

Metabolism and function of bile acids

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1. Introduction

Bile acids make up a group of sterol-derived compounds that act as detergents in the intestine to facilitate the digestion and absorption of fats and fat-soluble molecules. In mammalian species, the cholesterol side chain is trimmed to yield C₂₄-sterol derivatives. In other vertebrate species, the hydroxylation of the side chain does not lead to its removal and the products of the biosynthetic pathway are referred to as bile alcohols. Invertebrate species do not synthesize sterol bile acids. Over the last few years, much information has been gained about the function of bile acids and the mechanisms that regulate their synthesis. The focus of this chapter is to provide a general overview of bile acid biochemistry and to review recent discoveries that have advanced our understanding of bile acid metabolism and function in mammals.

The concept of bile was developed around the late 1600s to mid 1700s. It was early in the 1800s when bile solutes were crudely isolated. Among the components identified were the amino acid taurine (identified in ox bile, hence its name), cholesterol and a nitrogenous acid. The term 'cholic acid' was initially applied to the acidic component but this was changed to the generic term 'bile acid' shortly after. By the mid 1800s, taurine- and glycine-conjugated bile acids could be distinguished and it was also around this time that the idea that bile acids were responsible for solubilizing cholesterol in bile emerged. Nearly half a century ago, it became evident that bile acids are synthesized from cholesterol [1]. Bile acids are the major solutes in bile. The typical mammalian bile is comprised of about 82% water, 12% bile acids, 4% phospholipids (mostly phosphatidylcholines), 1% unesterified cholesterol and the remaining 1% as assorted solutes (including proteins).

2. Bile acid structure

The structure of bile acids holds the key for their ability to act as efficient detergents. In general, cholesterol is modified by epimerization of the pre-existing 3 β -hydroxyl group, saturation and hydroxylation of the steroid nucleus and trimming of the side chain [2]. Fig. 1 shows the positions of the carbons in the steroid nucleus that are modified during bile acid biosynthesis. Under normal physiological conditions, one or two hydroxyl groups are added to the steroid nucleus. This modification renders the sterol less hydrophobic, enabling it to interact with an aqueous environment more efficiently. The hydroxyl groups of many bile acids are oriented towards one face of the

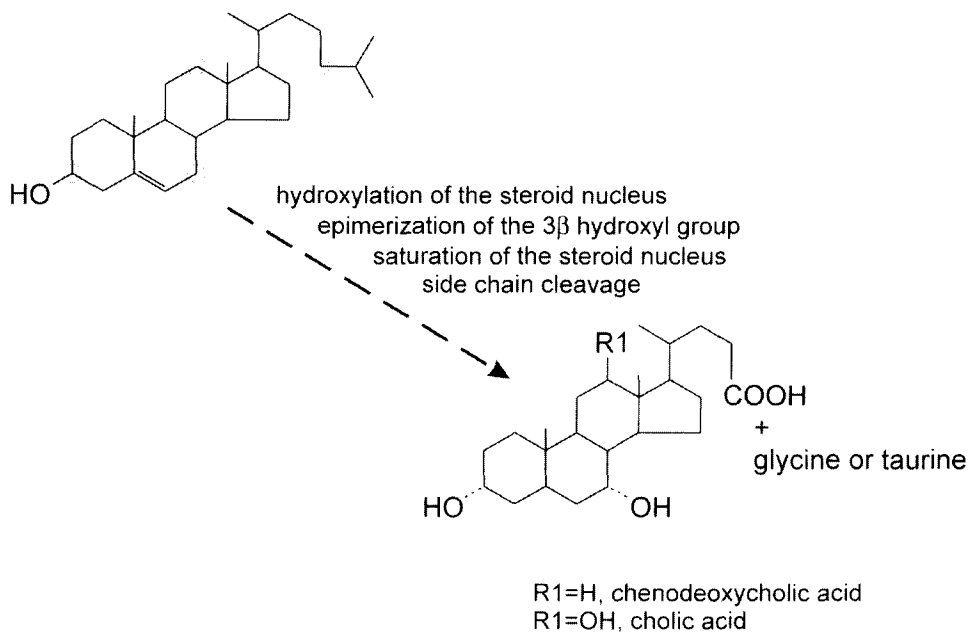


Fig. 1. Conversion of cholesterol into bile acids. The carbons in the cholesterol molecule that are modified during the conversion process are circled.

steroid nucleus giving the molecule an amphipathic character. After trimming 3 carbons from the side chain, the ‘free’ bile acids are covalently linked to one of two amino acids (either taurine or glycine) to form ‘conjugated’ bile acids. Conjugated bile acids readily ionize, allowing these polar molecules to efficiently interact with both hydrophobic and hydrophilic substances (Fig. 2).

The number and specific orientation of the hydroxyl groups added to the steroid nucleus vary according to animal species. Table 1 lists the bile acids that are commonly found in the bile of different mammalian species. Some bile acids, such as cholic and

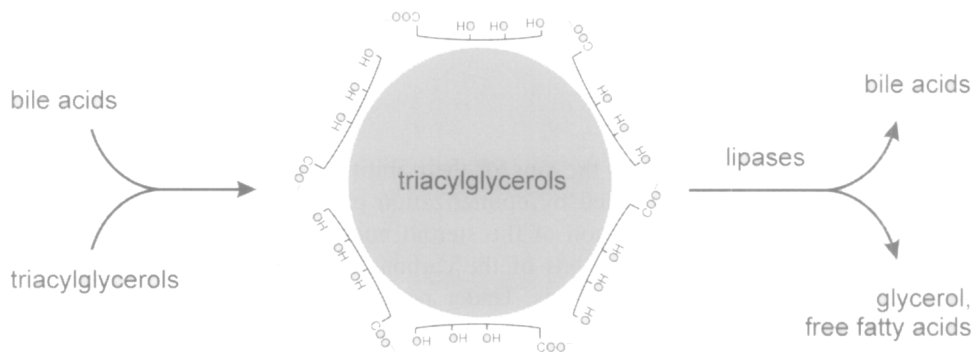


Fig. 2. Interaction of bile acids with triacylglycerols. Lipid-soluble nutrients may be present in the triacylglycerol droplet. Lipases hydrolyze the triacylglycerols to liberate free fatty acids and glycerol.

Table 1
Abundant bile acids found in the bile of selected mammalian species

Bile acid	Position and orientation of hydroxyl groups	Species
Chenodeoxycholic acid	3 α , 7 α	bear, hamster, human, pig
Ursodeoxycholic acid	3 α , 7 β	bear
Deoxycholic acid	3 α , 12 α	cat, human, rabbit
Hyochoic acid	3 α , 6 α , 7 α	pig
β -muricholic acid	3 α , 6 β , 7 β	mouse, rat
Cholic acid	3 α , 7 α , 12 α	bear, cat, hamster, human, mouse, pig, rabbit, rat

chenodeoxycholic acids, are common to many mammalian species whereas others are unique to certain species. Ursodeoxycholic acid, which is abundant in bear bile, has been found to be therapeutically useful for treating primary biliary cirrhosis and dissolving gallstones. It is chemically and biologically distinct from its isomer chenodeoxycholic acid, which differs only in the orientation of the hydroxyl group attached to carbon 7 of the steroid nucleus. Among the mammalian species that are commonly studied in the laboratory, the hamster is the only one that shows a biliary bile acid composition that is comparable to that of humans.

Fig. 3 shows the synthesis of taurine from cysteine via oxidation and decarboxylation reactions. Taurine is very rare in plants but is abundant in animal tissue, particularly in the brain. The bile acids of carnivores are mostly conjugated to taurine whereas those of herbivores are conjugated to glycine. Both taurine- and glycine-conjugated bile acids are found in the bile of omnivores. The bile of cats contains taurine-conjugated

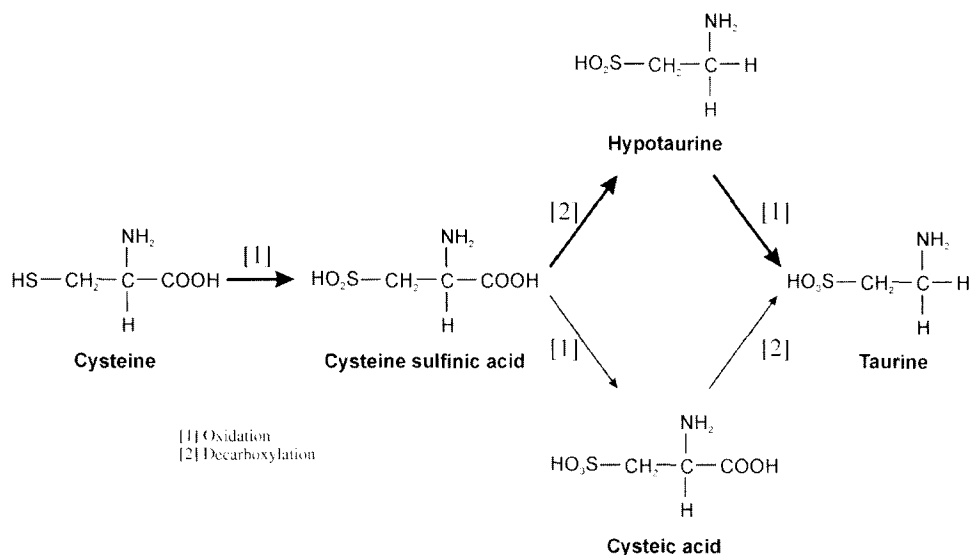


Fig. 3. Synthesis of taurine from cysteine. The major pathway for the formation of taurine is via hypotauroine.

bile acids exclusively. Cats appear to have a requirement for taurine as withdrawal of dietary taurine causes the degeneration of the retina leading ultimately to blindness. Interestingly, taurine deficiency does appear to have any significant consequences in other mammalian species. Conjugated bile acids are more acidic than unconjugated bile acids due to the additional carboxyl group contributed by the amino acid. Consequently, conjugated bile acids readily ionize and exist mainly as bile salts at physiological pH. The functional significance of the choice of amino acid used for conjugation is not clear. Cultured rat hepatoma cells show differential sensitivity to taurine- and glycine-conjugated bile acids [3]. In these cells, glycine-conjugated bile acids are toxic and induce cell death by apoptosis whereas taurine-conjugated bile acids are well-tolerated and even promote cell survival.

3. Biosynthesis of bile acids

Classical studies elucidated the major steps in the bile acid biosynthetic pathway mainly by analyzing the metabolites formed from labeled cholesterol and oxysterols [4]. At least 18 distinct reactions occurring in various subcellular compartments (cytosol, endoplasmic reticulum, mitochondria, and peroxisomes) are necessary to transform cholesterol into bile acids. Reactions involving modifications of the steroid nucleus occur in the endoplasmic reticulum and mitochondria. The removal of the cholesterol side chain involves peroxisomes. Many of the enzymes that catalyze these reactions have been purified, their cDNAs cloned and ectopically expressed in a variety of cultured cell lines. In addition, the impact of overactivity and deficiency of some of these enzymes in the formation of bile acids *in vivo* has been studied through the use of gene therapy, transgenic, and targeted gene disruption techniques.

3.1. The classical and alternative bile acid biosynthetic pathways

The classical pathway operates entirely in the liver (Fig. 4). It begins by α -hydroxylation of carbon 7 of the cholesterol steroid nucleus. This reaction is catalyzed by the microsomal cytochrome *P*-450 monooxygenase referred to as cholesterol 7 α -hydroxylase (cyp7a) and is the rate-limiting step of the classical pathway. Several of the enzymes that participate in the transformation of cholesterol into bile acids belong to the cytochrome *P*-450 family. In general, this class of enzymes catalyzes the hydroxylation of various organic compounds using molecular oxygen as a cosubstrate. The heme-containing monooxygenases recognize specific molecules, or a group of related compounds, and work in concert with NADPH:cytochrome *P*-450 oxidoreductase which supplies electrons for the reactions. Cyp7a shows a high degree of selectivity towards cholesterol. Bile acid output from the liver is correlated with the cyp7a activity, and it is generally considered that the classical pathway is the source of the bulk of the bile acids made by the liver.

The existence of an alternate pathway for the synthesis of bile acids was suspected because it was possible for oxysterols to be converted into bile acids (N. Wachtel, 1968). It is now recognized that a variety of oxysterols produced by an assortment of cell types

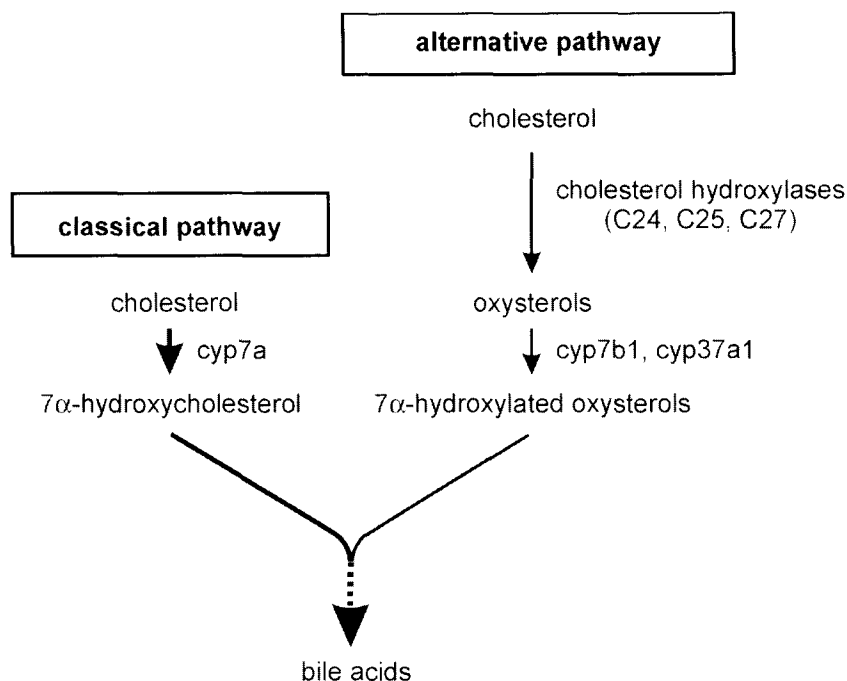


Fig. 4. The bile acid biosynthetic pathways. The classical pathway operates entirely in the liver. In other tissues, the entry of cholesterol into the alternate pathways is facilitated by cholesterol hydroxylases. The oxysterols generated by these enzymes are 7 α -hydroxylated by oxysterol hydroxylases and the products enter the latter steps of the classical pathway.

can be converted into bile acids. The production of these oxysterols is catalyzed by several sterol hydroxylases: sterol 27-hydroxylase (cyp27) (J.J. Cali, 1991), cholesterol 25-hydroxylase (E.G. Lund, 1998) and cholesterol 24-hydroxylase (cyp46) (E.G. Lund, 1999). Cholesterol 25-hydroxylase is not a cytochrome *P*-450 monooxygenase, unlike the two other enzymes. Almost all of the 24-hydroxycholesterol that ends up in the liver originates from the brain, and it has been suggested that the production of this oxysterol is the major mechanism responsible for eliminating excess cholesterol from this organ (I. Bjorkhem, 2001). Cyp27 is also important in the latter stages of bile acid synthesis in the liver, as it is the major enzyme that catalyzes the hydroxylation of the side chain to facilitate the cleavage of the sterol side chain. The oxysterols generated outside the liver are 7 α -hydroxylated, mainly by oxysterol hydroxylases distinct from cyp7a. The cyp7b1 oxysterol 7 α -hydroxylase prefers 25-hydroxycholesterol and 27-hydroxycholesterol, while the cyp37a1 oxysterol 7 α -hydroxylase is selective for 24-hydroxycholesterol (J. Li-Hawkins, 2000). Cyp7a can accept some oxysterols as a substrate, although it largely prefers cholesterol.

The latter steps required to complete the synthesis of bile acids occur only in the liver and are common to both the classical and alternative pathways. Consequently, the liver is the only organ in the body capable of producing bile acids. The isomerization of the 3 β hydroxyl group and the saturation of the steroid nucleus involve 3 β -hydroxy-

Δ^5 -C₂₇-steroid oxidoreductase (K. Wikvall, 1981), 3-oxo- Δ^4 -steroid 5 β -reductase (O. Berseus, 1967) and 3 α -hydroxysteroid dehydrogenase (A. Stolz, 1987). The activities of these enzymes are necessary for the formation of normal bile acids. The enzyme sterol 12 α -hydroxylase (cyp8b1) catalyzes the addition of a hydroxyl group to carbon 12 of the steroid nucleus, and therefore controls the production of the cholic and chenodeoxycholic acids. Changes in the ratio of cholic to chenodeoxycholic acid affect the overall hydrophobicity of the bile acid pool.

The cDNA for an enzyme capable of catalyzing the 6 α -hydroxylation of the steroid nucleus was cloned from pig liver (K. Lundell, 2001). This enzyme, named cyp4a21, is believed to be responsible for the formation of hyocholic acid, a bile acid typically found in porcine bile. The steps leading to the synthesis of β -muricholic acid (a 6 β -hydroxylated bile acid) are less understood [5]. This bile acid appears only in rat and mouse bile. The conversion of lithocholic acid (a 3 α -monohydroxylated bile acid) and chenodeoxycholic acid into β -muricholic acid has been observed, but the identities of the enzymes catalyzing the reactions are not known. Intestinal bacteria are thought to be responsible for modifying the steroid nucleus to form 7 β -hydroxylated bile acids. However it was recently noted that bear liver has the capacity to produce ursodeoxycholic acid, indicating the existence of hepatic enzymes that can catalyze the direct 7 β -hydroxylation of the steroid nucleus or epimerization of the 7 α -hydroxyl group (L.R. Hagey, 1993).

3.2. Mutations affecting key enzymes involved in bile acid biosynthesis

Bile acid synthesis represents a major pathway for cholesterol catabolism. In humans, bile acid excretion can account for the disposal of up to ~0.5 g of cholesterol per day. In animal studies, direct stimulation of bile acid synthesis by increasing the abundance of cyp7a enzyme in the liver through gene therapy, reduces the concentration of cholesterol in the plasma (D.K. Spady, 1995, 1998; L.B. Agellon, 1997). It was reasonably expected that inhibiting bile acid synthesis by repression of cyp7a would impair cholesterol catabolism and lead to an increased concentration of plasma cholesterol. In mice, the complete loss of cyp7a function results in the high incidence of neonatal lethality due mainly to inefficient absorption of fats and fat-soluble vitamins [6]. Cyp7a-deficient mice that manage to survive beyond the weaning period synthesize bile acids via the alternative pathway [7] but these mice do not develop hypercholesterolemia [8]. In contrast, a recently discovered mutation in the human *CYP7A1* gene that causes cyp7a deficiency appears to cause hypercholesterolemia (J.P. Kane, 2002). It is not yet known if the loss of cyp7a activity has an effect on human neonatal survival.

Mutations in human cyp27 cause cerebrotendinous xanthomatosis (CTX) (J.J. Cali, 1991). This disorder, which is characterized by neurological defects and premature atherosclerosis, may well be the consequence of sterol accumulation in neural and other tissues [9]. CTX patients have reduced capacity for normal bile acid synthesis but produce large amounts of bile alcohols. This is consistent with the importance of cyp27 in the removal of the cholesterol side chain. Interestingly, deficiency of cyp27 in mice does not elaborate the full complement of defects observed in humans with CTX [10]. The basis for the difference is not completely understood. It has been suggested

that other cytochrome *P*-450 enzymes, *cyp3a4* in particular, partially compensate for the missing functions supplied by *cyp27* in the murine species (A. Honda, 2001). Indeed, *cyp27*-deficient mice are still capable of producing normal C24 bile acids but overall bile acid synthesis is markedly diminished. This finding confirms that *cyp27* activity is quantitatively important in side chain cleavage, but that hydroxylation of another carbon in the side chain can permit some side chain cleavage to proceed. McArdle RH-7777 rat hepatoma cells are deficient in both *cyp7a* and *cyp27*, and no longer possess the capacity to synthesize bile acids. Reinstatement of *cyp7a* activity enables these cells to synthesize C24 bile acids despite the absence of *cyp27* activity (E.D. Labonté, 2000). *Cyp27* deficiency causes hypertriglyceridemia and hepatomegaly in mice, indicating that *cyp27* function affects other metabolic processes in this species (J.J. Repa, 2000).

The importance of the *cyp7b1* oxysterol 7α -hydroxylase in bile acid synthesis has also been studied in mice. Mice lacking this microsomal enzyme are viable and do not exhibit obvious defects in cholesterol or bile acid metabolism [11]. The notable feature in *cyp7b1*-deficient mice is the accumulation of 25- and 27-hydroxycholesterol in plasma and cells, suggesting that *cyp7b1* is important in the catabolism of these oxysterols into bile acids. In humans, mutations in *cyp7b1* results in severe neonatal liver disease characterized by cholestasis (arrest of bile flow) and cirrhosis (damage and scarring of liver tissue resulting from chronic impaired liver function) [12]. Mutations in 3β -hydroxy- Δ^5 -C₂₇-steroid oxidoreductase and 3-oxo- Δ^4 -steroid 5β -reductase are also known to cause progressive intrahepatic cholestasis [13–15].

The importance of peroxisomes in the cleavage of the cholesterol side chain during bile acid synthesis is well illustrated in Zellweger syndrome (Chapter 9). This genetic disorder is characterized by peroxisome deficiency and accumulation of large amounts of bile alcohols in the plasma of afflicted patients (R.J. Wanders, 1987). Mice carrying an induced mutation in the *Scp2* gene also accumulate bile alcohols similar to those seen in Zellweger patients [16]. The *Scp2* gene codes for two proteins: the cytosolic sterol carrier protein-2 (SCP2) and the peroxisomal sterol carrier protein-x (SCPx) (Chapter 17). SCPx contains the entire SCP2 sequence plus an N-terminal domain that has a β -ketothiolase activity (U. Seedorf, 1994). The basis for the accumulation of bile alcohols in mice homozygous for a mutant *Scp2* gene is the deficiency in peroxisomal β -ketothiolase activity supplied by SCPx.

4. Transport of bile acids

4.1. Enterohepatic circulation

Bile acids circulate between the liver and intestines via bile and portal blood. The path traced by bile acids between these two organs is depicted in Fig. 5, and is referred to as the enterohepatic circulation [17]. A number of transporters involved in the transport of bile acids have been described [18]. Hepatocytes recover bile acids from portal blood by an active process involving sodium/taurocholate co-transporting polypeptide (ntcp) (B. Hagenbuch, 1991). The recovered bile acids, along with newly synthesized bile

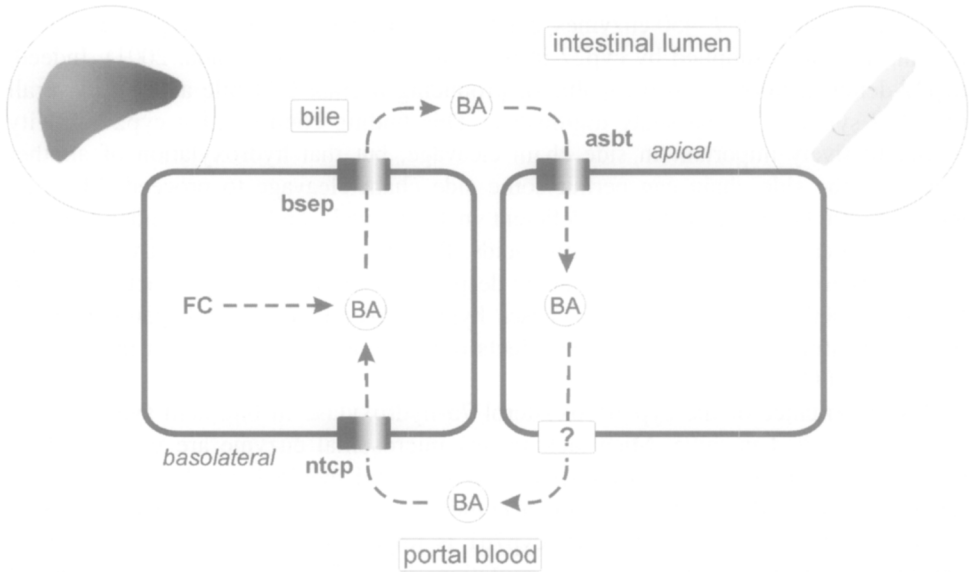


Fig. 5. Transport of bile acids in the enterohepatic circulation. The left and right sides of the figure depict a liver and intestinal cell, respectively. Note that the movement of bile acids in the enterohepatic circulation is vectorial. Abbreviations: asbt, apical/sodium bile acid cotransporter; BA, bile acids; bsep, bile salt export pump; FC, unesterified cholesterol; ntcp, sodium/taurocholate cotransporting polypeptide. The identity of the protein (depicted by '?') mediating the exit of bile acids from the basolateral pole of enterocytes is not yet known.

acids, are secreted into bile via the bile salt export pump (bsep, also known as sister of *p*-glycoprotein) (T. Gerloff, 1998). This protein belongs to the adenosine triphosphate binding (ABC) cassette family of transporters (M. Dean, 2001). Mutations in human bsep are known to cause progressive familial intrahepatic cholestasis type 2 [19]. However, targeted disruption of the murine gene encoding the bsep does not reproduce the human disease phenotype in mice [20]. This finding is another example illustrating a difference between human and murine bile acid metabolism.

The secreted bile acids are stored in the gallbladder prior to being released into the small intestine. An exception occurs in the rat (but not in the mouse), which lacks a gallbladder and thus continuously releases bile into the intestine. The primary bile acids (the products of bile acid biosynthesis in the liver) are metabolized by enteric bacteria to produce deconjugated (i.e., lacking taurine or glycine), and/or dehydroxylated derivatives referred to as secondary bile acids. The secondary bile acids may be further modified by sulfation and/or glucuronidation, but these modifications are not significant under normal physiological conditions. The deconjugated bile acids ('free' bile acids) are absorbed along the entire axis of the intestines. The majority of the conjugated bile acids are recovered in the terminal ileum via an active process involving the apical/sodium bile acid transporter (asbt) (M.H. Wong, 1994). In humans, mutations in asbt cause primary bile acid malabsorption (P. Oelkers, 1997). The identity of the intestinal bile acid exporter is not yet established.

As already mentioned, the major lipids found in bile are bile acids, phospholipids (mainly phosphatidylcholines, PC) and unesterified cholesterol. The solubility of cholesterol in bile is dependent upon the ratio of these lipids. The acyl chain composition of biliary PC (predominantly C16:0 at the *sn*-1 position and either C18:1 or C18:2 at the *sn*-2 position) differs from that normally found in bulk cell membranes (predominantly C18:0 at the *sn*-1 position and C20:4 at the *sn*-2 position). It is now known that the secretion of PC into bile requires a canalicular membrane protein referred to as *mdr2* (an ABC-type transporter encoded by the *Abcb4* gene in mice). Mice that are deficient in *mdr2* have a very low concentration of PC in bile (J.J.M. Smit, 1993). The secretion of bile acids into bile is not affected by *mdr2* deficiency. However, cholesterol concentration in the bile of *mdr2*-deficient mice is diminished, indicating that the secretion of cholesterol into bile is dependent on biliary PC. Mutations in MDR3 (the human equivalent of *mdr2*) cause progressive familial intrahepatic cholestasis type 3 (J.M. De Vree, 1998). It was recently proposed that *abca1* (Chapter 20), another ABC-type transporter, is involved in the cellular efflux of cholesterol. However, it is not yet clear if this transporter is directly responsible for mediating the transport cholesterol. *Abca1* is found in a variety of organs including the liver. It remains to be determined if this protein is found in canalicular membrane of hepatocytes.

4.2. Intracellular transport

The mechanism for the intracellular transport of bile acids is less understood than the uptake and secretion of bile acids by liver and intestinal cells. Several intracellular proteins capable of binding bile acids have been identified but it is not yet clear if these proteins are involved in the transcellular transport of bile acids [18]. The best candidate protein in the intestine is the ileal lipid binding protein (*ilbp*). This protein, a member of the intracellular lipid binding protein family (A.V. Hertzler, 2000), is abundantly expressed in the distal portion of the small intestine where *asbt* is found. It has been suggested that *ilbp* and *asbt* interact to form a macromolecular bile acid transport system in intestinal cells [21]. The protein providing the equivalent function in liver cells is not known. The liver-fatty acid binding protein does not bind bile acids efficiently. High level expression of the human bile acid binder in hepatoma cells capable of active bile acid uptake does not appear to influence bile acid transport [3].

5. Molecular regulation of key enzymes in the bile acid biosynthetic pathways

Bile acid synthesis is modulated by a variety of hormonal and nutrient factors. Alterations in bile acid metabolism have been documented in response to thyroid hormones, glucocorticoids and insulin. It is also well known that cholesterol and bile acids have opposite effects on the activity of the bile acid biosynthetic pathway (Fig. 6). A major advance into the understanding of the mechanisms that regulate bile acid synthesis came with the cloning of the rat *cyp7a* cDNA, which permitted the expression of the *cyp7a*

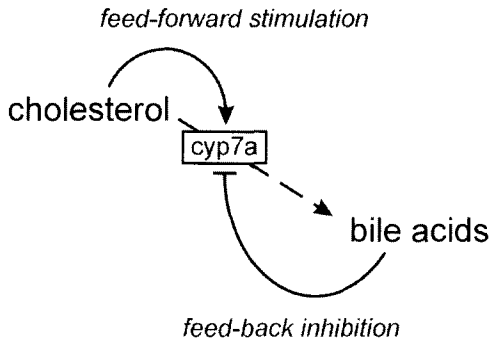


Fig. 6. Regulation of bile acid synthesis.

gene to be monitored at the molecular level [22]. Many of the details relating to the molecular mechanisms involved in regulating bile acid synthesis have been elucidated using both cultured cells and genetically modified mouse strains.

Feeding rats with a cholesterol-enriched diet induces bile acid synthesis. This increase is attributable to the rise in cyp7a activity, which catalyzes the rate-limiting step of the classical pathway (Fig. 6). Interrupting the return of bile acids to the liver, by diverting bile or by feeding a bile acid-binding resin, also stimulates the synthesis of bile acids. In contrast, reintroduction of bile acids into bile-diverted rats reverses the stimulatory effect, indicating that bile acid synthesis is subject to end-product inhibition. It was later discovered that cyp7a enzyme activity is closely correlated with cyp7a mRNA abundance, indicating that cyp7a gene transcription is the major determinant of cyp7a activity.

In many of the early studies, crystalline cholesterol was added directly to the standard rodent chow and fed to the animals. Although this experimental condition was useful in illustrating the stimulation of the cyp7a gene in response to dietary cholesterol, it does not normally exist in nature. The use of semi-purified diets has revealed that the composition of the fat in which cholesterol is presented has a marked influence on the ability of cholesterol to regulate cyp7a gene expression [23]. It has also become apparent that the fat component of the diet is capable of stimulating murine *Cyp7a1* gene expression, independent of exogenous cholesterol.

The regulation of the classical and alternative bile acid biosynthetic pathways has been studied mostly in mice and rats. The data indicate that the classical pathway is under stringent regulation, with much of the control exerted on the cyp7a gene. In contrast, the alternative pathway appears to operate constitutively. There is also emerging evidence indicating that the synthesis of bile acids in humans is only moderately regulated, unlike that in mice and rats.

5.1. Transcriptional control

Several transcription factor binding sites have been mapped in the cyp7a gene promoter, and many of these bind transcription factors that are members of the nuclear receptor superfamily (Table 2). Some of these receptors, notably the liver x receptor α (LXR α)

Table 2
Transcription factors shown to have functional interaction with the *cyp7a* gene promoter

Transcription factor	Cyp7a gene
BTEB	rat
C/EBP β	rat
COUP-TFII (ARP-1)	rat
DBP	rat
HNF-1	human
HNF-3	human, hamster, rat
HNF-4	human, hamster, rat
LRH-1 (also known as CPF, FTF)	human, rat
LXR α	rat
PPAR α	mouse
TR α and TR β	human

The data in this table are compiled from studies published by academic (L.B. Agellon, J.Y. Chiang, A.D. Cooper, E. De Fabiani, G. Gil, D.J. Waxman, U. Schibler) and pharmaceutical (Tularik Inc., Glaxo-Wellcome Research and Development) laboratories.

[NR1H3]) and peroxisome proliferator-activated receptor α (PPAR α [NR1C1]), bind to their target elements as heterodimers with retinoid x receptor α (RXR α [NR2B1]).

The stimulation of *cyp7a* gene expression by cholesterol involves LXR α (Fig. 7),

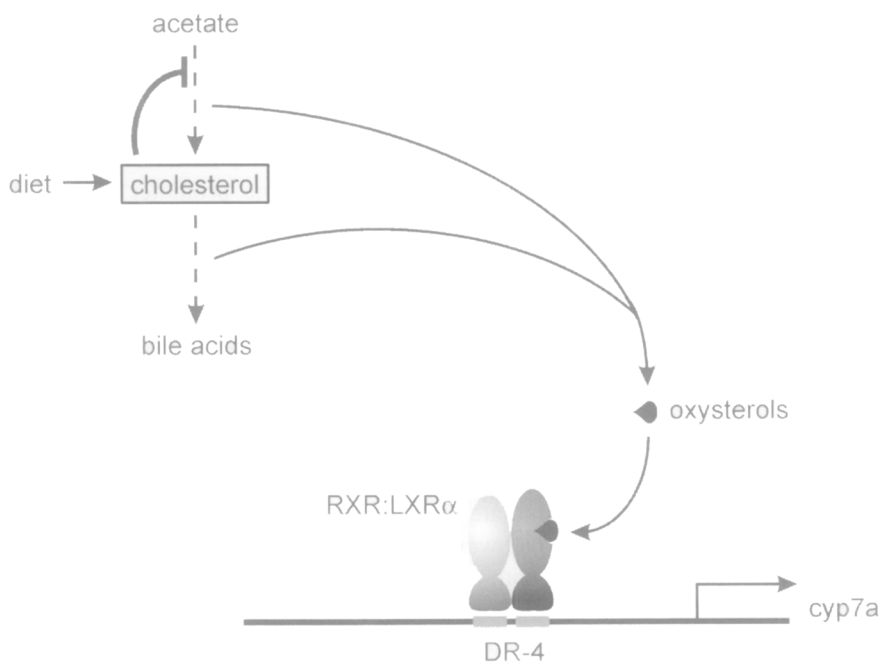


Fig. 7. Induction of *cyp7a* gene expression by oxysterol-activated LXR α :RXR α . Both the bile acid and cholesterol biosynthetic pathways generate oxysterols. The binding site of LXR α :RXR α in the *cyp7a* gene promoter is a DR-1 (a direct repeat of the hexanucleotide hormone response element separated by 4 nt).

an oxysterol-activated transcription factor (D.J. Mangelsdorf, 1996). In cultured cells, induction of the rat *Cyp7a1* gene promoter by oxysterols is dependent on LXR α (J.M. Lehman, 1997). In LXR α -deficient mice, the *Cyp7a1* gene is no longer induced by cholesterol feeding [24]. The oxysterols that serve as potent ligands for LXR α are likely generated by the early steps in the alternative bile acid biosynthetic pathway (i.e., 25-hydroxycholesterol and 27-hydroxycholesterol), and by the cholesterol biosynthetic pathway (i.e., 24(S),25-epoxycholesterol). It is notable that 24(S),25-epoxycholesterol is also capable of repressing 3-hydroxy-3-methyl-glutaryl coenzyme A reductase activity (T.A. Spencer, 1985).

Fatty acids and their metabolites can stimulate the murine *Cyp7a1* gene promoter via PPAR α : RXR α in hepatoma cells [25]. Interestingly, LXR α : RXR α and PPAR α : RXR α heterodimers bind to overlapping regions in the murine *CYP7a1* gene promoter. It is currently not known how these transcription factors interact with the *cyp7a* gene promoter when both are simultaneously activated, or whether this is relevant to the earlier finding that the type of fat in the diet influences the response of the murine *Cyp7a1* gene to dietary cholesterol. The corresponding region of the human *CYP7A1* gene promoter does not interact with either PPAR α : RXR α or LXR α : RXR α . In transgenic mice, the human *CYP7A1* gene is not stimulated by cholesterol feeding (L.B. Agellon, 2002). A differential interaction of thyroid hormone receptors (TR α [NR1A1] and TR β [NR1A2]) with the *cyp7a* gene promoters of different species has also been documented [26]. The human *CYP7A1* gene promoter binds and is inhibited by the thyroid hormone receptor. In contrast, the murine *Cyp7a1* gene promoter does not interact with the thyroid hormone receptor. These differences may indicate that the *cyp7a* gene promoters of different organisms are configured to respond to regulatory cues relevant to each species.

An indirect mechanism for the inhibition of *cyp7a* gene expression by bile acids has been proposed (Fig. 8). The liver receptor homolog protein-1 (LRH-1 [NR5A2]) is a monomeric orphan nuclear receptor bound to *cyp7a* gene promoter to enable expression in the liver (M. Nitta, 1999). It was recently discovered that bile acids are the physiological ligands of the farnesoid x receptor (FXR [NR1H4]), another transcription factor belonging to the nuclear receptor superfamily (M. Makashima, 1999; B.M. Forman, 1999). In the liver, FXR stimulates the expression of the gene encoding the nuclear factor known as small heterodimer partner (SHP; NR0B2) [27,28]. The interaction of SHP with LRH-1 renders the *cyp7a* gene promoter insensitive to stimulation by other transcription factors. However the proposed model (Fig. 8) cannot account for some observations. For example, the *Cyp7a1* gene is resistant to inhibition by bile acids in FXR-deficient mice (C.J. Sinal, 2000) even though it has been suggested that bile acids can stimulate SHP gene expression via an alternative mechanism involving the JNK/c-Jun pathway (J.H. Miyake, 2000; S. Gupta, 2001). Furthermore, feeding a diet containing both cholesterol and bile acids does not abolish *cyp7a* gene expression [29].

Bile acids inhibit the expression of the *cyp8b1* gene (encodes the sterol 12 α -hydroxylase) in parallel with the *cyp7a* gene (Z.R. Vlahcevic, 2000). Suppression of *cyp8b1* gene expression is likely mediated through SHP, as LRH-1 is also required for *cyp8b1* promoter activity (A. del Castillo-Olivares, 2000). The expression of the

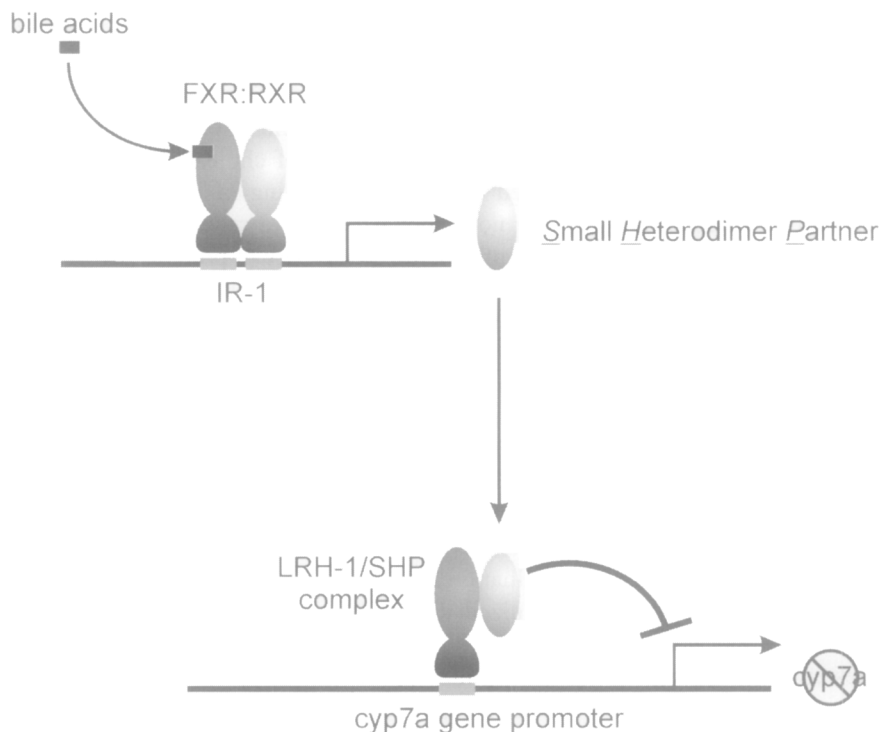


Fig. 8. Repression of *cyp7a* gene expression by bile acids. The liver receptor homolog-1 (LRH-1) binds to the *cyp7a* gene promoter to enable expression in the liver. A bile acid-activated FXR:RXR heterodimer binds to an IR-1 (an inverted repeat of the hexanucleotide hormone response element separated by 1 nt) in the promoter of the gene that encodes the small heterodimer partner (SHP) and stimulates its expression. Binding of SHP to LRH-1 arrests the expression of the *cyp7a* gene.

rat *Cyp8b1* gene is also inhibited by thyroid hormone, but it is not clear whether this effect involves the interaction of the thyroid hormone receptor with the rat *cyp8b1* gene promoter (U. Andersson, 1999). Moreover thyroid hormones, unlike bile acids, exhibit opposite effects on the expression of *Cyp8b1* and *Cyp7a1* genes in rats.

5.2. Post-transcriptional control

The majority of the studies described in the literature dealing with the regulation of bile acid synthesis have focused on *cyp7a* and it is apparent that most of the control is exerted at the level of gene transcription. The *cyp7a* mRNA has a short half-life, and this is attributable to the existence of multiple copies of the AUUUA motif in its 3'-untranslated region. However, some bile acids can further accelerate the decay of chimeric mRNAs containing the 3'-untranslated region of the murine *cyp7a* mRNA in hepatoma cells, and this effect is independent of the AUUUA element [30]. The regulation of *cyp7a* enzyme activity by phosphorylation/dephosphorylation has been suggested. However the results obtained by several studies are conflicting and the topic remains controversial.

6. Future directions

The well-known function of bile acids is to aid in the digestion and absorption of lipids and lipid-soluble nutrients in the intestine. Since bile acids are synthesized from cholesterol, they are also regarded as the terminal products of cholesterol catabolism. Research in the past decade has provided a new understanding of the physiological importance of bile acids. Bile acids are now known to be active regulators of cellular processes, such as signal transduction, by influencing the activity of proteins involved in signaling cascades, and gene expression by influencing the turnover of specific mRNA species as well as by serving as the natural ligand for the nuclear receptor FXR.

The synthesis of bile acids is under tight control, with both substrate and end-product actively participating in the regulatory process. The enzymes involved in bile acid synthesis are coordinately regulated with the proteins that transport bile acids. Much of the control is exerted at the level of transcription. It will be of interest to determine how bile acid metabolism is integrated into other processes, such as reverse cholesterol transport and perhaps fat metabolism. The use of targeted gene disruption technology to generate specific mutations in the bile acid biosynthetic pathway has been highly useful in uncovering new components and evaluation of their relative importance. It should be realized that there are already recognized differences in the way standard mouse strains metabolize bile acids. Nevertheless, surprising new information has emerged from studies employing engineered mouse strains. It has become apparent that gender dimorphism exists with respect to several components of bile acid metabolism in the mouse. In addition, these studies reveal that the metabolism of bile acids in humans and mice may be more dissimilar than previously believed. It will be important to explain how these differences arise. Significant advances have also been gained in the area of bile acid transport. Proteins that mediate the passage of bile acids across cell membranes have been identified, although some still await discovery. The transport of bile acids within cells remains poorly understood but this should become clear in the coming years.

Abbreviations

cyp7a	cholesterol 7 α -hydroxylase
cyp7b1	oxysterol 7 α -hydroxylase
cyp8b1	sterol 12 α -hydroxylase
cyp27	sterol 27-hydroxylase
FXR	farnesoid x receptor
LXR α	liver x receptor α
LRH-1	liver receptor homolog-1
PC	phosphatidylcholine
PPAR α	peroxisome proliferator-activated receptor α
SHP	small heterodimer partner
RXR	retinoid x receptor

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