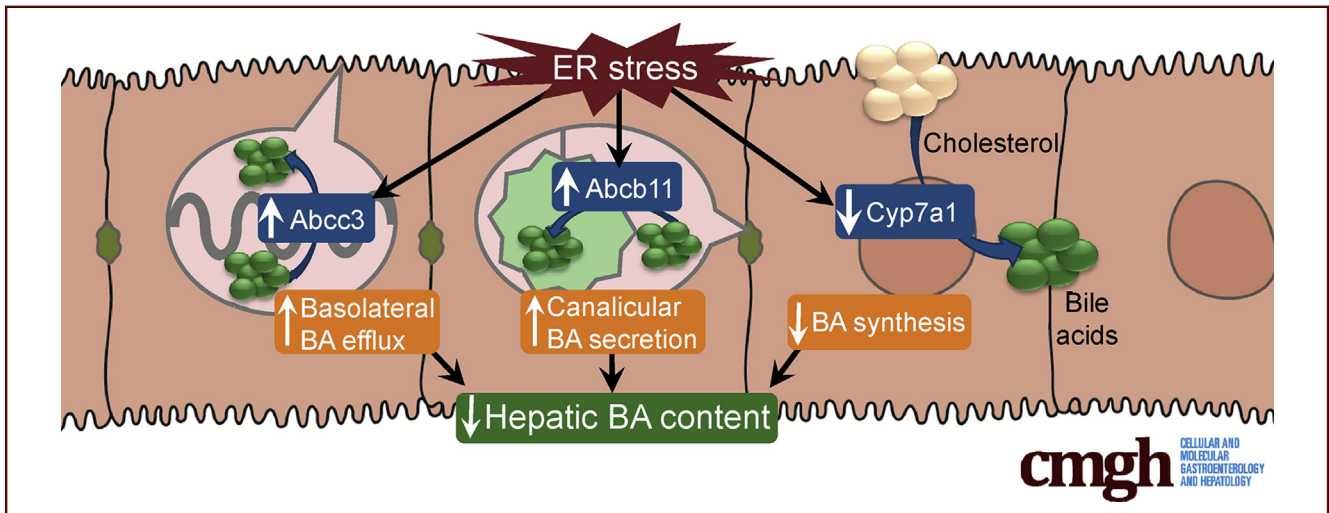## ORIGINAL RESEARCH

Endoplasmic Reticulum Stress Regulates Hepatic Bile Acid Metabolism in Mice

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SUMMARY

We show that induction of endoplasmic reticulum stress in mice suppresses the primary bile acid synthetic pathway controlled by cholesterol 7α-hydroxylase and activates hepatic bile acid transporters that promote the removal of excess bile acids from the liver.

BACKGROUND & AIMS: Cholestasis promotes endoplasmic reticulum (ER) stress in the liver, however, the effect of ER stress on hepatic bile acid metabolism is unknown. We aim to determine the effect of ER stress on hepatic bile acid synthesis and transport in mice.

METHODS: ER stress was induced pharmacologically in C57BL/6J mice and human hepatoma (HepG2) cells. The hepatic expression of genes controlling bile acid synthesis and transport was determined. To measure the activity of the primary bile acid synthetic pathway, the concentration of 7α-hydroxy-4-cholesten-3-1 was measured in plasma.

RESULTS: Induction of ER stress in mice and HepG2 cells rapidly suppressed the hepatic expression of the primary bile acid synthetic enzyme, cholesterol 7α-hydroxylase. Plasma levels of 7α-hydroxy-4-cholesten-3-1 were reduced in mice subjected to ER stress, indicating impaired bile acid synthesis. Induction of ER stress in mice and HepG2 cells increased expression of the bile salt export pump (adenosine triphosphate binding cassette [Abc]b11) and a bile salt efflux pump (Abcc3). The observed regulation of Cyp7a1, Abcb11, and Abcc3 occurred in the absence of hepatic inflammatory cytokine activation and was not dependent on activation of hepatic small heterodimer partner or intestinal fibroblast growth factor 15. Consistent with suppressed bile acid synthesis and enhanced bile acid export from hepatocytes, prolonged ER stress decreased the hepatic bile acid content in mice.

CONCLUSIONS: Induction of ER stress in mice suppresses bile acid synthesis and enhances bile acid removal from hepatocytes independently of established bile acid regulatory pathways. These data show a novel function of the ER stress response in regulating bile acid metabolism. *(Cell Mol Gastroenterol Hepatol 2017;3:261–271; http://dx.doi.org/10.1016/j.jcmgh.2016.11.006)*

The enterohepatic circulation of bile acids is a highly efficient process by which bile acid homeostasis is maintained. Bile acids are secreted from the liver into bile via the rate-limiting adenosine triphosphate binding cassette transporter, ATP-binding cassette subfamily B member 11 (ABCB11), and subsequently are transported in bile to the lumen of the small intestine where they aid in the digestion of dietary fat, cholesterol, and fat-soluble vitamins.[1,2] Bile acids ultimately are taken up in the ileum and returned to the liver via the portal circulation where they are transported across the hepatic sinusoidal membrane by the sodium/taurocholate cotransporter (NTCP).[3–5] Although the enterohepatic circulation of bile acids is a highly efficient process, de novo synthesis of bile acids must occur in the liver to replenish the small fraction of bile acids that are lost by fecal excretion. New bile acids are synthesized in the liver from cholesterol, which is catalyzed by the rate-limiting cytochrome P450 enzyme, cholesterol 7α hydroxylase (CYP7A1).[6–8] Disruption of the enterohepatic circulation of bile acids can result in accumulation of bile acids within the liver, leading to cholestatic liver injury. Numerous compensatory mechanisms are activated in response to cholestasis to reduce bile acid accumulation, including suppression of bile acid uptake, increased bile acid efflux, enhanced biliary bile acid secretion, and reduced bile acid synthesis.[9]

Extrahepatic biliary obstruction is the classic cause of cholestasis, however, intrahepatic cholestasis can result from impairment in bile acid transporter function or disruption of the compensatory mechanisms that counteract hepatic bile acid accumulation. Systemic inflammation is a well-described cause of cholestasis in the absence of mechanical biliary obstruction.[9–13] The pathogenesis of inflammation-induced cholestasis is incompletely defined but is thought to involve inflammatory cytokine-mediated inhibition of hepatic bile acid transporter expression.[11–13] Moreover, the interplay between bile acid metabolism and cellular stress responses in the liver is complex and incompletely understood.

Activation of the unfolded protein response (UPR), a highly conserved signaling cascade induced by endoplasmic reticulum (ER) stress, has been identified as a feature of many hepatic diseases including cholestatic liver disease.[14–21] It is unclear whether activation of the UPR in the setting of cholestasis is a manifestation of a generalized inflammatory response or whether the UPR directly functions in the pathogenesis of cholestatic liver disease. Although the UPR initially was identified as a mechanism to maintain proper protein folding and processing, it now is clear that this signaling cascade regulates a wide range of cellular processes. In particular, the UPR increasingly is recognized as a critical regulator of hepatic lipid metabolism, which is tightly linked to bile acid metabolism.[11,13–15] Accumulating evidence has suggested that bile acids modulate ER stress, however, it is unknown whether ER stress, in turn, alters bile acid homeostasis.[22–24] In this article we explore the effect of ER stress on the regulation of bile acid synthesis and transport in mice.

Materials and Methods

Animals and Treatments

Male C57BL/6J mice (8–10 weeks of age) were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were in the light 14 hours per day and in darkness 10 hours per day everyday of their life (per routine animal housing protocol) and were given free access to standard laboratory chow and water. To induce acute ER stress, mice were treated with a single intraperitoneal (IP) injection of tunicamycin (0.5 mg/kg) and were killed 6 hours later. To induce prolonged ER stress, mice were treated with daily injections of tunicamycin (0.1 mg/kg IP) for 5 days (cumulative dose, 0.5 mg/kg IP). Control mice were treated with vehicle (10% dimethyl sulfoxide [DMSO] IP). Blood was collected by cardiac puncture and immediately centrifuged to collect the plasma. The livers were excised rapidly, flushed with ice-cold saline, sectioned, and snap-frozen in liquid nitrogen. The small intestine was removed, flushed with ice-cold saline, and the terminal 5-cm segment was snap-frozen in liquid nitrogen. The livers and small intestine were stored at -80°C until analysis. Liver histology was prepared by the Northwestern University Mouse Histology and Phenotyping Laboratory. All animal protocols were approved by the Northwestern University Institutional Animal Care and Use Committee.

Plasma and Hepatic Biochemical Analysis

Hepatic bile acid concentration was measured using a BioQuant total bile acid colorimetric assay (BQ Kits, San Diego, CA). Plasma alanine aminotransferase level (Teco Diagnostics, Anaheim, CA) was measured using a spectrophotometric assay per the manufacturer's protocol. Total cholesterol content in liver homogenate was measured using an Infinity spectrophotometric assay (Fisher Scientific, Middletown, VA). Plasma 7α-hydroxy-4-cholesten-3-1 (C4) measurement was performed at the Mayo Clinic Immunochemical Core Lab (Rochester, MN).

High-Performance Liquid Chromatography Assay

Hepatic bile acid composition was measured by high-performance liquid chromatography as previously described.[25] Samples were spiked with glycocholic acid as an internal standard to control for extraction efficiency. The content of tauromuricholic acid, taurocholic acid, and

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taurochenodeoxycholic acid was calculated from a standard curve and reported as the percentage of total hepatic bile acids.

Cell Culture

Human hepatoma (HepG2) cells (ATCC, Mannasas, VA) were cultured in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum and maintained at 37°C in 5% CO_2. Cells were grown to 80% confluence in 6-well plates and treated with 12 μmol/L tunicamycin, 100 nmol/L thapsigargin (Sigma-Aldrich, St. Louis, MO), 5 mmol/L DL-homocysteine (Sigma-Aldrich), or vehicle (DMSO/saline) in serum-free DMEM for 6 hours. To determine whether the effects of tunicamycin are dependent on c-Jun-N-terminal kinase (JNK) or extracellular signaling-regulated kinase (ERK), HepG2 cells were treated with the JNK inhibitor SP600125 (Sigma-Aldrich) at a concentration of 25 μmol/L or the MAPK/ERK kinase inhibitor PD184352 (Santa Cruz Biotechnology, Dallas, TX) at 1 μmol/L or vehicle (DMSO/saline) as previously described.[26] One hour after exposure to SP600125 or PD184352, cells were treated with tunicamycin (12 μmol/L; Sigma-Aldrich) in serum-free DMEM and incubated for an additional 6 hours. Successful inhibition of JNK and ERK activation was confirmed by Western blot analysis as previously described.[26]

Analysis of Gene and Protein Expression

Total RNA from frozen liver, ileum, or cultured HepG2 cells was isolated using TRIzol reagent (Ambion Life Technologies, Carlsbad, CA), and real-time quantitative polymerase chain reaction was performed as previously described.[27,28] Total protein was isolated from frozen liver samples and Western blotting was performed as previously described.[27,28] Protein detection was performed using polyclonal rabbit antibodies to CYP7A1 (Proteintech, Rosemont, IL) and glyceraldehyde-3-phosphate dehydrogenase (Cell Signaling Technology, Danvers, MA). Bound antibody was detected using goat anti-rabbit polyclonal horseradish-peroxidase antibody (Cell Signaling Technology) and developed using enhanced chemiluminescence Western Blotting Substrate (Cell Signaling Technology). Representative Western blots of pooled samples are shown. Densitometry was performed on individual samples using ImageJ software (available: imagej.nih.gov/ij/; National Institutes of Health, Bethesda, MD).

Statistical Analysis

Data are presented as means ± SD. Comparisons between groups were performed using the Student t test analysis.

All authors had access to the study data and reviewed and approved the final manuscript.

Results
ER Stress Suppresses the Primary Bile Acid Synthetic Pathway

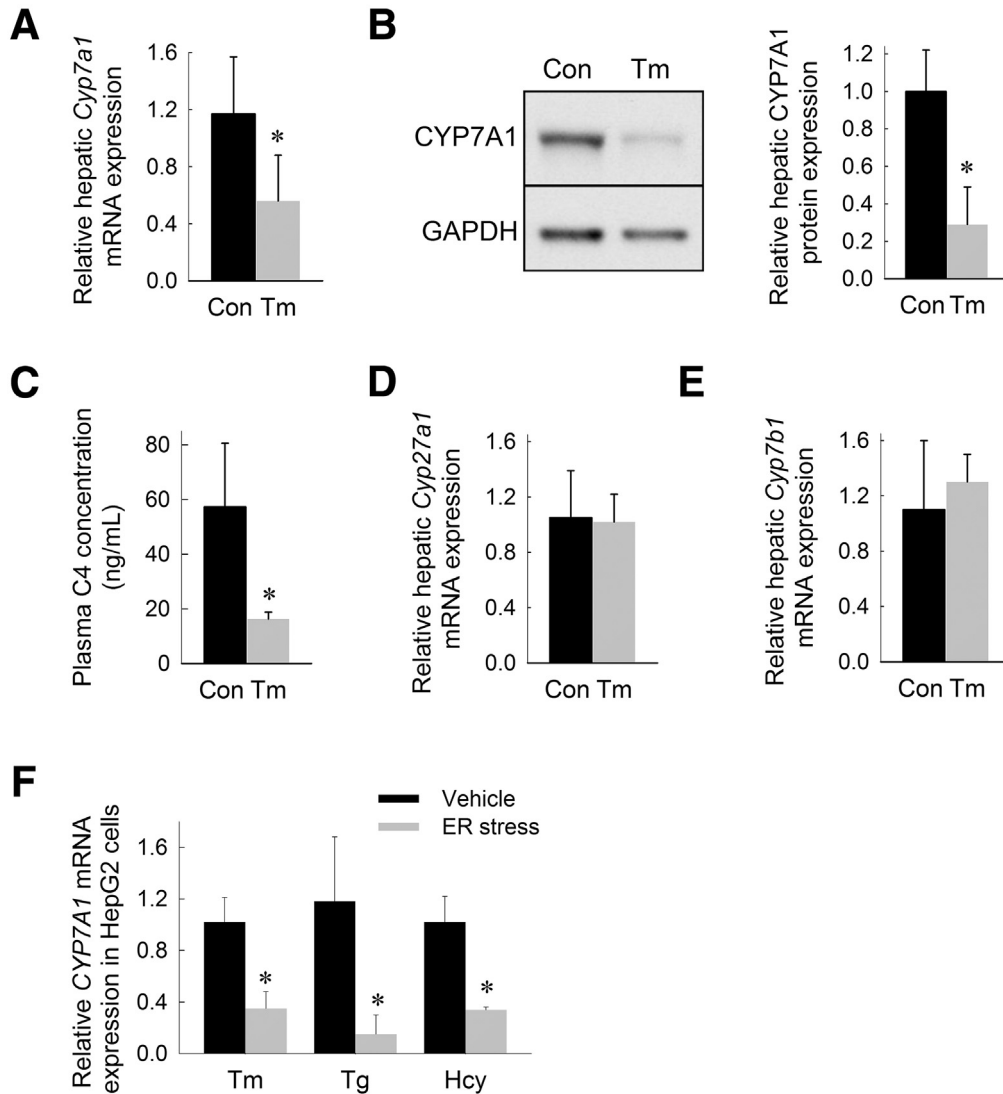CYP7A1 is the primary bile acid synthetic enzyme controlling the rate-limiting step in the conversion of hepatic cholesterol to bile acids.[29,30] To determine the effect of ER stress on hepatic *Cyp7a1* expression, mice were treated with tunicamycin (0.5 mg/kg IP), a well-established ER stress–inducing agent in mice.[31–33] Mice treated with tunicamycin showed robust hepatic UPR activation at 6 hours as evidenced by induction of glucose-regulated protein 78 kilodaltons, an ER chaperone and master regulator of the UPR, spliced X-box binding protein 1, a major mediator of the inositol requiring enzyme 1α (IRE1α) branch of the UPR, and CCAAT/enhancer binding protein homologous protein, a regulator of ER stress-induced apoptosis[34–37] (Table 1). Induction of hepatic ER stress resulted in significant suppression of hepatic *Cyp7a1* messenger RNA (mRNA) and CYP7A1 protein expression (Figure 1*A* and *B*). We next determined whether ER stress suppresses CYP7A1-dependent bile acid synthesis. The plasma concentration of C4, a stable intermediate generated in the synthesis of bile acids from cholesterol, is an indicator of the activity of the CYP7A1-dependent bile acid synthetic pathway. Consistent with the observed suppression of CYP7A1, induction of ER stress in mice reduced the plasma concentration of C4 (Figure 1*C*). Although CYP7A1 regulates the major pathway of bile acid synthesis, bile acid synthesis also occurs via the alternative (acidic) pathway of bile acid synthesis involving sterol 27-hydroxylase and oxysterol 7-α-hydroxylase.[38–40] We found that neither sterol 27-hydroxylase nor oxysterol 7-α hydroxylase mRNA expression was altered by induction of ER stress (Figure 1*D*).

There are significant differences in the expression levels and regulation of the *CYP7A1* gene in mice and human beings. Most notably, human beings lack an Liver X Receptor-response element in the *CYP7A1* promoter, rendering human *CYP7A1* unresponsive to dietary cholesterol.[41–43] To exclude the possibility that the effects of ER stress on *Cyp7a1* expression are specific to mice, we determined the effect of pharmacologic ER stress on *CYP7A1* expression in a human hepatoma cell line (HepG2). Paralleling our findings in vivo, induction of ER stress in HepG2 cells suppressed

Table 1. Activation of the Hepatic and Intestinal Unfolded Protein Response in Mice Treated With Tunicamycin for 6 Hours or 5 Days

	Relative hepatic expression		Relative ileal expression	
	6 hours	5 days	6 hours	5 days
Grp78/BiP	47.4[a] ± 20.5	19.3[a] ± 15.9	1.2 ± 0.5	8.4[a] ± 6.0
Xbp1s	5.7[a] ± 1.9	5.1[a] ± 4.9	1.1 ± 0.4	0.5[a] ± 0.2
Chop	197.7[a] ± 79.6	20.5[a] ± 21.1	1.2 ± 0.6	10.9[a] ± 4.8

NOTE. Hepatic and ileal mRNA expression levels relative to vehicle-treated controls. Means (n = 6) ± SD.
Chop, CCAAT/enhancer binding protein homologous protein; *Grp78/BiP*, glucose-regulated protein 78 kilodaltons; *Xbp1s*, X-box binding protein 1.
[a]$P < .05$ vs vehicle-treated control at same time point.

Figure 1. ER stress suppresses the primary bile acid synthetic pathway in mice. (A) Relative hepatic *Cyp7a*1 mRNA expression, (B) Western blot of hepatic CYP7A1 protein levels with corresponding densitometry analysis, (C) plasma C4 concentration (ng/mL), (D) relative hepatic sterol 27-hydroxylase (*Cyp27a1*) mRNA expression, and (E) relative hepatic oxysterol 7α hydroxylase (*Cyp7b1*) mRNA expression in mice treated with tunicamycin (Tm) for 6 hours. (F) Relative *CYP7A1* mRNA levels in HepG2 cells treated with Tm, thapsigargin (Tg), or homocysteine (Hcy) for 6 hours. Representative Western blot of hepatic protein from 6 individually treated mice pooled per lane. Gene expression and plasma analysis are shown as the means (n = 6) ± SD. For cell culture experiments, gene expression is reported as the means of 6 identically treated replicates. *$P < .05$ vs vehicle-injected controls. Con, control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

CYP7A1 expression (Figure 1*E*). To ensure that these effects are not specific to tunicamycin, we also treated HepG2 cells with 2 alternative pharmacologic ER stress–inducing agents, thapsigargin and homocysteine, for 6 hours. *CYP7A1* expression was suppressed by 87% and 67% in HepG2 cells treated with thapsigargin and homocysteine, respectively (Figure 1*E*).
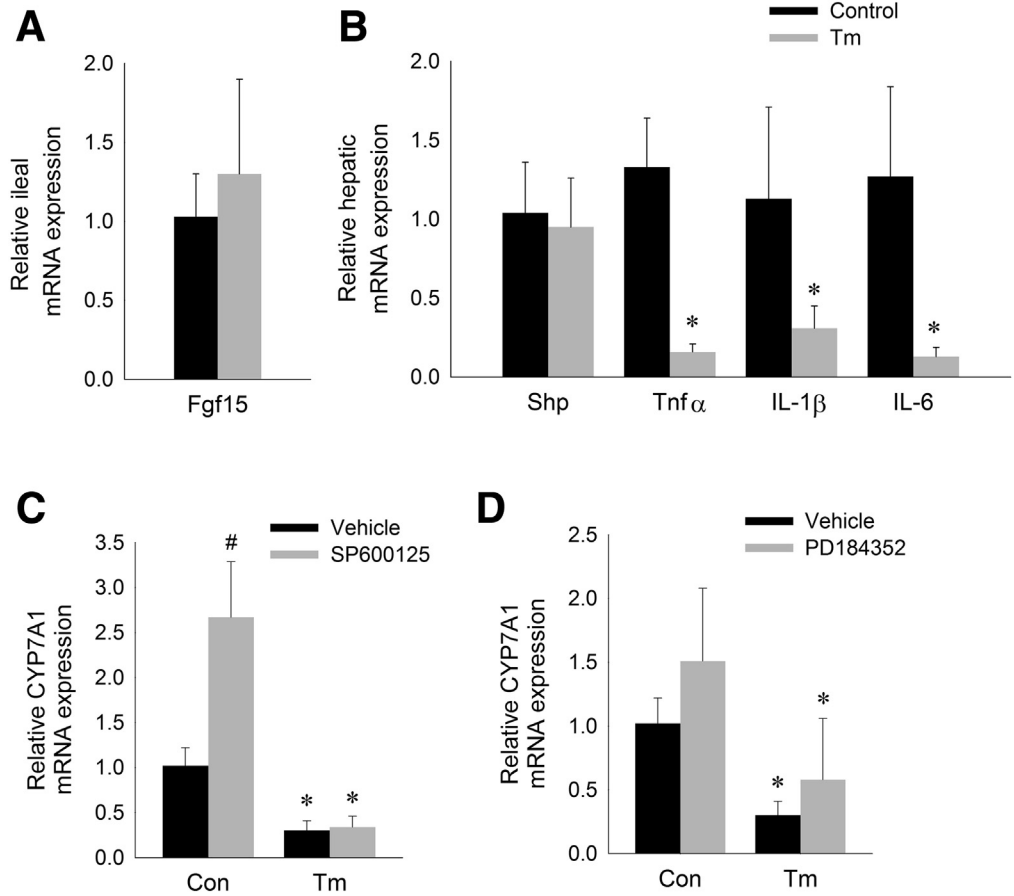
Suppression of Cyp7a1 by ER Stress Is Independent of Farnesoid X Receptor–Dependent Bile Acid Feedback Inhibition Pathways

A major mechanism of *Cyp7a1* regulation is via feedback inhibition from bile acids.[44–46] Specifically, bile acids bind to the farnesoid X receptor (FXR) in the ileum, stimulating release of fibroblast growth factor (FGF) 15/19 from the ileocyte, which subsequently acts in the liver to suppress *Cyp7a1* transcription.[47,48] Intestinal ER stress,

achieved through oral administration of tunicamycin to mice, has been shown to induce ileal *Fgf15* expression.[49] We found that administration of intraperitoneal tunicamycin did not induce intestinal ER stress at 6 hours (Table 1). Consistent with an absence of intestinal UPR activation, we found no induction of ileal *Fgf*15 expression in mice treated with intraperitoneal tunicamycin for 6 hours (Figure 2*A*). These data indicate that ER stress suppresses hepatic *Cyp7a1* independent of ileal *Fgf15* activation.

Although activation of intestinal FXR-FGF15/19 signaling now is considered the major mechanism of bile acid feedback inhibition of *Cyp7a1* transcription, bile acids also inhibit *Cyp7a1* via activation of FXR within the liver, leading to induction of the small heterodimer partner (*Shp*). We found that induction of ER stress in mice did not increase hepatic *Shp* expression, indicating that tunicamycin does not suppress *Cyp7a1* via a FXR-SHP–dependent mechanism (Figure 2*B*).

Figure 2. Suppression of CYP7A1 by ER stress is independent of known bile acid regulatory pathways. (A) Relative ileal *Fgf15* mRNA expression and (B) relative hepatic *Shp*, tumor necrosis factor (*Tnf*)α, *Il1β*, and *Il6* mRNA expression in mice treated with tunicamycin (Tm) for 6 hours. Means (n = 6) ± SD. *P < .05 vs control. (C and D) Relative *CYP7A1* mRNA levels in HepG2 cells treated with (C) Tm ± JNK-inhibitor (SP600125) or (D) tunicamycin ± MAPK/ERK kinase inhibitor (PD184352) for 6 hours. Means (n = 6) ± SD. *P < .05 vs control cells not treated with Tm, #P < .05 vs control cells not treated with inhibitor.

Suppression of Cyp7a1 by ER Stress Is Independent of Hepatic Inflammatory Cytokine Activation

Suppression of *Cyp7a1* expression is a feature of the hepatic inflammatory response.[11,12] Activation of cytokines such as tumor necrosis factor α, interleukin (IL)1β, and IL6 is associated with liver injury and has been shown to suppress hepatic *Cyp7a1* expression in vitro and in vivo.[11,50–53] Therefore, we considered the possibility that suppression of *Cyp7a1* expression by ER stress is a manifestation of a broader stress response associated with inflammatory cytokine activation. We found no significant inflammatory cytokine activation at 6 hours after induction of ER stress (Figure 2B). On the contrary, acute exposure to ER stress suppressed hepatic *Tnfα*, *Il1β*, and *Il6* expression. These data indicate that suppression of bile acid synthesis by ER stress is independent of inflammatory cytokine activation.

Suppression of CYP7A1 Expression by ER Stress Is Not Dependent on JNK and ERK Mitogen-Activated Protein Kinase Activation

Activation of JNK has been implicated in the inhibition of *CYP7A1* by inflammatory cytokines.[52,54,55] Furthermore, it is well established that JNK activation occurs in response to

ER stress.[26,56,57] To confirm whether ER stress–induced *CYP7A1* suppression is dependent on JNK activation, we determined the effects of tunicamycin on HepG2 cells treated with the JNK inhibitor SP600125. Inhibition of JNK signaling in HepG2 cells did not prevent suppression of *CYP7A1* by tunicamycin (Figure 2C).

FGF15/19-mediated suppression of *CYP7A1* expression is dependent on ERK activation.[58,59] We assessed whether ER stress–induced *CYP7A1* suppression is dependent on ERK activation. Inhibition of ERK in HepG2 cells using PD184352 did not prevent ER stress–induced suppression of *CYP7A1*, thus providing additional evidence that the effect of ER on hepatic bile acid synthesis is not dependent on FGF15/19 activation (Figure 2D).

ER Stress Regulates Hepatic Bile Acid Transporters in Mice

Suppression of *Cyp7a1* expression is a major compensatory mechanism to prevent bile acid accumulation in the setting of cholestatic liver injury. Several other compensatory mechanisms are activated in the liver in response to cholestasis, including suppression of bile acid uptake, induction of bile acid efflux, and enhanced biliary bile acid secretion.[9] We next examined the effects ER stress on these anticholestatic mechanisms. Acute ER stress increased

hepatic expression of adenosine triphosphate binding cassette (Abc)b11, the canalicular bile salt export pump, yet had no significant effect on the hepatic expression of Ntcp, the basolateral transporter controlling sinusoidal uptake of bile acids into hepatocytes (Figure 3A). The multidrug

Figure 3. ER stress regulates the expression of hepatic bile acid transporters in mice and HepG2 cells. (A) Relative hepatic mRNA levels of Abcb11, Ntcp, and Abcc3 in mice treated with tunicamycin (Tm) for 6 hours. Relative mRNA expression of (B) ABCB11 and (C) ABCC3 in HepG2 cells treated with Tm, thapsigargin (Tg), or homocysteine (Hcy) for 6 hours. Means (n = 6) ± SD. *P < .05 vs controls

resistance-associated protein 3 (ABCC3) is a basolateral bile acid efflux pump that is thought to have a protective role in the setting of cholestasis by removing bile acids from the cholestatic liver.[60,61] The expression of Abcc3 was increased in response to acute ER stress (Figure 3A).
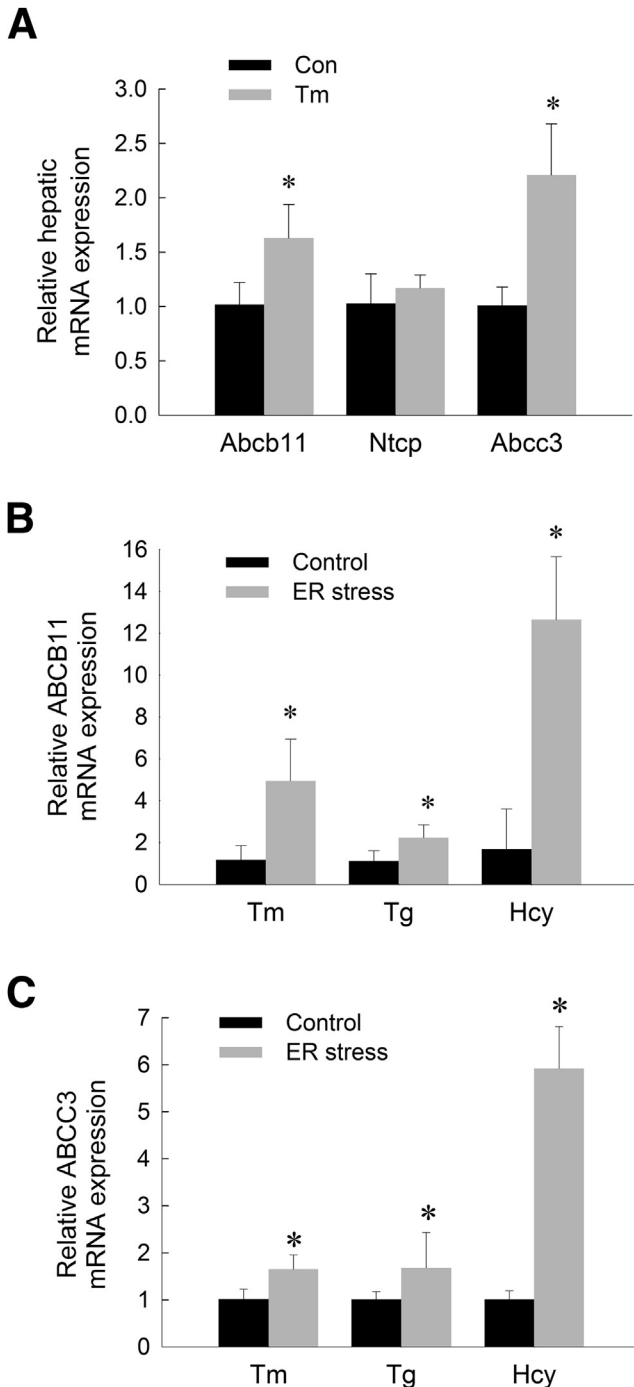
Having shown that hepatic Abcb11 and Abcc3 are activated in mice subjected to ER stress, we next measured ABCB11 and ABCC3 expression in human hepatoma cells (HepG2) treated with tunicamycin. Paralleling our findings in vivo, induction of ER stress with tunicamycin, thapsigargin, or homocysteine in HepG2 cells increased expression of ABCB11 and ABCC3 (Figure 3B and C).

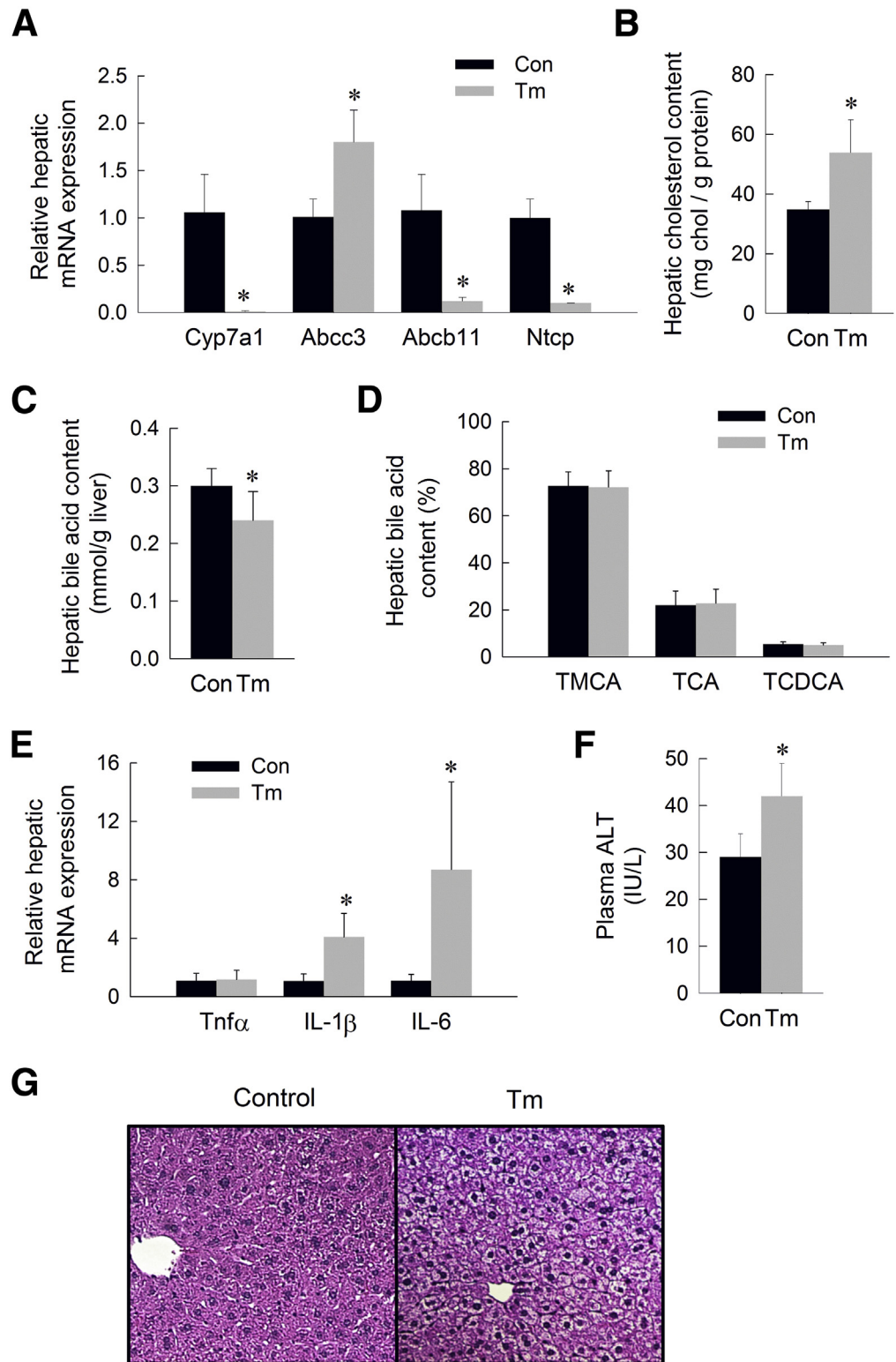## Prolonged ER Stress Reduces the Hepatic Bile Acid Content in Mice

We have shown that induction of ER stress suppresses the primary bile acid synthetic pathway and enhances expression of transporters responsible for the removal of bile acids from the liver. These pathways seemingly would converge to reduce the hepatic bile acid content over time. To test this hypothesis, we examined the effect of prolonged ER stress on hepatic bile acid content. Mice were treated with daily injections of low-dose tunicamycin (0.1 mg/kg IP) for 5 days. Similar to the pattern observed in mice acutely after induction of ER stress, mice subjected to prolonged ER stress showed induction of the hepatic UPR associated with marked suppression of Cyp7a1 and induction of Abcc3 (Table 1 and Figure 4A). Consistent with suppressed CYP7A1-dependent bile acid synthesis, hepatic cholesterol content was increased and hepatic bile acid content was decreased in response to prolonged ER stress (Figure 4B and C). The relative composition of the hepatic bile acids was unaffected by ER stress (Figure 4D). Unlike acute ER stress, prolonged ER stress suppressed hepatic expression of Abcb11 and Ntcp, bile acid transporters known to be suppressed by hepatic inflammation (Figure 4A). We found that prolonged ER stress resulted in induction of the inflammatory cytokines Il1β and Il6, suggesting activation of a more generalized hepatic inflammatory response in the setting of sustained ER stress (Figure 4E). Consistent with the development of hepatic inflammation, mice subjected to prolonged ER stress showed a mild increase in plasma alanine aminotransferase level (Figure 4F) and early ballooning degeneration on H&E-stained liver sections (Figure 4G). There was no overt hepatic steatosis or cholestasis evident histologically. Prolonged ER stress also resulted in induction of the intestinal UPR, suggesting activation of a broader systemic stress response over time (Table 1).

Discussion

Bile acids perform critical physiologic functions, however, when present in excess these molecules promote hepatotoxicity. In response to hepatocellular stress, protective mechanisms are activated to reduce hepatic accumulation of toxic bile acids. We show that ER stress activates anticholestatic mechanisms and decreases hepatic bile acid content. Potentially the most critical ER

Figure 4. Effect of prolonged ER stress on bile acid metabolism. (*A*) Relative hepatic mRNA expression of *Cyp7a1*, *Abcc3*, *Abcb11*, and *Ntcp*, (*B*) hepatic cholesterol content (mg chol/g liver), (*C*) hepatic bile acid content (mmol/g liver), (*D*) hepatic bile acid composition (% of total hepatic bile acids), (*E*) relative hepatic mRNA expression of tumor necrosis factor (*Tnf*)α, *IL1β*, and *IL6*, (*F*) plasma alanine aminotransferase (ALT) level (IU/L), and (*G*) H&E-stained liver sections of mice treated with tunicamycin for 5 days. Means (n = 6) ± SD. *P < .05 vs vehicle-injected controls. Con, control; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; Tm, tunicamycin; TMCA, tauromuricholic acid.

stress–induced anticholestatic mechanism we have identified is suppression of the CYP7A1-dependent bile acid synthetic pathway. We found that suppression of CYP7A1 occurs early after induction of ER stress, in the absence of inflammatory cytokine activation. In addition, we showed that CYP7A1 suppression by ER stress occurred in the absence of hepatic *Shp* or ileal *Fgf15* activation. These data show that CYP7A1 suppression by ER stress was independent of established FXR-dependent or cytokine-mediated pathways. Moreover, these data strongly suggest that the

observed effects of acute ER stress on hepatic bile acid metabolism were a direct result of ER stress and not simply a manifestation of a broader hepatic inflammatory response.

We found that ER stress also regulated the expression of hepatic basolateral and canalicular transporters. ER stress acutely increased hepatic expression of *Abcc3*, a basolateral bile salt efflux pump, and Abcb11, the canalicular bile salt export pump. These findings suggest that, as part of a co-ordinated protective response, the hepatocyte removed toxic bile acids from the liver in response to ER stress. Consistent with this assertion was the finding that pro-longed ER stress lead to a reduction in hepatic bile acid content.

Contrary to the acute response to ER stress, we found that sustained ER stress led to suppression of *Abcb11* and *Ntcp*. Furthermore, we found that sustained ER stress promoted a hepatic inflammatory response associated with cytokine activation. Suppression of *Abcb11* and *Ntcp* has been a well-established feature of the hepatic inflammatory response,[12,13,62–65] and we speculate that suppression of these transporters with prolonged ER stress is secondary to inflammatory cytokine activation.

We have speculated that suppression of CYP7A1 is a direct result of UPR activation yet it remains unclear which element(s) of this highly intricate signaling cascade may be responsible for the observed phenotype. The UPR is initiated through 3 ER transmembrane receptors, PKR-like ER kinase, activating transcription factor 6 α, and IRE1α and 1 master chaperone, glucose-regulated protein 78 kilodaltons. The IRE1α pathway is the most evolutionarily conserved branch of the UPR and is considered the major mediator of the adaptive response to cellular stress.[66–68] It therefore is reasonable to hypothesize that the IRE1α arm of the UPR may mediate an adaptive response aimed at maintaining bile acid homeostasis. Furthermore, although all 3 branches of the UPR have been shown to modulate metabolic pathways, the IRE1α pathway has emerged as the branch most strongly implicated in regulating hepatic lipid metabolism, which is linked intimately to bile acid metabolism.[69–71] Activated IRE1α, functioning as an endonuclease, induces nonconventional splicing of the transcription factor X-box binding protein 1, which has been implicated in the transcription of a myriad of genes involved in proteostasis and metabolic processes. It has become increasingly apparent, however, that the major regulatory effect of IRE1α activation is attributable to regulated IRE1α-dependent mRNA decay (RIDD).[72] A rapidly increasing number of mRNAs and microRNAs involved in a diverse array of cellular processes have been identified as RIDD targets.[72–75] At least 2 such mRNAs encode ER-localized cytochrome P450 enzymes, Cyp1a2 and Cyp2e1.[76] These data raise the possibility that in the setting of ER stress, Cyp7a1 mRNA may be targeted for destruction via RIDD.

The present work implicates ER stress as a novel regulator of the major bile acid synthetic pathway. Although the classic pathophysiologic consequence of disrupted bile acid homeostasis is cholestatic liver injury, altered bile acid metabolism now is known to impact numerous physiologic processes and may have broader implications for human metabolic disease. Impaired bile acid metabolism has been implicated in the pathogenesis of diabetes and obesity, both of which are associated with ER stress.[77–79] The possibility that altered bile acid homeostasis is a mechanistic link between ER stress and the development of metabolic disease warrants further investigation.

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