

University of Groningen

## Metabolic consequences of ileal interruption of the enterohepatic circulation of bile acids

van de Peppel, Ivo P; Verkade, Henkjan J; Jonker, Johan W

*Published in:*

American Journal of Physiology. Gastrointestinal and Liver Physiology

*DOI:*

[10.1152/ajpgi.00308.2020](https://doi.org/10.1152/ajpgi.00308.2020)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2020

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

van de Peppel, I. P., Verkade, H. J., & Jonker, J. W. (2020). Metabolic consequences of ileal interruption of the enterohepatic circulation of bile acids. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 319(5), G619-G625. <https://doi.org/10.1152/ajpgi.00308.2020>

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

## MINI-REVIEW

# Metabolic consequences of ileal interruption of the enterohepatic circulation of bile acids

 Ivo P. van de Peppel, Henkjan J. Verkade, and Johan W. Jonker

Section of Molecular Metabolism and Nutrition, Department of Pediatrics, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Submitted 20 August 2020; accepted in final form 15 September 2020

**van de Peppel IP, Verkade HJ, Jonker JW.** Metabolic consequences of ileal interruption of the enterohepatic circulation of bile acids. *Am J Physiol Gastrointest Liver Physiol* 319: G619–G625, 2020. First published September 16, 2020; doi:10.1152/ajpgi.00308.2020.—The enterohepatic circulation of bile acids comprises a tightly regulated process of hepatic bile acid secretion, intestinal reabsorption and transport back to the liver. Disruption of this process has significant consequences for gastrointestinal, liver and whole body homeostasis and therefore offers opportunities for therapeutic intervention. In this review we discuss the effects of (pharmacological) interruption of the enterohepatic circulation at different levels. Recently, several studies have been published on ileal interruption of the enterohepatic circulation of bile acids, targeting the apical-sodium dependent bile acid transporter (ASBT, *SLC10A2*), as therapy for various diseases. However, ambiguous results have been reported and in-depth mechanistic insights are lacking. Here we discuss these novel studies and review the current knowledge on the consequences of ASBT inhibition and its potential effects on physiology and metabolism.

ASBT; bile acids; enterohepatic circulation; FXR

## INTRODUCTION

The enterohepatic circulation (EHC) of bile acids (BAs) is a process that is tightly controlled by negative feedback regulation, resulting in maintenance of the BA pool size and adequate BA homeostasis. The classic role of BAs is to aid in the biliary secretion of hydrophobic compounds and to increase intestinal absorption of lipids and fat-soluble vitamins through formation of micelles. More recently, an additional role for BAs has emerged, namely as signaling molecules, through their identification as ligands for several receptors including the farnesoid X receptor (FXR, *NR1H4*) and the and the G protein-coupled bile acid receptor 1 (GPBAR1 or TGR5). BAs have been demonstrated to affect a variety of physiological pathways and have potential in the treatment of gastrointestinal, hepatic and metabolic disorders (7). In this review we discuss the effects of interrupting the EHC, specifically at the level of the apical sodium-dependent bile acid transporter (ASBT, *SLC10A2*), and its consequences for health and disease.

## THE ENTEROHEPATIC CIRCULATION OF BILE ACIDS

BAs are synthesized in the liver and enter the EHC comprising of hepatic secretion into the bile, biliary secretion into the duodenum, reabsorption in the distal intestine and transport back to the liver. Under physiological conditions ~95–98% of total BAs are reabsorbed every cycle and the remainder is excreted via the feces. With a daily cycling frequency of 5 to 10

times, this means that each day 25 to 50% of the total BA pool is lost which is replaced by hepatic synthesis from cholesterol. The rate of BA synthesis is controlled by the enzyme cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) which is highly regulated at both the transcriptional level and post-transcriptional level (10). Subsequently, BAs are secreted into bile, mainly via the bile salt export pump (BSEP, *ABCB11*), and stored in the gallbladder. Upon a meal, the gallbladder contracts in response to cholecystokinin and bile is secreted into the duodenum. In the intestine BAs facilitate efficient absorption of poorly soluble hydrophobic dietary components such as cholesterol, fatty acids (FAs) and fat-soluble vitamins (A, D, E and K) through formation of micelles. Reabsorption of BAs occurs mainly by the ileal enterocyte via the apical sodium-dependent bile acid transporter (ASBT, *SLC10A2*). In the enterocyte, BAs activate the FXR, a ligand-activated transcription factor of the family of nuclear receptors. Activation of FXR induces several genes involved in transcellular BA transport and leads to increased expression and subsequent secretion of fibroblast growth factor 19 (FGF19, FGF15 in mice) in the portal circulation (21). FGF15/19 travels to the liver where it binds to and activates the FGF receptor 4/ $\beta$ -Klotho (FGFR4/KLB) complex which exerts negative feedback on CYP7A1, thereby lowering BA synthesis. Reabsorbed BAs are secreted from the enterocyte into the portal circulation via the organic solute transporter- $\alpha/\beta$  (OST- $\alpha/\beta$ , *SLC51A/B*). In the liver BAs are taken up mainly via the Na<sup>+</sup>-taurocholate cotransporting polypeptide (NTCP, *SLC10A1*) and activate hepatic FXR, which regulates several genes important in cholesterol and BA homeostasis (e.g., BSEP and multidrug resistance

Correspondence: I. P. van de Peppel (i.p.van.de.peppel@umcg.nl).

protein 3 (MDR3), *ABCB4*) and results in negative feedback on BA synthesis via the small heterodimer partner (SHP, *NR0B2*). SHP represses expression of CYP7A1 by inhibiting the activity of liver receptor homolog 1 (LRH-1, *NR5A2*), an orphan nuclear receptor that positively regulates the expression of CYP7A1. However, studies in tissue-specific *Fxr* knockout mice indicated a much more prominent role for the intestinal FXR-FGF15/19 axis in CYP7A1 repression than the hepatic FXR-SHP-LRH-1 route (25).

#### INTERRUPTION OF THE ENTEROHEPATIC CIRCULATION OF BILE ACIDS

The EHC of BAs can be interrupted at different levels in the liver or the intestine (Fig. 1, A–E). The consequences for BA metabolism and (liver) disease are highly dependent on the level of interruption and can be either adverse or beneficial (Table 1). Below we will discuss the different levels of EHC interruption and its (patho)physiological consequences.

#### BA Sequestrants

One approach to reduce reabsorption of BAs from the intestine is using BA-binding resins or sequestrants (e.g., cholestyramine, colestevlam, colestimide) (Fig. 1A). BA sequestrants are currently used in (experimental) treatment of cholestatic liver disorders, BA diarrhea, dyslipidemia and hyperglycemia (27, 34). The effects of BA sequestration on non-alcoholic fatty liver disease and steatohepatitis (NAFLD/NASH) vary among studies

but are mostly limited (30, 50, 51). Intestinal binding of BAs decreases their reabsorption efficacy and results in a compensatory increase in BA synthesis thereby lowering (plasma) cholesterol levels. A recent meta-analysis showed that, combined with statin treatment, BA sequestrants resulted in an additional 16.2% decrease in LDL cholesterol (LDL-c) levels (1).

#### ASBT Inhibition

Soon after human ASBT was identified, it was shown that mutations in this gene could cause primary BA malabsorption and diarrhea, highlighting its significance for the EHC (39). ASBT is expressed at the apical membrane of ileal enterocytes and mediates uptake of conjugated BAs from the intestinal lumen (Fig. 1B). Pharmacological inhibition of ASBT has been explored as a therapy for constipation, dyslipidemia, atherosclerosis, type 2 diabetes mellitus (T2DM), NAFLD and cholestatic liver diseases. Table 2 gives a contemporary overview of the clinical trials using ASBT inhibitors and illustrates that most current research is focused on NAFLD/NASH and cholestatic liver disorders (reviewed in more detail in (31)).

#### OST $\alpha/\beta$ Inhibition

The heterodimeric OST $\alpha/\beta$  transporter facilitates BA export from the basolateral side of the ileal enterocyte to the blood (Fig. 1C) (3). These transporters are highly expressed in the small intestine but also present in other organs including the kidney and liver. While both ASBT and OST $\alpha/\beta$  are involved in ileal reabsorption of BAs, physiological consequences of inhibition are

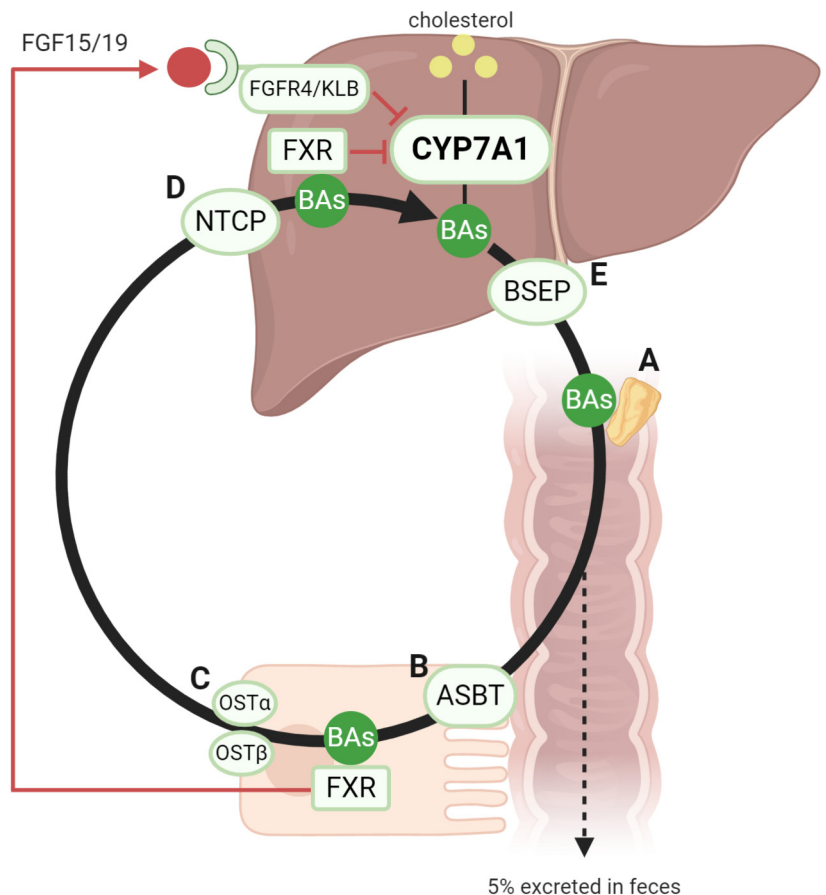


Fig. 1. Schematic representation of the enterohepatic circulation of bile acids with different levels of potential interruption. BA binding with sequestrants (A), ASBT inhibition (B), OST- $\alpha/\beta$  inhibition (C), NTCP inhibition (D), and BSEP inhibition (E). ASBT, apical sodium-dependent bile acid transporter; BAs, bile acids; BSEP, bile salt export pump; CYP7A1, cholesterol 7 $\alpha$ -hydroxylase; FGF15/19, fibroblast growth factor 15/19; FGFR4/KLB, fibroblast growth factor receptor 4 / $\beta$ -Klotho complex; FXR, farnesoid X receptor; NTCP, Na<sup>+</sup>-taurocholate cotransporting polypeptide; OST- $\alpha/\beta$ , organic solute transporter- $\alpha/\beta$ .

Table 1. Different levels of EHC interruption and their effect on bile acids and liver disease

Level of EHC Interruption	Bile Acid Synthesis	Total Serum Bile Acids	Bile Acid Pool Size	Effect of Liver Disease
BA sequestrants (A)	↑	↓	↓	Limited improvement in NAFLD in some (pre)clinical studies (30, 50, 51).
ASBT inhibition (B)	↑	↓	↓	Improved NAFLD in some pre-clinical studies (41, 44, 56) but did not reduce NASH in a clinical trial (37). Reduced PBC associated pruritis in a clinical trial (33).
OST $\alpha/\beta$ inhibition (C)	↓/↔	↓	↓	No clear hepatic effects (15).
NTCP inhibition (D)	↔	↑	↔	No clear clinical hepatic phenotype (55). Improves NAFLD in one preclinical study (13).
BSEP inhibition (E)	↓	↑	↔/↓	Results in (severe) intrahepatic cholestasis (54).

Letters in brackets at the level of interruption refer to the position in Fig. 1. ASBT, apical sodium-dependent bile acid transporter; BA, bile acid; BSEP, bile salt export pump; EHC, enterohepatic circulation; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NTCP, Na<sup>+</sup>-taurocholate cotransporting polypeptide; OST- $\alpha/\beta$ , organic solute transporter- $\alpha/\beta$ ; PBC, primary biliary cholangitis.

significantly different due to their different localization at the membrane. In contrast to inhibition of ASBT, upon OST $\alpha/\beta$  inhibition, BAs are still taken up by the enterocyte where they increase FGF15/19 secretion, leading to a subsequent downregulation of CYP7A1 expression. *Ost $\alpha$*  inactivation in *ApoE*<sup>-/-</sup> mice resulted in increased levels of LDL-c and increased atherogenesis while *Asbt* inactivation protected against atherosclerosis (29). A recent study demonstrated that *Ost $\alpha$*  inactivation in mice had several negative effects on the intestine, already present early in post-natal development, including BA accumulation and oxidative stress resulting in intestinal injury (15).

#### NTCP Inhibition

Uptake of BAs by the liver is mainly controlled by the Na<sup>+</sup>-taurocholate cotransporting polypeptide (NTCP) (Fig. 1D). Therefore, mutations in the *SLC10A1* gene coding for NTCP lead to conjugated hypercholanemia and elevated plasma total BAs (24, 55). However, the reported clinical phenotype of

*SLC10A1* mutations is mild, without overt symptoms of pruritis, liver dysfunction or steatorrhea. The expression of other hepatic BA transporters such as the solute carrier organic anion transporter family member 1B1 and 3 (*OATP1/3*), potentially mitigates some of the effects of NTCP dysfunction in humans (53).

NTCP has also been identified as the entry protein for the hepatitis B and D viruses (38). In line with this, the NTCP inhibitor Myrcludex B was shown to be effective in treatment of hepatitis D infection (4). While side effects were reported to be limited, Myrcludex B treatment did result in a prolonged elevation in plasma BAs (12). *Ntcp*<sup>-/-</sup> mice were protected against high-fat diet-induced obesity and hepatic steatosis (13). However, long-term (metabolic) consequences of NTCP inhibition using Myrcludex B are currently not known.

#### BSEP Inhibition

BSEP (or sister of P-glycoprotein (s-Pgp)) is responsible for BA export from the liver to the bile. Mutations in the *ABCB11* gene

Table 2. Current ASBT inhibitors under clinical investigation

Drug Name	Company	Indication	Status	Notes
Elobixibat (A3309)	Albireo	Constipation NAFLD/NASH	Approved for clinical use in Japan Phase II clinical trial	NCT04006145
Odevixibat (A4250)	Albireo	PFIC	Phase III clinical trial	NCT03566238 NCT03659916
Maralixibat (SHP625, formerly LUM001 or lopixibat)	Mirum	PFIC Alagille Syndrome, PSC PBC	Phase III clinical trial Phase III clinical trial	NCT04185363 NCT03905330 NCT04168385 No significant effect on pruritis in phase II PBC trial (33)
Linerixibat (GSK2330672)	Glaxosmithkline	PBC	Open label long-term safety trial	NCT04167358 Phase 2a trial showed efficacy in reducing PBC-associated pruritis (18)
Volixibat (SHP626, formerly LUM002)	Mirum	NASH	Phase II clinical trial	Completed, no reductions in hepatic fat content, serum alanine transferase or NAFLD activity score on histology (37)

ASBT, apical sodium-dependent bile acid transporter; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; PFIC, progressive familial intrahepatic cholestasis; PSC, primary sclerosing cholangitis.

encoding BSEP result in progressive familial intrahepatic cholestasis type 2 (PFIC2), an autosomal recessive disorder, frequently characterized by early onset intrahepatic cholestasis, pruritus and progression to hepatic fibrosis, cirrhosis and end-stage liver disease before adulthood (54). PFIC2 patients exhibit a 100-fold reduction in BA secretion into bile resulting in accumulation of BAs within hepatocytes, liver injury and cholestasis. Mutations in *ABCB11* have also been associated with two milder cholestatic syndromes: 1) benign recurrent intrahepatic cholestasis type 2 (BRIC2), which is characterized by intermittent episodes of cholestasis without progression to liver disease, and 2) intrahepatic cholestasis of pregnancy, which is associated with increased risk of intrauterine fetal death and prematurity (28). Several drugs also inhibit BSEP and are associated with drug-induced liver injury. Inhibition of BSEP has no known therapeutic potential.

#### METABOLIC CONSEQUENCES OF ILEAL INTERRUPTION OF THE ENTEROHEPATIC CIRCULATION OF BILE ACIDS

The initial (preclinical) studies on ASBT inhibitors explored their potential in treatment of atherosclerosis. Similar to BA sequestrants, preventing ileal BA reabsorption increases hepatic BA synthesis and cholesterol catabolism. In several animal models including mice, hamsters and monkeys, ASBT inhibition was effective in lowering plasma levels of LDL-c and in improving atherosclerotic outcomes (5, 26, 29). In humans, ASBT inhibition also lowers plasma LDL-c levels (9, 36, 37). However, due to the

effectiveness of cholesterol lowering drugs such as statins and BA sequestrants, research on the therapeutic use of ASBT inhibitors shifted to treatment of chronic constipation and (pruritus associated with) cholestatic disorders (23, 31). Recently, ASBT inhibition has also been reported to improve NAFLD, glucose metabolism and lipid metabolism (7). The mechanism underlying these improvements, however, has not been fully elucidated. In the following sections we discuss five important consequences of ASBT inhibition (Fig. 2) and discuss their potential role in the metabolic effects found in recent studies.

#### Changes in Bile Acid Pool Size and Composition

ASBT inhibition decreases intestinal BA reabsorption and increases hepatic BA synthesis. Studies in *Asbt*<sup>-/-</sup> mice demonstrated that the increase in synthesis is not sufficient to maintain a similar BA pool size as compared with wild type mice (11). At the same time, a shift toward a more hydrophobic BA and FXR agonistic composition occurs (11, 41, 52). In mice there is a relative decrease in muricholic acid (MCA) and increase in cholic acid (CA) upon ASBT inhibition. High colonic concentrations of CA are converted to deoxycholic acid (DCA) which is passively re-absorbed. We demonstrated that in *Asbt*<sup>-/-</sup> mice, taurine conjugated DCA (TDCA) accounted for ~50% of the biliary and plasma BA composition, while in wild type mice this was <5% (52). Both (T)CA and especially (T)DCA are potent activators of FXR and TGR5 while MCAs are FXR antagonists (19, 40, 45). Whereas ileal FXR activation is

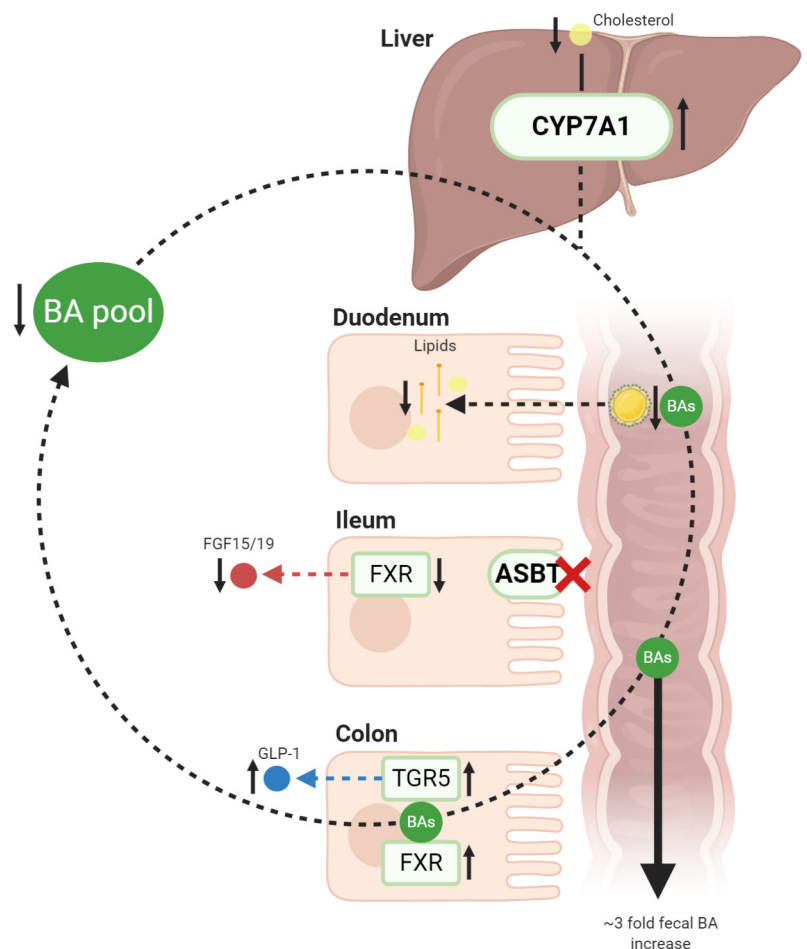


Fig. 2. Schematic representation of the main consequences of inhibition of ASBT. ASBT, apical sodium-dependent bile acid transporter; BAs, bile acids; CYP7A1, cholesterol 7 $\alpha$ -hydroxylase; FGF15/19, fibroblast growth factor 15/19; FXR, farnesoid X receptor; TGR5, G protein-coupled bile acid receptor 1.

consistently lower upon ASBT inhibition, the shift toward a more hydrophobic BA composition in bile and plasma could contribute to differential activation of BA activated receptors other tissues. Watanabe et al. demonstrated that BAs increase energy expenditure in brown adipocytes and skeletal myocytes through TGR5 activation (57). However, in their study, mice received dietary CA which not only increased relative concentrations of TGR5 agonists but also BA pool size and plasma BA concentrations, which are decreased upon ASBT inhibition. While there is a large relative increase in TGR5 agonistic BA species in plasma of *Asbt*<sup>-/-</sup> mice, absolute concentrations of (T)DCA were similar or only slightly increased (unpublished data from our group). The human BA composition differs from murine composition as it does not contain hydrophilic MCAs. Unfortunately, there is no data on the effect of ASBT inhibition on human BA pool size or composition. Lastly, patients with obesity, T2DM and/or NAFLD often display elevated plasma BA concentrations (7) and it was recently demonstrated that obesity is associated with increased ileal ASBT expression (49), although mechanistic insight and consequences are lacking.

#### *Increased Catabolism of Cholesterol*

The increase in cholesterol catabolism upon ASBT inhibition reduces levels of plasma LDL-c as well as hepatic cholesterol content. The accumulation of free cholesterol in the liver is toxic to hepatocytes and contributes to the pathophysiology of NASH (35). However, while the effects of ASBT inhibition on lowering total hepatic cholesterol content are robust among different dietary conditions and models, the effects on hepatic free cholesterol and triglyceride content vary among studies (37, 41, 42, 44, 56). More specifically, treatment of mice with an ASBT inhibitor (SC-435) on a choline-deficient L-amino acid-defined (CDAA) diet resulted in a significant decrease in free cholesterol content while liver triglyceride content and NASH were not improved (42). Although the pathophysiological mechanism of CDAA diet induced NAFLD is different (mainly impaired lipid secretion) from a high fat diet, it seems unlikely that the beneficial effects of ASBT inhibitor treatment on NASH are (entirely) due to its effects on lowering of hepatic cholesterol.

#### *Decreased Absorption of Lipids*

Genetic inactivation or inhibition of ASBT in mice resulted in lower absorption of FAs and cholesterol (42, 52). BAs are crucial for micellar solubilization which is most important for hydrophobic lipids such as cholesterol and (long-chain) saturated FAs. This is highlighted by studies using cholestatic and bile-deficient rat models where the absorption of long-chain saturated FAs was decreased more significantly than the absorption of (long chain) polyunsaturated FAs (22). Rao et al. (42) recently showed that the reduction in FA absorption in ASBT inhibitor treated mice, correlated with the beneficial effects on CDAA diet induced hepatic steatosis. These results combined with preliminary data from our group using different high fat diets demonstrates that the extend of the beneficial effects of ASBT inhibition are, at least partially, dependent on dietary FA composition and content.

#### *Decreased Secretion of FGF15/19*

In addition to its role in the negative feedback on BA synthesis, FGF15/19 has been implicated in cell proliferation (particularly

hepatocellular carcinoma) and various metabolic processes. Pharmacological administration of FGF19-mimetics (e.g., NGM282) or overexpression of FGF15/19 have shown to improve insulin sensitivity, reduce bodyweight gain, decrease plasma lipids and reduce NAFLD activity (17, 48). However, abrogation of endogenous FGF15/19 secretion or activity does not necessarily have opposing (negative) effects compared with FGF15/19 treatment (48). One study demonstrated that *Fgf15*<sup>-/-</sup> mice on a high-fat diet for 12 wk displayed increased bodyweight gain and hepatic steatosis (2). However, another study where *Fgf15*<sup>-/-</sup> mice were fed a high-fat diet for 24 wk reported a reduction in hepatic fibrosis with no effect on bodyweight gain or hepatic steatosis (47). The authors explained the effects by duration of the diet. Since hepatic fibrosis takes long to develop, it is possible that in the late stages of NAFLD/NASH development, the absence of *Fgf15* might slow down fibrogenesis. Knockout of *Fgfr4* in mice has generated conflicting results regarding diet-induced obesity and related changes in glucose metabolism, but has consistently shown to reduce NAFLD development (16, 20). However, one major difference between *Fgfr4* knockout and reduced FGFR4 activation upon ASBT inhibition is that BA reabsorption in *Fgfr4*<sup>-/-</sup> mice is still intact and therefore, the total BA pool is increased. It is thought that this increase in total BAs results in metabolically beneficial effects through increased (hepatic) FXR activation, TGR5 activation and/or increases in adipokines (48). This is in line with the observation that induction of hepatic but not intestinal FXR or FGF15 is important in prevention of NAFLD development in the context of a high cholesterol diet in mice (46).

ASBT inhibition consistently lowers FGF15/19 secretion, however, aside from the impact on hepatic BA synthesis, other physiological consequences are unclear. Based on results from *Fgf15*<sup>-/-</sup> and *Fgfr4*<sup>-/-</sup> mice, it is unlikely that a decrease in FGF15/19 per se accounts for the beneficial metabolic effects.

#### *Increased Colonic TGR5 Activation and GLP-1 Release*

Glucagon like peptide-1 (GLP-1) is released upon activation of TGR5 in enteroendocrine L-cells in the distal intestine and colon (6). GLP-1 improves (postprandial) glucose metabolism by increasing insulin secretion (14). While colonic gene expression levels of *Tgr5* and *proglucagon* (*Gcg*) in mice are not changed upon ASBT inhibition, high levels of the *ileal bile acid-binding protein* (*Ibabp*) due to FXR activation in the colon indicate increased intracellular BA levels in the colon (41, 42). Additionally, the change in BA composition upon ASBT inhibition results in a more potent TGR5 agonistic profile (6). Finally, long-chain FAs are able to increase GLP-1 release by L-cells via interaction with two distinct orphan G protein-coupled receptors (GPR40 and GPR120) (32). As intestinal FA absorption is reduced upon ASBT inhibition, more FAs reach the distal intestine where they could passively diffuse into L-cells increasing GLP-1 secretion. In line with this, Zucker rats treated with an ASBT inhibitor (264W94) displayed an increase in total plasma GLP-1 and improvement in glucose metabolism (8). Treatment of patients with constipation with the ASBT inhibitor (Elobixibat) also increased GLP-1 levels (43).

#### *Conclusion*

Interruption of the EHC of BAs is possible at various levels with different (patho)physiological consequences. Based on

current literature, only interruption at the intestinal luminal level (Fig. 1A) and the ileal level (Fig. 1B) seem to have beneficial metabolic effects. Especially ASBT inhibition has emerged as potential therapeutic strategy in a host of different hepatic and metabolic disorders. The consequences of ASBT inhibition are multifaceted and include a decrease in intestinal lipid absorption, an increase in cholesterol removal, an increase in GLP-1 secretion and changes in the BA pool/composition. Considering the effects on lipid absorption, the efficacy of ASBT inhibition is likely dependent on dietary composition which could explain some of the variation observed between studies. While there are multiple studies demonstrating promising results of ASBT inhibition on metabolic outcomes, more research on the exact effects on lipid absorption, the changes in BA composition in humans and the mechanism underlying potential increased GLP-1 secretion are required to fully evaluate its therapeutic potential.

#### ACKNOWLEDGMENTS

Figures were created using Biorender.com.

#### GRANTS

This study was supported by a grant from the European Foundation for the Study of Diabetes (award supported by EFSD/Novo Nordisk to J. W. Jonker).

#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

#### REFERENCES

- Alder M, Bavishi A, Zumpf K, Peterson J, Stone NJ. A meta-analysis assessing additional LDL-C reduction from addition of a bile acid sequestrant to statin therapy. *Am J Med* 000: 1–6, 2020. doi:10.1016/j.amjmed.2020.03.056.
- Alvarez-Sola G, Uriarte I, Latasa MU, Fernandez-Barrena MG, Urtasun R, Elizalde M, Barcena-Varela M, Jiménez M, Chang HC, Barbero R, Catalán V, Rodríguez A, Frühbeck G, Gallego-Escuredo JM, Gavaldà-Navarro A, Villarroja F, Rodríguez-Ortigosa CM, Corrales FJ, Prieto J, Berraondo P, Berasain C, Avila MA. Fibroblast growth factor 15/19 (FGF15/19) protects from diet-induced hepatic steatosis: development of an FGF19-based chimeric molecule to promote fatty liver regeneration. *Gut* 66: 1818–1828, 2017. doi:10.1136/gutjnl-2016-312975.
- Ballatori N, Christian WV, Lee JY, Dawson PA, Soroka CJ, Boyer JL, Madejczyk MS, Li N. OST $\alpha$ -OST $\beta$ : a major basolateral bile acid and steroid transporter in human intestinal, renal, and biliary epithelia. *Hepatology* 42: 1270–1279, 2005. doi:10.1002/hep.20961.
- Bogomolov P, Alexandrov A, Voronkova N, Macievich M, Kokina K, Petrachenkova M, Lehr T, Lempp FA, Wedemeyer H, Haag M, Schwab M, Haefeli WE, Blank A, Urban S. Treatment of chronic hepatitis D with the entry inhibitor myrcludex B: First results of a phase Ib/IIa study. *J Hepatol* 65: 490–498, 2016. doi:10.1016/j.jhep.2016.04.016.
- Braun A, Yesilaltay A, Acton S, Broschat KO, Krul ES, Napawan N, Stagliano N, Krieger M. Inhibition of intestinal absorption of cholesterol by ezetimibe or bile acids by SC-435 alters lipoprotein metabolism and extends the lifespan of SR-BI/apoE double knockout mice. *Atherosclerosis* 198: 77–84, 2008. doi:10.1016/j.atherosclerosis.2007.10.012.
- Brighton CA, Rievaj J, Kuhre RE, Glass LL, Schoonjans K, Holst JJ, Gribble FM, Reimann F. Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein-coupled bile acid receptors. *Endocrinology* 156: 3961–3970, 2015. doi:10.1210/en.2015-1321.
- Chávez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. *Gastroenterology* 152: 1679–1694.e3, 2017. doi:10.1053/j.gastro.2017.01.055.
- Chen L, Yao X, Young A, McNulty J, Anderson D, Liu Y, Nystrom C, Croom D, Ross S, Collins J, Rajpal D, Hamlet K, Smith C, Gedulin B. Inhibition of apical sodium-dependent bile acid transporter as a novel treatment for diabetes. *Am J Physiol Endocrinol Metab* 302: E68–E76, 2012. doi:10.1152/ajpendo.00323.2011.
- Chey WD, Camilleri M, Chang L, Rikner L, Graffner H. A randomized placebo-controlled phase IIb trial of a3309, a bile acid transporter inhibitor, for chronic idiopathic constipation. *Am J Gastroenterol* 106: 1803–1812, 2011. [Erratum in *Am J Gastroenterol* 109: 782, 2014.] doi:10.1038/ajg.2011.162.
- Chiang JYL, Ferrell JM. Up to date on cholesterol 7 alpha-hydroxylase (CYP7A1) in bile acid synthesis. *Liver Res* 4: 47–63, 2020. doi:10.1016/j.livres.2020.05.001.
- Dawson PA, Haywood J, Craddock AL, Wilson M, Tietjen M, Kluckman K, Maeda N, Parks JS. Targeted deletion of the ileal bile acid transporter eliminates enterohepatic cycling of bile acids in mice. *J Biol Chem* 278: 33920–33927, 2003. doi:10.1074/jbc.M306370200.
- Donkers JM, Appelman MD, van de Graaf SFJ. Mechanistic insights into the inhibition of NTCP by myrcludex B. *JHEP Rep* 1: 278–285, 2019. doi:10.1016/j.jhepr.2019.07.006.
- Donkers JM, Kooijman S, Slijepcevic D, Kunst RF, Roscam Abbing RLP, Haazen L, de Waart DR, Levels JHM, Schoonjans K, Rensen PCN, Oude Elferink RPJ, van de Graaf SFJ. NTCP deficiency in mice protects against obesity and hepatosteatosis. *JCI Insight* 5: e127197, 2019. doi:10.1172/jci.insight.127197.
- Drucker DJ. Mechanisms of action and therapeutic application of glucagon-like peptide-1. *Cell Metab* 27: 740–756, 2018. doi:10.1016/j.cmet.2018.03.001.
- Ferrebee CB, Li J, Haywood J, Pachura K, Robinson BS, Hinrichs BH, Jones RM, Rao A, Dawson PA. Organic solute transporter  $\alpha$ - $\beta$  protects ileal enterocytes from bile acid-induced injury. *Cell Mol Gastroenterol Hepatol* 5: 499–522, 2018. doi:10.1016/j.jcmgh.2018.01.006.
- Ge H, Zhang J, Gong Y, Gupte J, Ye J, Weiszmann J, Samayoa K, Coberly S, Gardner J, Wang H, Corbin T, Chui D, Baribault H, Li Y. Fibroblast growth factor receptor 4 (FGFR4) deficiency improves insulin resistance and glucose metabolism under diet-induced obesity conditions. *J Biol Chem* 289: 30470–30480, 2014. doi:10.1074/jbc.M114.592022.
- Harrison SA, Rossi SJ, Paredes AH, Trotter JF, Bashir MR, Guy CD, Banerjee R, Jaros MJ, Owers S, Baxter BA, Ling L, DePaoli AM. NGM282 improves liver fibrosis and histology in 12 weeks in patients with nonalcoholic steatohepatitis. *Hepatology* 71: 1198–1212, 2020. doi:10.1002/hep.30590.
- Hegade VS, Kendrick SFW, Dobbins RL, Miller SR, Thompson D, Richards D, Storey J, Dukes GE, Corrigan M, Oude Elferink RPJ, Beuers U, Hirschfield GM, Jones DE. Effect of ileal bile acid transporter inhibitor GSK2330672 on pruritus in primary biliary cholangitis: a double-blind, randomised, placebo-controlled, crossover, phase 2a study. *Lancet* 399: 1114–1123, 2017. doi:10.1016/S0140-6736(17)30319-7.
- Hu X, Bonde Y, Eggertsen G, Rudling M. Muricholic bile acids are potent regulators of bile acid synthesis via a positive feedback mechanism. *J Intern Med* 275: 27–38, 2014. doi:10.1111/joim.12140.
- Huang X, Yang C, Luo Y, Jin C, Wang F, McKeenan WL. FGFR4 prevents hyperlipidemia and insulin resistance but underlies high-fat diet induced fatty liver. *Diabetes* 56: 2501–2510, 2007. doi:10.2337/db07-0648.
- Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, Luo G, Jones SA, Goodwin B, Richardson JA, Gerard RD, Repa JJ, Mangelsdorf DJ, Kliewer SA. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab* 2: 217–225, 2005. doi:10.1016/j.cmet.2005.09.001.
- Kalivianakis M, Minich DM, Havinga R, Kuipers F, Stellaard F, Vonk RJ, Verkade HJ. Detection of impaired intestinal absorption of long-chain fatty acids: validation studies of a novel test in a rat model of fat malabsorption. *Am J Clin Nutr* 72: 174–180, 2000. doi:10.1093/ajcn/72.1.174.
- Kamath BM, Stein P, Houwen RHJ, Verkade HJ. Potential of ileal bile acid transporter inhibition as a therapeutic target in Alagille syndrome and progressive familial intrahepatic cholestasis. *Liver Int* 40: 1812–1822, 2020. doi:10.1111/liv.14553.
- Karpen SJ, Dawson PA. Not all (bile acids) who wander are lost: the first report of a patient with an isolated NTCP defect. *Hepatology* 61: 24–27, 2015. doi:10.1002/hep.27294.
- Kim I, Ahn S-H, Inagaki T, Choi M, Ito S, Guo GL, Kliewer SA, Gonzalez FJ. Differential regulation of bile acid homeostasis by the farnesoid X receptor in liver and intestine. *J Lipid Res* 48: 2664–2672, 2007. doi:10.1194/jlr.M700330-JLR200.
- Kitayama K, Nakai D, Kono K, van der Hoop AG, Kurata H, de Wit EC, Cohen LH, Inaba T, Kohama T. Novel non-systemic inhibitor of ileal apical Na<sup>+</sup>-dependent bile acid transporter reduces serum cholesterol levels in hamsters and monkeys. *Eur J Pharmacol* 539: 89–98, 2006. doi:10.1016/j.ejphar.2006.04.005.

27. Kriegermeier A, Green R. Pediatric cholestatic liver disease: review of bile acid metabolism and discussion of current and emerging therapies. *Front Med (Lausanne)* 7: 149, 2020. doi:10.3389/fmed.2020.00149.
28. Kubitz R, Dröge C, Stindt J, Weissenberger K, Häussinger D. The bile salt export pump (BSEP) in health and disease. *Clin Res Hepatol Gastroenterol* 36: 536–553, 2012. doi:10.1016/j.clinre.2012.06.006.
29. Lan T, Haywood J, Dawson PA. Inhibition of ileal apical but not basolateral bile acid transport reduces atherosclerosis in apoE<sup>-/-</sup> mice. *Atherosclerosis* 229: 374–380, 2013. doi:10.1016/j.atherosclerosis.2013.05.017.
30. Le TA, Chen J, Changchien C, Peterson MR, Kono Y, Patton H, Cohen BL, Brenner D, Sirlin C, Loomba R; San Diego Integrated NAFLD Research Consortium (SINC). Effect of colesvelam on liver fat quantified by magnetic resonance in nonalcoholic steatohepatitis: a randomized controlled trial. *Hepatology* 56: 922–932, 2012. doi:10.1002/hep.25731.
31. Li M, Wang Q, Li Y, Cao S, Zhang Y, Wang Z, Liu G, Li J, Gu B. Apical sodium-dependent bile acid transporter, drug target for bile acid related diseases and delivery target for prodrugs: Current and future challenges. *Pharmacol Ther* 212: 107539, 2020. doi:10.1016/j.pharmthera.2020.107539.
32. Lim GE, Brubaker PL. Glucagon-like peptide 1 secretion by the L-cell: The view from within. *Diabetes* 55, Suppl 2: S70–S77, 2006. doi:10.2337/db06-S020.
33. Mayo MJ, Pockros PJ, Jones D, Bowlus CL, Levy C, Patanwala I, Bacon B, Luketic V, Vuppalanchi R, Medendorp S, Dorenbaum A, Kennedy C, Novak P, Gu J, Apostol G, Hirschfield GM. A randomized, controlled, phase 2 study of maralixibat in the treatment of itching associated with primary biliary cholangitis. *Hepatol Commun* 3: 365–381, 2019. doi:10.1002/hep4.1305.
34. Mazidi M, Rezaie P, Karimi E, Kengne AP. The effects of bile acid sequestrants on lipid profile and blood glucose concentrations: A systematic review and meta-analysis of randomized controlled trials. *Int J Cardiol* 227: 850–857, 2017. doi:10.1016/j.ijcard.2016.10.011.
35. Musso G, Gambino R, Cassader M. Cholesterol metabolism and the pathogenesis of non-alcoholic steatohepatitis. *Prog Lipid Res* 52: 175–191, 2013. doi:10.1016/j.plipres.2012.11.002.
36. Nakajima A, Seki M, Taniguchi S, Ohta A, Gillberg PG, Mattsson JP, Camilleri M. Safety and efficacy of elobixibat for chronic constipation: results from a randomised, double-blind, placebo-controlled, phase 3 trial and an open-label, single-arm, phase 3 trial. *Lancet Gastroenterol Hepatol* 3: 537–547, 2018. doi:10.1016/S2468-1253(18)30123-7.
37. Newsome PN, Palmer M, Freilich B, Sheikh MY, Sheikh A, Sarles H, Herring R, Mantry P, Kayali Z, Hassanein T, Lee HM, Aithal GP; Volixibat in Adults study group. Volixibat in adults with non-alcoholic steatohepatitis: 24-week interim analysis from a randomized, phase II study. *J Hepatol* 73: 231–240, 2020. doi:10.1016/j.jhep.2020.03.024.
38. Ni Y, Lempp FA, Mehrle S, Nkongolo S, Kaufman C, Fälth M, Stindt J, Königer C, Nassal M, Kubitz R, Sültmann H, Urban S. Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes. *Gastroenterology* 146: 1070–1083.E6, 2014. doi:10.1053/j.gastro.2013.12.024.
39. Oelkers P, Kirby LC, Heubi JE, Dawson PA. Primary bile acid malabsorption caused by mutations in the ileal sodium-dependent bile acid transporter gene (SLC10A2). *J Clin Invest* 99: 1880–1887, 1997. doi:10.1172/JCI119355.
40. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Conslor TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, Lehmann JM. Bile acids: natural ligands for an orphan nuclear receptor. *Science* 284: 1365–1368, 1999. doi:10.1126/science.284.5418.1365.
41. Rao A, Kosters A, Mells JE, Zhang W, Setchell KDR, Amanso AM, Wynn GM, Xu T, Keller BT, Yin H, Banton S, Jones DP, Wu H, Dawson PA, Karpen SJ. Inhibition of ileal bile acid uptake protects against nonalcoholic fatty liver disease in high-fat diet-fed mice. *Sci Transl Med* 8: 357ra122, 2016. doi:10.1126/scitranslmed.aaf4823.
42. Rao A, van de Peppel IP, Gumber S, Karpen SJ, Dawson PA. Attenuation of the hepatoprotective effects of ileal apical sodium dependent bile acid transporter (ASBT) inhibition in choline-deficient L-amino acid-defined (CDAA) diet-fed mice. *Front Med (Lausanne)* 7: 60, 2020. doi:10.3389/fmed.2020.00060.
43. Rudling M, Camilleri M, Graffner H, Holst JJ, Rikner L. Specific inhibition of bile acid transport alters plasma lipids and GLP-1. *BMC Cardiovasc Disord* 15: 75, 2015. doi:10.1186/s12872-015-0070-9.
44. Salic K, Kleemann R, Wilkins-Port C, McNulty J, Verschuren L, Palmer M. Apical sodium-dependent bile acid transporter inhibition with volixibat improves metabolic aspects and components of non-alcoholic steatohepatitis in *Ldlr*<sup>-/-</sup> Leiden mice. *PLoS One* 14: e0218459, 2019. doi:10.1371/journal.pone.0218459.
45. Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, Angelin B, Hyötyläinen T, Orešić M, Bäckhed F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* 17: 225–235, 2013. doi:10.1016/j.cmet.2013.01.003.
46. Schmitt J, Kong B, Stieger B, Tschopp O, Schultze SM, Rau M, Weber A, Müllhaupt B, Guo GL, Geier A. Protective effects of farnesoid X receptor (FXR) on hepatic lipid accumulation are mediated by hepatic FXR and independent of intestinal FGF15 signal. *Liver Int* 35: 1133–1144, 2015. doi:10.1111/liv.12456.
47. Schumacher JD, Kong B, Pan Y, Zhan L, Sun R, Aa J, Rizzolo D, Richardson JR, Chen A, Goedken M, Aleksunes LM, Laskin DL, Guo GL. The effect of fibroblast growth factor 15 deficiency on the development of high fat diet induced non-alcoholic steatohepatitis. *Toxicol Appl Pharmacol* 330: 1–8, 2017. doi:10.1016/j.taap.2017.06.023.
48. Somme E, Jornayvaz FR. Fibroblast growth factor 15/19: from basic functions to therapeutic perspectives. *Endocr Rev* 39: 960–989, 2018. doi:10.1210/er.2018-00134.
49. Sundaram S, Palaniappan B, Nepal N, Chaffins S, Sundaram U, Arthur S. Mechanism of dyslipidemia in obesity-unique regulation of ileal villus cell brush border membrane sodium-bile acid cotransport. *Cells* 8: 1197, 2019. doi:10.3390/cells8101197.
50. Takahashi S, Luo Y, Ranjit S, Xie C, Libby AE, Orlicky DJ, Dvornikov A, Wang XX, Myakala K, Jones BA, Bhasin K, Wang D, McManaman JL, Krausz KW, Gratton E, Ir D, Robertson CE, Frank DN, Gonzalez FJ, Levi M, Pessin JE. Bile acid sequestration reverses liver injury and prevents progression of nonalcoholic steatohepatitis in Western diet-fed mice. *J Biol Chem* 295: 4733–4747, 2020. doi:10.1074/jbc.RA119.011913.
51. Taniai M, Hashimoto E, Tobari M, Yatsuji S, Haruta I, Tokushige K, Shiratori K. Treatment of nonalcoholic steatohepatitis with colestid. *Hepatol Res* 39: 685–693, 2009. doi:10.1111/j.1872-034X.2009.00507.x.
52. van de Peppel IP, Bertolini A, van Dijk TH, Groen AK, Jonker JW, Verkade HJ. Efficient reabsorption of transported intestinally excreted cholesterol is a strong determinant for cholesterol disposal in mice. *J Lipid Res* 60: 1562–1572, 2019. doi:10.1194/jlr.M094607.
53. van de Steeg E, Wagenaar E, van der Kruijssen CMM, Burggraaf JEC, de Waart DR, Elferink RP, Kenworthy KE, Schinkel AH. Organic anion transporting polypeptide 1a/1b-knockout mice provide insights into hepatic handling of bilirubin, bile acids, and drugs. *J Clin Invest* 120: 2942–2952, 2010. doi:10.1172/JCI42168.
54. van Wessel DBE, Thompson RJ, Gonzales E, Jankowska I, Sokal E, Grammatikopoulos T, Kadaristiana A, Jacquemin E, Spraul A, Lipinski P, Czubkowski P, Rock N, Shagrani M, Broering D, Algoufi T, Mazhar N, Nicastro E, Kelly DA, Nebbia G, Arnell H, Björn Fischer, Hulscher JBF, Serranti D, Arikian C, Polat E, Debray D, Laccaille F, Goncalves C, Hierro L, Muñoz Bartolo G, Mozer-Glassberg Y, Azaz A, Breclj J, Dezsófi A, Calvo PL, Grabhorn E, Sturm E, van der Woerd WJ, Kamath BM, Wang JS, Li L, Durmaz Ö, Onal Z, Bunt TMG, Hansen BE, Verkade HJ; NATURAL course and Prognosis of PFIC and Effect of biliary Diversion (NAPPED) consortium. Genotype correlates with the natural history of severe bile salt export pump deficiency. *J Hepatol* 73: 84–93, 2020. doi:10.1016/j.jhep.2020.02.007.
55. Vaz FM, Paulusma CC, Huidekoper H, de Ru M, Lim C, Koster J, Homok K, Bootsma AH, Groen AK, Schaap FG, Oude Elferink RPJ, Waterham HR, Wanders RJA. Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: conjugated hypercholanemia without a clear clinical phenotype. *Hepatology* 61: 260–267, 2015. doi:10.1002/hep.27240.
56. Wang Y, Gunewardena S, Li F, Matye DJ, Chen C, Chao X, Jung T, Zhang Y, Czerwiniski M, Ni HM, Ding WX, Li T. An FGF15/19-TFEB regulatory loop controls hepatic cholesterol and bile acid homeostasis. *Nat Commun* 11: 3612, 2020. doi:10.1038/s41467-020-17363-6.
57. Watanabe M, Houten SM, Matakai C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, Schoonjans K, Bianco AC, Auwerx J. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 439: 484–489, 2006. doi:10.1038/nature04330.