



**GASTROINTESTINAL, HEPATOBILIARY, AND PANCREATIC PATHOLOGY**

# Role of Bile Acids in Liver Injury and Regeneration following Acetaminophen Overdose

Bharat Bhushan, Prachi Borude, Genea Edwards, Chad Walesky, Joshua Cleveland, Feng Li, Xiaochao Ma, and Udayan Apte

From the Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas

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Address correspondence to  
Udayan Apte, Ph.D., DABT,  
4087 HLSIC, MS 1018, 3901  
Rainbow Blvd, Kansas City,  
KS 66160. E-mail: [uapte@kumc.edu](mailto:uapte@kumc.edu).

Bile acids play a critical role in liver injury and regeneration, but their role in acetaminophen (APAP)–induced liver injury is not known. We tested the effect of bile acid modulation on APAP hepatotoxicity using C57BL/6 mice, which were fed a normal diet, a 2% cholestyramine (CSA)–containing diet for bile acid depletion, or a 0.2% cholic acid (CA)–containing diet for 1 week before treatment with 400 mg/kg APAP. CSA-mediated bile acid depletion resulted in significantly higher liver injury and delayed regeneration after APAP treatment. In contrast, 0.2% CA supplementation in the diet resulted in a moderate delay in progression of liver injury and significantly higher liver regeneration after APAP treatment. Either CSA-mediated bile acid depletion or CA supplementation did not affect hepatic CYP2E1 levels or glutathione depletion after APAP treatment. CSA-fed mice exhibited significantly higher activation of c-Jun N-terminal protein kinases and a significant decrease in intestinal fibroblast growth factor 15 mRNA after APAP treatment. In contrast, mice fed a 0.2% CA diet had significantly lower c-Jun N-terminal protein kinase activation and 12-fold higher fibroblast growth factor 15 mRNA in the intestines. Liver regeneration after APAP treatment was significantly faster in CA diet–fed mice after APAP administration secondary to rapid cyclin D1 induction. Taken together, these data indicate that bile acids play a critical role in both initiation and recovery of APAP-induced liver injury. (*Am J Pathol* 2013, 183: 1518–1526; <http://dx.doi.org/10.1016/j.ajpath.2013.07.012>)

Bile acids are versatile biological molecules that regulate energy homeostasis, activate nuclear receptors and cell signaling pathways, and control cell proliferation and inflammatory processes in the liver and gastrointestinal tract.<sup>1,2</sup> Bile acids maintain their own homeostasis by activating a complex signaling network involving hepatic and intestinal farnesoid X receptor (FXR), small heterodimer partner, and intestinal fibroblast growth factor (FGF) 15 (FGF19 in human) expression, culminating in inhibition of the primary bile acid–synthesizing enzyme, CYP7A1.<sup>3–6</sup> Although bile acids are potent signaling molecules at pathophysiological concentrations, they cause apoptosis, necrosis, and oxidative stress.<sup>3,7–10</sup> Bile acids have also been implicated in stimulation of liver regeneration.<sup>11–14</sup> Studies in recent years indicate that the bile acid–mediated gut–liver signaling axis may play a critical role in regulation of liver homeostasis.<sup>6,15,16</sup>

Acetaminophen (APAP) is the most commonly used analgesic and antipyretic agent.<sup>17</sup> An overdose of APAP is the major cause of acute liver failure in the United

States.<sup>18,19</sup> The mechanisms of APAP-induced liver injury and subsequent liver regeneration are the focus of intense investigation.<sup>20–22</sup> In an overdose situation, excess APAP is mainly metabolized by CYP2E1 to a reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI). In hepatocytes, conjugation of NAPQI to GSH is the key mechanism for detoxification of NAPQI. Once the GSH is depleted, NAPQI attacks cellular proteins, especially mitochondrial proteins, to form protein adducts. This triggers a cascade of intracellular signaling events involving c-Jun N-terminal protein kinase (JNK) activation and mitochondrial permeability transition, finally culminating in necrotic cell death.<sup>20</sup> Liver injury is followed by compensatory liver regeneration, which is a critical determinant of final outcome of liver injury.<sup>23</sup> Despite decades of research, how these

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B.B. and P.B. contributed equally to this work.

intracellular events are affected by extracellular signaling is not known.

The current study was designed to explore the role of bile acids in initiation of liver injury and stimulation of liver regeneration after APAP overdose. These studies are highly significant because the data reveal a novel role of bile acids in cellular protection and liver regeneration after APAP overdose, and these studies investigate the effect of resin-mediated bile acid depletion, a commonly used therapy, on APAP toxicity.

## Materials and Methods

### Animals, Treatments, and Tissue Collection

The C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, MN). Two-month-old male mice ( $n = 5$  to 8 per group per time point) were used in these studies. All animal studies were approved by and performed in accordance with the Institutional Animal Care and Use Committee at University of Kansas Medical Center (Kansas City). Mice were fed a normal diet, a 2% cholestyramine (CSA)—containing diet, or a 0.2% cholic acid (CA)—containing diet for 1 week. After a 1-week feeding of specific diets, mice were injected i.p. with 400 mg/kg APAP (Sigma, St. Louis, MO) dissolved in warm sterile saline solution. Mice were not fasted before APAP treatment. Mice were sacrificed at 0, 1, 4, 8, 12, and 24 hours after APAP treatment, and livers, intestines, and serum were collected and processed as previously described.<sup>14</sup>

### Histological and IHC Data

Paraffin-embedded liver sections (4  $\mu$ m thick) were used for H&E staining and immunohistochemical (IHC) detection of proliferating cell nuclear antigen (PCNA), as previously described.<sup>14</sup> H&E-stained slides were used for necrosis scoring, as previously described,<sup>24</sup> and PCNA-stained slides were used for PCNA scoring, as previously described.<sup>25</sup>

### Protein Extraction, Microsome Preparation, and Western Blot Analysis

Protein extracts were prepared from frozen liver tissues using radioimmunoprecipitation assay buffer, containing fresh protease and phosphatase inhibitors, and used for Western blot analysis, as previously described.<sup>14</sup> Primary and secondary antibodies were purchased from Cell Signaling Technologies (Danvers, MA).

Microsomes were prepared from murine liver by homogenizing tissue using a Teflon homogenizer in phosphate buffer (pH 7.4) and centrifuged at  $9000 \times g$  for 30 minutes at 4°C. The supernatant from this centrifugation was centrifuged at  $100,000 \times g$  for 60 minutes at 4°C. The microsomal pellet was resuspended in phosphate buffer with 0.1 mmol/L EDTA (pH 7.4). Microsomes (50  $\mu$ g) were used for CYP2E1 Western blot analysis.

### Real-Time PCR Analysis

Total RNA was extracted from all murine intestines using TRIzol, per the manufacturer's protocol (Sigma), and reverse transcribed as previously described.<sup>14</sup> FGF15 mRNA levels were determined by real-time PCR assays on the Applied Biosystems Prism 7300 Instrument (Grand Island, NY), as previously described.<sup>14</sup> 18S RNA levels were used for data normalization.

### Serum ALT Measurement

Serum alanine aminotransferase (ALT) was measured by kinetic assay method using the Infinity ALT kit (Thermo Scientific, Waltham, MA), according to the manufacturer's protocol.

### Bile Acid Analysis

Serum, liver, and intestinal total bile acids were measured enzymatically using a Total Bile Acid Assay Kit (BQ kits, San Diego, CA), per the manufacturer's protocol. Briefly, in a 96-well plate, 20  $\mu$ L of bile acid standard and murine serum samples were added in triplicate. Diaphorase in phosphate buffer (150  $\mu$ L) was then added and incubated at 37°C for 4 minutes. After adding 30  $\mu$ L of 3- $\alpha$ -hydroxy steroid dehydrogenase in Tris buffer, absorbance was measured at 540 nm kinetically for 5 minutes. Specific hepatic bile acids were analyzed by Ultra Performance Liquid Chromatography-Mass Spectrometry (Waters, Milford, MA), as previously described.<sup>14</sup>

### Glutathione Analysis

Total GSH was measured in liver homogenates by the 5,5'-dithiobis-(2-nitrobenzoic acid)-glutathione reductase recycling assay (Sigma). Briefly, total glutathione disulfide (GSH) was extracted from liver homogenates in 5% sulfosalicylic acid and oxidized to GSSG by 5,5'-dithiobis-(2-nitrobenzoic acid). After adding NADPH, the rate of GSH formation in the presence of glutathione reductase was measured kinetically at 412 nm.

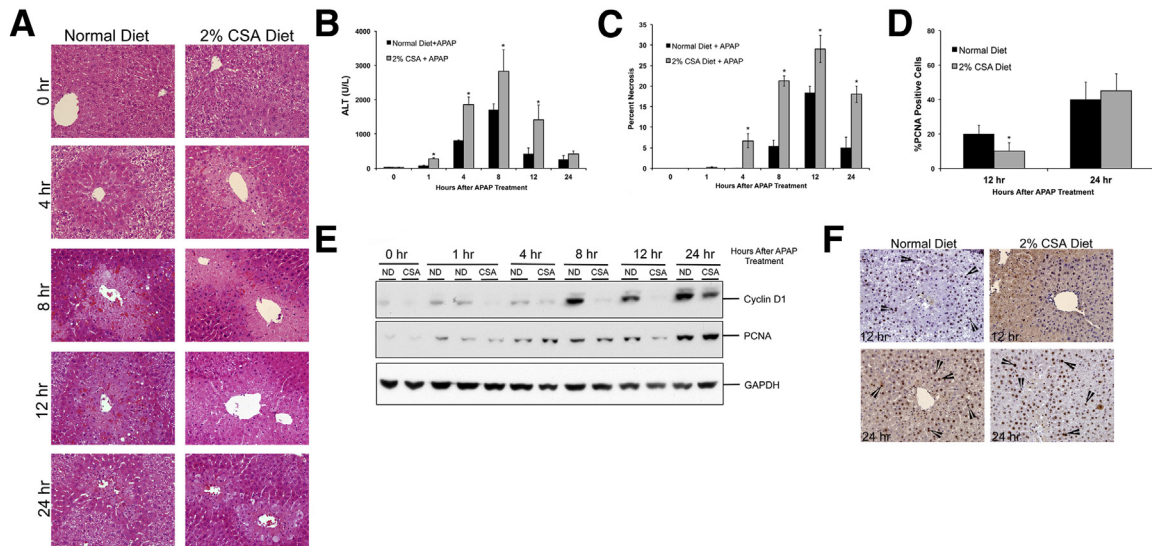
### Statistical Analysis

All data presented as bar graphs are expressed as means  $\pm$  SEM. An unpaired Student's *t*-test was used to evaluate statistically significant differences between groups.  $P < 0.05$  was considered statistically significant.

## Results

### Increase in APAP-Induced Liver Injury and Delayed Liver Regeneration after Cholestyramine-Mediated Bile Acid Depletion

The CSA-fed mice showed a rapid and higher liver injury within 1 hour after APAP treatment compared with control



**Figure 1** Increased acetaminophen (APAP)-induced liver injury after bile acid depletion. **A:** Representative photomicrographs of H&E-stained liver sections obtained over a time course of 0 to 24 hours after APAP treatment of mice fed either a normal diet or a 2% CSA diet. **B:** Serum ALT activity measured over a time course of 0 to 24 hours after APAP treatment of mice fed either a normal diet or a 2% CSA diet. **C:** Percentage necrosis after APAP treatment. **D:** Percentage PCNA-positive cells at 12 and 24 hours in liver sections of APAP-treated mice fed either a normal diet or a 2% CSA diet. **E:** Western blot analysis of cyclin D1 and PCNA using total liver cell extracts of normal diet- or CSA diet-fed mice treated with APAP. **F:** Representative photomicrographs of PCNA IHC staining of liver sections of APAP-treated mice fed either a normal diet or a 2% CSA diet at 12 and 24 hours after APAP treatment. **Arrowheads** indicate cells in the S phase. \**P* < 0.05 (**B–D**). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

mice, as demonstrated by histopathological analysis of H&E-stained liver sections (Figure 1A), serum ALT levels (Figure 1B), and necrosis scoring (Figure 1C). Centrilobular necrosis was clearly evident at 4 hours after APAP treatment in CSA-fed mice but not until 8 hours after APAP treatment in normal diet-fed mice. Peak liver injury was observed at 8 hours in both groups. Injury decreased significantly in the normal diet-fed mice at 24 hours after APAP treatment. Although serum ALT values declined at 24 hours in CSA-fed mice, necrosis scoring demonstrated that significant liver cell death was still evident in CSA-fed mice at 24 hours after APAP administration. Liver regeneration was studied using PCNA analysis for determining cell proliferation and Western blot analysis of cyclin D1 and PCNA (Figure 1, D–F). PCNA staining (Figure 1, D and F) and cyclin D1 Western blot data (Figure 1E) indicate that the CSA-fed mice experienced a delay in initiation of liver regeneration after APAP overdose and exhibited significantly lower cell proliferation at 12 hours after APAP administration. However, this delay in cell proliferation was overcome by 24 hours after APAP treatment, and both normal diet-fed and CSA-fed mice had similar liver regeneration.

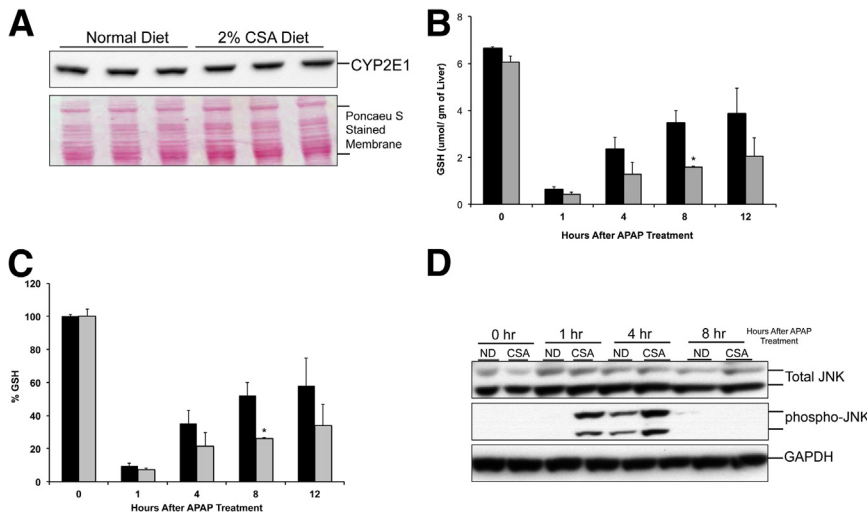
### Mechanisms of Increased APAP-Induced Injury after Bile Acid Depletion

To determine the mechanism behind increased APAP-induced cell injury in CSA-fed mice, we analyzed the levels of APAP-metabolizing enzyme, CYP2E1, antioxidant GSH, and JNK activation, which is known to be a critical step in APAP-induced liver injury. Western blot analysis showed no difference in microsomal CYP2E1 expression

between CSA diet-fed mice and normal diet-fed mice (Figure 2A). Analysis of total GSH levels in liver revealed that CSA diet feeding did not have any effect on hepatic GSH levels before APAP treatment. Furthermore, similar GSH depletion (90% for control) was observed at 1 hour after APAP treatment in both normal diet- and CSA-fed mice (Figure 2B). Interestingly, the recovery of hepatic GSH was significantly delayed in the CSA diet-fed mice after APAP administration (Figure 2C). By 12 hours, the normal diet-fed mice had recovered GSH up to 60% of the pre-APAP levels, but the CSA diet-fed mice had recovered only 30% of their pre-APAP levels. Western blot analysis of total and phosphorylated JNK indicated significant activation of JNK in CSA-fed mice within 1 hour after APAP treatment, which further increased and was higher than the normal diet-fed mice at 4 hours after APAP administration (Figure 2D). In contrast, JNK activation was evident in normal diet-fed mice only at 4 hours after APAP treatment.

### Changes in Bile Acid Composition in Livers of CSA-Fed Mice

Next, we determined changes in total bile acid levels in serum, liver, and intestine in normal diet-fed and CSA-fed mice. The data indicate a significant decrease in serum, hepatic, and intestinal bile acid levels after CSA treatment (Figure 3, A–C). We further quantified specific bile acid species in the livers of normal diet-fed and CSA-fed mice, which indicated a significant decrease in tauro-β-muricholic acid and ω-muricholic acid in CSA-fed mice (Figure 3, D–G).

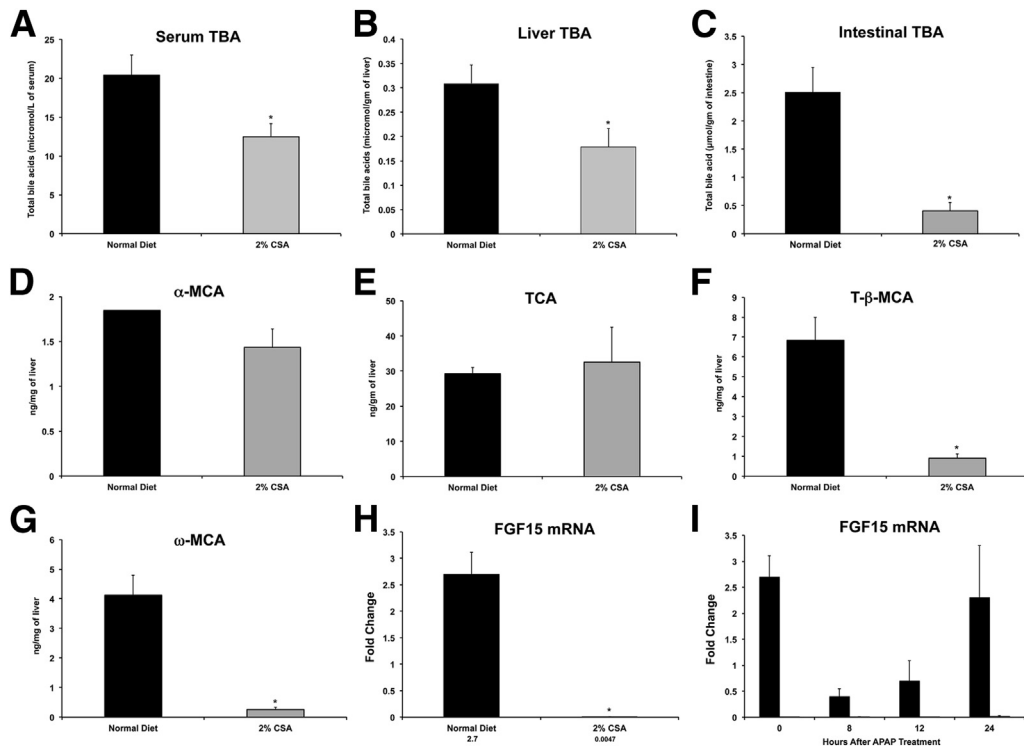


**Figure 2** Mechanisms of increased acetaminophen (APAP)-induced injury after bile acid depletion. **A:** Western blot analysis of microsomal CYP2E1 using total liver cell extract of normal diet (ND)-fed and cholestyramine (CSA)-fed murine livers obtained before APAP treatment at 0 hours. **B:** GSH levels in the livers of ND-fed (black bars) and CSA-fed (gray bars) mice during a time course of 0 to 12 hours after APAP treatment. **C:** Percentage of GSH depletion in the livers of control (black bars) and CSA-fed (gray bars) mice during a time course of 0 to 12 hours after APAP treatment. **D:** Western blot analysis of total and phosphorylated JNK using total liver cell extracts of ND-fed and CSA-fed murine livers obtained at various time points after APAP treatment. \**P* < 0.05 (**B** and **C**). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

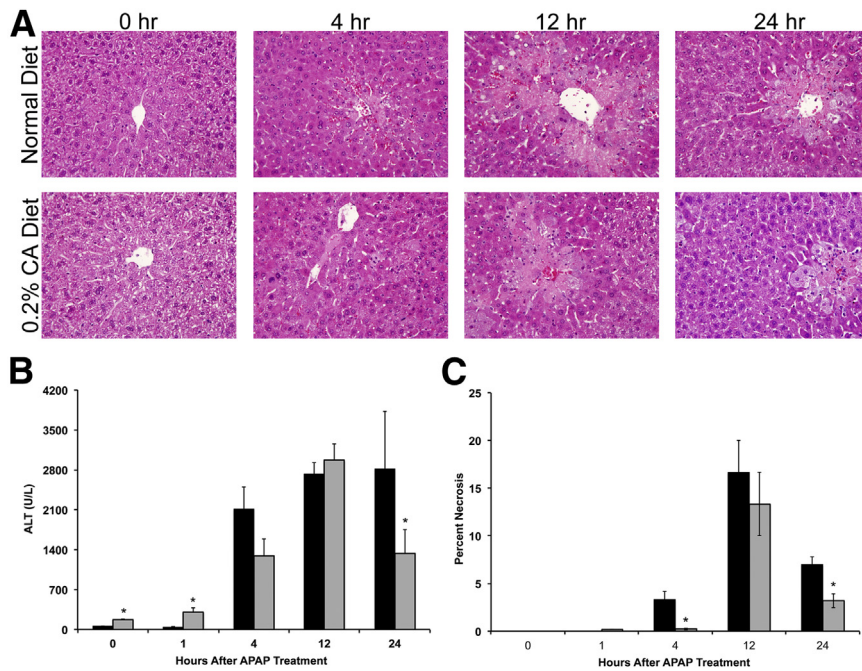
### Significant Inhibition of Intestinal FGF15 Expression after CSA Treatment

Bile acids are known to regulate their own biosynthesis via the liver-gut FXR-FGF15 signaling axis. Bile acids are absorbed in the ileal cells, where they activate FXR, which, in turn, stimulates FGF15 expression and secretion. FGF15 comes to liver via portal circulation and via binding to FGF receptor 4, inhibits hepatic CYP7A1 expression, and

down-regulates bile acid synthesis. The effect of CSA-mediated bile acid reduction on intestinal FGF15 expression in normal diet-fed and CSA diet-fed mice was determined using real-time PCR. The data indicate that CSA-fed mice had >500-fold lower intestinal FGF15 than normal diet-fed mice, even before APAP treatment (Figure 3H). After APAP treatment, *FGF15* gene expression decreased significantly by 8 hours after APAP treatment but returned to pre-APAP levels by 24 hours after APAP treatment in



**Figure 3** Changes in total bile acids (TBAs) and intestinal FGF15 mRNA after CSA treatment. Total bile acids in serum (**A**), liver (**B**), and intestines (**C**) in normal diet-fed (black bars) or CSA-diet (gray bars) fed mice. UPLC-MS-mediated quantification of specific hepatic bile acids, including  $\alpha$ -muricholic acid (MCA; **D**), tauro- $\beta$ -muricholic acid (TCA; **E**), tauro- $\beta$ -MCA (**F**), and  $\omega$ -MCA (**G**). Serum and liver samples at 0 hours before APAP administration were used for the analysis. **H:** Real-time PCR analysis of FGF15 mRNA in the intestine of control and CSA-fed mice before APAP treatment at 0 hours. **I:** Real-time PCR analysis of FGF15 mRNA in the intestine of control and CSA-fed mice at various time points after APAP treatment. \**P* < 0.05 (**A–C** and **F–H**).



**Figure 4** Cholic acid supplementation delays initiation of injury and stimulates rapid recovery after APAP treatment. **A:** Representative photomicrographs of H&E-stained liver sections of normal diet- and 0.2% CA diet-fed mice treated with APAP during a time course of 0 to 24 hours. **B:** Serum ALT levels in normal diet-fed (black bars) and 0.2% CA diet-fed mice (gray bars) treated with APAP. **C:** Percentage of cells in necrosis in the livers of normal diet-fed (black bars) and 0.2% CA diet-fed mice (gray bars) treated with APAP. \* $P < 0.05$  (B and C).

normal diet-fed mice. In contrast, FGF15 expression remained extremely low to undetectable throughout the time course after APAP treatment in CSA-fed mice (Figure 3I).

#### Delayed Initiation and Rapid Resolution of APAP-Induced Liver Injury in 0.2% CA Diet-Fed Mice

To determine whether bile acid supplementation modulates APAP hepatotoxicity, we fed C57BL/6 mice either a normal diet or a 0.2% CA diet for 1 week before treatment of 400 mg/kg of APAP. Liver injury was determined using serum ALT levels (Figure 4B) and necrosis scoring of H&E-stained paraffin sections (Figure 4, A and C). CA diet-fed mice showed a moderate increase in serum ALT levels before APAP treatment, which continued at 1 hour after APAP treatment. However, necrosis was not evident in histological examinations in CA diet-fed mice before (0 hours) and 1 hour after APAP treatment. After APAP treatment, CA diet-fed mice exhibited a delayed initiation of liver injury, as shown by a lower (but not statistically significant) serum ALT level and necrosis score at 4 hours, which were statistically significant. However, liver injury increased thereafter and was similar in normal diet-fed and CA diet-fed mice at its peak at 12 hours. Interestingly, APAP-induced liver injury decreased much faster and was significantly lower at 24 hours in CA diet-fed mice compared with normal diet-fed mice, as shown by lower serum ALT levels and necrosis scores.

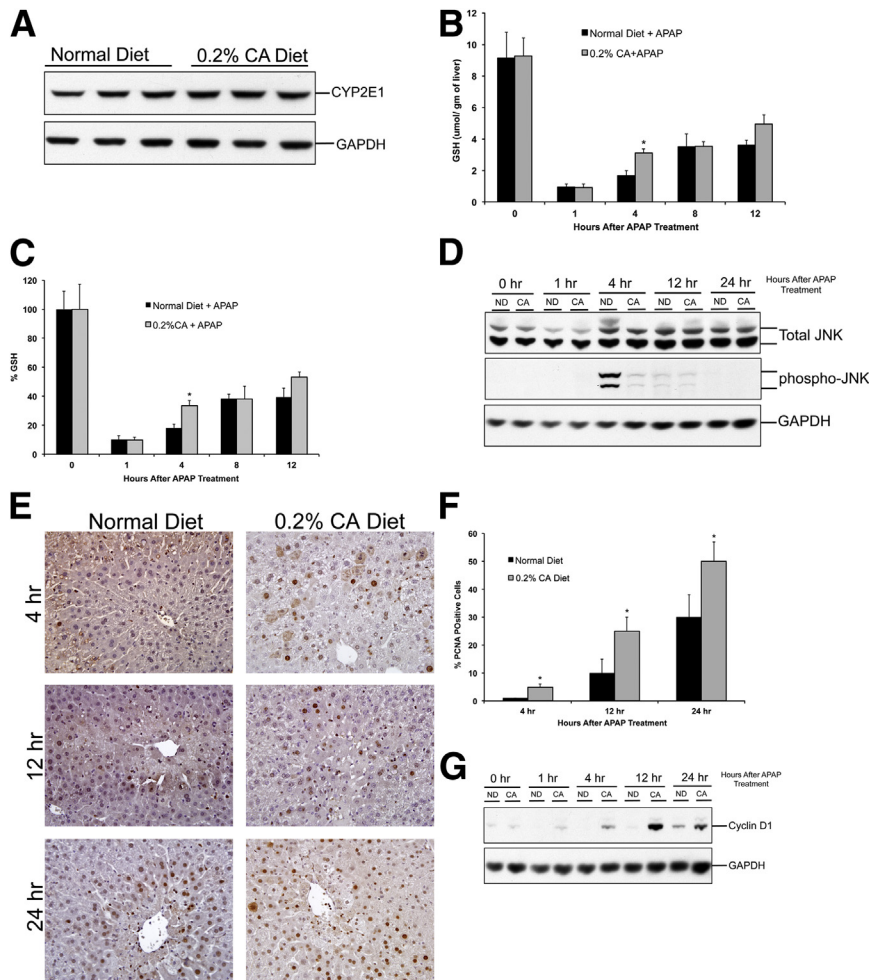
#### Mechanisms of Delayed Initiation of Liver Injury after CA Supplementation

To determine the mechanisms of delayed initiation of APAP-induced liver injury in CA diet-fed mice, we

determined hepatic CYP2E1 levels and GSH depletion after APAP treatment. Western blot analysis using total cell lysates indicated no difference in hepatic CYP2E1 protein expression (Figure 5A). Total GSH-level analysis revealed that cholic acid supplementation did not change hepatic GSH content before APAP treatment. Furthermore, an equal GSH depletion was observed in normal diet-fed and CA diet-fed mice 1 hour after APAP treatment (Figure 5B). GSH recovery was moderately faster in CA diet-fed mice after APAP administration (Figure 5C). We next determined activation of JNK using Western blot analysis, which showed that JNK activation was significantly lower in CA diet-fed mice compared with normal diet-fed mice after APAP treatment (Figure 5D). Substantial JNK phosphorylation was observed in normal diet-fed mice at 4 hours after APAP treatment. In contrast, only moderate JNK phosphorylation was observed in CA diet-fed mice.

#### Mechanisms of Rapid Recovery from APAP-Induced Liver Injury after CA Supplementation

We further determined the level of liver regeneration after APAP-induced liver injury using PCNA analysis and cyclin D1 protein expression. Our data indicate a rapid and higher increase in liver cell proliferation in CA diet-fed mice after APAP treatment (Figure 5, E and F). There was no ongoing cell proliferation in CA diet-fed mice before APAP administration (data not shown). However, a significant number of hepatocytes were PCNA positive at 4 hours after APAP treatment, which further increased at 12 and 24 hours in CA diet-fed mice. Normal diet-fed mice also showed an increase in liver regeneration, but it was significantly slower and at a lower extent compared with CA diet-fed mice. The increase in cell proliferation was consistent with a rapid and



**Figure 5** Mechanisms of acetaminophen (APAP)-induced liver injury and regeneration after cholic acid supplementation. Western blot analysis of CYP2E1 using total liver cell extract (A), GSH levels in the livers (B), percentage of GSH depletion (C), Western blot analysis of total and phosphorylated JNK using total liver cell extract (D), representative photomicrographs of PCNA IHC staining in the livers (E), bar graph showing percentage of PCNA-positive cells in livers (F), and Western blot analysis of cyclin D1 using total liver cell extracts (G) of normal diet (ND)-fed and 0.2% CA diet-fed mice after APAP treatment. \* $P < 0.05$  (B, C, and F). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

higher increase in cyclin D1 expression in CA diet-fed mice after APAP treatment (Figure 5G).

#### Increase in Total Bile Acids and FGF15 mRNA in CA Diet-Fed Mice

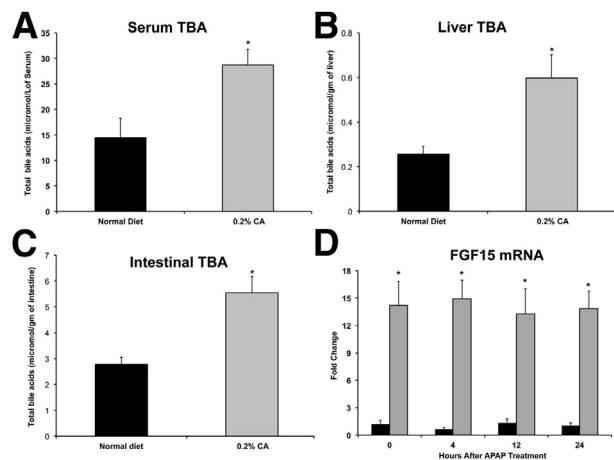
An analysis of total bile acid levels in serum, liver, and intestines of normal diet-fed and CA diet-fed mice revealed that CA supplementation substantially increased total bile acid content in all three compartments (Figure 6, A–C). Further analysis revealed that intestinal FGF15 mRNA was consistently 12- to 14-fold higher in CA diet-fed mice compared with normal diet-fed mice throughout the time course of 0 to 24 hours after APAP treatment (Figure 6D).

### Discussion

Overdose of APAP, the most commonly used analgesic drug, is the main cause of acute liver failure in the Western world.<sup>26</sup> Despite decades of research, the mechanisms of APAP-induced liver injury are not completely known.<sup>27</sup> Even more complicated and unknown are the mechanisms of APAP overdose on the background of existing disease

conditions.<sup>27</sup> Furthermore, the contribution of extrahepatic factors to APAP-induced liver injury and subsequent liver regeneration is completely unknown.

Extensive studies in the past decade have demonstrated that bile acids are excellent signaling molecules and that bile acid signaling is involved in several physiological and pathological conditions.<sup>1,4</sup> Previous studies have shown that bile acids can signal via multiple receptors, including FXR, TGR5, and epidermal growth factor receptor.<sup>3,4,28</sup> At a normal concentration, bile acids seem to be protective against cell stress and injury, but are known to be toxic at higher concentrations.<sup>4</sup> Reduction of bile acids using resins, such as cholestyramine, is a common therapy against hypercholesterolemia and in cholestasis.<sup>29</sup> Similarly, ursodeoxycholic acid has been used as a treatment for several biliary diseases.<sup>30</sup> However, the role of bile acids in APAP-induced liver injury is not known. Our data indicate that depletion of bile acids using CSA results in increased APAP-induced liver injury and slower recovery. In contrast, supplementation of cholic acid in diet resulted in delayed initiation of liver injury and rapid recovery after APAP treatment. Taken together, our data indicate that bile acids may be protective against APAP-induced liver injury.



**Figure 6** Changes in total bile acids and FGF15 mRNA after cholic acid supplementation. Total bile acids in serum (A), liver (B), and intestines (C) of normal diet–fed and 0.2% cholic acid (CA) diet–fed mice before acetaminophen (APAP) treatment. D: Intestinal FGF15 mRNA expression in normal diet–fed (black bars) and 0.2% CA diet–fed (gray bars) mice before and after APAP administration. \* $P < 0.05$  (A–D).

Our observations are also consistent with previous reports in long-lived Little mice, which were shown to be protected from APAP and bromobenzene hepatotoxicity.<sup>31</sup> These mice are also known to have increased bile acids, which have been implicated in several phenotypes observed in these mice, including prolongation of life span and protection from APAP. Furthermore, our data are also consistent with previous studies that activation of FXR, the primary bile acid receptor, provides protection from APAP-induced liver injury.<sup>32</sup> However, the mechanisms behind these observations may be different from those underlying our observations.

Our data indicate that neither bile acid depletion by CSA nor CA supplementation interferes with initial bioactivation of APAP to its reactive metabolite, NAPQI. This is evident from rapid and equal depletion of hepatic GSH after APAP treatment in all treatment groups. Previous studies have shown that depletion of GSH is a sensitive indicator of APAP metabolism, especially in overdose situations.<sup>21</sup> Recent studies indicate that GSH recovery also plays a critical role in injury development after APAP overdose.<sup>33</sup> It is known that GSH recovery, driven by *de novo* GSH synthesis catalyzed by the enzyme glutamate-cysteine ligase (Gclc), is delayed at higher doses of APAP, which exhibit higher liver injury.<sup>33</sup> Consistent with this observation, our results showed that GSH recovery is significantly delayed after APAP administration in mice with CSA-mediated bile acid depletion. Similarly, the GSH recovery was faster in mice fed a cholic acid diet. These data indicate that bile acids may be involved in GSH recovery after APAP overdose. The exact mechanisms behind this effect are not known. It is known that Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a major regulator of Gclc expression, and studies have shown that bile acids can affect Nrf2 activation.<sup>34</sup> This raises the possibility that bile acids may

stimulate Gclc expression via Nrf2, which may be involved in the protective effect shown by bile acids.

Hepatic JNK activation is a critical step in the intracellular signaling involved in APAP-induced liver injury.<sup>20,35,36</sup> Our data show that bile acid depletion results in rapid activation of JNK and that cholic acid supplementation results in a decreased activation of JNK compared with normal diet control mice. These data indicate that bile acids may suppress JNK activation during APAP-induced liver injury. Whether bile acids directly affect JNK activation or induce this effect via an upstream regulator is not known. Bile acids are known to signal via multiple pathways in the liver, including FXR, TGR5, and epidermal growth factor receptor. It is possible that bile acids may use one of these pathways to suppress JNK signaling after APAP-induced liver injury.

It is known that bile acids play a critical role in the gut-liver signaling axis by stimulating FGF15 (FGF19 in humans) expression in the ileal cells via an FXR-dependent mechanism.<sup>6</sup> This gut-liver axis of FGF15 signaling has been implicated in liver regeneration.<sup>13,14,37,38</sup> In our studies, CSA-mediated bile acid depletion, which resulted in exacerbation of APAP toxicity, reduced FGF15 expression to undetectable levels. Similarly, 0.2% CA supplementation, which resulted in delayed initiation and rapid recovery from APAP toxicity, resulted in 15-fold up-regulation of FGF15 mRNA. These data suggest a role for FGF15 in the bile acid–mediated protection from APAP hepatotoxicity. However, the exact role of FGF15 remains to be investigated. It is possible that bile acid–stimulated FGF15 has a cytoprotective role during the initiation phase of injury. This notion is supported by previous observations that FGF15-knockout mice exhibited extensive liver injury after partial hepatectomy (PHX).<sup>38</sup> It is known that the regenerating lobes after PHX do not normally exhibit necrotic cell death.<sup>39</sup> However, recent studies have demonstrated that FGF15-knockout mice have significant necrosis in regenerating lobes after PHX,<sup>38</sup> indicating that lack of FGF15 may remove an important cytoprotective mechanism in hepatocytes.

Several studies have demonstrated that compensatory liver regeneration after APAP-induced liver injury is a critical determinant of final outcome of APAP overdose.<sup>40–44</sup> Our data clearly demonstrate that bile acid depletion results in delayed liver regeneration and that bile acid supplementation stimulates rapid liver regeneration after APAP overdose. These data are consistent with previous observations that bile acid supplementation stimulates liver regeneration.<sup>13</sup> Recent studies have elegantly shown that FGF15 may be a primary mediator of the bile acid–induced stimulation of liver regeneration after PHX.<sup>38,45</sup> It remains to be seen whether these same mechanisms apply in stimulating regeneration after APAP overdose.

Taken together, our data indicate that bile acids play a central role in modulating APAP-induced liver injury and regeneration. The observation that cholestyramine-mediated

bile acid depletion exacerbates APAP-induced liver injury and delays regeneration is significant because of the routine use of cholestyramine in clinical practice. These data suggest a possible drug-drug interaction between APAP and CSA, and caution should be taken in administering APAP for patients taking CSA. Our data also indicate that bile acids have a cytoprotective and proregenerative role in APAP-induced liver injury, which could potentially be used for treatment of APAP-induced patients with acute liver failure.

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