DHA attenuates postprandial hyperlipidemia via activating PPARα in intestinal epithelial cells

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Abstract It is known that peroxisome proliferator-activated receptor (PPAR)α, whose activation reduces hyperlipidemia, is highly expressed in intestinal epithelial cells. Docosahexaenoic acid (DHA) could improve postprandial hyperlipidemia, however, its relationship with intestinal PPARα activation is not revealed. In this study, we investigated whether DHA can affect postprandial hyperlipidemia by activating intestinal PPARα using Caco-2 cells and C57BL/6 mice. The genes involved in fatty acid (FA) oxidation and oxygen consumption rate were increased, and the secretion of triacylglyceride (TG) and apolipoprotein B (apoB) was decreased in DHA-treated Caco-2 cells. Additionally, intestinal FA oxidation was induced, and TG and apoB secretion from intestinal epithelial cells was reduced, resulting in the attenuation of plasma TG and apoB levels after oral administration of olive oil in DHA-rich oil-fed mice compared with controls. However, no increase in genes involved in FA oxidation was observed in the liver. Furthermore, the effects of DHA on intestinal lipid secretion and postprandial hyperlipidemia were abolished in PPARα knockout mice. In conclusion, the present work suggests that DHA can inhibit the secretion of TG from intestinal epithelial cells via PPARα activation, which attenuates postprandial hyperlipidemia.—Kimura, R., N. Takahashi, S. Lin, T. Goto, K. Murota, R. Nakata, H. Inoue, and T. Kawada. DHA attenuates postprandial hyperlipidemia via activating PPARα in intestinal epithelial cells. J. Lipid Res. 2013. 54: 3258–3268.

Supplementary key words small intestine • fatty acid oxidation • triacylglyceride • cardiovascular diseases • polyunsaturated fatty acid • dyslipidemia • docosahexaenoic acid • peroxisome proliferator-activated receptor α

Over the past few decades, the prevalence of metabolic syndrome has markedly increased worldwide, particularly in wealthy industrialized countries (1). Metabolic syndrome includes multiple factors such as insulin resistance, dyslipidemia, and central obesity and increases the risk of developing serious metabolic disorders such as cardiovascular diseases and type 2 diabetes. Many epidemiological studies, including prospective cohort studies (2–4), cross-sectional studies (5, 6), and case-control studies (7), demonstrate that postprandial hyperlipidemia is an independent risk factor for cardiovascular disease. Therefore, attenuating postprandial hyperlipidemia could be a key factor for preventing cardiovascular diseases.

High intake of dietary fat significantly increases postprandial plasma triacylglyceride (TG) levels. The epithelial cells in the small intestine are constantly exposed to this dietary fat. Therefore, the regulation of lipid metabolism in intestinal epithelial cells could affect postprandial hyperlipidemia. Previous studies have demonstrated that peroxisome proliferator-activated receptor (PPAR)α is highly expressed in intestinal epithelial cells along the length of the small intestine as well as in the liver, skeletal muscle, and brown fat (8, 9). PPARα, which is a nuclear transcriptional factor, regulates the mRNA expression of fatty acid (FA) oxidation-related enzymes (10, 11). Synthetic PPARα agonists, such as fibrates, decrease circulating lipid levels and are commonly used as drugs for the treatment of hyperlipidemia (12). PPARα knockout (PPARα−/−) mice showed dyslipidemia (13, 14). Recently, we and others have reported that activation of PPARα in intestinal epithelial cells improves postprandial hyperlipidemia through enhancing FA oxidation (15, 16). PUFA s, such as docosahexaenoic acid (DHA) and

Acknowledgments: Acs, acyl-CoA synthetase; Aox, acyl-CoA oxidase; ASM, acid-soluble metabolite; AUC, area under the curve; Cpt1a, carnitine palmitoyltransferase 1A; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; Fabp, fatty acid binding protein; HFD, high-fat diet; OCR, oxygen consumption rate; PPAR, peroxisome proliferator-activated receptor; PPARα−/−, peroxisome proliferator-activated receptor α knockout; PPRE, peroxisome proliferator-activated receptor-response element; TG, triacylglyceride; Ucp2, uncoupling protein-2.

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The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of four figures and one table.
eicosapentaenoic acid (EPA), are known to lower plasma TG; the mechanism responsible for their hypolipidemic action is thought to be involved in the regulation of TG clearance from circulation and TG synthesis in the liver (17–19). Recent studies have found that PUFAs increase the mRNA expression levels of genes involved in FA oxidation in intestinal epithelial cells (20). However, it is unknown whether dietary lipids, such as DHA could affect the intestinal lipid metabolism, resulting in improvement of postprandial hyperlipidemia.

In this study, we investigated whether DHA improves postprandial hyperlipidemia by altering the lipid metabolism in intestinal epithelial cells. DHA induced FA oxidation in intestinal epithelial cells by activating PPARs, which attenuated postprandial hyperlipidemia by directly reducing TG secretion from intestinal epithelial cells. Furthermore, we confirmed that hepatic lipid metabolism is unlikely to contribute to these effects of DHA. These findings suggest that activating intestinal PPARs by dietary lipids such as DHA may shed light on postprandial hyperlipidemia-induced cardiovascular diseases.

MATERIALS AND METHODS

Chemicals and cell culture

DHA and EPA were purchased from Nacalai Tesque (Kyoto, Japan) and dissolved in ethanol. Bezafibrate was purchased from Sigma (St. Louis, MO) and dissolved in dimethylsulfoxide (DMSO) as a stock solution. Decanoic acid and palmitic acid were purchased from Nacalai Tesque and Wako Pure Chemicals (Osaka, Japan), respectively. All other chemicals used were from Sigma or Nacalai Tesque and were guaranteed to be reagent or tissue-culture grade.

Human Caco-2 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and were cultured in DMEM (100 mg/dl glucose) containing 10% fetal bovine serum, 1% nonessential amino acid solution, and 10 mg/ml penicillin/streptomycin (100 mg/dl glucose) containing 10% fetal bovine serum, 1% nonessential amino acid solution, and 10 mg/ml penicillin/streptomycin at 37°C in 5% CO₂/95% air under humidified conditions. Caco-2 cells were seeded at a density of 1.2 × 10⁵ cells/ml on 12-well Transwell plates (Corning Inc., Corning, NY) for 2 weeks for differentiation into intestinal epithelial-like cells. To evaluate differentiation of Caco-2 cells, we measured transepithelial electrical resistance. No significance in transepithelial electrical resistance was detected in any experiment (data not shown). The apical medium was changed any experiment (data not shown). The apical medium was changed before the measurement, cells were washed, and 675 μl of nonbuffered (sodium-carbonate-free, pH 7.4) DMEM medium supplemented with 0.2 mM palmitic acid, 0.2 mM oleic acid. Additionally, the basolateral medium was changed to serum-free DMEM. After 48 h, the basolateral medium was collected to measure TG and apolipoprotein B (apoB) secretion. Cell viability was measured in Caco-2 cells treated with DHA and bezafibrate based on cell titer (Promega, Fitchburg, WI).

Luciferase assays

Luciferase assays were performed using the modified dual luciferase system as previously described (21). Briefly, for luciferase assays using the GAL4/PPAR chimera system, CV-1 cells or Caco-2 cells were transfected with p4xUASg-tk-luc (reporter plasmid), pM-h PPARα ligand binding domain and human PPARα-ligand binding domain), pM-h PPARγ or pM-h PPARδ, and pRL-CMV (internal control plasmid for normalizing transfection efficiencies). Transfected cells were treated with DHA and EPA at the indicated concentrations for 24 h. Bezafibrate (50 μM), pioglitazone (1 μM), or GW501516 (1 μM) were used as positive controls. For luciferase assays using a PPAR full-length system, a reporter plasmid (p4xPPRE-kluc) and pRL-CMV were transfected into Caco-2 cells. Transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. Four hours after transfection, transfected cells were cultured in medium containing DHA for an additional 24 h. Luciferase assays were performed using the dual luciferase system according to the manufacturer’s protocol.

Real-time quantitative RT-PCR

Total RNA samples were prepared from Caco-2 cells, mouse intestinal epithelial cells, and hepatocytes using Sepasol Super-1 (Nacalai Tesque) and Qiazol lysis reagent (Qiagen, Hilden, Germany) according to the manufacturer’s instructions, respectively. Using M-MLV reverse transcriptase (Invitrogen), total RNA was reverse-transcribed following the manufacturer’s protocol using a thermal cycler (Takara, Shiga, Japan). To quantify mRNA expression, real-time PCR was performed using a LightCycler system (Roche Diagnostics, Mannheim, Germany) using SYBR Green fluorescence signals as described previously (22). Oligonucleotide primers of human and mouse 36b4 and PPARα target genes used in this study were designed using a PCR primer selection program found in the website of the Virtual Genomic Center from the GenBank database, as previously described (Table 1) (23). To compare mRNA expression levels among samples, copy numbers of all transcripts were divided by that of human and mouse 36b4, showing a constant expression level. All mRNA expression levels are represented relative to the control in each experiment.

Measurement of oxygen consumption rate in Caco-2 cells

The cellular oxygen consumption rate (OCR) was measured using a Seahorse Bioscience XF analyzer in 24-well plates at 37°C, with correction for positional temperature variations adjusted for the four empty wells in the plate (24, 25). Caco-2 cells were cultured for 2 weeks after seeding on the plate and were treated with PPARα agonist, 50 μM bezafibrate, or either 1 μM or 25 μM DHA. Immediately before the measurement, cells were washed, and 675 μl of nonbuffered (sodium-carbonate-free, pH 7.4) DMEM medium supplemented with 0.2 mM palmitic acid, 0.2 mM oleic acid was added to each well. Prior to the measurement, the oxygen consumption rate was adjusted to 10–15% of baseline and 10 μM rotenone was added to each well to inhibit mitochondrial respiration.

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<th>Gene</th>
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<tr>
<td>Human Acs</td>
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<tr>
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Fw, forward; Rev, reverse.
(Clone 12G10; Monosan, Uden, The Netherlands), affinity-purified anti-apoB (Rockland, Gilbertsville, PA), and horseradish peroxidase (HRP)-conjugated anti-goat IgG (Promega) as the capture, primary, and secondary antibodies, respectively. Details of these procedures have been previously described (15). HRP activity was detected using a 3,3′,5,5′-tetramethylbenzidine peroxidase substrate (KPL, Gaithersburg, MD).

Animal experiments

DHA-rich oil containing 25.4% DHA and 8% EPA was a gift from NOF Corporation (Kanagawa, Japan). EPA-rich oil containing 28.4% EPA and 12.3% DHA was a gift from Nippon Suisan Kaisha, Ltd. (Tokyo, Japan). All other chemicals were from Sigma or Nacalai Tesque and were guaranteed to be reagent or tissue-culture grade.

All mice were maintained separately in a temperature-controlled (23°C) facility under a constant 12 h light/dark cycle with free access to water. To analyze the effects of DHA on intestinal lipid metabolism and postprandial hyperlipidemia, 9-week-old male C57BL/6 mice (CLEA Japan, Tokyo, Japan) were fed a high-fat diet (HFD) consisting of 60% (kcal%) fat from dietary oil, 26% protein, and 14% carbohydrate for 1 week to induce postprandial hyperlipidemia (26), and were then divided into three groups with the same average serum TG level and body weight after 16 h fasting. Ten-week-old male C57BL/6 mice were maintained for 1 week either on a 60% HFD or on a diet containing 1.9% DHA or 3.7% L-carnitine, and 2% FA-free BSA was added to each well. After equilibration for 30 min, 2 min measurements were performed at 3 min intervals with inter-measurement mixing to homogenize the oxygen in the medium.

Measurement of TG and apoB secretion in Caco-2 cells

To measure TG secretion, we used the Triglyceride E Test Wako kit (Wako Pure Chemicals). To measure apoB secretion, an enzyme-linked immunosorbent assay (ELISA) was performed using an anti-human low density lipoprotein apoB antibody.

### TABLE 2. Composition of experimental diets

<table>
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<th>Experimental Diet</th>
<th>Control</th>
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<th>3.7% DHA</th>
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<tr>
<td>Corn oil (%)</td>
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<td>29.38</td>
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</tr>
<tr>
<td>Casein (%)</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Sucrose (%)</td>
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<td>23.29</td>
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<tr>
<td>Fat energy (en %)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>60.6</td>
<td>60.7</td>
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<tr>
<td>DHA (en %)&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
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<td>0</td>
<td>0.58</td>
<td>1.16</td>
</tr>
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</table>

<sup>a</sup>Percent of weight from total food weight.

<sup>b</sup>Percent of energy from total energy intake.

<sup>c</sup>Percent of energy from total energy intake.

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**Fig. 1.** DHA activated PPARα in luciferase assays using the GAL4 chimera and PPRE-luc systems. A: Luciferase assay using the GAL4/PPARα chimera system in CV-1 cells. Reporter plasmids (p4XUASg-luc and pRL-CMV) were transfected into CV-1 cells together with pM-h PPARα. Transfected cells were treated with bezafibrate (Beza) or DHA at the indicated concentrations for 24 h. B: Luciferase assay using a PPRE-luc system in Caco-2 cells. Reporter plasmids (p4XPPRE-k-luc and pRL-CMV) were transfected into Caco-2 cells. Transfected cells were treated with bezafibrate or DHA at the indicated concentrations for 24 h. C–E: Luciferase assay using the GAL4/PPARα, PPARγ, and PPARδ chimera system in Caco-2 cells. Reporter plasmids were transfected with pM-hPPARα, pM-hPPARγ, and pM-hPPARδ in Caco-2 cells. Transfected cells were treated with 25 μM DHA and EPA, and 50 μM bezafibrate, 1 μM pioglitazone (pio), or 1 μM GW501516 (GW) as positive controls (Cont) for 24 h. The average activity of luciferase in a vehicle control was set at 100% and the relative activities were represented as fold induction relative to that of control. Values are means ± SEM of 5–10 tests. *P < 0.05 and **P < 0.01 compared with each control.
DHA attenuates hyperlipidemia via intestinal PPARα

To clarify whether the effects of DHA-rich oil on intestinal lipid metabolism and postprandial hyperlipidemia involves PPARα, we used PPARα−/− mice with a C57BL/6 genetic background. PPARα−/− mice were fed a HFD consisting of 60% (kcal%) fat for 1 week, and were then divided into two groups with the same average serum TG level and body weight after 16 h fasting. Ten-week-old male PPARα−/− mice were maintained for 1 week either on a 60% HFD or on a 60% HFD containing 3.7% DHA or 0.2% bezafibrate.

For RNA analysis, the proximal intestine and the liver were harvested from the mice. After washing, intestinal epithelial cells were collected using a slide glass. Collected tissue was stored in RNAlater (Ambion, Austin, TX; Applied Biosystems, Foster City, CA) at −80°C until use.

Measurement of FA oxidation

FA oxidation with isolated intestinal epithelial cells and hepatocytes was analyzed as previously described (15, 25). Briefly, collected intestinal epithelial cells and hepatocytes were washed with 1% FBS/DMEM three times and used for experiments. Cells were incubated with a piece of filter paper containing 200 μl 3 N NaOH in DMEM containing 200 μM palmitic acid, 0.1% FA-free BSA, 200 μM L-carnitine, and [14C]palmitic acid (1 μCi/ml) (American Radiolabeled Chemicals, St. Louis, MO) at 37°C for 2 h. The tubes were gently shaken every 30 min during incubation. After 2 h of incubation, 200 μl of 12 N HCl was added to the cells to release [14C]CO2 and they were incubated at 37°C overnight to trap [14C] CO2. The saturated filter paper containing trapped [14C]CO2 was assessed for radioactivity in a liquid scintillation counter (LS6500; Beckman Coulter, Brea, CA). The acidified medium was centrifuged, and 200 μl of supernatant was assessed to determine the amount of [14C]-labeled acid-soluble metabolites (ASMs), which includes ketone bodies. Protein concentration was determined using a protein assay kit (Bio-Rad, Hercules, CA).

Postprandial TG and apoB secretion

To measure plasma TG concentration, mice were administered an oral gavage of 300 μl olive oil after a 16 h fast, and blood samples were collected every 30 min to 240 min after olive oil administration from the tail vein of nonanesthetized mice. To measure TG secretion from intestinal epithelial cells, mice were injected with 500 mg/kg body weight tyloxapol (T0307, Sigma) into the intraperitoneal cavity to block serum lipase activity after a 16 h fast (28). After 30 min, mice were administered an oral gavage of 300 μl olive oil. Blood samples were obtained before tyloxapol injection and every 30 min for 240 min after olive oil administration. Plasma TG concentration was determined using the Triglyceride E-Test Wako kit (Wako Pure Chemicals).

To measure postprandial apoB48 secretion, plasma collected at 120 min was mixed with Laemmli sample buffer (Bio-Rad) (1:8) and boiled for 5 min at 95°C. Plasma samples were subjected to SDS-PAGE on a 5% gel. Separated proteins were transferred electrophoretically to polyvinylidene fluoride membranes (Millipore).
Corporation, Billerica, MA), which were blocked with 5% nonfat dried milk in phosphate-buffered saline. The membranes were incubated with the anti-mouse apoB48/100 antibodies (Meridian Life Science, Memphis, TN), and then with peroxidase-conjugated anti-rabbit IgG antibodies (Santa Cruz Biotechnologies, Santa Cruz, CA), respectively. Protein bands were detected using an enhanced chemiluminescence (ECL) Western blotting detection system (Millipore Corporation). The bands were quantitatively evaluated using National Institutes of Health Image J software.

Measurement of intestinal TG in mice

Lipids in intestinal mucosa were extracted using the hexane/isopropanol (3:2) extraction methods (29). Briefly, intestinal mucosa were homogenized using hexane/isopropanol (3:2) for 1 min, the suspension was centrifuged, and the pellet was rinsed with the same solvent. The entire liquid phase was evaporated, the dried extract dissolved in isopropanol, and TG content was measured as above. Triolein dissolved in isopropanol was used as the standard for TG. The efficiency of extraction was measured by comparing the recovery of triolein in samples that had been spiked and samples that had not been spiked with known quantities of triolein standard (30). The assessed recovery was 81.2 ± 4.65%.

Measurement of TG in feces of mice

The feces were dried at 60°C overnight and the lipids were extracted using the Folch method (31). This analysis enables measurement of lipids extracted per gram of dried fecal samples. Briefly, lipids present in the feces were extracted using chloroform/methanol (2:1), dissolved in isopropanol, and TG content was measured as above.

Statistical analysis

Data are presented as means ± SEM. For analyses of two groups, unpaired Student’s t-test was used. To analyze three or more groups, ANOVA was used along with Tukey-Kramer’s multiple comparison tests to determine statistical significance. Differences were considered significant at P < 0.05.

RESULTS

DHA activated PPARα in CV-1 cells and Caco-2 cells

First, we investigated whether DHA activated PPARα based on a luciferase assay using the GAL4/PPARα chimera system. DHA activated luciferase activity of PPARα in CV-1 cells in a dose-dependent manner (Fig. 1A). Furthermore, DHA stimulated PPAR-response element (PPRE)-luciferase activity in Caco-2 cells (Fig. 1B). DHA also activated luciferase activity of PPARα in Caco-2 cells (Fig. 1C). The effects of DHA on PPARα activation were higher than those of EPA under our experimental conditions (approximately 5.9- and 2.6-fold increases at 25 μM DHA and EPA, respectively), as shown in Fig. 1C. Moreover, DHA enhanced the activation of PPARγ by approximately 1.7-fold (Fig. 1D). However, DHA did not increase PPARγ activation in Caco-2 cells (Fig. 1E, supplementary Fig. 1). Cytotoxicity was not observed following 25 μM DHA treatment of Caco-2 cells (data not shown). These results suggest that DHA induces PPARα activation in intact cells.

DHA induced the genes involved in FA oxidation and OCR in Caco-2 cells

To investigate the effects of PPARα activation by DHA on intestinal lipid metabolism, we measured mRNA expression levels of genes involved in FA oxidation in DHA-treated Caco-2 cells. DHA treatment induced mRNA expression of genes involved in FA oxidation, such as acyl-CoA synthetase (Acs), carnitine palmitoyltransferase 1A (Cpt1a), and acyl-CoA oxidase (Aox) and other PPARα target genes such as uncoupling protein-2 (Ucp2) and fatty acid binding protein (Fabp) (Fig. 2A–E). Moreover, the OCR, determined using extracellular flux analysis, was enhanced following DHA treatment as shown in Fig. 2F. In contrast, decanoic acid (C10), which had little activity toward PPARα, did not affect mRNA expression of Cpt1a, and palmitic acid (C16), which showed lower PPARα activity than DHA, did not significantly induce Cpt1a expression in Caco-2 cells (supplementary Fig. 2A, B). These findings suggest that DHA enhances FA oxidation in Caco-2 cells.

DHA decreased the secretion of TG and apoB from Caco-2 cells

To determine the effects of PPARα activation by DHA on lipid secretion from Caco-2 cells, we examined the amounts of lipid secreted from DHA-treated Caco-2 cells. TG secretion from DHA-treated Caco-2 cells was significantly decreased (to 77 and 72% with either 1 or 25 μM DHA treatment, respectively), as shown in Fig. 3A. DHA treatment reduced
the secretion of apoB, which is the primary apolipoprotein of chylomicrons, to 67 and 59% with either 1 or 25 μM DHA treatment, respectively (Fig. 3B). The effects of DHA on secretion were similar to those of bezafibrate, a potent PPARα agonist (Fig. 3A, B). While C10 did not inhibit TG secretion, C16 did decrease TG secretion from Caco-2 cells. However, the effect of C16 on decrease of TG secretion was lower than that of DHA (supplementary Fig. IIC). These results suggest that lipid secretion from intestinal epithelial cells is related to PPARα activity.

**DHA-rich oil enhanced FA oxidation in intestinal epithelial cells of C57BL/6 mice**

Next, we examined whether the effects of DHA in vitro also occurred in vivo. Because PPARα agonists are known to reduce food intake in rodents (32), all mice were housed in pair-fed conditions in each experiment; there was no difference in food intake between groups. The mRNA expression levels of FA oxidation-related genes such as Acs, Cpt1a, and Aox and other PPARα target genes such as Ucp2, Fabp, and Cd36 were increased in C57BL/6 mice fed a HFD containing DHA-rich oil for one week (Fig. 4A–F). When the cells were incubated with [14C]palmitic acid for 2 h, oxidation of [14C]palmitic acid to CO₂ and ASMs were enhanced in intestinal epithelial cells of DHA-rich oil-fed mice compared with control mice (Fig. 4G, H). However, surprisingly, DHA-rich oil-fed mice showed no increase in mRNA expression levels of FA oxidation-related genes in the liver under the same conditions as shown in Fig. 5A–C. Moreover, the production of CO₂ and ASM were not augmented in the liver of DHA-rich oil-fed mice compared with control mice (Fig. 5D, E). These findings suggest that DHA-rich oil enhances FA oxidation in intestinal epithelial cells of mice.

**Fig. 4.** FA oxidation was enhanced in intestinal epithelial cells of DHA-rich oil-fed C57BL/6 mice. A–F: The mRNA expression levels of FA oxidation-related genes (Acs, Cpt1a, and Aox) and other PPARα target genes (Ucp2, Fabp, and Cd36) in intestinal epithelial cells were quantified. G, H: Production of CO₂ and ASM in intestinal epithelial cells was determined using [14C]palmitic acid. Control values were set at 100% and the relative values are represented as fold induction relative to that of control. The values are means ± SEM of six tests. *P < 0.05 and **P < 0.01 compared with each control.
Effects of DHA on postprandial lipid metabolism were mediated by the activation of intestinal PPARα

To clarify the involvement of PPARα in the effects of DHA on postprandial lipid metabolism, we examined the effects of DHA in PPARα−/− mice. The baseline characteristics of PPARα−/− mice compared with control mice are shown in Table I. The mRNA expression levels of genes involved in FA oxidation (Acs, Cpt1a, and Aox), and the production of CO2 and ASM did not change significantly in intestinal epithelial cells of DHA-rich oil-fed PPARα−/− mice or bezafibrate-fed PPARα−/− mice (Fig. 7A–E, supplementary Fig. IVA–C). Moreover, there was no difference in intestinal TG levels between DHA-rich oil-fed PPARα−/− mice and control mice (Fig. 7F). Finally, the effects of DHA-rich oil on plasma TG and apoB levels after olive oil administration were abolished in PPARα−/− mice without and with tyloxapol, similar to the results of bezafibrate (Fig. 7G–I, supplementary Fig. IVD–F), suggesting that lipid secretion from intestinal epithelial cells is related to PPARα activity. These findings suggest that the activation of intestinal PPARα is a key factor for attenuating postprandial hypertriglyceridemia by decreasing TG secretion from intestinal epithelial cells.

DISCUSSION

Activation of PPARα is well-known to decrease plasma TG levels through FA oxidation in the liver and skeletal muscle (9–11). Although the role of PPARα expressed in intestinal epithelial cells remained obscure (8, 33), we and
DHA attenuates hyperlipidemia via intestinal PPAR activation in positive regulation of postprandial systemic lipid metabolism. Although it has been shown that PPAR activation in intestinal epithelial cells reduces postprandial hyperlipidemia, it was unknown whether postprandial hyperlipidemia is also improved by dietary lipids, which generally show lower PPAR activation than synthesized PPAR agonists (34, 35). Previous studies have indicated that DHA increases mRNA expression levels of FA oxidation-related genes in intestinal epithelial cells (36, 37) and that PUFAs including DHA enhance FA oxidation in hepatocytes (38). The present study has recently demonstrated that PPAR agonists improve postprandial hyperlipidemia through increasing FA oxidation in intestinal epithelial cells (15, 16). It is suggested that PPAR activation reduces TG secretion from intestinal epithelial cells, which attenuates postprandial hyperlipidemia (15, 16) (supplementary Fig. IV). To clarify the contribution of intestinal PPAR activation to postprandial systemic lipid metabolism, further investigation is necessary, including studies involving intestinal epithelial cell-specific PPAR knockout mice. However, these findings indicate that intestinal PPAR activation plays a critical role in positive regulation of postprandial systemic lipid metabolism.

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study showed that DHA enhanced FA oxidation and decreased TG secretion in Caco-2 cells and intestinal epithelial cells (Figs. 2–4, 6), resulting in reduction of postprandial hyperlipidemia via PPARα activation in mice (Figs. 6, 7). However, surprisingly, no induction of the genes involved in FA oxidation was observed in the liver of DHA-rich oil-fed mice under our experimental conditions (Fig. 5). Our findings presented here strongly indicate that effects of DHA in attenuating postprandial hyperlipidemia are attributed to the decrease of TG secretion from intestinal epithelial cells. During early stages after a meal, most TG secretion into circulation is thought to be derived from dietary fat absorbed in intestinal epithelial cells because they are directly exposed to dietary fat, while insulin prevents hepatic VLDL secretion during the postprandial state (39, 40). In DHA-rich oil-fed mice, plasma TG levels were decreased after olive oil administration with tyloxapol, which inhibits plasma lipoprotein lipase, suggesting that TG secretion from intestinal epithelial cells was reduced (Fig. 6D). This was supported by the results that DHA reduced TG and apoB secretion in Caco-2 cells, as shown in Fig. 3. Moreover, we observed that TG accumulation in intestinal epithelial cells was generally decreased (Fig. 6F) and the level in the weight of feces and fecal TG did not change in DHA-rich oil-fed mice (Fig. 6G, H). These findings suggest that DHA is a potent factor to reduce TG secretion from intestinal epithelial cells via FA oxidation by PPARα activation, resulting in attenuating postprandial hyperlipidemia.

In this study, mRNA expression levels of intestinal FA oxidation-related genes in DHA-rich oil-fed PPARα−/− mice
were increased, although the increases were not signifi-
cant (Fig. 7A, C). Previous reports have indicated that
PPARδ compensates for the lack of PPARα in the skeletal
muscles of PPARα−/− mice (41) and that PPARδ activates
FA oxidation (42). DHA and bezafibrate did not activate
PPARδ in our luciferase assays (Fig. 1E, supplementary
Fig. 1). However, the concentration of DHA exposed to
intestinal epithelial cells may have been much higher than
that used in Caco-2 cells. Therefore, the increase in intes-
tinal FA oxidation-related genes in Fig. 7A and C may be
related to the PPARα effect.

The present study showed higher mRNA expression lev-
els of Cd36 (Fig. 4), which is involved in FA transport in
intestinal epithelial cells of DHA-rich oil-fed mice. Cd36 is
thought to be involved in regulating chylomicron produc-
tion (43, 44). Interestingly, Cd36 knockout mice showed
both fasting and postprandial hyperlipidemia and have
been used as a model of postprandial hyperlipidemia (45).
A recent study showed that Cd36 critically regulates FA ox-
idation in skeletal muscle (46). Additionally, Cd36 is one of
PPARα target genes (47). Therefore, an increase of Cd36
may contribute to reduction of postprandial hyperlipidemia
via intestinal FA oxidation in DHA-rich oil-fed mice.

In conclusion, we found that DHA directly reduced TG
secretion from intestinal epithelial cells by activation of
PPARα-induced FA oxidation, resulting in improving post-
prandial hyperlipidemia. The present work suggests that
a dietary lipid such as DHA, which activates PPARα, is
a promising factor to attenuate postprandial hyperlipidemia
via intestinal FA oxidation.

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