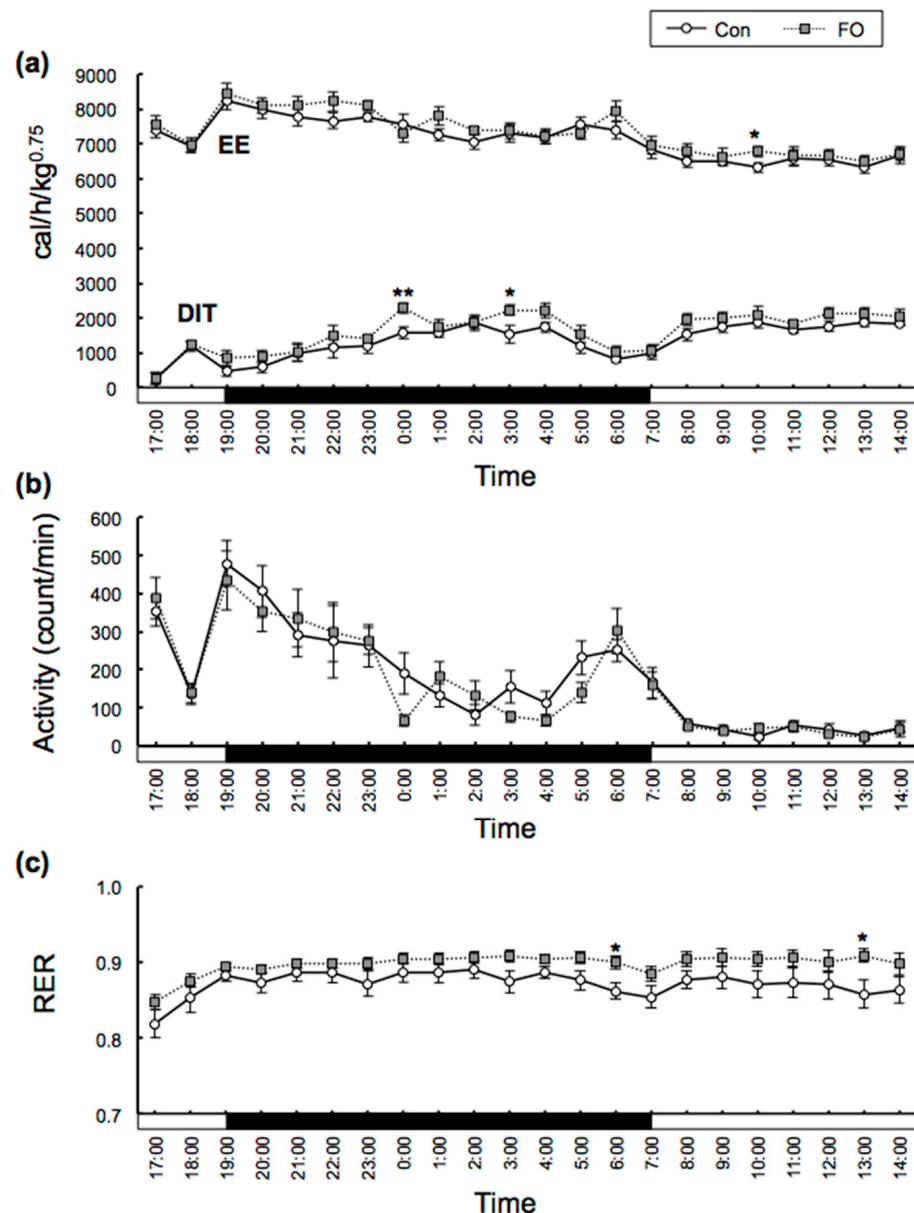


Figure 1. Time course of diet-induced thermogenesis (DIT), energy expenditure (EE), activity and respiratory exchange ratio (RER) in the control fat (Con)- and fish oil (FO)-fed male mice. The measurements were carried out over a 22-h period. The data of EE (upper lines), DIT (lower lines) (a), activity (b) and RER (c) are shown for every hour. White circles and gray squares represent data from the Con- and the FO-fed mice, respectively. The black and white bars on the x axis represent dark and light cycles, respectively. Values are mean \pm SEM (n = 7). * $p < 0.05$, ** $p < 0.01$ vs. Con-fed mice. Significant differences between two groups were tested by Student *t*-test.

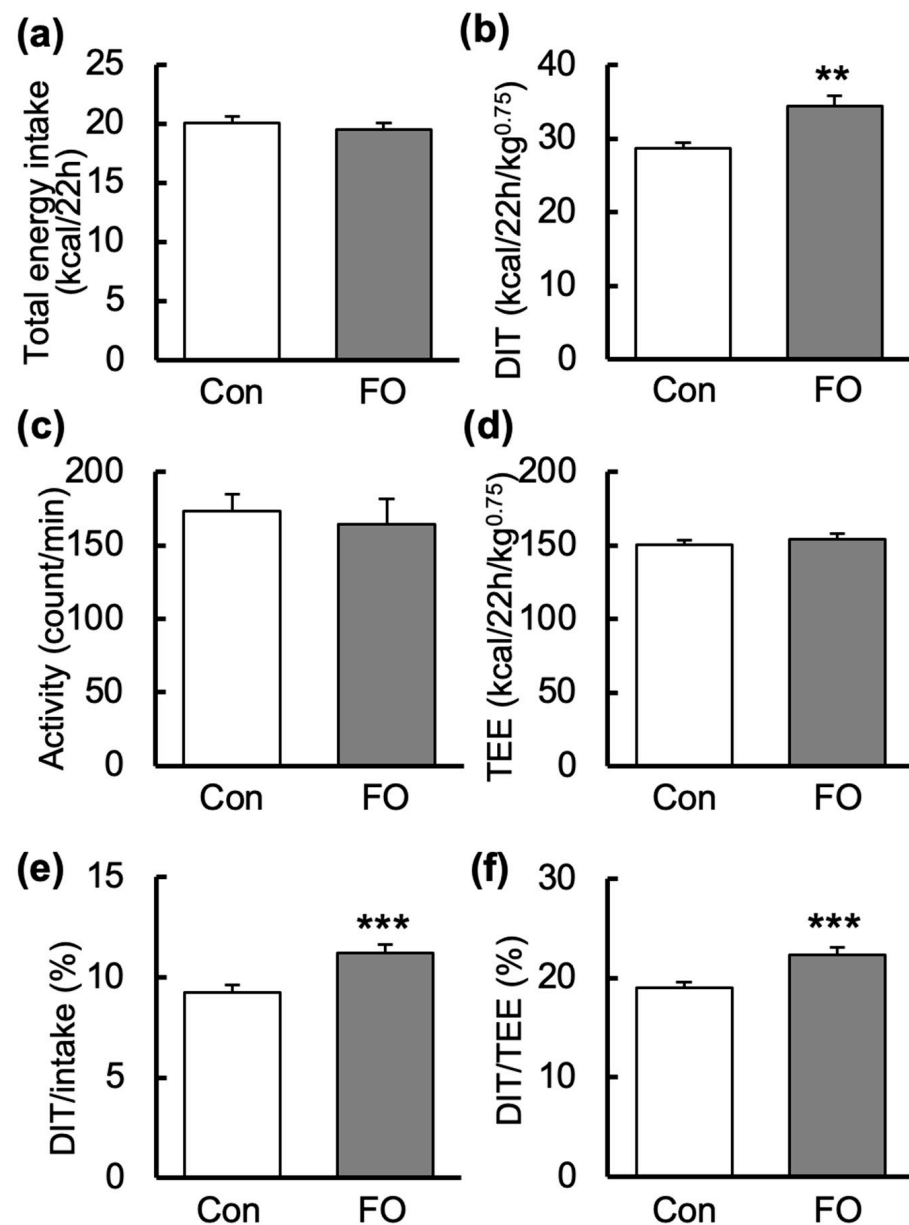


Figure 2. Values of total energy intake, diet-induced thermogenesis (DIT), activity and total energy expenditure (TEE) during DIT measurement in the control fat (Con)- and fish oil (FO)-fed male mice. Total energy intake (a) at measurement of energy metabolism was estimated by subtracting the food weight at the completion of measurement from the initial food weight measurement. The values of total DIT (b), activity (c) and TEE (d) were calculated from measurements taken over 22 h under the fed condition. DIT/intake (e) and DIT/TEE (f) were calculated by dividing total DIT by total calorie intake and by TEE, respectively. White and gray columns represent data from the Con- and FO-fed mice, respectively. Values are mean \pm SEM (n = 7). ** $p < 0.01$, *** $p < 0.001$ vs. Con-fed mice. Significant differences between two groups were tested by Student *t*-test.

2.2. Body Weight and Tissue Weights of Con- and FO-Fed Mice

The mean energy intake was similar between the Con- and the FO-fed mice during the 10-day administration period (Con, 17.6 ± 0.8 kcal/day; FO, 17.7 ± 0.6 kcal/day, $p = 0.97$). Although final body weight (BW) was not different between the Con- and FO-fed mice, the BW gain in the FO-fed mice was significantly lower than that in the Con-fed mice during the 10-day period (Con, $10.3 \pm 1.4\%$; FO, $6.6 \pm 1.0\%$, $p < 0.05$). The weights of subcutaneous WAT (subWAT) in the FO-fed mice were not different from those in the

FO-fed mice ($p = 0.07$, Table 1). However, the weights of epididymal WAT and mesenteric WAT in the FO-fed mice were lower than those in the Con-fed mice. The weight of BAT was not affected by FO supplementation.

Table 1. BW and weights of tissues in Con- and FO-fed mice.

BW/Tissues	Con-Fed	FO-Fed
BW at start (g)	23.1 ± 0.4	23.1 ± 0.2
Final BW (g)	25.9 ± 0.5	25.2 ± 0.4
BAT (g)	0.087 ± 0.006	0.083 ± 0.007
Subcutaneous WAT(g)	0.329 ± 0.029	0.251 ± 0.026
Epididymal WAT (g)	0.462 ± 0.041	0.326 ± 0.018 *
Mesenteric WAT (g)	0.149 ± 0.024	0.081 ± 0.008 *
Liver (g)	1.19 ± 0.03	1.22 ± 0.04

Values are mean ± SEM (n = 7). * $p < 0.05$ vs. Con-fed mice. Significant differences between two groups were tested by Student *t*-test. BW: body weight; Con: control; FO: fish oil; BAT: brown adipose tissue; WAT: white adipose tissue.

2.3. Serum Chemicals of Con- and FO-Fed Mice

Because the weights of the epididymal WAT and mesenteric WAT in the FO-fed mice were lower than those in the Con-fed mice, we analyzed serum concentrations of glucose, non-esterified fatty acid (NEFA), triglyceride (TG) and total cholesterol (TC). The concentrations of serum glucose in the Con- and the FO-fed mice were the same (Table 2). However, the serum concentrations of NEFA, TG and TC in the FO-fed mice were significantly lower than those in the Con-fed mice (Table 2).

Table 2. Serum chemicals of Con- and FO-fed mice.

	Con-Fed	FO-Fed
Glucose (mg/dL)	169.6 ± 15.8	177.1 ± 11.4
NEFA (mEq/L)	0.83 ± 0.07	0.49 ± 0.06 **
TG (mg/dL)	179.9 ± 25.1	70.3 ± 16.4 **
TC (mg/dL)	179.0 ± 13.4	102.3 ± 3.7 ***

Values are mean ± SEM (n = 7). ** $p < 0.01$, *** $p < 0.001$ vs. Con-fed mice. Significant differences between two groups were tested by Student *t*-test. Con: control; FO: fish oil; NEFA: non-esterified fatty acid; TG: triglyceride; TC: total cholesterol.

2.4. Effects of FO Supplementation on BAT

To confirm the mechanism of increase of DIT in the FO-fed mice, we examined expression profiling of the *Ucp1* gene and UCP1 protein. FO supplementation resulted in a 1.5-fold increase in *Ucp1* mRNA in BAT (Figure 3a). UCP1 protein expression was also analyzed (n = 7 in each group), and representative data (n = 2 in each group) indicating a 1.2-fold increase in expression are shown in Figure 3b. The mRNA expression of *Ppara*, which is one of the nuclear transcription factors whose activation leads to increased fatty acid β -oxidation [39], was significantly increased by FO supplementation (Figure 3a). However, FO supplementation did not affect the mRNA expressions of target genes carnitine palmitoyltransferase I (*Cpt 1*), acyl-CoA oxidase (*Aco*) and medium-chain acyl-CoA dehydrogenase (*Mcad*) (Figure 3a). Fibroblast growth factor 21 (*Fgf21*) expression was also not increased by FO administration (Figure 3a). The expression of the mitochondria biogenesis marker peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α) and that of crucial thermogenesis biomarker type 2 iodothyronine deiodinase (Dio2) in the mice was not different between the two groups (Figure 3a). No difference in β 3-adrenergic receptor (β 3-AR) mRNA was observed in BAT (Con, 100.0 ± 7.0%; FO, 93.0 ± 11.4%).

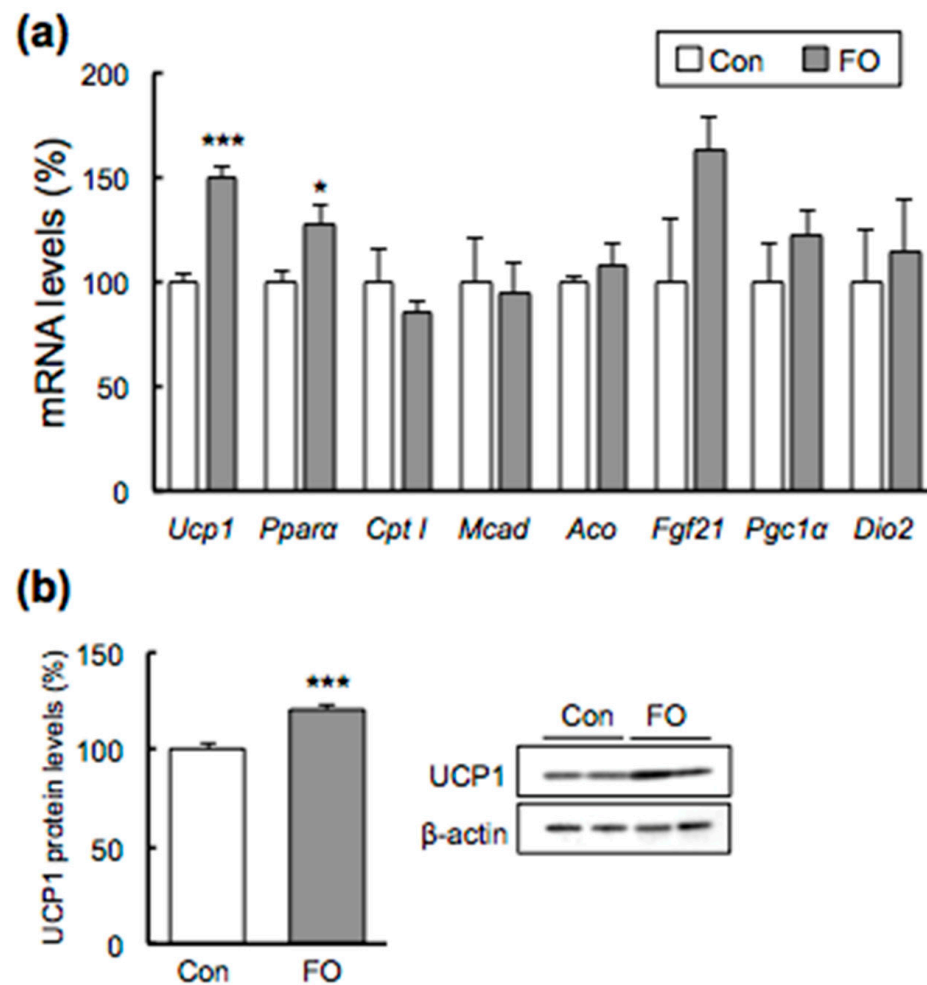


Figure 3. Effect of fish oil (FO) supplementation on gene expression and UCP1 protein levels in brown adipose tissue. mRNA levels of *Ucp1* and *Ppara* and its target genes (a) and UCP1 protein (b) were assessed by quantitative RT-PCR or western blotting. β -actin was used as the normalization control. The percent of mRNA and protein levels relative to those of Con-fed mice are indicated. White and gray columns represent data from the Con- and FO-fed mice, respectively. Values are mean \pm SEM (n = 7). * $p < 0.05$, *** $p < 0.001$ vs. Con-fed mice. Significant differences between two groups were tested by Student *t*-test.

2.5. Effects of FO Supplementation on Gene Expression in subWAT

FO dramatically increased *Ucp1* mRNA expression by 6.3-fold in the subWAT (Figure 4a). UCP1 protein levels were also analyzed (n = 7 in each group), and representative data (n = 2 in each group) indicating a 2.7-fold increase compared with those in the Con-fed mice are shown in Figure 4b. FO supplementation also caused higher expressions of *Ppara* and its target genes of *Cpt1*, *Aco* and *Mcad* in comparison to those in the Con-fed mice (Figure 4a). *Fgf21* expression in the FO-fed mice was also increased by 2.3-fold compared with that in the Con-fed mice (Figure 4a). β 3-AR mRNA expression was higher in subWAT from the FO-fed mice than that in the Con-fed mice (Con, $100.0 \pm 17.9\%$; FO, $246.1 \pm 40.4\%$, $p < 0.001$).

Among the brown fat-selective genes, expression of cell death-inducing DNA fragmentation factor α -like effector a (Cidea) was significantly increased by 3.9-fold in the FO-fed mice compared with that in the Con-fed mice, whereas that of PR domain containing 16 (Prdm16) mRNA was not different (Figure 4a).

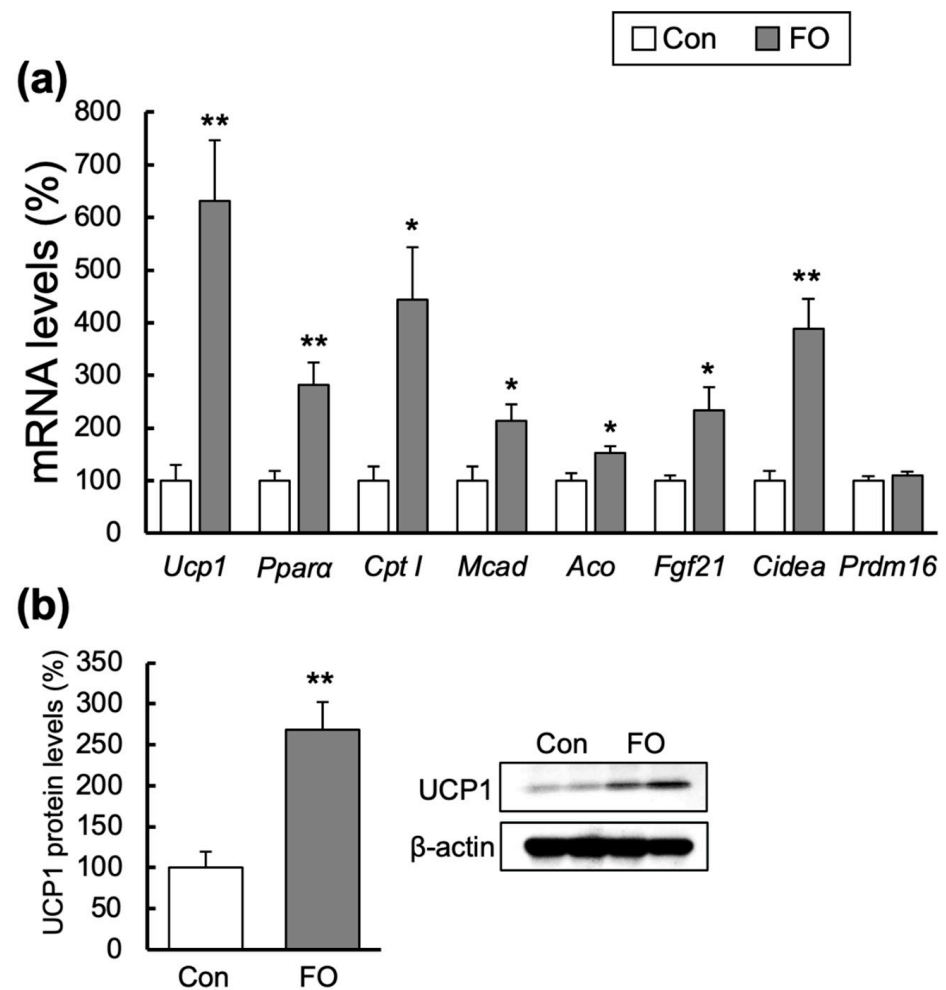


Figure 4. Effect of fish oil (FO) supplementation on gene expression and UCP1 protein levels in subcutaneous white adipose tissue. The mRNA levels of *Ucp1*, *Ppara* and its target genes and beige adipocyte-specific gene (a) and UCP1 protein (b) were assessed by quantitative RT-PCR or western blotting. β -actin was used as the normalization control. The percent of mRNA and protein levels relative to those of control fat (Con)-fed mice are indicated. White and gray columns represent data from the Con- and the FO-fed mice, respectively. Values are mean \pm SEM (n = 7). * $p < 0.05$, ** $p < 0.01$ vs. Con-fed mice. Significant differences between two groups were tested by Student *t*-test.

2.6. Effects of FO Supplementation on Gene Expression in Liver

As the effects of supplementation could also be caused by increased metabolism in the liver, we analyzed gene expressions in the liver related to fatty acid β -oxidation and fatty acid synthesis. As shown in Table S1, fatty acid β -oxidation was induced the FO-fed mice, and fatty acid synthesis was decreased.

3. Discussion

We found that FO increased DIT in mice by 1.2-fold along with the activation of BAT caused by the increased expression of UCP1 and the browning of subWAT. As females are reported to produce less heat than males, we used male mice for our experiment [40]. We observed DIT in both the light period and dark period, although it was higher in the latter because mice eat principally in the dark period. Actually, the Con and FO groups of mice took about 70–80% and 20–30% of their total food intake in the dark and light periods, respectively. It appeared that maintenance of DIT in the light period was caused by feeding in the light period (Figure 1a).

In this study, FO increased the expression of the *Ucp1* gene in BAT by 1.5-fold. Other researchers also reported that FO administration both in vitro and in vivo induced the increased expression of *Ucp1* in BAT. *Ucp1* mRNA expression and UCP1 protein levels were both significantly increased in brown progenitor cells isolated from interscapular BAT supplemented with EPA [35]. EPA also increased mitochondrial content in a dose-dependent manner in HIB 1B brown adipose cells [41]. EPA administration to C57BL/6J mice for 11 weeks, and DHA-enriched FO (DHA 25%, EPA 8%) or EPA-enriched FO (DHA 12%, EPA 28%) administration to mice for 10 weeks, significantly increased UCP1 protein levels and *Ucp1* mRNA expression in BAT [34,42]. UCP1 activity in BAT was significantly increased in rats fed with EPA or a mixture of EPA and DHA for 4 weeks by GDP binding [43]. These reports support our results that UCP1 expression was significantly increased by FO administration in BAT (Figure 3a,b). The nuclear receptor PPAR α regulates the expression of *Cpt 1*, *Mcad* and *Aco*, which are involved in the fatty acid β -oxidation [41,44,45]. FO administration increased the expression of *Ppar α* mRNA by 1.3-fold ($p < 0.05$), but expressions of its target genes *Cpt 1*, *Mcad* and *Aco* were not affected in BAT, although that of the other PPAR α target gene, *Ucp1*, increased (Figure 3a). Kim et al. reported that the expression of *Cpt 1* mRNA in BAT of mice fed EPA-enriched FO increased significantly compared with that of control mice. In contrast, the expression of *Cpt 1* mRNA did not increase in mice fed DHA-enriched FO, which has a similar fatty acid ratio as in the present study [34]. The reason why EPA-enriched FO could, but DHA-enriched FO could not, induce *Cpt 1* expression in BAT is currently not clear. Further study will be required to reveal the mechanism.

FO also increased the expressions of the *Ucp1* gene and other genes related to the fatty acid β -oxidation in subWAT (Figure 4a). In terms of the marker of browning, *Cidea* mRNA was increased by 3.9-fold, but the expression of *Prdm16* was not increased (Figure 4a). FO enhances fatty acid oxidation through PPAR α activation in WAT and causes browning of subWAT [34,46]. When cells derived from subcutaneous adipocytes from overweight females were treated with 200 μ M EPA, expressions of UCP1 and *CIDEA* mRNA increased significantly. The mRNA expression of *PRMD16* increased significantly with 100 μ M EPA treatment but not with 200 μ M EPA treatment [31]. When the stromal vascular cells isolated from subWAT of C57BL/6J mice were treated with 200 μ M EPA during a differentiated process, the expressions of fatty acid β -oxidation-related genes *Ucp1*, 2, 3 and *Cpt 1*, and *Cidea*, increased significantly, but that of *Prdm16* was still not increased as in our results [32]. The reasons for FO causing different expressions of *Cidea* and *Prdm16* are currently unknown. Due to the increased expressions of *Ucp1* and *Cpt 1* mRNA in FO-fed mice, the brown adipocyte-like phenotype was induced in subWAT [33]. The PPAR α agonist is known to promote browning in subWAT [47,48] and increase the body temperature [48]. Contrary to these reports, UCP1 protein is reported to be very low or undetectable in subWAT even though mice were fed FO [42]. Our results supported the findings that FO administration markedly increased UCP1 expression in subWAT and induced subWAT browning. Beige adipocytes were shown to have potent thermogenic ability comparable to classical BAT [30], and the thermogenic density and total quantitative contribution in subWAT were maximally one-fifth and one-third of all BAT mitochondria, respectively [49]. Thus, the classical BAT depots would still be predominate in thermogenesis, but the browning of WAT would also contribute to thermogenesis. Sato et al. recently showed that phospholipase A2 group IID, which is expressed in M2-type macrophages in WAT, released n-3 fatty acid and increased energy expenditure and rectal temperature by facilitating subWAT browning, which ameliorated diet-induced obesity [50]. Thus, FO-caused browning of WAT might also contribute to inducing DIT.

FGF21 is reported to have an endocrinological role in BAT and WAT [51,52]. The expression of *Fgf21* mRNA in subWAT increased dramatically in mice after exposure to cold [52]. Moreover, the differentiated primary subWAT treated with β -agonist synthesized and secreted FGF21, suggesting that adipose FGF21 may act mainly in a paracrine/autocrine manner [52]. However, similar to the previous research concerning FO [34], the expression

of *Fgf21* did not increase with FO administration in BAT (Figure 3a). However, contrary to that report, *Fgf21* expression in the present study was significantly increased by FO administration in subWAT (Figure 4a). This result leads us to the hypothesis that increased *Fgf21* of subWAT might induce the browning of WAT observed in the present study.

FO is reported to induce UCP1 expression in BAT and WAT via the sympathetic nervous system and transient receptor potential vanilloid 1 [34]. In the present study, β 3-AR mRNA expression was higher in subWAT from the FO-fed mice than that in the Con-fed mice. However, no difference in β 3-AR mRNA was observed in BAT. Although we did not determine the direct influence of fish oil on sympathetic flow, over the short term of 10 days, FO intake might induce UCP1 expression in subWAT via the sympathetic nervous system at least in part.

G-protein-coupled receptor 120 (GPR120), a receptor for n-3 polyunsaturated fatty acids, was also suggested to contribute to thermogenic activation in BAT and WAT by n-3 fatty acids by suppressing tissue inflammation induced by macrophages, especially in obese mice [53–56]. We used non-obese mice, and the expression of *Gpr120* was not affected in BAT and subWAT by FO supplementation (data not shown).

A systematic review indicated that EPA and DHA lowered serum lipid levels such as TG concentration [57]. Some mechanisms of serum lipid lowering by FO have been reported. EPA increased lipid oxidation in rat liver and reduced serum lipids [58]. FO also lowered serum lipids in adult human subjects [59]. We previously reported that FO administration at the same dose as in the present study decreased fatty acid synthesis genes such as acetyl-CoA carboxylase and increased fatty acid oxidation genes such as *Cpt 1*, *Mcad* and *Aco* in mouse liver [60]. The rate limiting step in mitochondrial fatty acid oxidation is mediated by CPT I [61]. Even though CPT I activity in WAT was still low compared with that in liver and BAT in rat [62], activation of CPT I by overexpression of CPT I in 3T3-L1 adipocytes reduced NEFA release [63]. These FO-induced mechanisms in liver and WAT may contribute to lowering of the serum lipid levels. In general, enhanced fatty acid oxidation in the whole body is related to decreased RER. However, in human, RER correlated negatively with plasma palmitate concentrations [64]. In the present study, FO administration caused decreased serum concentrations of NEFA and TG (Table 2). We showed here that the RER of the FO-fed mice was slightly higher than that of the Con-fed mice (Figure 1c), although not significantly so. This was probably due to the reduced serum lipid levels in the FO-fed mice.

The short period of FO administration of 10 days in the present study did not result in weight loss, but weight gain and the weights of epididymal and mesenteric WAT were significantly reduced. Mice fed 21.42 or 42.84 energy% (en%) FO for 6 weeks significantly reduced BW by about 1.5 g or 4 g, respectively [36]. It is likely that mice need to be fed FO for a longer period of time to reduce their weight. BAT-positive subjects would undergo higher DIT than BAT-negative subjects [65]. Thus, BAT activation is expected to have an anti-obesity effect. Interestingly, BAT-positive subjects (young healthy men) showed an increase in EE after oral ingestion of capsinoids (9 mg) [15]. Moreover, capsinoids 6 mg/day taken orally for 12 weeks promoted loss of human abdominal fat [66]. FO supplementation in the present study resulted in a 1.2-fold increase in $DIT_{/intake}$ (Figure 2e). In human, DIT uses 10% of the daily energy intake [67]. The estimated energy requirement for adult men is about 2500 kcal/day [68], and the energy consumed by DIT was calculated to be about 250 kcal/day, and 300 kcal/day if multiplied by 1.2. Thus, a 1.2-fold increase in DIT was estimated to maximally increase energy consumption by 50 kcal/day. Adult human adipose tissue contains 71.6% crude fat [69]. Therefore, an increase in DIT by 1.2-fold was estimated to indicate fat burning of 500 g of adipocytes over about 2 months.

In conclusion, we first showed that FO supplementation significantly increased DIT by 1.2-fold. $DIT_{/intake}$ and $DIT_{/TEE}$ for the FO-fed mice were 11.2% and 22.3%, respectively. The FO-increased DIT was complemented by the increased expression of UCP1, activation of BAT and subWAT browning. FO may be a promising dietary fat for the prevention of overweight and obesity.

4. Materials and Methods

4.1. Animals

Seven-week-old male C57BL/6J mice were obtained from Tokyo Laboratory Animal Science (Tokyo, Japan). They were fed a standard laboratory diet (CE2) from CLEA Japan, Inc. (Tokyo, Japan) for 1 week for stabilization of their metabolism. Mice were maintained under a controlled environment at 22 °C in a 12-h light (0700–1900 h)/12-h dark (1900–0700 h) cycle. They were housed individually and allowed access to the experimental diets and water ad libitum. Care of the mice followed guidelines of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The National Institutes of Biomedical Innovation, Health and Nutrition, Japan, reviewed and approved all animal procedures (Approval no. DS27-52R3).

4.2. Diet

Mice received a fat-rich diet (30 en%) containing either mixed fat with safflower oil and butter (control) or FO (n = 7 in each group). Diets were prepared as described previously [60,70], and the composition of the diet is listed in Table 3. Butter and safflower oil were purchased from Snow Brand Milk Corp. (Hokkaido, Japan) and Benibana Food (Tokyo, Japan), respectively. FO (containing 7% EPA and 24% DHA) was kindly provided by the NOF Corporation (Tokyo, Japan). The food was provided to the mice every day. To estimate daily food intake, the food weight of each day was subtracted from the initial food weight of the previous day. Mean food intake over the entire experimental period in the two groups of mice was calculated using these data. The diets were offered for 10 days.

Table 3. Dietary composition.

Dietary Constituents	Con		FO
		g/100 g	
Safflower oil (high oleic)	3.46		0.00
Butter	10.38		0.00
Fish oil	0.00		13.84
Casein	22.2		22.2
α-Starch	52.98		52.98
Vitamin mix (AIN-93)	1.12		1.12
Mineral mix (AIN-93)	3.92		3.92
Cellulose powder	5.60		5.60
L-Cystine	0.34		0.34
		en%	
Fat	30		30
Carbohydrate	50		50
Protein	20		20

Con: control; FO: fish oil; en%: energy %.

4.3. Measurement of O₂ Consumption and CO₂ Production to Calculate DIT and Energy Production

Mice on the 9th day of the experimental diet administration were used for the experiment. The method for calculating DIT was described previously [37]. Briefly, mice were placed in the calorimeter without food 6 days before starting the experiment at 1700 h, and then energy metabolism was measured for the 11-h period from 0000–1100 h. Oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were monitored with a system that measures O₂/CO₂ metabolism in small animals (MK-5000RQ; Muromachi Kikai Co., Ltd., Tokyo, Japan), and their values were used to calculate DIT and EE. The EE was calculated as follows: EE (kcal/min) = 3.9 VO₂ + 1.1 VCO₂ [71]. For the measurements made after feeding, the same mice used in the fasted measurements were placed in the calorimeter at 1600 h. The research diet was provided at 1700 h, and energy metabolism was measured over the 22-h period from 1700–1500 h. VO₂, VCO₂ and activity were monitored by the system at 3-min intervals, and every four data points were averaged. The average value

over the 12-min period was considered the mean value. The data were normalized to the square root of the activity count. Under the fasting conditions, 55 (5/h \times 11 h) values each for EE and activity were selected from the measurements obtained over the 11-h period; we then plotted EE against the square root of activity and identified a linear regression equation by simple linear regression analysis. Under the fed conditions, 110 (5/h \times 22 h) values each for EE and activity were selected from the measurements obtained over the 22-h period, and EE was then plotted against the square root of activity.

4.4. Quantitative Real-Time PCR

On the 10th day of the experimental diet, mice were sacrificed by cervical dislocation, and BAT, subWAT and liver were extracted from the mice. RNA was extracted from these tissues with TRIzol Reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) following manufacturer's instructions. RNA was isolated and quantified with a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA isolated from BAT and subWAT was reverse transcribed, and quantitative real-time RT-PCR was performed as described previously [60,72]. The primers for quantitative real-time PCR are listed in Table 4.

Table 4. Primers used for quantitative real-time PCR.

Gene	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>36b4</i>	GGCCCTGCACTCTCGCTTTC	TGCCAGGACGCGCTTGT
<i>Aco</i>	GCCCAACTGTGACTTCCATT	GGCATGTAACCCGTAGCACT
<i>β3-AR</i>	TCTAGTTCCCAGCGGAGTTTTCATCG	CGCGCACCTTCATAGCCATCAAACC
<i>Cidea</i>	ATCACAACCTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
<i>Cpt 1</i>	GCACTGCAGCTCGCACATTACAA	CTCAGACAGTACCTCCTTCAGGAAA
<i>Dio2</i>	GCACGTCTCCAATCCTGAAT	TGAACCAAAGTTGACCACCA
<i>Fgf21</i>	ATGGAATGGATGAGATCTAGAGTTGG	TCTTGGTCGTCATCTGTGTAGAGG
<i>Mcad</i>	GATCGCAATGGGTGCTTTTGATAGAA	AGCTGATTGGCAATGTCTCCAGCAAA
<i>Pgc1α</i>	AAGTGTGGAACCTCTGGAACCTG	GGGTTATCTTGGTTGGCTTTATG
<i>Pparα</i>	CCTCAGGGTACCACTACGGAGT	GGTCTTCTTCTGAATCTTGACGCT
<i>Prdm16</i>	GACATTCCAATCCCACCAGA	CACCTCTGTATCCGTCAGCA
<i>Ucp1</i>	GGCCCTTGTAACAACAATAATAC	GGCAACAAGAGCTGACAGTAAAT

4.5. Serum Chemistry

Blood was obtained from the mice, and serum glucose was measured with an Ascensia autoanalyzer (Bayer Medical, Ltd., Tokyo, Japan). Serum levels of NEFA, TG and TC were measured by enzymatic colorimetry with NEFA C, TG E and TC E test kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively.

4.6. Western Blotting

To prepare tissue lysates, BAT and subWAT were homogenized on ice in ice-cold lysis buffer consisting of 25 mM Tris-HCl, pH 7.4, 10 mM sodium orthovanadate, 50 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM EGTA, 1 mM phenylmethylsulfonyl fluoride and 1% NP-40 that supplemented with a protease inhibitor cocktail and phosphatase inhibitor cocktail (both, Roche Diagnostics, Mannheim, Germany). After centrifugation of the tissue homogenates at 14,000 \times g for 10 min at 4 °C, the supernatants were collected for determination of protein concentrations by Bradford protein assay using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Proteins (5 μ g for BAT or 25 μ g for subWAT) were separated by SDS-PAGE (7.5% gel) and then transferred electrophoretically onto Clear Blot Membrane-P (ATTO, Tokyo, Japan) and immunoblotted with specific primary antibodies: UCP1 (ab10983, 1:2000 dilution; Abcam,) and β -actin (C4) (sc-47778, 1:5000 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The secondary antibodies included goat anti-rabbit IgG (sc-2005, 1:8000 dilution) and m-IgG κ BP-HRP (sc-516102, 1:6000 dilution; both from Santa Cruz

Biotechnology, Inc.). ECL detection reagents (Amersham Biosciences, Buckinghamshire, UK) were used to detect the desired proteins, which were then quantified with the NIH Image software program (NIH, Bethesda, MD, USA).

4.7. Statistical Analysis

Values are shown as the mean \pm SEM. Significant differences between the mean values of the two groups were evaluated by Student *t*-test with IBM SPSS Statistics 23. Statistical significance was indicated by a *p* value < 0.05 .

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/md19050278/s1>, Table S1: Effect of fish oil (FO) supplementation on gene expression in liver.

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References

- Lam, Y.Y.; Ravussin, E. Analysis of energy metabolism in humans: A review of methodologies. *Mol. Metab.* **2016**, *5*, 1057–1071. [[CrossRef](#)] [[PubMed](#)]
- Tappy, L. Thermic effect of food and sympathetic nervous system activity in humans. *Reprod. Nutr. Dev.* **1996**, *36*, 391–397. [[CrossRef](#)] [[PubMed](#)]
- Diepvens, K.; Westerterp, K.R.; Westerterp-Plantenga, M.S. Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2007**, *292*, R77–R85. [[CrossRef](#)] [[PubMed](#)]
- Cannon, B.; Nedergaard, J. Brown adipose tissue: Function and physiological significance. *Physiol. Rev.* **2004**, *84*, 277–359. [[CrossRef](#)]
- Heaton, J.M. The distribution of brown adipose tissue in the human. *J. Anat.* **1972**, *112*, 35–39.
- Van Marken Lichtenbelt, W.D.; Vanhommerig, J.W.; Smulders, N.M.; Drossaerts, J.M.; Kemerink, G.J.; Bouvy, N.D.; Schrauwen, P.; Teule, G.J. Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* **2009**, *360*, 1500–1508. [[CrossRef](#)] [[PubMed](#)]
- Virtanen, K.A.; Lidell, M.E.; Orava, J.; Heglind, M.; Westergren, R.; Niemi, T.; Taittonen, M.; Laine, J.; Savisto, N.J.; Enerbäck, S.; et al. Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* **2009**, *360*, 1518–1525. [[CrossRef](#)]
- Heaton, G.M.; Wagenvoort, R.J.; Kemp, A., Jr.; Nicholls, D.G. Brown-adipose-tissue mitochondria: Photoaffinity labelling of the regulatory site of energy dissipation. *Eur. J. Biochem.* **1978**, *82*, 515–521. [[CrossRef](#)]
- Aquila, H.; Link, T.A.; Klingenberg, M. The uncoupling protein from brown fat mitochondria is related to the mitochondrial ADP/ATP carrier. Analysis of sequence homologies and of folding of the protein in the membrane. *EMBO J.* **1985**, *4*, 2369–2376. [[CrossRef](#)]
- Von Essen, G.; Lindsund, E.; Cannon, B.; Nedergaard, J. Adaptive facultative diet-induced thermogenesis in wild-type but not in UCP1-ablated mice. *Am. J. Physiol. Endocrinol. Metab.* **2017**, *313*, E515–E527. [[CrossRef](#)] [[PubMed](#)]
- Kontani, Y.; Wang, Y.; Kimura, K.; Inokuma, K.I.; Saito, M.; Suzuki-Miura, T.; Wang, Z.; Sato, Y.; Mori, N.; Yamashita, H. UCP1 deficiency increases susceptibility to diet-induced obesity with age. *Aging Cell* **2005**, *4*, 147–155. [[CrossRef](#)] [[PubMed](#)]
- Nagai, N.; Sakane, N.; Ueno, L.M.; Hamada, T.; Moritani, T. The -3826 A→G variant of the uncoupling protein-1 gene diminishes postprandial thermogenesis after a high fat meal in healthy boys. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 5661–5667. [[CrossRef](#)] [[PubMed](#)]
- Saito, M.; Okamatsu-Ogura, Y.; Matsushita, M.; Watanabe, K.; Yoneshiro, T.; Nio-Kobayashi, J.; Iwanaga, T.; Miyagawa, M.; Kameya, T.; Nakada, K.; et al. High incidence of metabolically active brown adipose tissue in healthy adult humans: Effects of cold exposure and adiposity. *Diabetes* **2009**, *58*, 1526–1531. [[CrossRef](#)]

14. Cypess, A.M.; Weiner, L.S.; Roberts-Toler, C.; Elia, E.F.; Kessler, S.H.; Kahn, P.A.; English, J.; Chatman, K.; Trauger, S.A.; Doria, A.; et al. Activation of human brown adipose tissue by a β 3-adrenergic receptor agonist. *Cell Metab.* **2015**, *21*, 33–38. [[CrossRef](#)] [[PubMed](#)]
15. Yoneshiro, T.; Aita, S.; Kawai, Y.; Iwanaga, T.; Saito, M. Nonpungent capsaicin analogs (capsinoids) increase energy expenditure through the activation of brown adipose tissue in humans. *Am. J. Clin. Nutr.* **2012**, *95*, 845–850. [[CrossRef](#)]
16. Yoneshiro, T.; Aita, S.; Matsushita, M.; Kayahara, T.; Kameya, T.; Kawai, Y.; Iwanaga, T.; Saito, M. Recruited brown adipose tissue as an antiobesity agent in humans. *J. Clin. Investig.* **2013**, *123*, 3404–3408. [[CrossRef](#)]
17. Ramel, A.; Martínéz, A.; Kiely, M.; Morais, G.; Bandarra, N.M.; Thorsdottir, I. Beneficial effects of long-chain n-3 fatty acids included in an energy-restricted diet on insulin resistance in overweight and obese European young adults. *Diabetologia* **2008**, *51*, 1261–1268. [[CrossRef](#)]
18. Thorsdottir, I.; Tomasson, H.; Gunnarsdottir, I.; Gisladdottir, E.; Kiely, M.; Parra, M.D.; Bandarra, N.M.; Schaafsma, G.; Martínéz, J.A. Randomized trial of weight-loss-diets for young adults varying in fish and fish oil content. *Int. J. Obes.* **2007**, *31*, 1560–1566. [[CrossRef](#)] [[PubMed](#)]
19. Gunnarsdottir, I.; Tomasson, H.; Kiely, M.; Martínéz, J.A.; Bandarra, N.M.; Morais, M.G.; Thorsdottir, I. Inclusion of fish or fish oil in weight-loss diets for young adults: Effects on blood lipids. *Int. J. Obes.* **2008**, *32*, 1105–1112. [[CrossRef](#)] [[PubMed](#)]
20. Calder, P.C. Mechanisms of action of (n-3) fatty acids. *J. Nutr.* **2012**, *142*, 592S–599S. [[CrossRef](#)]
21. Arterburn, L.M.; Hall, E.B.; Oken, H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am. J. Clin. Nutr.* **2006**, *83*, 1467S–1476S. [[CrossRef](#)] [[PubMed](#)]
22. Muskiet, F.A.; Fokkema, M.R.; Schaafsma, A.; Boersma, E.R.; Crawford, M.A. Is docosahexaenoic acid (DHA) essential? Lessons from DHA status regulation, our ancient diet, epidemiology and randomized controlled trials. *J. Nutr.* **2004**, *134*, 183–186. [[CrossRef](#)] [[PubMed](#)]
23. Forman, B.M.; Chen, J.; Evans, R.M. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4312–4317. [[CrossRef](#)] [[PubMed](#)]
24. Krey, G.; Braissant, O.; L'Horset, F.; Kalkhoven, E.; Perroud, M.; Parker, M.G.; Wahli, W. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* **1997**, *11*, 779–791. [[CrossRef](#)] [[PubMed](#)]
25. Escher, P.; Braissant, O.; Basu-Modak, S.; Michalik, L.; Wahli, W.; Desvergne, B. Rat PPARs: Quantitative analysis in adult rat tissues and regulation in fasting and refeeding. *Endocrinology* **2001**, *142*, 4195–4202. [[CrossRef](#)] [[PubMed](#)]
26. Barbera, M.J.; Schluter, A.; Pedraza, N.; Iglesias, R.; Villarroya, F.; Giralt, M. Peroxisome proliferator-activated receptor alpha activates transcription of the brown fat uncoupling protein-1 gene. A link between regulation of the thermogenic and lipid oxidation pathways in the brown fat cell. *J. Biol. Chem.* **2001**, *276*, 1486–1493. [[CrossRef](#)]
27. Ishibashi, J.; Seale, P. Medicine. Beige can be slimming. *Science* **2010**, *328*, 1113–1114. [[CrossRef](#)]
28. Seale, P.; Bjork, B.; Yang, W.; Kajimura, S.; Chin, S.; Kuang, S.; Scimè, A.; Devarakonda, S.; Conroe, H.M.; Erdjument-Bromage, H.; et al. PRDM16 controls a brown fat/skeletal muscle switch. *Nature* **2008**, *454*, 961–967. [[CrossRef](#)]
29. Wu, J.; Boström, P.; Sparks, L.M.; Ye, L.; Choi, J.H.; Giang, A.H.; Khandekar, M.; Virtanen, K.A.; Nuutila, P.; Schaart, G.; et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* **2012**, *150*, 366–376. [[CrossRef](#)]
30. Okamatsu-Ogura, Y.; Fukano, K.; Tsubota, A.; Uozumi, A.; Terao, A.; Kimura, K.; Saito, M. Thermogenic ability of uncoupling protein 1 in beige adipocytes in mice. *PLoS ONE* **2013**, *8*, e84229. [[CrossRef](#)]
31. Laiglesia, L.M.; Lorente-Cebrián, S.; Prieto-Hontoria, P.L.; Fernández-Galilea, M.; Ribeiro, S.M.; Sáinz, N.; Martínez, J.A.; Moreno-Aliaga, M.J. Eicosapentaenoic acid promotes mitochondrial biogenesis and beige-like features in subcutaneous adipocytes from overweight subjects. *J. Nutr. Biochem.* **2016**, *37*, 76–82. [[CrossRef](#)] [[PubMed](#)]
32. Zhao, M.; Chen, X. Eicosapentaenoic acid promotes thermogenic and fatty acid storage capacity in mouse subcutaneous adipocytes. *Biochem. Biophys. Res. Commun.* **2014**, *450*, 1446–1451. [[CrossRef](#)] [[PubMed](#)]
33. Bargut, T.C.; Souza-Mello, V.; Mandarim-de-Lacerda, C.A.; Aguila, M.B. Fish oil diet modulates epididymal and inguinal adipocyte metabolism in mice. *Food Funct.* **2016**, *7*, 1468–1476. [[CrossRef](#)] [[PubMed](#)]
34. Kim, M.; Goto, T.; Yu, R.; Uchida, K.; Tominaga, M.; Kano, Y.; Takahashi, N.; Kawada, T. Fish oil intake induces UCP1 upregulation in brown and white adipose tissue via the sympathetic nervous system. *Sci. Rep.* **2015**, *5*, 18013. [[CrossRef](#)] [[PubMed](#)]
35. Kim, J.; Okla, M.; Erickson, A.; Carr, T.; Natarajan, S.K.; Chung, S. Eicosapentaenoic Acid Potentiates Brown Thermogenesis through FFAR4-dependent Up-regulation of miR-30b and miR-378. *J. Biol. Chem.* **2016**, *291*, 20551–20562. [[CrossRef](#)] [[PubMed](#)]
36. Bargut, T.C.; Silva-e-Silva, A.C.; Souza-Mello, V.; Mandarim-de-Lacerda, C.A.; Aguila, M.B. Mice fed fish oil diet and upregulation of brown adipose tissue thermogenic markers. *Eur. J. Nutr.* **2016**, *55*, 159–169. [[CrossRef](#)]
37. Yamazaki, T.; Ikaga, R.; Li, D.; Nakae, S.; Tanaka, S. A novel method for measuring diet-induced thermogenesis in mice. *MethodsX* **2019**, *6*, 1950–1956. [[CrossRef](#)]
38. Hanssen, M.J.; Hoeks, J.; Brans, B.; van der Lans, A.A.; Schaart, G.; van den Driessche, J.J.; Jörgensen, J.A.; Boekschoten, M.V.; Hesselink, M.K.; Havekes, B.; et al. Short-term cold acclimation improves insulin sensitivity in patients with type 2 diabetes mellitus. *Nat. Med.* **2015**, *21*, 863–865. [[CrossRef](#)]
39. Lefebvre, P.; Chinetti, G.; Fruchart, J.C.; Staels, B. Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. *J. Clin. Investig.* **2006**, *116*, 571–580. [[CrossRef](#)]

40. Leblanc, J.; Dussault, J.; Lupien, D.; Richard, D. Effect of diet and exercise on norepinephrine-induced thermogenesis in male and female rats. *J. Appl. Physiol.* **1982**, *52*, 556–561. [[CrossRef](#)]
41. Gulick, T.; Cresci, S.; Caira, T.; Moore, D.D.; Kelly, D.P. The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 11012–11016. [[CrossRef](#)] [[PubMed](#)]
42. Pahlavani, M.; Razafimanjato, F.; Ramalingam, L.; Kalupahana, N.S.; Moussa, H.; Scoggin, S.; Moustaid-Moussa, N. Eicosapentaenoic acid regulates brown adipose tissue metabolism in high-fat-fed mice and in clonal brown adipocytes. *J. Nutr. Biochem.* **2017**, *39*, 101–109. [[CrossRef](#)] [[PubMed](#)]
43. Oudart, H.; Groscolas, R.; Calgari, C.; Nibbelink, M.; Leray, C.; Le Maho, Y.; Malan, A. Brown fat thermogenesis in rats fed high-fat diets enriched with n-3 polyunsaturated fatty acids. *Int. J. Obes. Relat. Metab. Disord.* **1997**, *21*, 955–962. [[CrossRef](#)] [[PubMed](#)]
44. Mascaró, C.; Acosta, E.; Ortiz, J.A.; Marrero, P.F.; Hegardt, F.G.; Haro, D. Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. *J. Biol. Chem.* **1998**, *273*, 8560–8563. [[CrossRef](#)]
45. Leone, T.C.; Weinheimer, C.J.; Kelly, D.P. A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: The PPARalpha-null mouse as a model of fatty acid oxidation disorders. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 7473–7478. [[CrossRef](#)] [[PubMed](#)]
46. Goto, T.; Lee, J.Y.; Teraminami, A.; Kim, Y.I.; Hirai, S.; Uemura, T.; Inoue, H.; Takahashi, N.; Kawada, T. Activation of peroxisome proliferator-activated receptor-alpha stimulates both differentiation and fatty acid oxidation in adipocytes. *J. Lipid. Res.* **2011**, *52*, 873–884. [[CrossRef](#)]
47. Rachid, T.L.; Penna-de-Carvalho, A.; Bringhenti, I.; Aguila, M.B.; Mandarim-de-Lacerda, C.A.; Souza-Mello, V. Fenofibrate (PPARalpha agonist) induces beige cell formation in subcutaneous white adipose tissue from diet-induced male obese mice. *Mol. Cell. Endocrinol.* **2015**, *402*, 86–94. [[CrossRef](#)]
48. Rachid, T.L.; Silva-Veiga, F.M.; Graus-Nunes, F.; Bringhenti, I.; Mandarim-de-Lacerda, C.A.; Souza-Mello, V. Differential actions of PPAR- α and PPAR- β/δ on beige adipocyte formation: A study in the subcutaneous white adipose tissue of obese male mice. *PLoS ONE* **2018**, *13*, e0191365. [[CrossRef](#)]
49. Shabalina, I.G.; Petrovic, N.; de Jong, J.M.; Kalinovich, A.V.; Cannon, B.; Nedergaard, J. UCP1 in brite/beige adipose tissue mitochondria is functionally thermogenic. *Cell Rep.* **2013**, *5*, 1196–1203. [[CrossRef](#)]
50. Sato, H.; Taketomi, Y.; Miki, Y.; Murase, R.; Yamamoto, K.; Murakami, M. Secreted Phospholipase PLA2G2D Contributes to Metabolic Health by Mobilizing ω 3 Polyunsaturated Fatty Acids in WAT. *Cell Rep.* **2020**, *31*, 107579. [[CrossRef](#)]
51. Hondares, E.; Iglesias, R.; Giralt, A.; Gonzalez, F.J.; Giralt, M.; Mampel, T.; Villarroya, F. Thermogenic activation induces FGF21 expression and release in brown adipose tissue. *J. Biol. Chem.* **2011**, *286*, 12983–12990. [[CrossRef](#)]
52. Fisher, F.M.; Kleiner, S.; Douris, N.; Fox, E.C.; Mepani, R.J.; Verdeguer, F.; Wu, J.; Kharitonov, A.; Flier, J.S.; Maratos-Flier, E.; et al. FGF21 regulates PGC-1 α and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev.* **2012**, *26*, 271–281. [[CrossRef](#)]
53. Quesada-López, T.; Cereijo, R.; Turatsinze, J.V.; Planavila, A.; Cairó, M.; Gavaldà-Navarro, A.; Peyrou, M.; Moure, R.; Iglesias, R.; Giralt, M.; et al. The lipid sensor GPR120 promotes brown fat activation and FGF21 release from adipocytes. *Nat. Commun.* **2016**, *7*, 13479. [[CrossRef](#)] [[PubMed](#)]
54. Oh, D.Y.; Talukdar, S.; Bae, E.J.; Imamura, T.; Morinaga, H.; Fan, W.; Li, P.; Lu, W.J.; Watkins, S.M.; Olefsky, J.M. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* **2010**, *142*, 687–698. [[CrossRef](#)] [[PubMed](#)]
55. Huber, J.; Löffler, M.; Bilban, M.; Reimers, M.; Kadl, A.; Todoric, J.; Zeyda, M.; Geyeregger, R.; Schreiner, M.; Weichhart, T.; et al. Prevention of high-fat diet-induced adipose tissue remodeling in obese diabetic mice by n-3 polyunsaturated fatty acids. *Int. J. Obes.* **2007**, *31*, 1004–1013. [[CrossRef](#)] [[PubMed](#)]
56. Todoric, J.; Löffler, M.; Huber, J.; Bilban, M.; Reimers, M.; Kadl, A.; Zeyda, M.; Waldhäusl, W.; Stulnig, T.M. Adipose tissue inflammation induced by high-fat diet in obese diabetic mice is prevented by n-3 polyunsaturated fatty acids. *Diabetologia* **2006**, *49*, 2109–2119. [[CrossRef](#)]
57. Innes, J.K.; Calder, P.C. The Differential Effects of Eicosapentaenoic Acid and Docosahexaenoic Acid on Cardiometabolic Risk Factors: A Systematic Review. *Int. J. Mol. Sci.* **2018**, *19*, 532. [[CrossRef](#)]
58. Madsen, L.; Rustan, A.C.; Vaagenes, H.; Berge, K.; Dyrøy, E.; Berge, R.K. Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference. *Lipids* **1999**, *34*, 951–963. [[CrossRef](#)]
59. Buckley, R.; Shewring, B.; Turner, R.; Yaqoob, P.; Minihane, A.M. Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects. *Br. J. Nutr.* **2004**, *92*, 477–483. [[CrossRef](#)] [[PubMed](#)]
60. Wada, S.; Yamazaki, T.; Kawano, Y.; Miura, S.; Ezaki, O. Fish oil fed prior to ethanol administration prevents acute ethanol-induced fatty liver in mice. *J. Hepatol.* **2008**, *49*, 441–450. [[CrossRef](#)] [[PubMed](#)]
61. Houten, S.M.; Violante, S.; Ventura, F.V.; Wanders, R.J. The Biochemistry and Physiology of Mitochondrial Fatty Acid β -Oxidation and Its Genetic Disorders. *Annu. Rev. Physiol.* **2016**, *78*, 23–44. [[CrossRef](#)]
62. Warfel, J.D.; Vandanmagsar, B.; Dubuisson, O.S.; Hodgeson, S.M.; Elks, C.M.; Ravussin, E.; Mynatt, R.L. Examination of carnitine palmitoyltransferase 1 abundance in white adipose tissue: Implications in obesity research. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2017**, *312*, R816–R820. [[CrossRef](#)]

63. Gao, X.; Li, K.; Hui, X.; Kong, X.; Sweeney, G.; Wang, Y.; Xu, A.; Teng, M.; Liu, P.; Wu, D. Carnitine palmitoyltransferase 1A prevents fatty acid-induced adipocyte dysfunction through suppression of c-Jun N-terminal kinase. *Biochem. J.* **2011**, *435*, 723–732. [[CrossRef](#)] [[PubMed](#)]
64. Jensen, M.D.; Bajnárek, J.; Lee, S.Y.; Nielsen, S.; Koutsari, C. Relationship between postabsorptive respiratory exchange ratio and plasma free fatty acid concentrations. *J. Lipid. Res.* **2009**, *50*, 1863–1869. [[CrossRef](#)] [[PubMed](#)]
65. Hibi, M.; Oishi, S.; Matsushita, M.; Yoneshiro, T.; Yamaguchi, T.; Usui, C.; Yasunaga, K.; Katsuragi, Y.; Kubota, K.; Tanaka, S.; et al. Brown adipose tissue is involved in diet-induced thermogenesis and whole-body fat utilization in healthy humans. *Int. J. Obes.* **2016**, *40*, 1655–1661. [[CrossRef](#)] [[PubMed](#)]
66. Snitker, S.; Fujishima, Y.; Shen, H.; Ott, S.; Pi-Sunyer, X.; Furuhashi, Y.; Sato, H.; Takahashi, M. Effects of novel capsinoid treatment on fatness and energy metabolism in humans: Possible pharmacogenetic implications. *Am. J. Clin. Nutr.* **2009**, *89*, 45–50. [[CrossRef](#)] [[PubMed](#)]
67. Westerterp, K.R. Diet induced thermogenesis. *Nutr. Metab.* **2004**, *1*, 5. [[CrossRef](#)]
68. Trumbo, P.; Schlicker, S.; Yates, A.A.; Poos, M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J. Am. Diet. Assoc.* **2002**, *102*, 1621–1630. [[CrossRef](#)]
69. Forbes, R.M.; Cooper, A.R.; Mitchell, H.H. The composition of the adult human body as determined by chemical analysis. *J. Biol. Chem.* **1953**, *203*, 359–366. [[CrossRef](#)]
70. Li, D.; Ikaga, R.; Yamazaki, T. Soya protein β -conglycinin ameliorates fatty liver and obesity in diet-induced obese mice through the down-regulation of PPAR γ . *Br. J. Nutr.* **2018**, *119*, 1220–1232. [[CrossRef](#)]
71. Weir, J.B. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* **1949**, *109*, 1–9. [[CrossRef](#)] [[PubMed](#)]
72. Yamazaki, T.; Okawa, S.; Takahashi, M. The effects on weight loss and gene expression in adipose and hepatic tissues of very-low carbohydrate and low-fat isoenergetic diets in diet-induced obese mice. *Nutr. Metab.* **2016**, *13*, 78. [[CrossRef](#)] [[PubMed](#)]