



Activation of peroxisome proliferator-activated receptor- α (PPAR α) suppresses postprandial lipidemia through fatty acid oxidation in enterocytes

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ABSTRACT

Activation of peroxisome proliferator-activated receptor (PPAR)- α which regulates lipid metabolism in peripheral tissues such as the liver and skeletal muscle, decreases circulating lipid levels, thus improving hyperlipidemia under fasting conditions. Recently, postprandial serum lipid levels have been found to correlate more closely to cardiovascular diseases than fasting levels, although fasting hyperlipidemia is considered an important risk of cardiovascular diseases. However, the effect of PPAR α activation on postprandial lipidemia has not been clarified. In this study, we examined the effects of PPAR α activation in enterocytes on lipid secretion and postprandial lipidemia. In Caco-2 enterocytes, bezafibrate, a potent PPAR α agonist, increased mRNA expression levels of fatty acid oxidation-related genes, such as *acyl-CoA oxidase*, *carbamite palmitoyl transferase*, and *acyl-CoA synthase*, and oxygen consumption rate (OCR) and suppressed secretion levels of both triglycerides and apolipoprotein B into the basolateral side. *In vivo* experiments revealed that feeding high-fat-diet containing bezafibrate increased mRNA expression levels of fatty acid oxidation-related genes and production of CO₂ and acid soluble metabolites in enterocytes. Moreover, bezafibrate treatment suppressed postprandial lipidemia after oral administration of olive oil to the mice. These findings indicate that PPAR α activation suppresses postprandial lipidemia through enhancement of fatty acid oxidation in enterocytes, suggesting that intestinal lipid metabolism regulated by PPAR α activity is a novel target of PPAR α agonist for decreasing circulating levels of lipids under postprandial conditions.

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1. Introduction

Peroxisome proliferator-activated receptor (PPAR)- α is involved in regulation of fatty acid oxidation in various peripheral tissues including the liver and skeletal muscle [1]. PPAR α is among nuclear receptors that are ligand-dependent transcriptional factors inducing mRNA expression of target genes [2]. PPAR α activation enhances fatty acid oxidation by inducing mRNA expression of fatty acid oxidation-related genes such as *acyl-CoA synthase* (ACS), *carbamite palmitoyl transferase* (CPT), and *acyl-CoA oxidase* (AOX) [2,3]. The PPAR α -dependent enhancement of fatty acid oxidation decreases the levels of circulating and accumulating lipids. This is why synthetic PPAR α agonists such as fibrates have been widely used as anti-hyperlipidemic drugs [4]. We have identified and analyzed food-derived compounds that activate PPAR α [5–8]. These compounds decrease the amounts of lipids accumulated in

hepatocytes *in vitro* and suppress development of fatty liver *in vivo*. Moreover, we have reported that fatty acid oxidation in adipocytes is induced by PPAR α activation, thereby suppressing lipid accumulation [9]. Therefore, PPAR α activation is considered to be valuable for prevention and improvement of metabolic syndrome.

High serum lipid levels under fasting conditions have been considered a risk of cardiovascular diseases [10]. Many studies have revealed that serum lipid levels under postprandial conditions, rather than under fasting conditions, strongly correlate with the risk of cardiovascular diseases [11]. After absorption and resynthesis of triglycerides (TGs) in enterocytes, TGs are assembled into chylomicrons together with apolipoprotein B (ApoB). The chylomicrons assembled in enterocytes are transported through lymph vessels. It has been elucidated that intestinal lipid absorption and chylomicron production require CD36 [12]. Disruption of CD36 suppresses the secretion of lipids from enterocytes [13]. Therefore, intestinal lipid metabolism is indispensable for regulation of serum lipid levels in addition to hepatic lipid metabolism.

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In this study, we examined the effects of PPAR α activation in enterocytes on intestinal fatty acid oxidation and postprandial lipidemia. Treatment with bezafibrate, a synthetic PPAR α agonist, increased the mRNA expression levels of fatty acid oxidation-related genes and oxygen consumption rate (OCR) and inhibited lipid transport into the basolateral side in *in vitro* experiments using Caco-2 cells. Moreover, administration of bezafibrate also enhanced fatty acid oxidation in enterocytes and suppressed postprandial lipidemia in *in vivo* experiments using high-fat-diet (HFD)-fed mice. These findings indicate that PPAR α activation in enterocytes results in suppression of postprandial lipidemia by enhancement of fatty acid oxidation, suggesting that intestinal fatty acid oxidation is a novel target of PPAR α treatment for prevention and improvement of hyperlipidemia.

2. Materials and methods

2.1. Chemicals and cell culture

Bezafibrate was purchased from Sigma (MO, USA) and dissolved in DMSO as a stock solution. All other reagents were from Sigma or Nacalai Tesque (Kyoto, Japan) and guaranteed to be of reagent or tissue-culture grade.

Human Caco-2 cells were purchased from American Type Culture Collection (ATCC) and cultured in DMEM (100 mg/dL glucose) containing 10% FBS, 1% nonessential amino acid solution, and 10 mg/mL penicillin/streptomycin at 37 °C in 5% CO₂/95% air under a humidified condition. After seeding, Caco-2 cells were seeded at a density of 340,000 cells/mL on 12-well Traswell plates (Corning Inc., MA, USA) for 2 weeks for differentiation into enterocyte-like cells. For the evaluation of differentiation of Caco-2 cells, we measured intestinal alkaline phosphatase activity and transepithelial electrical resistance (TER). There was no significant change in these differentiation markers in all experiments (data not shown). Twenty-four hours before starting the experiments, apical medium was changed to DMEM containing 50 μ M bezafibrate, 600 μ M taurocholic acid Na salt hydrates, and 500 μ M oleic acid. At the same time, basolateral medium was also changed to serum-free DMEM. For ApoB and TG measurements, basolateral medium was collected.

2.2. Animal experiments

Nine-week-old male C57BL/6 mice were purchased from Clea Japan (Tokyo, Japan). The mice were maintained under a constant 12-h light/dark cycle. The mice were maintained for 7 days on a standard diet and then divided into two groups with the same average body weight and serum TG level. Each group was maintained on 60% HFD or HFD containing 0.2% (w/w) bezafibrate for 7 days. The energy intake of all the mice was adjusted by pair feeding. Thus, the levels of food intake of each group were almost the same (average food intakes were 2.88 ± 0.18 and 2.60 ± 0.17 g/day in the control HFD-fed and 0.2% bezafibrate-fed mice, respectively). For real-time quantitative RT-PCR, proximal 1/4 of the intestine was harvested from mice. After washing twice with cold PBS, intestinal epithelial tissue, which mainly contains enterocytes, was collected with a slide glass. The collected tissue was stored in RNAlater (Ambion/Applied Biosystems, TX, USA) at –80 °C until use. For measurement of fatty acid oxidation, enterocytes were collected from the proximal 1/4 of the intestine and incubated in 1 mg/mL collagenase IA/HBSS for 40 min. The collected enterocytes were washed with 1% FBS/DMEM three times and used for experiments. For measurement of plasma TG concentration, blood samples were collected from the tail vein of nonanesthetized mice. Anesthesia was induced using sevoflurane in all experiments.

2.3. Real-time quantitative RT-PCR

Total RNA samples were prepared from Caco-2 cells and enterocytes using Sepasol Super-I (Nacalai Tesque) and an SV total RNA isolation system (Promega, WI, USA), respectively, in accordance with each manufacturer's protocol as previously described [14]. To quantify mRNA expression, PCR was performed using a fluorescence temperature cycler (LightCycler System: Roche Diagnostics, Mannheim, Germany), as described previously [15]. Primer sets were designed using a PCR primer selection program at the web site of the Virtual Genomic Center from the GenBank database and the sequences were described in our previous reports [5,6]. To compare mRNA expression level among samples, the copy number of each transcript was divided by that of 36B4 showing a constant expression level. All mRNA expression levels are presented as the percentage of the control in each experiment.

2.4. Measurements of ApoB and TG

For measurement of ApoB amount, ELISA was performed using an anti-human low-density lipoprotein APO-B antibody (Clone 12G10; Monosan, Uden, Netherlands), affinity purified anti-apolipoprotein B (Rockland, PA, USA), and HRP-conjugated anti-goat IgG (Promega) as the capture, primary, and secondary antibodies, respectively. Details of procedures were previously described [16]. HRP activity was detected using TMB peroxidase substrate (KPL, MD, USA). Purified human very low-density lipoprotein (VLDL; Chemicon Millipore, MA, USA) was used as the standard protein. For measurement of TG amount, we used triglyceride E Test WAKO (Wako, Osaka, Japan).

2.5. Determination of OCR

OCR indicative of mitochondrial respiration was determined using an XF24 Extracellular Flux Analyzer (Seahorse Bioscience, MA, USA). The XF24 device created a transient 7 μ L chambers above target cells in which cells were monitored in real-time as previously reported [17]. Caco-2 cells were cultured for 2 weeks on the customized Seahorse 24-well plates (Seahorse Bioscience, MA, USA). Differentiated Caco-2 cells were incubated in pre-warmed XF24 assay medium for 1 h. The assay media consisted of DMEM containing 10% FBS, 1 mM L-glutamine, 1 mM sodium pyruvate, 3.7 g/L NaCl, and 25 mM glucose. OCR was calculated by plotting the oxygen tension of the medium in the chamber as a function of time (pmol/min). OCR (pmol/min) was divided by protein amount in each well.

2.6. Immunoblottings

Immunoblottings were carried out as previously described [14,15]. The anti-mouse AOX antibody was from Abcam (MA, USA). Anti-mouse b-actin and horseradish peroxidase-conjugated anti-rabbit IgG were purchased from Cell Signaling Technology (MA, USA) and Santa Cruz Biotechnology (CA, USA), respectively. Protein bands were detected by chemiluminescence using an enhanced chemiluminescence (ECL) system (NEN Lifescience Products) in accordance with the manufacturer's instructions.

2.7. Measurement of fatty acid oxidation

The collected enterocytes harvested from the control or bezafibrate-containing HFD-fed mice were incubated in DMEM containing 200 μ M palmitic acid, 0.005% fatty-acid-free BSA, 200 μ M L-carnitine, and [¹⁴C]palmitic acid (1 μ Ci) (American Radiolabeled Chemicals, MO, USA) for 2 h. Fatty acid oxidation products were assessed as previously described [9] with modification.

Briefly, the labeling medium containing enterocytes was transferred to a 50-mL polypropylene tube. An uncapped 2-mL sample tube containing a piece of filter paper soaked in 3 N NaOH was placed inside a 50-mL sample tube. After the tube was sealed, 200 μ L of 12 N HCl was added to the medium sample to release [14 C]-CO₂. The tube was then incubated at 37 °C for 24 h. The saturated filter paper containing trapped [14 C]-CO₂ was assessed for radioactivity in a liquid scintillation counter (LS6500, Beckman Coulter, CA, USA). The acidified medium was centrifuged and 200 μ L of supernatant was assessed for the amount of [14 C]-labeled acid soluble metabolites, which include labeled ketone bodies. Protein concentration was determined using a Protein Assay kit (Bio-Rad, CA, USA).

2.8. Statistical analysis

The data were presented as means \pm S.E.M. and statistically analyzed using one-way ANOVA when their variances were heterogeneous and unpaired *t*-test. Differences were considered significant at *P* < 0.05.

3. Results

3.1. Treatment with bezafibrate increases mRNA expression levels of fatty acid oxidation-related genes in Caco-2 cells and suppresses TG secretion into the basolateral side

To examine the effects of PPAR α activation on enterocytes, we first performed *in vitro* experiments using Caco-2 cells.

Expression of PPAR α mRNA was detectable and constant throughout our experimental period (data not shown). Differentiated Caco-2 cells treated with 50 μ M bezafibrate for 24 h showed significant increases in mRNA expression levels of fatty acid oxidation-related genes such as ACS, CPT1A, and AOX, as shown in Fig. 1A. These findings suggest that bezafibrate treatment increased fatty acid oxidation in Caco-2 cells. Indeed, OCR was significantly increased by bezafibrate treatment (Fig. 1B). Under the same conditions, TG secretion into the basolateral side of Transwell plates was suppressed (Fig. 1C). Secretion of the ApoB protein, which is a component of chylomicrons that transports food-derived lipids, was also suppressed by bezafibrate treatment (Fig. 1D). These findings indicate that bezafibrate treatment suppressed TG secretion by increasing mRNA expression levels of fatty acid oxidation-related genes in Caco-2 cells, suggesting that enhancement of fatty acid oxidation suppresses TG secretion in enterocytes.

3.2. Feeding of bezafibrate enhances mRNA expression levels of fatty acid oxidation-related genes and fatty acid oxidation in intestinal epithelial cells of mice

To examine the *in vivo* effects of PPAR α activation on TG secretion, C57BL mice were fed HFD containing 0.2% bezafibrate for 7 days. Bezafibrate treatment resulted in the increase in the mRNA expression levels of fatty acid oxidation-related genes such as ACS, CPT1A, and AOX in enterocytes, although the increase of CPT1A and AOX mRNA expression was not statistically significant, as shown in Fig. 2. Protein expression level of AOX was increased as shown in Fig. 2D. Measurements of fatty acid oxidation using [14 C]-labeled

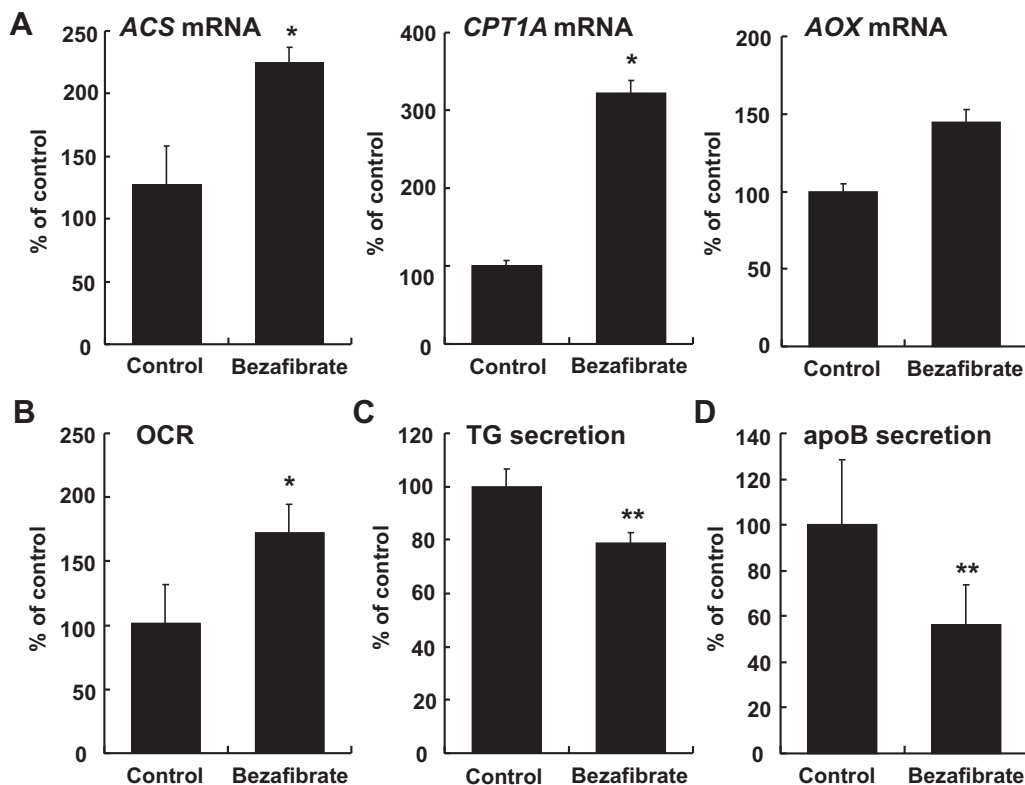


Fig. 1. Effects of bezafibrate treatment on lipid metabolism in Caco-2 cells. (A) Increased mRNA expression levels of fatty acid oxidation-related genes (ACS, CPT1A, and AOX) in bezafibrate-treated Caco-2 cells. The copy number of each transcript was divided by that of 36B4 for normalization. (B) Increase in oxygen consumption rate (OCR) induced by bezafibrate treatment. (C) Suppression of TG secretion into the basolateral side. (D) Suppression of ApoB secretion into the basolateral side. The value of a vehicle control was set at 100% and relative value is presented as fold induction compare with that of the vehicle control. The values are means \pm S.E.M. of 3–4 samples. **P* < 0.05 and ***P* < 0.01 compared with each vehicle control.

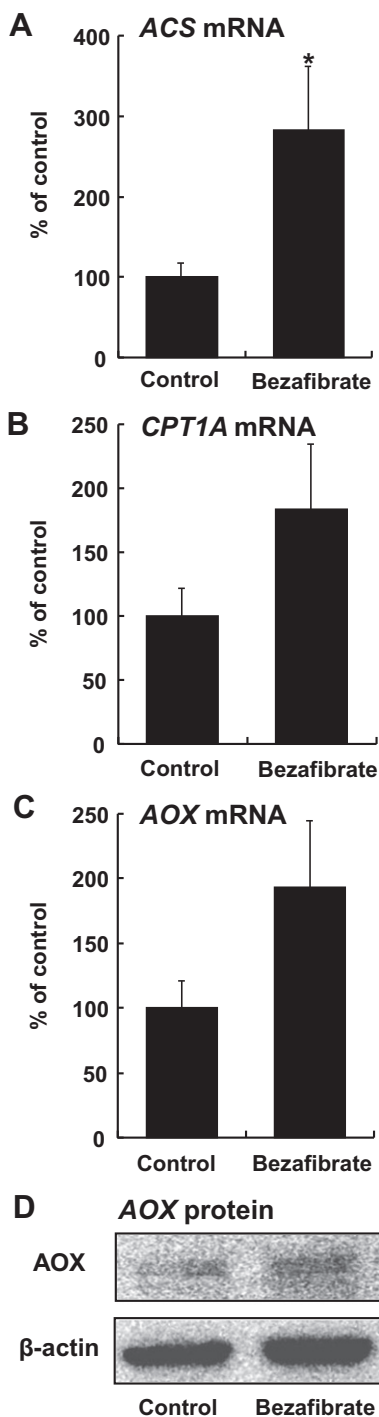


Fig. 2. Effects of bezafibrate treatment on mRNA expression of fatty acid oxidation-related genes in enterocytes. Increased mRNA expression levels of fatty acid oxidation-related genes, ACS (A), CPT1A (B), and AOX (C) in enterocytes of bezafibrate-fed mice. The copy number of each transcript was divided by that of 36B4 for normalization. The value of a vehicle control was set at 100% and relative value is presented as fold induction compared with that of the vehicle control. The values are means \pm S.E.M. of 6–7 samples derived from different mice. * $P < 0.05$ compared with each vehicle control. (D) Immunoblottings of AOX expressing in enterocytes. A representative data are presented. Immunoblottings of β -actin are indicated as a control.

palmitic acid revealed that the bezafibrate-treated enterocytes showed increased productions of CO_2 and ketone bodies, which are products of fatty acid oxidation (Fig. 3). These findings indicate that bezafibrate feeding for 7 days enhances fatty acid oxidation in enterocytes.

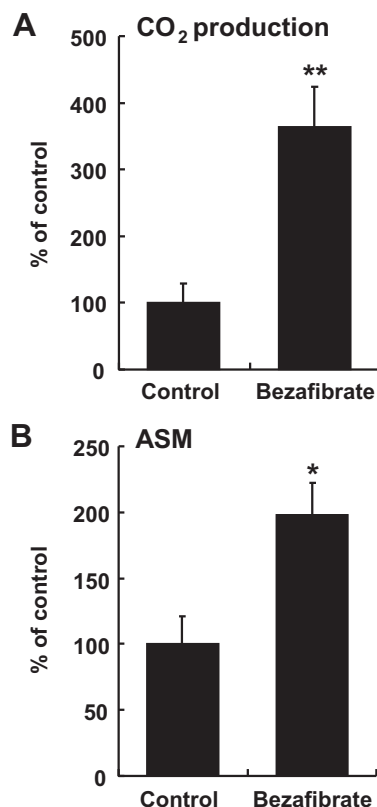


Fig. 3. Effects of bezafibrate administration on fatty acid oxidation in enterocytes. Increased fatty acid oxidation in enterocytes of bezafibrate-fed mice. The value of a vehicle control was set at 100% and relative value is presented as fold induction compared with that of the vehicle control. The values are means \pm S.E.M. of 6–7 samples derived from different mice. * $P < 0.05$ and ** $P < 0.01$ compared with each vehicle control.

3.3. Feeding of bezafibrate suppresses postprandial hyperlipidemia in mice

To study the effects of bezafibrate-induced enhancement of fatty acid oxidation on postprandial lipidemia, plasma TG concentration was measured after oral administration of olive oil (300 $\mu\text{L}/\text{mouse}$). After 16 h fasting to decrease basal TG concentration, olive oil was orally administered and plasma TG concentration was determined every 30 min after the administration. The plasma TG concentration was lower in bezafibrate-fed mice than in control mice throughout the experimental periods as shown in Fig. 4A. Initial peaks of plasma TG concentration 90 min after the administration were 198 and 91.9 mg/dl in the control and bezafibrate-fed mice, respectively. Area under curve (AUC) of plasma TG concentration in bezafibrate-fed mice was smaller than that in control mice (Fig. 4B). The findings suggest that bezafibrate feeding suppressed postprandial lipidemia through the enhancement of fatty acid oxidation in enterocytes.

4. Discussion

Hyperlipidemia is considered to be a risk factor for cardiovascular diseases [10,11]. The liver mainly regulates circulating amounts of lipids under normal conditions through production and absorption of lipoproteins. However, under postprandial conditions, lipid absorption into and transport from intestinal epithelial tissue are also important for regulating serum lipid levels. Postprandial serum lipid levels have been shown to have a stronger positive correlation with coronary artery disease than the fasting serum lipid

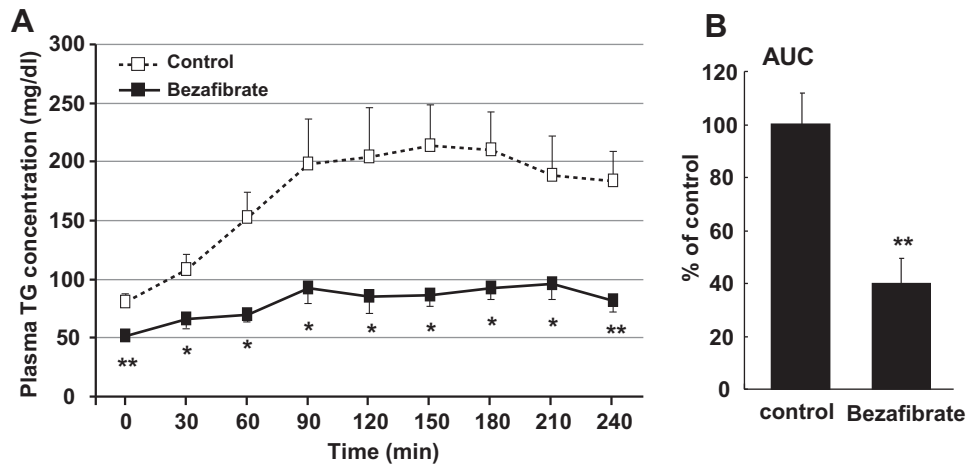


Fig. 4. Effects of bezafibrate administration on postprandial hyperlipidemia. (A) Decreased plasma TG concentration in bezafibrate-fed mice. Olive oil (300 μ L/mouse) was orally administered at time 0. After the administration, plasma TG concentration (mg/dL) was determined for 240 min at intervals of 30 min. (B) Areas under curves (AUC) were calculated and indicated. The values are means \pm S.E.M. of 6–7 serum samples derived from different mice. * P < 0.05 and ** P < 0.01 compared with each plasma TG concentration at the same time.

levels [11]. Thus, decreasing postprandial serum lipid levels is valuable for prevention of cardiovascular diseases. For suppression of absorption of dietary lipids, pancreatic lipase inhibitors have been used [18,19]. Orlistat, a pancreatic lipase inhibitor used for the treatment of obesity, is an effective drug for suppressing lipid absorption through intestinal epithelia and preventing weight gain in the treatment of obesity in the primary care setting. However, such inhibition of pancreatic lipase causes fecal urgency, oily spotting, and fatty/oily stool [20]. Our present study indicates that enhancement of fatty acid oxidation in enterocytes suppresses postprandial lipidemia through PPAR α activation in mice. Thus far, there has been no report showing such fecal side effects in the case of PPAR α agonist administration. Therefore, the present study suggests a possibility that suppression of fatty acid oxidation by PPAR α activation is a novel target for prevention of cardiovascular diseases.

We have demonstrated that many food-derived compounds function as PPAR α agonists with anti-diabetic and anti-hyperlipidemic effects in various tissues such as the liver and skeletal muscle [5–8,21]. Thus far, it has been considered that the activity of such compounds as PPAR α agonists contributes to the decrease in TG concentration in the liver, skeletal muscle, and plasma in such mice fed food-derived compound. As described above, enhancing fatty acid oxidation in enterocytes suppresses postprandial lipidemia in mice. Thus, enhancing intestinal fatty acid oxidation might contribute to the improvement of lipid accumulation in the liver and skeletal muscle and/or hyperlipidemia caused by metabolic abnormalities, which was observed in our previous studies using obese diabetic mice, although further investigations are needed to elucidate the contribution. Natural compounds activating PPAR α generally show lower activities than synthetic agonists such as bezafibrate [22]. This is because natural compounds are transformed into metabolites that have no effect on the liver before entering the whole-body circulation. In this sense, the intestinal effects of natural compounds before being metabolized in the liver are very significant when considering the functions of food-derived compounds. The present study indicates that we should consider the importance of intestinal lipid metabolism as a primary target of PPAR α agonists.

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