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## RESEARCH LETTER

### The Apical Sodium-Dependent Bile Acid Transporter in Cholangiocytes Is Not Required for the Generation of Bile Flow in Mice



Bile flow is generated by the osmotic activity exerted by the secretion of bile acids and other solutes from hepatocytes into bile canaliculi. The bile duct system, lined by cholangiocytes, can subsequently alter the bile composition, but it has remained unclear whether bile acid transporters in cholangiocytes quantitatively contribute to the generation of bile flow *in vivo*. Classically, bile flow has been subclassified into a *bile acid-dependent* component, indicating the linear positive correlation between bile acid secretion and bile flow generation, and a *bile acid-independent* component, the Y-intercept of the bile acid secretion to bile flow correlation. The magnitude of bile acid-dependent bile flow (the slope of the linear correlation between bile flow and bile acid secretion rate, also termed “apparent choleric activity”) depends on the physicochemical properties of different bile acid species, which determine their osmotic pressure. Unconjugated ursodeoxycholic acid (UDCA) and its C<sub>23</sub> side chain-shortened analogue *nor*-UDCA can induce hypercholerisis, that is, much more bile flow than expected based on bile acid secretion rate. This characteristic is suggested to be beneficial in the treatment of cholestatic disorders. The proposed mechanism for hypercholerisis is the cholehepatic shunt, involving (passive) reuptake of these unconjugated bile acids by cholangiocytes, subsequent return to hepatocytes, and resecretion into canaliculi (Figure 1A).<sup>1,2</sup>

Conjugation of bile acids prevents their passive reabsorption across epithelia. Unconjugated bile acids,

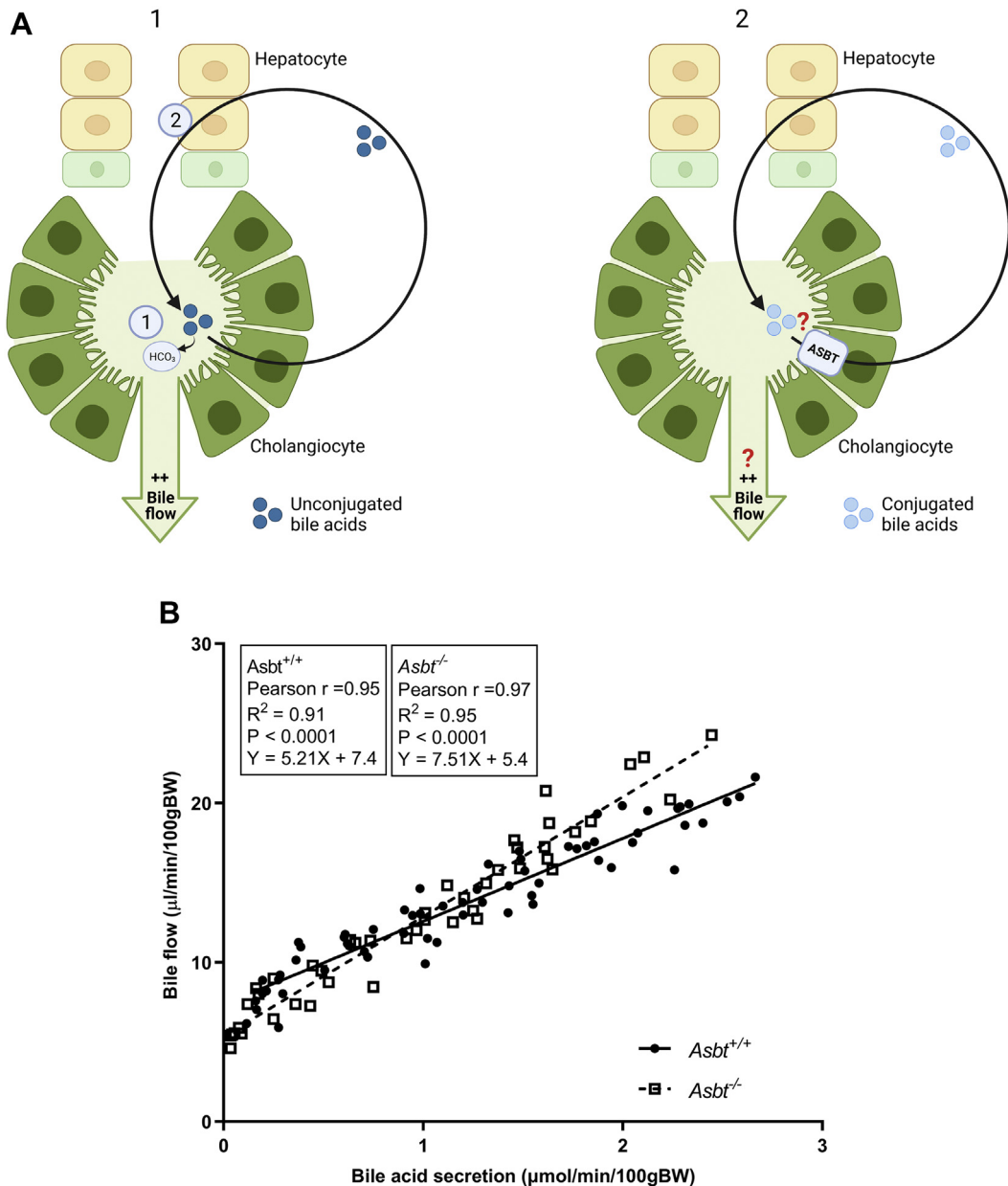
which have the potential to undergo cholehepatic shunting, are rarely found in bile, due to highly efficient hepatic conjugation of newly synthesized and of intestinally (re)absorbed bile acids. Because the apical sodium-dependent bile acid transporter (ASBT), the major bile acid transporter in the intestine, is expressed at the apical membrane of mouse and rat cholangiocytes, it has been hypothesized that ASBT in cholangiocytes is involved in the uptake of *conjugated* bile acids, thereby mediating their cholehepatic shunting, even under physiological conditions.<sup>3,4</sup> This hypothesis is supported by experiments in bile duct-ligated rats, where secretin induced ASBT translocation to the cholangiocyte plasma membrane (which would increase the bile acid uptake capacity of cholangiocytes), increased bile flow, and prolonged biliary transit time of the administered conjugated bile acid.<sup>5</sup> Pharmacological inhibition of ileal ASBT to interrupt the enterohepatic circulation of bile acids is being considered for the treatment of a number of liver and metabolic diseases, including cholestasis.<sup>6</sup> It has remained unclear, however, whether ASBT in *cholangiocytes* contributes to bile flow generation. Here, we investigated the contribution of cholangiocyte ASBT in the generation of bile flow *in vivo* (Figure 1A) by determining whether absence of ASBT decreases the choleric capacity of tauro-UDCA (TUDCA), a conjugated bile acid with affinity for ASBT.<sup>7</sup> To this end, we administered intravenous TUDCA to *Asbt*<sup>-/-</sup> and *Asbt*<sup>+/+</sup> mice and quantified bile flow and bile acid secretion rate, both with and without secretin treatment (Supplementary Methods, mice characteristics in Table A1).

Before TUDCA administration, bile flow and bile acid secretion rate were lower in *Asbt*<sup>-/-</sup> mice than those in *Asbt*<sup>+/+</sup> mice, in line with previous studies<sup>8</sup> (Figure A1A and B, 5.5 vs 7.6  $\mu\text{l}/\text{min}/100\text{-g BW}$ , respectively,  $P = .003$ ; and, 0.043 vs 0.195  $\mu\text{mol}/\text{min}/100\text{-g BW}$ , respectively,  $P = .003$ ). This

observation can be explained by a smaller bile acid pool secondary to the reduced ileal bile acid reabsorption in *Asbt*<sup>-/-</sup> mice.<sup>8</sup> To obtain similar bile acid secretion rates in the 2 genotypes, we infused TUDCA at stepwise increasing dosages. At a TUDCA infusion rate of 0.3  $\mu\text{mol}/\text{min}$ , bile flow was similar in *Asbt*<sup>-/-</sup> and *Asbt*<sup>+/+</sup> mice (Figure A1A and B). In both groups, bile flow and bile acid secretion rate increased with escalating TUDCA doses (Figure A1A and B), and bile flow correlated linearly and positively with bile acid secretion rate (Figure 1). The apparent choleric activity of TUDCA (the slope of the regression line in Figure 1) was *higher* in *Asbt*<sup>-/-</sup> mice than in *Asbt*<sup>+/+</sup> mice (Figure A1C, 7.2 vs 5.2  $\mu\text{l}/\mu\text{mol}$ ,  $P = .003$ ), demonstrating that ASBT absence did not decrease the choleric activity of TUDCA.

Previous research showed that secretin induces ASBT translocation to the plasma membrane, which has been proposed to increase the bile acid reuptake capacity of cholangiocytes.<sup>5</sup> To test whether the action of secretin on ASBT uptake capacity is required to induce hypercholerisis via cholehepatic shunting of TUDCA, we conducted an additional experiment with constant rate TUDCA infusion, followed by secretin treatment after reaching a stable biliary bile acid secretion rate (Supplementary Methods, mice characteristics in Table A2, Figure A2A). While secretin administration did not affect bile acid-dependent bile flow (Figure 2A, Figure A2A), it increased non-bile acid-dependent (probably ductal) bile flow (Figure 2B, Figure A2B), in accordance with previous literature.<sup>9</sup> This, however, was not different between *Asbt*<sup>-/-</sup> and *Asbt*<sup>+/+</sup> mice (Figure 2B, Figure A2B, +3.3 vs +3.0  $\mu\text{l}/\text{min}/100\text{-g BW}$ ,  $P = .4$ ), indicating that the increase in bile flow caused by secretin is independent of ASBT presence or activation.

Although our study does not prove nor disprove the occurrence of cholehepatic shunting of conjugated bile

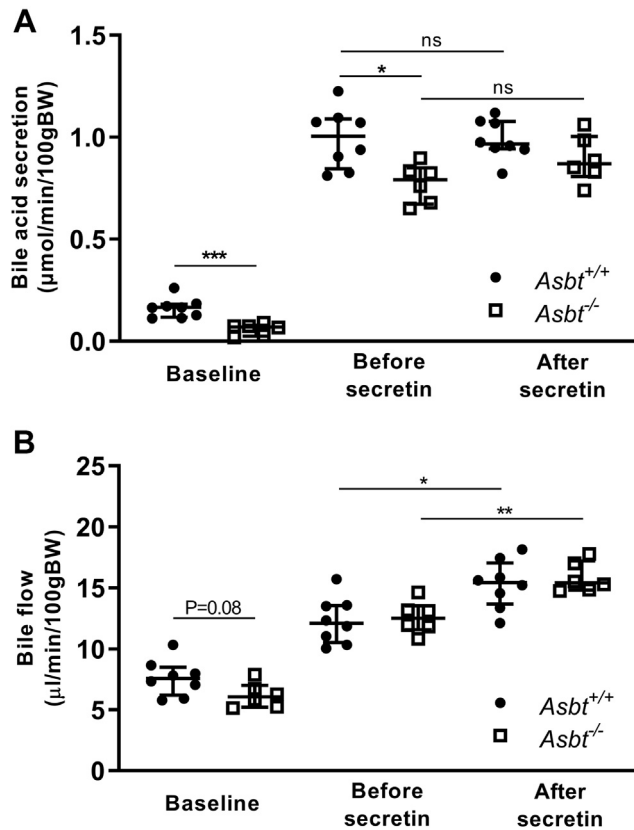


**Figure 1.** (A) Cholehepatic shunting of (1) unconjugated bile acids by passive diffusion across cholangiocytes, and (2) hypothesis tested, of conjugated bile acids via ASBT-mediated transport into cholangiocytes. (1) Unconjugated bile acids (such as nor-UDCA) can induce hypercholeresis thanks to their ability to undergo cholehepatic shunting. Cholehepatic shunting likely increases bile flow via the following mechanisms: (i) After their secretion from hepatocytes into bile, unconjugated bile acids in biliary ducts can be passively taken up by cholangiocytes, leaving a bicarbonate ion behind upon protonation, and (ii) following passive uptake by cholangiocytes, unconjugated bile acids can be returned to hepatocytes, which resecretate them into the canaliculus, thereby providing additional osmotic drive (bile acid-dependent bile flow). (2) The hypothesis tested in this study: ASBT expression on the apical membrane of cholangiocytes is responsible for cholehepatic shunting of conjugated bile acids (such as TUDCA), thereby contributing to bile flow. Due to their high polarity, conjugated bile acids do not readily cross membranes by passive transport. Our results do not offer support for the tested hypothesis. The figure was created with [BioRender.com](https://www.biorender.com). (B) Bile flow in relation to bile acid secretion rate in *Asbt*<sup>-/-</sup> and *Asbt*<sup>+/+</sup> mice. Data points represent different time points of individual mice.

acids, our results do not support a role for ASBT in mediating cholehepatic shunting of conjugated bile acids under physiological conditions, as absence of ASBT did not decrease the choleretic capacity of TUDCA, neither

with nor without secretin. Instead, absence of ASBT slightly increased the choleretic capacity of TUDCA. We speculate that compensatory mechanisms, such as differences in the expression and function of proteins

involved in the creation of bile flow arising as adaptations to the absence of ASBT, could potentially explain this observation. We cannot exclude that ASBT expressed by cholangiocytes lining the *gallbladder* in the WT mice



**Figure 2.** Bile acid secretion rate (A) and bile flow (B) in *Asbt*<sup>-/-</sup> and *Asbt*<sup>+/+</sup> mice. Data of individual mice before TUDCA infusion (baseline); averages of datapoints during stable TUDCA infusion (before secretin); and after secretin administration during stable TUDCA infusion (after secretin). \**P* < .05, \*\**P* < .01, \*\*\**P* < .001.

does not mediate cholehepatic shunting, as the gallbladder was cannulated and thus unavailable for potential bile acid uptake in this study. Our conclusion that cholangiocyte ASBT does not mediate cholehepatic shunting thus applies to intrahepatic and extrahepatic, but not gallbladder, cholangiocytes. ASBT in cholangiocytes may have other functions that are not tested in our experiment, such as mediating bile acid-modulated nuclear receptor signaling.<sup>10</sup> Our *in vivo* results do not support the conclusions from bile duct-ligated rat experiments,<sup>5</sup> which presented indications for cholehepatic shunting of conjugated bile acids. Because we performed our study only under physiological conditions, it is still possible that cholehepatic shunting of conjugated bile acids via ASBT occurs

under pathological conditions, such as cholestasis.<sup>5</sup> Further studies are required to investigate whether this is the case.

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## Supplementary Materials

Material associated with this article can be found in the online version at

<https://doi.org/10.1016/j.gastha.2022.02.003>.

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**Abbreviations used in this paper:** ASBT, apical sodium-dependent bile acid transporter; TUDCA, tauro-UDCA; UDCA, unconjugated ursodeoxycholic acid

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The authors disclose no conflicts.

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**Ethical Statement:**

The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

**Data Transparency Statement:**

Data, analytic methods, and study materials will be available upon request.