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Intestinal function in cholestasis and essential fatty acid deficiency

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CHAPTER 1

General introduction

INTRODUCTION

Cholestatic liver diseases comprises a wide range of conditions characterized by defective bile formation and, therefore, impaired bile salt transport from the liver to the intestinal lumen. Physiological consequences include retention of bile salts in the hepatocytes, limited availability of bile salts in the intestinal lumen, hyperbilirubinemia and elevated plasma bile salt levels. These consequences eventually lead to liver injury, lipid malabsorption, jaundice, pruritus and potentially peripheral tissue injury. As a result of malabsorption, cholestatic patients, especially children, can develop serious nutritional defects. For instance, cholestatic disorders are frequently associated with essential fatty acid (EFA) deficiency. The resultant malnutritional state strongly affects prognosis and treatment outcome of cholestatic patients¹. Identifying specific nutritional defects associated with cholestasis and EFA deficiency and the mechanism(s) underlying these defects will open possibilities towards successful therapies.

In this general introduction, an overview of bile salt metabolism in health and disease (cholestasis) as well as bile salt functions in lipid metabolism will be provided. Processes involved in lipid and carbohydrate absorption from the intestine will be discussed and, finally, the aim of the work described in this thesis will be defined.

BILE SALT SYNTHESIS

Bile acids are present in the form of sodium salts under physiological conditions prevailing in the body (pH 5-7.5) and therefore will be referred to as bile salts. Bile salts are synthesized from cholesterol via the classical (neutral) pathway or the alternative (acidic) pathway. A schematic image of bile salt synthesis is depicted in **Figure 1**. The classical pathway involves modification of the sterol nucleus including saturation of the double bond, epimerization of the 3 β -hydroxyl group and hydroxylation at the 7 α and 12 α -positions, preceding oxidative cleavage of the side chain. Microsomal cholesterol 7 α -hydroxylase (CYP7A1) catalyzes the first and rate-controlling step, yielding 7 α -hydroxycholesterol which is subsequently converted into 7 α -hydroxy-4-cholesten-3-one by microsomal HSD3B7 (3 β -hydroxy- Δ^5 -C₂₇-steroid dehydrogenase/isomerase). 7 α -Hydroxy-4-cholesten-3-one can either enter the pathway towards synthesis of cholic acid (CA) or the pathway towards synthesis of chenodeoxycholic acid (CDCA). Microsomal sterol 12 α -hydroxylase (CYP8B1) catalyzes the hydroxylation of 7 α -hydroxy-4-cholesten-3-one into 7 α ,12 α -dihydroxy-4-cholesten-3-one, the precursor of CA. The cytosolic enzymes AKR1D1 (Δ^{4-3} -oxosteroid-5 β -reductase) and AKR1C4 (3 α -hydroxysteroid dehydrogenase) catalyze reduction of 7 α -hydroxy-4-cholesten-3-one and 7 α ,12 α -dihydroxy-4-cholesten-3-one, yielding 5 β -cholestan-3 α ,7 α ,12 α -triol (CA precursor) and 5 β -cholestan-3 α ,7 α -diol (CDCA precursor), respectively. Mitochondrial sterol 27-hydroxylase (CYP27A1) oxidizes the side chains of both precursors, yielding 3 α ,7 α ,12 α -trihydroxy-5 β -cholestanic acid and 3 α ,7 α -dihydroxy-5 β -cholestanic acid. These precursors are ligated to coenzyme A by bile acid CoA synthetase (BACS) activity and are subsequently transported into the peroxisomes for side chain cleavage. Side chain cleavage eventually leads to the formation of CA (3 α ,7 α ,12 α) and CDCA (3 α ,7 α). In the alternative pathway, cholesterol is hydroxylated into oxidized intermediates (oxysterols) through the actions of CYP27A1 and oxysterol 7 α -hydroxylase (CYP7B1). While the classical pathway is exclusive to the liver, the alternative pathway also occurs in peripheral tissues²⁻⁴. In rodents, the majority of CDCA is converted to the more hydrophilic bile salt α -

muricholic acid (α -MA) and subsequently to β -muricholic acid (β -MA) in the liver. In rodents, CA and β -MA are the main bile salts present in bile ^{5,6}.

Nearly all bile salts undergo conjugation with glycine or taurine in liver peroxisomes, catalyzed by bile acid-CoA:amino acid *N*-acetyltransferase (BAAT). In humans, the majority of bile salts are conjugated with glycine, while rodent bile salts mainly consist of taurine-conjugates ^{2-4,7}.

A small fraction of the (conjugated) primary bile salts enter the colon, where they can be deconjugated and converted to hydrophobic secondary bile salts by colonic bacteria, or are excreted into the feces. After deconjugation, the primary bile salt CA can be 7α -dehydroxylated into deoxycholic acid (DCA) and CDCA can be 7α -dehydroxylated into lithocholic acid (LCA) ⁸. In addition, CDCA can be converted to the more hydrophilic bile salt ursodeoxycholic acid (UDCA) by colonic bacterial oxidation of the 7α -hydroxyl group and stereospecific reduction of the 7-keto group, generating the corresponding 7β -hydroxyl group ⁹. In mice and rats, β -MA is converted to hyodeoxycholic acid (HDCA) through 7β -hydroxylation and 6β -hydroxy epimerization and or to ω -muricholic acid (ω -MA) through epimerization of the 6-hydroxy group ^{5,6,10}.

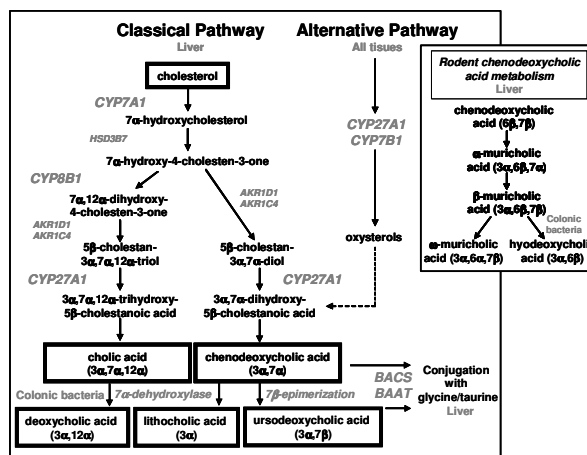


Figure 1 Classical and alternative pathway of bile salt synthesis and rodent chenodeoxycholic acid metabolism.

ENTEROHEPATIC CIRCULATION

Under non-cholestatic conditions, conjugated primary and secondary bile salts are secreted into the bile. Bile functions as a route for the excretion of endogenous and exogenous compounds such as bile salts, bilirubin, phospholipids, cholesterol, drugs and toxins. In humans and mice, bile salts are stored and concentrated in the gallbladder during the interdigestive period and are excreted postprandially to aid in the absorption of lipids, cholesterol and fat-soluble vitamins. The turnover of the bile salt pool is ~5%, that is ~5% is lost in the feces per cycle, and ~95% is (passively and actively) reabsorbed by the intestine. The majority of bile salts is absorbed via active transport in the terminal ileum and transported back to the liver via the portal circulation ¹¹⁻¹³.

Hepatic bile formation is driven by the osmotic gradient generated by secretion of relatively non-permeant solutes into the canalicular lumen. Thus, accumulation of osmotically active molecules promotes the movement of water and electrolytes across the canalicular

membrane. Bile salts account at least for 40-50% of bile flow, while the remainder depends on electrolytes^{14,15}, bicarbonate and mostly glutathione and its conjugates¹⁶⁻¹⁸. Most of the glutathione (70-80%) in bile is in the reduced form (GSH)¹⁵. **Figure 2** represents a schematic image of the enterohepatic circulation and the bile salt transporters involved.

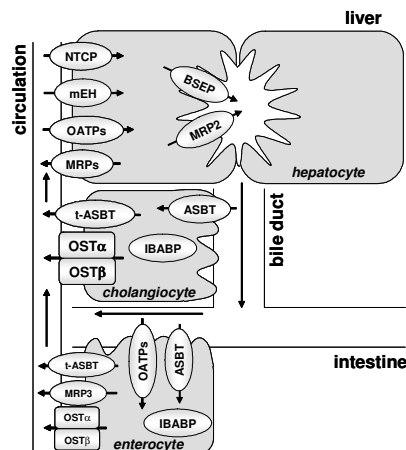


Figure 2 Schematic image of the enterohepatic circulation of bile salts with the bile salt transporters involved.

Bile salt transport across the canalicular membrane into the bile canaliculi occurs against a 100-1000 fold concentration gradient¹⁹. Canalicular secretion of bile salts involves the actions of transporters whose function depends on ATP hydrolysis. The major transporters involved are Bile Salt Export Pump (BSEP/ABCB11), responsible for transport of monovalent bile salts, and the Multidrug Resistance-associated Protein 2 (MRP2/ABCC2), the main transporter of divalent bile salts^{12,13,19-23}. BSEP is essential for canalicular bile secretion, evidenced by knockout mice exhibiting impaired bile secretion and intrahepatic cholestasis. Wang *et al.* demonstrated that secretion of CA (monovalent) was greatly reduced (6% of wild-type), while total bile salt output was about 30% of wildtype mice in mice lacking BSEP²⁴. BSEP is exclusively expressed in the liver²⁵. BSEP has high affinity for TCDCA > TCA > TUDCA > GCA^{26,27}. MRP2 is localized to the canalicular membrane of hepatocytes, the apical membrane of renal proximal tubule epithelial cells, and the apical membrane of duodenal and jejunal enterocytes²⁸. Besides divalent bile salts like sulphated TLCA and GLCA, MRP2 mediates the export of bilirubin conjugates, glutathione, glucuronide and sulphate conjugates, and some unconjugated drugs^{12,28-30}. MRP2 does not have the capacity to transport monovalent bile salts^{12,31}. MRP2-deficient rats (GY/TR⁻ and EHBR rats) are characterized by permanent hyperbilirubinemia and strong induction of MRP3^{12,32-34}.

Bile salts are actively absorbed by cholangiocytes lining the bile ducts to recycle back to the hepatocytes for their re-secretion (cholehepatic shunt pathway)^{12,13,35}. Apical Sodium-dependent Bile Salt Transporter (ASBT) mediates active transport of bile salts from the bile ducts across the apical membrane^{36,37}. Ileal Bile Acid Binding Protein (IBABP) is also expressed in cholangiocytes, though its exact function is still unclear³⁶. It is suggested that IBABP modulates transcellular transport and prevents intracellular bile salt toxicity by binding bile salts, however, evidence is still lacking³⁵. Bile salt efflux via the basolateral

membrane is mediated by t-ASBT, an alternatively spliced form of ASBT³⁸, Multidrug resistance protein 3 (MDR3/ABCB4) is also expressed at the basolateral membrane of cholangiocytes³⁹. However, there is no direct evidence that MDR3 is involved in cholangiocyte bile salt efflux. Recently, the heterodimeric Organic Solute Transporter α/β (OST α/β) has been identified in cholangiocytes. OST α/β can transport bile salts via facilitated diffusion^{40,41}.

Bile salts that have not been transported back to the liver via the cholehepatic shunt pathway enter the intestinal lumen and are passively and actively absorbed by the enterocytes. Apical uptake of bile salts in the enterocytes occurs via passive diffusion of unconjugated bile salts in the small intestine and the colon (after deconjugation by intestinal microflora), which accounts for a small fraction of intestinal bile salt conservation^{42,43}. ASBT is also expressed in the terminal ileum and is responsible for absorption of the majority of luminal bile salts⁴⁴⁻⁴⁶. Human ASBT efficiently transports conjugated and unconjugated bile salts with a preference for the taurine and glycine conjugates^{47,48}. ASBT mediated transport is electrogenic with 2:1 Na⁺/bile salt coupling stoichiometry⁴⁹. ASBT exhibits a higher affinity for dihydroxy bile salts (CDCA and DCA) compared to trihydroxy bile salts such as CA, TCA and GCA⁴⁷. The phenotype of ASBT knockout mice involves intestinal bile salt malabsorption and interruption of the enterohepatic circulation, emphasizing the importance of ASBT⁵⁰. A Na⁺-independent bile salt transporter (Organic anion transporting polypeptide; Oatp1a5) was found to be expressed in rat jejunal enterocytes¹². OATP1A2 has been suggested as the human Oatp1a5 ortholog^{51,52}. OATPs transport anions via a Na⁺-independent mechanism. Jejunal uptake of bile salts occurs via in-to-out HCO₃⁻ gradient; however the role of OATPs herein remains unclear⁵³. Intracellular transport of bile salts in enterocytes is thought to occur via IBABP. Intestinal IBABP expression is restricted to the terminal ileum^{12,54}. No data is yet available about IBABP knockout models, but the observation that downregulation of IBABP expression resulted in increased fecal bile salt loss is suggestive of a function in ileal bile salt absorption⁵⁵. However, Kok *et al.* demonstrated that intestinal bile salt reabsorption was markedly increased in *Fxr*^{-/-} mice despite complete absence of *Ibabp*, suggestive of a function as a negative regulator of bile salt reabsorption rather than a positive⁵⁶. Concluding, IBABP function in bile salt reabsorption remains unclear. t-ASBT MRP3 and OST α/β have been proposed as candidates for basolateral bile salt efflux transporters in enterocytes; their specific roles, however, have remained undefined so far¹².

To complete their enterohepatic circulation, absorbed bile salts are transported back to the liver via the portal blood. The basolateral membrane of the hepatocytes is in direct contact with the space of Disse that receives its content from the portal blood through large pores of the sinusoidal endothelium. The majority of bile salts reaches the space of Disse bound to albumin and needs to be dissociated for translocation across the membrane⁵⁷. Na⁺-dependent Taurocholate Co-transporting Polypeptide (NTCP) is the main transporter mediating bile salt uptake. NTCP has been shown to mediate the transport of both conjugated and unconjugated bile salts in a Na⁺-dependent manner with a stoichiometry of 2:1⁵⁸. Several studies have also suggested a role for microsomal epoxide hydrolase (mEH) in Na⁺-dependent bile salt uptake¹². However, since mice lacking mEH expression have no apparent abnormalities in bile salt homeostasis, the contribution of mEH to hepatocyte bile salt uptake has become debated⁵⁹. Though, hypercholanemia was associated with a 85% decrease of mEH protein due to a point mutation in a patient with no alteration in NTCP expression⁶⁰. Zhu *et al.* proposed that mEH is more efficient for transporting bile salts

conjugated with glycine compared to NTCP in humans⁶⁰. Since the majority of bile salts in humans are conjugated with glycine, in contrast to rats and mice, it is possible that mEH is responsible for hepatic uptake of a higher percentage of bile salts compared to NTCP⁶⁰. Most unconjugated bile salts are transported in a Na⁺-independent manner via passive diffusion or carrier-mediated transport^{57,61}. Several members of the OATP family have been implicated in basolateral bile salt influx; OATP1A2, OATP1B1 and OATP1B3^{12,57,62}. OATPs mediate the exchange of extracellular HCO₃⁻ or glutathione, indicating that they are also involved in GSH efflux⁶³.

Under physiological conditions, bile salt transport from the hepatocyte across the basolateral membrane into the portal blood is negligible⁵¹. Transport is mediated by members of the MRP family. MRP1 (ABCC1), 3 (ABCC3) and 4 (ABCC4) are localized to the basolateral membrane and can transport bile salts and other compounds in a ATP-dependent fashion^{12,64,65}. Zelcer *et al.* demonstrated that bile salt homeostasis was unaltered in mice lacking Mrp3, indicating that Mrp3 is not solely responsible for bile salt efflux or may not be involved⁶⁶.

Hepatic transcellular transport of bile salts can occur via intracellular trafficking and vesicle-mediated transport. Under physiological conditions, the majority of bile salts is transported based on the basolateral to canalicular concentration gradient, possibly be mediated by intracellular bile salt-binding proteins. The involvement of vesicle-mediated transport was concluded from several observations that indicated the partitioning of hydrophobic bile salts into membraneous intracellular organs such as endoplasmic reticulum and Golgi apparatus^{42,51}.

PHYSIOLOGICAL FUNCTIONS OF BILE SALTS

Physiological functions of bile salts include cholesterol elimination, stimulation of bile flow, stimulation of biliary phospholipid secretion, regulation of bile salt and cholesterol biosynthesis and enhancement of lipid absorption⁶⁷. Apart from their role in dietary lipid absorption and cholesterol homeostasis, bile salts also function as signaling molecules. Through activation of diverse signaling pathways, bile salts can regulate their own enterohepatic circulation, and metabolism of triglycerides, cholesterol, energy and glucose⁶⁸. Bile salts can activate nuclear hormone receptors such as the farnesoid X receptor (FXR)⁶⁹⁻⁷¹, involved in the regulation of bile salt metabolism, lipid metabolism and glucose metabolism. The role of FXR in bile salt homeostasis and enterohepatic circulation will be discussed below in combination with cholestasis.

Bile salts have also been shown to affect the microflora and the integrity of the small intestine. Obstruction of bile flow in humans and rodents causes proliferation of intestinal bacteria and mucosal injury, which can lead to bacterial translocation across the mucosal barrier and systemic infection⁷². Bacterial overgrowth and translocation caused by biliary obstruction in rats was inhibited by oral administration of bile salts^{73,74}. In addition, oral administration of bile salts blocked endotoxemia in patients with obstructive jaundice⁷⁵⁻⁷⁷. Recently, Inagaki *et al.* demonstrated that FXR induces genes involved in enteroprotection, such as iNOS, IL18 and angiogenin, and thereby inhibits bacterial growth and ileal mucosal injury in cholestatic mice. Moreover, mice lacking FXR had increased ileal levels of bacteria and a compromised epithelial barrier⁷⁸.

In addition to FXR, bile salts have also been shown to activate other nuclear receptors, the pregnane X receptor, the vitamin D receptor and the constitutive androstane receptor,

leading to a reduction of bile salt toxicity⁷⁹⁻⁸². Bile salts can also activate MAPK pathways, usually by activating cellular membrane receptors, and thereby affect proliferation and apoptosis⁸³⁻⁸⁸. Recently, bile salts were also found to be ligands for a G protein-coupled membrane receptor (TGR5/GPBAR1)^{89,90}. Watanabe *et al.* provided data to suggest that bile salts regulate energy metabolism via binding to GPBAR1^{91,92}.

CHOLESTASIS

Cholestatic liver disease has been associated with numerous nutritional deficiencies, including EFA deficiency, and subsequent failure to thrive (reviewed in chapter 2)¹. Despite numerous treatment options to alleviate the clinical manifestations of cholestasis, cholestatic liver disease can necessitate a liver transplantation^{93,94}. In children, cholestasis-induced malnutrition or failure-to-thrive is associated with a worse outcome of their disease⁹⁵⁻⁹⁹. Cholestatic disorders can be divided in hereditary (genetic) disorders and acquired disorders. The majority of hereditary cholestatic disorders are characterized by defects in transporters involved in bile formation¹⁰⁰⁻¹⁰².

Etiology of cholestatic disorders

Progressive Familial Intrahepatic Cholestasis (PFIC) comprises a group of autosomal recessively inherited disorders associated with transporter defects. Mutations in the *FIC-1* (*ATP8B1*) gene, encoding FIC-1, can cause PFIC type 1 (PFIC-1) or Benign Recurrent Intrahepatic Cholestasis (BRIC)^{100,102,103}. PFIC-1 is most commonly diagnosed in newborns and often begins with cholestatic episodes progressing to permanent cholestasis with fibrosis, cirrhosis and liver failure in the first two decades of life. PFIC-1 is also associated with jaundice, pruritus, diarrhea and a failure to thrive. Plasma levels of bile salts and cholesterol are elevated¹⁰⁴. BRIC is characterized by recurrent spells of cholestasis associated with pruritus, jaundice, fatigue, loss of appetite, anorexia and elevated plasma bile salt levels^{103,104}. The age of onset, severity and number of these episodes vary greatly¹⁰⁵. FIC-1 profoundly acts as an aminophospholipid translocase, 'flipping' specific phospholipids (phosphatidylserine and phosphatidylethanolamine) from the outer leaflet to the inner leaflet of the membrane^{106,107}. FIC-1 and other homologues have been detected all over the body, including the bile canalicular membrane of hepatocytes and, with higher abundance, in the intestine and cholangiocytes^{106,108,109}. Studies with FIC-1 knockout mice revealed disturbed bile salt homeostasis, but only mildly impaired bile secretion, despite elevated plasma bile salt levels^{110,111}. The former findings are suggestive of an important role for the intestine in PFIC-1. This was confirmed by the observation that biliary diversion is a successful treatment for PFIC-1 patients, alleviating intrahepatic and extrahepatic symptoms^{112,113}. Chen *et al.* demonstrated that *FIC-1* mRNA was absent in the ileum of PFIC-1 patients, coinciding with strongly reduced *FXR* expression and fourfold higher *ASBT* expression. They also showed that BSEP promoter activity was diminished while ASBT promoter activity was greatly enhanced in *FIC-1* antisense treated Caco-2 cells, presumably via the loss of FXR¹¹⁴. Therefore, it is likely that bile salt uptake from the ileum is enhanced and bile salt secretion from the liver is diminished in PFIC-1 patients, explaining the manifestation of cholestasis. Studies with *Fic1* (*Atp8b1*) knockout mice, however, did not reveal enhanced bile salt absorption or increased *Asbt* expression, suggesting that the accumulation of bile salts in the plasma is not caused by increased intestinal bile salt absorption¹¹⁰. Moreover, Paulusma *et al.* showed that *Atp8b1* deficiency resulted in a loss

of canalicular phospholipid asymmetry that in turn renders the canalicular membrane less resistant toward hydrophobic bile salts, which may impair bile salt transport and cause cholestasis¹¹⁵.

PFIC-2 is caused by mutations in the *BSEP (ABCB11)*, encoding BSEP, the canalicular bile salt efflux pump¹¹⁶. Jansen *et al.* demonstrated that BSEP was absent from the canalicular membrane in PFIC-2 patients¹¹⁷. The clinical symptoms of PFIC-2 overlap with PFIC-1, but with fewer extrahepatic abnormalities and generally with a less episodic character, though often with more severe symptoms¹⁰²⁻¹⁰⁴. Bsep knockout mice show a clear decrease in bile salt secretion^{24,103}, explaining the manifestation of cholestasis.

PFIC-3 is associated with mutations in the *MDR3 (ABCB4)* gene, encoding MDR3¹⁰²⁻¹⁰⁴. MDR3 is a phospholipid floppase at the canalicular membrane, facilitating the secretion of phosphatidylcholine in bile¹¹⁸. The clinical symptoms of PFIC-3 are different from PFIC-1 and PFIC-2 as in that symptoms usually present somewhat later in life and also liver failure occurs at a later age. The histological picture shows strong bile duct proliferation and cirrhosis. PFIC-3 is also associated with jaundice, pruritus and elevated plasma bile salt levels¹⁰²⁻¹⁰⁴. A mouse model in which the mouse homolog of the human MDR3 gene, *Mdr2*, was disrupted revealed absence of biliary phospholipids despite normal bile salt secretion, progressive liver disease and histology similar to that in PFIC-3 patients¹¹⁹. Biliary phospholipids form mixed micelles with bile salts and prevent crystallization of cholesterol. In the absence of phospholipid secretion, the detergent bile salts tend to solubilize lipids from the biliary epithelium and cholangiocytes, leading to bile duct damage and secondary biliary fibrosis and cirrhosis¹⁰⁴.

Inborn errors of bile salt biosynthesis can also lead to cholestasis. Seven defects have been characterized so far, including mutations in the genes encoding for HSD3B7¹²⁰⁻¹²², AKR1D1^{123,124}, CYP7A1¹²⁵, CYP27A1¹²⁶, CYP7B1¹²⁷, BAAT¹²⁸ and 2-Methylacyl-CoA racemase¹²⁹. The phenotype of these defects is highly variable and they do not all lead to cholestasis. Liver disease tends to be more severe in defects that involve modifications of the cholesterol side chain than defects responsible for catalyzing reactions in the steroid nucleus (except for CYP7A1 deficiency)¹³⁰.

Finally, the autosomal dominant hereditary Alagille syndrome is the most common form of hereditary cholestatic liver disease in children. Alagille syndrome is associated with mutations in the human *JAGGED* gene, leading to hypoplasia of the intrahepatic bile ducts among various other defects^{131,132}. Clinical manifestations include chronic cholestasis, characteristic facial features, pulmonary artery hypoplasia and congenital heart disease. Severely affected children account for about 20% of the patients and may require liver transplantation¹³³. Jagged 1 encodes a ligand for Notch 1, which seems essential for remodeling of embryonic vasculature^{134,135}.

Acquired forms of cholestasis can be caused by changes in transporter expression or function and, more commonly, by obstructions or destruction of the bile ducts. Changes in transporter or function are usually due to cholestatic agents such as drugs, hormones or pro-inflammatory cytokines. Gallstones and tumors can cause obstruction of extrahepatic bile ducts, while vanishing bile duct syndromes such as biliary atresia, primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are associated with the destruction of bile ducts.

Biliary atresia is characterized by the immune-mediated destruction of the extrahepatic and intrahepatic bile ducts and development of biliary cirrhosis. Biliary atresia is the leading cause of liver transplantation in children. The disorder occurs in 1 in 16,000-18,000 live

births and is slightly more common in females (1.2:1). Biliary atresia can be distinguished in perinatal (acquired) and the embryonic (fetal) types. The majority (80%) of biliary atresia cases are of the perinatal type. These otherwise normal infants are presumably born with a patent biliary system which undergoes progressive inflammation initiated by a perinatal insult. The embryonic form of biliary atresia is associated with other congenital anomalies, most commonly involving abdominal situs inversus (major visceral organs are inverted or mirrored from their normal positions)¹³⁶. The clinical symptoms of both types are similar, though the etiologies may differ^{131,137}. Several potential mechanisms involved in the pathogenesis of both forms have been suggested, including defects in morphogenesis, defects in prenatal circulation (embryonic), immunologic dysregulation, viral infection and toxin exposure (perinatal)¹³⁷⁻¹⁴⁰.

PBC is an autoimmune disease marked by slow but progressive disappearance of the intrahepatic bile ducts. Both autoreactive T cells and natural killer cells have been associated with the pathogenesis of PBC^{141,142}. Also PSC is an inflammatory autoimmune disease leading to the progressive destruction of the biliary tree. Though several mechanisms contributing to PSC have been postulated, including portal bacteremia, viral infections, toxins and ischemic injury, the etiology of PSC remains unknown¹³¹.

Treatment of cholestatic disorders

Current surgical treatment options include biliary drainage for obstructive cholestasis and Kasai-portoenterostomy for biliary atresia. Despite good results, however, the need for liver transplantation remains for many cases.

A conventional treatment for cholestasis-induced pruritus is the anion-exchange resin cholestyramine. Cholestyramine is a hydrophilic, water-insoluble, non-absorbable agent that binds bile salts, preventing their absorption from the terminal ileum. Recently, a new bile salt-binding polymer, colesevelam hydrochloride has been reported to be more effective than cholestyramine¹⁴³. Alternative anti-pruritus treatments include rifampicine and naltrexon.

UDCA, at present, is the only approved drug for cholestatic disorders⁹⁴, and has been proven beneficial for PBC¹⁴⁴⁻¹⁴⁹, PSC¹⁵⁰⁻¹⁵², PFIC¹⁵³ and some forms of drug-induced cholestasis¹⁵⁴. The beneficial effects of UDCA have been attributed to three major actions: protection of cholangiocytes against cytotoxicity of hydrophobic bile salts, stimulation of hepatobiliary and renal secretory routes, and protection of hepatocytes against bile salt-induced apoptosis.

Hydrophobic bile salts are known to damage cell membranes at high micromolar to millimolar concentrations *in vitro*. UDCA conjugates counteract these effects, by modulating the structure and composition of phospholipid-rich micelles in bile^{155,156}. Moreover, enrichment with UDCA renders bile more hydrophilic and less cytotoxic in humans, decreasing the degree of cholangiocellular injury¹⁵⁷. Ca²⁺ and PKC-dependent mechanisms have been shown to contribute to anticholestatic actions of UDCA conjugates in hepatocytes¹⁵⁸⁻¹⁶⁰. In cholangiocytes, UDCA might work in a similar fashion.

The disturbance common to all forms of cholestasis is impaired bile production. This results in a retention of bile salts and other potentially toxic biliary constituents in the liver, which can lead to or aggravate liver cell injury with further implication for bile formation and hepatocellular apoptosis¹⁶¹. UDCA has been shown to stimulate biliary secretion of bile salts and other organic anions and prevent cholestasis induced by hydrophobic bile salts in the rat liver^{160,162}.

Apoptosis is the major form of hepatocyte cell death in cholestatic liver diseases such as PBC, and has been attributed to the actions of accumulating hydrophobic bile salts in liver cells^{160,162,163}. *In vitro* and *in vivo* studies in rats revealed that UDCA inhibits apoptosis via reduction of mitochondrial membrane permeability transition and of mitochondrial cytochrome c release^{164,165}. In addition, UDCA induced survival signaling in hepatocytes via activation of the epidermal growth factor receptor (EGFR) and mitogen activated protein kinase (MAPK)¹⁶⁶.

LIPID ABSORPTION AND METABOLISM

Cholestasis is frequently accompanied by lipid malabsorption and essential fatty acid (EFA) deficiency. Lipids are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents and general insolubility in water. Lipids serve many physiological functions, including energy storage, structural components of cell membranes and signaling molecules. Fahy *et al.* developed a well-defined classification system for lipids. Lipids were divided into eight categories; fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides. The lipids that are discussed here are triglycerides (glycerolipids), phospholipids (glycerophospholipids), fatty acids (fatty acyls) and cholesterol (sterol lipids). Bile salts are also included in the sterol lipid category¹⁶⁷. Lipid absorption and metabolism can be divided in several steps; lipolysis, solubilization, mucosal uptake, re-esterification and chylomicron formation, and lipoprotein metabolism. **Figure 3** shows the chemical structures of triglycerides, phospholipids, cholesterol and bile salts and a schematic overview of lipid and cholesterol absorption and metabolism is given in **Figure 4**.

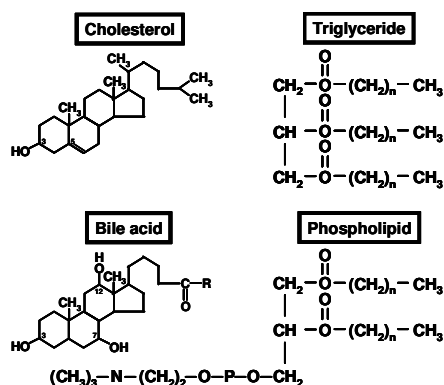


Figure 3 Chemical structures of cholesterol, a bile acid (cholic acid), a triglyceride and a phospholipid (phosphatidylcholine).

Lipolysis

Triglycerides (triacylglycerols) are the major lipid contents in the human diet and constitute about 40% of the energy intake in Western diets. Other dietary lipids include phospholipids, glycolipids, sterols and vitamins A, D, E and K. Prior to lipolysis, the chewing process disperses the lipids, whereby the surface area is increased and a food bolus is formed. The food bolus is transferred to the stomach where partial enzymatic hydrolysis of triglycerides

into diacylglycerol and free fatty acids takes place^{168,169}. Hydrolysis is performed by gastric lipase or lingual lipase. Lingual lipase is released from von Ebner's glands, the lingual serous glands on the tongue, and is transferred with the food bolus from the mouth into the stomach where its activity is exerted^{170,171}. Gastric lipase is released from the gastric mucosa¹⁷². The relative contribution of these lipases to hydrolysis depend on the species considered. Rodents have high activity of lingual lipase and low activity of gastric lipase, while primates and humans have high activity of gastric lipase¹⁷³. About 10-30% of dietary triglycerides are hydrolyzed in the stomach in humans and rats^{174,175}. The predominant fraction of dietary triglycerides is hydrolyzed by pancreatic lipase. The entry of triglyceride degradation products in the duodenum causes gallbladder emptying and cholecystokinin release with subsequent pancreatic lipase secretion¹⁷⁶⁻¹⁷⁸. Pancreatic lipase binds to the surface of the food bolus with co-lipase as a mediator to overcome the expulsion of the lipase into the water-phase caused by bile salts and digests the triglycerides. This lipolytic process results in the formation of monoacylglycerol and free fatty acids¹⁷⁹. Phospholipids in the duodenum are derived from diet, bile and intestinal epithelial sloughing. Pancreatic phospholipase A₂ catalyzes the hydrolysis of a variety of phospholipids in the duodenum, yielding lysophospholipids and free fatty acids¹⁸⁰. Cholesterol in the intestinal lumen can be derived from diet, from bile and from epithelial sloughing. In response to a meal, carboxyl ester lipase is secreted from the pancreas and catalyzes de-esterification of cholesteryl esters. The pool of unesterified cholesterol (mainly derived from bile) in the intestinal lumen is relatively much larger than the esterified dietary pool of cholesterol¹⁸⁰. Accordingly, targeted disruption of the gene encoding for carboxyl ester lipase only has a slight inhibitory effect on intestinal cholesterol absorption in mice^{181,182}.

Solubilization

The products of pancreatic lipolysis of dietary lipids (fatty acids, monoacylglycerol, lysophospholipids, unesterified cholesterol) are more polar than their parent lipids, but still have limited solubility in the aqueous environment of the intestinal lumen. Bile provides the ideal detergent for the solubilization of these lipolytic products. The amphipathic nature of bile salts allows them to form micelles. Micelles are water-soluble poly-molecular aggregates with a discoid configuration in which the polar groups of the lipid molecules are at the surface projecting into the aqueous medium, while the apolar hydrocarbon parts are at the core. Bile salt micelles desorb polar lipids from the surface of emulsified droplets, thus allowing hydrolysis to proceed¹⁸³⁻¹⁸⁵. A substantial proportion of dietary triglycerides can be absorbed even in the absence of bile salts (even up to 75%), as in biliary obstruction and diversion¹⁸⁶, suggesting that a mechanism of lipid solubilization can compensate for bile salt deficiency. Phospholipids for instance, are relatively independent from bile components for their efficient intestinal absorption. Phospholipids have a higher tendency than triglycerides to interact with water and can associate into liquid crystals, which have been suggested to play a role in luminal lipid solubilization under bile-deficient conditions¹⁸⁷.

Mucosal uptake

Lipolytic products are mainly absorbed taken up by proximal jejunal enterocytes¹⁸⁸. During the process intestinal absorption, lipids solubilized in micelles must dissociate from them, and this occurs at a thin water layer adjacent to the luminal surface of the enterocytes, the unstirred water layer^{189,190}. The existence of an acidic microclimate in this water layer (pH 5.3-6.0), promotes both micellar dissociation and fatty acid protonation, facilitating diffusion

of fatty acids across the cellular lipid membrane¹⁹¹. Schoeller *et al.* identified a sodium/hydrogen exchanger in the brush border membrane of rat jejunal enterocytes, which is probably responsible for the acidification of the unstirred water layer¹⁹². Passive diffusion of fatty acid monomers across the microvillous membrane is very rapid^{188,193}.

In addition to rapid diffusion some fatty acid transport proteins have been identified. Fatty acid transport protein 4 (FATP4) and fatty acid translocase (FAT/CD36) are present on intestinal brush border membrane transporters and have been suggested to facilitate transport of fatty acids across the cellular membrane^{194,195}. Recently, Nassir *et al.* postulated that Fat is important for fatty acid and cholesterol uptake by the proximal intestine but not the distal intestine, based on reduced uptake of fatty acids (50%) and cholesterol (60%) in Fat-deficient primary proximal enterocytes¹⁹⁶. However, Goudriaan *et al.* demonstrated that lipid absorption was not affected in Fat knockout mice¹⁹⁷. Targeted disruption of *Fatp4* in mice was shown to be lethal. Heterozygous *Fatp4* deletion mice showed decreased fatty acid uptake by enterocytes *ex vivo*, but not *in vivo*, possibly due to the large excess capacity of the small intestine for lipid absorption¹⁹⁸. The subcellular localization of FATP4 has been a subject of debate. Stahl *et al.* showed significant amounts of FATP4 on the apical membrane of enterocytes¹⁹⁵, while Milger *et al.* found that FATP4 was localized exclusively intracellularly in enterocytes¹⁹⁹. Milger *et al.* showed increased fatty acid uptake rate in case of FATP4 overexpression. However, they proposed that FATP4 may not be involved in fatty acid translocation at the plasma membrane, but may rather drive fatty acid uptake indirectly by stimulating its intracellular esterification¹⁹⁹.

Cholesterol uptake by the enterocyte can occur via diffusion or active transport systems^{180,200-202}. Two groups identified two adjacent genes *ABCG5* and *ABCG8* encoding membrane transporters in the intestine and the liver^{203,204}. Studies with transgenic and knockout mice showed that heterodimerization of *ABCG5* and *ABCG8* results in a functional sterol transporter, transporting cholesterol and plant sterols out of the cell across the apical membrane of enterocytes or hepatocytes. *ABCG5* and *ABCG8* are localized at the apical brush border membrane of enterocytes and at the canalicular membrane of hepatocytes²⁰⁵⁻²⁰⁹. Altmann *et al.* identified Niemann Pick C1 Like1 (NPC1L1) as a cholesterol uptake transporter. NPC1L1 is predominantly expressed in the intestine, with peak expression in the jejunum, corresponding with the efficiency of intestinal cholesterol absorption^{210,211}. Davies *et al.* showed that NPC1L1 is localized at the apical membrane of enterocytes²¹². NPC1L1 is essential for intestinal cholesterol absorption, evidenced by the observation that *Npc1l1* knockout mice are defective in intestinal cholesterol uptake^{210,213}.

Re-esterification and chylomicron formation

Once taken up by via the apical membrane of the enterocyte, fatty acids bind to IFABP and diffuse to the endoplasmic reticulum. Induction of cytosolic intestinal fatty acid binding protein (IFABP) expression in differentiated intestinal cells correlated with increased re-esterification, but not with enhanced fatty acid uptake²¹⁴. In the endoplasmic reticulum fatty acids are activated to acyl-CoA and are subsequently re-esterified into triglycerides¹⁸⁸. Two major pathways exist for synthesizing diacylglycerol: the glycerol phosphate pathway and the monoacylglycerol pathway. In the glycerol pathway, which functions in most cells, diacylglycerol is derived by the dephosphorylation of phosphatidic acid produced by sequential acylations of glycerol phosphate. In the monoacylglycerol pathway, reported predominantly in the intestine, diacylglycerol is formed directly from monoacylglycerol and fatty acyl-CoA in a reaction catalyzed by acyl-CoA:monoacylglycerol acyltransferases

(MGATs)^{215,216}. MGAT1 is expressed in most tissues but not the intestine. MGATs 2 and 3 are primarily expressed in the intestine and may be the key contributors to triglyceride packaging within the enterocytes²¹⁷⁻²¹⁹. Esterification of diacylglycerol into triglycerides is catalyzed by acyl-CoA:diacylglycerol acyltransferase (DGAT) 1 and 2. In humans, DGAT1 is highly expressed in the small intestine and the colon^{220,221}. Mice lacking Dgat1 were found to have normal plasma triglyceride levels, suggesting alternative mechanisms by which triglycerides can be synthesized²²². DGAT2 possesses widespread expression in humans, with particularly high levels in liver and adipose tissue²²³. The expression patterns of DGAT1 and DGAT 2 indicate that they might have different functions within different tissues. DGAT1 likely plays a role in intestinal triglyceride resynthesis, whereas DGAT2 may function primarily in triglyceride synthesis and export from the liver²²⁴. Esterification of cholesterol into cholesteryl esters is catalyzed by acyl-CoA cholesterol acyltransferase 2 (ACAT2). ACAT2 expression is limited to the liver and small intestine²²⁵. The importance of ACAT2 in cholesteryl ester synthesis was evidenced by the observation that Acat2 knockout mice have impaired dietary cholesterol absorption and are resistant to diet-induced hypercholesterolemia and gallstone formation^{226,227}. Microsomal triglyceride transfer protein (MTTP) is responsible for the assembly of cholesterol, triglycerides and phospholipids together with one apolipoprotein ApoB48 molecule (among other lipoproteins) to form a chylomicron particle, and with one ApoB100 molecule (among other lipoproteins) to form a very low density lipoprotein (VLDL) particle^{228,229}. Humans only have ApoB48 in the intestine, thus VLDL particles of intestinal origin contain ApoB48. Phospholipids of luminal origin are predominantly used by enterocytes for the assembly of the surface coat of chylomicrons^{230,231}. Mice with a conditional intestine-specific Mttp deletion had impaired cholesterol absorption, large cytoplasmic triglyceride droplets and no chylomicron-sized particles²³², stressing the significance of MTTP in chylomicron formation. Moreover, Wetterau et al. demonstrated that a defect absence of MTTP is responsible for the disorder abetalipoproteinemia, characterized by a defect in assembly or secretion of VLDLs and chylomicrons²³³.

VLDLs (diameter 30-80 nm, $0.93 < \rho < 1.006$ g/ml) are the predominant lipoproteins secreted during the fasting state^{234,235}. In the postprandial state, chylomicrons (diameter 75-450 nm, $\rho < 0.93$ g/ml) secretion is induced after fat digestion^{236,237}. Chylomicrons and VLDLs are secreted through the basolateral membrane of enterocytes into the interstitium, enter the lymphatic capillaries of intestinal microvilli that drain into omental lymphatic channels, eventually reaching the systemic circulation through the thoracic duct¹⁸⁸.

Lipoprotein metabolism

Once entered the circulation, chylomicrons and VLDLs interact with other lipoproteins such as LDL and HDL. Lipid transfer between these plasma lipoproteins is mediated by plasma lipid transfer proteins, cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP)²³⁸.

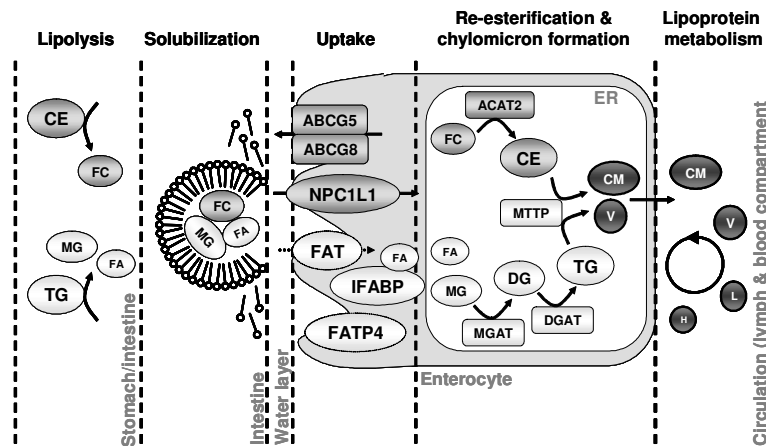


Figure 4 Schematic image of the specific phases of lipid absorption. CE = cholesteryl ester, FC = free cholesterol, TG = triglyceride, DG = diacylglycerol, MG = monoacylglycerol, FA = fatty acid, CM = chylomicron, V = VLDL, L = LDL, H = HDL.

ESSENTIAL FATTY ACIDS

The essential fatty acids (EFAs), linoleic acid (LA) and α -linolenic acid (ALA), are not synthesized *de novo* and thus must be derived from the diet. Since EFAs are abundantly available in the normal diet, EFA deficiency is relatively rare in humans. LA can be elongated and desaturated to dihomo- γ -linolenic acid (DGLA) and arachidonic acid (AA), and ALA to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EFAs are polyunsaturated fatty acids (PUFAs), which are characterized by a long carboxyl chain and the presence of double bond. There are four independent families of PUFAs, depending on the parent fatty acid from which they are synthesized. The ω -3 series is derived from ALA (18:3 ω -3), the ω -6 series from *cis*-LA (18:2 ω -6), the ω -9 series from oleic acid (OA; 18:1 ω -9) and the ω -7 series is derived from palmitoleic acid (PA; 16:1 ω -7). The first number designates the amount of carbon atoms, the second number designates the amount of double bonds, and the (ω -X) or (n-X) indicates position of the double bond carbon closest to the methyl end. The latter two series are not EFAs, since they can be synthesized in mammals.

EFAs and their metabolites have been shown to affect cell membrane fluidity, induce second messenger action and exhibit antibiotic actions. Cell membrane fluidity is determined by its lipid composition among other factors. Increased incorporation of saturated fatty acids and cholesterol into the cell membrane phospholipids renders the cell membrane more rigid, while increased incorporation of unsaturated fatty acids will make it more fluid²³⁹⁻²⁴¹. Moreover, EFA metabolites are precursors to prostaglandins, thromboxanes, leukotrienes, lipoxins and resolvins. EFAs and EFA-derived compounds have been shown to play significant roles in the pathobiology of many disorders, including collagen vascular diseases, hypertension, diabetes mellitus, metabolic syndrome, psoriasis, eczema, atopic dermatitis, coronary heart disease, atherosclerosis and cancer²³⁹. **Figure 5** shows an overview of EFAs and their metabolites.

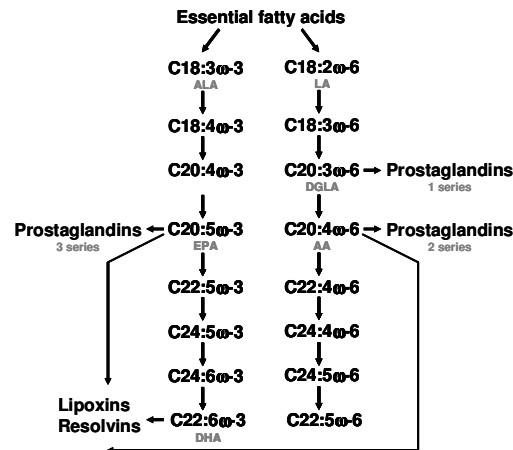


Figure 5 Dietary essential fatty acids and their metabolites.

FAT MALABSORPTION IN CHOLESTASIS AND EFA DEFICIENCY

High incidence of fat malabsorption and EFA deficiency has been reported in cholestatic patients^{1,97,242-244}. Fat malabsorption is defined by an intestinal absorption efficiency lower than 95% of the amount ingested via the diet¹⁸⁸. Interestingly, EFA deficiency can not only be caused by fat malabsorption, it can also itself induce fat malabsorption^{243,245,246}. For reasons of clarity, fatty acid absorption will be referred to as fat absorption.

Previous studies in our laboratory revealed that both cholestasis and bile-deficiency are associated with impaired plasma LA status in relevant rat models (bile duct ligation and permanent bile diversion, respectively)^{247,248}. Interestingly, impaired plasma LA status in cholestatic rats was due to decreased net absorption²⁴⁷, while in bile-deficient rats postabsorptive metabolism of LA (increased plasma AA) was increased²⁴⁸.

The mechanism underlying EFA deficiency-induced fat malabsorption has been extensively studied, but has not been entirely elucidated yet. Fat digestion and fat uptake were not affected in EFA deficient rats^{249,250}. Levy *et al.* demonstrated that bile flow and biliary secretion of bile salts, phospholipids and cholesterol were significantly decreased in EFA deficient rats^{250,251}. In addition, intracellular events such as re-esterification and chylomicron production were impaired during EFA deficiency in rats^{249,250}. Thus, EFA deficiency-induced fat malabsorption in rats can be ascribed to both intraluminal (bile formation) and intracellular events (fat processing). In contrast to rats, however, EFA deficiency in mice was associated with increased bile flow and biliary secretion of bile salts, phospholipids and cholesterol²⁵².

Biliary phospholipids can stimulate dietary fat absorption by facilitating intraluminal lipid solubilization and by providing surface components for chylomicron assembly. Biliary secretion of phospholipids with a different composition could theoretically be involved in EFA deficiency-induced malabsorption in mice²⁵³. Voshol *et al.* showed that postprandial appearance of chylomicrons was impaired in mice deficient for phospholipid floppase *Mdr2*. Net absorption of dietary lipids, however, was unchanged in *Mdr2*^{-/-} mice²⁵⁴. Werner *et al.* demonstrated that absence of biliary phospholipid secretion (*Mdr2*^{-/-}) did not change fat malabsorption in EFA deficient mice, compared with control (*Mdr2*^{+/+}) EFA deficient mice.

²⁵². Werner *et al.* also demonstrated that absence of biliary phospholipid secretion (*Mdr2*^{-/-}) increased chylomicron size, while enhanced biliary phospholipid secretion (EFA deficient) yielded smaller chylomicrons in mice lymph ²⁵³, further emphasizing the importance of biliary phospholipid secretion in fat absorption.

Some pathological changes have been noted in the intestine of EFA deficient rats, i.e. a restricted surface area due to villi shortening and a lack of cellular differentiation ²⁵⁵. This effect has been ascribed to decreased EFA content of biliary phosphatidylcholine found in EFA deficient rats. Acyl chain analysis of biliary phosphatidylcholine revealed strongly decreased LA and AA content, compensated by increased oleic acid and palmitoleic acid content ^{249,250}. The concentration and composition of fatty acids in biliary phosphatidylcholine was also found to affect chylomicron assembly, secretion and clearance ^{256,257}.

In normal diets, 90% of EFAs are present as acyl esters in triglycerides (90%), and only 10% as acyl esters in phospholipids and cholesteryl esters. Biliary phospholipids can facilitate dietary lipid absorption under bile-deficient conditions (e.g. cholestasis) ²⁵⁸. Indeed, oral EFA supplementation in the form of phospholipids was found more effective than in the form of triglycerides in increasing LCPUFA concentrations in liver and brain of cholestatic EFA deficient mice ²⁵⁹.

INTESTINAL CARBOHYDRATE DIGESTION AND ABSORPTION

Besides lipids, dietary carbohydrates serve as an important source of energy. **Figure 6** shows a schematic overview of disaccharidase digestion and absorption. Dietary carbohydrates include polysaccharides (starches) and sugars (di- and monosaccharides). Starches from plants make up 75% of dietary carbohydrates and are composed of amylase (linear α -1-4-linked D-glucose polymer) and amylopectin (linear α -1-4-linked D-glucose polymer, with additional α -1-6 linkages). Intestinal digestion commences with salivary and pancreatic amylases. Alpha-amylase produces linear maltose (glucose- α -1-4-glucose) and isomaltose (glucose- α -1-6-glucose) oligosaccharides as well as some large oligomers. Final hydrolysis occurs in the small intestine where brush-border membrane maltase-glycoamylase and sucrase-isomaltase hydrolyze oligosaccharides to glucose, which is subsequently taken up by the sodium-dependent glucose transporter SGLT1. Sucrase-isomaltase digests all of the sucrose and 80% of the dietary maltose, while maltase-glycoamylase digests the remaining maltose and glucose oligomers. Dietary sugars (disaccharides) include lactose (milk), sucrose (sugar beet and sugar cane) and minor amounts of trehalose (mushrooms). These sugars are hydrolyzed by intestinal brush border membrane disaccharidases; lactase, sucrase and trehalase. Lactose is hydrolyzed into glucose and galactose, while hydrolysis of sucrose yields glucose and fructose. Dietary monosaccharides (mostly derived from fruits) are transported into the enterocytes directly by brush-border membrane transporters. Fructose is transported by the uniporter glucose transporter GLUT5 and glucose and galactose by SGLT1. Transport into the circulation of all monosaccharides occurs via GLUT2 ^{202,260}. Regulation of sucrase and lactase gene transcription has been shown to be exerted by cooperative action of transcription factors HNF1 α , GATA4 and CDX2 ²⁶¹⁻²⁶⁴.

It is rather unexplored whether cholestasis affects intestinal carbohydrate digestion and absorption. Borges *et al.* reported that obstructive jaundice did not affect jejunal absorption of the monosaccharide glucose in rats ²⁶⁵. In addition, sucrase enzyme activity was shown to

be unaffected in cholestyramine-fed and bile-diverted rats, based on determinations in intestinal tissue samples²⁶⁶.

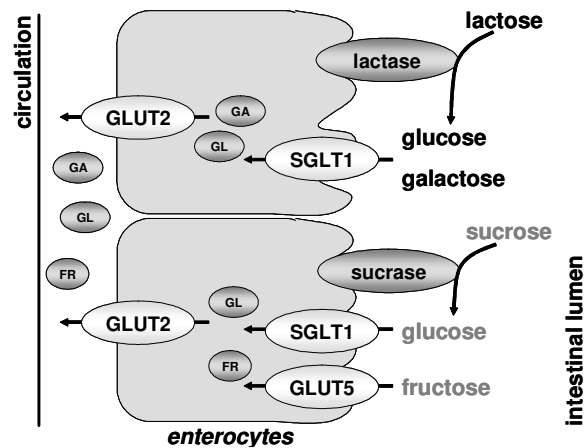


Figure 6 Schematic image of enterocytes digesting lactose and sucrose and subsequently absorbing the hydrolysis products; monosaccharides glucose

FARNESOID X RECEPTOR

As stated before, FXR is involved in the regulation of the enterohepatic circulation of bile salts and the feedback regulation of bile salt synthesis. FXR activation has been suggested to serve as a protective mechanism, because potential toxicity of bile salt accumulation, evident in cholestatic disorders, is counteracted by bile salt-activated FXR²⁶⁷. Three groups simultaneously identified bile salts as ligands for FXR⁶⁹⁻⁷¹. There are two known FXR genes, commonly referred to as *FXR α* and *FXR β* . In humans and mice, *FXR α* encodes four isoforms, *FXR α 1-4*, result from different promoters and transcription initiation sites as well as alternative splicing of the RNA^{268,269}. *FXR β* encodes a functional member of the nuclear receptor family in rodents, rabbit and dogs, but is a pseudogene in humans and primates²⁷⁰. *FXR α* is mainly expressed in the liver, gut, kidney and adrenal glands, with much lower levels in adipose tissue^{268,269,271,272}. Many FXR target genes are regulated in an isoform-independent manner, however, IBABP and fibroblast growth factor 19 (FGF19) are more responsive to the *FXR α 2* and *FXR α 4*^{269,273,274}. FXR isoforms modulate expression of genes by binding either as a monomer or as a heterodimer with retinoid X receptor (RXR) to DNA sequence motifs²⁶⁷. FXR isoforms can be activated by structurally different ligands, including several primary and secondary bile salt species conjugated to either taurine or glycine, CDCA being the most potent ligand^{69,71}. The ligand binding domain is not conserved between species. For instance, mouse FXR is more responsive to CA than its human counterpart²⁷⁵. In the next sections, the role of *FXR α* (referred to as FXR) in bile salt metabolism, lipid metabolism and glucose metabolism will be discussed.

FXR in control of bile salt metabolism

During the last decade, it has become clear that FXR has a crucial role in regulating bile salt metabolism. FXR regulates expression of genes involved in (1) bile salt synthesis, (2) hepatic secretion of bile salts and (3) phospholipid into the bile, (4) remaining (apart from hepatic secretion) bile salt transport, and (5) bile salt conjugation and (subsequent) bile salt

detoxification²⁷⁶. **Figure 7** is a schematic image of the effect of bile salt-induced FXR activation on its target genes involved in bile salt metabolism.

- (1) Expression and induction of Cyp7a1 in rodents are dependent on oxysterol-activated liver X receptor α (LXR α) and liver receptor homolog 1 (LRH1)²⁷⁷. The human CYP7A1 gene, in contrast, lacks a LXR α response element²⁷⁸. FXR activation induces expression of small heterodimer partner (SHP)^{279,280}, a member of the nuclear receptor family that lacks a DNA-binding domain²⁸¹. SHP, in turn, can dimerize with and inactivate both LXR α and LRH1, resulting in a decrease in Cyp7a1 expression^{279,280,282}. Support for this regulatory cascade comes from studies showing that treatment of Shp knockout mice with a potent, synthetic FXR agonist (GW4064) fails to repress Cyp7a1^{283,284}. Another pathway that regulates bile salt synthesis is initiated after activation of FXR in enterocytes. This activation results in enhanced transcription and secretion of fibroblast growth factor 15 (Fgf15; FGF19 in humans)²⁸⁵. Subsequent binding of Fgf15 to fibroblast growth factor receptor 4 (FGFR4) on the hepatocyte cell membrane results in the activation of the JNK pathway and repression of Cyp7a1 and Cyp8b1²⁸⁵. Moreover, FXR was found to induce expression of intestinal fibroblast growth factor 19 (FGF19; Fgf15 in rodents), a secreted growth factor that signals through the FGFR4 cell-surface receptor tyrosine kinase. FGF19/Fgf15 strongly suppresses expression of CYP7A1 in primary cultures of human hepatocytes and mouse liver through a JNK-dependent pathway²⁸⁶. Mice lacking Fgf15 have increased hepatic CYP7A1 mRNA, protein levels and enzyme activity²⁸⁵.
- (2) Bile salt-activated FXR activates transcription of the canalicular bile salt export pump BSEP, and thus facilitates bile salt excretion into the bile²⁸⁷. Also MRP2 expression was induced upon FXR activation²⁸⁸.
- (3) Huang *et al.* demonstrated that FXR activation by CDCA and GW4064 induces expression of the human MDR3 gene²⁸⁹. The mouse MDR3 homolog Mdr2 was also shown to be induced upon FXR activation²⁹⁰. Induction of MDR3/Mdr2 expression by bile salt-activated FXR increases phospholipid excretion into bile.
- (4) Negative feedback regulation rat Ntcp by bile salt-activated Fxr has been shown to occur via Shp induction, leading to decreased transport of bile salt into the hepatocytes²⁹¹. Ileal expression of ASBT in the rat is unaffected by bile salts, while in humans and mice it is under negative feedback regulation via a FXR mediated, SHP dependent effect²⁹². Thus, bile salt-activated FXR reduces the amount of bile salts reabsorbed in cholangiocytes and terminal ileal enterocytes in humans and mice. In contrast to ABST, human IBABP was shown to be induced upon FXR activation *in vitro* and *in vivo*²⁹³. Ballatori *et al.* demonstrated that bile salt transporter OST α/β is localized to the basolateral membrane in humans and rodents⁴⁰. Apical to basolateral transport of TCA was shown to be increased by expression of Ost α/β in polarized canine kidney cells, while basolateral to apical transport was unaffected²⁹⁴. This indicates that OST α/β transports bile salts out of the cell. Human and mice OST α/β was shown to be positively regulated by bile salts via FXR^{274,295}. Strikingly, Frankenberg *et al.* found both Fxr response elements and a Lrh1 response element in the mouse Ost α and Ost β promoters. Mouse Ost α/β is thus positively and negatively regulated by bile salts. Although the positive regulatory pathway appears to be dominant, this arrangement provides a mechanism to finely titrate Ost α/β expression to the bile salt flux²⁹⁵.
- (5) FXR is also involved in the regulation of bile salt conjugation with glycine or taurine, evidenced by the observation that Bacs (bile acid CoA synthetase) and Baat (bile acid-

CoA:amino acid *N*-acetyltransferase) are induced by Fxr in the rat liver. Furthermore, Bacs and Baat mRNA levels were upregulated by treatment with the Fxr agonist GW4064 in rats²⁹⁶, facilitating bile salt conjugation. SULT2A1 (dehydroepiandrosterone-sulfotransferase) is a cytosolic enzyme that mediates sulphate conjugation of bile salts. SULT2A1 is expressed in the liver and the intestine, and its expression has been shown to be increased upon FXR activation by CDCA. Song *et al.* speculated that increased solubility of sulphated bile salts facilitates their intracellular transport and clearance²⁹⁷. Finally, expression of human UGT2B4 (uridine 5'-diphosphate-glucuronosyltransferase 2B4), which catalyzes the conversion of hydrophobic bile salts into more hydrophilic glucuronide derivatives, was increased upon CDCA and GW4064 induced FXR activation²⁹⁸.

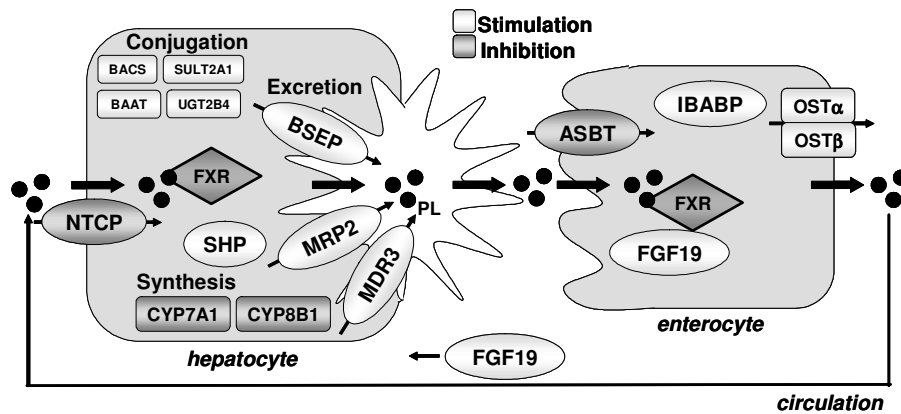


Figure 7 Schematic image of stimulatory and inhibitory actions of FXR with respect to bile salt homeostasis [adapted from 68].

FXR in control of lipid metabolism

The involvement of bile salts in the regulation of lipid metabolism became apparent when treatment of patients suffering from gallstones or hypertriglyceridemia with CDCA reduced plasma triglycerides^{299,300}. Some important FXR actions related to lipid metabolism are listed below. Sinal *et al.* demonstrated that FXR is involved in the control of plasma lipid levels, evidenced by increased plasma levels of triglycerides and cholesterol in *Fxr*^{-/-} mice³⁰¹. *Fxr*^{-/-} mice were also shown to have higher levels of HDL cholesterol, consistent with reduced expression of SR-B1, the scavenger receptor that facilitates clearance of HDL cholesterol from blood^{302,303}. Moreover, administration of FXR agonists to healthy rats and mice reduced plasma triglyceride levels^{304,305}. FXR activation also alters transcription of genes involved in fatty acid and triglyceride synthesis and lipoprotein metabolism³⁰⁶. Bile salt activated Fxr led to lower plasma triglyceride levels via induction of Shp and subsequent repression of mouse Srebp-1c, a transcription factor that controls genes involved in fatty acid and triglyceride synthesis³⁰⁷. These decreased levels of plasma lipids were associated with decreased VLDL secretion, caused by reduced expression of MTTP in hamsters fed with CDCA and in HepG2 cells exposed to CDCA^{308,309}.

SCOPE OF THIS THESIS

As stated before, cholestatic liver disease is frequently associated with nutritional defects, including EFA deficiency. The resultant malnutritional state strongly affects prognosis and treatment outcome in cholestatic children. Our ultimate goal is to improve the prognosis of cholestatic children by optimization of their nutritional status. As one of the strategies to achieve this, we herewith aimed to elucidate the effects of cholestasis and EFA deficiency on intestinal function, with emphasis on nutrient absorption.

This thesis focusses on the intestinal digestion and absorption of fat and carbohydrates in cholestasis and EFA deficiency. In **chapter 2**, the nutritional status of children with cholestatic liver disease and potential treatment options are discussed. The majority of cholestatic disorders are associated with elevated plasma bile salt levels. Bile salts have been shown to induce proliferation and apoptosis in intestinal cells *in vitro*. Theoretically, high plasma bile salt levels could affect intestinal epithelial cells and their capacity to absorb nutrients. In **chapter 3**, intestinal function in cholestatic rats is compared to intestinal function in control rats to elucidate the intestinal effects of cholestasis. With stable isotope methodology intestinal digestion and absorption of sucrose and glucose were assessed. To be able to pinpoint possible effects to high plasma bile salt levels, cholestatic and control rats were compared to bile-deficient rats. In **chapter 4** the effects of cholestatic conditions on enterocytes in different developmental stages are investigated, by exposing human intestinal epithelial cells to bile salts in cholestatic concentrations *in vitro*.

Cholestasis-induced fat malabsorption is often associated with EFA deficiency. EFA deficiency itself can also induce fat malabsorption. The underlying mechanism, however, has not been elucidated. Since EFA deficiency has been associated with pathological changes in small intestine, i.e., a restricted surface area due to villi shortening and a lack of cellular differentiation, intestinal absorption of other nutrients could theoretically also be affected. To test this theory, intestinal function and its capacity to digest and absorb carbohydrates will be investigated during EFA deficiency. In **chapter 5** intestinal function and digestion and absorption of lactose and glucose was assessed in EFA deficient mice.

Finally, like EFAs, the nuclear receptor FXR has been shown to be involved in bile salt homeostasis and lipid homeostasis. We speculate that FXR is involved in EFA deficiency-induced fat malabsorption. In **chapter 6** the involvement of FXR in bile formation and fat malabsorption was assessed by comparing these parameters in EFA deficient Fxr knockout mice and EFA deficient control mice.

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