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Bile Acid Regulation of Hepatic Physiology
III. Regulation of bile acid synthesis:
past progress and future challenges

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Fuchs, Michael. Bile Acid Regulation of Hepatic Physiology. III. Regulation of bile acid synthesis: past progress and future challenges. *Am J Physiol Gastrointest Liver Physiol* 284: G551–G557, 2003;10.1152/ajpgi.00468.2002.—Bile acids, amphipathic detergent-like molecules synthesized from cholesterol, are highly conserved by means of enterohepatic circulation. They participate in the generation of bile flow and biliary lipid secretion and also promote absorption of fat-soluble vitamins and lipids. Conversion of cholesterol to bile acids represents a quantitatively important route to eliminate cholesterol from the body. Regulation of bile acid synthesis involves a complex and interrelated group of transcription regulators that link bile acid synthesis to cholesterol and fatty acid metabolism. Targeting key steps of bile acid synthetic pathways as well as the metabolic network that maintains homeostatic levels of lipids should provide exciting novel opportunities for the treatment of cardiovascular and liver diseases.

cholesterol; sterol carrier protein 2; steroid acute response; transcription factors; atherosclerosis

BILE ACIDS ARE NATURAL end products of cholesterol. They are exclusively synthesized in the liver in a process that is regulated by many factors including nutrients, hormones, and bile acids. It is well established that regulation of bile acid synthesis occurs in a feed-forward mechanism by modulating intracellular cholesterol availability. After their absorption from the intestine, bile acids return to the liver and inhibit their own synthesis in a feedback regulatory loop. Recently, both regulatory mechanisms have been shown to mainly occur at the transcriptional level. The synthesis of bile acids was initially thought to involve a single major pathway. In fact, employing modern biochemical and molecular biological tools have facilitated the cloning of key regulatory enzymes of additional bile acid synthesis pathways. Each pathway differs in the initial steps, and cholesterol from extrahepatic tissues (e.g. macrophages, brain) may be used for bile acid formation too. Studying the regulation of bile acid synthetic enzymes in vitro and in vivo revealed that not only well-known key enzymes, but also cholesterol delivery to mitochondria may represent a rate-limiting step in bile acid synthesis. Extensive research into nuclear receptor-mediated regulation of bile acid synthesis highlighted a complex interrelationship between bile acid and oxysterol regulation of additional enzymes of bile acid, cholesterol, and fatty acid metabolism. The purpose of this review is to highlight new developments and to point out common molecular and biochemical principles that broaden our understanding of the complex biological processes that link bile acid synthesis to lipid metabolism.

## PATHWAYS OF BILE ACID SYNTHESIS

Cholic acid (CA) and chenodeoxycholic acid (CDCA), the two major human bile acids, are synthesized from cholesterol in a series of reactions catalyzed by enzymes located in the endoplasmic reticulum, mitochondria, cytosol, and peroxisomes. In the past, it has become clear that synthesis of CA and CDCA occurs via several pathways (Fig. 1). Each pathway differs in the initial steps and involves distinct hydroxylases that modify the steroid nucleus and the steroid side chain, thereby converting water-insoluble cholesterol molecules into water-soluble bile acids. Initial branching in early steps of bile acid synthesis involves 7α-hydroxylation of either cholesterol or one of three oxysterols containing a hydroxyl group at the C24, C25, or C27 position of the side chain (11).

Cholesterol 7α-Hydroxylase Pathway

This pathway is commonly referred to as the "neutral" bile acid synthesis pathway, which in humans results in the synthesis of approximately equal amounts of CA and CDCA. The first, rate-limiting and extensively regulated enzyme is cholesterol 7α-hydroxylase (CYP7A1), a microsomal cytochrome *P*-450 enzyme exclusively expressed in hepatocytes. In a second microsomal step catalyzed by 3β-hydroxy-Δ^5-C_{27} steroid oxidoreductase (C_{27} 3β-HSD), the 3β-hydroxyl group is oxidized, allowing a shift in double-bond position in the steroid nucleus to form 7α-hydroxy-4-cholesten-3-one, which represents the common precursor of both CA and CDCA. There exists only a single C_{27} 3β-HSD (27), which achieves convergence of initially branched steps of bile acid synthesis. Microsomal sterol 12α-hydroxylase (CYP8B1) converts 7α-hydroxy-4-cholesten-3-one to $7\alpha,12\alpha$-dihydroxy-4-cholesten-3-one, a precursor of CA. Cytosolic 3-oxosteroid-5β-reductase and 3α-hydroxysteroid dehydrogenase subsequently reduce $7\alpha,12\alpha$-dihydroxy-4-cholesten-3-one into 5β-cholestane $3\alpha,7\alpha,12\alpha$-triol. Alternatively, the latter two enzymes

Fig. 1. Bile acid synthesis pathways. In the classic pathway, cholesterol is initially converted in the liver to 7α-hydroxycholesterol by the rate-limiting enzyme cholesterol 7α-hydroxylase (CYP7A1). In the 3 alternative pathways, cholesterol is first converted to oxysterols. In liver and extrahepatic tissues (e.g. macrophages), this involves sterol 27-hydroxylase (Cyp27A1) or cholesterol 25-hydroxylase (CH25H), whereas in the brain, this step is exclusively catalyzed by cholesterol 24-hydroxylase (CYP46). The oxysterols are then 7α-hydroxylated by oxysterol 7α-hydroxylases CYP7B1 and CYP39A1, respectivly. Whereas the classic bile acid synthesis pathway forms preferentially cholic acid (CA), the alternative pathways produce mainly chenodeoxycholic acid (CDCA).
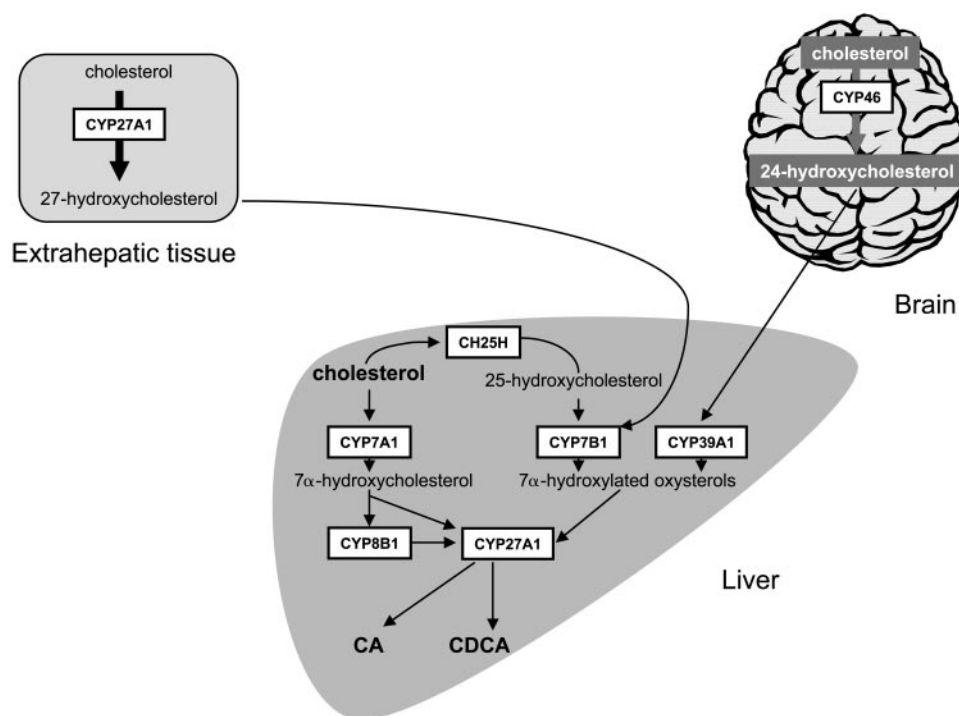
reduce 7α-hydroxy-4-cholesten-3-one producing 5β-cholestane-3α,7α-diol, a precursor of CDCA. After modification of the steroid nucleus, mitochondrial sterol 27-hydroxylase (CYP27A1) forms 5β-cholestane 3α,7α,27-triol and 5β-cholestane-3α,7α,12α,27-tetrol, respectively. These products are subsequently oxidized by alcohol and aldehyde dehydrogenases and CoA-esterified by bile acid CoA-ligase. After introduction of a C24–C25 double bond in peroxisomes, hydration and hydroxylation at C24 occurs. The final step of side-chain oxidation involves the β-ketothiolase activity of sterol carrier protein X (SCPX) catalyzing the cleavage of 24-keto-THCA-CoA into CA and propionate. Finally, cholic acid is conjugated with glycine or taurine. The importance of SCPX for bile acid synthesis is highlighted by studies with *Scp2* knockout mice (15). These animals accumulate 3α,7α,12α,-trihydroxy-27-nor-5β-cholestane-24-one, a bile alcohol derivative that is formed by decarboxylation of the CA intermediate 24-keto-THCA-CoA. In addition, *Scp2* knockout mice had elevated levels of 23-norcholic acid and 23-norchenodeoxycholic acid, which are less efficiently secreted into bile. Induction of α-oxidation, with formation of 23-nor-bile acids, may represent a compensatory mechanism associated with inefficient β-oxidation of the steroid side chain.

The Sterol 27-Hydroxylase Pathway

This biosynthetic pathway is commonly referred to as the "acidic" bile acid synthesis pathway. CDCA is the main product synthesized by this pathway in which oxidative cleavage of the side chain precedes modifications of the steroid nucleus. The initial step is catalyzed by CYP27A1, which resides on inner mitochondrial membranes and also catalyzes 27-hydroxylation

of bile acid intermediates of the CYP7A1 pathway, 5β-cholestane 3α,7α-diol, and 5β-cholestane-3α,7α,12α-triol, respectively. The possibility of a reverse sequence, initial mitochondrial 27-hydroxylation of cholesterol followed by 7α-hydroxylation, has been excluded. The intracellular routing that follows generation of 27-hydroxycholesterol is not entirely clear, but it is assumed that 27-hydroxycholesterol undergoes successive oxidation to the C27 acid before being exported from the mitochondria. The monohydroxy C27 bile acid is then metabolized to 3β-hydroxy-5-cholestenoic acid, which also can be hydroxylated by microsomal oxysterol 7α-hydroxylase (CYP7B1). Then, C_{27} 3β-HSD initiates sterol nuclear modifications similar to those in the neutral pathway.

In the adrenal gland, delivery of cholesterol to the inner mitochondrial membrane by steroid acute response (StAR) protein is essential for steroidogenesis. StAR protein also has been shown to stimulate CYP27A1 activity (33). It is therefore attractive to speculate that increasing cholesterol delivery to and into mitochondria may increase bile acid synthesis via CYP27A1. Sterol carrier protein 2 (SCP2) has been implicated in cholesterol transport to mitochondria (5), where it may stimulate 27-hydroxylation of cholesterol. Localization of SCP2 in liver cytosol and mitochondria is fully consistent with this concept. In addition, hepatic overexpression as well as homozygous disruption of *Scp2* demonstrated that both CYP27A1 and CYP7B1 are under control of SCP2-mediated intracellular trafficking events (20, 37). Recent elegant in vitro studies demonstrated that elevated StAR protein levels are associated with increased CDCA synthesis that is more pronounced than achieved by elevated CYP27A1 expression (22). In addition, by overexpress-

ing StAR in the bile fistula rat, a model believed to achieve maximal bile acid synthesis rates, a significant increase in bile acid synthesis occured. These findings are in line with the concept that cholesterol transport to the inner mitochondrial membrane is the predominant rate-limiting step in this pathway. Although the liver lacks StAR expression, transport of cholesterol to the inner mitochondrial membrane is absolutely required for 27-hydroxylation, which may be accomplished by StAR-like proteins such as StAR4 and StAR5 (30).

CYP27A1 is not only expressed in hepatocytes, but also in peripheral tissues such as macrophages and vascular endothelium. Most of the 27-hydroxycholesterol generated is packed into lipoproteins and transported to the liver. After receptor-mediated uptake and generation of free sterols, 7α-hydroxylation occurs in the endoplasmic reticulum. Subsequent transformation to 5β-cholestane-3α,7α,27-triol and 5β-cholestane-3α,7α,12α,27-tetrol occurs before completion of side-chain oxidation in the mitochondria and peroxisomes to yield CDCA and CA, respectively.

The Cholesterol 25-Hydroxylase Pathway

Cholesterol 25-hydroxylase is expressed at low levels in the endoplasmic reticulum and Golgi apparatus in most tissues. Regulation of this enzyme not belonging to the cytochrome *P*-450 family is unknown. Cholesterol 25-hydroxlase is a substrate for oxysterol 7α-hydroxylase (CYP7B1), which produces 7α-hydroxylated oxysterols that are funnelled into downstream steps of the biosynthetic bile acid pathway. More recently, in vitro studies (21) demonstrated that CYP7A1 was active toward 25- and 27-hydroxycholesterol. Whether this overlapping substrate specificity is of physiological relevance in vivo is unknown at present.

The Cholesterol 24-Hydroxylase Pathway

Brain cholesterol resides mainly in plasma membranes of myelin sheaths and contains ~25% of total body cholesterol mass. Because the blood-brain barrier prevents cholesterol exchange with circulating lipoproteins, cholesterol removal from brain appears to require side-chain hydroxylation and formation of oxysterols, which facilitate the ability to pass plasma membranes and the blood-brain barrier. Cholesterol 24-hydroxlase (CYP46), a cytochrome *P*-450 enzyme, almost exclusively expressed in the endoplasmic reticulum of brain, catalyzes the formation of 24-hydroxycholesterol (17). So far, mechanisms regulating the expression and activity of CYP46 are not well known. The fact that the daily flux of 24-hydroxycholesterol from the brain into the jugular vein is similar to the uptake of 24-hydroxycholesterol by the liver is consistent with the brain as the major producer and the liver as the major excretory organ of 24-hydroxycholesterol. Recent studies (4) suggest that approximately one-half of 24-hydroxycholesterol is excreted into bile following conjugation with sulphuric and glucuronic acid. Formation of bile acids from the other half of 24-hydroxycholesterol requires 7α-hydroxylation. Although CYP7A1

may have some activity toward 24-hydroxycholesterol, this step is primarily catalyzed by CYP39A1, another cytochrome *P*-450 enzyme expressed exclusively in liver (16).

Relative Contributions of the Different Synthetic Pathways

The relative contribution of CYP7A1 and CYP27A1 to total bile acid synthesis was studied in adult patients that had their gallbladders removed. The efficient conversion of [³H]7α-hydroxycholesterol but not [³H]27-hydroxycholesterol to CA and CDCA in these otherwise healthy individuals demonstrated that <20% of total bile acids (~350 mg/day) were synthesized from [³H]27-hydroxycholesterol (34). Because the hepatic uptake of ~20 mg/day of 27-oxygenated products is almost identical with a production rate for 27-hydroxycholesterol of ~18 mg/day, most, if not all, 27-hydroxycholesterol may be produced outside the liver (8). This is in line with a quantitatively less important contribution of the CYP27A1 pathway in adults. It has been calculated that, in humans, no more than 5–16% of total bile acids are synthesized from 25-hydroxycholesterol (9). Compared with 25-hydroxycholesterol, production of bile acids from 24-hydroxycholesterol in healthy humans may not exceed 7 mg/day and thus indicates an even minor contribution to daily bile acid synthesis (3).

Relatively little data are available in humans to generate a complete picture of bile acid synthesis at neonatal age where CYP7A1 is absent (28). In contrast to maternal bile, fetal gallbladder bile is composed of a high proportion of CDCA that cannot be attributed to placental exchange alone. This supports the concept of a metabolic pathway that begins with CYP27A1 and generates monohydroxy bile acids. Proper expression of CYP7B1 at this age is critical, as evident from severe cholestatic liver disease in the absence of CYP7B1 activity (28). It is unknown, however, at what time during development that the CYP7A1 pathway contributes to and later on dominates bile acid synthesis.

In patients with chronic liver disease, however, the situation appears to be quite different. Numerous acidic but few neutral pathway intermediates are present in plasma of cirrhotic patients, which almost exclusively synthesize CDCA, albeit at low levels (11). Bile acid and cholesterol metabolism may be quite different between humans and rodents. This becomes evident with respect to the acidic pathway, which may contribute >50% to total bile acid synthesis in the rat and mouse. Thus, when it comes to bile acid and cholesterol metabolism, findings in rodents may not necessarily reflect the situation in humans.

TRANSCRIPTION REGULATORS AT THE CROSSROADS BETWEEN BILE ACID SYNTHESIS AND LIPID METABOLISM: A MARRIED LIFE

Liver X Receptor

Bile acid levels in the enterohepatic circulation are under tight negative feedback control, mainly achieved

through transcriptional control of bile acid synthetic enzymes involving farnesoid X receptor (FXR) (6) (Fig. 2). Liver X receptor (LXR) is a master regulator of a feed-forward mechanism that helps to maintain cholesterol homeostasis. On activation by oxysterols, LXR forms heterodimers with the retinoid X receptor, which induce the expression of several genes that facilitate intestinal sterol excretion and reverse cholesterol transport. In the liver, 7α-hydroxylation of cholesterol is regulated in an LXR-dependent manner, thereby increasing bile acid synthesis. However, humans do not respond with upregulation of CYP7A1 in response to excess dietary cholesterol, probably attributable to the absence of an LXR-responsive element in the *CYP7A1* promoter (1). CYP7A1 unresponsiveness to dietary cholesterol but not CA in mice lacking the heparin sulfate-regulated transmembrane tyrosine kinase receptor FGFR4 was surprising but may be explained by activation of receptor-interacting protein 140, a repressor of LXR-mediated transcription (36).

Sterol Regulatory Element Binding Proteins

Sterol regulatory element binding proteins (SREBPs) are membrane-bound transcription factors that play crucial roles in controlling cholesterol homeostasis, fatty acid synthesis, and carbohydrate metabolism. Two sequential proteolytic cleavage steps allow binding of mature SREBPs to sterol regulatory elements within the promoter region of target genes. SREBP1 appears to regulate genes primarily involved in fatty acid synthesis, and SREBP2 is more relevant for cholesterogenic genes. With regard to bile acid synthesis, it is of interest that dietary cholesterol on one hand may activate 7α-hydroxylation but on the other hand may inhibit 12α-hydroxylation. An explanation may be
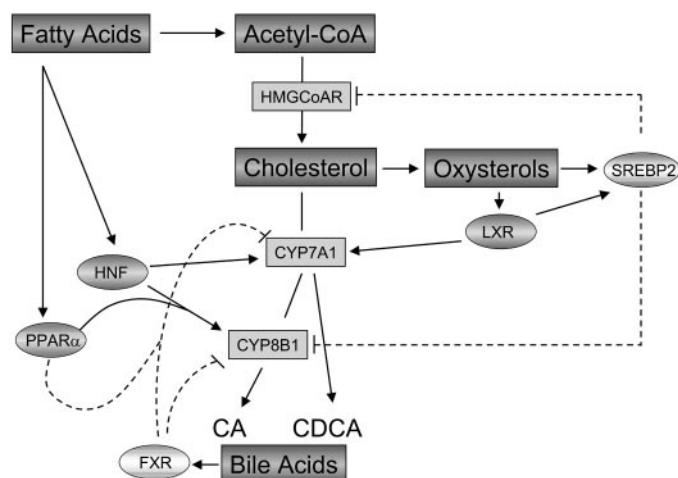

Fig. 2. Simplified model of the complex interrelationship between bile acid synthesis and lipid metabolism. Synthesis of cholesterol is governed by SREBP2 by a classic feedback regulatory loop (dash line). Conversion of cholesterol to bile acids is regulated by transcription activators, e.g. liver X receptors (LXR), hepatocyte nuclear factor (HNF; solid line), or repressors such as farnesoid X receptor (FXR; dashed line). Peroxisomal proliferation activating receptors (PPARα) appears to have a dual effect as an activator and repressor. SREBP2, sterol regulatory element-binding protein.

provided by the fact that SREBPs have opposite effects on the 12α-hydroxylase promoter SREBP1 activating and SREBP2 suppressing CYP8B1 activity (7). The physiological role of this finding remains speculative. It is possible that under conditions with low intracellular cholesterol contents, a higher SREBP2-SREBP1 ratio would increase the hydrophobicity of the bile acid pool enriched with CA. This, in turn, would decrease 7α-hydroxylation of cholesterol, thereby decreasing conversion of cholesterol to bile acids and saving cholesterol for other needs. In contrast, cholesterol accumulation is not only prevented by increasing the formation of bile acids, but also by inhibition of the proteolytic cleavage of SREBP2, thereby reducing synthesis or uptake of cholesterol.

Peroxisomal Proliferator Activator Receptors

Peroxisomal proliferator activator receptors (PPARs) are nuclear receptors activated by polyunsaturated fatty acids and various synthetic ligands such as fibrates. They play a dual role in the regulation of fatty acid metabolism as well as in regulating cholesterol metabolism by modulating the expression of enzymes involved in the reverse cholesterol transport pathway and bile acid synthesis. Predominantly expressed in macrophages, PPARγ induces LXR, which, in turn, promotes cellular efflux of phospholipids and cholesterol into high-density lipoproteins with subsequent cholesterol delivery to hepatocytes. In the liver, PPARα appears to stimulate CYP8B1 and to decrease CYP7A1 expression (14). This is in line with the observation that fibrates changed the bile acid pool composition by increasing CA and decreasing CDCA (32). So far, the physiological role of bile acids on PPAR regulation is unknown.

Hepatocyte Nuclear Factors

This family of nuclear receptors is highly expressed in liver and important for liver function. Hepatocyte nuclear factors (HNF)1α and 4α appear to be important for basal transcription of *CYP7A1*, *CYP27A1*, and *CYP8B1* (13,29). HNF4α also appears to be crucial for bile acid-mediated repression of *CYP8B1* (38). Mutations in the *HNF4α* gene have been reported in patients with diabetes (35) and may be linked to the increased CA synthesis in diabetes. The enrichment of the bile acid pool with CA may further augment intestinal cholesterol absorption, contributing to gallstone formation. The recent identification of an HNF3β-responsive element in the hamster *Cyp7a1* promoter suggests that yet another nuclear factor may be involved in transactivation of *Cyp7a1*. The physiological importance of HNF3β for bile acid synthesis now can be studied in a transgenic mouse model (24).

PRIMARY DEFECTS IN BILE ACID SYNTHESIS

Liver disease due to defects of bile acid synthesis is related to deficiencies of CYP7B1, 3β-hydroxy-Δ^5-C_{27} steroid oxidoreductase, and 3-oxo-Δ^4-steroid 5β-reductase enzymes (11). Until recently, detection of accumu-

lated bile acid precursors in serum and urine has facilitated diagnosis of these rare disorders. Clinical manifestations include neonatal hepatitis, progressive cholestasis, fat malabsorption, and neurological symptoms. Because these diseases may be treated with bile acids, early diagnosis is important.

Contrary to these defects, CYP7A1 deficiency in adults apparently is not associated with severe liver disease but rather with hyperlipidemia, premature atherosclerosis, and gallstone formation (23). As expected, upregulation of the CYP27A1 pathway occurred but could not prevent accumulation of hepatic cholesterol and probably also of oxysterols. Hypertriglyceridemia under these conditions may be explained by LXR-mediated induction of SREBP1c with subsequent elevated synthesis of triglycerides (25). Because low bile acid levels may downregulate apoC-II in a FXR-dependent fashion, this may cause insufficient removal of triglycerides by hepatic lipase.

In patients with cerebrotendinous xanthomatosis, *CYP27A1* mutations decrease total bile acid synthesis, particularly of CDCA. Despite normal serum cholesterol levels these patients develop tendon xanthomatosis, accumulation of cholesterol in tissue, progressive neurological dysfunction, and premature atherosclerosis, most likely reflecting reduced elimination of cholesterol from macrophages. This is in line with a flux of 27-hydroxycholesterol to the liver for further transformation into bile acids. Early diagnosis of cerebrotendinous xanthomatosis is important, because progression of this disease may be prevented by treatment with bile acids and inhibitors of cholesterol synthesis.

The availability of mice with disruption of genes encoding bile acid synthetic enzymes such as *Cyp7a1*, *Cyp7b1*, and *Cyp27a1* has provided novel insights into bile acid synthesis pathways and cholesterol homeostasis. Due to species differences in genes that govern bile acid synthesis, however, knockout mouse models may be of limited use when extrapolating results to the situation in humans.

WHERE NO ONE HAS GONE BEFORE

Having summarized in brief some of the achievements of bile acid research in the past, I will also attempt to highlight two important challenges for the future: one is bile acid biochemistry and the other is bile acids as modulators of cholesterol metabolism.

From Biochemical Pathways to Detection of Genetic Defects

Healthy humans synthesize approximately twice as much CA as CDCA. This is intriguing, because 7α-hydroxycholesterol is converted equally to CA and CDCA and CDCA is the preferred product of 27-hydroxycholesterol. Neither initial 12α- nor initial 7β-hydroxylation of cholesterol provides an explanation. Because up to 40% of CA synthesis may bypass initial 7α-hydroxylation (10), another as yet unidentified pathway appears to contribute to the preferred production of CA. Uncovering such an additional pathway

may open new avenues for the development of lipid-lowering treatment strategies. Appreciation that genetic defects of bile acid synthesis may present as cholestatic liver disease that can be treated by administration of bile acids is well established. Isolation of all genes involved in bile acid synthesis and their structural characterization should pave the way to provide simple and rapid screening methods. A molecular genetic analysis may be coupled with urinary bile acid analysis by fast-atom bombardment ionization mass spectrometry assays and chemical colorimetric tests to obtain an unambiguous diagnosis of the genetic defect in bile acid synthesis of every infant with unexplained cholestasis or fat malabsorption. Someday, gene therapy may become feasible and less costly, thereby providing an alternative to oral bile acid replacement therapy.

Targeting Bile Acid Synthesis to Prevent Deposition of Excess Cholesterol

A second area of future research that will generate much interest is the liver as a therapeutic target for atherosclerotic vascular disease, the leading cause of death in the industrialized world. Formation of 27-hydroxycholesterol returning to the liver for bile acid synthesis removes cholesterol deposited in the vascular endothelium and may be regarded as an antiatherogenic mechanism able to reduce the accumulation of cholesterol. This is supported by the fact that premature atherosclerosis and accumulation of excess cholesterol in xanthoma is observed in individuals with cerebrotendinous xanthomatosis. The relative importance of this mechanism in relation to the high-density lipoprotein-mediated reverse cholesterol transport, however, is unknown. It is reasonable to hypothesize that persistence of the CYP27A1 pathway into adulthood would protect against later development of atherosclerosis. Increasing bile acid synthesis by targeting biosynthetic enzymes directly may represent an antiatherogenic mechanism. CYP27A1 overexpression in a transgenic mouse model demonstrated no significant increase in bile acid synthesis (18), suggesting that this may not be a suitable approach to decrease serum cholesterol levels. However, one has to consider that substrate delivery to mitochondria is rate limiting and, therefore, increasing cholesterol delivery to mitochondria may be required for sufficient upregulation of bile acid synthesis. Employing mice with overexpression of StAR or StAR-like protein should give the answer. Nevertheless, increasing net cholesterol movement from extrahepatic tissues to the liver may not necessarily augment cholesterol flux through the entire reverse cholesterol transport pathway (2), indicating that multiple steps have to be targeted at the same time to achieve a sufficient metabolic response. Alternatively, targeting 7α-hydroxylation of cholesterol may provide a possibility to increase conversion of cholesterol to bile acids, thereby lowering serum cholesterol levels. Indeed, augmentation of CYP7A1 expression proved to be an effective strategy for lowering choles-

terol even in animals with a genetic absence of low-density lipoprotein receptors (31), and constitutive transgenic expression of CYP7A1 in mice conferred resistance to atherosclerosis (19).

Strategies targeted at LXR may hold great promise as future treatments for atherosclerosis. Although LXR activation is associated with upregulation of SREBP1 target genes (25), causing fatty livers and elevated triglyceride levels, the latter may be short lived. The answer may be LXR modulators that induce the cholesterol-metabolizing pathway but not the fatty acid pathway. Although this will require a diligent approach, there is a precedent in selective estrogen-receptor modulators. Unfortunately, the *CYP7A1* gene is not stimulated by dietary cholesterol, which is attributable to the inability of the *CYP7A1* gene promoter to interact with LXR (1). It thus remains a significant challenge to achieve induction of CYP7A1 expression in a therapeutic intervention. FXR antagonists should prevent feedback regulation of bile acid synthesis and stimulate CYP7A1 expression. Because an increase in bile acid synthesis may, in turn, stimulate FXR, thereby inhibiting bile acid synthesis, a combination therapy of FXR antagonists and LXR agonists or compounds that activate both nuclear receptors may be used alternatively. Reducing intestinal bile acid absorption by administration of bile acid sequestrants is yet another possibility to induce bile acid synthesis. More potent forms of these drugs have recently been developed, but inhibitors of the ileal bile acid transporter may be more effective in increasing bile acid synthesis (26). With recent concern over rhabdomyolysis with statins, particularly when combined with fibrates, there may be renewed interest in inhibitors of bile acid absorption as an alternative or complementary approach.

Over the past decades, the field of bile acid research has witnessed a number of both basic and clinical discoveries. Initially, it was believed that measurements of serum bile acids are of diagnostic value. Then physicians' attention was caught by the discovery that bile acid administration promotes dissolution of gallstones. In the past decade the use of ursodeoxycholic acid in cholestatic liver disease reawakened interest in the field of bile acid research. Detection of genetic defects in bile acid synthesis has been followed by successful replacement treatment as well as cloning some of the key enzymes in bile acid synthesis. Although the lipid research community has been delighted, so far only a small number of patients have benefited from these advances. Pharmacologically manipulating the conversion of cholesterol to bile acids could lead to new therapeutic modalities for atherosclerosis and likely will generate great interest in the years to come. Physicians will have no choice but to be aware of the potential advantages and pitfalls of such interventions, which certainly will have impact on their future patient care. Although I have made some cautious predictions about the future, one should always be aware of the remarkable number of erroneous state-ments that have been made before even by famous scholars (5).

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