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Rationale

Bile acids are amphipathic molecules synthesized by the liver and secreted into the intestine. The “traditional” functions of bile acids have been to stimulate bile production and the biliary secretion of endogenous and exogenous compounds, and to aid in the intestinal absorption of fat, cholesterol and fat-soluble vitamins by their solubilization into mixed micelles. In recent years, research on bile acids has extended beyond the traditional scope of bile secretion and lipid absorption and has shown involvement of bile acids as signaling molecules in many other processes in the body. Altering bile acids and their receptors affect gastrointestinal function, microbiota composition, liver function, cholesterol homeostasis, and glucose metabolism. This has led to a surge of novel therapeutic options modulating bile acid homeostasis in an attempt to affect disorders related to these functions.

Among these therapeutic options is the modulation of intestinal reabsorption of bile acids and subsequent effects on receptor activation. Prevention of reabsorption of bile acids in the intestine has been studied as potential therapy for a variety of metabolic and hepatic conditions. Malabsorption of bile acids reduces farnesoid X receptor (FXR) activation and induces changes in bile acid pool size and composition. However, bile acid malabsorption can also cause unwanted symptoms and is a hallmark of diseases such as primary bile acid diarrhea and cystic fibrosis. Therefore, further exploration of the effects of bile acid malabsorption on both ends of the spectrum is necessary. This thesis addresses bile acid malabsorption as consequence of disease, specifically in cystic fibrosis, and as potential treatment in hypercholesterolemia, obesity and non-alcoholic fatty liver disease.
General introduction

Bile acid synthesis

Bile acids are amphipathic steroid molecules synthesized from cholesterol by the liver. They act as detergents and are required for efficient intestinal absorption of poorly soluble nutrients such as cholesterol, fatty acids and fat-soluble vitamins (A, D, E, and K). Synthesis of bile acids in the liver is achieved via two main pathways, the classical pathway, resulting in synthesis of cholic acid (CA) and chenodeoxycholic acid (CDCA), and the acidic or alternative pathway resulting in production CDCA (Fig. 1). In humans it was estimated that the alternative pathway contributes only about 9% to total bile acid synthesis (1).

Figure 1. Hepatic bile acid synthesis in humans and mice. Bile acids are synthesized from cholesterol via two main pathways, the classic (‘neutral’) and the alternative (‘acidic’) pathway. The classic pathway is responsible of synthesis of both chenodeoxycholic acid (CDCA) and cholic acid (CA) via conversion of cholesterol by cholesterol 7α-hydroxylase (CYP7A1). Cholesterol 12 α-hydroxylase (CYP8B1) is necessary for synthesis of CA and its activity is important in determining the ratio of CA to CDCA. Synthesis via the alternative pathway results in chenodeoxycholic acid (CDCA) via by sterol 27-hydroxylase (CYP27A1) and oxysterol 7α-hydroxylase (CYP7B1). In mice CDCA as well as ursodeoxycholic acid (UDCA) are subsequently converted into α- and β-muricholic acid (MCA) respectively.
The rate of bile acid synthesis is controlled by the enzyme cholesterol 7α-hydroxylase (CYP7A1), the first step in the classical pathway. Synthesis of CA is completed by the sterol 12α-hydroxylase (CYP8B1). The alternative pathway starts with conversion by sterol 27-hydroxylase (CYP27A1) and subsequent conversion by oxysterol 7α-hydroxylase (CYP7B1) to form CDCA. Bile acid composition varies greatly between species (see for review (2)). In humans, CA and CDCA are the main primary bile acid species while in mice CDCA is converted to α- and β-muricholic acid (MCA). Ursodeoxycholic acid (UDCA), which represents only a minor component of the bile acid pool, is generated from CDCA by the gut microbiota in humans (3). In mice, however, UDCA is considered a primary bile acid as it can be synthesized in the absence of gut microbiota (4). In mice both CDCA and UDCA undergo 6β-hydroxylation by the cytochrome P450 Cyp2c70, to generate α-MCA and β-MCA, respectively (5). These 6-hydroxylated bile acid species are more water soluble and therefore relatively poor detergents as compared to CA or CDCA (6). This is important to note when using mice to study bile acid metabolism as MCAs make up almost 50% of their biliary bile acids, resulting in a significantly lower biliary hydrophobicity as compared to humans.

After secretion into the intestine bile acids are subject to transformation by intestinal microbiota, mainly in the distal small intestine and colon. Many intestinal bacteria harbor the ability to deconjugate bile acids via the bile salt hydrolase enzyme (7). After deconjugation, some bacterial species perform additional modifications, such as 7α-dehydroxylation, to create secondary bile acid species. In humans, conversion of CA and CDCA results in deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. DCA and LCA are more hydrophobic than primary bile acids and have different affinities for bile acid activated receptors in the intestine. Additionally, in rats (and likely also in mice) β-MCA can be converted to ω-MCA. Subsequently, α-, β-, and ω-MCA can undergo 7α- or β-hydroxylation to form hyodeoxycholic acid (HDCA) or murideoxycholic acid (MDCA) (8). However, in general these bile acid species only represent minor quantities of cecal or fecal bile acid content in mice (9).

The enterohepatic circulation of bile acids

In the physiological situation, bile acid homeostasis is achieved by the enterohepatic circulation resulting in ~95% of total bile acids being reabsorbed and ~5% being excreted via the feces every cycle (Fig. 2).
**Figure 2. The enterohepatic circulation of bile acids (taken from (10)).** Bile acids (BAs) are synthesized and conjugated in the liver after which they are actively secreted via the bile into the duodenum. In the ileum BAs are reabsorbed by the enterocytes through the apical sodium-dependent bile acid transporter (ASBT). Here bile acids activate the nuclear farnesoid X receptor (FXR) which leads to transcriptional regulation of genes involved in bile acid homeostasis including fibroblast growth factor 19 (FGF19 in humans or Fgf15 in mice). After its secretion into the plasma FGF15/19 travels to the liver where it binds fibroblast growth factor receptor 4 (FGFR4) β-Klotho complex, activation of which ultimately results in suppression of CYP7A1, the rate controlling enzyme of bile acid synthesis. Reabsorbed BAs can also travel to the liver and directly inhibit CYP7A1 via activating hepatic FXR. Microbiota in the intestine can biotransform BAs to deconjugated and secondary bile acid species. The non-reabsorbed fraction of intestinal BA (~5% per cycle in the physiological situation) is excreted in feces.
Reabsorption is mediated by active transport of conjugated bile acids into the ileal enterocyte via the apical sodium-dependent bile acid transporter (ASBT, SLC10A2) (11). In the ileal enterocyte bile acids activate the farnesoid X receptor (FXR, NR1H4), a ligand-activated transcription factor of the family of nuclear receptors, which leads to increased expression and subsequent release into the circulation of fibroblast growth factor 19 (FGF19, Fgf15 in mice) (12). In the liver FGF19 can bind to and activate the FGF receptor 4 (FGFR4)/β-Klotho complex which in turn exerts negative feedback on the rate controlling enzyme of bile acid synthesis, cholesterol 7α-hydroxylase (CYP7A1). Reabsorbed bile acids can also cause negative feedback by directly activating hepatic FXR. However, studies in tissue-specific Fxr knockout mice indicated a much more prominent role for the FXR-FGF15/19 axis in CYP7A1 repression (13).

Microbiota also play an important role in the enterohepatic bile acid feedback system. A bidirectional relationship between microbiota and FXR modulation exists. Both germfree and antibiotic treated mice have an increased CYP7A1 activity, likely due to increased levels of TβMCA, a naturally occurring FXR antagonist (14,15). In turn, a recent study showed in both mice and humans that treatment with an FXR agonist changes microbiota (16). This makes the intestinal microbiome an interesting but complex potential target for modulating bile acid homeostasis and its related effects.

The role of bile acids in cholesterol and lipid metabolism

Cholesterol homeostasis is a tightly regulated and complex process involving synthesis, absorption, excretion and transport via lipoproteins (17,18). Cholesterol enters the body either via synthesis or via intestinal absorption from dietary sources. At the level of the enterocyte, cholesterol is absorbed via the Nieman-Pick C1-Like 1 (NPC1L1) protein (19). In the circulation cholesterol trafficking is mediated via lipoproteins. The liver secretes cholesterol in very low density lipoproteins (VLDL) and low density lipoprotein (LDL) that transport cholesterol to the peripheral tissues. Plasma levels are controlled by the rate of lipoprotein secretion and removal of lipoproteins by tissues expressing their corresponding receptors. High density lipoprotein (HDL) is regarded to be mainly involved in reverse cholesterol transport, the transport of cholesterol back from the periphery to the liver for subsequent secretion into bile and fecal disposal. However, this concept was challenged by data in mice that showed that the absence of HDL did not impair biliary or fecal cholesterol disposal, suggesting compensatory mechanisms to sustain reverse cholesterol transport in the absence of HDL (20).
There are significant species differences which are important to note when studying cholesterol homeostasis. Contrary to humans, mice carry most of their cholesterol in HDL particles and have a higher rate of LDL clearance (21,22).

Elimination of cholesterol from the body occurs mainly via the feces either as neutral sterols (cholesterol and its bacterial metabolites) or as acid sterols (i.e. bile acids). Cholesterol is excreted into the intestine either via bile or via transintestinal cholesterol excretion (TICE) (23). TICE is at least partly mediated through active excretion by the ATP-binding cassette sub-family G members 5 and 8 (ABCG5/8). The concept of TICE has been based on multiple conditions in which fecal neutral sterol output exceeded biliary and dietary cholesterol input into the intestine (reviewed in (23,24)). However, to what degree reabsorption of a continuous (physiological) flux of cholesterol contributed to the observed effects of increased net TICE remains unclear. While treatment with ezetimibe, an NPC1L1 inhibitor, was shown to increase fecal neutral sterol excretion, it remained unexplained whether this was the result of activated secretion or inhibition of reabsorption (25–27). Various other conditions including high-fat diet feeding, liver X receptor (LXR) activation and intestinal FXR activation have been shown to affect TICE (28–30).

Conversion of cholesterol to bile acids represents about 45% of the daily total sterol elimination in both mice and humans (22). Bile acids are also required for solubilization of dietary cholesterol into mixed micelles to help it efficiently travel along the unstirred water layer for subsequent absorption into the enterocyte (31). Intestinal bile acids need to be present in a critical micellar concentration (CMC) to efficiently aid in the solubilization of cholesterol and hydrophobic fatty acids. When concentrations of bile acids are below the CMC in the small intestine, cholesterol absorption strongly decreases. Aside from the absolute concentration of intestinal bile acids, the composition of different bile acid species contributes to the efficacy of micellar solubilization (32). Hydrophobic bile acids such as DCA and CA have a lower CMC and are therefore more effective detergents than hydrophilic bile acids (33).

Modulating bile acid activated receptor activity of mainly FXR has also been implicated in the regulation of lipid metabolism (see for overview Fig. 3). Mice with genetic inactivation of Fxr have elevated serum and hepatic levels of cholesterol and triglycerides (34,35). Conversely, activation of FXR via the synthetic agonist GW4064 decreased plasma cholesterol and triglycerides in db/db and wildtype mice (36). A derivative of the naturally occurring FXR agonist CDCA, 6α-ethyl-CDCA (obeticholic acid, OCA), was shown to reduce plasma cholesterol levels in mice and this effect was abrogated upon liver specific knockout of Fxr, implying an
hepatic FXR mediated effect on reverse cholesterol transport (37). Intestine specific FXR activation by the synthetic agonist PX20606 induced Fgf15 expression and also lowered plasma cholesterol levels through increasing intestinal cholesterol excretion (30). In humans, treatment with the FXR agonist OCA resulted in an increase in plasma cholesterol, mainly in the low density lipoprotein (LDL) fraction (38). DCA and CDCA treatment also resulted in an increase in plasma LDL cholesterol in humans (39,40). The likely explanation provided for these effects is that activation of FXR leads to inhibition of bile acid synthesis via CYP7A1 suppression. Therefore, less cholesterol is catabolized to bile acids, increasing hepatic cholesterol content and inhibiting LDL receptor (LDLR) activity. This is supported by the observation that LDLR gene expression decreased with CDCA treatment in humans (40). Conversely, when FXR activity was decreased, by interrupting the enterohepatic bile acid circulation with cholestyramine, a bile acid sequestrant, LDL receptor gene expression was increased. FXR activation also reduces very-low-density lipoprotein (VLDL) secretion and increases VLDL receptor expression (41,42). Expression of apolipoprotein A1, a major protein component of high density lipoprotein (HDL) cholesterol, was shown to be decreased upon FXR activation (43). In the same study, patients with cholestasis due to progressive familial intrahepatic cholestasis and biliary atresia displayed lower serum HDL levels. In mice, FXR activation increased reverse cholesterol transport, via upregulation of the scavenger receptor class B type 1, thereby also reducing HDL levels (44).

FXR modulation does not only affect cholesterol and lipoproteins but is also involved in triglyceride metabolism. In mice, administration of CA resulted in an FXR dependent decrease in triglycerides (42,45). Lipogenesis is decreased through repression of the sterol responsive element binding protein 1c (SREBP1c) both directly via hepatic FXR activation and indirectly via FGF15/19 action following intestinal FXR activation (42,46). Additionally, activation of FXR increases the activity of lipoprotein lipase (LPL), by induction of the obligatory cofactor apoC-II and reducing apoC-III, an inhibitor of LPL (45,47). This in turn increases hydrolysis of triglycerides in chylomicrons and VLDL contributing to the triglyceride lowering effect of FXR agonists.
* Contradicting results for the effects of FXR activation on total cholesterol and LDL cholesterol in humans exist. Total cholesterol and LDL went up in humans with FXR agonist treatment while in mice these parameters went down.

Figure 3. Effects of FXR activation (intestinal and hepatic) on lipoprotein metabolism

Bile acids in control of glucose metabolism

Prevalence rates of obesity and related disorders such as type 2 diabetes mellitus and non-alcoholic fatty liver disease are rising worldwide at an alarming rate (48,49). Current treatment options are not sufficient to control this epidemic. Therefore, studies aimed at understanding alternative mechanisms involved in metabolism, subsequently leading to potential novel therapies, have emerged. Among the possible targets are microbiota, nuclear receptors and bile acid homeostasis. Bile acids and their receptors have been implicated in important signaling pathways eliciting various metabolic effects including lipid, glucose and energy metabolism (50,51). The most important bile acid activated receptors involved in these effects are nuclear receptors such as FXR, the vitamin D receptor (VDR, NR1I1) and the pregnane X receptor (PXR, NR1I2), and the G-protein coupled bile acid receptor 1 (GPBAR1, GPCR19 also known as TGR5) (52).
FXR is expressed in several organs including the liver, intestine, kidneys, adrenal glands, adipose tissue and immune cells. Parks et al. discovered that bile acids act as natural ligands for FXR but with varying potency for different species (53). In order of decreasing potency the hydrophobic bile acid species CDCA, DCA, CA and LCA are the most effective FXR activators in vitro (54). In contrast, hydrophilic MCA species have been shown to antagonize FXR (4,55). Pharmacological modulation of FXR, especially in the intestine, has been extensively explored as a potential target for therapy in disorders of glucose metabolism. Activation of intestinal FXR leads to induction and release of the hormone FGF15/19 that, aside from regulating bile acid synthesis, has been implicated in liver regeneration and glucose metabolism (56–58). In humans, both obesity and T2DM are associated with lower plasma levels of FGF19 (59,60). The FXR agonist OCA was recently approved by the Food and Drug Administration (FDA) for the treatment of primary biliary cholangitis (PBC), and is in clinical trials for treatment of non-alcoholic steatohepatitis (NASH). Administration of OCA to patients with NAFLD and T2DM increased plasma FGF19 levels, decreased liver enzymes (alanine aminotransferase and γ-glutamyltransferase) and improved insulin sensitivity (61). In a phase 3 clinical trial, OCA ameliorated histology scores of NASH patients (38). Animal studies, however, have not generated unambiguous results regarding the effects of the FXR-FGF15/19 axis on metabolism. Transgenic mice with hepatic overexpression of FGF19 display an increased metabolic rate and decreased adiposity (62). Kir et al. found that administration of FGF19 to WT mice improved glucose metabolism by inducing hepatic glycogen and protein synthesis (63). Conversely, Fgf15−/− mice showed glucose intolerance and a reduced hepatic glycogen content. Administration of FGF19 was even found to reverse diabetes mellitus in ob/ob mice (64). A direct involvement of Fxr-Fgf15 signaling was demonstrated by intestinal inactivation of Fxr and by reducing intestinal Fxr signaling through remodeling the intestinal microbial composition by the anti-oxidant tempol, both conditions that reduced diet induced obesity and hepatic triglyceride accumulation in mice (65,66). The metabolic improvements by tempol were explained by the reduced presence of microbial species of Lactobacillus and their BSH activity, resulting in increased levels of tauro-βMCA, a naturally occurring FXR antagonist. This claim was further supported by a study in which administration of glyco-β-muricholic acid (Gly-MCA), a selective FXR inhibitor, reduced obesity and improved related metabolic abnormalities in mice (67). Paradoxically, intestine-specific FXR activation using the intestinally restricted FXR agonist fexaramine, also reduced diet-induced obesity, insulin resistance and
hepatic steatosis in mice fed a high fat diet (HFD) (68). In a recent study, however, these beneficial effects of fexaramine were explained by an indirect effect on the microbiota and pronounced alterations in bile acid metabolism, resulting in more of the secondary bile acids LCA and DCA, the most potent naturally occurring TGR5 agonists (69).

TGR5 is expressed by a variety of cell types including adipocytes, myocytes, immune cells, bile duct epithelium, Kupffer cells and enterocytes. In brown adipose tissue TGR5 activation increases energy expenditure (70,71). Intestinal TGR5 is activated by bile acids and luminal nutrients in both the ileum and colon, and it enhances the secretion of glucagon like peptide-1 (GLP-1) (72). GLP-1 is an incretin hormone that induces insulin synthesis and release by the pancreas and acts to preserve β-cell function.

The vitamin D receptor (VDR) is mainly known for its role in regulating calcium homeostasis. However, VDR activation induces more changes including effects on drug metabolism, bile acid homeostasis and inflammation (73). LCA is a potent ligand for VDR and is possibly involved in mediating bile acid induced toxicity and colon cancer risk (74). A role of VDR in regulating bile acid synthesis has been proposed but results have not been unambiguous. In earlier studies, VDR activation decreased CYP7A1 dependent bile acid synthesis both via intestinal Fgf15 levels and via direct effects in the hepatocyte (75,76). A more recent study showed that VDR activation induced a SHP dependent increase in CYP7A1 expression both in vitro, in human hepatocytes, and in vivo, in mice (77). Despite these conflicting results, it is tempting to speculate that changes in VDR activation are involved in some of the pleiotropic metabolic effects observed when modulating bile acid homeostasis.

The xenobiotic receptor PXR is activated by a wide variety of ligands including prescription drugs, environmental contaminants and steroids (78,79). It is also activated by bile acids, most notably by LCA, and involved in hepatic protection of liver toxicity (80). There is some evidence for both intestinal and hepatic PXR mediated repression of CYP7A1 (81). PXR is also implicated in energy metabolism by promoting lipogenesis and suppressing fatty acid β-oxidation and gluconeogenesis (79,82). However, similar to VDR, the role of PXR is complex and its effects are dependent on a variety of cofactors and receptors. Therefore, direct consequences of bile acid mediated PXR effects for metabolism have been difficult to assess.
**Bile acid malabsorption – disease or therapy?**

The importance of FXR-FGF15/19 signaling in controlling bile acid homeostasis is illustrated by various bile acid malabsorption syndromes. Clinically, bile acid malabsorption in patients causes diarrhea due to high bile acid concentrations in the colon that lead to secretion of water and electrolytes and stimulation of propulsive contractions (83). Patients with primary bile acid diarrhea, a condition in which bile acid malabsorption occurs in the absence of ileal or other obvious gastrointestinal disease, display lower levels of FGF19 and higher levels of 7α-hydroxy-4-cholesten-3-one (C4), a surrogate marker for BA synthesis (84,85). Bile acid diarrhea patients are usually treated with bile acid sequestrants that bind free bile acids in the intestine, preventing them from exerting their colonic stimulatory effects that cause diarrhea (86). In a murine model of bile acid malabsorption it has been shown that Fxr activation or Fgf15 administration reduces fecal bile acid excretion (87). As patients with bile acid malabsorption display lower plasma levels of FGF19, it is tempting to speculate that treatment with an FXR agonist or FGF19 could be a useful novel therapy in treatment of bile acid malabsorption (88).

Bile acid malabsorption is also one of the features of the genetic disease cystic fibrosis (CF) (89–91). Although the mechanism underlying bile acid malabsorption in CF is not fully understood, it potentially affects other aspects of the disease phenotype which will be reviewed in more detail in Chapter 3.

While bile acid malabsorption in the cases described above is a manifestation of disease resulting in unwanted symptoms, induction of BA malabsorption has also been studied as a potential therapy for several conditions. Induction of bile acid malabsorption either by directly inhibiting ASBT or by intestinal binding of bile acids via sequestrants, potentially improves outcomes of various metabolic and hepatic disorders (51,92). Both bile acid sequestrants and ASBT inhibitors have shown to improve liver outcomes in preclinical studies as treatment for cholestatic disorders by reducing toxic biliary bile acid concentrations (93–95). Unfortunately, while the bile acid sequestrant colesvelam successfully reduced serum bile acid concentrations, this did not result in a clinical reduction of cholestasis induced pruritis in humans (96). On the other hand, recent studies with ASBT inhibitor treatment showed potential benefits for treatment of cholestasis induced pruritus in patients with primary biliary cholangitis and Alagille syndrome (97,98). Both ASBT inhibition and bile acid sequestrants have been studied for their effect on hypercholesterolemia and atherosclerosis (99). Bile acid sequestrants such as cholestyramine and colesvelam are registered as adjunct therapy to statins for
lowering plasma cholesterol levels. Bile acid sequestrants lower plasma LDL cholesterol and elevate HDL cholesterol while increasing triglyceride levels in humans (92). The presumed underlying mechanism of lowering cholesterol is that interruption of the enterohepatic circulation of bile acids leads to increased catabolism of hepatic cholesterol to bile acids and their subsequent excretion. Subsequently, it results in a compensatory increased expression of hepatic LDL receptor and decreased levels of plasma LDL cholesterol.

Although bile acid sequestrants are well studied and clinically available, data on interrupting the enterohepatic circulation of bile acids with ASBT inhibitors for improving lipid and glucose metabolism is more limited. Nevertheless, in several animal models, ASBT inhibition has shown to reduce hypercholesterolemia and atherosclerosis (100–103). Asbt<sup>−/−</sup> mice have increased fecal bile acid excretion, a smaller total bile acid pool size and subsequently a lower intestinal cholesterol absorption and increased fecal lipid excretion (104). Experiments with ASBT inhibition and plasma lipid profiles have not created unambiguous results and are dependent on experimental conditions. For example, Dawson et al. showed that in chow fed conditions Asbt<sup>−/−</sup> mice have decreased hepatic cholesterol levels but increased plasma cholesterol levels, mainly in the HDL fraction (104). However, when combining Asbt<sup>−/−</sup> with apolipoprotein E-deficiency (ApoE<sup>−/−</sup>), creating a more humanized lipoprotein profile with more VLDL and LDL compared to HDL, and an atherogenic diet in mice, Asbt inactivation decreased plasma cholesterol, mainly in the VLDL and LDL fractions (100). In ob/ob mice, treatment with an ASBT inhibitor for 11 days decreased plasma triglycerides but did not affect total hepatic or plasma cholesterol levels (105). In WT mice fed a HFD with added cholesterol (0.2% w/w) combined with an ASBT inhibitor for 16 weeks, robust effects were observed in preventing hepatic cholesterol and triglyceride accumulation while plasma cholesterol was not changed compared to untreated WT mice (106). Differences in (nuclear) receptor signaling are possibly involved in the different changes observed in these experiments. ASBT inhibition clearly decreases intestinal FXR activation and subsequently FGF15/19 signaling in all experimental conditions. However, the effects on hepatic FXR signaling are less clear and Rao et al. speculated that a more FXR agonistic bile acid composition upon ASBT inhibition (more CA and DCA) could increase hepatic FXR signaling. Therefore, the effects on lipid profile resulting from low levels FGF15/19 could potentially be counteracted by increased hepatic FXR activation (Fig. 3).
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Interruption of the enterohepatic circulation of bile acids has also been studied for its effects on glucose metabolism and related disorders. Both bile acid sequestration and ASBT inhibition have shown beneficial effects on glucose homeostasis in animal models and humans (51,92). Interestingly, partial disruption of the enterohepatic circulation by bile diversion to the ileum in mice also resulted in metabolic improvements that were similar to bariatric surgery (107).

ASBT inhibitors have also shown to improve lipid and glucose metabolism in Zucker Diabetic Fatty (ZDF) rats, a model for type 2 diabetes, and ob/ob mice (105,108,109). In T2DM, the ASBT inhibitor GSK2330672 lowered serum glucose and LDL cholesterol levels (110). More recently, treatment with an ASBT inhibitor has shown to prevent development of NAFLD and improve glucose metabolic outcomes in long-term HFD feeding in C57BL/6J mice (106). The precise mechanism underlying these beneficial changes of interruption of the enterohepatic circulation on glucose homeostasis and related disorders, however, remains unclear. In T2DM patients treated with a bile acid sequestrant, there was no correlation between changes in measured parameters of bile acid homeostasis and improvements in glucose metabolism (111). Chen et al. showed an increase in GLP-1 release after ASBT inhibitor treatment in ZDF rats, suggesting possible involvement of increased TGR5 signaling as a consequence of increased colonic bile acid concentrations (109). Hoffman argued that beneficial improvements in glucose homeostasis upon bile acid sequestrant therapy might be the result of defective fatty acid absorption, leading to higher ileal concentrations of triglycerides, subsequently entering enteroendocrine L-cells and resulting in higher GLP-1 release from the intestine (112). This is supported by the observation that medium and long chain fatty acids are sensed through G-protein-coupled receptors 40 and 120 in the distal intestine to stimulate the release of GLP-1 (113,114). However, no direct proof of involvement of these mechanisms in the benefits of bile acid sequestrants nor ASBT inhibitors exist. Further studies are therefore needed to specifically address the underlying mechanisms of interruption of the enterohepatic circulation and its beneficial effects on metabolic homeostasis.
The aim of this thesis was to study the effects of bile acid malabsorption in physiology, disease and as potential therapy. Under physiological conditions, the enterohepatic circulation of bile acids is a highly efficient and tightly regulated system. Normally 95% of bile acids secreted into the intestine are reabsorbed in the terminal ileum every cycle. Interruption of this enterohepatic circulation induces various changes, including effects on hormones, lipid absorption and microbiota. These changes may in some situations be beneficial to health while in others they might not. This thesis specifically explored the effects of interruption of the enterohepatic circulation of bile acids in several models. The objective of the first part of this thesis (Chapter 3-5) was to delineate the mechanism of bile acid malabsorption in cystic fibrosis (CF), a state in which bile acid malabsorption is part of the disease phenotype. The second part (Chapter 6-8) explores targeted induction of bile acid malabsorption through inhibition of the main intestinal uptake transporter for bile acids, the apical sodium-dependent bile acid transporter (ASBT), and its potential beneficial metabolic effects.

CF is a genetic disease caused by a defect in the cystic fibrosis transmembrane conductance regulator (CFTR). CF is characterized by dysfunction of various organ systems due to defective transmembrane ion transport leading to thickening of mucus. With the increasingly effective treatment of pulmonary infections and the associated increase in patient survival, gastrointestinal problems have become more in focus. Liver involvement is relatively common in CF and displays a heterogeneous phenotype. Chapter 2 reviews how the liver can be affected and how to approach CF related liver involvement from a clinical perspective. Aside from liver involvement, CF patients also exhibit a disruption of bile acid homeostasis characterized by bile acid malabsorption and a subsequent increase in hepatic synthesis. In the early 1990s studies were performed to characterize this defect and explore its potential role in nutrient malabsorption. Life expectancy of CF patients has increased significantly over the last two decades and aside from pulmonary and nutritional issues, other complications such as diabetes, gastrointestinal problems and liver disease are more common. In chapter 3 the potential role of bile acid homeostasis in the development of these CF related complications is reviewed, together with possible therapies.

In chapter 4 we aimed to characterize bile acid homeostasis in CF patients using novel biomarkers and assess to what extent this was affected upon CFTR modulator treatment. These results will provide us with more insight into the gastrointestinal effects of CFTR modulator therapies and the potential of using
these bile acid related biomarkers in future CF studies. To this end we measured plasma markers of bile acid absorption and synthesis in CF patients with a so called class III gating mutation of the CFTR protein. We investigated whether these markers are affected in the CF condition, consistent with the known bile acid malabsorption phenotype in CF patients, and whether they change upon treatment with ivacaftor, a novel CFTR potentiator.

Improving bile acid homeostasis in CF could potentially affect many other gastrointestinal and metabolic complications (reviewed in chapter 3). Therefore, we studied in chapter 5 whether we could improve bile acid homeostasis in a mouse model of CF using polyethylene glycol, a commonly used laxative. Cfitr knockout mice display a severe intestinal phenotype and, like CF patients, also exhibit bile acid malabsorption. Polyethylene glycol is widely used in CF mice to prevent intestinal obstruction and has been shown to improve certain intestinal features. However, the effects on bile acid homeostasis in the CF condition have remained unknown.

The second part of the thesis focuses on inducing bile acid malabsorption to improve specific features of metabolic conditions, such as hypercholesterolemia and obesity. To this end, ASBT inactivation, another bile acid malabsorption model, was used to explore implications of an interrupted enterohepatic circulation of bile acids. Cholesterol metabolism is highly intertwined with bile acid homeostasis. Therefore, studying the effects of bile acid malabsorption on cholesterol metabolism could provide us with novel insights in mechanisms and therapies for hypercholesterolemia. In chapter 6 we studied intestinal cholesterol excretion in models of (partial) impaired cholesterol absorption. We evaluated the effects of inhibition of the ASBT, responsible for ileal bile acid reabsorption, on intestinal cholesterol fluxes and compared this to directly inhibiting intestinal cholesterol absorption using the drug ezetimibe. Bile acids are crucial for the efficient intestinal absorption of lipids and involved in other processes regarding glucose and lipid metabolism. In chapter 7 we investigated the role of ASBT inhibition in diet induced obesity, insulin resistance and non-alcoholic fatty liver disease (NAFLD). We also measured the absorption of individual fatty acids upon ASBT inhibition. Subsequently, we modified fat composition of a high fat diet and assessed differential effects on obesity, insulin resistance and hepatic fat accumulation in Asbt⁻/⁻ mice and their littermate controls.

It was previously shown that ASBT inhibition prevents hepatic steatosis in high fat diet fed mice, highlighting its potential role in treatment of NAFLD. However, it is not known whether ASBT inhibition can actually prevent the
progression of steatosis to the more clinically relevant steatohepatitis and fibrosis. Therefore, in chapter 8 we use a dietary choline deficiency model to induce hepatic steatosis and subsequent fibrosis in mice with and without an ASBT inhibitor.

In chapter 9, we discuss the findings of the different studies in relation to the current literature on the enterohepatic circulation in health and disease and conclude with the implications of the present findings for our evolving understanding and future research.
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