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# Pregnane X receptor (PXR) deficiency improves high fat diet-induced obesity via induction of fibroblast growth factor 15 (FGF15) expression

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ABSTRACT

Obesity has become a significant global health problem, and is a high risk factor for a variety of metabolic diseases. Fibroblast growth factor (FGF) 15 plays an important role in the regulation of metabolism. Xenobiotic-sensing nuclear receptors pregnane X receptor (PXR/NR1I2) and constitutive androstane receptor (CAR/NR1I3) play important roles in xenobiotic detoxification and metabolism, and also are involved in the regulation of energy metabolism. However, the effects that PXR and CAR have on the regulation of FGF15 are unknown. Here, we found that body weight, hepatic triglyceride levels, liver steatosis, and hepatic mRNA expression levels of cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1), the key enzymes in the bile acid classical synthesis pathway, were significantly decreased in high fat diet (HFD)-fed PXR knockout (KO) mice compared to HFD-fed wild-type mice. Interestingly, intestinal FGF15 expression levels were significantly elevated in HFD-fed PXR KO mice compared with HFD-fed wild-type mice. Additionally, serum total bile acid levels were significantly decreased in PXR KO mice than those in wild-type mice when fed a control diet or HFD. Total lipids in feces were significantly increased in HFD-fed PXR KO mice compared to HFD-fed wild-type mice. However, these alterations were not found in HFD-fed CAR KO mice. These results indicate that PXR deficiency improves HFD-induced obesity via induction of FGF15 expression, resulting in suppression of bile acid synthesis and reduction of lipid absorption, hepatic lipid accumulation and liver triglyceride levels. Our findings suggest that PXR may negatively regulate FGF15 expression and represent a potential therapeutic target for the treatment for metabolic disorders such as obesity.



## 1. Introduction

Excessive intake of dietary fat highly contributes to the development of obesity. Obesity has become a significant global health problem and is a high risk factor for a variety of metabolic diseases such as nonalcoholic fatty liver, nonalcoholic steatohepatitis, and diabetes. Exploration for improving obesity outcomes has been a concern throughout the world.

Fibroblast growth factor (FGF) 15 and FGF19 are mouse and human orthologs, respectively. FGF15/19 has been found to be expressed in ileal enterocytes of the small intestine where it functions as a hormone and has important effects on the regulation of metabolism. FGF15/19 plays a crucial role in the regulation of bile acid homeostasis via enterohepatic circulation, primarily by negatively regulating bile acid synthesis through suppression of hepatic cholesterol 7α-hydroxylase (CYP7A1), which is the first and rate-limiting enzyme in the classical bile acid synthesis pathway [1–3]. Due to its importance in the regulation of the bile acid synthesis pathway, FGF15/19 is a potential pharmaceutical target for the treatment for metabolic disorders such as obesity, type 2 diabetes, and dyslipidemia [4,5]. Additionally, the metabolic effects of FGF15/19 have been investigated in experimental mouse models. Tomlinson et al. reported that FGF19 transgenic *ob/ob* mice were resistant to diet-induced obesity and diabetes [6]. Fu et al. reported that treatment of *ob/ob* mice with FGF19 significantly reduced body weight gain and liver triglycerides [7]. FGF19 treatment also decreased liver triglycerides of high fat diet (HFD)-fed

*Abbreviations:* FGF, fibroblast growth factor; PXR, pregnane X receptor; NR, nuclear receptor; CAR, constitutive androstane receptor; CYP, cytochrome P450; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; HFD, high fat diet; KO, knockout; ob, obesity; FXR, farnesoid X receptor; H&E, hematoxylin-eosin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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mice. They further found that the body weights and liver triglycerides of the FGF19 transgenic *ob/ob* mice were significantly less than the non-transgenic *ob/ob* mice.

Farnesoid X receptor (FXR), vitamin D receptor and vitamin A receptor (retinoid X receptor) have been reported to transcriptionally regulate FGF15 expression [1,8]. Recently, Diet1 has been reported to play a role in the regulation of FGF15/19 expression resulting in modulated bile acid and lipid levels [9]. Although some nuclear receptors and proteins have been identified as regulators of FGF15/19 expression, factors for regulation of FGF15/19 are not fully understood.

Pregnane X receptor (PXR/NR1I2) and constitutive androstane receptor (CAR/NR1I3) are identified as xenobiotic-sensing nuclear receptors [10,11]. Both of them have important roles in xenobiotic detoxification and metabolism by regulating phase I and phase II drug-metabolizing enzymes and transporters [12–19]. Additionally, PXR and CAR have been found to be involved in the regulation of energy metabolism [20]. He et al. and Spruiell et al. reported that under HFD feeding, PXR knockout (KO) mice showed less body weight gain, and they further elucidated that PXR deficiency inhibited insulin resistance and impaired glucose homeostasis, respectively [21,22]. These results indicated that PXR inhibited HFD-induced obesity in mice. However, whether FGF15 is involved in the suppressive effect of PXR on obesity is not known.

In this report, to our knowledge, we are the first to demonstrate that PXR deficiency improves HFD-induced obesity via induction of FGF15 expression. Here, we found that PXR KO mice were resistant to HFD-induced obesity. HFD-fed PXR KO mice showed significantly lower levels of liver triglycerides, hepatic steatosis, serum total bile acids, and hepatic gene expression of enzymes involved in bile acid synthesis pathway, but markedly higher levels of intestinal FGF15 expression and fecal total lipids in comparison with HFD-fed wild-type mice. These alterations were not found in HFD-fed CAR KO mice. Our findings suggest that PXR may play roles in negative regulation of FGF15 expression. Thus, high levels of FGF15 suppress bile acid synthesis, resulting in reducing the absorption of lipids, hepatic lipids accumulation and liver triglycerides.

## 2. Materials and methods

### 2.1. Animals and treatments

PXR KO and CAR KO mice on the C57BL/6 genetic background were kindly provided by Dr. Masahiko Negishi (National Institute of Environmental Health Sciences/National Institutes of Health, Research Triangle Park, NC) [23,24]. Male PXR KO, CAR KO, or their littermate wild-type mice (5–6 mice/group) of 6-week-old were fed a high fat diet (HFD) consisting of 45% of calories from fat or control diet consisting 10% of calories from fat (Medicience Ltd., Yangzhou, China) for 4 weeks. Mice were housed in a specific pathogen-free and environmentally temperature-controlled facility with a 12-h light/dark cycle with access to rodent food and water *ad libitum*. All protocols for animal handling were approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University.

### 2.2. Blood and tissues collection

All mice were sacrificed after four weeks of control diet or high fat diet feeding. Serum samples were separated from blood by centrifugation. Liver and kidneys were collected and weighed. A segment of fresh liver tissue was fixed in 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, MO) for histological examination. The remaining liver tissue was stored at −80 °C until analysis. Small intestine was harvested and divided into two equal lengths. Distal part of the small intestine which contains ileum was cut open longitudinally. The distal intestinal mucosa was gently scraped and collected. Samples were stored at −80 °C until use.

### 2.3. Hepatic triglyceride levels analysis

Hepatic triglyceride levels were quantified by a triglyceride assay kit (Cayman, Ann Arbor, MI) according to the manufacturer's instructions. A segment of liver tissue was homogenized in the diluted Standard Diluent which was provided by the kit and centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatants were collected and diluted with the diluted Standard Diluent. Then the samples were subjected to triglyceride analysis.

### 2.4. Serum total bile acids analysis

Serum total bile acids were quantified by an enzymatic total bile acids assay (Joyin, Shanghai, China) according to the manufacturer's instructions.

### 2.5. Histological study

A segment of liver tissue was fixed in 10% neutral buffered formalin, embedded in paraffin, cut in four-micrometer-thick sections, and stained with hematoxylin-eosin (H&E) (Beyotime, Shanghai, China).

### 2.6. Quantitative real-time PCR analysis

Total RNA was prepared from the liver or distal small intestinal mucosa using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Total RNA of 1 μg was synthesized to cDNA by reverse transcription using iScript Select cDNA Synthesis Kit (Bio-Rad, Hercules, CA) according to the manufacturer's protocol. mRNA levels were quantified by quantitative real-time PCR with iTaq™ Universal SYBR Green Supermix (Bio-Rad) and CFX96 Touch Real-Time PCR Detection system (Bio-Rad). Primer sequences are listed in Table 1. Relative mRNA expression levels were normalized against housekeeping gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same sample and were calculated using the $2^{-\Delta\Delta Ct}$ method.

### 2.7. Western blot analysis

Tissues were homogenized in lysis buffer (Beyotime) and centrifuged. Protein concentration of the supernatant was determined with Bio-Rad protein assay reagent. Samples were diluted with SDS sample buffer (Beyotime), subjected to 10% polyacrylamide gel electrophoresis and then transferred to a polyvinylidene difluoride (PVDF) membrane (Invitrogen). The membrane was blocked with 5% nonfat milk blocking buffer for one hour at room temperature and blotted with FGF15 (1:500, Santa Cruz Biotechnology, Santa Cruz, CA) or GAPDH (1:1000, Cell Signaling Technology, Danvers, MA) primary antibody, overnight at 4 °C. The blots were incubated with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (1:1000, Cell Signaling Technology) for one hour at room temperature. The protein bands on the membranes were developed using SuperSignal Western Femto kit (Pierce Biotechnology, Rockford, IL) and quantified by ChemiDoc XRS$^+$ system (Bio-Rad). GAPDH is used as a loading control.

### 2.8. Fecal lipids content analysis

During four weeks of diet treatment, mice were housed in individual metabolic cages with food (control diet or HFD) and water