Original article

MOLECULAR METABOLISM

# 11β-Hydroxysteroid dehydrogenase-1 is involved in bile acid homeostasis by modulating fatty acid transport protein-5 in the liver of mice[a]

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## ABSTRACT

11β-Hydroxysteroid dehydrogenase-1 (11β-HSD1) plays a key role in glucocorticoid receptor (GR) activation. Besides, it metabolizes some oxysterols and bile acids (BAs). The GR regulates BA homeostasis; however, the impact of impaired 11β-HSD1 activity remained unknown. We profiled plasma and liver BAs in liver-specific and global 11β-HSD1-deficient mice. 11β-HSD1-deficiency resulted in elevated circulating unconjugated BAs, an effect more pronounced in global than liver-specific knockout mice. Gene expression analyses revealed decreased expression of the BA-CoA ligase Fatp5, suggesting impaired BA amidation. Reduced organic anion-transporting polypeptide-1A1 (*Oatp1a1*) and enhanced organic solute-transporter-β (*Ostb*) mRNA expression were observed in livers from global 11β-HSD1-deficient mice. The impact of 11β-HSD1-deficiency on BA homeostasis seems to be GR-independent because intrahepatic corticosterone and GR target gene expression were not substantially decreased in livers from global knockout mice. Moreover, Fatp5 expression in livers from hepatocyte-specific GR knockout mice was unchanged. The results revealed a role for 11β-HSD1 in BA homeostasis.



**Keywords** 11β-Hydroxysteroid dehydrogenase; Bile acids; Glucocorticoids; Bile acid conjugation; Bile acid transport

## 1. INTRODUCTION

Bile acids (BAs) are essential for the absorption of dietary nutrients and the solubilization and excretion of toxic lipophilic compounds [1]. Moreover, they have been implicated in the regulation of inflammation, energy homeostasis and glucose and lipid metabolism [2–4]. BA synthesis and transport are chiefly controlled by the farnesoid X receptor (Fxr). Activation by BAs represses BA synthesis via induction of the short heterodimer partner (Shp) and stimulation of BA conjugation and canalicular efflux [5–7]. Other nuclear receptors, including the glucocorticoid receptor (GR), are also involved in the maintenance of BA homeostasis [8–12]. A role for the GR has been shown in the induction of the $Na^+$-taurocholate cotransporting polypeptide (Ntcp) expression [8]. This was supported by low Ntcp expression levels in loss-of-GR-function mouse models [9], strongly suggesting that Ntcp is a GR target gene. Ntcp is considered to be the most relevant sodium-dependent BA uptake system in hepatocytes [9,13]. Its reduced expression upon loss-of-GR-function causes impaired trans-hepatic BA flow, resulting in the accumulation of circulating BAs [9].

GR activity depends on the intracellular availability of active glucocorticoids, which is controlled by the uptake from the circulation and the activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). 11β-HSD1 is a NADPH-dependent microsomal enzyme regenerating cortisol from inactive cortisone (corticosterone from 11-dehydrocorticosterone in rodents) [14]. It is highly expressed in liver, adipose, skeletal muscle and macrophage [15]. Besides glucocorticoids, 11β-HSD1 metabolizes other endogenous compounds including the oxysterol 7-ketocholesterol [16,17] and the BA 7-oxolithocholic acid (7-oxoLCA) [18,19]. The physiological relevance

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*Abbreviations:* 11β-hydroxysteroid dehydrogenase 1, 11β-HSD1; bile acids, BAs; BA coenzyme A: amino acid N-acyltransferase, Baat; cholesterol 7α-hydroxylase, Cyp7a1; farnesoid X receptor, Fxr; fatty acid transport protein, Fatp; glucocorticoid receptor, GR; $Na^+$-taurocholate cotransporting polypeptide, Ntcp; Organic solute transporter, Ost; Organic anion-transporting polypeptide, Oatp; short heterodimer partner, Shp; sterol-regulatory element-binding protein 1C, Srebp1c

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of 11β-HSD1 in the metabolism of these latter substrates remains largely unexplored.

Studies with transgenic mice and clinical observations revealed an association between elevated 11β-HSD1 expression and the development of metabolic diseases [20—22] and suggested pharmacological inhibition as a promising therapeutic approach [23—25]. Global 11β-HSD1 knockout mice showed improved lipid profiles, seemingly driven by increased hepatic fat catabolism [26]. In contrast, the expression of genes involved in hepatic lipid homeostasis was unaffected in liver-specific 11β-HSD1 knockout mice except for a reduced expression of sterol regulatory element-binding protein 1C (Srebp1c) upon high-fat feeding, an observation remaining elusive at present [27]. Because it has been previously shown that Fxr modulates lipogenesis by decreasing Srebp1c expression *via* the induction of Shp [28], it remained to be investigated whether enhanced Fxr signaling mediated by elevated BAs could explain, at least in part, Srebp1c modulation upon liver-specific 11β-HSD1 disruption.

Because of the advanced development of 11β-HSD1 inhibitors to treat glucocorticoid excess associated with the metabolic syndrome [22,29,30], and the recent discovery of a role of this enzyme in BA metabolism [18,19], we investigated a possible link between 11β-HSD1 activity, GR function and BA homeostasis using mouse models of 11β-HSD1-deficiency.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and reagents

Cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), deoxycholylglycine (DC-Gly), chenodeoxycholylglycine (CDC-Gly), chenodeoxycholyltaurine (CDC-Tau), [2,2,4,4-$^2H_4$]-CA (98% isotopic purity), [2,2,4,4-$^2H_4$]-CDCA (>98% isotopic purity) and [2,2,4,4-$^2H_4$]-LCA (98% isotopic purity) were purchased from Sigma—Aldrich (St. Louis, MO). 7-Oxodeoxycholic acid (7-oxoDCA), hydeoxycholic acid (HDCA), α-muricholic acid (αMCA), β-muricholic acid (βMCA), ω-muricholic acid (ωMCA), ursodeoxycholylglycine (UDC-Gly), lithocholyltaurine (LC-Tau), α-muricholyltaurine (αMC-Tau), β-muricholyltaurine (βMC-Tau), ω-muricholyltaurine (ωMC-Tau) and [2,2,4,4-$^2H_4$]-DCA (98% isotopic purity) were obtained from Steraloids (Newport, RI). Cholylglycine (C-Gly), cholyltaurine (C-Tau), deoxycholyltaurine (DC-Tau) and ursodeoxycholyltaurine (UDC-Tau) were purchased from Calbiochem (Läufelfingen, Switzerland), and [2,2,4,4-$^2H_4$]-UDCA (>98% isotopic purity), [2,2,4,4-$^2H_4$]-C-Gly (>98% isotopic purity), [2,2,4,4-$^2H_4$]-CDC-Gly (>98% isotopic purity) and [2,2,4,4-$^2H_4$]-UDC-Gly (>98% isotopic purity) from C/D/N Isotopes Inc. (Pointe-Claire, Canada). All other chemicals were from Fluka AG (Buchs, Switzerland) of the highest grade available.

### 2.2. Animal experimentation

To determine the impact of 11β-HSD1 on BA homeostasis, 15 week-old wild-type ($n = 16$), liver-specific 11β-HSD1 knockout ($n = 16$) and global 11β-HSD1 knockout mice ($n = 8$) (previously described in Ref. [27]) were fasted overnight and anaesthetized with isoflurane prior to collection of blood samples by intra-cardiac puncture and isolation of livers. Plasma was prepared immediately by centrifugation and stored at −80 °C until further processing. Livers were frozen in liquid nitrogen and stored at −80 °C. These animal studies were conducted under the Home Office license, following approval of the Joint Ethics and Research Governance Committee of the University of Birmingham (Birmingham, United Kingdom) in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986, and the EU Directive 2010/63/EU for animal experiments. Mice were kept in a climate-controlled facility, housed under standard conditions on a 12-h light/dark cycle and fed *ad libitum* with standard chow and free access to drinking water.

To assess the influence of the GR on the expression of fatty acid transport protein 5 (Fatp5), livers were taken from adult male hepatocytes-specific GR-deficient mice ($GR^{alfpCre}$) ($n = 6$) and wild-type controls ($n = 6$) on a 129-C57BL6-FVB/N background. These mice were bred and housed within the German Cancer Research Center Heidelberg as reported previously [9,31,32].

### 2.3. Quantification of BAs and glucocorticoids in serum and liver tissue by LC—MS/MS

Extraction and quantification of circulating BAs were conducted as described previously [33], with the exception that ωMC-Tau was included in the method and that quantification of αMC-Tau and βMC-Tau was combined due to insufficient separation and overlapping peaks in some samples. The extraction of BAs and glucocorticoids from liver tissue was performed using 100 mg of tissue that was homogenized in 200 μL 50% methanol. Samples were spiked with 300 μL of deuterium labeled BAs (CA-d4, CDCA-d4, DCA-d4, LCA-d4, UDCA-d4, C-Gly-d4 and UDC-Gly-d4) at a final concentration of 1000 nM in acetonitrile, followed by protein precipitation with 1.5 mL of ice-cold alkaline (5% $NH_4OH$) acetonitrile. Thereafter, samples were mixed continuously for 1 h and centrifuged at 11,000 × g for 10 min. The supernatants were transferred into new tubes, the solvent was evaporated and the residuals were reconstituted in 100 μL of 50% methanol, followed by further centrifugation and removal of insoluble particles. The method was validated on the basis of extraction efficiency, intra-day accuracy and precision for representative BAs, including CA, CDCA, DCA, LCA, αMCA, CDC-Gly, UDC-Gly, C-Tau, αMC-Tau. The method presented acceptable extraction efficiency, accuracy and precision for the BAs studied (Supplementary Tables 1—3). The amounts of corticosterone and 11-dehydrocorticosterone in serum and liver tissue samples were semi-quantitatively assessed by monitoring the ion transitions of $m/z$ 347.2 → 329.3 and $m/z$ 345.2 → 121.1, respectively. C-Gly-d4 was used as a surrogate internal standard.

### 2.4. Analysis of mRNA expression

Total mRNA was extracted from liver tissue using Trizol reagent according to the manufacturer's instructions (Life Technologies, Carlsbad, CA). RNA concentration and purity was determined spectrophotometrically on a NanoDrop™ 1000 spectrophotometer (Thermo Scientific, Waltham, MA) by measuring fluorescence at 260 nm, 230 nm and 280 nm. Total RNA (2 μg) was reverse transcribed to cDNA using the Superscript III First-Strand Synthesis System and oligo dT (Life Technologies). Relative quantification of mRNA expression levels was performed by real-time RT-PCR on a RotorGene 6000 (Corbett, Sydney, Australia) and using the KAPA SYBR® FAST qPCR Kit (Kapasystems, Boston, MA). The relative expression of each gene compared with the internal control cyclophilin was determined using the delta-delta-CT method. Primers were either obtained from Sigma or synthesized from validated sequences obtained at Primerbank [34] (Supplementary Table 4). The primer quality was validated by determination of melting curves. Samples were measured in triplicates.

### 2.5. Western blot analysis

Frozen, powdered liver tissue (100 mg) was homogenized in 1 mL buffer containing 0.25 M sucrose, 10 mM Tris—HCl (pH 7.5) and protease inhibitors (Complete mini, Roche Diagnostics, Rotkreuz,

  

Switzerland) using a Dounce homogenizer. Homogenates were centrifuged at 1000 × g for 10 min (to obtain the nuclear fraction, which was discarded), followed by further centrifugation at 100,000 × g for 1 h (to obtain the microsomal/membraneous fraction). The resulting pellet was dissolved in resuspension buffer (0.25 M sucrose, 10 mM HEPES, pH 7.5, and protease inhibitors). Equal amounts of protein were separated by SDS-PAGE, followed by transfer of proteins onto 0.2 μm polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA). Membranes were incubated for 1 h in Tris-buffered saline, pH 7.4, containing 0.1% Tween-20 and 5% non-fat milk. The membranes were incubated overnight at 4 °C with primary antibodies against BA CoA:amino acid N-acyltransferase (Baat; ab83882; Abcam, Cambridge, UK), Fatp5 (ab166698; Abcam, Cambridge, UK); fatty acid transport protein 2 (Fatp2, SAB2102193, Sigma–Aldrich, Saint Louis, USA), Ntxp/Slc10a1 (kindly provided by Dr. Bruno Stieger, Department of Medicine, University Hospital, Zurich, Switzerland), and valosin-containing protein (Vcp, ab11433, Abcam, Cambridge, UK; for plasma membrane loading control) and endoplasmic reticulum protein 57 (Erp57, kindly provided by Dr. Ari Helenius, Institute of Biochemistry, ETH Zurich, Switzerland; for endoplasmic reticulum membrane loading control), respectively. After washing, blots were incubated with goat anti-rabbit IgG-horseradish peroxidase or anti-mouse IgG horseradish peroxidase for 2 h, followed by visualization of bands using enhanced chemiluminescence horseradish peroxidase substrate (Millipore, Billerica, MA, USA) according to the manufacturer's instructions using a Fujifilm LAS-4000 detection system (Bucher Biotec, Basel, Switzerland). Quantification of signals from immunoblotting was carried out using ImageJ software (National institute of Health, Bethesda, MD, US).

### 2.6. Statistical analysis

Data are presented as mean ± SEM. Statistical significance was assessed by Student's *t*-test and one-way ANOVA where appropriate. A *p*-value $\leq 0.05$ was considered to be significant.

## 3. RESULTS

### 3.1. Circulating levels of BAs in liver-specific and global 11β-HSD1-deficient mice

In order to investigate the impact of liver-specific and global 11β-HSD1-deficiency on BA homeostasis, we determined the concentrations of circulating and intrahepatic BAs using UPLC–MS/MS [33]. Disruption of 11β-HSD1 caused significant disturbances in BA homeostasis. Circulating unconjugated BAs tended to increase approximately 3-fold in liver-specific 11β-HSD1 knockout mice and were significantly elevated (10-fold) in global knockout mice, when compared with wild-type controls (Figure 1A). The quantification of individual BAs in plasma from global 11β-HSD1-deficient mice revealed that all unconjugated BAs studied, with the exception of βMCA and ωMCA, were significantly elevated (up to 25-fold, Table 1). The most abundant BAs in plasma from global knockout mice were CA and 7-oxoDCA. These two BAs were also increased in plasma from liver-specific 11β-HSD1-deficient mice; however, the values did not reach statistical significance. The taurine conjugated BAs tended to increase 3-fold in global knockout but not liver-specific knockout mice (Figure 1B, Table 1). The main taurine conjugated BA C-Tau tended to increase 4.5-fold in plasma from 11β-HSD1 global knockout, compared with wild-type littermates, in contrast to liver-specific 11β-HSD1 disruption where no change was observed. The glycine conjugated BAs were present at very low concentrations in mice (Figure 1C). A comparison of the total circulating BAs revealed a 4-fold increase in the circulation of global knockout mice but no significant change in liver-specific knockout mice (Table 1).

### 3.2. Intrahepatic levels of BAs in liver-specific and global 11β-HSD1-deficient mice

The assessment of hepatic BA concentrations revealed a significant 3-fold increase of unconjugated BAs in liver-specific 11β-HSD1-deficient mice compared with wild-type controls (Figure 1D). The quantification of individual BAs showed that both primary and secondary BAs such as

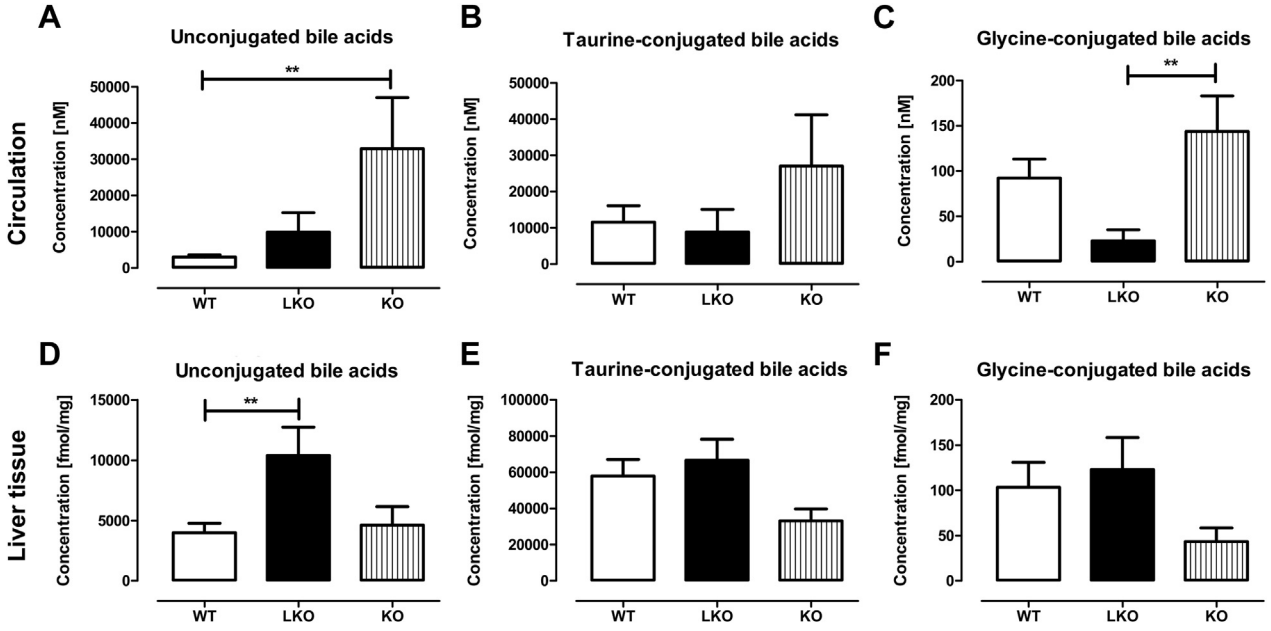


**Figure 1:** 11β-HSD1-deficiency alters circulating and intrahepatic BA concentrations. Circulating (A–C) and intrahepatic concentrations (D–F) of unconjugated (A, D), taurine-conjugated (B, E) and glycine-conjugated BAs (C, F) in wild-type (WT) ($n = 16$), liver-specific (LKO) ($n = 16$) and global 11β-HSD1-deficient mice (KO) ($n = 8$). Data represent mean ± SEM. $^{**}p \leq 0.01$.

 

| | WT | LKO | KO |
|---|---|---|---|
| CA | 636 ± 174 | 4188 ± 2395 | 9439 ± 4758* |
| CDCA | 17 ± 3 | 56 ± 17 | 259 ± 151* |
| DCA | 227 ± 42 | 267 ± 122 | 1598 ± 731* |
| 7-oxoDCA | 646 ± 268 | 3074 ± 1952 | 16,454 ± 7026** |
| HDCA | 28 ± 4 | 44 ± 14 | 264 ± 82***/## |
| LCA | N.D. | N.D. | N.D. |
| αMCA | 95 ± 29 | 567 ± 375 | 2996 ± 1455** |
| βMCA | 755 ± 114 | 402 ± 136 | 614 ± 246 |
| ωMCA | 787 ± 126 | 1248 ± 618 | 1868 ± 1153 |
| UDCA | 83 ± 11 | 103 ± 48 | 537 ± 280**/# |
| C-Tau | 3494 ± 1513 | 3344 ± 2822 | 15,853 ± 7772 |
| CDC-Tau | 185 ± 99 | 113 ± 75 | 644 ± 410 |
| DC-Tau | 848 ± 465 | 385 ± 192 | 2475 ± 1488 |
| LC-Tau | 9 ± 5 | 3 ± 1 | 44 ± 16**/## |
| αMC-Tau + βMCA-Tau | 3152 ± 1327 | 4186 ± 2743 | 5577 ± 2846 |
| ωMCA-Tau | 3784 ± 1636 | 2058 ± 1727 | 4409 ± 2876 |
| UDC-Tau | 227 ± 103 | 23 ± 14 | 38 ± 6 |
| C-Gly | 66 ± 15 | 26 ± 15 | 114 ± 38 |
| CDC-Gly | 13 ± 3 | 1.2 ± 0.2 | 10 ± 2 |
| DC-Gly | 15 ± 3 | 1 ± 0.2* | 10 ± 2 |
| LC-Gly | N.D. | N.D. | N.D. |
| UDC-Gly | 19 ± 4 | 4 ± 3 | 13 ± 2 |
| Sum of all BAs | 14,713 ± 4936 | 18,766 ± 11,442 | 60,071 ± 27,448* |

**Table 1:** Profiles of circulating bile acids from wild-type and from liver-specific and global 11β-HSD1-deficient mice.

The results are expressed in nM as mean ± SEM. WT, wild-type ($n = 16$); LKO, liver-specific 11β-HSD1-deficient mice ($n = 16$); KO, global 11β-HSD1 deficient mice ($n = 8$). Statistics: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p < 0.0001$ KO compared with WT and $^{\#}p \leq 0.05$, and $^{\#\#}p \leq 0.01$ compared with LKO.

| | WT | LKO | KO |
|---|---|---|---|
| CA | 945 ± 218 | 2794 ± 636** | 1123 ± 369 |
| CDCA | 11 ± 2 | 14 ± 3 | 19 ± 4* |
| DCA | 64 ± 15 | 48 ± 16 | 159 ± 44*/## |
| 7-oxoDCA | 564 ± 167 | 3239 ± 840** | 2087 ± 905 |
| HDCA | 24 ± 4 | 41 ± 12 | 105 ± 34***/# |
| LCA | 18 ± 4 | 4 ± 2 | 23 ± 7 |
| αMCA | 173 ± 33 | 1342 ± 374*** | 897 ± 271 |
| βMCA | 1479 ± 259 | 1212 ± 256 | 261 ± 52* |
| ωMCA | 667 ± 138 | 1589 ± 382* | 465 ± 164# |
| UDCA | 62 ± 11 | 124 ± 26* | 55 ± 14 |
| C-Tau | 25,279 ± 5705 | 33,235 ± 5876 | 18,895 ± 4035 |
| CDC-Tau | 934 ± 188 | 1333 ± 268 | 672 ± 242 |
| DC-Tau | 2253 ± 636 | 1184 ± 367 | 2766 ± 441 |
| LC-Tau | 27 ± 4 | 20 ± 4 | 32 ± 3 |
| αMC-Tau + βMC-Tau | 13,432 ± 2248 | 14,976 ± 3028 | 6743 ± 1728 |
| ωMC-Tau | 10,633 ± 1768 | 14,000 ± 2484 | 5574 ± 1460 |
| UDC-Tau | 1190 ± 189 | 472 ± 260 | 15 ± 4** |
| C-Gly | 96 ± 27 | 110 ± 35 | 45 ± 16 |
| CDC-Gly | 5 ± 1 | 7 ± 1 | 1 ± 0.1**/### |
| DC-Gly | 6 ± 2 | 6 ± 1 | 1 ± 0.8 |
| LC-Gly | 3 ± 2 | 1 ± 1 | 1 ± 0.2* |
| UDC-Gly | 6 ± 1 | 4 ± 1 | 1 ± 1 |
| Sum of all BAs | 61,992 ± 9806 | 75,716 ± 13,225 | 39,905 ± 8709 |

**Table 2:** Profiles of hepatic bile acids from wild-type and from liver-specific and global 11β-HSD1-deficient mice.

The results are expressed in fmol/mg of tissue as mean ± SEM. WT, wild-type ($n = 16$); LKO, liver-specific 11β-HSD1-deficient mice ($n = 16$); KO, global 11β-HSD1-deficient mice ($n = 8$). Statistics: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$ compared with WT, and $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$, and $^{\#\#\#}p \leq 0.0001$ KO compared with LKO. Comparison of "Sum all BAs" was done by paired Student's *t*-test.

CA (3-fold), 7-oxoDCA (6-fold), αMCA (8-fold), ωMCA (2-fold), and UDCA (2-fold) were significantly elevated in liver tissue from liver-specific knockout mice (Table 2). The profile of unconjugated BAs in liver tissue from global knockout mice more closely resembled that from wild-type animals. Intrahepatic taurine- and glycine-conjugated BAs were mostly unchanged in both liver-specific and global 11β-HSD1 knockout mice (Figure 1E and F). Interestingly, intrahepatic total BA concentrations tended to be lower in global 11β-HSD1-deficient mice compared with wild-type mice; however, the value did not reach statistical significance (Table 2).

### 3.3. Analysis of the expression of genes involved in BA homeostasis

To begin to understand the changes in BA levels observed in plasma and liver tissue from 11β-HSD1-deficient mice, we determined the expression of genes involved in BA homeostasis. First, we measured the expression of genes with a role in BA synthesis and conjugation in liver tissue from liver-specific 11β-HSD1-deficient mice (Figure 2A and B). The mRNA of *Cyp7a1*, the rate-limiting enzyme in BA synthesis, was decreased, suggesting an adaptive response to the elevated hepatic concentration of unconjugated BAs. In line with this assumption, we observed an enhanced *Shp* gene expression (Figure 2C), suggesting an enhanced activity of the BA sensing receptor Fxr. Next, we analyzed the expression of BA conjugating enzymes and found significantly lower mRNA levels of *Fatp2* (also known as very long-chain coenzyme A synthetase, *Vlcs*) and *Fatp5* (also known as very long-chain coenzyme A synthetase homolog 2, *Vlcsh2*) (Figure 2B), two enzymes possessing BA coenzyme A synthetase activity, an intermediate step in BA conjugation [35]. The expression of the BA coenzyme A:amino acid N-acetyltransferase (*Baat*) was not altered. Furthermore, we determined the mRNA levels of several transporters involved in the uptake and basolateral or canalicular efflux of BAs. *Oatp1b2* was the only gene for which a weak but significant down regulation was detected (Figure 2D).

In liver tissue from global 11β-HSD1-deficient mice the altered *Cyp7a1* and *Shp* expression was not recapitulated (Figure 3), which may be explained by the fact that BA concentrations were not altered in liver tissues from global knockout mice. *Fatp5* and *Baat* expression levels were significantly down regulated, whereas *Fatp2* expression tended to decrease (Figure 3B). Moreover, we observed a significantly reduced expression of the BA uptake transporter *Oatp1a1* and trends to reduced expression of *Oatp1b2* and *Ntcp* (Figure 3D). Ntcp is known to preferentially transport conjugated BAs [36], which may provide an explanation for the trend to increased levels of conjugated BAs in plasma from global 11β-HSD1 knockout mice (Figure 1B and C). Among the other BA transporters, a two-fold increase in *Ostb* mRNA expression was observed (Figure 3E).

To evaluate whether the observed changes in *Fatp5* mRNA levels translated into protein expression, we conducted Western blotting using liver membrane preparations from wild-type, liver-specific and global 11β-HSD1-deficient mice. Consistent with the gene expression data, Fatp5 protein levels were significantly decreased in livers from both genetically modified mouse models (Figure 4A and B). Of note, the reduction of Fatp5 protein content in livers from global 11β-HSD1-deficient mice was more pronounced than that in liver-specific knockout mice. The endoplasmic reticulum membrane loading control Erp57 was not affected by the genotype. In line with its mRNA expression levels, the Ntcp protein content showed only a trend to decrease in liver tissue from global 11β-HSD1 knockout mice (Figure 4C). The plasma membrane loading control Vcp was not affected by the genotype. Although *Fatp2* and *Baat* mRNA expression

 

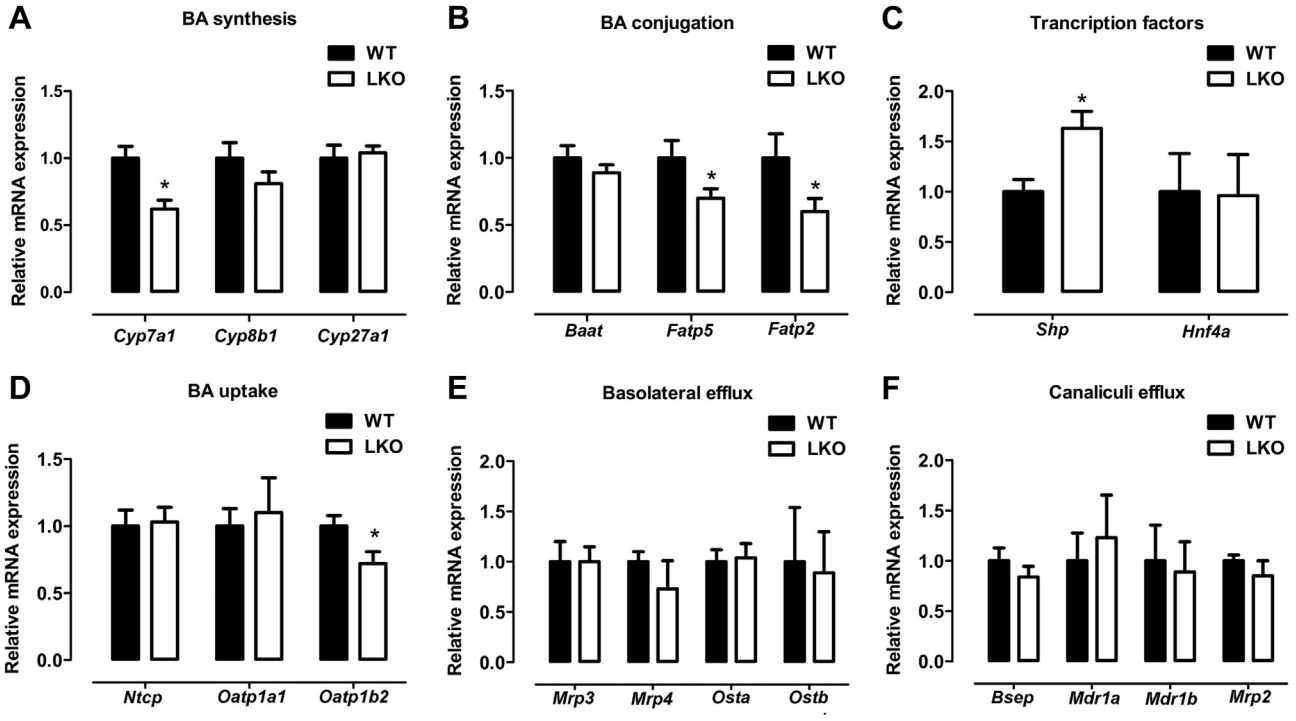


**Figure 2:** Liver-specific disruption of 11β-HSD1 alters the expression of genes involved in BA homeostasis. Gene expression profiles in liver tissues from wild-type (WT) and liver-specific 11β-HSD1-deficient mice (LKO) were determined by real-time RT-PCR. Data ($n > 8$) represent mean ± SEM. *$p \leq 0.05$. Bile acid CoA:amino acid N-acyltransferase, *Baat*; Bile salt export pump, *Bsep*; Cholesterol 7α-hydroxylase, *Cyp7a1*; fatty acid transport protein 2, *Fatp2*; fatty acid transport protein 5, *Fatp5*; Hepatocyte nuclear factor 4 alpha, *Hnf4a*; multidrug-resistance protein 1a, *Mdr1a*; multidrug-resistance protein 1b, *Mdr1b*; multidrug-resistance protein 2, *Mrp2*; multidrug-resistance protein 3, *Mrp3*; multidrug-resistance protein 4, *Mrp4*; $Na^+$-taurocholate cotransporting polypeptide, *Ntcp*; organic anion-transporting polypeptide 1a1, *Oatp1a1*; organic anion-transporting polypeptide 1b2, *Oatp1b2*; organic solute transporter-α, *Osta*; organic solute transporter-β, *Ostb*; sterol 12-alpha-hydroxylase, *Cyp8b1*; sterol 27-hydroxylase, *Cyp27a1*; small heterodimer partner, *Shp*.

levels were reduced in liver tissues from liver-specific and global 11β-HSD1-deficient mice, respectively (Figures 2B and 3B), these changes did not translate into protein content (data not shown), and the significance of these observations is unclear.

### 3.4. Circulating and intrahepatic levels of glucocorticoids in liver-specific and global 11β-HSD1-deficient mice

Because the most widely accepted function of 11β-HSD1 is the conversion of inactive to active glucocorticoids, and several genes involved in BA homeostasis are known to be regulated by glucocorticoids, we determined the circulating and intrahepatic glucocorticoid concentrations in wild-type, liver-specific and global 11β-HSD1-deficient mice. The circulating corticosterone levels were unchanged upon liver-specific and global 11β-HSD1 disruption (as reported previously [27]); however, we observed an elevation of circulating 11-dehydrocorticosterone (3-fold) in global but not liver-specific 11β-HSD1-deficient mice compared with controls (Figure 5A and B). This finding is in line with measurements of tetrahydro metabolites in urine reported earlier, showing an elevation in global but not liver-specific 11β-HSD1-deficient [27]. On the other hand, we found 2-fold reduced intrahepatic corticosterone levels in global but not in liver-specific 11β-HSD1-deficient mice when compared with wild-type controls (Figure 5C). Unexpectedly, intrahepatic 11-dehydrocorticosterone levels were significantly reduced in liver-specific (3-fold) and even more pronounced in global 11β-HSD1-deficient mice (10-fold) (Figure 5D).

To begin to understand these changes, we measured the mRNA expression levels of the genes encoding the glucocorticoid catabolizing enzymes steroid 5α-reductase 1 (*Srd5a1*), steroid 5α-reductase 2 (*Srd5a2*) and steroid 5β-reductase (*Akr1d1*) in liver tissue from global 11β-HSD1 knockout mice. We found a significantly decreased *Srd5a1* expression, while the other enzymes were not altered (Figure 5E).

### 3.5. Fatp5 is not down regulated in GR knockout mice

If the down regulation of *Fatp5* gene expression in 11β-HSD1-deficient mice is a result of decreased active glucocorticoids in the liver, then a reduced expression should be present in GR-deficient mice. As shown in Figure 6A, the BA conjugating genes *Baat*, *Fatp2* and *Fatp5* were expressed at comparable levels in livers from hepatocyte-specific GR-deficient mice. Importantly, the mRNA and protein expression of *Ntcp*, a glucocorticoid-dependent gene was almost completely abolished in livers from GR-deficient mice (Figure 6A–C), consistent with results reported earlier [9].

## 4. DISCUSSION

The present work provides evidence for a novel role of 11β-HSD1 in the maintenance of BA homeostasis. Disruption of 11β-HSD1 led to a tendency for elevated levels of unconjugated BAs in liver-specific 11β-HSD1 knockout mice and significantly higher levels in global 11β-HSD1-deficient mice. Intrahepatic unconjugated BAs were increased in liver-specific but not global knockout mice, suggesting compensatory adaptation. Expression analyses revealed markedly reduced Fatp5 mRNA and protein levels in both mouse models. Fatp5 is a member of the solute carrier family 27. Its expression is almost exclusively restricted to the liver, where it localizes to the basal membrane of hepatocytes. Fatp5 not only mediates the hepatic uptake of fatty acids but also exhibits bile acid-CoA ligase activity, hence activating BAs to CoA thioesters, which is essential for proper BA conjugation [37–40].

 

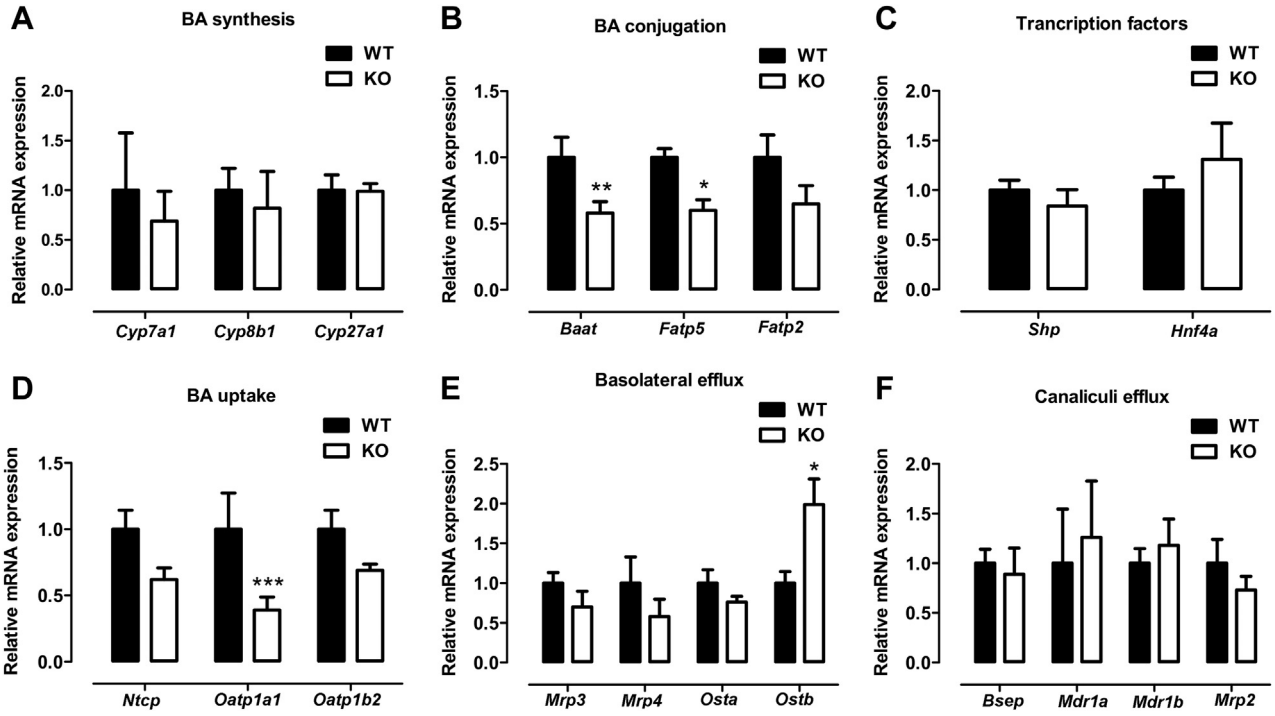


**Figure 3:** Global 11β-HSD1-deficiency alters the expression of genes involved in BA homeostasis. Gene expression profiles in liver from wild-type (WT) and global 11β-HSD1-deficient mice (KO) were determined by real-time RT-PCR. Data ($n > 8$) represent mean ± SEM. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$.

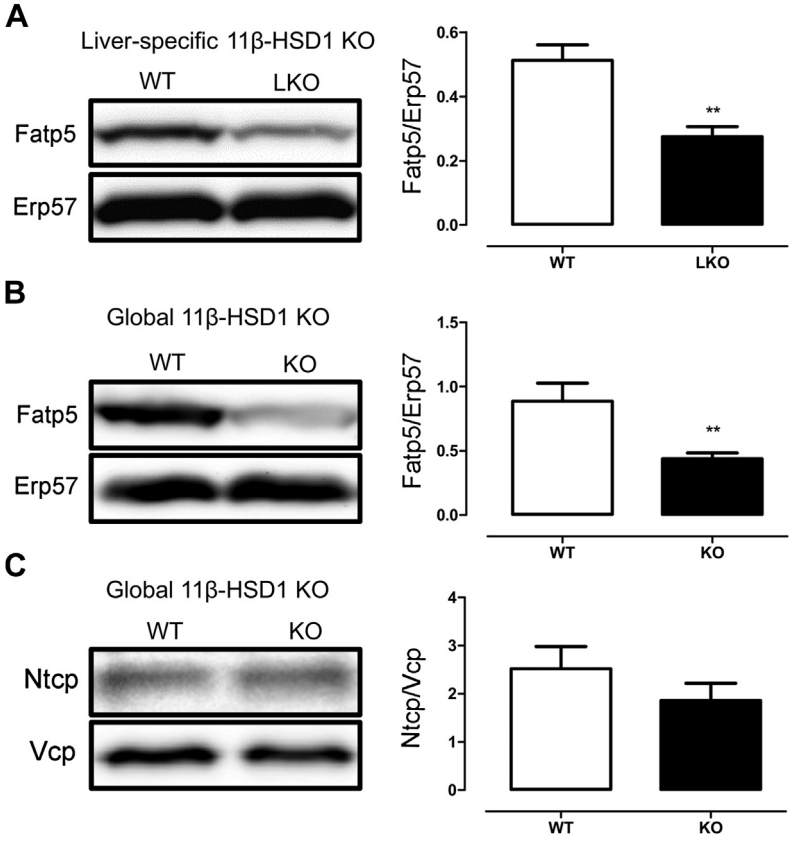


**Figure 4:** 11β-HSD1-deficiency reduces hepatic Fatp5 protein content. (A–C) Representative images and semi-quantitative analyses of Western blots of Fatp5 (A, B) and Ntcp (C) in liver membrane preparations from wild-type (WT) ($n = 16$), liver-specific (LKO) ($n = 16$) and global 11β-HSD1-deficient mice (KO) ($n = 8$) are shown. $^{**}p \leq 0.01$. Fatp5, fatty acid transport protein 5; Erp57, endoplasmic reticulum protein 57; Ntcp, $Na^{+}$-taurocholate cotransporting polypeptide; Vcp, valosin-containing protein.

 

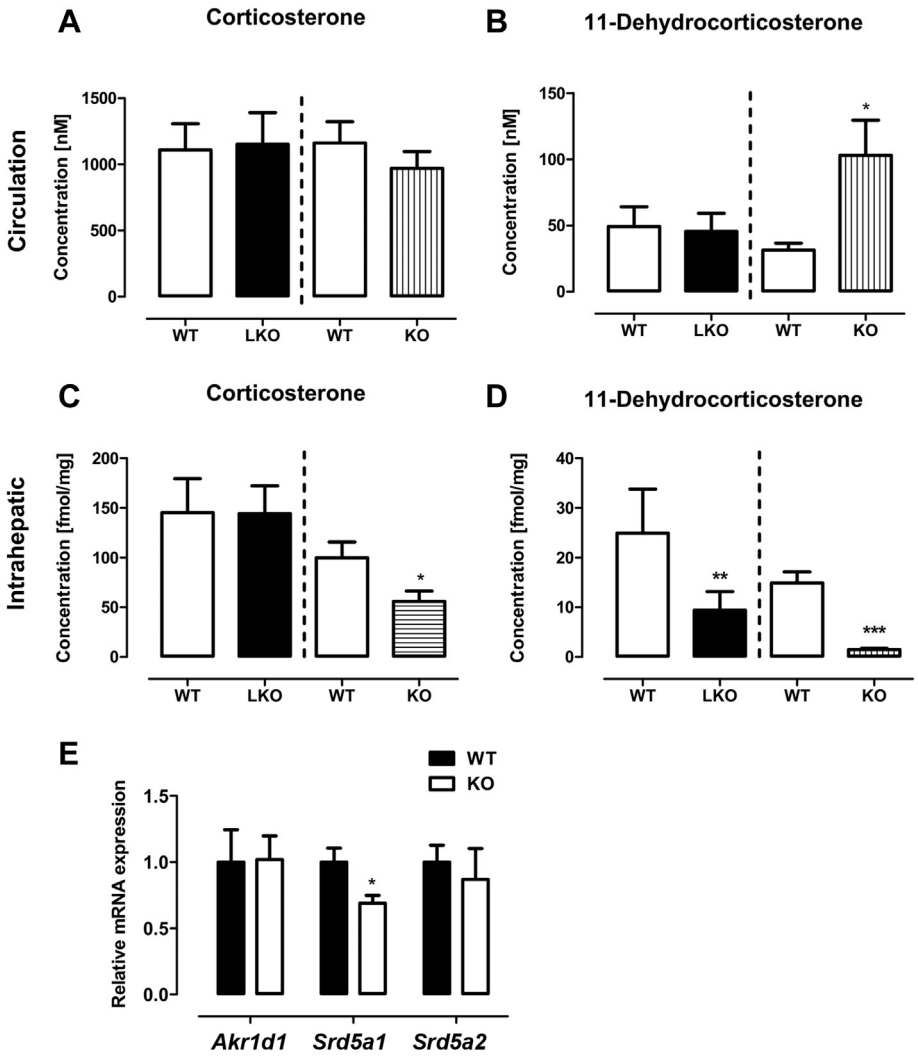


**Figure 5:** 11β-HSD1-deficiency alters the levels of glucocorticoids in plasma and liver tissue. (A–D) Circulating and intrahepatic concentrations of corticosterone and 11-dehydrocorticosterone in wild-type (WT) ($n = 16$), liver-specific (LKO) ($n = 16$) and global 11β-HSD1-deficient mice (KO) ($n = 8$) were determined by LC–MS/MS. (E) Expression levels of sterol-5α-reductases 1 and 2 (*Srd5a1* and *Srd5a2*) and 5β-reductase (*Akr1d1*) in livers from global 11β-HSD1-deficient mice were analyzed by real-time RT-PCR. Data represent mean ± SEM. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$.

Mice deficient in *Fatp5*, albeit having normal total BA concentrations in gall bladder bile, feces, liver, serum, and urine, show a dramatically increased percentage of unconjugated BAs in bile, suggesting that *Fatp5*-deficiency leads to a defect in BA conjugation [41]. Moreover, an accumulation of unconjugated BAs (>85%) in plasma and urine of neonatal humans with homozygous missense mutations in *Fatp5 (Slc27a5)* has been reported [42]. Thus, the decreased Fatp5 expression in mouse models of 11β-HSD1-deficiency may explain the elevated circulating concentrations of unconjugated BAs.

Consistent with the intrahepatic accumulation of unconjugated BAs observed in liver-specific 11β-HSD1-deficient mice, the mRNA expression levels of *Cyp7a1* and *Shp* were altered, suggesting a feedback regulation by enhanced Fxr activity [7,43–45]. Since it has been shown that Shp can attenuate *Srebp1c* expression [28], and reduced hepatic *Srebp1c* mRNA levels were found in liver-specific 11β-HSD1 knockout mice upon high-fat feeding [27], we hypothesized that elevated hepatic BAs might explain this observation. However, hepatic *Srebp1c* mRNA was not altered in 11β-HSD1-deficient mice that were fed standard rodent chow and in the fasted state (data not shown), suggesting that the observed change in *Shp* expression was not sufficient to alter *Srebp1c* levels. It will be interesting to study the impact of high-fat feeding on BA homeostasis in mouse models of 11β-HSD1-deficiency to see whether this leads to an enhanced activation of Fxr-Shp and thus more pronounced attenuation of *Srebp1c* expression. An adaptation preventing further hepatic uptake of unconjugated BAs in liver-specific 11β-HSD1-deficient mice was indicated by a modest decrease in the expression of *Oatp1b2*, which mainly mediates the uptake of unconjugated BAs into hepatocytes [46]. Interestingly, the changes in BA metabolism and expression of genes involved in BA homeostasis in livers from global 11β-HSD1 knockout mice were different from those observed in liver-specific 11β-HSD1-deficient mice. The hepatic levels of unconjugated BAs were similar to those of wild-type controls and there was no change in *Cyp7a1* and *Shp* expression levels, suggesting basal Fxr transcriptional activity. On the other hand, global 11β-HSD1 disruption led to a remarkable 10-fold accumulation of circulating unconjugated BAs. These differences may be caused by adaptive mechanisms involving an up regulation of Ostb. Ostb is a single-transmembrane domain protein of the heteromeric organic transporter Osta–Ostb, which mediates the cellular efflux or uptake of BAs, depending on the electrochemical gradient of a

 

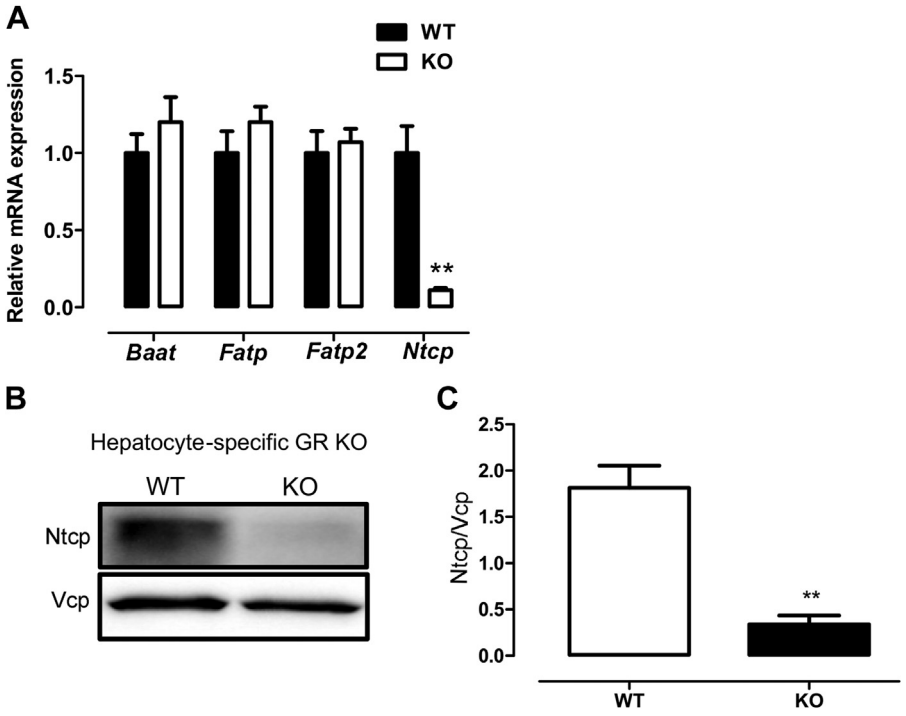


**Figure 6:** The GR does not regulate hepatic *Fatp5* expression. (A) Gene expression of *Fatp5* measured by real-time RT-PCR is not reduced in liver tissue from hepatocyte-specific GR-deficient mice despite of the significant reduction of the expression of the GR-dependent gene *Ntcp*. (B) Representative images of Western blots and semi-quantitative analysis of Ntcp protein in liver membrane preparations from wild-type (WT) ($n = 6$) and hepatocytes-specific GR-deficient mice (KO) ($n = 6$). **$p \leq 0.01$. *Baat*, Bile acid CoA:amino acid N-acyltransferase; *Fatp5*, fatty acid transport protein 5; *Fatp2*, fatty acid transport protein 2; *Ntcp*, $Na^+$-taurocholate cotransporting polypeptide; *Vcp*, valosin-containing protein.

given substrate [47]. It has been shown that for proper delivery to the plasma membrane and function the expression of both subunits Osta and Osta is essential [47–51]. Thus, elevated Ostb expression may contribute to remove the excess of unconjugated BAs from the liver into the circulation upon global 11β-HSD1 disruption by enhancing the stability of the Osta–Ostb complex [47,52]. In line with this hypothesis is the fact that although Ostb is expressed at low levels in mouse liver [47], it is dramatically up regulated in mouse models of bile duct obstruction [52]. Interestingly, in this model the Ostb transcript seem to be preferentially up regulated in comparison to Osta [52]. Also, in patients with primary biliary cirrhosis Ostb transcripts are preferentially elevated, whereas Osta levels were only modestly increased [52]. Concerning substrate specificity, Osta–Ostb mediate the transport of the major taurine and glycine conjugated BAs [47]; however, a systematic screening for substrates in different species has not yet been conducted [53]. In addition, down regulation of *Oatp1a1* might also play a role in the accumulation of unconjugated BAs in the circulation of global 11β-HSD1-deficient mice. A trend for increased levels of unconjugated BAs such as deoxycholic acid (DCA), αMCA, ωMCA and βMCA was detected in serum of mice lacking *Oatp1a1* [54]. Further studies are needed to clarify whether altered Osta–Ostb and Oatp1a1 expression is indeed involved in the accumulation of unconjugated BAs in plasma from global 11β-HSD1-deficient mice.

We initially anticipated that decreased hepatic concentrations of active glucocorticoids in 11β-HSD1-deficient mice would result in impaired GR activation and would be responsible for altered BA homeostasis and Fatp5 expression. However, hepatic corticosterone levels were not changed in liver-specific 11β-HSD1-deficient mice, indicating that the observed effects on BA profiles and Fatp5 expression are independent of GR activity. A GR-independent mechanism underlying these observations is further supported by the fact that *Fatp5* mRNA expression in livers from hepatocyte-specific GR knockout mice ($GR^{alfpCre}$) was comparable to that of wild-type controls, whereas the glucocorticoid-dependent gene *Ntcp* was dramatically reduced [9]. Moreover, we observed a modest but significant decrease of *Fatp5* in livers of 8 weeks old C57/bl6 mice after treatment with 1 mg dexamethasone/kg body weight for 24 h (data not shown). In line with a GR-independent mechanisms underlying the altered Fatp5 expression in liver-specific 11β-HSD1 knockout mice, the expression of the classical glucocorticoid-dependent gluconeogenic target genes phosphoenolpyruvate carboxykinase, glucose-6-phosphatase catalytic subunit, glucokinase, and peroxisome proliferator-activated receptor gamma coactivator 1α [27] were not or only minimally affected. Although, we observed about 50% lower corticosterone levels in livers from global 11β-HSD1-deficient mice, *Ntcp* mRNA expression and protein content only tended to be decreased, a finding consistent with the trend to elevated taurine and glycine conjugated BAs in plasma from global 11β-HSD1-deficient mice. These observations suggest that a 50% reduction of hepatic active glucocorticoids is not sufficient to significantly alter the GR-dependent transactivation of *Ntcp* or that counteracting mechanisms of enhanced GR sensitivity may exist.

Rather unexpectedly, we found markedly decreased hepatic concentrations of the inactive 11-dehydrocorticosterone in both mouse models of 11β-HSD1-deficiency. This decrease could not be attributed to an increased expression of sterol-5α- and sterol-5β-reductases. In fact, the expression of sterol-5α-reductase 1 was significantly lower than in wild-type mice. The reason for this observation remains unclear. Thus, our results suggest that a GR-independent mechanism is responsible for the reduced expression of Fatp5 in livers from mouse models of 11β-HSD1-deficiency. Besides glucocorticoids 11β-HSD1 plays a role in the metabolism of 7-ketocholesterol and 7-oxoLCA, and reduced 11β-HSD1 activity due to pharmacological inhibition or

 

genetic manipulation led to the accumulation of 7-ketocholesterol and 7-oxoLCA in rats and mice, respectively [16,19]. It is not clear at present whether the accumulation of 7-ketocholesterol or 7-oxoLCA may be responsible for the effects on gene expression and the alterations in BA homeostasis observed in the present study. 7-ketocholesterol has been shown to modulate the activity of the aryl-hydrocarbon receptor (Ahr)[55]. In contrast, little is known on possible effects of 7-oxoLCA on BA receptors and other nuclear receptors [56]. We did not observe any effect of 7-oxoLCA on human Fxr and vitamin-D receptor in assays using recombinant receptors and corresponding reporter gene constructs; however, we cannot rule out that 7-ketocholesterol or 7-oxoLCA may have caused the observed effects through binding to a yet unidentified nuclear receptor. Alternatively, a novel substrate of 11β-HSD1 might be responsible for the observed decrease in Fatp5 expression and subsequent disturbances of BA homeostasis.

Liver-specific over expression of 11β-HSD1 in mice led to the development of steatosis, enhanced hepatic lipid synthesis through an up regulation of fatty acid synthase (Fas) and impaired hepatic lipid clearance [57]. Whether increased hepatic Fatp5 activity upon over expression of 11β-HSD1 contributes to steatosis is currently unknown and this deserves further investigation. Fatp5 plays an essential role in fatty acid partitioning among metabolic active organs [38], and *Fatp5*-deficient mice display a redistribution of lipids from the liver to other tissues. Fatp5 has been proposed as a potential target for treatment of non-alcoholic fatty liver disease [37,58]. It is appealing to infer that down regulation of Fatp5 upon therapeutic intervention with selective 11β-HSD1 inhibitors may contribute to the observed beneficial effects on lipid profiles in humans [29,59]. In addition, the elevated circulating BAs upon abolishing 11β-HSD1 activity may stimulate energy expenditure in brown adipose tissue [3], ameliorate atherosclerosis [60] and enhance glucose tolerance through the activation of Tgr5 [4]. On the other hand, future studies need to address whether long-term intervention with 11β-HSD1 inhibitors may alter BA homeostasis and cause drug-induced liver injury.

## 5. CONCLUSIONS

Our findings reveal a role of 11β-HSD1 in BA homeostasis. Using transgenic mice, we showed that 11β-HSD1-deficiency resulted in elevated concentrations of unconjugated BAs in the circulation. The effects were caused, at least in part, by a decreased hepatic expression of Fatp5, likely by a glucocorticoid-independent mechanism. The elucidation of novel 11β-HSD1 functions is important with respect to the development of 11β-HSD1 inhibitors for clinical applications.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2014.04.008.

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