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2.3.6 Metabolism of bile acids

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Introduction

Bile acids are synthesized in the liver from cholesterol; they are secreted in bile and stored in the gallbladder. After a meal, the gallbladder contracts, and stored bile is transferred to the duodenum and via the jejunum to the ileum. This movement is stimulated by intestinal propulsion. In the ileum, 90–95% of bile salts are reabsorbed and returned to the liver. The remainder is lost to the colon, where primary bile salts are transformed by bacterial metabolism into secondary bile salts. Some of the secondary bile salts are also reabsorbed, and the rest is removed with the faeces. Primary and secondary bile salts return to the liver via the portal circulation. In the liver, bile salts are taken up into hepatocytes, thereby completing the enterohepatic cycle.

Bile acids serve a number of functions: (i) they are the main solutes in bile and, as such, they are important for the generation of the so-called bile salt-dependent bile flow; (ii) bile salts are indispensable for the secretion of cholesterol and phospholipids from the liver; (iii) in bile, bile salts form mixed micelles that keep fat-soluble organic compounds in solution, including fat-soluble vitamins; (iv) in the intestine, bile salts promote the dissolution and hydrolysis of triglycerides by pancreatic enzymes; (v) bile salts act as signalling molecules in the regulation of enzymes and transporters of drug and intermediary metabolism.

The adult human liver produces about 500 mg of bile acids per day [1,2]. About three times this amount represents the total bile acid pool size that cycles through the enterohepatic circulation [2]. Bile acids complete an enterohepatic cycle about eight times per day. Enterohepatic cycling represents an efficient system for reuse of active components. Enterohepatic cycling not only serves to reclaim bile acids, but it also enables bile acids to act as messengers that carry signals from intestine to liver. Thus, they regulate their own synthesis and transport rates. Bile acids are also able to repress hepatic fatty acid and triglyceride synthesis [3,4].

Biosynthesis and metabolic defects

At least 16 different enzymes are involved in the biosynthesis of bile salts [1,5,6]. Most of these enzymes are active in the neutral (or classic) and acidic (or alternative) pathways, the two main

routes for the conversion of cholesterol to the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) (Fig. 1). The neutral pathway starts with the hydroxylation of the sterol nucleus of cholesterol by 7α -hydroxylase (CYP7A1) in the endoplasmic reticulum. CYP7A1 is regarded as the rate-limiting enzyme in bile acid biosynthesis, exemplified by the fact that mice deficient for Cyp7a1 have a 75% reduced bile acid pool size causing vitamin deficiencies, lipid malabsorption and liver failure [7–9]. The acidic pathway starts with the hydroxylation of the cholesterol side-chain by sterol 27-hydroxylase (CYP27). The CYP27 product, 5-cholesten- 3β -27-diol, is not a substrate for CYP7A1, but is hydroxylated at the C7 position by an alternative P450 enzyme, CYP7B1. From here on, the neutral and acidic pathways largely overlap. Double hydroxylated CDCA and triple hydroxylated CA are the principal bile acids. Their ratio depends on the activity of sterol 12α -hydroxylase (CYP8B1). Bile acid synthesis is completed in hepatocyte peroxisomes, where bile acid coenzyme A:amino acid N-acyltransferase (BAAT) conjugates either taurine or glycine to CA or CDCA. At least 95% of the bile acid pool is generated through these two pathways. Extensive intracellular transport of bile acid intermediates occurs between various organelles. Transport in and out of these organelles may be mediated by transport proteins, but these have not been characterized in detail yet.

Bile acid synthesis defects (BASD) are rare genetic disorders that are the underlying cause of approximately 2% of persistent cholestasis in infants (see also Chapter 16.10, Genetic cholestatic diseases). BASDs are recognized by the absence or reduction of normal primary bile salts in serum and/or urine. Instead, non-typical bile acids and sterols are often detected in the body fluids of these patients. These can be identified by fast atom bombardment ionization–mass spectrometry (FAB-MS) and gas chromatography–mass spectrometry (GC-MS). Disease-causing mutations have been identified in 9 out of the 16 bile acid biosynthesis enzymes (Table 1). Cholestasis is a common clinical presentation of these diseases. The associated liver diseases may vary from mild to life-threatening but, in many cases, can be managed by replacement of deficient primary bile salts. This not only leads to restoration of normal bile function, but also induces feedback inhibition on the production of toxic bile acid intermediates.

Patients with CYP7A1 deficiency have a markedly reduced bile acid synthesis rate [10]. Symptoms include hyperlipidaemia, premature vascular disease and gallstones. A mutation in the CYP7A gene that results in truncation of the enzyme has been detected in these patients. Only one case of CYP7B1 deficiency has been reported to date [11]. This child produced no primary bile acids, and serum concentrations of the toxic 27α -hydroxy cholesterol were increased. A mutation was identified in the CYP7B1 gene that truncates and inactivates the enzyme. In addition, it was found that expression of CYP7A, at both the mRNA and activity level, was absent. Bile acid treatment was ineffective, suggesting that the biosynthesis of toxic 27α -hydroxy cholesterol cannot be suppressed.

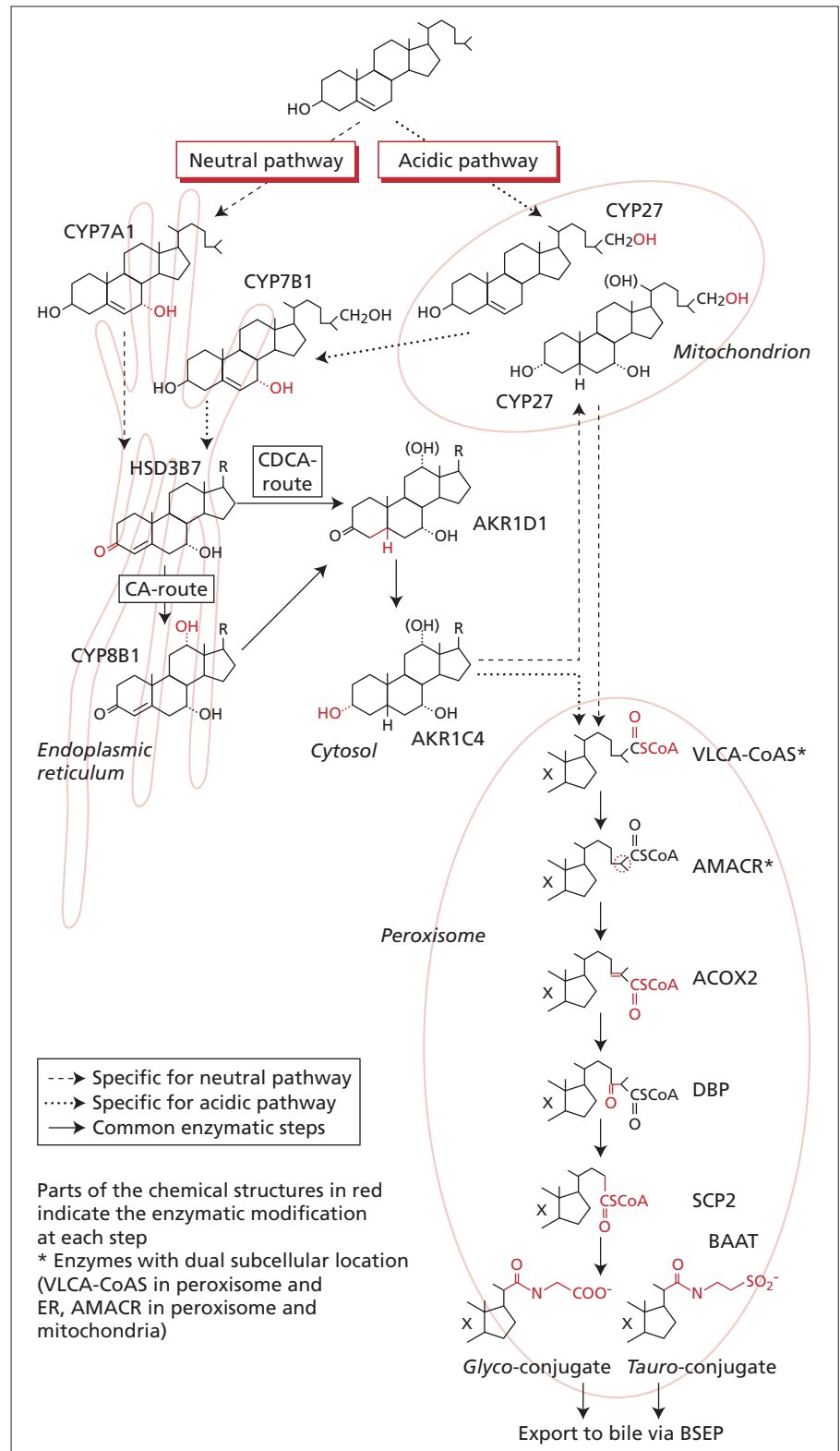


Fig. 1 Biosynthesis of bile acids in the liver. AMACR, α -methylacyl-CoA racemase; BSEP, bile salt export pump; ER, endoplasmic reticulum.

Table 1 Enzymes of the neutral and acidic pathways of bile acid synthesis.

Enzyme	Abbreviation	Mw	Tissue	Organelle	Disease symptoms	Mouse knock-out [ref.]
Cholesterol 7 α -hydroxylase	CYP7A1	57 660	Liver	ER	Hypercholesterolemia, premature gallstone disease, NH	[7,8]
Sterol 27-hydroxylase	CYP27A1	56 900	Many	Mito	CTX; progressive CNS neuropathy, cholestanol and bile alcohol accumulation	[65]
Oxysterol 7 α -hydroxylase	CYP7B1	58 255	Many, liver enriched	ER	Hyperoxysterolaemia, NLF	[66]
3 β -hydroxy- Δ^5 -C ₂₇ steroid oxidoreductase	HSD3B7	40 929	Many	ER	NLF, hepatotoxic bile acid intermediate accumulation	
Sterol 12 α -hydroxylase	CYP8B1	58 078	Liver	ER	ND	
Δ^4 -3-oxosteroid 5 β -reductase	AKR1D1	37 377	Many	Cytosol	NLF, hepatotoxic bile acid intermediate accumulation	
3 α -hydroxysteroid dehydrogenase	AKR1C4	37 095	Many	Cytosol	ND	
Bile acid CoA synthetase	BACS	70 312	Many	ER/Perox	ND	
Very long-chain acyl-coenzyme A synthetase	VLCA-CoAS					
Alpha-methylacyl-CoA racemase	AMACR	42 359	Liver	Perox/Mito	Adult onset sensory motor neuropathy, neonatal liver disease, pristanic acid accumulation	
2-methylacyl-CoA racemase	ACOX2	76 826	Kidney	Perox	Nd	
Branched-chain acyl CoA oxidase	DBP	79 686	Many	Perox	Hypotonia, liver enlargement, developmental defects, pristanic acid/C ₂₇ bile acid accumulation	[67]
D-bifunctional enzyme						
Thiolase 2 sterol carrier protein-2/sterol carrier protein-x	SCP2 SCPx	58 993	Liver enriched	Perox	ND	[68]
Bile acid CoA: amino acid N-acyltransferase	BAAT	46 296	Liver only	Perox	Familial hypercholanaemia, fat malabsorption, vitamin K deficiency	[69]
<i>Enzymes of alternative routes</i>						
Cholesterol 24-hydroxylase	CYP46A1	56 821	Brain	ER	ND	
Cholesterol 25-hydroxylase	CH25H	31 700	Many	ER	NH with fibrosis	Cited in ref. 1
Oxysterol 7 α -hydroxylase	CYP39A1	54 129	Many	ER	ND	Cited in ref. 1

Mito, mitochondrion; ER, endoplasmic reticulum; Perox, peroxisome; CTX, Cerebrotendinous xanthomatosis; ND, no disease; NLF, neonatal liver failure; NH, neonatal hepatitis.

Mutations in the gene encoding 3 β -hydroxy C₂₇-steroid dehydrogenase/isomerase (3 β HSD) represent the most common disorders of bile acid biosynthesis [12–16]. Clinical manifestations may start at any age and include cholestasis, fat malabsorption, vitamin deficiency, pruritus and poor growth. Urine and plasma bile acid levels are high and consist of abnormal conjugates of the unoxidized precursors di- and tri-hydroxy- Δ -5-cholenic acids. These abnormal bile acids are poorly transported across the canalicular membrane and interfere with the adenosine triphosphate (ATP)-dependent transport of cholic acid. 3 β HSD deficiency can be treated successfully by administration of primary bile acids. Patients with Δ^4 -3-oxosteroid 5 β reductase deficiency (AKR1D1) present with neonatal cholestasis [17,18]. Urine and serum levels of primary bile acids were low but Δ^4 -3-oxo bile acid concentrations were elevated. Administration of primary bile acids constitutes successful therapy in these patients. Treatment by ursodeoxycholic acid is not sufficient, probably because this bile acid does not feedback to inhibit bile acid synthesis and thus does not prevent the production of hepatotoxic Δ^4 -3-oxo bile acids.

Cerebrotendinous xanthomatosis (CTX) is caused by a deficiency of mitochondrial CYP27 [19,20]. CTX is a slowly progressive chronic disease characterized by early dementia and xanthomata. Bile acid synthesis is reduced, but the clinical manifestations are caused by the accumulation of cholesterol and cholestenol in the brain. This gradually disrupts the myelin sheets surrounding the neurons. If diagnosed early, CTX can be treated effectively with bile acid therapy. Deficiency of the conjugation enzyme BAAT has been reported to cause familial hypercholeanaemia (FHCA). Patients present with high serum bile salt concentrations, fat malabsorption and vitamin K deficiency [21].

The final enzymatic steps of bile acid biosynthesis take place in peroxisomes. Zellweger syndrome (ZS) is a genetic disorder that affects the formation of these organelles. Mutations in over a dozen different genes have been shown to be the molecular cause of ZS or the related disorders neonatal adrenoleucodystrophy and Refsum disease [22]. These genes encode proteins that are involved in transporting newly synthesized enzymes to peroxisomes or are essential for the formation of the peroxisomal membrane. Indirectly, these mutations also affect the enzymes in peroxisomes. This may also affect bile acid synthesis. Patients present with cerebral neuronal migration disorder, craniofacial dysmorphism, psychomotor retardation and chronic liver disease. ZS is generally fatal in the first 2 years of life. Biochemically, these patients are characterized by increased levels of very-long-chain fatty acids, atypical mono-, di- and tri-C₂₇ hydroxy bile acids (such as cholestanic acid) and hyperpipecolic acidemia.

Hepatic secretion and enterohepatic cycling of bile salts

Although bile salts can diffuse through membranes, hepatocytes and ileal mucosal cells express proteins that efficiently pump bile salts in and out of these cells. The sodium-dependent taurocholate cotransporting polypeptide (NTCP, SLC10A1) is located at the sinusoidal plasma membrane domain of hepatocytes (Fig. 2). The apical sodium-dependent bile salt transporter (ASBT, SLC10A2) is similar to NTCP, but is specifically expressed at the luminal surface of mucosa cells in the ileum [23,24]. These are high-affinity bile salt transport systems that allow the absorption of bile salts from the portal blood or the bowel lumen respectively. Both are sodium dependent with an out-to-in sodium gradient that drives this transport. In

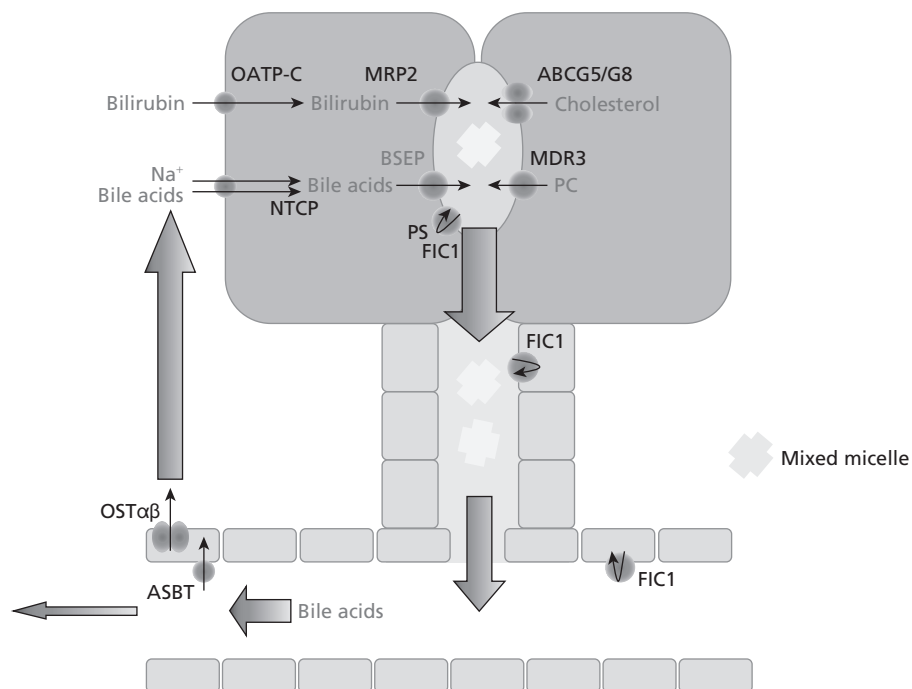


Fig. 2 Transporters involved in bile formation in liver and intestine. PC, phosphatidylcholine; PS, phosphatidylserine; GGT, gamma-glutamyltransferase.

addition, hepatocytes contain other transport proteins that may import bile salts, including the organic anion transporting polypeptide-C (OATP-C, SLC21A6), OATP-A (SLC21A3), OATP-B (SLC21A9) and OATP8 (SLC21A8) [25]. These transporters have a broad substrate specificity, they are bidirectional and do not need sodium for their transport activity. NTCP is believed to be the predominant bile salt transporter responsible for efficient hepatic uptake. Only a small fraction of portal blood bile salts spills over in the general circulation. Even the high bile salt concentrations that enter the portal circulation after a meal are efficiently dealt with by the liver.

No clinically important genetic defects of NTCP have been recognized thus far. In two children with familial hypercholelanaemia, NTCP was normal [26]. In acquired forms of cholestasis, such as autoimmune, alcoholic and drug-induced hepatitis, obstructive cholestasis and primary biliary cirrhosis, NTCP levels are reduced [27–29]. This is a meaningful adaptation as it prevents the intracellular accumulation of bile salts.

How bile salts traverse the interior of the hepatocyte is not clear. In the simplest model, bile salts just dissolve in the cytosol. In this model, the cytosolic bile salt concentration is governed by the net balance between passive influx and active efflux. As bile salts are cytotoxic, influx and efflux of bile salts has to be well coordinated in order to avoid accumulation. This calls for a well-organized short-term regulation of influx and efflux transport proteins.

Bile salt secretion from hepatocytes to bile is mediated by the bile salt export pump BSEP (ABCB11). This is a bile salt-specific pump with the highest affinity for tauro- and glycochenodeoxycholic acid [30,31]. However, it also transports taurocholic acid, glycocholic acid and tauroursodeoxycholic acid and even unconjugated bile acids, albeit with lower affinity [31,32]. BSEP is a member of a large family of proteins known as the ATP-binding cassette (ABC) transporters, which have a wide range of transport functions. These proteins use ATP to pump their substrates against steep concentration gradients. This enables BSEP to build up a biliary bile salt concentration into the millimolar range, a concentration well above the critical micellar concentration. Pure bile salt micelles are extremely cytotoxic because of a detergent-like membranolytic activity. This activity has to

be neutralized, and this is accomplished by incorporation of phospholipids, cholesterol and other organic molecules.

In the canalicular lumen, bile salt micelles interact with the lumen-facing leaflet of the canalicular membranes that is enriched with phosphatidylcholine and cholesterol [33]. These are extracted into the bile salt micelle and contribute to the formation of the characteristic bile salt–phospholipid–cholesterol mixed micelle. Phospholipid transfer from the inner to the outer leaflet that faces the canalicular lumen is mediated by multidrug-resistant protein 3, MDR3 (ABCB4; in rodents *mdr2*, *abcb4*) [34]. Genetic deficiency of MDR3 causes a disease called progressive familial intrahepatic cholestasis (PFIC) type 3 [35,36] (Table 2). In this disease, phospholipid transfer through the canalicular membrane is abrogated, and this results in bile without phospholipids. Bile salt transfer is undisturbed, and the bile that is produced under these conditions is extremely cytotoxic. It damages surrounding hepatocytes and bile duct epithelial cells. Hence, liver histology of these patients shows portal inflammation, bile duct proliferation and periportal fibrosis.

BSEP gene mutations are the cause of PFIC type 2 or benign recurrent intrahepatic cholestasis (BRIC) type 2 (see also Chapter 16.10, Genetic cholestatic diseases). PFIC type 2 is characterized by neonatal hepatitis and persistent cholestasis with malabsorption, stunted growth and haemorrhagic diathesis as a result [37,38]. Patients often present with subdural haematoma in the first months of life. This can be prevented by early vitamin K supplementation. This disease provides proof for the functional importance of BSEP. Less severe defects cause a more benign type of relapsing cholestasis, BRIC type 2 [39].

PFIC type 1 and BRIC type 1 result from mutations of the *FIC1* gene affecting the FIC1 (ATP8B1) protein [40,41]. As an aminophospholipid translocator, malfunction of FIC1 affects the distribution of phosphatidylserine (PS) across the two plasma membrane leaflets. Too much PS in the outer membrane leaflet makes the outer membrane leaflet unstable. *Fic1*^{−/−} mice have excessive outer leaflet-anchored proteins in their bile [42]. How cholestasis develops from FIC1 deficiency is not well understood.

Drug-induced cholestasis is a frequently observed, clinically relevant adverse effect of drugs. A great number of drugs and complementary medication can cause this disease. Many *BSEP*

Table 2 Transport defects.

Transport protein	Abbreviation	Membrane domain	Tissue	Disease symptoms	Mouse knock-out [ref.]
FIC1	ATP8B1	Canalicular	Liver, bile duct, intestine, pancreas	PFIC type 1, cholestasis first episodic later permanent, low GGT; BRIC type 1, episodic cholestasis; intrahepatic cholestasis of pregnancy	[70]
BSEP	ABCB11	Canalicular	Liver	PFIC type 2, permanent cholestasis, low GGT; BRIC type 2, episodic cholestasis	[71]
MDR 3	ABCB4	Canalicular	Liver	PFIC type 3, cholestasis, pruritus, high GGT; intrahepatic cholestasis of pregnancy; intrahepatic cholelithiasis	[34]

gene mutations and polymorphisms have been described [43–46]. However, a relation between these polymorphisms and drug-induced cholestasis or hepatitis remains difficult to prove and, to date, no clear connection between these and drug-induced cholestasis has been established.

The ASBT (SLC10A2) mediates the uptake of bile salts in the terminal ileum and is responsible for the preservation of bile salts in the enterohepatic circulation [24,47]. Mutations that affect the function of this protein cause bile acid-induced diarrhoea [48]. Jejunum also expresses OATP-B. However, OATP-B has a rather narrow substrate specificity and is not a good bile salt carrier [49,50]. Rat jejunum expresses Oatp3 that can serve as alternative transporter for glycine- and taurine-conjugated bile salts.

Organic solute transporter (OST) α and β act as heterodimers and mediate bile salt transport across the serosal membrane, thus allowing bile salt entry into the portal circulation [51]. They are specifically expressed in the ASBT-containing cells of the terminal ileum but also in hepatocytes. ASBT and OST $\alpha\beta$ are responsible for the vectorial transport of bile salts from the intestinal lumen to the portal circulation.

Bile acids as signalling molecules

It has long been known that bile acid synthesis and enterohepatic cycling are highly regulated processes. In recent years, important progress has been made in understanding the molecular mechanism involved in this process, recognizing that bile salts themselves are the crucial signalling molecules.

The nuclear hormone receptors (NHR) belong to a family of proteins that, upon binding an appropriate ligand, can activate or suppress gene expression [52] (see also Chapter 3.4, Cellular cholestasis). Promoter regions of genes contain characteristic nucleotide sequences for binding NHRs. These consist of two hexamers separated by a spacer of 0–8 nucleotides. Class II NHRs function as heterodimers in which the common retinoid X receptor RXR α complexes with a partner that can be farnesoid X receptor (FXR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), the liver X receptor (LXR), the retinoic acid receptor (RAR), the peroxisomal proliferator-activated receptor (PPAR) or the vitamin D receptor (VDR) [53]. These members of the NHR family have received their names from the first ligands identified. Natural and much more potent ligands have been characterized for these NHRs, making their historic names deceptive as they do not reflect their actual function. A typical example is FXR, which is strongly activated by bile acids. NHRs reside either in the nucleus (PXR) or in the cytoplasm and move to the nucleus upon binding a ligand (CAR) [54]. Drugs, bile acids and intermediates of bile acid biosynthesis, the oxysterols, are major ligands for these NHRs. FXR binds bile salts with high affinity and thus serves as a bile acid biosensor. FXR affects a great number of target genes, with an emphasis on genes related to bile acid synthesis and transport, lipid and carbohydrate metabolism.

In the human small intestine, ASBT expression is negatively regulated by bile salts; at high bile salt concentrations, the

expression of ASBT is downregulated [55,56]. This is mediated by the FXR-dependent induction of a protein called small heterodimer partner-1 (SHP-1). This protein negatively interferes with the RAR:RXR-dependent transcription of ASBT. Expression of the export proteins OST $\alpha\beta$ is also controlled by FXR [57]. Activation of FXR leads to an increased expression of OST $\alpha\beta$. Thus, bile acids regulate their own intestinal absorption, and FXR regulation protects ileum cells from high bile salt concentrations.

After intestinal absorption, bile acids are taken up from the portal blood into the liver. Here they also bind and activate FXR, which induces SHP-1 expression. SHP-1 then interferes with LXR-dependent transcription of *CYP7A1* and with RAR:RXR-dependent transcription of *NTCP*, thus reducing bile acid biosynthesis as well as bile acid uptake [52,58]. *NTCP* is downregulated during cholestasis, and this is caused by SHP-dependent and SHP-independent mechanisms. For the short-term regulation, it is relevant to note that *Ntcp* is a cAMP-dependent phosphoprotein. This allows the rapid regulation of *Ntcp* activity by phosphorylation [59].

While hepatic bile acid synthesis and uptake are negatively regulated by FXR, bile acid export is positively regulated. The *BSEP* gene contains a bile acid response element that provides a site for interaction with FXR:RXR α that increases transcription of this gene [60,61]. The combined downstream effects of bile acid-activated FXR results in the protection of hepatocytes against bile acid toxicity. Bile acids and drugs also serve as ligands for PXR, CAR and VDR [62]. The main function of these proteins is to provide protection against bile salt and drug toxicity. They have overlapping ligand specificity and regulate the transcription of an overlapping number of target genes, which includes many members of the cytochrome P450 family and ABC transport proteins. Induction of these proteins allows detoxification by biotransformation and secretion of harmful substances. Proof of these concepts comes from studies with *PXR* $^{-/-}$ and *CAR* $^{-/-}$ knockout mice, which are quite vulnerable to bile salt and drug toxicity [63,64].

Conclusions

Bile acids are important as emulsifiers of fat in the intestine, and an intact enterohepatic cycling of bile salts is indispensable for daily nutrition. Without bile, patients rapidly lose weight and become catabolic. Bile acids are also necessary for the intestinal uptake of fat-soluble vitamins. Therefore, infants with cholestasis often present with haemorrhagic complications due to vitamin K deficiency.

In view of the many proteins associated with bile acid metabolism, it is perhaps not surprising that there are many genetic diseases that affect bile acid biosynthesis or secretion. Cholestasis is a common phenotype in these diseases. A proper admixture of the three main components of bile – bile acids, phospholipids and cholesterol – is needed not only to prevent gallstone formation but also to avoid hepatocyte and bile duct damage. Retention of bile acids due to impaired secretion may

result in hepatocyte injury. However, the liver possesses a number of adaptations that function to prevent the accumulation of bile acids. Liver injury occurs when adaptation fails.

For the treatment of cholestatic liver disease, the repertoire of drugs and interventions is limited. Primary bile salts are used for the treatment of the genetic bile acid synthesis defects. Ursodeoxycholic acid is useful for the treatment of chronic cholestatic liver diseases, in particular primary biliary cirrhosis. Also, patients with intrahepatic cholestasis of pregnancy and patients with MDR3 deficiency benefit from ursodeoxycholic acid treatment. Partial external biliary diversion is a therapeutic option for patients with PFIC types 1 and 2. For severe genetic forms of cholestasis, liver transplantation is a life-saving procedure. Gene therapy and hepatocyte transplantation remain unfulfilled promises. New insights into transcriptional regulation by NHRs offer an opportunity for drug development, and this will be likely to expand the number of drugs available for the treatment of cholestatic liver disease.

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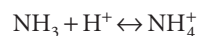
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2.3.7 Ammonia, urea production and pH regulation

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Ammonia plays a central role in nitrogen metabolism. It is a major byproduct of protein and nucleic acid catabolism, and its nitrogen can be incorporated into urea, amino acids, nucleic acids and many other nitrogenous compounds. Ammonia is present in body fluids as both NH_3 and NH_4^+ , and these are in equilibrium according to the equation:



The $\text{p}K_a$ of this reaction is 9.25, so that at physiological pH there is a great excess of the ionized form. NH_3 can diffuse freely across membranes via aquaporins [1] and NH_4^+ is carried in liver by an active transport system, the RhB glycoprotein [2].

The blood ammonia concentration is normally below 35 $\mu\text{mol/L}$; this is important as ammonia is neurotoxic at higher concentrations. Excessive cerebral ammonia uptake in hyperammonaemic states leads to astrocytic glutamine accumulation and cerebral oedema, which is important in the pathogenesis of hepatic encephalopathy [3–5].