

Role of the Pregnane X Receptor in Binge Ethanol-Induced Steatosis and Hepatotoxicity

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ABSTRACT

The pregnane X receptor (PXR, NR1I2) is a xenobiotic-sensing nuclear receptor that defends against toxic agents. We have shown that PXR promotes chronic ethanol (EtOH)-induced steatosis. Therefore, we examined the role of PXR in binge EtOH-induced hepatotoxicity. Male wild-type (WT) and *Pxr*-null mice were orally administered three binge doses of EtOH (4.5 g/kg, every 12 hours) and were euthanized 4 hours after the final dose. *Pxr*-null mice displayed higher basal mRNA levels of hepatic lipogenic transcription factor sterol regulatory element binding protein 1c (*Srebp-1c*) and its target stearoyl-CoA desaturase 1 and the lipid peroxide-detoxifying aldo-keto reductase 1b7 (*Akr1b7*) and higher protein levels of EtOH-metabolizing alcohol dehydrogenase 1 (ADH1). In both genotypes, binge EtOH-induced triglyceride accumulation was associated with inhibition of fatty acid β -oxidation and upregulation of *Srebp-1c*-regulated lipogenic genes and hepatic

CYP2E1 protein. Unexpectedly, gene expression of *Cyp2b10*, a constitutive androstane receptor target gene implicated in EtOH hepatotoxicity, was PXR-dependent upregulated by binge EtOH. In addition, in WT mice, binge EtOH-induced inhibition of hepatic *Akr1b8* mRNA, and protein levels of aldehyde dehydrogenase 1A1 and antiapoptotic Bcl-2 but increased proapoptotic Bax protein expression, leading to increases in residual EtOH concentration and the cellular oxidative stress marker, malondialdehyde. In contrast, *Pxr*-null mice displayed increased *Akr1b7* gene and ADH1 protein expression and hypertriglyceridemia after binge EtOH exposure. Taken together, this study demonstrates that PXR ablation prevents EtOH-induced upregulation of *Cyp2b10* and that PXR potentiates binge EtOH-induced oxidative stress and inhibition of EtOH catabolism but protects against alcoholic hyperlipidemia.

Introduction

Alcoholic liver disease (ALD) is a common pathology associated with excessive alcohol use. Despite intense research efforts to unravel the molecular basis of ethanol (EtOH)-induced liver damage, there are currently no Food and Drug Administration-approved treatments for ALD. Interestingly, published reports have implicated the involvement of ligand-activated transcription factors called nuclear

receptors in ALD (Pang et al., 2009; Gyamfi and Wan, 2010; Chen et al., 2011; Wu et al., 2014).

The pregnane X receptor (PXR, NR1I2) is a xenobiotic-sensing nuclear receptor that coordinates the metabolic response to drugs and toxic agents and is highly expressed in the liver and intestine (Pavek and Dvorak, 2008). A recent report established that loss of PXR is protective against chronic EtOH-induced hepatic triglyceride accumulation and steatosis, whereas PXR signaling aggravates hepatotoxicity (Choi et al., 2018). Chronic EtOH-induced hepatotoxicity in wild-type (WT) mice was associated with gene induction of transcription factors involved in lipogenesis and upregulation of *Pxr*, constitutive androstane receptor (*Car*), and CAR target gene *Cyp2b10* (Choi et al., 2018). Importantly, PXR deficiency suppressed these changes. Similar to the effect of chronic EtOH on WT mice, activation of mouse CAR by 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene and EtOH infusion enhanced

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ABBREVIATIONS: ACC, acetyl-CoA carboxylase; ADH, alcohol dehydrogenase; AKR, aldo-keto reductase; ALT, alanine aminotransferase; ALDH, aldehyde dehydrogenase; ApoB, apolipoprotein B; AST, aspartate aminotransferase; BEC, blood ethanol concentration; CAR, constitutive androstane receptor; CPT, carnitine palmitoyltransferase; DGAT, diacylglycerol acyltransferase; EtOH, ethanol; FAS, fatty acid synthase; FXR, farnesoid X receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GRP, glucose-regulated protein; HMGCR, 3-hydroxy-3-methylglutaryl CoA reductase; LFABP, liver fatty acid binding protein; LPO, lipid peroxidation; MDA, malondialdehyde; PCN, pregnenolone 16 α -carbonitrile; PCR, polymerase chain reaction; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; ROS, reactive oxygen species; RXRa, retinoid X receptor α ; SCD, stearoyl-CoA desaturase; SHP, small heterodimer partner; SREBP, sterol regulatory element binding protein; UCP, uncoupling protein; WT, wild-type.

both acute and chronic EtOH-induced hepatotoxicity (Chen et al., 2011; Wang et al., 2017). Furthermore, EtOH can increase both PXR and its target *Cyp3a11/CYP3A* gene in rodents, in human primary hepatocytes, and in the liver of human alcoholics (Kostrubsky et al., 1995; Niemelä et al., 2000; He et al., 2006). Taken together, it appears that PXR/CAR crosstalk mediates and exacerbates EtOH-induced steatosis and ALD pathogenesis.

Intriguingly, both ligand PXR activation and PXR knockdown in mice, human hepatoma HepG2 cells, and human hepatocytes led to hepatic lipid accumulation (Nakamura et al., 2007; Moreau et al., 2009; Spruiell et al., 2014b; Bitter et al., 2015). However, the underlying mechanisms involved in the dual role of PXR activation and ablation in steatosis are still being unraveled. Interestingly, steatosis induced by PXR activation in mice implicated the involvement of sterol regulatory element binding protein (SREBP)-independent activation of the free fatty acid uptake transporter CD36 and several lipogenic enzymes, including stearoyl-CoA desaturase 1 (SCD1) and long-chain free fatty acid elongase, as well as suppression of peroxisome proliferator-activated receptor α (PPAR α) target genes (Zhou et al., 2006; Nakamura et al., 2007). In contrast, human PXR activation by rifampicin in HepG2 cells was reported to involve the SREBP1-dependent lipogenic pathway (Bitter et al., 2015), suggesting species differences in PXR ligand-induced steatosis. However, ligand-dependent PXR activation and steatosis induction in primary human hepatocytes did not involve CD36, but upregulation of the thyroid hormone responsive SPOT 14 homolog, which activates lipogenic fatty acid synthase (FAS) expression (Moreau et al., 2009).

On the other hand, PXR ablation results in increased hepatic lipid accumulation in untreated *Pxr*-null mice, possibly involving upregulation of the *Srebp1c* target *Scd1* (Nakamura et al., 2007; Spruiell et al., 2014b). Furthermore, PXR knockdown in HepG2 cells also resulted in lipid accumulation; however, it was not associated with SCD1 upregulation but instead increased expression of aldo-keto reductase 1B10 (AKR1b10), which has been implicated in steatosis and steatohepatitis (Ma et al., 2008; Tsuzura et al., 2014; Bitter et al., 2015). Understanding the mechanisms connecting PXR and EtOH-induced steatosis will provide important insights into potential therapeutic targets.

Binge (acute) alcohol drinking is a more common form of alcohol consumption than chronic alcoholism, with more than 75% of all alcohol consumed by bingeing (Sarkar and Chang, 2013; Shukla et al., 2013). Binge drinking contributes significantly to the incidence of cirrhosis seen in patients with ALD (Parrish et al., 1993). Furthermore, binge EtOH administration to mice produced behavioral abnormalities and changes in blood EtOH concentration (BEC), recapitulating some of the changes in human binge drinkers (Carson and Pruett, 1996). Acute EtOH exposure to both humans and rodent models also produced steatosis, lipid peroxidation (LPO), and mitochondrial dysfunction (Meagher et al., 1999; Shukla et al., 2013; Spruiell et al., 2015). Compared with chronic EtOH exposure, relatively little is known regarding the molecular mechanisms of binge EtOH-induced hepatotoxicity. PXR signaling promotes chronic EtOH-induced steatosis (Choi et al., 2018). However, whether PXR is also involved in binge EtOH-induced hepatotoxicity is unknown.

Therefore, we binge dosed male WT and *Pxr*-null mice and evaluated them for hepatotoxicity, lipid profiles, and changes in lipid, alcohol metabolism, and PXR target gene expression.

Materials and Methods

Animal Care and Treatment. This study used male C57BL/6J mice (which served as the WT) and *Pxr*-null mice that were developed by Staudinger et al. (2001) and characterized previously (Staudinger et al., 2001; Spruiell et al., 2014b). Mice (three to five per cage) were housed at the Animal Resources Complex at North Carolina Central University in polycarbonate cages on racks directly vented via the facility's exhaust system at 22°C with a 12-hour/12-hour light/dark cycle. Age-matched (aged 10–12 weeks) male WT and *Pxr*-null mice were each randomly separated into two groups ($n = 8$ to 9 for each group) and gavaged with three doses of 4.5 g/kg EtOH or saline solution every 12 hours at 9:00 AM, 9:00 PM, and 9:00 AM the next day. Four hours after the final dose, mice were anesthetized with isoflurane and euthanized. The liver was rapidly dissected, weighed, snap-frozen in liquid nitrogen, and stored at -80°C . Blood samples collected by cardiac puncture from anesthetized mice were centrifuged at 3000 rpm for 15 minutes to collect serum and were stored at -80°C until they were assayed for EtOH concentration, liver enzymes, triglycerides, and cholesterol. All procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the North Carolina Central University Institutional Animal Care and Use Committee.

Hematoxylin and Eosin Staining of Liver Sections. Liver tissues were fixed in 10% formalin/phosphate-buffered saline and used for hematoxylin and eosin staining and for histologic examination.

Quantification of Alanine Aminotransferase, Aspartate Aminotransferase, Cholesterol, and Triglyceride in Serum. Serum was prepared from whole blood after centrifugation and stored at -80°C . Levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum were determined using the Cholestech LDX analyzer (Cholestech Corporation, Hayward, CA) as reported previously (Spruiell et al., 2014a,b). Serum triglyceride and cholesterol levels were measured using commercially available kits (Wako Pure Chemical Industries, Richmond, VA).

Determination of Serum Alcohol Concentration. Blood samples were collected after three doses of binge EtOH administration and were centrifuged at 3000 rpm for 15 minutes to collect serum for the measurement of BEC. The EtOH L3K assay kit for quantitative measurement of EtOH concentration (Sekisui Diagnostics P.E.I. Inc., Charlottetown, PE, Canada) was used according to the manufacturer's instructions as described previously (Spruiell et al., 2015). The reaction is based on the enzymatic conversion of EtOH by alcohol dehydrogenase (ADH) to acetaldehyde and NADH. EtOH concentration in the serum was quantified as the rate of increase in NADH absorbance due to the reduction of NAD^{+} at 380 nm.

Determination of Hepatic Triglyceride, Cholesterol, and Nonesterified Fatty Acid Levels. Total hepatic lipids were isolated from 100 mg liver tissue as previously described (Gyamfi et al., 2008). Hepatic triglyceride, cholesterol, and nonesterified fatty acid levels were measured using commercially available test kits (Wako Pure Chemical Industries).

Determination of LPO in Liver Tissues. LPO in liver was measured as the thiobarbituric acid reactive product, malondialdehyde (MDA), using a thiobarbituric acid reactive substances kit (ZeptoMetrix, Buffalo, NY) according to the manufacturer's instructions. Protein contents in liver homogenates were determined by the BCA protein assay kit (Thermo Scientific, Rockford, IL).

Western Blot Analyses from Liver Extracts. Western blot analysis was performed using frozen livers as described previously (Spruiell et al., 2014a). The protein concentrations in liver

homogenates were quantified by the BCA protein assay kit (Thermo Scientific). Briefly, 40 μ g liver homogenates was mixed with Laemmli loading buffer containing β -mercaptoethanol and denatured at 100°C. Proteins were separated on 10% or 15% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. The membranes were incubated overnight with rabbit polyclonal primary antibody for CYP2E1 (1:1000, ab28146, lot no. GR182611-20; Abcam, Cambridge, MA). Primary antibodies from Santa Cruz Biotechnology Inc. (Dallas, TX) were all used at a concentration of 1:200 for mouse monoclonal ADH1 (Sc-137078 lot no. L3015), rabbit polyclonal antibody for glucose-regulated protein GRP78 (Sc-13968, lot no. H1809), and goat polyclonal antibody for CY3A11 (SC-30621, lot no. D2109). Rabbit monoclonal primary antibody for aldehyde dehydrogenase ALDH1A1 (1:1000, NB110-55451, lot no. GR182706-7) were from Novus Biologicals (Littleton, CO). Rabbit polyclonal primary antibody for CYP2B10 (1:5000, AB9916, lot no. 2794496) was from Sigma-Aldrich (St. Louis, MO). Rabbit polyclonal primary antibodies for Bax (no. 2772, lot no. 10), caspase-12 (no. 2202, lot no. 8), Bcl-2 (no. 2870, lot no. 5), and phosphorylated-eIF2 α (Ser51) (no. 9721, lot no. 12) were from Cell Signaling Technology (Danvers, MA) and were all used at a concentration of 1:1000. Blots were then incubated with the appropriate peroxidase-conjugated anti-rabbit (no. 7074, lot no. 26) or anti-mouse (no. 7076, lot no. 32) IgG secondary antibodies (Cell Signaling

Technology) or donkey anti-goat (Sc-2020, lot no. 2113) IgG secondary antibody (Santa Cruz Biotechnology Inc.) diluted at 1:5000 in Tris-buffered saline/Tween 20 plus 1% milk for 60 minutes at room temperature. After initial probing, blots were stripped and reprobed with α -tubulin antibody (1:1000, no. 2125, lot no. 9; Cell Signaling Technology). Enhanced chemiluminescence was used to identify protein bands. ImageJ software (National Institutes of Health, Bethesda, MD) was used to quantify band intensity.

Total RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction. Total RNA isolation, cDNA synthesis, and real-time quantitative polymerase chain reaction (PCR) were performed as previously described (Spruiell et al., 2014b). mRNA expression of the following genes was quantified: *Ppara*, carnitine palmitoyltransferase 1 (*Cpt1*), liver fatty acid binding protein 1 (*Lfabp1*), apolipoprotein B-1 (*Apob1*), *Srebp1c*, *Scd1*, *Fas*, acetyl-CoA carboxylase 1a (*Acc1a*), diacylglycerol acyltransferase 1 (*Dgat1*), *Dgat2*, *Srebp2*, 3-hydroxy-3-methyl-glutaryl CoA reductase (*Hmgcr*), *Pxr*, *Car*, *Cyp2b10*, farnesoid X receptor (*Fxr*), small heterodimer partner (*Shp*), and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*). The primer sequences (Table 1) for mRNA encoding *Ppara*, *Cpt1*, *Lfabp1*, *Apob1*, *Srebp2*, *Srebp1c*, *Scd1*, *Acc1a*, *Fas*, *Hmgcr*, *Cyp2b10*, *Fxr*, *Shp*, and *Gapdh* were used for the SYBR Green assay. The following proprietary TaqMan Gene Expression Assays were also

TABLE 1
Sequences of primers used for real-time quantitative PCR

Name	Sequence	Accession Number
PPAR α		
Sense	GATTCAGAAGAAGAACCGGAACA	NM011144
Antisense	TGCTTTTTTCAGATCTTGGCATTTC	
CPT1		
Sense	CGATCATCATGACTATGCGCTACT	NM013495
Antisense	GCCGTGCTCTGCAAACATC	
LFABP		
Sense	TGCATGAAGGGAAGAAAATCAA	NM017399
Antisense	CCCCCAGGGTGAACTCATT	
APOB1		
Sense	GGTGTGTTTTCCATACCACGTTT	XM 137955
Antisense	TCGTAACGCCATGTCACTCTCT	
MTP		
Sense	CCGCTGTGCTTGCAGAAGA	NM008642
Antisense	TTTGACACTATTTTTCTGCTATGGT	
SREBP2		
Sense	ATGATCACCCCGACGTTTCAG	NM 033218
Antisense	GCTGCGTTCTGGTATATCAAAGG	
SREBP1c		
Sense	CATGCCATGGGCAAGTACAC	NM011480
Antisense	TGTTGCCATGGAGATAGCATCT	
SCD1		
Sense	CGTTCCAGA ATGACGTGTACGA	NM009127
Antisense	AGGGTCGGCGTGTGTTTC	
ACC1a		
Sense	ATGTCCGCACTGACTGTAACCA	NM133360
Antisense	TGCTCCGCACAGATTCTTCA	
FAS		
Sense	CCCGAGTCGCTTGAGTATATT	NM007988
Antisense	GGACCGAGTAATGCCATTTCAG	
HMGCR		
Sense	GGCAGTCAGTGGGAACATTGTC	NM 008255
Antisense	CAGTCTTTCCTCGTCCTTCGA	
CYP2B10		
Sense	TTTCTGCCCTTCTCAACAGGAA	NM 009999
Antisense	ATGGACGTGAAGAAAAGGAACAAC	
FXR		
Sense	CACGAAGATCAGATTGCTTTGC	NM 009108
Antisense	CCGCCGAACGAAGAAACAT	
SHP		
Sense	CAGGCACCCCTTCTGGTAGATCT	NM 011850
Antisense	GTCTTCAAGGAGTTTCAGTGATGTCA	
GAPDH		
Sense	TGTGTCCGTCGTGGATCTGA	NM001001303
Antisense	CCTGCTTACCACCTTCTTGA	

purchased from Applied Biosystems/Life Technologies (Grand Island, NY) and used for real-time quantitative PCR: *Adh1* (class I Adh, no. Mm00507711_m1), *Adh4* (class II Adh, no. Mm00478838_m1), *Aldh2* (no. Mm00477463_m1), *Aldh1a1* (no. Mm00657317_m1), *Pxr* (no. Mm01344139_m1), *Car* (no. Mm01283978_m1), retinoid X receptor a (*Rxra*) (no. Mm00441185_m1), uncoupling protein 2 (*Ucp2*) (no. Mm00627599_m1), *Dgat1* (no. Mm00515643_m1), *Dgat2* (no. Mm00499536_m1), *Akr1b7* (no. Mm00477605_m1), *Akr1b8* (no. Mm00484314_m1), and *Gapdh* (housekeeping gene; no. 99999915_g1). The amplification reactions were carried out in an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) as described previously (Gyamfi et al., 2008). Results are presented as levels of expression relative to that of controls after normalizing with *Gapdh* mRNA using the comparative threshold cycle method.

Statistical Analysis. Data are presented as means \pm S.E.M. and were analyzed using SigmaPlot 13 software (Systat Software Inc., San Jose, CA). The effect of genotype, binge EtOH, and their interaction was analyzed using a two-way analysis of variance with the Bonferroni post hoc test. A P value < 0.05 was considered statistically significant.

Results

Both WT and *Pxr*-Null Mice Are Susceptible to Binge EtOH-Induced Steatosis. The saline (control) or binge EtOH treatment had no effect on body weight, liver weight, or liver-to-body weight ratios in either genotype (data not shown). Histopathological analysis of liver sections by hematoxylin and eosin staining indicated the presence of many lipid droplets in both genotypes fed EtOH compared with their respective controls (Fig. 1). Similar to our previous report, basal hepatic triglyceride levels were elevated in control-fed *Pxr*-null mice (1.9-fold) (Spruiell et al., 2014b); however, these differences were not statistically significant (Fig. 2A). Furthermore, the genotype or the interaction between the genotype and EtOH treatment had no effect on hepatic triglyceride levels ($P = 0.715$). However, binge EtOH ingestion increased hepatic triglyceride levels ($P < 0.001$) in both WT (5.9-fold)

and *Pxr*-null (3.3-fold) mice but did not increase hepatic cholesterol levels (Fig. 2, A and B). Notably, the genotype influenced hepatic nonesterified fatty acid levels ($P = 0.002$), which were significantly higher only in EtOH-fed WT mice (Fig. 2C).

Basal Hepatic mRNA Levels of *Srebp1c* and Its Target Gene *Scd1* Are Upregulated in *Pxr*-Null Mice.

The increased steatosis and hepatic triglyceride content we observed in both genotypes fed EtOH (Figs. 1 and 2A) prompted us to examine the expression of mRNAs encoding *Srebp1c* and hepatic fatty acid and triglyceride synthesis enzymes. The genotype influenced basal hepatic mRNA levels of both *Srebp1c* and its target gene *Scd1*, which were significantly increased 2-fold and 2.5-fold in *Pxr*-null mice, respectively, compared with saline-treated (control) WT mice (Fig. 3, A and B). In contrast, EtOH administration had no effect on *Srebp1c* mRNA levels in either genotype but diminished *Scd1* mRNA ($P < 0.05$) in *Pxr*-null mice (Fig. 3, A and B). Basal hepatic gene expression of other *Srebp1c* targets, *Fas* and *Acc1a*, was not different between the two genotypes (Fig. 3, C and D). EtOH increased *Fas* mRNA levels in both WT (3.1-fold) and *Pxr*-null (2.8-fold) mice (Fig. 3C), suggesting a significant effect of treatment ($P = 0.001$) but not interaction between the genotype and EtOH on *Fas* mRNA levels. EtOH had no effect on *Acc1a* mRNA levels in WT mice (Fig. 3D). In contrast, EtOH increased hepatic *Acc1a* gene expression in *Pxr*-null mice (1.7-fold) ($P = 0.01$) (Fig. 3D), leading to significantly higher *Acc1a* mRNA levels in EtOH-fed *Pxr*-null mice compared with similarly treated WT mice (Fig. 3D). Pairwise multiple-comparison analysis revealed that both genotype and treatment significantly influenced *Acc1a* mRNA levels. Constitutive *Dgat1* and *Dgat2* mRNA levels were not significantly different between the two genotypes (Fig. 3, E and F). Both hepatic *Dgat1* and *Dgat2* mRNA levels were decreased by EtOH in *Pxr*-null mice ($P = 0.004$); however, only *Dgat1* mRNA levels were decreased in WT mice ($P < 0.001$) (Fig. 3, E and F). Basal levels of *Srebp2* mRNA and

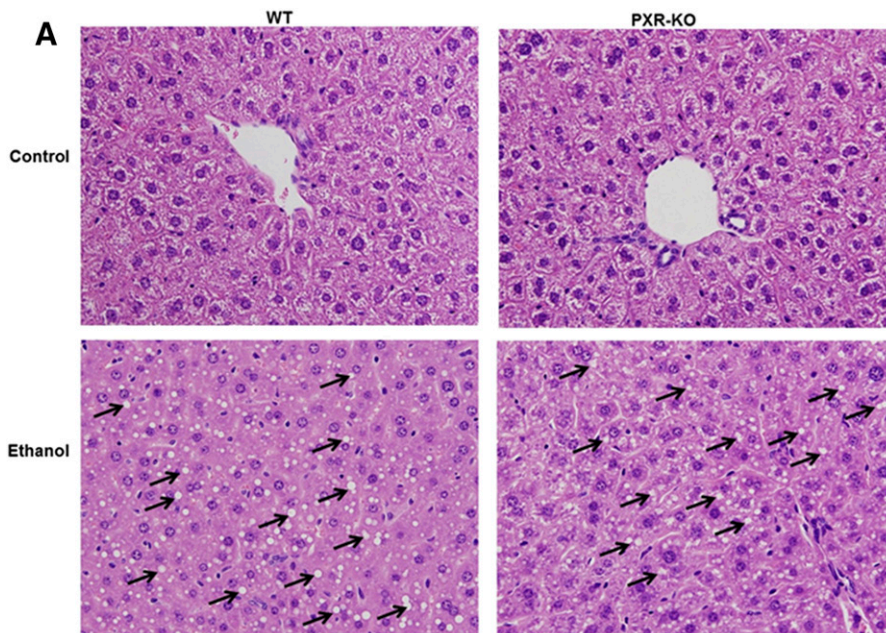


Fig. 1. Representative H&E staining of livers in control and binge EtOH-fed male WT and *Pxr*-null mice. Male WT and *Pxr*-null mice were orally administered saline (control) or EtOH (4.5 g/kg) every 12 hours for a total of three doses and were euthanized 4 hours after the final dose. H&E stains were assayed as described in the *Materials and Methods*. Binge EtOH administration produced steatosis (indicated by arrows) in both WT and *Pxr*-null mice fed EtOH. H&E, hematoxylin and eosin; KO, knockout. Original magnification, 400 \times .

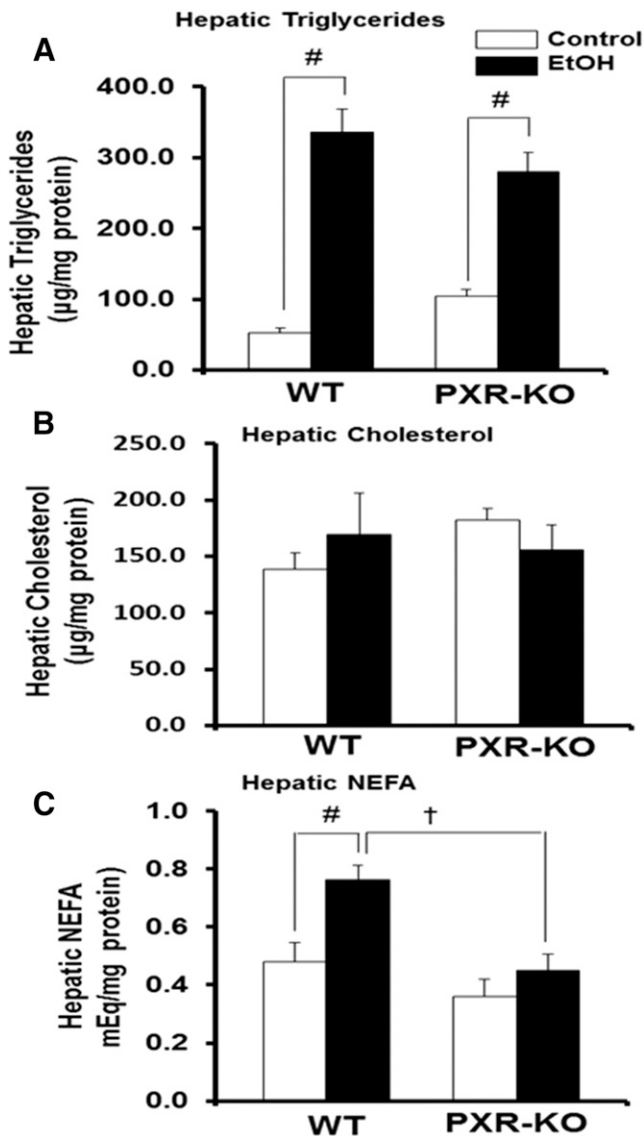


Fig. 2. Quantification of hepatic lipids in control and binge EtOH-fed male WT and *Pxr*-null mice. Male WT and *Pxr*-null mice were orally administered saline (control; white bars) or EtOH (4.5 g/kg; black bars) every 12 hours for a total of three doses and were euthanized 4 hours after the final dose. (A–C) Hepatic triglycerides (A), hepatic cholesterol (B), and hepatic NEFAs (C) were assayed as described in the *Materials and Methods*. Data represent the mean \pm S.E.M. ($n = 5$ to 6). [†] $P < 0.05$ (significantly different from treatment-matched WT group); [#] $P < 0.05$ (significantly different from genotype-matched saline control). KO, knockout; NEFA, nonesterified fatty acid.

its target gene *Hmgcr* were similar between control-fed WT and *Pxr*-null mice; however, EtOH significantly decreased the *Hmgcr* gene only in *Pxr*-null mice (Fig. 3, G and H).

Binge EtOH Ingestion Similarly Affected Hepatic Fatty Acid Oxidation Genes in Both Genotypes. Since alcohol-induced lipid accumulation may also result from inhibition of hepatic fatty acid β -oxidation, we monitored fatty acid uptake and oxidation genes. There was a significant effect of treatment ($P = 0.001$) but not genotype or interaction between the genotype and EtOH on *Ppara* mRNA levels. Basal hepatic *Ppara* mRNA levels were not different between saline-fed WT and similarly treated *Pxr*-null mice (Fig. 4A). Hepatic *Ppara* mRNA levels in both WT and *Pxr*-null mice

were significantly decreased by EtOH by 55% and 41%, respectively, compared with their corresponding controls (Fig. 4A). Similarly, basal *Cpt1a* gene expression did not vary between the two genotypes (Fig. 4B). However, there was a significant effect of both genotype ($P = 0.004$) and treatment ($P = 0.001$), in which binge EtOH treatment significantly decreased *Cpt1a* mRNA levels only in WT mice (Fig. 4B). Constitutive expression levels of *Lfabp1* mRNA were similar between both genotypes and EtOH did not significantly affect *Lfabp1* gene expression in either genotype (Fig. 4C). Basal hepatic levels of *Ucp2* mRNA were similar between both control-fed WT and *Pxr*-null mice (Fig. 4D). However, there was a significant effect of treatment ($P = 0.005$) but not genotype on *Ucp2* mRNA levels, such that EtOH significantly upregulated *Ucp2* mRNA levels only in WT mice (1.8-fold) (Fig. 4D). Constitutive levels of *Apob1* and microsomal triglyceride transfer protein *Mtp* mRNAs in the liver did not vary between the two genotypes and their mRNA levels were not different after EtOH treatment (Fig. 4, E and F).

Binge EtOH Upregulation of the *Cyp2b10* Gene Is PXR Dependent. Nuclear receptors including PXR, FXR, CAR, and SHP have also been implicated in lipid homeostasis (Tanaka et al., 2017). Furthermore, due to the crosstalk between PXR and CAR, we evaluated the effect of these nuclear receptors on the development of lipid accumulation by binge EtOH. As expected, *Pxr* mRNA was absent in the livers of *Pxr*-null mice (Fig. 5A). Interestingly, EtOH did not alter *Pxr* gene expression in either genotype (Fig. 5A). There was a significant effect of genotype on basal hepatic *Car* mRNA levels, which were significantly lower in *Pxr*-null mice compared with WT controls (Fig. 5B). Interestingly, EtOH did not alter *Car* gene expression in either genotype (Fig. 5B). Basal mRNA levels of *Rxra*, *Fxr*, and *Shp* were not different between both genotypes and *Rxra* and *Fxr* mRNA levels were not affected by EtOH (Fig. 5, C and D). However, there was a significant effect of treatment on *Shp* mRNA levels ($P = 0.001$), such that binge EtOH administration significantly reduced *Shp* mRNA levels in both WT and *Pxr*-null mice (Fig. 5E). Unexpectedly, binge EtOH induced the CAR target gene *Cyp2b10* (17-fold) only in WT mice (Fig. 5F). There was a statistically significant interaction between genotype and treatment on both *Cyp2b10* mRNA and CYP2B10 protein levels ($P = 0.035$). Basal *Cyp2b10* mRNA levels were somewhat higher in *Pxr*-null mice compared with WT mice but were not statistically different (Fig. 5F). Western blot analyses further confirmed that CYP2B10 protein levels were significantly higher in EtOH-fed WT mice (4-fold) compared with WT controls (Fig. 5G). However, EtOH did not alter CYP2B10 protein expression in *Pxr*-null mice (Fig. 5G). Constitutive hepatic protein expression of CYP3A11 was significantly induced in *Pxr*-null mice (2.0-fold) compared with saline-fed WT mice as previously reported (Fig. 5H) (Staudinger et al., 2001). Hepatic CYP3A11 protein levels were significantly increased by EtOH in WT mice (1.8-fold) but not in similarly treated *Pxr*-null mice (Fig. 5H).

Antiapoptotic Protein Bcl-2 Expression Is Decreased in EtOH-Fed WT Mice. Binge EtOH treatment had a significant effect on protein levels of hepatic endoplasmic reticulum stress markers GRP78 ($P = 0.015$) and phospho-eIF2 α ($P = 0.001$) in WT mice. Hepatic GRP78 and phospho-eIF2 α protein expression was upregulated 1.9- and 5.0-fold, respectively, in EtOH-treated WT mice versus saline-treated

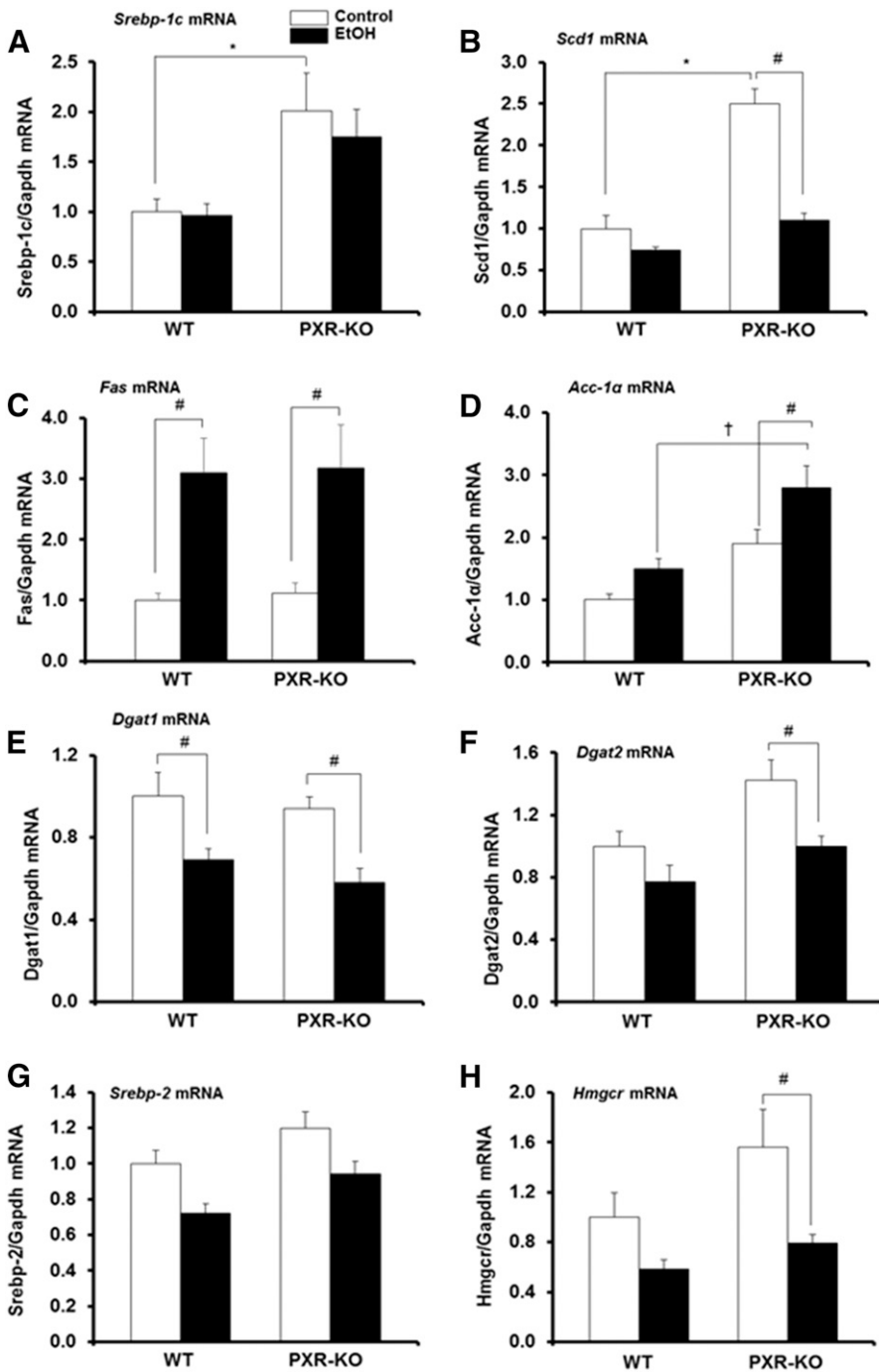


Fig. 3. Gene expression of mouse hepatic enzymes that promote lipogenesis. Male WT and *Pxr*-null mice were orally administered saline (control; white bars) or EtOH (4.5 g/kg; black bars) every 12 hours for a total of three doses and were euthanized 4 hours after the final dose. (A–H) Total hepatic mRNA levels of *Srebp1c* (A), *Scd1* (B), *Fas* (C), *Acc1α* (D), *Dgat1* (E), *Dgat2* (F), *Srebp2* (G), *Hmgcr* (H), and *Gapdh* were quantified by the SensiFast SYBR Hi-ROX Kit (Bioline, Taunton, MA). Data represent the mean \pm S.E.M. ($n = 5$ to 6). * $P < 0.05$ (significantly different from saline-treated WT control); † $P < 0.05$ (significantly different from treatment-matched WT group); # $P < 0.05$ (significantly different from genotype-matched saline control). KO, knockout.

WT mice (Fig. 6, A and B). Phospho-eIF2 α protein levels were similar in the livers of both genotypes fed saline (Fig. 6B). EtOH exposure increased hepatic phospho-eIF2 α protein levels (2.6-fold) but not GRP78 in *Pxr*-null mice (Fig. 6, A and B). Furthermore, basal expression of the endoplasmic-specific apoptotic protein caspase-12 was increased 3.6-fold in saline-fed *Pxr*-null mice compared with WT controls ($P = 0.002$) (Fig. 6C). EtOH nonsignificantly increased caspase-12 protein in WT mice (1.8-fold); however, caspase-12 protein was significantly decreased approximately 40% in *Pxr*-null mice (Fig. 6C). There was a statistically significant interaction between genotype and treatment on Bax protein levels

($P = 0.002$). Hepatic Bax protein expression levels were significantly higher in saline-fed *Pxr*-null mice compared with saline-fed WT mice (Fig. 6D). EtOH ingestion decreased Bax protein levels in *Pxr*-null mice ($P = 0.015$) but significantly increased Bax protein in WT mice (2.3-fold) to a level higher than that in EtOH-fed *Pxr*-null mice ($P = 0.009$) (Fig. 6D). Expression of the antiapoptotic protein Bcl-2 did not differ between the two genotypes fed saline; however, EtOH markedly inhibited the Bcl-2 protein only in WT mice (Fig. 6E). Furthermore, Bcl-2 protein levels were significantly higher in EtOH-fed *Pxr*-null mice than in similarly treated WT mice (Fig. 6E).

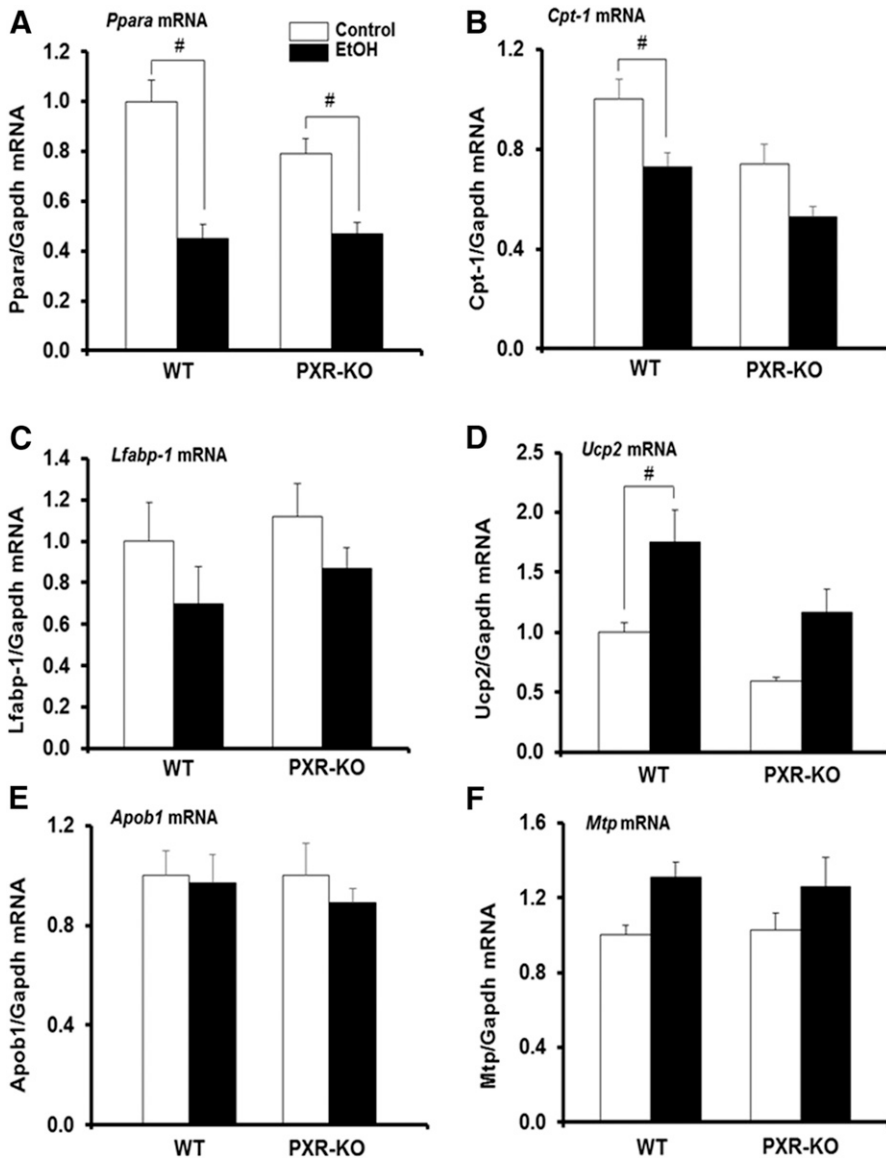


Fig. 4. Hepatic gene expression of mouse *Ppara* and its target genes. Male WT and *Pxr*-null mice were orally administered saline (control; white bars) or EtOH (4.5 g/kg; black bars) every 12 hours for a total of three doses and were euthanized 4 hours after the final dose. (A–F) Total hepatic mRNA levels of *Ppara* (A), *Cpt1* (B), *Lfabp1* (C), *Ucp2* (D), *Apob1* (E), *Mtp* (F), and *Gapdh* were quantified by the SensiFast SYBR Hi-ROX Kit (Bioline). Data represent the mean \pm S.E.M. ($n = 5$ to 6). # $P < 0.05$ (significantly different from genotype-matched saline control). KO, knockout, MTP, microsomal triglyceride transfer protein.

Basal and EtOH-Exposed Hepatic ADH1 Protein Levels Are Significantly Higher in *Pxr*-Null Mice. Published reports indicate that nuclear receptors regulate *Adh1* expression (Gyamfi et al., 2006; Langhi et al., 2013). Furthermore, EtOH metabolism is known to alter the intracellular redox state due to the generation of increased NADH levels, which promotes excess fatty acids synthesis leading to steatosis (Zakhari and Li, 2007), a phenomenon corroborated in our study (Figs. 1 and 2). Therefore, we examined the effect of PXR deficiency, binge EtOH, and their interaction on mRNAs of *Adh1*, *Adh4*, *Aldh2*, and *Aldh1a1* as well as the protein levels of ADH1, CYP2E1, and ALDH1A1, which are known to be involved in EtOH metabolism (Heit et al., 2015). The EtOH treatment had a significant effect on mRNA levels of hepatic *Adh1*, *Adh4*, and *Aldh2* ($P = 0.001$) but not the genotype or their interaction. Basal mRNA levels of hepatic *Adh1*, *Adh4*, and *Aldh2* did not vary significantly between the two genotypes treated with saline (Fig. 7, A–C). EtOH significantly decreased *Adh1*, *Adh4*, and *Aldh2* mRNA levels

by 34%–59% in WT mice but decreased only *Aldh2* mRNA (43%) in *Pxr*-null mice (Fig. 7, A–C). Genotype, treatment, and the interaction between genotype and treatment significantly affected *Aldh1a1* mRNA levels. Interestingly, basal *Aldh1a1* mRNA levels were not significantly different between the two genotypes (Fig. 7D). However, *Aldh1a1* mRNA levels were significantly lower after EtOH ingestion only in WT mice (46% of control) (Fig. 7D). Genotype, but not treatment or the interaction between genotype and EtOH, significantly affected ADH1 protein levels. Contrary to the mRNA data, Western blotting indicated a marked increase in basal hepatic ADH1 protein levels in *Pxr*-null mice (2.5-fold) compared with WT controls (Fig. 7E). Binge EtOH did not have any significant effect on ADH1 protein levels in WT mice but maintained the higher levels of ADH1 protein in *Pxr*-null mice (Fig. 7E). There was no significant effect of genotype or interaction between genotype and treatment on hepatic CYP2E1 protein levels; in agreement, basal CYP2E1 protein expression did not vary between WT and *Pxr*-null mice (Fig. 7F). However, there

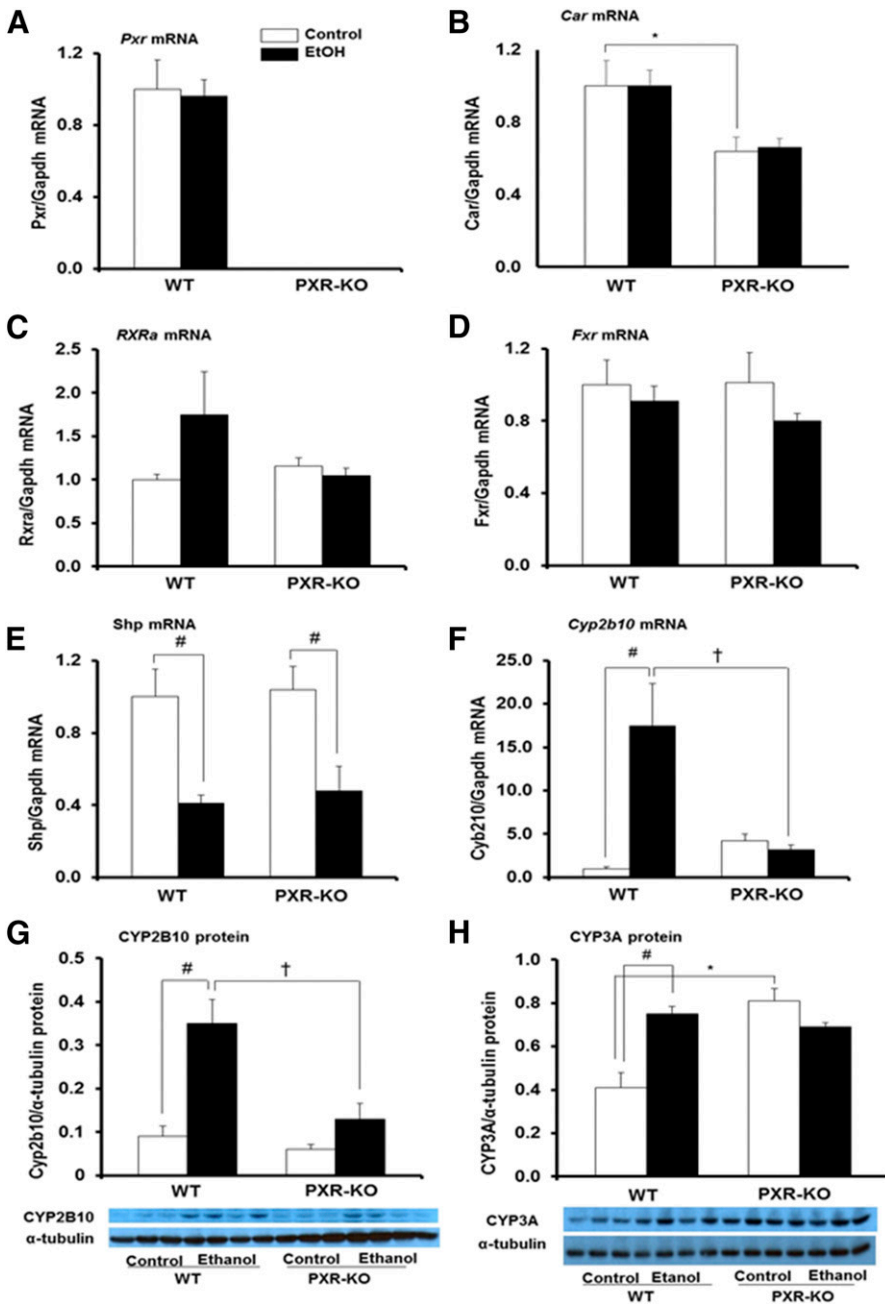


Fig. 5. Hepatic gene expression and immunoblot analysis of mouse type II nuclear receptors and their target genes important for xenobiotic metabolism and lipid homeostasis. Male WT and *Pxr*-null mice were orally administered saline (control; white bars) or EtOH (4.5 g/kg; black bars) every 12 hours for a total of three doses and were euthanized 4 hours after the final dose. (A–F) Total hepatic mRNA levels of *Pxr* (A), *Car* (B), *Rxra* (C), *Fxr* (D), *Shp* (E), *Cyp2b10* (F), and *Gapdh* were quantified by the SensiFast SYBR Hi-ROX Kit (Bioline). Data represent the mean \pm S.E.M. ($n = 5$ to 6). (G and H) Furthermore, Western blots of liver homogenate (40 μ g/lane) were probed with antibodies to CYP2B10 (G) and CYP3A11 (H). Bands were quantified and normalized to α -tubulin. Data represent the mean \pm S.E.M. ($n = 3$ to 4). * $P < 0.05$ (significantly different from saline-treated WT control); † $P < 0.05$ (significantly different from treatment-matched WT group); # $P < 0.05$ (significantly different from genotype-matched saline control). KO, knockout.

was a significant effect of EtOH treatment on CYP2E1 protein expression, which was upregulated (1.6-fold) in both genotypes (Fig. 7F). In agreement with the marked inhibition of *Aldh1a1* mRNA levels, ALDH1A1 protein expression was also markedly inhibited by EtOH only in WT mice (Fig. 7, D and G). Furthermore, both hepatic *Aldh1a1* mRNA and ALDH1A1 protein levels in EtOH-fed *Pxr*-null mice were higher compared with EtOH-fed WT mice ($P < 0.001$) (Fig. 7, D and G).

Basal Hepatic Gene Expression of *Akr1b7*, But Not *Akr1b8*, Is Elevated in *Pxr*-Null Mice. Both *Akr1b7* and *Akr1b8* (or its human ortholog AKR1B10) have been implicated in lipid metabolism (Tirard et al., 2007; Bitter et al., 2015). Interestingly, *Akr1b7* is both a CAR and PXR target gene (Liu et al., 2009). It was therefore of interest to examine the effect of PXR deficiency and binge EtOH exposure on the

hepatic expression of *Akr1b7* and *Akr1b8* genes using quantitative real-time-PCR. Genotype, but not treatment or the interaction between the genotype and treatment, had a major effect on *Akr1b7* mRNA levels, such that basal hepatic *Akr1b7* mRNA levels were significantly higher in *Pxr*-null mice (8.4-fold) than in saline-treated WT controls (Fig. 8A). Binge EtOH had no effect on hepatic levels of *Akr1b7* gene expression in both genotypes but maintained the higher *Akr1b7* mRNA levels in *Pxr*-null mice, such that *Akr1b7* mRNA levels were significantly higher in *Pxr*-null mice than in WT mice (Fig. 8A). Unlike *Akr1b7* mRNA, basal hepatic *Akr1b8* gene expression was similar between the two genotypes; however, EtOH significantly decreased hepatic *Akr1b8* mRNA levels only in WT mice (33%) (Fig. 8B). Induction of oxidative stress is involved in binge/acute

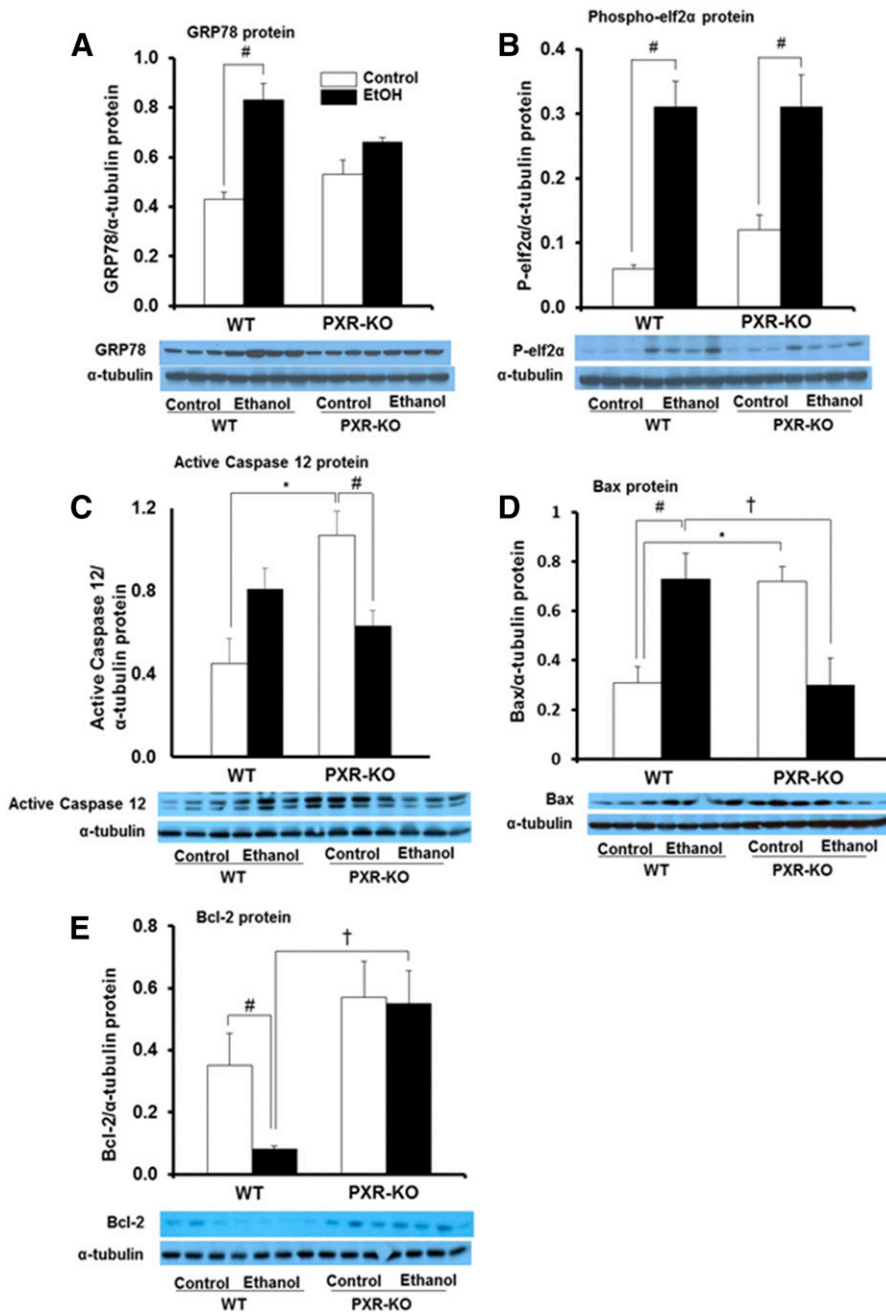


Fig. 6. Immunoblot analysis of hepatic endoplasmic reticulum stress and apoptotic markers. Male WT and *Pxr*-null mice were orally administered saline (control; white bars) or EtOH (4.5 g/kg; black bars) every 12 hours for a total of three doses and were euthanized 4 hours after the final dose. (A–E) Western blots of liver homogenate (40 μ g/lane) were probed with antibodies to GRP78 (A), phospho-elf2 α (B), active caspase-12 (C), Bax (D), and Bcl-2 (E). Bands were quantified and normalized to α -tubulin. Data represent the mean \pm S.E.M. ($n = 3$ to 4 mice/group). ^{*} $P < 0.05$ (significantly different from saline-treated WT control); [†] $P < 0.05$ (significantly different from treatment-matched WT group); [#] $P < 0.05$ (significantly different from genotype-matched saline control). KO, knockout; P, phosphorylated.

EtOH-induced hepatotoxicity (Meagher et al., 1999). Both *Akr1b7* and *Akr1b8* can detoxify toxic aldehydes generated during LPO (Spite et al., 2007; Liu et al., 2009). Furthermore, *ALDH1A1* is a major cytosolic ALDH involved in cellular defense against oxidative stress in the mouse liver (Makia et al., 2011). Therefore, we measured LPO, an early biochemical event of EtOH hepatotoxicity and a major indicator of oxidative stress, by quantifying the thiobarbituric acid reactive product, MDA. Consistent with reduced hepatic *Aldh1a1* and *Akr1b8* genes and *ALDH1A1* protein expression in EtOH-fed WT mice, hepatic MDA levels were elevated by binge EtOH only in the livers of WT mice (3.8-fold) (Figs. 7, D and G, and 8, B and C). Furthermore, the increase in hepatic MDA levels by EtOH was higher in WT mice compared with *Pxr*-null mice ($P = 0.003$) (Fig. 8C).

PXR Deficiency Leads to Increased Binge EtOH-Induced Hyperlipidemia. There was a significant interaction between treatment and genotype on serum triglyceride levels ($P = 0.010$). Basal serum triglyceride levels tended to increase in *Pxr*-null mice (1.5-fold) compared with saline-fed WT mice (Fig. 9A). Binge EtOH ingestion tended to increase serum triglyceride levels in WT mice (2.0-fold) (Fig. 9A). By contrast, serum triglyceride levels were markedly increased in EtOH-fed *Pxr*-null mice (4.8-fold) (Fig. 9A). Similarly, serum cholesterol levels were significantly elevated by EtOH in *Pxr*-null mice by 13% (Fig. 9B). Furthermore, after EtOH treatment, both serum triglyceride and cholesterol levels in *Pxr*-null mice were higher ($P < 0.05$) than in EtOH-fed WT mice (Fig. 9, A and B). Serum ALT and AST activities were measured as indices of

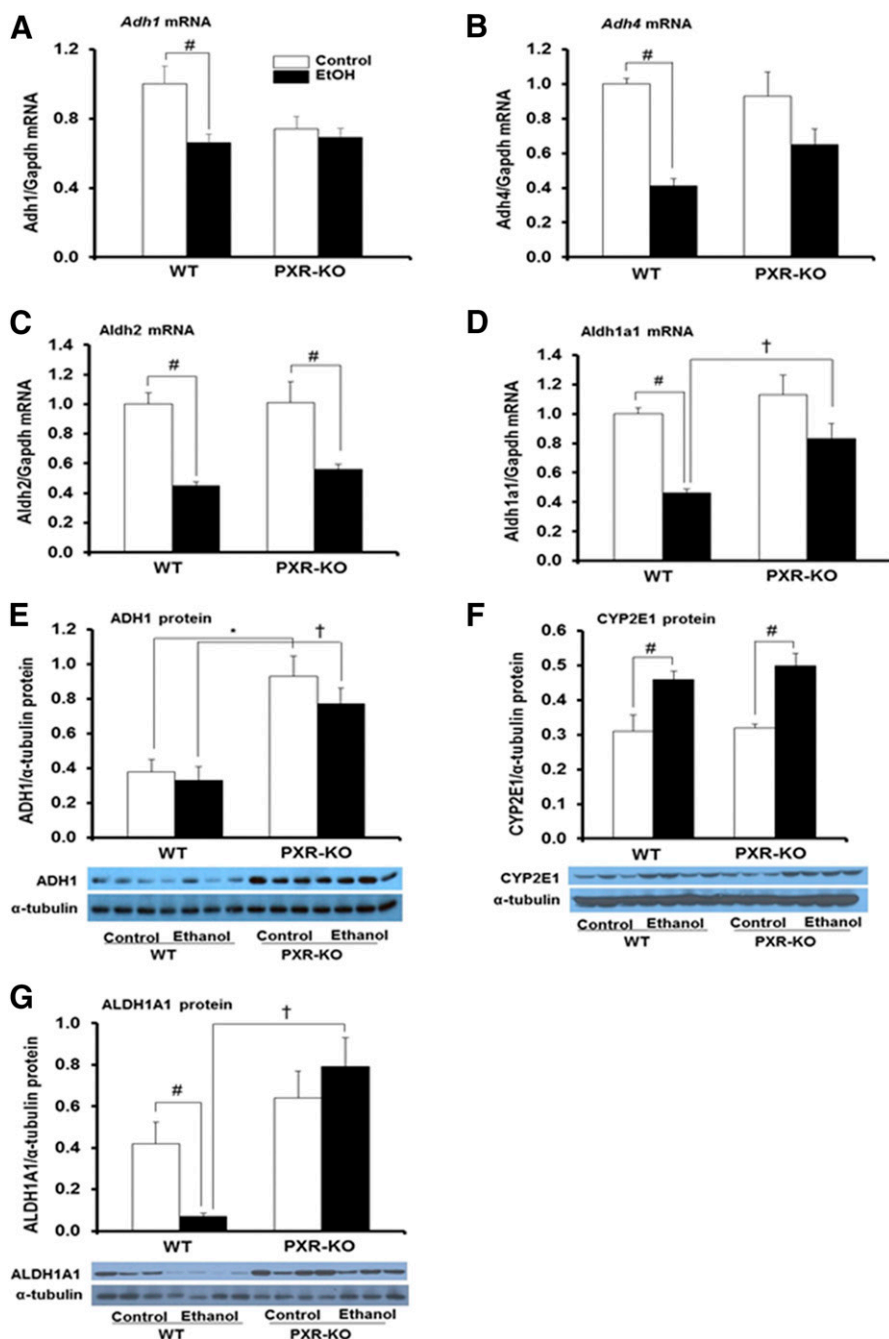


Fig. 7. Gene expression and immunoblot analysis of mouse hepatic enzymes involved in EtOH metabolism. Male WT and *Pxr*-null mice were orally administered saline (control; white bars) or EtOH (4.5 g/kg; black bars) every 12 hours for a total of three doses and were euthanized 4 hours after the final dose. (A–D) Total hepatic mRNA levels of *Adh1* (A), *Adh4* (B), *Aldh2* (C), *Aldh1a1* (D), and *Gapdh* were quantified by the SensiFast SYBR Hi-ROX Kit (Bioline). Data represent the mean \pm S.E.M. ($n = 5-7$). (E–G) Western blots of liver homogenate (40 μ g/lane) were probed with antibodies to ADH1 (E), CYP2E1 (F), and ALDH1A1 (G). Data represent the mean \pm S.E.M. ($n = 3$ to 4). * $P < 0.05$ (significantly different from saline-treated WT control); † $P < 0.05$ (significantly different from treatment-matched WT group); # $P < 0.05$ (significantly different from genotype-matched saline control). KO, knockout.

hepatocyte/organ injury. Unexpectedly, serum ALT and AST were not significantly induced in either genotype exposed to EtOH (Fig. 9, C and D). Due to the marked decreases in EtOH-metabolizing enzymes (especially hepatic *Aldh1a1* expression in EtOH-fed WT mice) but higher ADH1 protein levels in *Pxr*-null mice, serum EtOH concentrations were measured after administration of three doses of binge EtOH. There was an interaction between EtOH treatment and genotype on the residual serum EtOH concentration ($P = 0.014$). As expected, the EtOH concentration was higher ($P < 0.001$) in WT mice compared with similarly treated *Pxr*-null mice (Fig. 9E).

Discussion

In this report, we characterized the hepatotoxic responses of WT mice and *Pxr*-null mice to acute EtOH exposure. CAR and PXR are xenobiotic receptors that share ligands and regulate drug-inducible expression of the same target genes, such as CYP2B and CYP3A, as well as genes unique to each receptor (Wang and Negishi, 2003). CAR preactivation and alcohol administration increases EtOH-induced hepatotoxicity in a CAR-dependent manner (Chen et al., 2011). Although the *Car* gene was not induced by binge EtOH, our study demonstrates a robust PXR-dependent upregulation of *Cyp2b10*, a CAR target gene (Ding et al., 2006), in binge EtOH-fed WT mice. In

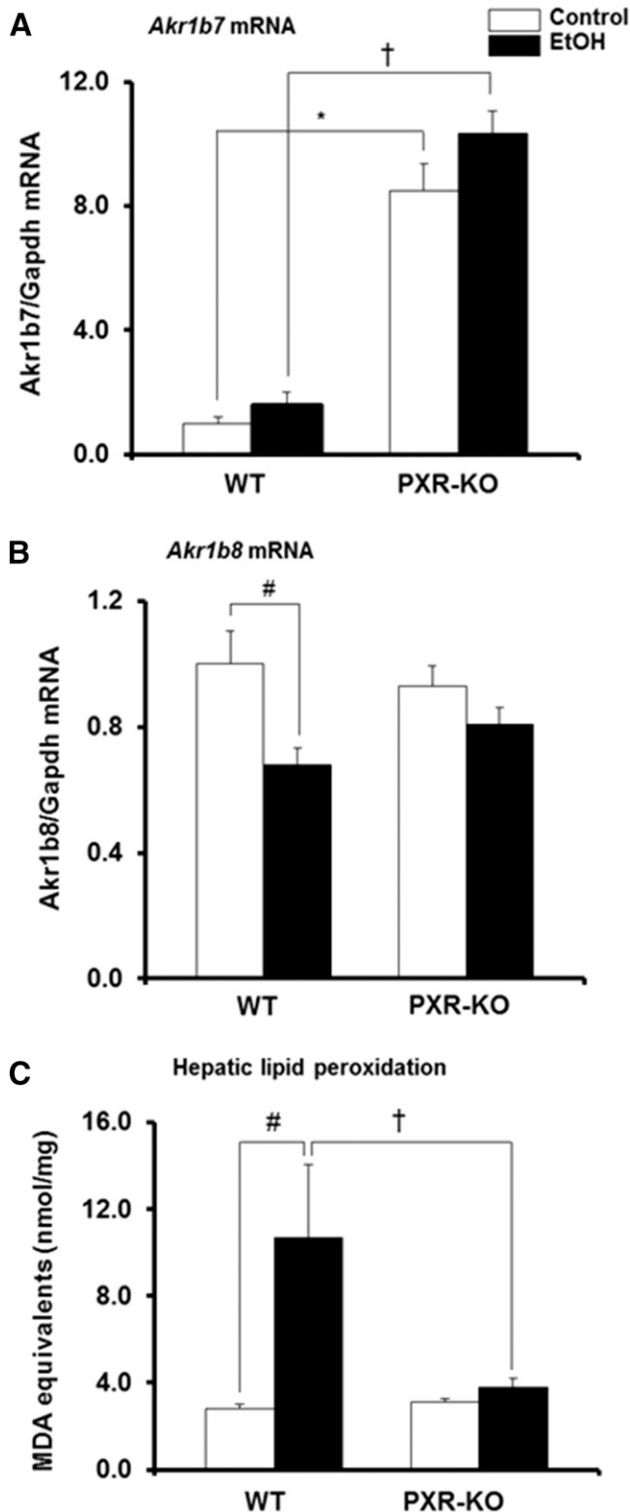


Fig. 8. Gene expression of mouse hepatic enzymes involved in the detoxification of lipid peroxides and the extent of lipid peroxidation in liver tissues. Male WT and *Pxr*-null mice were orally administered saline (control; white bars) or EtOH (4.5 g/kg; black bars) every 12 hours for a total of three doses and were euthanized 4 hours after the final dose. (A and B) Total hepatic mRNA levels of *Akr1b7* (A), *Akr1b8* (B), and *Gapdh* were quantified by the SensiFast SYBR Hi-ROX Kit (Bioline). Data represent the mean \pm S.E.M. ($n = 5-7$). (C) The extent of lipid peroxidation in liver tissues was quantified by measuring the thiobarbituric acid reactive product, MDA. Data represent the mean \pm S.E.M. ($n = 5$ to 6). * $P < 0.05$ (significantly different from saline-treated WT control); † $P < 0.05$ (significantly different from treatment-matched WT group); # $P < 0.05$

addition, protein expression of CYP3A11, whose expression can be regulated by both PXR and CAR, was also induced by binge EtOH only in WT mice (Wang and Negishi, 2003). Although the molecular basis for *Cyp2b10* upregulation by binge EtOH seen here is not fully understood, we confirm that PXR is required for optimal *Cyp2b10* upregulation by CAR (Wang and Negishi, 2003; Ding and Staudinger, 2005). Interestingly, in agreement with our findings, a PXR-dependent increase in *Cyp2b10* expression by chronic EtOH administration has been reported (Choi et al., 2018). Both PPAR γ coactivator 1 α and hepatocyte nuclear factor 4 α positively regulate *Cyp2b10* independent of CAR (Wiwi et al., 2004; Koga et al., 2016). Hepatic hepatocyte nuclear factor 4 α and PPAR γ coactivator 1 α mRNA levels were also examined in this study, but they were not induced by EtOH in both genotypes (data not shown). Together, our results indicate that although *Pxr*-null mice express CAR, binge EtOH could not induce *Cyp2b10* in this model, suggesting that PXR influences EtOH induction of *Cyp2b10*.

EtOH is metabolized mainly by oxidation to acetaldehyde by multiple enzymes, including ADH, CYP2E1, and catalase (Zakhari and Li, 2007). In this study, EtOH significantly decreased the gene expression of both *Adh1*, the principal enzyme important for EtOH oxidation, and *Adh4*, an isoform efficient at high EtOH concentrations (Zakhari and Li, 2007) only in WT mice. Although *Adh1* mRNA expression did not differ, basal hepatic protein expression of ADH1 was higher in *Pxr*-null mice than in WT mice. These findings confirm published reports indicating that hepatic ADH is regulated by a post-transcriptional mechanism (Gyamfi et al., 2006), possibly due to increased serum leptin levels in *Pxr*-null mice (Mezey et al., 2005; Spruiell et al., 2014b). Acetaldehyde, a toxic product generated from EtOH metabolism, is then oxidized to acetate by three ALDH isoforms (ALDH2, ALDH1B1, and ALDH1A1) (Heit et al., 2015). Among the cytosolic ALDH1 isoforms, ALDH1A1 also plays a major role in the removal of many reactive carbonyl species (Makia et al., 2011). One mechanism identified in the activation of CAR and enhanced EtOH hepatotoxicity involves inhibition of EtOH-metabolizing enzymes including ALDH1A1 expression (Chen et al., 2011). In our study, while *Aldh2* mRNA levels were inhibited in both genotypes, PXR deficiency prevented EtOH-induced suppression of both *Aldh1a1* gene and ALDH1A1 protein expression in *Pxr*-null mice, but not in WT mice. In agreement with decreased *Adh1*, *Adh4*, and *Aldh1a1* mRNAs as well as ADH1 and ALDH1A1 protein expression in EtOH-fed WT mice, the serum EtOH concentration was elevated in WT mice compared with EtOH-fed *Pxr*-null mice. Thus, our results indicate that PXR ablation results in increases in ADH1 protein expression and prevents binge EtOH-induced suppression of EtOH metabolism, thereby increasing EtOH elimination in *Pxr*-null mice.

In this study, EtOH-fed mice of both genotypes developed steatosis with higher hepatic triglyceride content. Importantly, expression of hepatic fatty acid β -oxidation genes including *Ppara* and *Cpt1* was suppressed by binge EtOH in WT mice, but only *Ppara* was suppressed in EtOH-fed *Pxr*-null mice. Moreover, binge EtOH also upregulated genes involved

(significantly different from genotype-matched saline control). KO, knockout.

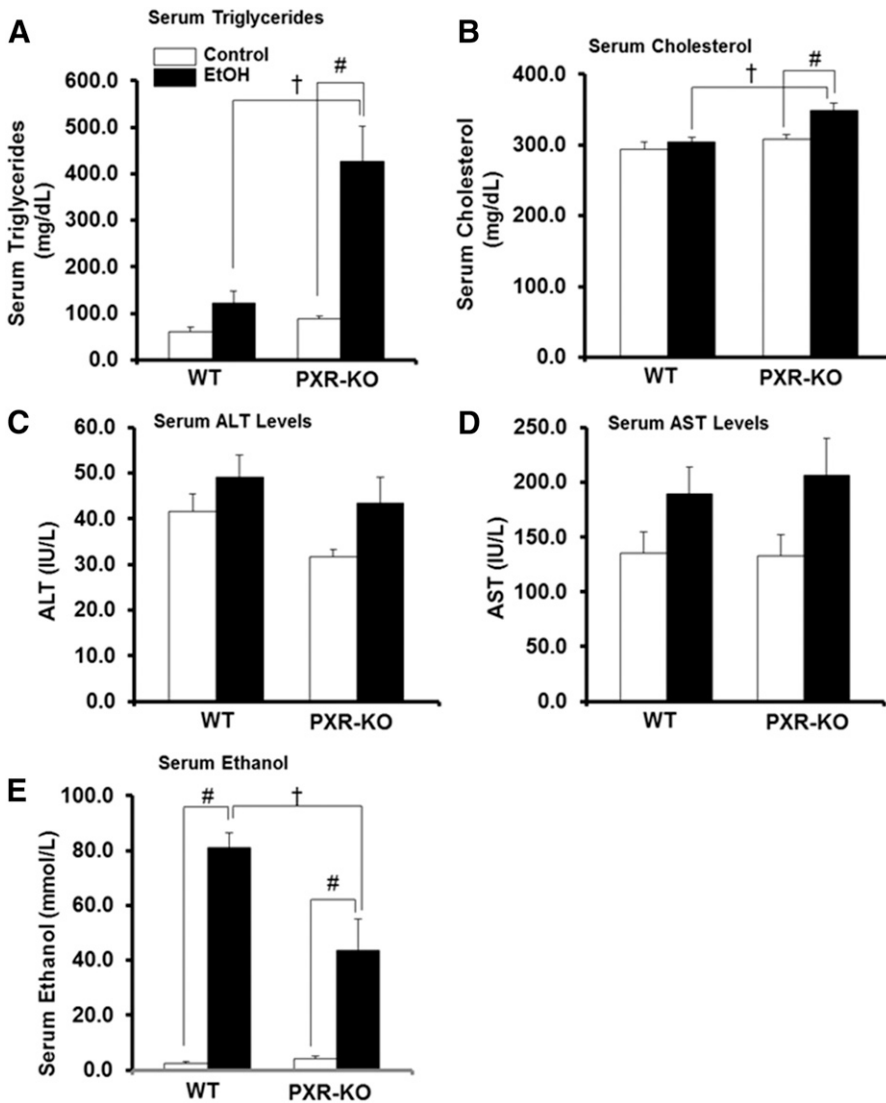


Fig. 9. Serum lipid profile, markers of hepatotoxicity, and serum EtOH concentration. Male WT and *Pxr*-null mice were orally administered saline (control; white bars) or EtOH (4.5 g/kg; black bars) every 12 hours for a total of three doses and were euthanized 4 hours after the final dose. (A–E) Serum was assayed for triglycerides (A), cholesterol (B), ALT (C), AST (D), and serum EtOH concentration (E) as described in the *Materials and Methods*. Data represent the mean \pm S.E.M. ($n = 5$ to 6). $^\dagger P < 0.05$ (significantly different from treatment-matched WT group); $^\# P < 0.05$ (significantly different from genotype-matched saline control). KO, knockout.

in fatty acid synthesis, including *Acc1a* and *Fas* in *Pxr*-null mice, whereas it induced only *Fas* in WT mice. Unexpectedly, binge EtOH treatment resulted in somewhat much larger hepatic triglyceride levels in WT mice (5.9-fold) compared with *Pxr*-null mice (3.3-fold). In this study, basal hepatic triglyceride levels were somewhat elevated in saline-fed *Pxr*-null mice (1.9-fold) compared with WT mice. Therefore, despite the fold increase being greater in WT mice, the absolute levels of hepatic triglyceride accumulation were similar between WT and *Pxr*-null mice. In a previous report, PXR knockdown-induced steatosis in HepG2 cells was associated with elevation of *AKR1B10* gene expression (Bitter et al., 2015). However, in this study, basal hepatic mRNA levels of *Akr1b8*, the mouse ortholog of human *AKR1B10*, were not different between WT and *Pxr*-null mice. Taken together, although EtOH-induced inhibition of fatty acid catabolic gene expression is the major mechanism for steatosis in both WT and the *Pxr*-null mice, marked upregulation of genes involved in fatty acid synthesis may also account for EtOH-induced steatosis in *Pxr*-null mice.

Acute alcohol consumption can cause hypertriglyceridemia (Zemánková et al., 2015). PPAR α activation in rodents and humans markedly reduces hepatic triglyceride synthesis and

promotes plasma triglyceride clearance (Kersten, 2008). Although serum triglycerides were not increased in EtOH-fed WT mice, it was unexpected to observe hyperlipidemia in the EtOH-fed *Pxr*-null mice. It is possible that the concomitant induction of lipogenic genes involved in triglyceride synthesis and suppression of PPAR α and its target genes by EtOH contributed to the hypertriglyceridemia seen in *Pxr*-null mice.

Cyp2b10 upregulation by EtOH in WT mice, as seen in our study, produces reactive oxygen species (ROS) and increased oxidative stress (Dostalek et al., 2007), an important feature of ALD. Interestingly, CYP2E1 only plays a minor role in the mechanism of early alcoholic liver injury, whereas both CYP2B and CYP3A can be major sources of ROS in alcoholic hepatitis (Kono et al., 1999). While EtOH administration caused upregulation of CYP2E1 protein in both genotypes, markers of oxidative stress including thiobarbituric acid reactive substances were elevated only in EtOH-fed WT mice in association with decreased *Aldh1a1* and *Akr1b8* expression. Unexpectedly, mRNA levels of *Akr1b7*, another common transcriptional target of both PXR and CAR that is known to detoxify lipid peroxides (Spite et al., 2007; Liu et al., 2009), was massively upregulated in both control and binge

EtOH-fed *Pxr*-null mice compared with EtOH-fed WT mice. It is not clear why basal *Akr1b7* mRNA levels are higher in *Pxr*-null mice. However, in our study, mRNA levels of the liver X receptor, a nuclear receptor known to regulate *Akr1b7* (Volle et al., 2004), did not differ between the two genotypes fed saline (data not shown). Furthermore, both *Akr1b7* and *Akr1b8* are nuclear factor E2-related factor-2 target genes (Beneš et al., 2013; Luo et al., 2015). Whether the elevated *Akr1b7* mRNA levels seen in saline-fed *Pxr*-null mice involve nuclear factor E2-related factor-2 activation remains to be determined. Nevertheless, it is reasonable to propose that elevated *Akr1b7* gene expression and resistance to EtOH-induced suppression of both *Aldh1a1* and *Akr1b8* gene/protein expression may protect against EtOH-induced oxidative stress in *Pxr*-null mice.

The antiapoptotic protein Bcl-2 can protect cells against ROS (Li et al., 2004). In agreement, Bcl-2 protein was suppressed in EtOH-exposed WT mice, whereas the proapoptotic Bax protein and other oxidative stress or endoplasmic reticulum stress markers including the *Ucp2* gene, GRP78, and phospho-elf2 α protein (Carmiel-Haggai et al., 2003; Ji and Kaplowitz, 2003) were increased in EtOH-exposed WT mice. While EtOH-induced activation of LPO was noted in EtOH-fed WT mice, unexpectedly, markers of liver damage, ALT and AST were not significantly increased in EtOH-fed WT mice. Similarly, in a previous study, three binge doses of EtOH (6 g/kg, every 12 hours) also failed to induce ALT activity (Abdelmegeed et al., 2013). In contrast with our findings, concomitant with increased LPO, serum ALT activity was significantly increased in male *PXR*-humanized mice after the same dose of acute EtOH (4.5 g/kg) was administered (Spruiell et al., 2015). Taken together, these findings are consistent with previous studies demonstrating the hepatotoxic effects of EtOH-induced activation of *Cyp2b10* due to increased oxidative stress and suppression of EtOH-metabolizing enzymes (Chen et al., 2011; Koga et al., 2016; Wang et al., 2017).

In summary, our findings suggest that PXR ablation prevents binge EtOH-induced upregulation of *Cyp2b10* mRNA and CYP2B10 protein expression, suppresses EtOH metabolism, increases BEC, and activates oxidative stress and apoptosis but promotes alcoholic hypertriglyceridemia. However, both PXR and PXR ablation enhance binge EtOH-induced steatosis. Furthermore, our results also suggest that PXR is involved in the transcriptional suppression of *Akr1b7* expression and that the PXR-dependent effects of EtOH as determined using *Pxr*-null mice have clinical relevance to EtOH-induced oxidative stress.

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Authorship Contributions

Participated in research design: M. A. Gyamfi.

Conducted experiments: S. Choi, A. A. Gyamfi, P. Neequaye, S. Addo, M. A. Gyamfi.

Contributed new reagents or analytic tools: F. Gonzalez, M. A. Gyamfi.

Performed data analysis: S. Choi, M. A. Gyamfi.

Wrote or contributed to the writing of the manuscript: F. Gonzalez, M. A. Gyamfi.

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