Thematic Review Series: Bile Acids

Bile acid transporters

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Abstract

In liver and intestine, transporters play a critical role in maintaining the enterohepatic circulation and bile acid homeostasis. Over the past two decades, there has been significant progress toward identifying the individual membrane transporters and unraveling their complex regulation. In the liver, bile acids are efficiently transported across the sinusoidal membrane by the Na\(^+\)taurocholate cotransporting polypeptide (NTCP), the bile salt export pump (BSEP), the apical sodium-dependent bile acid transporter (ASBT), and the organic solute transporter OST\(\alpha\)-OST\(\beta\). In addition to the hepatocyte and enterocyte, subgroups of these bile acid transporters are expressed by the biliary, renal, and colonic epithelium where they contribute to maintaining bile acid homeostasis and play important cytoprotective roles. This article will review our current understanding of the physiological role and regulation of these important carriers.

Supplementary key words   bile acids • cholesterol • nuclear receptors • cholestasis • enterohepatic circulation

Transporters have always assumed a prominent place in biology and human lore. Perhaps the most well known transporter is Charon, the ill-tempered old ferryman of Greek mythology whose task was to transport souls of the deceased across the river Acheron into Hades. Charon would ferry into Hades only those souls that would pay his toll while others were left to wander the shore for a hundred years before being delivered. This review focuses on another group of ferrymen: the Na\(^+\)taurocholate cotransporting polypeptide (NTCP), the bile salt export pump (BSEP), the apical sodium-dependent bile acid transporter (ASBT), and the organic solute transporter OST\(\alpha\)-OST\(\beta\), the major bile acid transporters that control the fate of bile acids, either absorption and enterohepatic cycling or excretion and elimination from the body. The role of these transporters in maintaining bile acid homeostasis and their regulation under physiological and pathophysiological conditions is discussed. This article complements recent comprehensive reviews of bile acid chemistry and physiology (1), bile acid transporter structure and function (2, 3), bile acid synthesis (4), and bile acid signaling (5–7).

Bile acids are synthesized from cholesterol in the liver and secreted into the small intestine where they facilitate absorption of fat-soluble vitamins and cholesterol (1). The majority of bile acids are reabsorbed from the intestine and returned to the liver via the portal venous circulation. At the hepatocyte sinusoidal membrane, bile acids are extracted and resorbed into bile (8). This enterohepatic circulation of bile acids is an extremely efficient process; less than 10% of the intestinal bile acids escapes resorption and is eliminated in the feces. In the small intestine, bile acids are absorbed by both passive and active mechanisms (9). Whereas passive absorption occurs down the length of the intestine, active absorption of bile acids is restricted to the ileum (10, 11). In man and all other vertebrates examined to date, the ileal epithelium has developed an efficient transport system for the active reclamation of bile acids.

Abbreviations: ASBT, apical sodium-dependent bile acid transporter; BRIC, benign recurrent intrahepatic cholestasis; BSEP, bile salt export pump; FXR, farnesoid X receptor; HNF, hepatocyte nuclear factor; IL, interleukin; JNK, Jun N-terminal kinase; LPS, lipopolysaccharide; LRH, liver receptor homolog; MRP, multidrug resistance protein; NTCP, Na\(^+\)taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptide; OST, organic solute transporter; PFIC, progressive familial intrahepatic cholestasis; RAR/RXR, retinoic acid receptor/retinoid X receptor; SHP, small heterodimer partner; TNF\(\alpha\), tumor necrosis factor alpha.

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of bile acids (1). The enterohepatic circulation maintains a bile acid pool size of approximately 4 mg in mice and 2 to 4 g in humans. This pool cycles multiple times per meal (12, 13) and as such, the intestinal bile acid absorbed may be as much as 20 mg/day in mice and 30 g/day in humans. Hepatic conversion of cholesterol to bile acid balances fecal bile acid excretion and this process represents a major route for elimination of cholesterol from the body (14, 15).

Evolution of an ileal active transport system and gallbladder improved the efficiency of intestinal lipid absorption by dissociating hepatic bile acid secretion from bile acid synthesis. This ensures a continuous supply of bile acids to be used repeatedly for lipid absorption during the digestion of a single meal or multiple meals throughout the day (16). Efficient intestinal reabsorption and hepatic extraction of bile acids also enables a very effective recycling and conservation mechanism that largely restricts these potentially cytotoxic detergents to the intestinal and hepatobiliary compartments. At a fundamental level, the bile acid enterohepatic circulation can be viewed as a series of storage chambers (gallbladder, small intestine), valves (sphincter of Oddi, ileocecal valve), mechanical pumps (canaliculi, biliary tract, small intestine), and chemical pumps (hepatocytic and enterocytic transporters). Over the past two decades, significant progress has been made to identify the major hepatic and intestinal transporters that function to maintain the enterohepatic circulation of bile acids (3, 17). These are summarized in Fig. 1.

HEPATOCELLULAR TRANSPORT OF BILE ACIDS

Approximately 95% of the bile acids secreted into bile are derived from the recirculating pool. To maintain this process, liver parenchymal cells must transport bile acids efficiently from the portal blood into bile. This vectorial trans-hepatocellular movement of bile acids is a remarkably concentrative transport process that is driven by a distinct set of primary (ATP-dependent), secondary (Na⁺ gradient-dependent), and tertiary (OH⁻ or HCO₃⁻ dependent anion exchange) transport systems at the sinusoidal and canicular plasma membranes (2, 3). Hepatocellular uptake of bile acids occurs against a presumed unfavorable electrochemical gradient (18, 19) via sodium-dependent and independent mechanisms. The uptake of conjugated (i.e., N-acyl amidated with taurine or glycine) bile acids, such as taurocholate, at the sinusoidal membrane is mediated predominantly (>75%) by secondary active Na⁺-dependent transport. In contrast to conjugated bile acids, Na⁺-dependent uptake accounts for less than half of the uptake of unconjugated bile acids (20–22). The Na⁺-dependent sinusoidal membrane uptake is mediated by the NTCP, whereas members of the organic anion transporting polypeptide (OATP) family are responsible for the Na⁺-independent transport.

After uptake, conjugated bile acids are shuttled across the hepatocyte to the canicular membrane for secretion into bile; unconjugated bile acids follow a similar path after first undergoing N-acyl amidation to taurine or glycine (1). Whereas bile acid concentrations within the hepatocyte are presumed to be in the micromolar range, canicular bile acid concentrations are as much as 1000-fold higher, necessitating active transport across the canicular membrane (23). BSEP is the transporter responsible for canicular transport of the major bile acid species (24), whereas secretion of sulfated bile acids or unusual bile acid such as tetra-hydroxylated forms is mediated by other ABC transporters, including the multidrug resistance protein (MRP)-2 (ABCC2) (25, 26) and multidrug resistance protein (MDR)-1 (ABCB1) (27).

An area of active investigation is the identification and analysis of transporters responsible for the sinusoidal efflux of bile acids and other organic solutes from the interior of the hepatocyte into the space of Disse. Whereas sinusoidal membrane bile acid transport is overwhelmingly in the direction of uptake under normal physiological conditions, bile acids can also be effluxed as an important protective mechanism to reduce bile acid overload under cholestatic conditions (28). Much of this work has focused on members of the MRP (ABCC) family of ATP-dependent transporters, in particular, MRP3 and MRP4 (29–31), and on the heteromeric transporter, OSTα-OSTβ (32, 33). MRP3 and MRP4 are expressed on the sinusoidal membrane of hepatocytes (29, 34) and have bile acid transport (30) or glutathione-bile acid cotransport activity (35). The expression of these transporters is induced under cholestatic conditions to promote bile acid efflux (3, 33, 36–40), thus lowering the concentration in the hepatocyte and decreasing the likelihood of apoptosis or necrosis (41). Our understanding of the relative contribution of the different efflux transporters is still developing; however, the most recent results point toward especially important roles of MRP4 and OSTα-OSTβ (33, 39, 42). The increased expression of these transporters is an important part of the adaptive response to conditions of bile acid overload that also includes downregulation of the major liver bile acid uptake transporters, NTCP, and members of the OATP family (28).

NTCP

NTCP (gene name SLCO1A1) is the founding member of the SLC10 family of solute carrier proteins, which includes two bile acid carriers (SLC10A1/NTCP and SLC10A2/ASBT), one steroid sulfate transporter (SLC10A6/SOAT), and four orphan carriers (SLC10A3, SLC10A4, SLC10A5, SLC10A7) (43–45). NTCP, a 349 amino acid membrane glycoprotein, functions as an electrogenic sodium-solute cotransporter and moves 2 or more Na⁺ ions per molecule of solute (46, 47). NTCP’s major physiological substrates include all the major glycine and taurine-conjugated bile acids (48–52). Depending on the structure of the bile acid and NTCP ortholog, unconjugated bile acids are moderate or weak substrates (50, 53, 54) and sulfated bile acids appear to be only weakly transported (55). In contrast to NTCP, members of the OATP
family expressed on the hepatocyte sinusoidal membrane, such as Oatp1a1 (Slco1a1), efficiently transport unconjugated or sulfated bile acids, suggesting that these carriers participate in the hepatic uptake of those bile acid species in vivo (50, 56, 57). The high level of NTCP expression at the sinusoidal membrane of hepatocytes and NTCP’s high affinity for conjugated bile acids promotes their efficient extraction from portal blood. Thus, NTCP functions to maintain the enterohepatic circulation of bile acids and keeps plasma concentrations at a minimum. Unlike the related ileal apical sodium bile acid transporter (ASBT or IBAT; SLC10A2), NTCP also interacts with a variety of drugs and steroids (51, 58–60). Although it is not yet clear how many of these compounds are competitive or non-competitive inhibitors of NTCP versus actual substrates, this broader inhibitor profile supports a possible role for NTCP in the clearance of some drugs or drug metabolites.

The properties of NTCP satisfied all the functional criteria for hepatocytic Na+-coupled bile acid uptake, including: 1) preferential high affinity transport of conjugated bile acids; 2) kinetics for taurocholate transport similar to isolated hepatocytes; 3) electrogenic Na+-taurocholate uptake (46); 4) appropriate tissue-specific expression in the liver; 5) similar ontogeny for Na+-dependent bile acid uptake and NTCP expression in development (61); and 6) the finding that NTCP-specific antisense oligonucleotides decreased Na+-dependent taurocholate uptake by more than 90% in rat liver mRNA-injected Xenopus oocytes (62). Although the presence of additional Na+-dependent bile acid transporters, such as microsomal epoxide hydrolase, has been suggested (63), the current evidence indicates that NTCP accounts for most, if not all, hepatic sinusoidal membrane Na+-dependent bile acid transport (43). However, genetic evidence supporting a primary role of NTCP in hepatic sinusoidal bile acid uptake is still lacking. NTCP null mice have not yet been described. Polymorphisms that interfere with bile acid transport in vitro were reported as part of an analysis of human NTCP genotypes in different ethnic groups (64). Unfortunately, there was no description of the in vivo bile acid phenotype associated with those polymorphisms. In addition, whereas inherited disorders characterized by a relatively isolated elevated plasma bile acid level (hypercholanemia; part of the pre-
dicted phenotypes for a defect in hepatic bile acid uptake) have been described, mutations in genes other than NTCP, such as the tight junction proteins ZO-2 and claudin-1, were found to be responsible for these diseases (65, 66). These findings leave open the possibility that an isolated NTCP gene defect may be asymptomatic as hepatocytes also express Na⁺-independent bile acid transporters.

**Regulation of NTCP expression and activity**

Substrate, cytokines, liver injury, and hormones all regulate transcription of the NTCP gene (2, 67). Although the molecular mechanisms and transcription factors may differ, a common theme among these major regulatory pathways is the suppression of NTCP gene expression as an adaptive response to reduce bile acid entry into the hepatocyte. In fact, the short-term modulation of NTCP activity to match Na⁺-dependent bile acid uptake to the bile acid load under physiological conditions may be largely controlled by posttranscriptional mechanisms (68). Under pathophysiological conditions, numerous mechanisms exist to decrease NTCP transcription. As described below, bile acids feed back to downregulate NTCP gene transcription by FXR-dependent and independent mechanisms to prevent cytotoxic bile acid accumulation. NTCP transcription is also decreased with different forms of cholestatic liver disease, including those associated with inflammation, ethinylestradiol and pregnancy, obstruction, and drugs or toxins, again as part of a coordinated response to reduce the extent of liver injury (67).

With regard to substrate, NTCP transcription is downregulated in a complex fashion by bile acids via direct or indirect mechanisms that potentially involve farnesoid X receptor (FXR), small heterodimer partner (SHP), retinoic acid receptor alpha (RARα), liver receptor homolog (LRH)-1, and forkhead box A2 (FOXa2) (69–76). FXR does not interact directly with the NTCP promoter but induces expression of other factors to indirectly repress NTCP expression. In the rat, bile acids act via FXR to induce the expression of SHP, which in turn can interfere with RXRα:RARα activation of the NTCP promoter. SHP may also be acting via HNF-4α and HNF-1α to repress rat NTCP expression (72, 73). In the mouse, NTCP expression is significantly reduced in HNF-4α null mice (77) and direct promoter analysis revealed HNF-4α strongly activates NTCP expression (78). HNF-4α, working in conjunction with peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), strongly activates NTCP expression and may be important for the increase in NTCP expression and liver sinusoidal bile acid uptake observed during fasting (79). In addition to the pathways involving RXRα:RARα and HNF-1α, several lines of evidence indicate that other mechanisms must exist for bile acid-dependent downregulation of NTCP. First, although HNF-1α and RXRα:RARα bind and activate the rat NTCP promoter, these factors do not appear to activate the mouse or human NTCP minimal promoters (73). Second, NTCP mRNA expression is still decreased by cholic acid feeding in SHP null mice, indicating that SHP-independent mechanisms must exist to mediate this repression of NTCP expression (80). The finding that the rat, mouse, and human NTCP promoters all include a consensus Foxa2 binding motif led to the suggestion that this may be a common pathway for NTCP regulation (73). However, recent studies argue against a direct role of Foxa2 in mediating the repression of NTCP by bile acids (81). Finally, some of the regulatory actions of bile acids are mediated through activation of the c-Jun N-terminal kinase (JNK) (82). JNK-dependent phosphorylation of RXRα has been shown to reduce RXRα:RARα binding and activation of the rat NTCP promoter (83), providing a potential mechanism to regulate NTCP expression independent of SHP.

With regard to inflammation, NTCP expression is rapidly downregulated following administration of lipopolysaccharide (LPS) or the inflammatory cytokines tumor necrosis factor alpha (TNFα), interleukin (IL)-1β, or IL-6 (84–87). Under conditions of inflammation-induced cholestasis, IL-1β appears to play a major role in regulating NTCP expression (88), although there must be some redundancy because LPS still downregulated NTCP expression in IL-1β receptor and TNFα receptor1 knockout mice (89). In contrast to IL-1β or TNFα, IL-6 does not appear to be important for downregulation of NTCP during the early stages of LPS-induced inflammation (85, 89). The downregulation of NTCP is mediated by FXR-independent mechanisms (90, 91) that potentially involve decreased RXRα expression, inactivation of RXRα by phosphorylation via mitogen-activated protein (MAP) kinase 4 and JNK (83, 92), and decreased nuclear levels of RXRα (93–95). In addition to affecting RXRα:RARα, LPS could downregulate NTCP expression by reducing HNF-1α expression and activity (86, 88). The regulation via HNF-1α may be secondary to LPS or IL-1β downregulation of HNF-4α (96), an important positive regulator of HNF-1α expression (97, 98).

In addition to regulation by substrate or under conditions of inflammation-induced cholestasis, NTCP is also downregulated in other forms of cholestatic liver injury, including obstructive, drug- or toxin-induced, and estradiol-induced cholestasis (67). In the early stages of obstructive cholestasis, NTCP transcription is downregulated by SHP-dependent mechanisms similar to those involved in the regulation by bile acids (90, 91, 99). At later stages, proinflammatory cytokines may contribute to the sustained downregulation of NTCP expression via RXRα:RARα-dependent mechanisms (91). NTCP expression is regulated during drug- or toxin-induced liver injury and during the liver regeneration that occurs in response to injury. After partial heparctomy or in the carbon tetrachloride model of toxic liver injury, NTCP mRNA expression is rapidly decreased (100, 101). Inactivation of TNFα but not IL-1β prevented this downregulation of NTCP during liver regeneration, underscoring the central role of TNFα in the regulation of NTCP during this process (88).

With regard to hormones, one of the best-characterized mechanisms of transcriptional regulation is the induction
by prolactin and growth hormone via recruitment of phosphorylated Stat5 to the NTCP promoter (102, 103). Glucocorticoids directly induce expression of NTCP via glucocorticoid receptor binding to the promoter in a mechanism that is enhanced by PGC-1α and suppressed by SHP (76). Finally, NTCP mRNA expression is decreased by estrogen and increased by thyroid hormone (104).

NTCP transport activity is also regulated posttranscriptionally. Cyclic AMP (cAMP) rapidly (within minutes) stimulates Na\(^+\)-taurocholate cotransport in hepatocytes by increasing the transport maximum (V\(_{\text{max}}\)) (105, 106). This increase in transport activity is due to cAMP-induced movement of NTCP from an intracellular compartment to the plasma membrane (68). The sinusoidal plasma membrane expression of NTCP is regulated by serine/threonine phosphorylation and dependent on microfilaments (107–109). NTCP is dephosphorylated at Serine 226 (110) in response to cAMP, leading to increased plasma membrane NTCP retention. The cAMP-induced dephosphorylation of NTCP is dependent on its ability to increase cytosolic Ca\(^{2+}\) and activate protein phosphatase 2B (PP2B), a calcium/calmodulin dependent serine/threonine protein phosphatase (111). In addition to this PP2B-mediated pathway, NTCP’s microfilament-dependent insertion and retrieval from the plasma membrane is regulated by PI3-kinase (68). This PI3-kinase stimulated translocation is thought to be mediated in part via protein kinase C delta, as well as other signaling pathways (112–115).

**BSEP**

Functional evidence of ATP-dependent bile acid transport by the canalicular membrane existed since the early 1990s (23). After cloning and expression studies identified a novel canalicular ABC transporter closely related to the multidrug resistance protein (MDR1)/P-glycoprotein, this 160 kDa protein (originally named “sister of P-glycoprotein”) was shown to transport conjugated bile acids efficiently and was subsequently renamed the bile salt export pump (BSEP; ABCB11) (24). The properties of BSEP satisfied all the functional criteria for hepatocytic canalicular bile acid export, including: 1) preferential high affinity transport of conjugated bile acids (116, 117); 2) appropriate tissue-specific expression in the liver; and 3) appropriate ontogeny of expression (118, 119). The role of BSEP as the major canalicular bile acid efflux pump was confirmed by the identification of ABCB11 mutations in patients with progressive familial intrahepatic cholestasis (PFIC) type 2, a hepatic cholestasis disorder characterized by biliary bile acid concentrations less than 1% of normal (120, 121). In addition to unconjugated, taurine-conjugated, and glycine-conjugated species of monovalent bile acids, human BSEP (but not rodent) also transports some sulfated bile acids such as tauroliothospholylcholate (122). Whereas bile acids are the major physiological substrate, BSEP appears to be able to transport a limited number of drugs such as the HMG-CoA reductase inhibitor, pravastatin (123). Other drugs, such as cyclosporin, rifamycin, troglitazone, and glibenclamide, are not substrates but interact with BSEP and inhibit bile acid export. Direct inhibition of BSEP may be an important mechanism of hepatotoxicity for drugs inducing cholestasis (24, 117, 124).

Defects in BSEP are responsible for inherited forms of liver disease. Depending on the clinical course, BSEP deficiency is categorized as PFIC2 or benign recurrent intrahepatic cholestasis (BRIC) type 2. PFIC2 is associated with low bile acid secretion, failure to thrive, intractable pruritus, progressive cholestasis, and a significantly increased risk for hepatobiliary malignancy (121, 125). In patients with PFIC2, BSEP protein is typically absent or significantly reduced in liver biopsy specimens, suggesting that these mutations impair BSEP synthesis, cellular trafficking, or stability (121). BRIC2 is associated with milder forms of liver disease and with less severe BSEP defects such as missense mutations (126). However, it should be noted that some missense mutations cause severe forms of BSEP deficiency by altering premRNA splicing as described in a recent comprehensive analysis of the major BSEP sequence variants (127). The BSEP mutations associated with milder disease may also confer increased risk for development of acquired forms of cholestasis, including intrahepatic cholestasis of pregnancy (ICP) and drug-induced cholestasis (128, 129).

**Regulation of BSEP expression and activity**

BSEP mRNA expression is induced when hepatocyte bile acid levels are elevated, such as following dietary challenge with bile acids (130), or under certain cholestatic conditions (32, 131, 132). This is due to a direct activation of the human and rodent BSEP genes by bile acids acting via FXR (133, 134). In addition to identification of an FXR-responsive element in the BSEP promoter, inactivation of FXR in mice results in low levels of BSEP expression that are not induced by bile acid feeding (135, 136). The induction of BSEP expression is not a universal property of all species of bile acids and correlates with the FXR ligand specificity (137, 138). In fact, lithocholic acid may act as a partial FXR antagonist to reduce BSEP expression (139), thereby providing another potential mechanism for lithocholic acid’s cholestatic effects (140). The FXR stimulation of BSEP expression appears to require recruitment of the chromatin-modifying enzymes, coactivator-associated arginine methyltransferase 1 (CARM1) and arginine methyltransferase (PRMT1), and subsequent chromatin remodeling (141, 142). Another potential regulator of BSEP is vitamin A. FXR functions as a heterodimer with RXR, and in vitro studies suggest that 9-cis retinoic acid activation of RXR inhibits the FXR-induced transcription of human BSEP (143). This observation was recently confirmed and diet studies in mice showed that the combination of vitamin A-deficiency and cholic acid feeding resulted in the highest levels of BSEP expression (144).

In addition to transcriptional regulation, there is considerable evidence for posttranscriptional regulation of the BSEP protein localization to the canalicular membrane (24). This short-term regulation enables the hepatocyte to rapidly modulate bile acid secretion in response...
to bile acid flux and to pathophysiological conditions such as cholestasis (68). Another rapidly developing area of study is the role of membrane lipid composition in the regulation of BSEP and other canalicular membrane transporters (145, 146). There is emerging evidence that the phosphatidyserine flipase, ATP8B1, plays a critical role in maintaining the phospholipid asymmetry and rigid cholesterol and sphingomyelin-rich exoplasmic leaflet of the canalicular membrane (147). In humans, ATP8B1 deficiency causes PFIC Type 1 (PFIC1) and BRIC Type 1 (BRIC1) (145). Among the mechanisms responsible for the cholestasis, inactivation of ATP8B1 results in a reduced cholesterol to phospholipid ratio for the canalicular membrane. This reduced cholesterol content is associated with a dramatic reduction in the V_{max} for taurocholate transport by BSEP (146) but not reduced canalicular levels of BSEP protein (148).

INTESTINAL TRANSPORT OF BILE ACIDS

Bile acids are reclaimed through a combination of passive absorption in the proximal small intestine, active transport in the distal ileum, and passive absorption in the colon. Bile acids are actively transported in the terminal ileum by the well-characterized ASBT (SLC10A2) (43, 149). This sodium- and potential-driven transporter moves bile acids from the lumen of the small intestine across the apical brush border membrane. Bile acids are then shuttle to the basolateral membrane and effluxed into the portal circulation by OST (147). This sodium- and potential-driven transporter moves bile acids from the basolateral membrane into the enterocyte apical brush border membrane. This was confirmed by analysis of ASBT null mice in which intestinal bile acid absorption was largely eliminated. A key observation was that feeding ASBT null mice a diet containing cholestyramine resulted in no further increase in fecal bile acid excretion. Because a bile acid binding resin would reduce alternative (nonASBT) passive or active mechanisms for intestinal bile acid absorption, these findings supported the conclusion that nonASBT mechanisms contribute little to intestinal bile acid absorption in the mouse (157).

In humans and many other species, the bile acid pool includes glycine conjugates, unconjugated bile acids, and more hydrophobic species (1). A fraction of the glycine conjugates and unconjugated bile acids are protonated under conditions of the acidic luminal surface pH in the intestine (158) and absorbed by passive diffusion across the apical brush border membrane (155). In addition to passive membrane diffusion, there is evidence for carrier-mediated transport of bile acids in the proximal small intestine. Studies using photoactivated bile acid derivatives specifically labeled an ~87 kDa brush border membrane protein that was present along the entire length of rabbit small intestine (159). Elegant in vivo uptake and cis-inhibition studies using perfused jejunum demonstrated bile acid transport specificity consistent with a facilitative carrier (160, 161). In vitro studies using rat jejunal brush border membrane vesicles extended those findings and clearly demonstrated conjugated bile acid uptake operating through an anion exchange mechanism (162). The properties of this transport system are similar to those ascribed to the OATP family transporters and a candidate for the jejunal bile acid transporter was cloned from rat (163). This ~80 kDa membrane glycoprotein, Oatp1a5 (Oatp3), exhibits expression properties, substrate specificity, and apical membrane localization consistent with the in vivo and in vitro studies of proximal small intestine (161, 162). Rodent Oatp1a5 is encoded as part of an OATP gene cluster and is syntenic with a region on human chromosome 12p12 that includes OATP1A2 (164). Human OATP1A2, which shares ~72% amino acid identity with rodent Oatp1a5, is expressed at the apical brush border membrane of human small intestinal epithelial cells and transports bile acids as well as a variety of drugs (165, 166) (167). Although these findings are suggestive, it should be noted that the intestinal level of expression for Oatp1a5/OATP1A2 is very low compared with the ASBT (163, 168) and their contribution to intestinal bile acid absorption is thought to be small as compared with ASBT-mediated transport in the ileum (157, 169).

Regardless of the mechanism of bile acid uptake, these weak acids will ionize at the neutral pH of the cytosolic compartment, potentially trapping bile acids in the cell and necessitating the presence of efflux carriers. Recent studies have identified OSTα-OSTβ as a major mechanism responsible for intestinal basolateral membrane bile acid export (170, 171). Although highly expressed in terminal ileum, OSTα-OSTβ is also expressed at lower levels in proximal small intestine of rodents and humans (150, 151, 172) where it can serve to export bile acids that were passively absorbed across the apical brush border membrane of the enterocyte.
ASBT

Bile acids are transported actively across the ileal brush-border membrane by the well-characterized ASBT (SLC10A2). The relationship between the hepatic, biliary, ileal, and renal Na⁺-bile acid cotransporter systems was resolved with the cloning of the bile acid carriers from those tissues. The liver and ileum express distinct but related Na⁺ bile acid cotransporter genes, NTCP (SLC10A1) and ASBT (SLC10A2), whereas the ileal enterocyte, renal proximal tubule cell, and cholangiocyte all express the same Na⁺ bile acid cotransporter (ASBT) (2). The inwardly directed Na⁺ gradient maintained by the basolateral Na⁺/K⁺-ATPase as well as the negative intracellular potential provide the driving force for ASBT-mediated bile acid uptake (173). The properties of the ASBT satisfied all the functional criteria for ileal active bile acid uptake, including: 1) a strict sodium-dependence for bile acid transport (55); 2) specific transport of all the major monovalent species of bile acids with negligible uptake of other solutes (51); 3) specific intestinal expression in the terminal ileum; 4) similar ontogeny for rat ileal sodium-dependent taurocholate uptake and ASBT expression at fetal day 22 and postnatal day 17 (174); 5) targeted inactivation of the ASBT gene eliminates enterohpatic cycling of bile acid in mice (157); and 6) loss-of-function mutations in the human ASBT gene are associated with intestinal bile acid malabsorption (169).

Regulation of ASBT expression and activity

ASBT is expressed in tissues that serve to facilitate the enterohepatic circulation of bile acids, including the apical membrane of ileal enterocytes, proximal renal convoluted tubule cells, epithelial cells lining the biliary tract (cholangiocytes), and gallbladder epithelial cells (175–179). The molecular mechanisms controlling the tissue-specific expression of ASBT are not known. In the intestine, Na⁺-dependent bile acid transport activity and ASBT expression are found in villus but not crypt enterocytes (174, 180). Expression is largely restricted to the ileum in mice, hamster, rats, and humans (181–185). Regulation of ASBT expression along the longitudinal axis of the intestine is not fully understood although recent studies showed that the transcription factor GATA4 is essential for this process. Remarkably, intestine-specific inactivation of GATA4 in mice results in a dramatic induction of ASBT expression in proximal intestine (186, 187). After injury or resection of distal small intestine, therapeutic options for restoring bile acid absorption have been limited as compensatory increases in ASBT expression appear to occur only in those remaining intestinal regions that natively expressed ASBT (i.e., ileum) (185, 188, 189). However, the exciting finding that GATA4 is critical for establishing the functional gradient of bile acid absorption along the cephalocaudal axis of the small intestine reveals a novel opportunity for inducing proximal bile acid absorption by modulating the GATA4 pathway.

The enterohepatic circulation efficiently conserves bile acids, thereby maintaining bile flow and adequate intraluminal bile acid concentrations for micellar solubilization and absorption of lipids. Considering its central role in the enterohepatic circulation, inherited defects or dysfunctional regulation of the ASBT may play a role in the pathogenesis or clinical presentation of a variety of gastrointestinal disorders. For example, ASBT mutations were identified as a cause of primary bile acid malabsorption, a rare idiopathic disorder associated with interruption of the enterohepatic circulation of bile acids. Patients with primary bile acid malabsorption present with chronic diarrhea beginning in early infancy, steatorrhea, fat-soluble vitamin malabsorption, and reduced plasma cholesterol levels (169). Although dysfunctional mutations were not found in the ASBT gene from patients with adult-onset forms of idiopathic bile acid malabsorption (190), aberrant regulation of the ASBT may still contribute to the phenotype in a subset of those patients (172). Indeed, a recent genetic study identified an ASBT haplotype associated with significantly reduced ileal expression of ASBT mRNA and protein (191). Other disorders associated with intestinal bile acid malabsorption that could potentially involve the ASBT include hypertriglyceridemia (192, 193), idiopathic chronic diarrhea (194), chronic ileitis (195), gallstone disease (196, 197), postcholecystectomy diarrhea, Crohn’s disease (198–202), and irritable bowel syndrome (203).

Substrate, cytokines, hormones, and sterols all regulate transcription of the ASBT gene. The regulation of ASBT expression by bile acids remains an area of controversy. Conflicting observations have been published, reporting that ASBT and/or ileal bile acid transport activity is induced, repressed, or unaffected by bile acids (204–208). Differences between experimental paradigms, methods for measuring ASBT protein or activity, species, and genetic background account for some of the discrepancies that have been observed (209, 210). The dose and mode of delivery of bile acid is also an important consideration, as cell models do not recapitulate the in vivo dynamic flux of bile acids. In addition, bile acids are cytotoxic in high concentrations or under conditions of static exposure, and can activate a variety of signaling pathways (6). As such, it is important to try to distinguish between “basal” feedback regulation of ASBT expression and more complex responses to protect the cell from bile acid-induced injury.

Some of the earliest support for regulation of ASBT by bile acids was obtained from intestinal perfusion studies in the guinea pig; those studies showed that the ileal bile acid transport capacity was decreased after bile acid feeding and increased following the administration of a bile acid binding resin (205). In the mouse and rabbit, bile acids can also repress ASBT expression, apparently by acting through FXR and SHP to antagonize LRH-1, a competence factor important for ASBT transcription (210, 211). A positive role for LRH-1 is supported by the finding that ASBT expression is decreased in ileum of intestine-specific LRH-1 null mice (75). In contrast, FXR is not essential for ASBT’s basal expression as ASBT expression is not decreased in ileum of FXR null mice (212, 213). For humans, in vitro studies using Caco-2 cells or ileal biopsies have
Identified several different potential mechanisms for bile acid regulation of ASBT. In one mechanism, bile acids were found to act through the FXR-SHP pathway to antagonize RARα and decrease human ASBT transcription (214). Conversely, bile acids were also found to increase ASBT transcription through an FXR-independent pathway involving the epidermal growth factor receptor and mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK) signaling through an AP-1 element in the ASBT promoter (208). In vivo, several lines of evidence indirectly suggest that negative feedback regulation of intestinal bile acid transport is operational in humans, including the findings that retention of the bile acid analog 75Se-homocholic acid-taurine is increased in primary biliary cirrhosis (215) and intestinal ASBT expression is increased in patients with obstructive cholestasis (216). However, these are pathophysiological states and clearly more needs to be done to understand how bile acids regulate their own active intestinal absorption under physiological conditions. A new potential pathway for the bile acid-mediated repression of ASBT transcription has recently begun to emerge. Bile acids act through FXR to induce expression of FGF15/19, which feeds back in an autocrine or paracrine fashion to repress ASBT expression (217). In this model, the newly secreted FGF15/19 binds to its receptor, the FGFR4/β-klotho complex, expressed on basolateral surface of enterocytes and signals to stabilize the SHP protein by inhibiting its ubiquitin/proteasome-dependent degradation (218). The elevated levels of SHP could then antagonize LRH-1 or RARα to downregulate ASBT expression.

In addition to regulation by bile acids, ASBT mRNA expression appears to be negatively regulated by cholesterol. In vitro studies using Caco-2 cells showed that sterols such as 25-hydroxycholesterol downregulated ASBT mRNA expression and promoter activity (219). As elucidated using a variety of in vivo and in vitro approaches, the apparent underlying mechanism involves an indirect effect of SREBP-2 on HNF-1α-mediated stimulation of ASBT transcription (220). In general agreement with that work, the addition of cholesterol to a cholic acid-containing diet appeared to downregulate ASBT expression and intestinal bile acid absorption by an FXR-independent mechanism (221).

Ileal inflammation is associated with bile acid malabsorption (199), and ASBT expression is decreased in animal models of ileitis (222) and in ileal biopsies from patients with Crohn’s disease (223). Investigation of the underlying mechanism revealed that cytokines decrease ASBT gene expression by acting through an activator-protein-1 (AP-1) site, which binds a c-jun/c-fos heterodimer (224, 225). In the setting of intestinal inflammation, c-fos is phosphorylated and translocates into the nucleus where binding of the downstream AP-1 element leads to transcriptional repression of ASBT expression (225). Corticosteroids have had a long-standing use in the treatment of inflammatory bowel disease. Exogenous corticosteroids can induce precocious expression of ASBT and also upregulate ASBT expression in mature animals (226). In humans, exogenous corticosteroid (Budesonide) treatment increased ASBT expression in ileal biopsies, and this effect is mediated through glucocorticoid receptor binding to specific response elements in the human ASBT promoter (225). Thus, the beneficial effects of corticosteroids in inflammatory bowel disease may involve not only an effect on inflammatory cytokines but also a direct effect on ASBT expression (227). With regard to other nuclear receptors, ASBT gene expression is activated by peroxisome proliferator-activated receptor α (PPARα) (228) and by vitamin D nuclear receptor (VDR) (229) binding to their respective response elements in the ASBT promoter.

In addition to transcriptional regulation, ASBT is regulated posttranscriptionally by modulating protein stability or transporter activity. The ASBT protein resides on the plasma membrane in lipid rafts and depleting membrane cholesterol using methyl-β-cyclodextrin significantly reduced ASBT’s association with rafts and taurocholate transport (230). The cholesterol-depleted cells exhibited no change in ASBT protein expression at the plasma membrane, suggesting that the decreased taurocholate uptake was due to reduced ASBT activity. Finally, proteosomal-mediated degradation of ASBT is stimulated by cytokines such as IL-1β (231). The downregulation of ASBT protein expression correlated with a decrease in the half-life of ASBT from ~6 h to ~3 h, and the enhanced degradation was blocked using a c-Jun N-terminal kinase inhibitor or proteasome inhibitors.

**OSTα-OSTβ**

The proteins responsible for bile acid export across the basolateral membrane of the ileal enterocyte, cholangiocytes, and renal proximal tubule cell have only recently been identified. In contrast to apical transport, little was known regarding the mechanism and regulation of bile acid export across the basolateral membrane of these epithelia. Several candidate ileal basolateral transporters had been partially characterized or implicated over the years, including a basolateral sodium-independent anion exchange activity (232), a bile acid photoprobe-labeled 54 kDa protein enriched in the ileal basolateral membrane fraction (233), an alternatively spliced form of the ASBT (234), and MRP3 (ABCC3) (235). However, none of these candidates fulfilled all the predicted criteria (236, 237) and the identity of the basolateral bile acid transporter remained an important missing link in our understanding of the enterohepatic circulation. The break-through in this area came with the elegant expression cloning of an unusual transporter, OSTα-OSTβ, from the little skate (Raja erinacea) by Ned Ballatori and coworkers (238). Subsequently, the human and mouse orthologs of skate OSTα-OSTβ were cloned and expressed in Xenopus oocytes where they transport bile acids as well as a variety of steroids (239). As with the skate, solute transport by the mammalian orthologs required coexpression of two different subunits: OSTα, a 340 amino acid polytopic membrane protein, and OSTβ, a 128 amino acid predicted type I.
membrane protein. Soon after their cloning, the mouse OSTα-OSTβ was identified as a candidate ileal basolateral bile acid transporter using a transcriptional profiling approach (150). Support for a role of OSTα-OSTβ in basolateral bile acid transport includes: 1) intestinal expression of OSTα and OSTβ mRNA that generally follows that of the ASBT with the highest levels in ileum (150, 151, 172), 2) appropriate cellular localization on the lateral and basal membranes of ileal enterocyte (150), 3) expression of OSTα-OSTβ on the basolateral membrane of hepatocytes, cholangiocytes, and renal proximal tubule cells, other membranes exhibiting bile acid efflux (151), 4) efficient transport of the major bile acid species (150, 151), and 5) expression of OSTα-OSTβ is positively regulated by bile acids (213, 240). Although the functional role of the individual subunits has not yet been determined, coexpression and assembly of both subunits into a complex is required for their trafficking to the plasma membrane and solute transport (150, 241).

No inherited defects have been reported for the OSTα or OSTβ genes in humans; however, targeted inactivation of the OSTα gene in mice resulted in impaired intestinal bile acid absorption and altered bile acid metabolism (170, 171). In contrast to ASBT null mice that exhibited the predicted sequelae associated with intestinal bile acid malabsorption (157, 242), the OSTα null mice exhibited a more complex phenotype. Studies using everted gut sacs (171) or intra-ileal administration of [3H]taurocholate (170) demonstrated a significant reduction in trans-ileal transport in OSTα null mice. However, fecal bile acid excretion was not increased as had been observed in ASBT null mice (157) or patients with ASBT mutations (169). These results were particularly perplexing because the whole body bile acid pool size was significantly decreased, a hallmark of intestinal bile acid malabsorption. Examination of the FGF15/19 signaling pathway provided a solution to this conundrum (Fig. 2). As predicted from this model, OSTα null mice had significantly increased FGF15 expression and reduced hepatic Cyp7a1 expression (170, 171). These findings further supported a central role of FGF15/19 in regulating hepatic bile acid synthesis (243, 244) as recently confirmed in the liver and intestine-specific FXR and LRH-1 null mice and reviewed by the late Dr. Roger Davis (75, 245, 246).

In addition to demonstrating an important role for OSTα-OSTβ in bile acid transport and metabolism, this work had important physiological implications. Whereas blocking apical bile acid uptake using bile acid sequestrants or ASBT inhibitors dramatically reduces the ileal expression of FGF15 and increases hepatic Cyp7a1 expression (242), a block in basolateral bile acid transport increases FGF15 expression and reduces hepatic bile acid synthesis. This combination of reduced return of bile acids in the enterohepatic circulation and reduced bile acid synthesis may have therapeutic benefit in various forms of cholestatic liver disease. Conversely, the reduction in ileal bile acid synthesis associated with inhibition of basolateral transport could predispose to elevated plasma cholesterol levels in marked contrast to inhibition of apical ileal bile acid transport (247, 248). Of particular interest with regard to human disease, this phenotype of decreased bile acid absorption coupled with an inability of the liver to synthesize additional bile acids to maintain a normal pool that was found in the OSTα null mice had been described by the late Z. Reno Vlahcevic (249) in a study of gallstone patients that was published in 1970. Whether OSTα-OSTβ or the FGF15/19 signaling pathway play a role in the pathophysiology of gallstone disease is an interesting question that is beginning to be explored (250).

**Regulation of OSTα-OSTβ expression and activity**

Expression of both subunits is essential for function (150, 241) and as such, the two subunit genes appear to be regulated coordinately. Bile acid feeding (213), administration of a synthetic FXR agonist (213, 245, 251), or induction of cholestasis (33, 252) increases expression of OSTα and OSTβ mRNA and multiple groups identified functional FXR responsive elements in the mouse (213, 251) and human (240, 251) OSTα and OSTβ promoters. The promoters for OSTα and OSTβ include functional responsive elements for LRH-1 as well as FXR, providing a mechanism for both negative as well as positive regulation by bile acids (213). Whereas positive regulation by bile acids is dominant, this push-pull form of dynamic regulation would enable the cell to finely titrate expression of OSTα-OSTβ to match the flux of bile acids. The predominantly positive regulation would also ensure efficient export of bile acids thereby preventing cellular injury due to intracellular accumulation. In support of a potential role for LRH-1, ileal expression of OSTα and OSTβ was decreased in the intestine-specific LRH-1 null mice (75). In addition to being regulated by FXR, LXR potentially induces OSTα and OSTβ expression by operating via an inverted repeat-1 element shared with FXR (253).

**RENAL BILE ACID TRANSPORT**

A fraction (10% to 50%, depending on the bile acid species) of the bile acids returning in the portal circulation escapes hepatic extraction and spills into the systemic circulation. The binding of bile acids to plasma proteins reduces glomerular filtration and minimizes urinary excretion of bile acids. In healthy humans, the kidney filters approximately 100 µmol of bile acids each day. Remarkably, only 1 to 2 µmol is excreted in the urine because of a highly efficient tubular reabsorption (254). Even in patients with cholestatic liver disease in whom plasma bile acid concentrations are elevated, the 24-h urinary excretion of nonsulfated bile acids is significantly less than the quantity that undergoes glomerular filtration (254–257). Subsequent studies have shown that bile acids in the glomerular filtrate are actively reabsorbed from the renal tubules by a sodium-dependent mechanism (258, 259) and this process contributes to the rise in serum bile acid concentrations in patients with cholestatic liver disease.

As in the ileum, the renal proximal tubule epithelium expresses the ASBT as a salvage mechanism to conserve...
FUTURE RESEARCH

With the identification of all the major plasma membrane bile acid transporters that maintain the enterohepatic circulation, the focus is shifting toward how these carriers control the extracellular and intracellular levels of bile acids under different physiological and pathophysiological conditions. In addition to its role as a detergent to solubilize biliary and dietary lipids, bile acids activate a variety of nuclear receptors and signaling pathways (5, 6). There is a growing appreciation of the role of bile acids as “hormones” to modulate lipid and glucose metabolism (7). By controlling the flux of bile acids in the enterohepatic circulation, the bile acid transporters have an opportunity to modulate bile acid signaling and metabolic regulation. As such, it will be important to further understand the regulation of these carriers and their relationship to metabolism and human disease. Investigators have also begun to explore the effects of genetic single-nucleotide polymorphisms and epigenetic alterations on transporter expression (266) but much work still needs to balanced against any risk of increased bile acid-induced kidney cell injury (265).
be done. In addition to the bile acid binding resins and ursodeoxycholic acid, new compounds are in development that target bile acid signaling pathways, such as nor-ursodeoxycholic acid, synthetic FXR agonists, FXR modulators, and agonists for the bile acid-activated G-protein coupled receptors (5, 267). It will be important to understand how these compounds affect the bile acid transporters in animal models and patients. Other fertile areas for translational investigation include the interaction of drugs with BSEP as a mechanism for drug-induced cholestasis, the therapeutic utility of ASBT inhibitors to lower plasma cholesterol levels and improve insulin resistance, the utility of ASBT or OSTα-OSTβ inhibitors to relieve the hepatic bile acid burden in some forms of cholestatic liver disease, and the modulation of intestinal GATA4 activity to restore intestinal bile acid absorption following ileal disease or resection. So, although we have learned a great deal about the bile acid transporters, important questions remain about the role of these important ferrymen in human health and disease.

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