DMD Fast Forward. Published on May 25, 2023 as DOI: 10.1124/dmd.123.001311
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Adipocyte-derived PXR signaling is dispensable for diet-induced obesity and metabolic disorders in mice

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DMD Fast Forward. Published on May 25, 2023 as DOI: 10.1124/dmd.123.001311
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Running Title: Adipocyte PXR does not play an essential role in obesity.

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Abbreviations: ACOX1: acyl-CoA oxidase 1; AP2: adipocyte protein 2; BAT: brown adipose tissue; CPT1b: carnitine palmitoyltransferase 1b; CVD: cardiovascular disease; epiWAT: epididymal white adipose tissue; FASN: fatty acid synthase; G6Pase: glucose 6-phosphatase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GLUT2: glucose transporter 2; GLUT4: glucose transporter 4; HFD: high-fat diet; IPGTT: intraperitoneal glucose tolerance test; IPITT: intraperitoneal insulin tolerance test; IRS1: insulin receptor substrate 1; KRB: BSA-Krebs-Ringer Bicarbonate; LDLR: low density lipoprotein receptor; ND: normal chow diet; OCT: optimal cutting temperature; PPARα: peroxisome proliferator-activated receptor α; PPARγ : peroxisome proliferator-activated receptor γ; PCN: pregnenolone 16α-carbonitrile; PEPCK: phosphoenolpyruvate carboxykinase; PRDM16: PR domain zinc finger protein 16; PXR: pregnane X receptor; retroWAT: retroperitoneal white adipose tissue; RIPA: radioimmunoprecipitation assay; SCD1: stearoyl-Coenzyme A desaturase 1; subWAT: subcutaneous white adipose tissue; SV: stromal vascular; SXR: steroid and xenobiotic receptor; UCP1: uncoupling protein 1; WAT: white adipose tissue; WT: wild type.

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Abstract

Pregnane X receptor (PXR) is a xenobiotic receptor that can be activated by numerous chemicals including endogenous hormones, dietary steroids, pharmaceutical agents, and environmental chemicals. PXR has been established to function as a xenobiotic sensor to coordinately regulate xenobiotic metabolism by regulating the expression of many enzymes and transporters required for xenobiotic metabolism. Recent studies have implicated a potentially important role for PXR in obesity and metabolic disease beyond xenobiotic metabolism, but how PXR action in different tissues or cell types contributes to obesity and metabolic disorders remains elusive. To investigate the role of adipocyte PXR in obesity, we generated a novel adipocyte-specific PXR deficient mouse model ($PXR^{\Delta Ad}$). Notably, we found that loss of adipocyte PXR did not affect food intake, energy expenditure, and obesity in high-fat diet-fed male mice. $PXR^{\Delta Ad}$ mice also had similar obesity-associated metabolic disorders including insulin resistance and hepatic steatosis as control littermates. PXR deficiency in adipocytes did not affect expression of key adipose genes in $PXR^{\Delta Ad}$ mice. Our findings suggest that adipocyte PXR signaling may be dispensable in diet-induced obesity and metabolic disorders in mice. Further studies are needed to understand the role of PXR signaling in obesity and metabolic disorders in the future.

Significance Statement: We demonstrate that deficiency of adipocyte PXR does not affect diet-induced obesity or metabolic disorders in mice and infer that adipocyte PXR signaling may not play a key role in diet-induced obesity. More studies are needed to understand the tissue-specific role of PXR in obesity.

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Introduction

The prevalence of obesity has rapidly increased over the past three decades, and the numbers of obese and overweight individuals are expected to increase to more than half of the world's population by 2030 (Kelly et al., 2008; Zhou, 2016). Obesity is a major risk factor for developing other chronic diseases including diabetes and cardiovascular disease (CVD) (Van Gaal et al., 2006; Gregor and Hotamisligil, 2011). While the contributions of diet and lifestyle to obesity have been well-recognized, the chemical environment to which we are exposed has also changed substantially and recent studies from us and others have implicated that certain environmental chemicals may act as "obesogens" to increase the risk of obesity (Grun et al., 2006; Grun and Blumberg, 2007; Diamanti-Kandarakis et al., 2009; Casals-Casas and Desvergne, 2011; Heindel and Blumberg, 2019; Egusquiza and Blumberg, 2020).

Many environmental chemicals have been demonstrated to activate the nuclear receptor pregnane X receptor (PXR; also known as steroid and xenobiotic receptor, or SXR; NR1I2 for standard nomenclature) (Bertilsson et al., 1998; Blumberg et al., 1998; Kliewer et al., 1998; Kliewer et al., 2002; Zhou et al., 2009b). PXR is a unique nuclear receptor that can be activated by numerous chemicals including dietary steroids, pharmaceutical agents, environmental chemicals and endogenous hormones (Blumberg et al., 1998; Kliewer et al., 1998; Zhou et al., 2009b; Zhou, 2016). PXR plays a key role in regulating the expression of many enzymes and transporters required for xenobiotic metabolism in the liver and intestine (Blumberg et al., 1998; Kliewer et al., 2002; Zhou et al., 2009b; Zhou, 2016). In the past two decades, the role of PXR as a xenobiotic sensor to regulate xenobiotic metabolism have been well studies by many groups (Zhou et al., 2009b).

Recent studies from our laboratory and others revealed novel functions of PXR in cardiometabolic disease including obesity, diabetes and cardiovascular disease (CVD) beyond xenobiotic metabolism (Bhalla et al., 2004; Kodama et al., 2004; Kodama et al., 2007; de Haan et al., 2009; Zhou et al., 2009a; Cheng et al., 2012; He et al., 2013; Spruiell et al., 2014a; Spruiell et al., 2014b; Sui et al., 2015; Zhou, 2016; Helsley and Zhou, 2017; Gwag et al., 2019; Meng et al., 2019; Zhang et al., 2023). Several studies have also investigated the function of PXR in obesity and metabolic disorders. For example, He et al. previously revealed an important role of PXR in obesity by feeding wild-type (WT) and PXR-deficient ($PXR^{-/-}$) mice with a high-fat diet

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(HFD) (He et al., 2013). Interestingly, they found that deficiency of PXR protected mice from diet-induced obesity and metabolic disorders such as insulin resistance and hepatic steatosis (He et al., 2013). Another study also reported that male $PXR^{-/-}$ mice were resistant to diet-induced obesity (Spruiell et al., 2014b). Paradoxically, male but not female human PXR transgenic mice on PXR-null background (PXR-humanized mice) were also protected from diet-induced obesity (Spruiell et al., 2014a; Spruiell et al., 2014b). Despite reduced diet-induced obesity, both male $PXR^{-/-}$ and PXR-humanized mice had increased insulin resistance phenotypes (Spruiell et al., 2014b) which were not consistent with the results from He et al.'s study (He et al., 2013). While these studies indicate a potential role of PXR in obesity and metabolic disorders, the results were not consistent, suggesting that more studies are needed to establish the function of PXR in diet-induced obesity.

In addition to liver and intestine, PXR is widely expressed in many other tissues and cell types (Owen et al., 2004; Albermann et al., 2005; Siest et al., 2008; Zhou et al., 2009a; Dubrac et al., 2010; Casey et al., 2011; Casey and Blumberg, 2012; Zhou, 2016). As a major energy storate site, adipose tissue also has essential regulatory roles in glucose homeostasis and energy balance during the emergence of obesity (Rosen and MacDougald, 2006). In addition, adipose tissue is also a potential site for toxicant accumulation and may play an important role in the storage and overall toxicokinetics of many xenobiotic chemicals (Jackson et al., 2017). However, the functions of adipocyte PXR remain elusive, partially due to the lack of tissue-specific PXR knockout mice. To investigate the function of adipocyte-derived PXR in obesity and metabolic disorders, we generated a novel adipocyte-specific PXR deficient mouse model. Here we demonstrate that deficiency of adipocyte PXR does not affect diet-induced obesity or metabolic disorders in male mice and infer that adipocyte PXR may not play an important role in diet-induced obesity in male mice.

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Materials and Methods

Animals

To study cell-specific functions of PXR, we previous generated PXR flox mice ($PXR^{F/F}$) on C56BL/6 background carrying loxP-flanked PXR alleles ($PXR^{F/F}$) (Gwag et al., 2019; Sui et al., 2021). To investigate the role of adipocyte PXR signaling, adipocyte-specific PXR knockout ($PXR^{\Delta Ad}$) mice were generated by crossing $PXR^{F/F}$ mice with Adiponectin-Cre (Adipoq-Cre) transgenic mice (Eguchi et al., 2011; Park et al., 2016). In this study, $PXR^{F/F}$ and $PXR^{\Delta Ad}$ had the same PXR flox background except for one allele of $PXR^{\Delta Ad}$ mice carrying Adipoq-Cre. Four-week-old male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ littermates were fed with either a normal chow diet (ND) or a western-type high-fat diet (HFD) containing 21.2% fat by weight (42% kcal from fat) (TD88137, Harlan Teklad) for 12 weeks until euthanasia at 16 weeks old (Sui et al., 2014; Park et al., 2016; Liu et al., 2023). In addition, mallet C56BL/6 WT mice were also fed with ND or HFD for 9 weeks. All animal studies were performed in compliance with approved protocols by Institutional Animal Care and Use Committees of University of California, Riverside and University of Kentucky.

Metabolic analyses

For obesity and metabolic phenotypic analyses, bodyweight was measured weekly and body composition was also analyzed by EchoMRI (Echo Medical System) the day before euthanasia (Helsley et al., 2016; Lu et al., 2019). Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin resistance test (IPITT) were performed as described previously (Helsley et al., 2016; Hernandez et al., 2023; Liu et al., 2023). Food intake, total activity, oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio, and energy expenditure were measured in mice with a LabMaster caging system (TSE Systems) using a separate cohort of male mice (Park et al., 2016). Each mouse was housed individually and monitored for 4–5 consecutive days. VO_2, VCO_2 and energy expenditure data were normalized by lean body mass and average from 4-day measurements (Park et al., 2016).

Insulin stimulation and glucose uptake studies

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For insulin administration studies, insulin (0.35 U/kg body weight) was injected into the inferior vena cava of 16-week-old male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (Park et al., 2016). Mice were euthanized after 5 minutes, and adipose tissues were collected for further protein isolation and Western blotting analysis. For glucose uptake studies, adipose tissue explants were used as previously described (Varlamov et al., 2010; Helsley et al., 2016). HFD-fed male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice were used for these studies. Mice were fasted for 7 h and epididymal white adipose tissue (WAT) was collected and minced into small pieces. The minced tissue explants were then incubated in 1% BSA-Krebs-Ringer Bicarbonate (KRB) buffer for 2 h in 24-well plates. The explants were then washed before incubating with saline or 17 nM insulin at 37 ℃ for 40 min. After the incubation, 1 μCi [^{3}H]-2-deoxyglucose (Perkin Elmer) was then added to the wells and incubated at 37 ℃ for 20 min. The tissues were washed with cold 1% BSA-KRB buffer and tissue weights were measured. The tissues were incubated in 1 ml 1N NaOH at 65 ℃ for 1 h. Half of the cell lysate was used for the radioactivity measurement by using a scintillation counter.

RNA isolation and quantitative Real-Time RT-PCR analyses

Total RNAs were isolated from mouse tissues or cells using TRIzol Reagent (Thermo Fisher Scientific) as previously described (Sui et al., 2015; Sui et al., 2021; Satta et al., 2022). Adipose stromal vascular (SV) cells and mature adipocytes were isolated from white adipose tissue as we previously described (Sui et al., 2014; Helsley et al., 2016). Quantitative Real-Time RT-PCR (QPCR) was performed on a Bio-Rad CFX Real-Time-PCR Machine by using gene-specific primers and SYBR Green PCR kit (Bio-Rad Laboratories) (Meng et al., 2019; Sui et al., 2021). The sequences of primer sets used in this study are listed in Supplemental Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the reference gene to normalize the mRNA expression.

Western blotting

Proteins were isolated from mouse tissues by homogenization in radioimmunoprecipitation assay (RIPA) buffer with complete mini protease inhibitor cocktail (Roche) as previously described (Meng et al., 2019; Sui et al., 2021). Protein concentrations were measured using the Pierce BCA protein assay kit (Thermo Fisher Scientific). Equal amount of proteins (30 μg) isolated

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from different tissues including WAT and liver were used for Western blotting analyses by using antibodies against PXR (Santa Cruz Techology, SC-7739), phosphor-AKT (Cell signaling technology, 9271S), AKT (Cell signaling technology, 9272) or Actin (Sigma, A2066).

Tissue staining and lipid analyses

Liver and WAT were fixed in 4% neutral buffered formalin (Thermo Fisher Scientific, 22-046-361) and embedded in paraffin. Five μm tissue sections were stained with hematoxylin (MilliporeSigma; 1.05175) and eosin (MilliporeSigma; R03040) as previously described (Sui et al., 2014; Park et al., 2016; Gwag et al., 2019). Oil-Red O staining of hepatic neutral lipids was also performed (Helsley et al., 2016; Gwag et al., 2019). Liver tissues were embedded in OCT and sectioned at 10 μm. Liver sections were then dried, fixed in 4% PFA, incubated for 5 min in 60% isopropanol then in 0.3% Oil Red O (Sigma-Aldrich; St. Louis, MO) for 20 min. Hepatic total cholesterol and triglyceride contents were also measured (Sui et al., 2014; Gwag et al., 2019). Briefly, 30-50 mg liver tissues were collected and homogenized in 300-500 μl Krebs-Ringer buffer (NaCl 118mM, KCl 5mM, $CaCl_2$ 13.8mM, $MgSO_4$ 1.2mM, K_2HPO_4 0.95mM, $NaHCO_3$ 25mM) by sonication for 30 seconds for 10 times. Samples were kept on ice during homogenization. The homogenates were then used for cholesterol and triglyceride assays (Sui et al., 2014; Gwag et al., 2019).

Statistical analysis

All data are presented as the mean ± SD. Individual pairwise comparisons were analyzed by 2-sample, 2-tailed Student's t test. Two-way ANOVA was used when multiple comparisons were performed (with Bonferroni correction method). Data analyses were performed using GraphPad Prism software and a P value < 0.05 was considered significant.

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Results

Generation of adipocyte-specific PXR knockout mice.

PXR has been implicated in diet-induced obesity and metabolic disorders but the functions of PXR in adipose tissues are poorly understood. We found the PXR is expressed in both white adipose tissue (WAT) including subcutaneous (sub) and epididymal (epi) WAT and brown adipose tissue (BAT) of wild-type (WT) mice (Supplemental Figure 1A). While adipose PXR expression levels were lower than PXR-abundant tissues such as liver and small intestine but were comparable to or even higher than other tissues including kidney and spleen (Supplemental Figure 1A). In addition, the PXR expression in WAT and BAT was not significantly affected by HFD feeding in WT mice (Supplemental Figure 1B). To further investigate the role of adipocyte-derived PXR in HFD-induced obesity and metabolic disorders, we generated adipocyte-specific PXR knockout mice ($PXR^{\Delta Ad}$) by crossing mice carrying loxP-flanked PXR alleles ($PXR^{F/F}$) (Gwag et al., 2019; Sui et al., 2021) with adiponectin (Adipoq)-Cre transgenic mice (Eguchi et al., 2011; Lee et al., 2013; Park et al., 2016). Previous studies demonstrated that Adipoq-Cre was specifically expressed in the adipose tissue (Eguchi et al., 2011; Lee et al., 2013; Park et al., 2016) and PCR analysis of genomic DNA confirmed that the recombination was specific to the adipose tissues of $PXR^{\Delta Ad}$ mice (Figure 1A). Western blotting analysis also demonstrated that PXR protein levels were substantially reduced in WAT of $PXR^{\Delta Ad}$ mice as compared with control $PXR^{F/F}$ mice (Figure 1B). Consistently, the mRNA levels of PXR were significantly decreased in both WAT and BAT but not in other major tissues of $PXR^{\Delta Ad}$ mice as compared with $PXR^{F/F}$ mice (Figure 1C).

Adipoq-Cre has also been shown to be present only in mature adipocytes but not in adipose stromal vascular (SV) cells (Park et al., 2016). Consistently, we found that the PXR mRNA levels were significantly reduced from mature adipocytes but not SV cells isolated from WAT of $PXR^{\Delta Ad}$ mice (Figure 1D). Collectively, these results demonstrated the specific and efficient PXR deletion in adipocytes and adipose tissue of $PXR^{\Delta Ad}$ mice.

Deficiency of PXR in adipocytes does not affect high-fat diet-induced obesity in mice.

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To determine the contribution of adipocyte-derived PXR to diet-induced obesity, 4-week-old male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ littermates were fed a ND or HFD for 12 weeks. After 6 weeks of feeding, HFD-fed mice started to have significantly increased body weight as compared with ND-fed mice (Figure 2A). However, deficiency of adipocyte PXR did not affect diet-induced body weight gain (Figure 2A). At the end of the study, body composition of these male mice was analyzed by Echo MRI. While the percentages of fat mass were significantly increased following HFD feeding in both $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice, adipocyte PXR deficiency had no effects on body composition of $PXR^{\Delta Ad}$ mice under ND or HFD feeding conditions (Figure 2B). As expected, HFD feeding also led to increased adipocyte size or adipocyte hypertrophy in WAT of obese $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice but deficiency of PXR did not cause observable morphology changes in WAT of $PXR^{\Delta Ad}$ mice (Figure 2C).

Next, we examined different adipose pads of those mice. Consistently, the weights both subWAT and visceral WAT including epiWAT and retroperitoneal (ret) WAT were significantly increased in both $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice after HFD feeding but loss of adipocyte PXR did not affect WAT weight (Figure 2D). HFD feeding also led to similarly increased BAT weight in $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (Figure 2D -right panel). Taken together, our results suggest that deficiency of PXR in adipocytes does not affect HFD-induced adiposity or obesity.

To determine the impact of adipocyte PXR deficiency on energy balance, we also conducted metabolic cage studies using a LabMaster system (Sui et al., 2014; Park et al., 2016). We found that male $PXR^{\Delta Ad}$ mice had similar food intake, total activity, oxygen consumption, carbon dioxide production, and respiratory exchange ratio as control male $PXR^{F/F}$ mice (Figure 2E). Therefore, the energy expenditure was not affected by PXR deficiency (Figure 2E). Taken together, our results suggest that deficiency of PXR in adipocyte does not affect HFD-induced adiposity or obesity in male mice.

Ablation of adipocyte PXR does not affect obesity-associated insulin resistance in $PXR^{\Delta Ad}$ mice

Obesity is often associated with increased incidence of metabolic disturbances such as glucose intolerance and insulin resistance. To assess this, we performed glucose tolerance test

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(GTT) and insulin tolerance test (ITT) in those mice. Indeed, we found that obese $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice had worse glucose tolerance (Figure 3A) and exhibited reduced hypoglycemic response to administered insulin (Figure 3B). However, deficiency of PXR did not affect GTT and ITT results in either ND-fed or HFD-fed mice.

To further investigate the impact of adipocyte PXR deficiency on adipose insulin signaling, HFD-fed male mice were injected with insulin prior to euthanasia and phosphorylation of Akt in WAT was analyzed (Helsley et al., 2016). Insulin treatment led to enhanced phosphorylation of Akt in WAT but deficiency of PXR did not affect insulin-stimulated phosphorylation of Akt (Figure 3C). Next, we performed ex vivo adipose glucose uptake assays. As expected, insulin treatment also increased glucose uptake by adipose tissue of both $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (Figure 3D). However, deletion of PXR did not affect the adipose glucose uptake under basal or insulin-stimulated conditions. Taken together, these studies indicated that adipocyte PXR does not play a significant role in obesity-associated insulin resistance in mice.

Ablation of adipocyte PXR does not affect expression of key adipose tissue genes

We next investigated how deficiency of adipocyte PXR affect key adipose gene expression in WAT (Figure 4A) and BAT (Figure 4B) of male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice. As expected, we found that HFD feeding led to increased expression of several key genes mediating adipose tissue differentiation (e.g., peroxisome proliferator-activated receptor gamma (PPARγ), adipocyte protein 2 (AP2)), β-oxidation (e.g., carnitine palmitoyltransferase 1b (CPT1b)), and lipogenesis (e.g., peroxisome proliferator-activated receptor alpha (PPARα)) in WAT and BAT in both $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice. However, deletion of adipocyte PXR had no significant effects on the expression of these genes in $PXR^{\Delta Ad}$ mice (Figure 4A). In addition, obese mice also had reduced expression of several WAT genes regulating glucose metabolism including glucose transporter 4 (GLUT4) and insulin receptor substrate 1 (IRS1), and key BAT genes including uncoupling protein 1(UCP1) and PR domain zinc finger protein 16 (PRDM16) of $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice, but PXR deficiency did not affect HFD-mediated reduction of those genes in $PXR^{\Delta Ad}$ mice (Figure 4B).

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Deficiency of adipocyte PXR does not alleviate high-fat diet-induced hepatic steatosis in mice

In addition to insulin resistance, obesity is frequently accompanied by other disorders such as hepatic steatosis. As expected, high-fat feeding led to increased lipid accumulation and hepatic steatosis in the liver of $PXR^{F/F}$ mice as indicated by hematoxylin & eosin and Oil-red O staining, but deficiency of adipocyte PXR did not affect HFD-induced hepatic steatosis in $PXR^{\Delta Ad}$ mice (Figure 5A, B). Hepatic cholesterol and triglyceride contents were also significantly increased in HFD-fed male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice as compared with ND-fed mice (Figure 5C). Consistently, there is no significant differences for hepatic cholesterol and triglyceride levels between male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice under either ND-fed or HFD-fed conditions. Hepatic gene expression analysis then revealed that deficiency of PXR did not affect the expression of several key hepatic genes regulating lipogenesis (e.g., stearoyl-Coenzyme A desaturase 1 (SCD-1)) and gluconeogenesis (e.g., phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase)) in male $PXR^{\Delta Ad}$ mice (Figure 5D). These results indicate that ablation of PXR in adipocytes has no significant effects on HFD-induced hepatic steatosis and altered key hepatic gene expression.

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Discussion

As a key xenobiotic receptor, the functions of PXR in regulating xenobiotic metabolism have been extensively investigated by many groups and the role of PXR as a xenobiotic sensor has been established (Blumberg and Evans, 1998; Kliewer et al., 2002; Zhou et al., 2009b). New evidence has suggested novel functions of PXR beyond xenobiotic metabolism, and several studies have also indicated that PXR may play an important role in obesity and metabolic disorders (Zhou, 2016; Zhang et al., 2023). However, the cell type-specific functions of PXR in obesity have not been investigated. Adipose tissue is the main body energy storage site and adipocyte dysfunction contributes significantly to obesity-associated metabolic disorders. Adipocytes have also been considered as a prominent storage site of lipophilic chemicals and adipose tissue has been implicated to play an important role in the storage and toxicokinetics of hydrophobic xenobiotics (Jackson et al., 2017). In the current study, we generated novel adipocyte-specific PXR deficient mice to explore the functions of adipocyte PXR in diet-induced obesity and metabolic disorders. Interestingly, we found that loss of adipocyte PXR did not significantly affect food intake, energy expenditure, or diet-induced obesity in male mice. In addition, deficiency of adipocyte PXR did not alleviate obesity-associated metabolic disorders including insulin resistance and hepatic steatosis in mice. Our results suggested that adipocyte PXR signaling plays only a minimal role in diet-induced obesity and metabolic disorders in mice (Figure 6).

Previous studies using whole-body PXR knockout or transgenic mice suggested that PXR plays complex role in diet-induced obesity and metabolic disorders. For example, He et al. also found that deficiency of PXR did not affect food intake in $PXR^{-/-}$ mice (He et al., 2013). Consistently, we also found that $PXR^{\Delta Ad}$ mice had similar food intake as control littermates. However, the whole-body PXR-deficient mice were resistant to diet-induced obesity, likely due to the increased oxygen consumption and mitochondrial beta-oxidation in those mice (He et al., 2013). In addtion, $PXR^{-/-}$ mice were also protected from obesity-associated insulin resistance and hepatic steatosis (He et al., 2013). Those authors also crossed $PXR^{-/-}$ mice with leptin-deficient ob/ob mice to investigate the role of PXR under genetic obesity condition. They found that PXR-deficient, ob/ob mice had increased oxygen consumption and energy expenditure (He et al., 2013). Consistently, deficiency of PXR led to decreased obesity and diabetic condition in

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ob/ob mice (He et al., 2013). In our study, deficiency of adipocyte PXR did not affect oxygen consumption, carbon dioxide production, or energy expenditure. It is plausible that PXR signaling in other cell types than adipocytes play an important role in regulating energy expenditure.

In contrast to He et al.'s findings, another study showed that activation of PXR by treating mice with the PXR ligand pregnenolone 16α-carbonitrile (PCN) prevented diet-induced obesity, insulin resistance, and hepatic steatosis (Ma and Liu, 2012). However, these studies were conducted in a different mouse strain, AKR/J mice instead of C57BL/6J mice. In addition, Ma et al. also found that PCN-mediated PXR activation can affect adipocyte differentiation and lipid metabolism-related gene expression in adipose tissue of AKR/J mice (Ma and Liu, 2012). We found that deficiency of PXR did not affect the expression of key adipose genes in either WAT or BAT. It is well-known that different strains have different susceptibilities to various diseases including obesity and diabetes. Therefore, it is possible that PXR signaling may interact with different pathways or regulate different genes in AKR/J mice than in C57BL/6J mice. Ma et al. also used relatively high concentrations of PCN (50 mg/kg) and did not conduct a similar study in PXR-deficient mice. Therefore, the observed beneficial effects of PCN treatment may not be completely attributable to PXR signaling, but may involve other signaling pathways such as glucocorticoid receptor signaling.

In addition to genetic backgrounds, Spruiell et al. showed that PXR signaling may have sex-specific effects on obesity and diabetes. They found that deficiency of PXR protected male mice from diet-induced obesity (Spruiell et al., 2014b), which was consistent with He et al.'s findings (He et al., 2013). Surprisingly, male PXR-humanized mice expressing human PXR instead of mouse PXR were also resistant to obesity. However, the same group found that female PXR-humanized mice were not resistant but rather susceptible to diet-induced obesity (Spruiell et al., 2014a). Despite different obesity phenotypes, both male and female PXR-humanized mice had exacerbated diabetic phenotypes including elevated plasma glucose or insulin levels and impaired glucose tolerance (Spruiell et al., 2014a; Spruiell et al., 2014b). In the current study, all $PXR^{F/F}$ and $PXR^{\Delta Ad}$ littermates used for obesity and metabolic function studies were males. One of reasons to select male mice for this study is that female C56BL/6 mice are relatively resistant to diet-induced obesity as compared to male mice (Hong et al., 2009).

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Estrogen has been shown to play an important role in the regulation of energy homeostasis and may have protective effects from obesity complications (Clegg, 2012; Lizcano and Guzman, 2014), which make studying obesity phenotype in female mice more complicated. Indeed, ovariectomized female C56BL/6 mice can gain more weight and adipose tissue as compared to sham female mice (Rogers et al., 2009). However, this is also an important limitation of our study as sex differences have been well recognized in murine and human obesity and metabolic disease studies. It would be interesting to study whether adipocyte PXR may have different effects on obesity and metabolic functions in female mice in the future.

Liver plays a key role in regulating whole body glucose homeostasis (Nordlie et al., 1999; Lin and Accili, 2011). Spruiell et al. previously suggested that the diabetic phenotypes in male and female PXR-humanized mice were partially due to dysregulated glucose metabolism in the liver (Spruiell et al., 2014a; Spruiell et al., 2014b). He et al. also demonstrated that transgenic activation of PXR in the liver exacerbated the diabetic phenotypes of ob/ob mice (He et al., 2013). PXR has been shown to regulate hepatic glucose and energy homeostasis through multiple mechanisms, including crosstalk with forkhead box protein O1 (FoxO1) and FoxA2 pathways (Kodama et al., 2004; Nakamura et al., 2007) and regulating genes involved in glucose uptake and gluconeogenesis (Bhalla et al., 2004; Kodama et al., 2007; Hassani-Nezhad-Gashti et al., 2018). We found that PXR deficiency in adipocytes did not cause observable liver phenotypes and the expression of several key hepatic genes regulating lipogenesis and gluconeogenesis was also unaffected in $PXR^{\Delta Ad}$ mice. Therefore, it is possible that hepatic PXR, but not adipocyte PXR, plays an important role in diet-induced metabolic disorders. In addition to liver, PXR is also expressed in many immune cells including macrophages (Owen et al., 2004; Albermann et al., 2005; Zhou et al., 2009a; Sui et al., 2020). Macrophages also play important roles in obesity-associated insulin resistance and diabetes (Olefsky and Glass, 2010; Park et al., 2016; Ying et al., 2017). We previously generated myeloid-specific PXR deficient mice and revealed the important function of myeloid PXR signaling in regulating atherosclerosis development (Sui et al., 2020). Under low-fat diet feeding condition, deficiency of myeloid PXR did not affect body weight, body composition, or metabolic phenotypes in lean mice (Sui et al., 2020). However, it is not clear whether PXR signaling functions in macrophages to regulate metabolic phenotypes in obese mice. Future studies are required to investigate how PXR

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functions differently in different cell types to modulate obesity and metabolic functions (Figure 6).

It is also important to note that our study investigated the adipocyte PXR function under ND and HFD feeding conditions without exposure the mice to PXR ligands. Many xenobiotic chemicals including some obesogens have been shown to target nuclear receptor signaling to affect adipose tissue expansion and function, leading to increased obesity and metabolic disorders (Grun and Blumberg, 2006; Heindel and Blumberg, 2019; Egusquiza and Blumberg, 2020). Since many of those chemicals have been identified as PXR ligands (Kretschmer and Baldwin, 2005; Zhou, 2016; Helsley and Zhou, 2017), it would be interesting to study whether adipocyte PXR plays a role in diet-induced obesity and metabolic disorders when the mice are under xenobiotic stress or exposed to PXR-activating xenobiotics in the future.

In summary, we generated a novel $PXR^{\Delta Ad}$ mouse model to investigate the role of adipocyte PXR in diet-induced obesity and metabolic disorders. We found that deficiency of adipocyte PXR did not affect body weight gain or body composition in HFD-fed male mice. Obese $PXR^{\Delta Ad}$ mice also had similar metabolic disorders including insulin resistance and hepatic steatosis as control littermates. Further, deficiency of PXR in adipocytes did not affect key adipose gene expression in $PXR^{\Delta Ad}$. These results suggest that adipocyte PXR signaling may not have indispensable function in diet-induced obesity and metabolic disorders. Although the negative results obtained from this study may make the study of PXR and obesity more challenging, we believe that these null results are still important and informative for future research directions on this topic. Further investigations are needed to better understand the role of PXR signaling in obesity and metabolic disorders.

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Acknowledgements:

We thank all lab members for their technical assistance and Dr. Wendy Katz for assisting the metabolic cage studies.

Data Availability:

All the data supporting the findings of this study are contained within the paper.

Authorship Contribution:

Participated in research designs: F.W., B.B., C.Z.

Conducted experiments: F.W., J.L., R.H., S-H. P., Y-J. L.

Performed data analysis: F.W., J.L., R.H., S-H. P., Y-J., L., S.W. C.Z.

Contributed new reagents or analytic tools: S.W., B.B., C.Z.

Wrote or contributed to the writing of the manuscript: F.W., J.L., R.H., B.B., C.Z.

Footnote:

This work was supported in part by National Institutes of Health grants (R01ES023470, R01HL167206, R01HL123358, R01HL131925, and P30GM127211) and American Heart Association grant (19TPA34890065). R.H. was supported by a National Institutes of Health T32 training grant (T32ES018827) and an American Heart Association predoctoral fellowship (23PRE1018751). No author has an actual or perceived conflict of interest with the contents of this article.

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Reference

Albermann N, Schmitz-Winnenthal FH, Z'Graggen K, Volk C, Hoffmann MM, Haefeli WE, and Weiss J (2005) Expression of the drug transporters MDR1/ABCB1, MRP1/ABCC1, MRP2/ABCC2, BCRP/ABCG2, and PXR in peripheral blood mononuclear cells and their relationship with the expression in intestine and liver. *Biochem Pharmacol* **70:**949-958.

Bertilsson G, Heidrich J, Svensson K, Asman M, Jendeberg L, Sydow-Backman M, Ohlsson R, Postlind H, Blomquist P, and Berkenstam A (1998) Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci U S A* **95:**12208-12213.

Bhalla S, Ozalp C, Fang S, Xiang L, and Kemper JK (2004) Ligand-activated pregnane X receptor interferes with HNF-4 signaling by targeting a common coactivator PGC-1alpha. Functional implications in hepatic cholesterol and glucose metabolism. *J Biol Chem* **279:**45139-45147.

Blumberg B and Evans RM (1998) Orphan nuclear receptors--new ligands and new possibilities. *Genes Dev* **12:**3149-3155.

Blumberg B, Sabbagh W, Jr., Juguilon H, Bolado J, Jr., van Meter CM, Ong ES, and Evans RM (1998) SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev* **12:**3195-3205.

Casals-Casas C and Desvergne B (2011) Endocrine disruptors: from endocrine to metabolic disruption. *Annu Rev Physiol* **73:**135-162.

Casey SC and Blumberg B (2012) The steroid and xenobiotic receptor negatively regulates B-1 cell development in the fetal liver. *Mol Endocrinol* **26:**916-925.

Casey SC, Nelson EL, Turco GM, Janes MR, Fruman DA, and Blumberg B (2011) B-1 cell lymphoma in mice lacking the steroid and xenobiotic receptor, SXR. *Mol Endocrinol* **25:**933-943.

Cheng J, Krausz KW, Tanaka N, and Gonzalez FJ (2012) Chronic exposure to rifaximin causes hepatic steatosis in pregnane x receptor-humanized mice. *Toxicol Sci* **129:**456-468.

Clegg DJ (2012) Minireview: the year in review of estrogen regulation of metabolism. *Mol Endocrinol* **26:**1957-1960.

de Haan W, de Vries-van der Weij J, Mol IM, Hoekstra M, Romijn JA, Jukema JW, Havekes LM, Princen HM, and Rensen PC (2009) PXR agonism decreases plasma HDL levels in ApoE3-Leiden.CETP mice. *Biochim Biophys Acta* **1791:**191-197.

Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, and Gore AC (2009) Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev* **30:**293-342.

Dubrac S, Elentner A, Ebner S, Horejs-Hoeck J, and Schmuth M (2010) Modulation of T lymphocyte function by the pregnane X receptor. *J Immunol* **184:**2949-2957.

Eguchi J, Wang X, Yu S, Kershaw EE, Chiu PC, Dushay J, Estall JL, Klein U, Maratos-Flier E, and Rosen ED (2011) Transcriptional Control of Adipose Lipid Handling by IRF4. *Cell Metab* **13:**249-259.

Egusquiza RJ and Blumberg B (2020) Environmental Obesogens and Their Impact on Susceptibility to Obesity: New Mechanisms and Chemicals. *Endocrinology* **161**.

Gregor MF and Hotamisligil GS (2011) Inflammatory mechanisms in obesity. *Annu Rev Immunol* **29:**415-445.

Grun F and Blumberg B (2006) Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. *Endocrinology* **147:**S50-55.

Grun F and Blumberg B (2007) Perturbed nuclear receptor signaling by environmental obesogens as emerging factors in the obesity crisis. *Rev Endocr Metab Disord* **8:**161-171.

Grun F, Watanabe H, Zamanian Z, Maeda L, Arima K, Cubacha R, Gardiner DM, Kanno J, Iguchi T, and Blumberg B (2006) Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates. *Mol Endocrinol* **20:**2141-2155.

Gwag T, Meng Z, Sui Y, Helsley RN, Park SH, Wang S, Greenberg RN, and Zhou C (2019) Non-nucleoside reverse transcriptase inhibitor efavirenz activates PXR to induce hypercholesterolemia and hepatic steatosis. *Journal of hepatology* **70:**930-940.

Hassani-Nezhad-Gashti F, Rysa J, Kummu O, Napankangas J, Buler M, Karpale M, Hukkanen J, and Hakkola J (2018) Activation of nuclear receptor PXR impairs glucose tolerance and dysregulates GLUT2 expression and subcellular localization in liver. *Biochem Pharmacol* **148:**253-264.

He J, Gao J, Xu M, Ren S, Stefanovic-Racic M, O'Doherty RM, and Xie W (2013) PXR ablation alleviates diet-induced and genetic obesity and insulin resistance in mice. *Diabetes* **62:**1876-1887.

Heindel JJ and Blumberg B (2019) Environmental Obesogens: Mechanisms and Controversies. *Annu Rev Pharmacol Toxicol* **59:**89-106.

Helsley RN, Sui Y, Park SH, Liu Z, Lee RG, Zhu B, Kern PA, and Zhou C (2016) Targeting IkappaB kinase beta in Adipocyte Lineage Cells for Treatment of Obesity and Metabolic Dysfunctions. *Stem Cells* **34:**1883-1895.

Helsley RN and Zhou C (2017) Epigenetic impact of endocrine disrupting chemicals on lipid homeostasis and atherosclerosis: a pregnane X receptor-centric view. *Environ Epigenet* **3:**dvx017.

Hernandez R, Shi J, Liu J, Li X, Wu J, Zhao L, Zhou T, Chen Q, and Zhou C (2023) PANDORA-Seq unveils the hidden small noncoding RNA landscape in atherosclerosis of LDL receptor-deficient mice. *J Lipid Res* **64:**100352.

Hong J, Stubbins RE, Smith RR, Harvey AE, and Nunez NP (2009) Differential susceptibility to obesity between male, female and ovariectomized female mice. *Nutr J* **8:**11.

Jackson E, Shoemaker R, Larian N, and Cassis L (2017) Adipose Tissue as a Site of Toxin Accumulation. *Compr Physiol* **7:**1085-1135.

Kelly T, Yang W, Chen CS, Reynolds K, and He J (2008) Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)* **32:**1431-1437.

Kliewer SA, Goodwin B, and Willson TM (2002) The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr Rev* **23:**687-702.

Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstrom RH, Perlmann T, and Lehmann JM (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92:**73-82.

Kodama S, Koike C, Negishi M, and Yamamoto Y (2004) Nuclear receptors CAR and PXR cross talk with FOXO1 to regulate genes that encode drug-metabolizing and gluconeogenic enzymes. *Mol Cell Biol* **24:**7931-7940.

Kodama S, Moore R, Yamamoto Y, and Negishi M (2007) Human nuclear pregnane X receptor cross-talk with CREB to repress cAMP activation of the glucose-6-phosphatase gene. *Biochem J* **407:**373-381.

Kretschmer XC and Baldwin WS (2005) CAR and PXR: xenosensors of endocrine disrupters? *Chem Biol Interact* **155:**111-128.

Lee KY, Russell SJ, Ussar S, Boucher J, Vernochet C, Mori MA, Smyth G, Rourk M, Cederquist C, Rosen ED, Kahn BB, and Kahn CR (2013) Lessons on conditional gene targeting in mouse adipose tissue. *Diabetes* **62:**864-874.

Lin HV and Accili D (2011) Hormonal regulation of hepatic glucose production in health and disease. *Cell Metab* **14:**9-19.

Liu J, Shi J, Hernandez R, Li X, Konchadi P, Miyake Y, Chen Q, Zhou T, and Zhou C (2023) Paternal phthalate exposure-elicited offspring metabolic disorders are associated with altered sperm small RNAs in mice. *Environ Int* **172:**107769.

Lizcano F and Guzman G (2014) Estrogen Deficiency and the Origin of Obesity during Menopause. *BioMed research international* **2014:**757461.

Lu W, Park SH, Meng Z, Wang F, and Zhou C (2019) Deficiency of Adipocyte IKKbeta Affects Atherosclerotic Plaque Vulnerability in Obese LDLR Deficient Mice. *Journal of the American Heart Association* **8:**e012009.

Ma Y and Liu D (2012) Activation of pregnane X receptor by pregnenolone 16 alpha-carbonitrile prevents high-fat diet-induced obesity in AKR/J mice. *PLoS One* **7:**e38734.

Meng Z, Gwag T, Sui Y, Park SH, Zhou X, and Zhou C (2019) The atypical antipsychotic quetiapine induces hyperlipidemia by activating intestinal PXR signaling. *JCI Insight* **4**.

Nakamura K, Moore R, Negishi M, and Sueyoshi T (2007) Nuclear pregnane X receptor cross-talk with FoxA2 to mediate drug-induced regulation of lipid metabolism in fasting mouse liver. *J Biol Chem* **282:**9768-9776.

Nordlie RC, Foster JD, and Lange AJ (1999) Regulation of glucose production by the liver. *Annual review of nutrition* **19:**379-406.

Olefsky JM and Glass CK (2010) Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol* **72:**219-246.

Owen A, Chandler B, Back DJ, and Khoo SH (2004) Expression of pregnane-X-receptor transcript in peripheral blood mononuclear cells and correlation with MDR1 mRNA. *Antivir Ther* **9:**819-821.

Park SH, Liu Z, Sui Y, Helsley RN, Zhu B, Powell DK, Kern PA, and Zhou C (2016) IKKbeta Is Essential for Adipocyte Survival and Adaptive Adipose Remodeling in Obesity. *Diabetes* **65:**1616-1629.

Rogers NH, Perfield JW, 2nd, Strissel KJ, Obin MS, and Greenberg AS (2009) Reduced energy expenditure and increased inflammation are early events in the development of ovariectomy-induced obesity. *Endocrinology* **150:**2161-2168.

Rosen ED and MacDougald OA (2006) Adipocyte differentiation from the inside out. *Nature reviews Molecular cell biology* **7:**885-896.

Satta S, Meng Z, Hernandez R, Cavallero S, Zhou T, Hsiai TK, and Zhou C (2022) An engineered nano-liposome-human ACE2 decoy neutralizes SARS-CoV-2 Spike protein-induced inflammation in both murine and human macrophages. *Theranostics* **12:**2639-2657.

Siest G, Jeannesson E, Marteau JB, Samara A, Marie B, Pfister M, and Visvikis-Siest S (2008) Transcription factor and drug-metabolizing enzyme gene expression in lymphocytes from healthy human subjects. *Drug Metab Dispos* **36:**182-189.

Spruiell K, Jones DZ, Cullen JM, Awumey EM, Gonzalez FJ, and Gyamfi MA (2014a) Role of human pregnane X receptor in high fat diet-induced obesity in pre-menopausal female mice. *Biochem Pharmacol* **89:**399-412.

Spruiell K, Richardson RM, Cullen JM, Awumey EM, Gonzalez FJ, and Gyamfi MA (2014b) Role of pregnane X receptor in obesity and glucose homeostasis in male mice. *J Biol Chem* **289:**3244-3261.

Sui Y, Helsley RN, Park SH, Song X, Liu Z, and Zhou C (2015) Intestinal pregnane x receptor links xenobiotic exposure and hypercholesterolemia. *Mol Endocrinol* **29:**765-776.

Sui Y, Meng Z, Chen J, Liu J, Hernandez R, Gonzales MB, Gwag T, Morris AJ, and Zhou C (2021) Effects of Dicyclohexyl Phthalate Exposure on PXR Activation and Lipid Homeostasis in Mice. *Environ Health Perspect* **129:**127001.

Sui Y, Meng Z, Park SH, Lu W, Livelo C, Chen Q, Zhou T, and Zhou C (2020) Myeloid-specific deficiency of pregnane X receptor decreases atherosclerosis in LDL receptor-deficient mice. *J Lipid Res* **61:**696-706.

Sui Y, Park SH, Xu J, Monette S, Helsley RN, Han SS, and Zhou C (2014) IKKbeta links vascular inflammation to obesity and atherosclerosis. *J Exp Med* **211:**869-886.

Van Gaal LF, Mertens IL, and De Block CE (2006) Mechanisms linking obesity with cardiovascular disease. *Nature* **444:**875-880.

Varlamov O, Somwar R, Cornea A, Kievit P, Grove KL, and Roberts CT, Jr. (2010) Single-cell analysis of insulin-regulated fatty acid uptake in adipocytes. *Am J Physiol Endocrinol Metab* **299:**E486-496.

Ying W, Riopel M, Bandyopadhyay G, Dong Y, Birmingham A, Seo JB, Ofrecio JM, Wollam J, Hernandez-Carretero A, Fu W, Li P, and Olefsky JM (2017) Adipose Tissue

Macrophage-Derived Exosomal miRNAs Can Modulate In Vivo and In Vitro Insulin Sensitivity. *Cell* **171:**372-384 e312.

Zhang J, Jia Q, Li Y, and He J (2023) The Function of Xenobiotic Receptors in Metabolic Diseases. *Drug Metab Dispos* **51:**237-248.

Zhou C (2016) Novel functions of PXR in cardiometabolic disease. *Biochim Biophys Acta* **1859:**1112-1120.

Zhou C, King N, Chen KY, and Breslow JL (2009a) Activation of PXR induces hypercholesterolemia in wild-type and accelerates atherosclerosis in apoE deficient mice. *J Lipid Res* **50:**2004-2013.

Zhou C, Verma S, and Blumberg B (2009b) The steroid and xenobiotic receptor (SXR), beyond xenobiotic metabolism. *Nucl Recept Signal* **7:**e001.

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Figure legends

Figure 1. Generation of adipocyte-specific PXR knockout mice.

(A) PCR analysis of genomic DNA isolated from major tissues of $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice showing that Adipoq-Cre-mediated recombination was specific to adipose tissues of $PXR^{\Delta Ad}$ mice. epiWAT, epididymal white adipose tissue; subWAT, subcutaneous white adipose tissue; BAT, brown adipose tissue. (B) Western blot analysis of PXR protein levels in WAT and liver of $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (n=3). (C) mRNA levels of PXR of major tissues, white adipose tissue (WAT), BAT from $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (n=3, two-sample, two tailed Student's t-test, * p<0.05). (D) mRNA levels of PXR in isolated adipocytes and stromal vascular (SV) cells from WAT of $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (n=3, two-sample, two tailed Student's t-test, *** p<0.001).

Figure 2. Deficiency of PXR in adipocyte does not affect high-fat diet-induced obesity and metabolic phenotypes in mice.

Four-week of old male of $PXR^{F/F}$ and $PXR^{\Delta Ad}$ male littermates were fed a ND or HFD for 12 weeks. (A-C) Growth curves (A), fat and lean mass (percentage of body weight) (B), representative hematoxylin & eosin staining images of WAT (scale bar, 100 μm) (C), and weight of different adipose pads (epiWAT, subWAT, retroperitoneal WAT (retroWAT), and BAT) (D) of ND or HFD-fed male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (n=5-10). (E) Metabolic cage analyses of food intake, total activity, oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio, and energy expenditure of HFD-fed $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (n=4-7). Two-way ANOVA followed by Bonferroni's multiple comparison test (*P<0.05, **P<0.01, and ***P<0.001). Statistically significant differences between ND and HFD-fed $PXR^{F/F}$ mice were indicated with * (*P<0.05, **P<0.01, and ***P<0.001). Statistically significant differences between ND and HFD-fed $PXR^{\Delta Ad}$ mice were indicated with † (†P<0.05 and †††P <0.001). ns, not significant.

Figure 3. Ablation of PXR does not affect obesity-associated insulin resistance in $PXR^{\Delta Ad}$ mice.

Four-week-old male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ male littermates were fed a ND or HFD for 12 weeks. (A and B) Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin resistance

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test (IPITT) of $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (n=5-10). (C) Western blotting analysis of phosphorylated-AKT and total AKT in adipose tissue after stimulated with saline or 0.35 units/kg body weight insulin for 5 min (n=3). (D) Glucose uptake was measured in primary adipose tissues from HFD-fed $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (n=4-6). Two-way ANOVA followed by Bonferroni's multiple comparison test (***P<0.001). Statistically significant differences between ND and HFD-fed $PXR^{F/F}$ mice were indicated with * (*P<0.05, **P<0.01, and ***P<0.001). Statistically significant differences between ND and HFD-fed $PXR^{\Delta Ad}$ mice were indicated with † (†P<0.05, ††P<0.01, and †††P <0.001).

Figure 4. Ablation of PXR does not affect key adipose tissue gene expression.

Four-week-old male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ male littermates were fed a HFD for 12 weeks. QPCR analyses of indicated genes in WAT (A) and BAT (B) (n=3). GAPDH was used as the reference gene to normalize the mRNA expression. Two-way ANOVA followed by Bonferroni's multiple comparison test (*P<0.05, **P<0.01, and ***P<0.001). ns, not significant. PPARγ: peroxisome proliferator-activated receptor γ; AP2: adipocyte protein 2; FASN: fatty acid synthase; SCD1: stearoyl-Coenzyme A desaturase 1; CPT1b: carnitine palmitoyltransferase 1b; PPARα: peroxisome proliferator-activated receptor α; GLUT4: glucose transporter 4; IRS1: insulin receptor substrate 1; UCP1: uncoupling protein 1; PRDM16: PR domain zinc finger protein 16; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Figure 5. Deficiency of adipocyte PXR does not alleviate high-fat diet-induced hepatic steatosis in mice.

Four-week-old male $PXR^{F/F}$ and $PXR^{\Delta Ad}$ male littermates were fed a ND or HFD for 12 weeks. (A and B) Representative H&E (bar=50 μm) (A) and Oil red O (bar=100 μm) (B) stained liver sections of ND and HFD-fed $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice. (C and D) Hepatic cholesterol (C) and triglyceride (D) levels from $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice fed a ND or HFD (n=5-8). (E) QPCR analysis of indicated hepatic genes of $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (n=3). GAPDH was used as the reference gene to normalize the mRNA expression. Two-way ANOVA followed by Bonferroni's multiple comparison test (**P<0.01 and ***P<0.001). FASN: fatty acid synthase; SCD1: stearoyl-Coenzyme A desaturase 1; LDLR: low density lipoprotein receptor; ACOX1: acyl-CoA oxidase 1; PEPCK: phosphoenolpyruvate carboxykinase; G6Pase: glucose 6-phosphatase; GLUT2: glucose transporter 2; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

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Figure 6. Schematic of the potential role of PXR signaling in diet-induced obesity and metabolic disorders.

PXR signaling has been implicated in diet-induced obesity, insulin resistance, and hepatic steatosis. Results from the current study demonstrate that deficiency of adipocyte PXR does not affect diet-induced obesity or metabolic disorders in male mice, indicating that adipocyte PXR may not play an important role in obesity. However, PXR is expressed in many other tissues and cell types. Future studies are required to investigate how PXR signaling in those tissues or cell types including liver, intestine, skeletal muscle, pancreas, macrophages, and adipose progenitors regulates diet-induced obesity and metabolic disorders.

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Figure 1

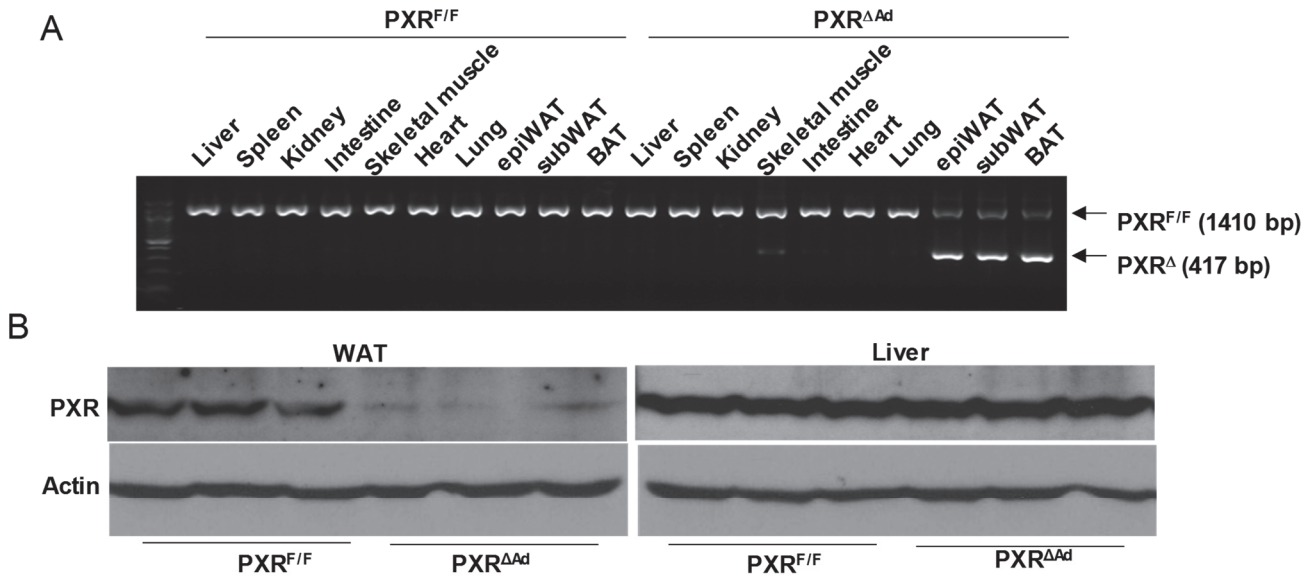

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Figure 2

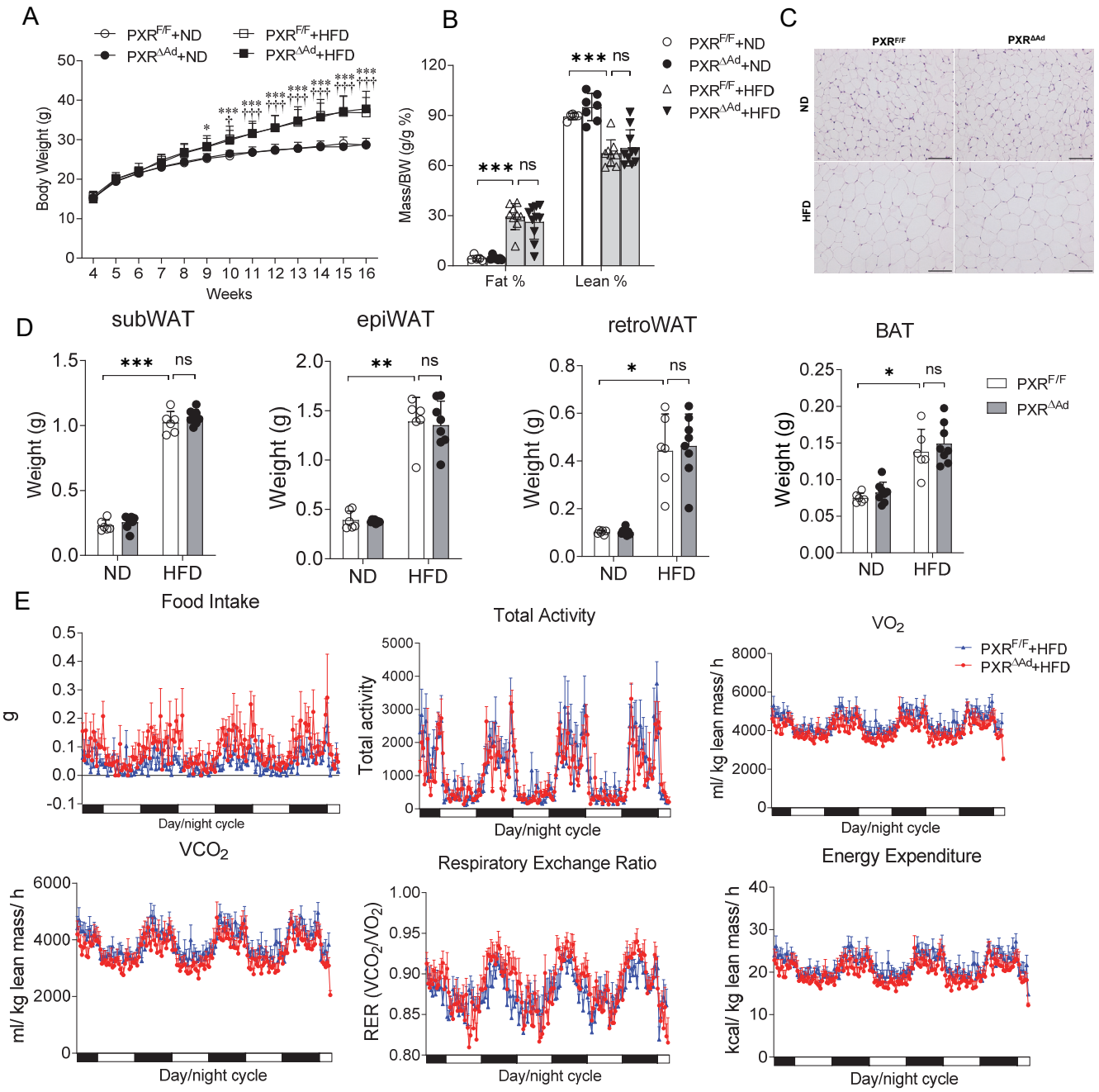

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Figure 3

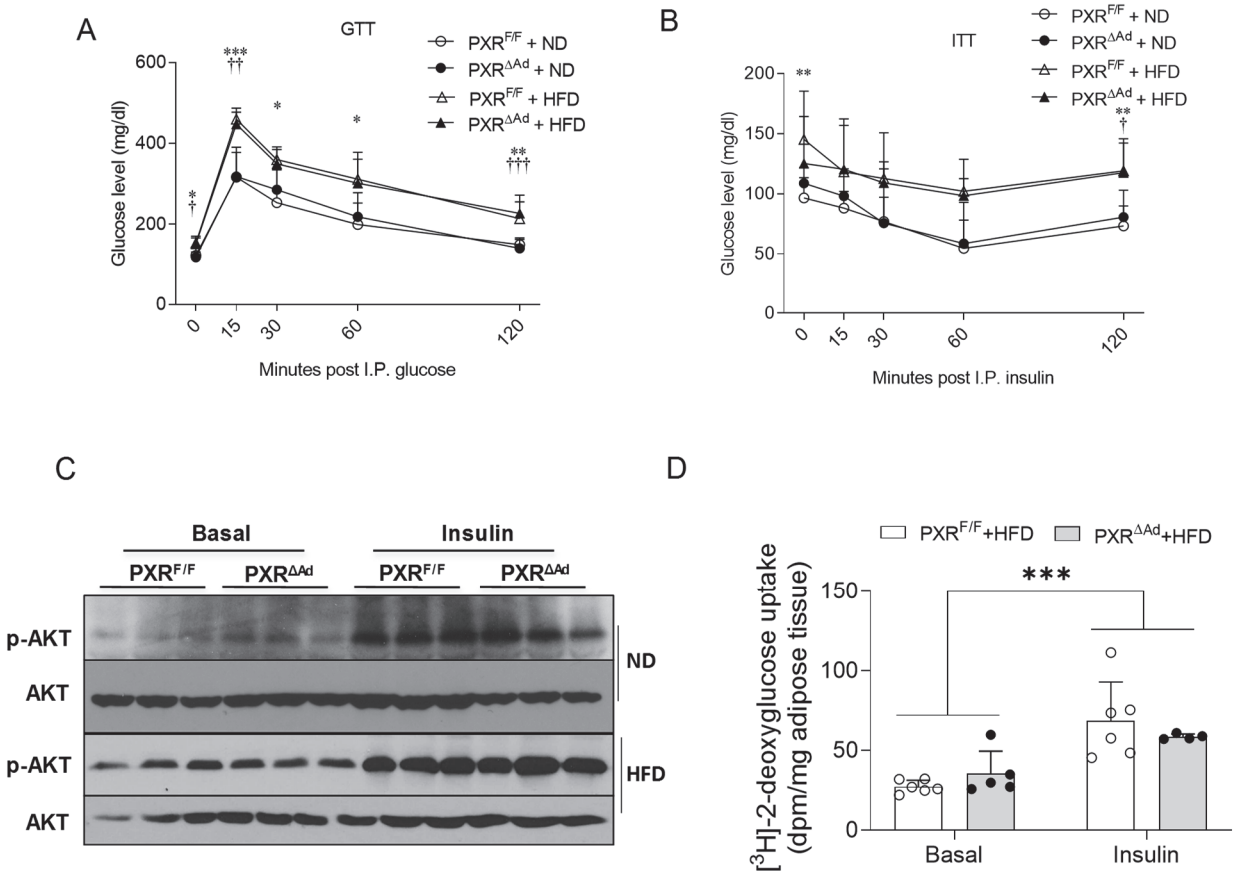

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Figure 4

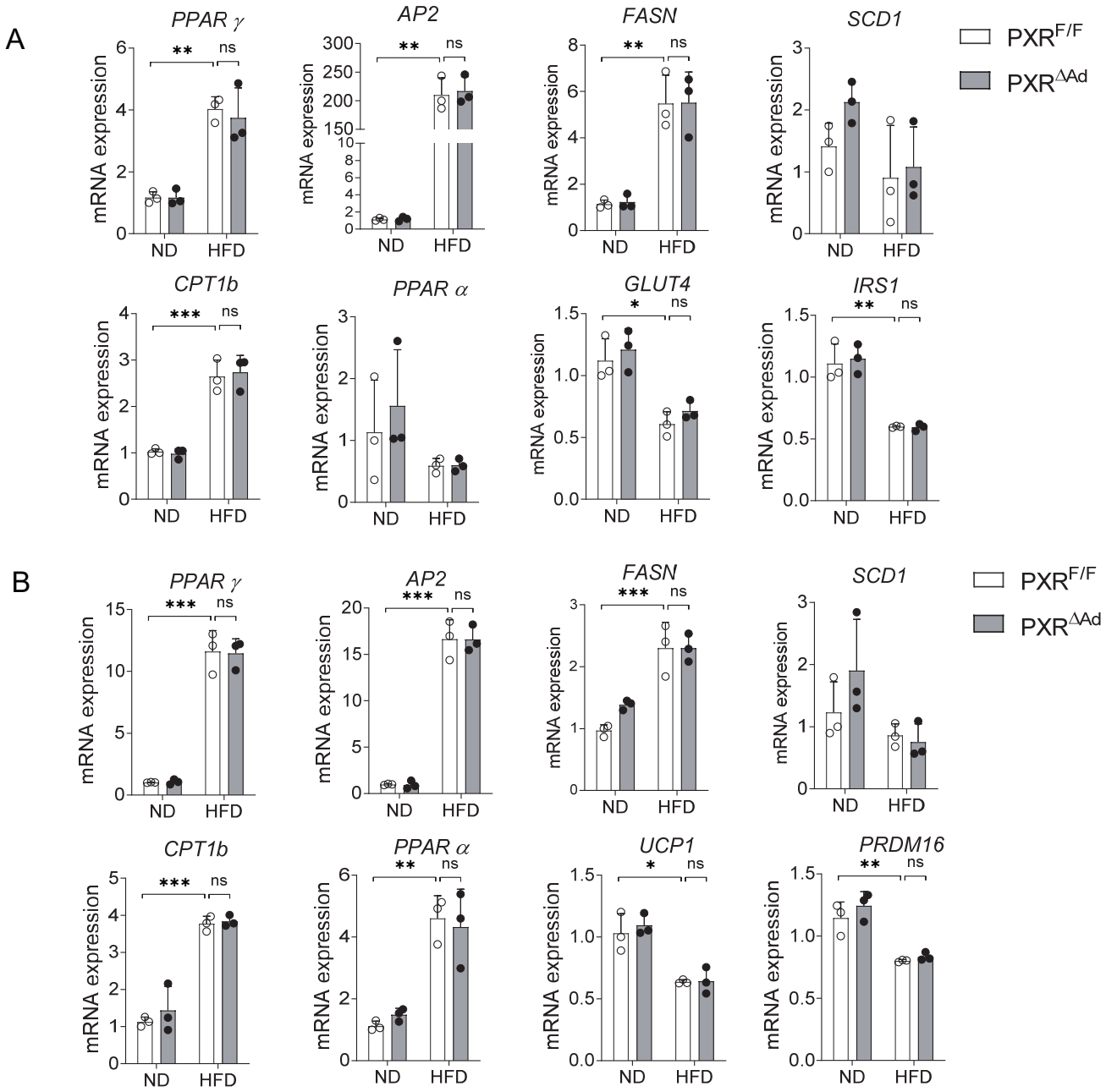

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Figure 5

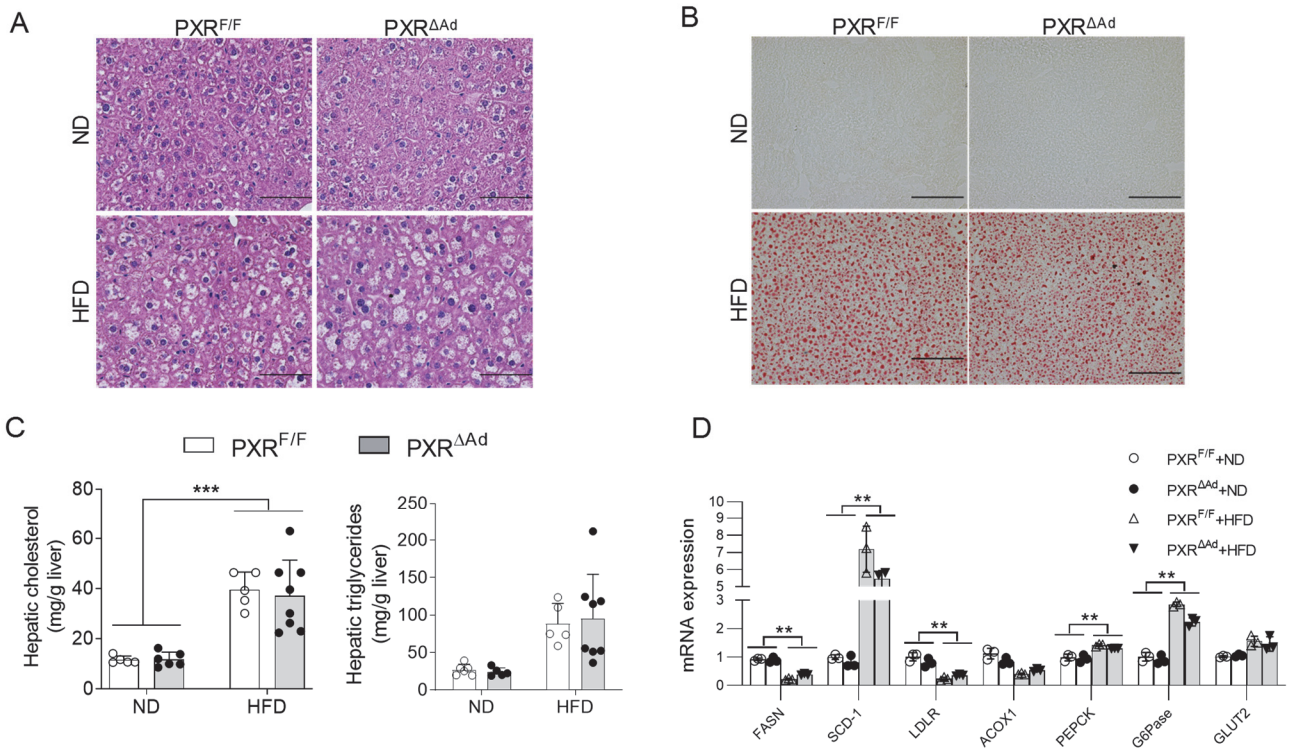
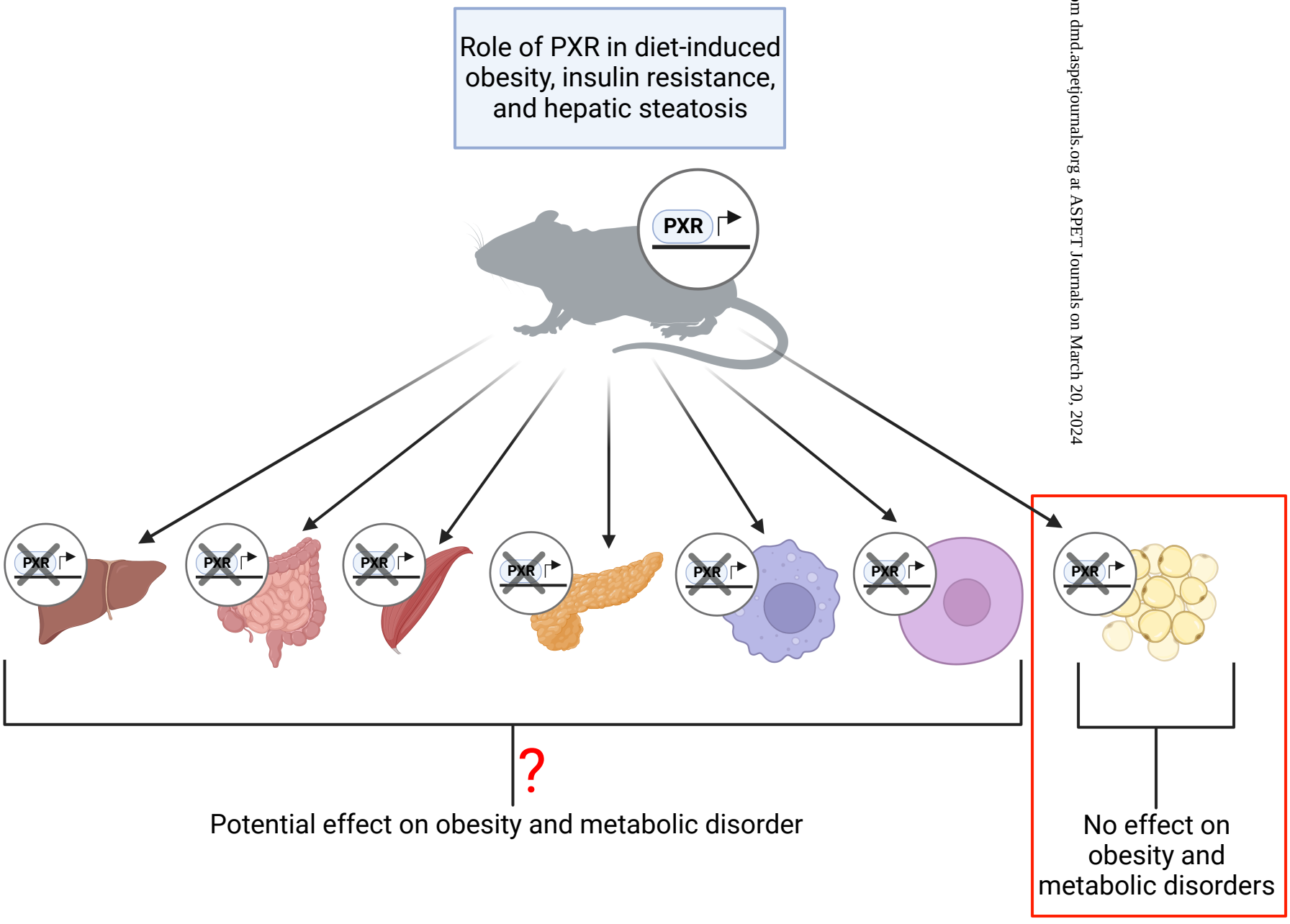


Figure 6



Supplemental Material for

Adipocyte-derived PXR signaling is dispensable for diet-induced obesity and metabolic disorders in male mice

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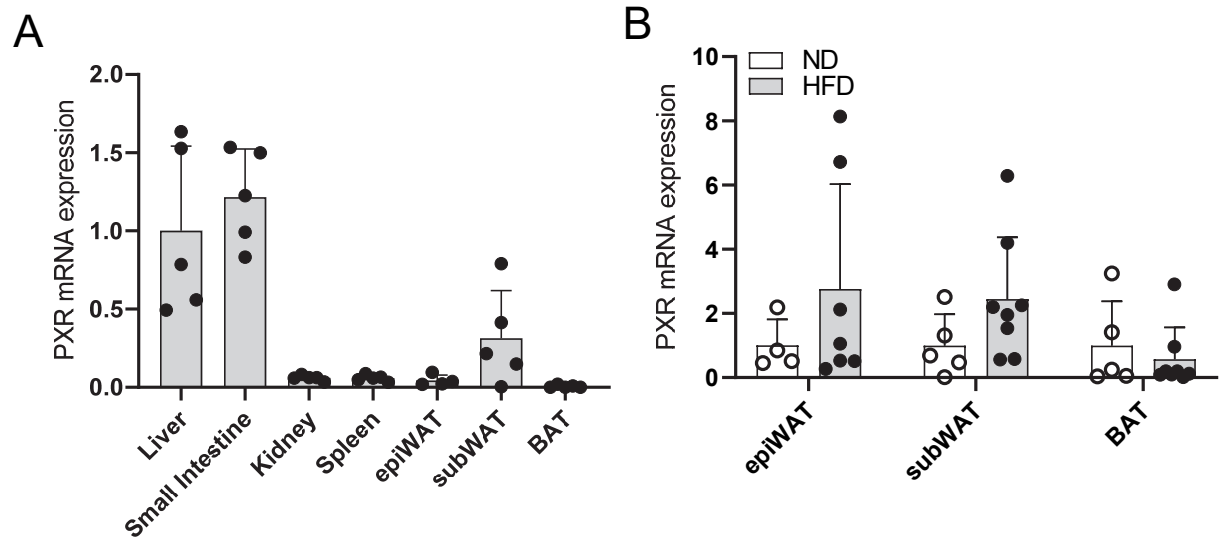

Supplemental Figure 1. PXR is expressed in adipose tissues of wild-type mice. (A) mRNA levels of PXR in major tissues, epididymal white adipose tissue (epiWAT); subcutaneous WAT (subWAT), and brown adipose tissue (BAT) of C57BL/6J wild-type mice were analyzed by QPCR m $PXR^{F/F}$ and $PXR^{\Delta Ad}$ mice (n=4-5). (B) Three-week of old male wild-type mice were fed a ND or HFD for nine weeks. QPCR analysis of PXR mRNA levels in epiWAT, subWAT, and BAT (n=4-8).

Supplemental Table 1. Primer sequences for QPCR analyses

Genes	Forward primer sequences (5'-3')	Reverse primer sequences (5'-3')
PXR	GACGCTCAGATGCAAACCTT	TCTTCTCCGCGCAGCTGCA
PPARγ	GTGCCAGTTTCGATCCGTAGA	GGCCAGCATCGTGTAGATGA
AP2	GGACCTGATGCAACCCTATGA	CCATCTAGGGTTATGATGCTC TTCA
FASN	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
SCD1	TTCTTGCGATACACTCTGGTGC	CGGGATTGAATGTTCTTGTCGT
CPT1b	CTCCGCCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
PPARα	TGTCGAATATGTGGGGACAA	AATCTTGCAGCTCCGATCAC
GLUT4	CAGAAGGTGATTGAACAGAG	AATGATGCCAATGAGAAAGG
IRS1	AGTGGCGGCACAAGTCGAGC	GCCTCGCTATCCGCGGCAAT
UCP1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
PRDM16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
LDLR	GAAGTCGACACTGTACTGACCACC	CTCCTCATTCCCTCTGCCAGCCAT
ACOX1	GGATGGTAGTCCGGAGAACA	AGTCTGGATCGTTCAGAATCAAG
PEPCK	CCACAGCTGCTGCAGAACA	GAAGGGTCGCATGGCAAA
G6Pase	CCGGATCTACCTTGCTGCTCACTTT	TAGCAGGTAGAATCCAAGCGCGAAAC
GLUT2	GGCTAATTTCAGGACTGGTT	TTTCTTTGCCCTGACTTCCT
GAPDH	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT