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# FXR deficiency alters bile acid pool composition and exacerbates chronic alcohol induced liver injury

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ABSTRACT

Recent studies have investigated the roles of FXR deficiency in the pathogenesis of alcoholic liver disease (ALD). However, the underlying molecular mechanisms remain unclear. In this study, FXR knockout ($FXR^{-/-}$) and wild-type (WT) mice were subjected to chronic-plus-binge alcohol feeding to study the effect of FXR deficiency on ALD development. The degree of liver injury was greater in $FXR^{-/-}$ mice compared to WT mice. Ethanol feeding enhanced hepatic steatosis in $FXR^{-/-}$ mice, accompanied by decreased mRNA levels of *Pparα* and *Srebp-1c*. The expression of *Lcn2* was increased by ethanol treatment, despite unchanged expression of pro-inflammatory cytokines *Tnfα*, *Il6* and *Il-1β*. Furthermore, ethanol treatment altered bile acid (BA) homeostasis to a greater extent in $FXR^{-/-}$ mice, as well as serum and hepatic BA pool composition. The mRNA levels of hepatic *Cyp7a1* and *Shp*, as well as intestinal *Fgf15*, were decreased in WT mice with ethanol feeding, which were further reduced in $FXR^{-/-}$ mice. Levels of both primary and secondary BAs were markedly elevated in $FXR^{-/-}$ mice, which were further increased after ethanol treatment. Moreover, hepatic MAPK signaling pathways were disturbed presumably by increased hepatic BA levels. In summary, FXR deficiency increased hepatic steatosis and altered BA pool composition, contributing to worsened liver toxicity.

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## 1. Introduction

Alcoholic liver disease (ALD) could be caused by chronic and overt alcohol consumption, and is currently one of the major causes of liver morbidity and mortality worldwide. ALD represents a wide spectrum of morphological changes that can occur in varying degrees, including fatty liver, hepatic inflammation, alcoholic hepatitis, and alcoholic cirrhosis [1]. The mechanisms by which alcohol consumption leads to ALD remain elusive. Currently, ethanol is considered to generate hepatotoxicity by altering hepatic lipid metabolism and increasing endotoxin release from the gut, contributing to inflammation and oxidative stress [1–4]. Additionally, environmental and genetic factors, including gender, ethnicity, nutritional factors, oxidative stress, and hepatic co-morbidities, may play a crucial role in ALD development [4,5].

Bile acids (BAs) are critical for facilitating the absorption of dietary lipids and lipid-soluble vitamins in the intestines [6,7]. At physiological levels, BAs function as signaling molecules and activate specific nuclear receptors [farnesoid X receptor (FXR), pregnane X receptor, and vitamin D receptor] [8–13] and membrane receptors [G protein-coupled bile acid receptor 1 (TGR5) and sphingosine-1 phosphate receptor 2 [14,15]. Specifically, FXR is a ligand-activated transcription factor belonging to the nuclear receptor superfamily, and is essential for regulating BA and lipid homeostasis. FXR has been shown to suppress liver steatosis [16–19], inflammation and fibrosis [20,21], thus playing a critical role in the prevention and treatment of the nonalcoholic steatohepatitis [18,19,21]. FXR has also been shown to promote liver regeneration [22], and therefore, enhances recovery from chemical-induced liver injury or liver resection. Previous studies suggest alcohol may down-regulate the expression and function

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of FXR [23,24], which may be responsible for altered BA synthesis and total BA pool size. Deficiency of FXR is unable to suppress BA synthesis by FXR-mediated negative feedback mechanisms, and thereby increases BA levels in the body [6]. Therefore, high concentrations of BAs can damage cells, induce apoptosis, and promote tumorigenesis [7,25].

FXR has previously been shown to protect the liver from chronic alcohol-induced injury and cellular damage, as activation of FXR by synthetic agonists WAY-362450 or obeticholic acid attenuated chronic alcohol-induced liver injury, steatosis, and oxidative stress [23,24]. However, the mechanisms are not clear. Current evidence suggests that activation of intestinal FXR protects against ethanol-induced liver disease by inhibiting BA synthesis and reducing BA toxicity in the liver [26]. Moreover, FXR is involved in maintaining and preserving the integrity and function of the intestinal barrier, and preventing bacterial translocation from the intestinal tract [27,28]. Ethanol exposure has been shown to induce bacterial translocation and increase gut permeability, which promotes endotoxemia and the development of ALD [29]. In agreement with the importance of intestinal FXR to mitigate ALD, our previous study found that hepatic FXR plays only a minor role in ameliorating ethanol-induced injury, therefore FXR in extra-hepatic tissues may be responsible for effects on ALD development [30]. In this study we used whole-body FXR knockout mice ($FXR^{-/-}$) and a chronic-plus-binge ethanol feeding model to study the effects of whole-body FXR deficiency on BA metabolism in ALD development.

## 2. Materials and methods

### 2.1. Animals and treatments

Age-matched male C57Bl/6J (WT) and $FXR^{-/-}$ mice (8–12 weeks old, n = 5–7/strain/diet) were fed *ad libitum* a Lieber-DeCarli ethanol-containing diet (5% (v/v) ethanol liquid diet) or isocaloric maltose-dextrin liquid diet (Vehicle, Veh) for 10 days. Mice were then administered a single binge (oral gavage) of ethanol (5 g/kg body weight) or maltose solution (9 g maltose/kg of body weight) [31], before euthanized for tissue collection 9 h following binge gavage. All animal experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the Rutgers University. Methods for serum biochemical assays, liver histopathological examination and gene expression analysis have been described in detail previously [30].

### 2.2. Western blot analysis

Livers were homogenized and lysed in 1X RIPA buffer with protease inhibitors (Thermo Fisher Scientific). Proteins (20 μg per well) were separated on 12% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. After blocking with 5% milk, the blots were incubated with primary antibodies overnight and visualized using ECL substrates (Thermo Fisher Scientific). Primary antibody targeting CYP2E1 was purchased from Abcam. Antibodies targeting total JNK, phosphorylated JNK, total ERK, phosphorylated ERK, nuclear factor-kappa B (NF-κB), P65, phosphorylated P65, NF-κB Inhibitor α (IκBα), and phosphorylated IκBα were purchased from Cell Signaling. Band density was quantified by ImageJ software and normalized to levels of β-actin or α-tubulin.

### 2.3. Lipid peroxidation assay

Liver samples were homogenized in the RIPA buffer with protease inhibitors and the levels of malondialdehyde (MDA), an indicator of oxidative stress in liver tissue, were measured by the TBARS Assay Kit (Cayman Chemical), according to manufacturer's protocol.

### 2.4. Analysis of serum and liver BA composition

Total serum and liver BAs were extracted and analyzed by Thermo Finnigan Ultra Performance Liquid Chromatography (UPLC) system coupled with a Thermo Finnigan LTQ XL Ion Trap Mass Spectrometer (Thermo Fisher Scientific). Detailed protocols were described in our previous publications [25,32]. The percent composition of specific BAs in the serum or liver was calculated. In total, 21 BA species were evaluated, including: cholic acid (CA), taurocholic acid (TCA), glycocholic acid (GCA), chenodeoxycholic acid (CDCA), taurochenodeoxycholic acid (TCDCA), glycochenodeoxycholic acid (GCDCA), α-muricholic acid (α-MCA), β-muricholic acid (β-MCA), ω-muricholic acid (ω-MCA), tauromuricholic acid (TMCA), deoxycholic acid (DCA), taurodeoxycholic acid (TDCA), glycodeoxycholic acid (GDCA), ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), glycoursodeoxycholic acid (GUDCA), hyodeoxycholic acid (HDCA), taurohyodeoxycholic acid (THDCA), lithocholic acid (LCA), taurolithocholic acid (TLCA), and glycolithocholic acid (GLCA).

### 2.5. Statistical analysis

The data are expressed as mean ± SD. Statistical significance was determined by the SigmaStat software. Difference among groups was assessed by two-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test. Findings were considered significant with $p < 0.05$.

## 3. Results

### 3.1. FXR deficiency exacerbated alcohol-induced liver injury

Ethanol feeding increased liver-to-body-weight ratios in both WT and $FXR^{-/-}$ mice, with the increase greater in $FXR^{-/-}$ mice (Fig. 1A). Ethanol decreased body weight (BW) in both strains with less BW loss in $FXR^{-/-}$ compared to WT mice (Fig. 1A). In WT mice, ethanol feeding increased serum activity of AST while decreased that of ALP. Compared to WT, $FXR^{-/-}$ mice presented higher increase in serum activity of ALT, AST and ALP, as well as BA levels (Fig. 1B), indicating more severe liver damage in $FXR^{-/-}$ mice following ethanol feeding. There were no difference in serum levels of triglycerides between WT and $FXR^{-/-}$ mice fed either diet. Interestingly, ethanol feeding decreased serum levels of total cholesterol in WT, but not in $FXR^{-/-}$ mice.

No difference between WT and $FXR^{-/-}$ mouse livers was found with vehicle feeding by H&E staining (Fig. 1C). In WT mice, ethanol feeding resulted in increased accumulation of small lipid droplets in hepatocytes (microvesicular steatosis), accompanied by a small degree of inflammatory cell infiltration. Severe hepatocyte ballooning, steatosis (mixed microvesicular and macrovesicular) and lobular inflammation with inflammatory cell infiltration were observed in $FXR^{-/-}$ mice (Fig. 1C).

### 3.2. FXR deficiency contributed to alcohol-induced liver inflammation

Lipopolysaccharide (LPS) released from gut bacteria is a strong driver for ethanol-induced liver inflammation. Hepatic mRNA levels of the LPS receptor, *Tlr4,* did not show a significant increase with ethanol feeding (Fig. 2A). However, the mRNA levels of another LPS receptor, *Cd14,* were significantly induced by ethanol in WT and $FXR^{-/-}$ mice. The induction of *Cd14* was higher in $FXR^{-/-}$ mice, although without statistical significance. Only *Tnfα* mRNA levels were significantly increased in $FXR^{-/-}$ mice compared to WT mice following ethanol feeding (Fig. 2A). The mRNA levels of other pro-inflammatory markers, *Lipocalin-2* (*Lcn2*), *Il-6* and *Il-1β*, were not

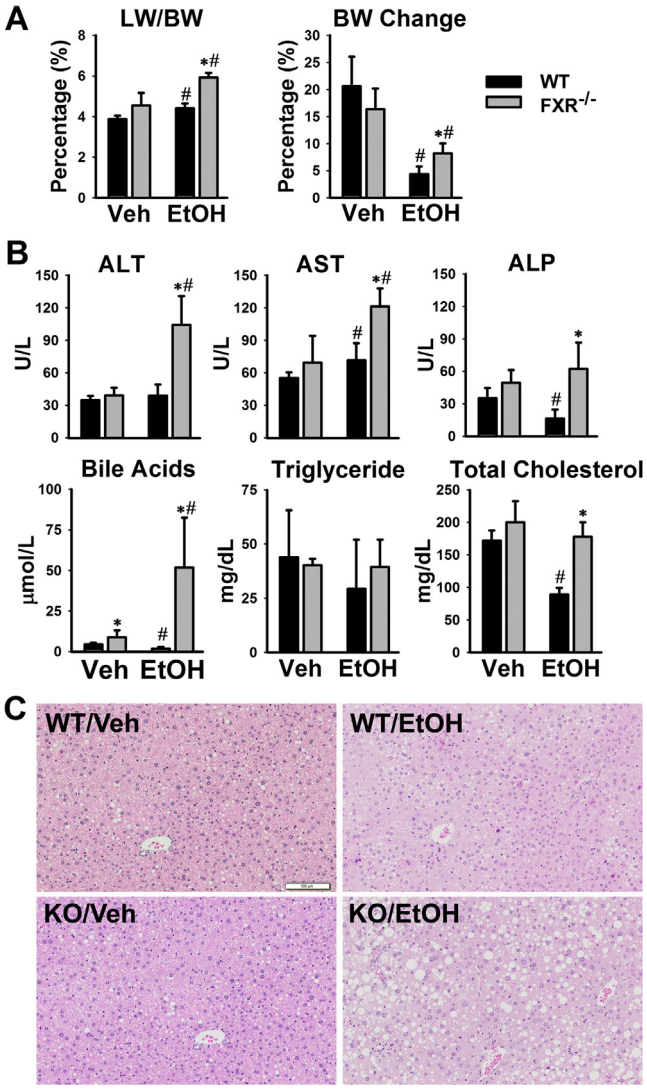


**Fig. 1.** Ethanol induced liver toxicity in FXR deficient mice. (A) Liver weight (LW) to body weight (BW) ratio and percentage of BW change in WT and FXR$^{-/-}$ mice after ethanol feeding. (B) Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and serum total bile acids, total triglyceride and total cholesterol levels. WT/Veh and FXR$^{-/-}$/Veh (n = 5), WT/EtOH and FXR$^{-/-}$/EtOH (n = 7). (C) Representative hepatic H&E staining. Differences were considered significant at $p < 0.05$; * indicates difference between strains (WT vs FXR$^{-/-}$); # indicates difference between treatments (vehicle vs ethanol) within the same strain.

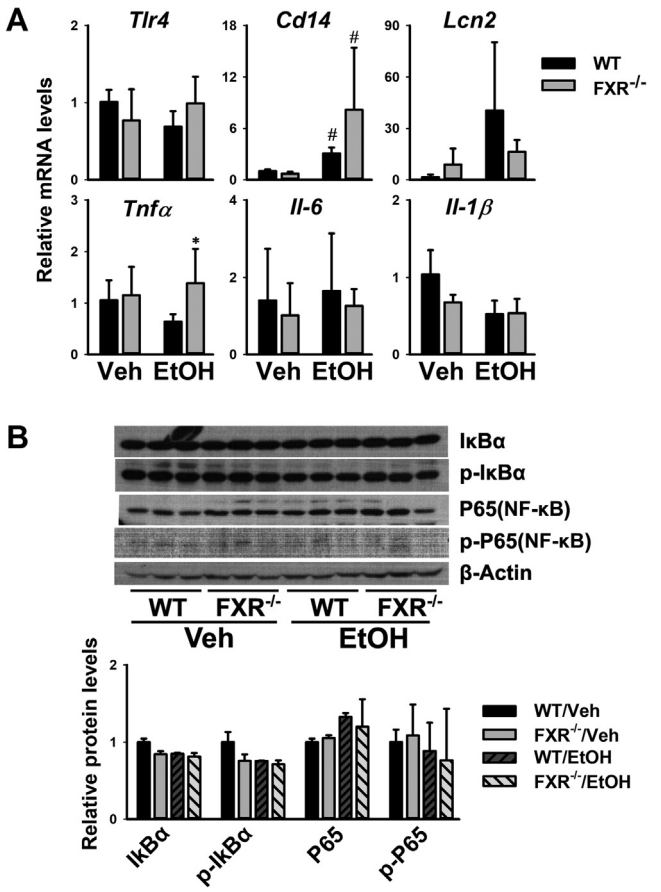


**Fig. 2.** Ethanol exposure on liver inflammation. (A) Relative hepatic gene expression of LPS receptors *Tlr4* and *Cd14*, acute phase protein, Lcn2, and inflammatory cytokines *Tnfα*, *Il-6* and *Il-1β*. (B) Representative Western blot image and semi-quantification of for NF-κB related proteins in WT and FXR$^{-/-}$ livers after ethanol feeding. Differences were considered significant at $p < 0.05$; * indicates difference between different strains (WT vs FXR$^{-/-}$); # indicates difference between treatments (vehicle vs ethanol) within the same strain.

significantly different between WT and FXR$^{-/-}$ mice after ethanol feeding.

NF-κB signaling pathway plays a critical role in control of the inflammatory response. WT mice on ethanol diet showed a trend for decreased IκBα and phosphorylated IκBα (p-IκBα). While the baseline levels were also decreased in vehicle-fed FXR$^{-/-}$ mice compared to WT mice, they were not decreased further by ethanol feeding (Fig. 2B). A component of the NF-κB complex, P65, was significantly induced by ethanol feeding in both WT and FXR$^{-/-}$ mice, as well as the levels of phosphorylated P65 (p-P65; Fig. 2B).

### 3.3. FXR deficiency increased hepatic oxidative stress and disrupted lipid homeostasis after ethanol feeding

Lipid peroxidation by oxidative stress has been shown to contribute to the hepatic inflammatory response. The byproduct, MDA, of lipid peroxidation was measured in the liver (Fig. 3A). No significant difference was noted between WT and FXR$^{-/-}$ mice when fed the vehicle, or in WT mice following ethanol feeding. Levels of MDA in FXR$^{-/-}$ mice were increased following ethanol feeding (Fig. 3A), indicating increased ethanol-induced oxidative stress in FXR$^{-/-}$ mice only.

CYP2E1-mediated ethanol oxidation plays a critical role in ethanol-induced oxidative stress, which causes liver injury. Ethanol feeding significantly induced CYP2E1 protein levels in WT mice, and FXR$^{-/-}$ mice fed the ethanol diet had similar levels. Interestingly, FXR$^{-/-}$ mice showed higher basal CYP2E1 protein levels when fed the vehicle, compared to WT mice (Fig. 3B).

The expression of genes involved in hepatic lipid homeostasis was measured to determine what the effects of FXR deficiency and ethanol exposure have (Fig. 3C). The mRNA levels of *Cd36* were unchanged by FXR deficiency or feeding. The mRNA levels of *Srebp-1c*, *Fas* and *Acss2* were similar between WT and FXR$^{-/-}$ mice under vehicle treatment, which were markedly decreased by ethanol feeding regardless of FXR deficiency. The mRNA levels of *Mttp* gene were only significantly down-regulated in ethanol-fed WT mice. The mRNA levels of *Pparα*, and its target gene, *Cyp4a10*, were decreased in FXR$^{-/-}$ mice on vehicle treatment, but ethanol feeding decreased only *Pparα* expression significantly in both WT and FXR$^{-/-}$ mice (Fig. 3C).

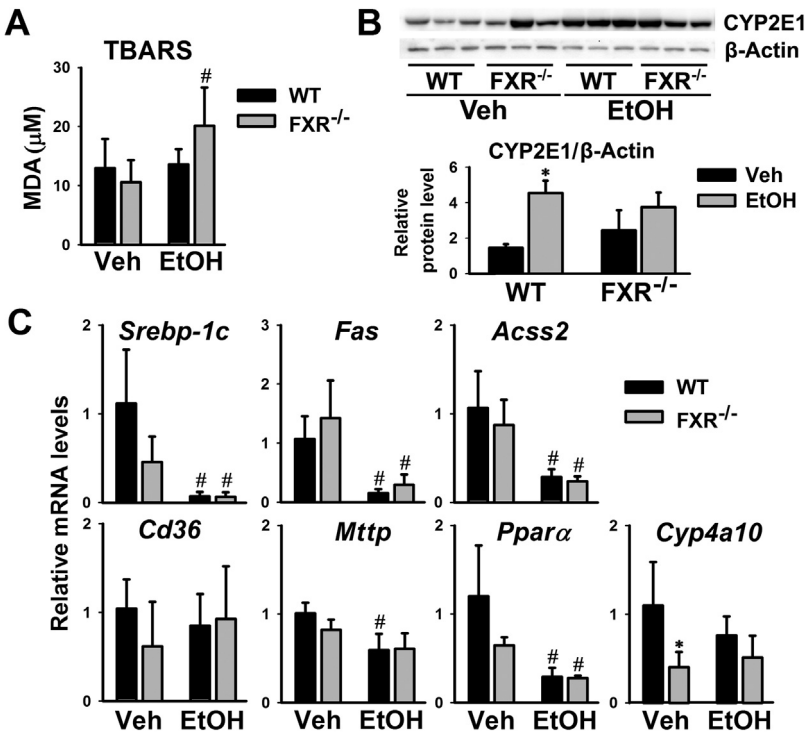


**Fig. 3.** Ethanol exposure disrupted lipid metabolism. (A) Malondialdehyde (MDA) level in WT and FXR$^{-/-}$ livers after ethanol feeding. (B) Relative CYP2E1 protein level in WT and FXR$^{-/-}$ liver after ethanol feeding. (C) Relative expression level of genes involved in hepatic fatty acid synthesis (*Fas*, *Acss2*, *Srebp-1c*), fatty acid uptake (*Cd36*), VLDL secretion (*Mttp*), and fatty acid β-oxidation (*Pparα* and its target gene *Cyp4a10*). Differences were considered significant at $p < 0.05$; * indicates difference between different strains (WT vs FXR$^{-/-}$); # indicates difference between treatments (vehicle vs ethanol) within the same strain.

### 3.4. BA homeostasis in FXR$^{-/-}$ mice was disrupted upon ethanol feeding

FXR functions as a BA sensor, and transcriptionally regulates the expression of genes involved in BA homeostasis. As expected, the expression of a FXR hepatic target gene, *Shp*, was lower in FXR$^{-/-}$ mice, and was further reduced by ethanol feeding in both WT and FXR$^{-/-}$ mice (Fig. 4A). There were no significant difference in the expression levels of key genes encoding enzymes for BA synthesis, *Cyp7a1* and *Cyp8b1*, between WT and FXR$^{-/-}$ mice, and ethanol feeding down-regulated the expression of both genes. When fed vehicle, the mRNA levels of genes encoding hepatic BA transporters, *Ntcp*, *Ostβ* and *Bsep*, were lower in FXR$^{-/-}$ mice compared to WT mice (Fig. 4A). Ethanol feeding down-regulated the expression of these genes in WT mice and further decreased their expression levels in FXR$^{-/-}$ mice.

The FXR/FGF15/FGFR4 axis is a major pathway to regulate BA synthesis in mice, in which the intestinal FGF15 acts as a hormone to suppress BA synthesis in hepatocytes. FXR target genes in the intestine, *Shp*, *Fgf15* and *Ibabp*, were down-regulated in FXR$^{-/-}$ mice (Fig. 4B). *Fgf15* gene expression in WT mice was decreased following ethanol feeding, but was not further decreased in FXR$^{-/-}$ mice. Ethanol feeding did not significantly affect the gene expression of *Fxr*, *Shp*, and *Ibabp* in the intestines of either WT or FXR$^{-/-}$ mice.

### 3.5. Ethanol feeding altered serum and liver BA profiles in FXR deficient mice

The concentrations of 21 BAs were measured in serum samples from vehicle and ethanol fed mice using the UPLC–MS method (Table 1A). As expected, tauro-conjugates were the dominant conjugated BAs in mice. β-MCA and its derivatives were the major primary BAs in mice compared to CA. In vehicle fed mice, the total serum levels of the 21 measured BA species were 2.5 fold higher in FXR$^{-/-}$ mice compared to WT mice. The increase in serum BAs observed in FXR$^{-/-}$ mice was even more pronounced with ethanol treatment with serum levels reaching 7 fold higher in FXR$^{-/-}$ mice compared to those of WT mice. Serum BA composition was not altered in WT mice after ethanol treatment. Compared to WT mice, serum concentrations of CA, TCA, TCDCA, TMCA and TUDCA were elevated in FXR$^{-/-}$ mice regardless of ethanol treatment. Ethanol feeding predominantly increased serum CA, TCA and TMCA in FXR$^{-/-}$ mice. In addition to elevated levels of primary BAs, FXR$^{-/-}$ mice had increased levels of secondary BAs, including TUDCA, HDCA, and THDCA. The levels of two hydrophobic and toxic secondary BAs, DCA and LCA, were significantly changed, with DCA increased in FXR$^{-/-}$ mice and further increased after ethanol feeding, while LCA increased after ethanol treatment in both WT and FXR$^{-/-}$ mice.

The percentage of individual BA species comprised serum BA pool was changed as well. In WT mice after ethanol feeding, the percentage of each BA remained similar except for a small percentage decrease in β-MCA and an increase in ω-MCA. TCA was the prevalent serum BA in FXR$^{-/-}$ mice, and ethanol treatment further increased the percentage of TCA in the pool. Moreover, the percentage of conjugated BAs in both WT and FXR$^{-/-}$ mice increased with ethanol feeding, but to a different degree. The percentage of total primary BAs in WT and FXR$^{-/-}$ mice was relatively unchanged. WT mice had a slight decrease, whereas FXR$^{-/-}$ mice had an increase after ethanol feeding.

The total liver BA concentration in the liver varied as well (Table 1B), specifically, FXR$^{-/-}$ mice following ethanol feeding had BA concentrations 1.7-fold higher compared to WT mice. TCA and TUDCA levels increased in FXR$^{-/-}$ mice with ethanol feeding, whereas CDCA, TCDCA, α-, β-, ω-MCA, UDCA, HDCA and THDCA decreased. Levels of secondary BAs LCA, conjugated TLCA, and GLCA were not detected in mouse livers following vehicle or ethanol feed-

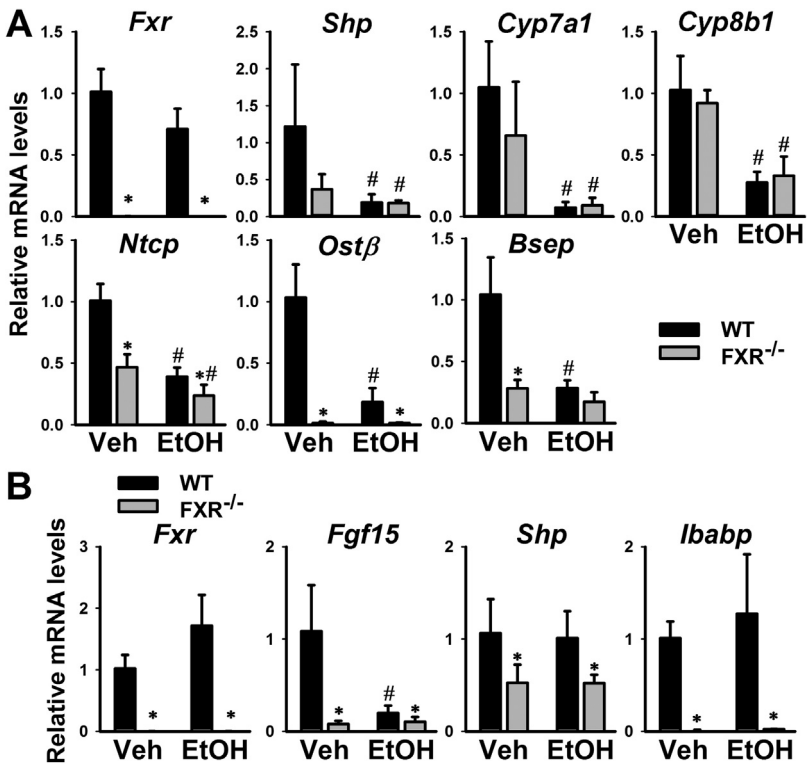


**Fig. 4.** Ethanol exposure disrupted BA homeostasis. Relative expression level of (A) hepatic and (B) intestinal genes involved in BA biosynthesis (*Cyp7a1*, *Cyp8b1*), regulation (*Fxr*, *Shp*, *Fgf15*) and transport (*Ntcp*, *Ostβ*, *Besp*, *Ibabp*). Differences were considered significant at $p < 0.05$; * indicates difference between different strains (WT vs FXR$^{-/-}$); # indicates difference between treatments (vehicle vs ethanol) within the same strain.

ing. In congruence with the changes in concentration of individual BA species, the contribution of each BA species to the total liver BA pool changed. The percentage of TCA increased, reaching 85.1% in FXR$^{-/-}$ mice after ethanol feeding. Ethanol did not significantly alter the percentage of conjugated BAs or total primary BAs in WT or FXR$^{-/-}$ mice.

Emerging evidence suggests that TCA induces liver damage through JNK signaling by causing endoplasmic reticulum stress [33]. To assess the changes in MAPK signaling cascade by ethanol treatment, Western blot analysis was performed (Fig. 5). The protein levels of MAPK, including pJNK, JNK, pERK, ERK, p-P38 and P38, showed a trend for decrease in FXR$^{-/-}$ mice. However, JNK and ERK dependent proteins, c-Jun and c-Fos, had higher basal levels in FXR$^{-/-}$ mice. Ethanol feeding significantly suppressed the MAPK kinase cascade, revealed by decreased levels of p-P38, pERK, and pJNK in WT mice. In contrast, ethanol feeding caused less or no significant changes in the phosphorylation of these proteins in FXR$^{-/-}$ mice. Furthermore, ethanol feeding significantly induced cJun and suppressed cFos protein expression in both WT and FXR$^{-/-}$ mice, but the suppression of cFos was lesser in FXR$^{-/-}$ mice.

## 4. Discussion

ALD presents as a spectrum of varying severities of liver pathology, ranging from simple steatosis, inflammation with hepatocyte ballooning, variable grades of fibrosis, to cirrhosis [1]. In this study, we have shown that ethanol induced more severe liver injury in FXR$^{-/-}$ mice than in WT mice, revealed by lipid accumulation, hepatocyte ballooning, and increased serum ALT levels. However, after ethanol feeding, FXR$^{-/-}$ mice only showed a slight increase in the hepatic expression of a pro-inflammatory cytokine, *Tnfα*, increased basal expression of acute phase protein, *Lcn2*, but no significant disturbance of NF-κB signaling pathway. FXR deficiency led to increased lipid peroxidation in the liver, suggesting increased oxidative stress following ethanol-feeding. Therefore, lipid accumulation might be the major cause for liver injury in this chronic-binge ethanol feeding model.

As an essential nuclear receptor regulating BA homeostasis [7,18], FXR is generally thought to be suppressed by ethanol, and thus resulting in increased BA synthesis, BA levels, liver injury, and inflammation during ALD [26]. However, not all BAs are toxic. Previous reports suggest that BA toxicity to the liver is related to the hydrophobicity of the individual BA specie, with hydrophobic BA species being more cytotoxic and hydrophilic BA species being less toxic. Emerging evidence suggests that TCA contributes to endoplasmic reticulum stress and thus induces liver damage mediated by JNK pathway [33]. In this study, ethanol exposure increased TCA levels in WT mice, and to a greater extent, in FXR$^{-/-}$ mice. However, no relationship between ethanol-induced liver injury and increased JNK signaling in FXR$^{-/-}$ mice after chronic-ethanol feeding was observed in this study. The downstream transcription factor, cJun, in the JNK pathway was induced in WT and FXR$^{-/-}$ mice by ethanol, indicating that JNK pathway might be involved in the ethanol-induced liver injury. Moreover, our previous study showed that deletion of FXR in hepatocytes only did not worsen liver injury under chronic-binge ethanol feeding [30]. These combined data suggest a greater role of intestinal FXR signaling in the protection against ethanol-induced liver injury.

In the intestine, ethanol exposure can change the composition of gut microbiota and promotes the growth of intestinal gram-negative bacteria, which results in the accumulation of endotoxins such as, LPS. Ethanol also impacts the integrity of the gastrointestinal mucosal barrier, increasing intestinal permeability of a variety of substances, including endotoxin, to the liver through portal circulation, therefore activating the innate immune response [1,4]. Intestinal FXR is critical in the preservation of integrity and function of the intestinal mucosal barrier to prevent bacterial translocation [27,28], and a recent study has demonstrated that intestinal

**Table 1**
Ethanol feeding and FXR deficiency altered BA profiles in the (A) serum and (B) liver. Relative levels of the 21 BAs measured in the serum of WT and FXR$^{-/-}$ mice treated with vehicle or ethanol. Data were presented as concentration μg/ml in serum and μg/g in liver (mean ± SD) and related BAs level was expressed as a percentage of total bile acids in serum.

A

| Bile acids | Mean ± SD (μg/ml) | | | | Percentage (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | WT/Veh | WT/EtOH | FXR KO/Veh | FXR KO/EtOH | WT/Veh | WT/EtOH | FXR KO/Veh | FXR KO/EtOH |
| CA | 0.7 ± 0.4 | 0.6 ± 0.3 | 0.8 ± 1.5 | 4.3 ± 9.5 | 7.6 | 6.7 | 3.9 | 3.9 |
| TCA | 1.2 ± 1.0 | 1.0 ± 0.8 | 11.2 ± 18.6 | 39.5 ± 83.6 | 12.7 | 12.6 | 40.3 | 48.0 |
| GCA | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 | 0.1 |
| CDCA | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.1 | 0.9 | 0.0 | 0.0 |
| TCDCA | 0.1 ± 0.0 | 0.1 ± 0.1 | 0.4 ± 0.7 | 0.4 ± 0.2 | 0.7 | 1.4 | 1.6 | 1.9 |
| GCDCA | 0 | 0 | 0 | 0 | 0.0 | 0.1 | 0.0 | 0.1 |
| α-MCA | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.2 | 0.1 ± 0.2 | 1.1 | 1.1 | 0.3 | 0.2 |
| β-MCA | 2.1 ± 0.9 | 1.2 ± 0.6 | 1.3 ± 1.6 | 2.5 ± 4.4 | 23.5 | 14.7 | 8.0 | 5.0 |
| ω-MCA | 3.1 ± 1.3 | 3.2 ± 1.6 | 1.0 ± 1.7 | 1.1 ± 1.4 | 34.1 | 37.8 | 5.0 | 4.1 |
| TMCA | 0.7 ± 0.6 | 0.8 ± 0.4 | 5.4 ± 8.4 | 8.2 ± 11.4 | 7.2 | 10.2 | 20.1 | 22.4 |
| DCA | 0.1 ± 0.1 | 0.0 ± 0.1 | 0.6 ± 0.5 | 1.1 ± 2.0 | 0.9 | 0.4 | 5.2 | 2.3 |
| TDCA | 0.4 ± 0.1 | 0.5 ± 0.1 | 1.0 ± 0.5 | 1.6 ± 0.7 | 4.7 | 6.0 | 10.6 | 8.1 |
| GDCA | 0 | 0 | 0 | 0 | 0.6 | 0.6 | 0.7 | 0.4 |
| UDCA | 0.3 ± 0.2 | 0.1 ± 0.0 | 0.0 ± 0.1 | 0.1 ± 0.0 | 3.0 | 1.8 | 0.2 | 0.2 |
| TUDCA | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.5 ± 0.8 | 0.3 ± 0.1 | 1.3 | 2.6 | 2.0 | 1.7 |
| GUDCA | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 | 0.0 |
| HDCA | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.0 | 0.3 | 0.3 | 0.5 | 0.4 |
| THDCA | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.0 | 0.3 | 0.4 | 0.3 | 0.6 |
| LCA | 0.0 ± 0.0 | 0.1 ± 0.1 | 0.0 ± 0.0 | 0.1 ± 0.0 | 0.5 | 1.7 | 0.7 | 0.5 |
| TLCA | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.4 | 0.5 | 0.6 | 0.1 |
| GLCA | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| Total bile acids | 9.0 ± 3.6 | 8.2 ± 1.7 | 22.6 ± 34.3 | 59.6 ± 113.0 | 100.0 | 100.0 | 100.1 | 100.0 |
| Primary bile acids | 8.0 ± 3.2 | 7.0 ± 1.7 | 20.2 ± 32.5 | 56.2 ± 110.5 | 88.0 | 85.6 | 79.2 | 85.7 |
| Secondary bile acids | 1.0 ± 0.4 | 1.1 ± 0.2 | 2.4 ± 1.9 | 3.4 ± 2.7 | 12.0 | 14.4 | 20.9 | 14.3 |
| Unconjugated bile acids | 6.5 ± 2.9 | 5.5 ± 2.5 | 4.0 ± 5.5 | 9.4 ± 17.4 | 72.1 | 65.6 | 23.7 | 16.5 |
| Conjugated bila acids | 2.5 ± 1.8 | 2.7 ± 1.5 | 18.6 ± 29.0 | 50.2 ± 95.6 | 27.9 | 34.4 | 76.4 | 83.5 |

B

| Bile acids | Mean ± SD (μg/g LW) | | | | Percentage (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | WT/Veh | WT/EtOH | FXR KO/Veh | FXR KO/EtOH | WT/Veh | WT/EtOH | FXR KO/Veh | FXR KO/EtOH |
| CA | 0.8 ± 0.4 | 0.5 ± 0.2 | 0.6 ± 0.2 | 0.5 ± 0.3 | 0.4 | 0.3 | 0.3 | 0.1 |
| TCA | 108.3 ± 42.7 | 103.9 ± 89.4 | 127.5 ± 44.8 | 275.0 ± 307.5 | 53.7 | 55.3 | 70.2 | 85.1 |
| GCA | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 | 0.0 |
| CDCA | 0.1 ± 0.0 | 0.1 ± 0.1 | 0.1 ± 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| TCDCA | 2.5 ± 0.4 | 3.4 ± 3.1 | 1.6 ± 1.3 | 0.7 ± 0.7 | 1.2 | 1.8 | 0.9 | 0.2 |
| GCDCA | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 | 0.0 |
| α-MCA | 0.3 ± 0.1 | 0.2 ± 0.2 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.2 | 0.1 | 0.1 | 0.0 |
| β-MCA | 5.4 ± 0.9 | 3.8 ± 2.1 | 4.8 ± 1.8 | 3.3 ± 1.0 | 2.7 | 2.0 | 2.6 | 1.0 |
| ω-MCA | 3.7 ± 1.3 | 3.5 ± 2.4 | 1.4 ± 0.5 | 1.0 ± 0.5 | 1.9 | 1.9 | 0.8 | 0.3 |
| TMCA | 77.4 ± 24.4 | 70.0 ± 74.6 | 42.5 ± 11.0 | 39.7 ± 23.6 | 38.4 | 37.3 | 23.4 | 12.3 |
| DCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TDCA | 0.7 ± 0.8 | 0.4 ± 0.3 | 1.8 ± 1.4 | 2.0 ± 0.7 | 0.3 | 0.2 | 1.0 | 0.6 |
| GDCA | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0 | 0 | 0 | 0 |
| UDCA | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 | 0.1 | 0.1 | 0.0 |
| TUDCA | 2.1 ± 0.5 | 1.8 ± 1.9 | 0.6 ± 0.2 | 0.5 ± 0.2 | 1.1 | 1.0 | 0.3 | 0.1 |
| GUDCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| HDCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| THDCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TLCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GLCA | 0 | 0<br>0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total bile acids | 201.8 ± 67.9 | 187.9 ± 174.1 | 181.6 ± 56.1 | 323.3 ± 328.9 | 100.0 | 100.0 | 100.0 | 100.0 |
| Primary bile acids | 198.6 ± 67.8 | 185.4 ± 171.9 | 178.7 ± 55.7 | 320.4 ± 328.9 | 98.4 | 98.7 | 98.4 | 99.1 |
| Secondary bile acids | 3.2 ± 1.2 | 2.5 ± 2.3 | 2.8 ± 1.5 | 2.8 ± 0.7 | 1.6 | 1.3 | 1.6 | 0.9 |
| Unconjugated Bile acids | 10.8 ± 2.4 | 8.3 ± 5.1 | 7.3 ± 2.4 | 5.2 ± 1.4 | 5.3 | 4.4 | 4.0 | 1.6 |
| Conjugated bila acids | 191.0 ± 66.0 | 179.6 ± 169.0 | 174.2 ± 53.9 | 318.1 ± 329.5 | 94.7 | 95.6 | 96.0 | 98.4 |

FXR, FGF15 and microbiota contribute to ALD [26]. The most significant finding here was an increased hepatic expression of *Cd14*, the LPS receptor, supporting increased LPS signaling and potential disruption of the intestinal barrier.

In summary, ethanol intake disturbs lipid metabolism and BA homeostasis, and FXR deficiency exacerbates ethanol-induced liver injury. The mechanism by which FXR protects the liver against ALD has yet to be determined. This study demonstrates FXR may regulate liver steatosis and inflammation through the modulation of BA homeostasis. These findings, in combination with those from our previous study, indicate a potential mechanism by which intestinal FXR helps to maintain intestinal epithelial integrity against gut-derived endotoxin and bacterial dislocation and protects against ethanol-induced liver injury [30]. Therefore, further studies using intestine-specific FXR deficient mice may shed further mechanistic insight into how FXR regulates the development of ALD. As

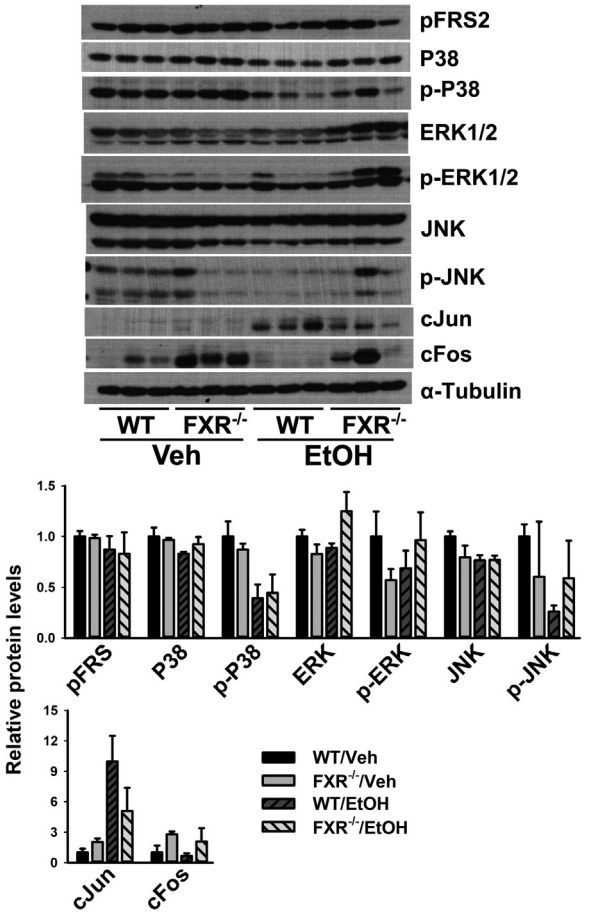


**Fig. 5.** Representative Western blot and semi-quantification of MAPK related proteins in WT and FXR$^{-/-}$ liver after ethanol feeding. Protein levels were analyzed by densitometric analysis using ImageJ and represented as mean ± SD of the three liver samples in the same group.

FXR agonists are currently in clinical development, the findings from intestine-specific FXR knockout mice may provide scientific basis for a set of preventive strategies and provide therapeutic approaches for the treatment of ALD.

## Conflict of interest

None declared.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2018.12.026.

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