Bile Acids: The Good, the Bad, and the Ugly

Alan F. Hofmann

Bile acids, amphipathic end products of cholesterol metabolism, are “good” in the infant because they enhance lipid absorption and thereby promote growth. Bile acids also induce bile flow and biliary lipid secretion. The enterohepatic circulation of bile acids is “bad” in the adult because it downregulates hepatocyte low-density lipoprotein receptor activity and thereby elevates plasma cholesterol levels. Defects in bile acid metabolism such as impaired biosynthesis or transport are “ugly” because they cause morbidity and death. New approaches for treating these defects are being developed.

In vertebrates, cholesterol balance is achieved by modulating both synthesis and excretion. Cholesterol excretion is mediated by bile acids, the water-soluble amphipathic molecules formed from cholesterol in the hepatocyte. In addition to their role in cholesterol homeostasis, bile acids also are functional detergents that induce bile flow and transport lipids as mixed micelles in the biliary tract and small intestine. Bile acid functions in health and dysfunctions in disease are the focus of this article.

The good

Bile as a secretory and excretory fluid. Bile, a fluid secreted by the liver into the intestine in vertebrates, is both a digestive and an excretory fluid. As a digestive fluid, bile contains bile acids, potent digestive surfactants that promote lipid absorption. As an excretory fluid, bile contains substances that cannot be eliminated efficiently in urine because they are insoluble or protein bound. These include not only bile acids (which are not only digestive surfactants but also end products of cholesterol metabolism), bilirubin (the end product of heme metabolism), cholesterol (derived from synthesis exceeding body needs), and heavy metals such as iron and copper (derived from absorption exceeding body needs). Biliary secretion also provides an excretory route for lipophilic steroids and drug metabolites. Bile also has a high concentration of phospholipids, which consist mostly of phosphatidylcholine (PC) and which form mixed micelles with bile acids. These mixed micelles contain amphipathic microdomains that can solubilize cholesterol. Mixed micelle formation also lowers the monomeric activity of bile acids and prevents their destroying the apical membrane of the biliary epithelial cells. IgA, an immunoglobulin, and mucus are secreted into bile, where their role is to prevent bacterial growth and adhesion. Finally, bile contains tocopherol, which may prevent oxidative damage to the biliary and small intestinal epithelium.

Bile acid metabolism. Bile acids are formed in the pericentral hepatocytes from cholesterol by a multienzyme process. In the biosynthesis of C24 bile acids (which are present in most vertebrates), the side chain of cholesterol undergoes oxidative cleavage resulting in the conversion of an isocyclic moiety into an isopentanoic acid moiety. One or two hydroxy groups are added to the nucleus. Although the pattern of hydroxylation varies between species, the hydroxylation is always on one face of the molecule, and the final product invariably has a hydrophobic face and a hydrophilic face, resulting in an amphipathic molecule. [Not all vertebrates form C24 bile acids. In ancient mammals such as the elephant and manatee, as well as in cartilaginous fish, C27 bile alcohols are formed. In reptiles, C27 bile acids are formed (9). The topology of a typical C24 bile acid is shown in Fig. 1.]

The composition of the bile acid molecules that circulate is often complex and is beyond the scope of this article. The complexity results from the circulating bile acids having two inputs. The first input consists of the bile acids formed from cholesterol in the hepatocyte; these are termed primary bile acids and usually consist of at least two bile acids. The second input consists of bile acids formed by bacteria in the colon by removal of the hydroxy group at C-7. Such bile acids are termed secondary bile acids. These are absorbed
from the colon and circulate together with the primary bile acids. Bile is thus a mixture of primary bile acids formed in the hepatocyte and secondary bile acids formed in the colon. All bile acids, whether primary or secondary, that are secreted into bile are conjugated with either glycine or taurine. Such conjugation increases the aqueous solubility at acidic pH, increases resistance to precipitation by Ca\(^{2+}\), and renders bile acids impermeable to cell membranes and paracellular junctions. The amphipathic nature of the molecule is responsible for its forming mixed micelles with amphipathic but water-insoluble lipids such as phosphatidylcholine. From Ref. 15 with kind permission of Kluwer Academic Publishers.

Enhancement of dietary lipid absorption by bile acids. Dietary triglyceride is hydrolyzed by pancreatic lipase to fatty acid and 2-monoglyceride molecules that are insoluble at physiological pH. The ability of bile acids to solubilize these lipolytic products efficiently has been known for nearly a century, and the discovery that such solubilization could be explained by mixed micelle formation was a superb application of concepts of colloid science to a physiological process. Recent work using neutron scattering indicates that the mixed micelles of bile (containing bile acids, PC, and cholesterol) and of small intestinal content (containing bile acids, fatty acids, and monoglyceride) have an identical molecular arrangement. In both types of micelles, the shape of the polymeric aggregate is cylindrical. The polar lipids are arranged radially with their hydrophilic heads facing outward toward the aqueous phase. The bile acid molecules are arranged perpendicularly between their polar heads. The hydrophobic face of the bile acid molecule rests like a wedge between the heads of the alkyl chains of the PC (or fatty acid) molecules; the hydrophilic face of the bile acid molecule faces the aqueous environment (4). The transformation of lipid bilayers or vesicles to mixed micelles by bile acids is illustrated in Fig. 2.

In the small intestine, micellar solubilization increases the aqueous concentration of fatty acids and monoglycerides by a factor of ~1,000. The mixed micelle diffuses more slowly than monomers, but the greatly increased aqueous concentration caused by micelle formation accelerates diffusion by a factor of at least 100. This is important because fatty acid uptake by the enterocyte is so rapid that diffusion becomes rate limiting in the overall absorptive process (8). Efficient fat digestion and absorption is especially important in the nursing infant, in whom fat is a major source of calories.

Bile acids will not solubilize dietary lipids in the form of mixed micelles unless bile acids are above a critical concentration, termed the critical micellization concentration. The relatively high concentration of bile acids in the small intestinal lumen during digestion is the result of several factors. First, conjugated bile acids are strong acids that are fully ionized at intestinal pH and are therefore impermeable to cell membranes, and the bile acid molecule is too large to pass across the paracellular junctions. Second, efficient conservation of bile acids by active (carrier mediated) absorption from the small intestine results in a pool of bile acids that cycles several times with each meal. (This movement of molecules from the biliary tract to the small intestine and back to the liver followed by resecretion into bile is termed the enterohepatic circulation.) The enterohepatic circulation of bile acids provides a large flux of surfactant molecules that greatly exceeds the rate at which bile acids are synthesized from cholesterol. For example, bile acid secretion with a meal averages 5 mmol/h. Synthesis of bile acids from cholesterol is ~0.02 mmol/h. The final factor contributing to the high luminal concentration of bile acids is the extremely high concentration of gallbladder bile (up to 300 mM), at least in those species that have gallbladders. The high concentration of gallbladder bile is the result of water removal by the gallbladder epithelium when bile is stored in the gallbladder.

The large recycling pool of bile acids thus serves a lipid transport function in both the biliary tract and the small intestine. In bile, the transport function of the “excretory” micelle (bile acids, PC, and cholesterol) helps in the excretion of cholesterol and other lipophilic molecules. In small intestinal content, the “absorptive” micelle helps in the absorption of triglyceride and fat-sol-
uble vitamins. Both types of micelles can bind polyvalent cations and promote their transport.

**Bile acid functions in the liver and biliary tract.** The large recycling pool of bile acids also has functions within the hepatocyte. Bile acids returning from the intestine are efficiently removed from portal venous blood by the hepatocyte. The first-pass extraction depends on the structure of the bile acid but for all bile acids exceeds 60%. Uptake is mediated by basolateral carrier proteins that are now being characterized at a molecular level (10). After uptake, bile acids do not remain in the hepatocyte but are rapidly pumped into the biliary canaliculus by an ATP-energized canalicular transporter that has recently been cloned (2). Transport across the canalicular membrane is extraordinarily concentrative, because the monomeric concentration of bile acids in the hepatocyte is thought to be 1–2 μM whereas that of canalicular bile is at least 1,000 μM. Bile acids are pumped into the canalicular space, which is semipermeable. As a result of the osmotic effects of the transiently elevated concentrations of bile acids, water and filtrate ions flow into the canalicular space, mostly via the paracellular junctions. In this manner, canalicular bile is generated. (The osmotic theory of bile formation was proposed about sixty years ago by Ivor Sperber and has been confirmed by multiple lines of experimental evidence; the mechanism of bile formation is identical to the pumping mechanism of the Alza pump.)

The canaliculi, which have a blind end at the pericentral region of the hepatic lobule, are surrounded by a spiral of actin microfilaments. These contract, driving canalicular bile toward the biliary ductules and initiating bile flow. After inducing bile flow into the canalicular lumen, bile acids also induce biliary lipid secreting by adsorbing to PC molecules that have been transported across the canalicular membrane by a PC-"flippase" (11). The PC molecules form vesicular bubbles that bud from the luminal face of the canalicular membrane, the energy for vesicle formation perhaps being provided by the canalicular contractions. Bile acids adsorb to these vesicles, detaching them from the luminal face of the canalicular membrane (11). As bile flows down the biliary tract, the vesicles are converted to mixed micelles by the continued adsorption of bile acid molecules. How cholesterol is transported into canalicular bile remains poorly understood. The conversion of vesicles to mixed micelles by bile acids is shown in Fig. 3.

About one-half of the bile secreted by the liver enters the gallbladder, where it is concentrated by

---

**FIGURE 2.** Schematic depiction of the enterohepatic circulation of bile acids. Flux of bile acids is depicted by black lines and organs by stippled areas.
a factor of three to six. During gallbladder storage and concentration, water and electrolytes are removed and bile is acidified by Na+/H+ exchange. With a meal, the gallbladder contracts, the sphincter of Oddi relaxes under neurohormonal stimuli, and bile enters the small intestine.

PC is hydrolyzed and absorbed; cholesterol precipitates from solution, enhancing its elimination. The bile acids now form mixed micelles with fatty acids and monoglycerides. The excretory micelle has become an absorptive micelle.

Bile acid transport. The enterohepatic circulation thus involves transcellular transport of bile acid molecules, mediated by membrane transporters, and interorgan flow, mediated by intestinal motility and blood flow. Vectorial membrane transport occurs in the hepatocyte and the ileal enterocyte, and interorgan flow occurs in the portal venous and systemic circulation, the biliary tract, and the small intestine.

The chemical pumps present in the hepatocyte have already been described. Evidence for the presence of an active transport system in the ileal enterocyte was provided by Lack and Weiner, who showed, using everted intestinal sacs, that conjugated bile acids move uphill in a Na+-dependent manner. Recently, work by Dawson and colleagues (1) has led to the cloning of the Na+-bile acid cotransporter present in the apical membrane of the ileal enterocyte. The ileal bile acid transporter shares homology with the hepatocyte Na+-dependent bile acid transporter located in the sinusoidal membrane but is a different protein. Exit of bile acids from the ileal enterocyte across the basolateral membrane and into portal venous blood involves a second carrier protein with anion-exchange products that remains poorly characterized.

The enterohepatic circulation of bile acids is under homeostatic control at the level of both the hepatocyte, with respect to bile acid biosynthesis, and the ileal enterocyte, with respect to bile acid transport. At the hepatocyte level, a decreased return of bile acids to the hepatocyte is followed by increased bile acid biosynthesis; the signal appears to be the intracellular concentration of bile acids. Normally, bile acid synthesis is downregulated. With interruption of the enterohepatic circulation, bile acid biosynthesis increases up to 15-fold. Because bile acids are derived from cholesterol, increased bile acid biosynthesis must be accompanied by an equivalent amount of cholesterol biosynthesis.

Homeostasis at the ileal enterocyte level is not well understood. There is convincing evidence for negative feedback (decreased transport with increased load) in the hamster and guinea pig; negative feedback of ileal transport is also present in humans, because bile acid secretion does not increase when bile acids are fed.

The bad

Inappropriate retention of bile acids and cholesterol metabolism. The physiological utility of the enterohepatic circulation of bile acids has fas-
cinated physiologists for centuries. Borelli, the legendary animal physiologist, predicted the existence of the enterohepatic circulation in the 17th century. Experimental proof of the enterohepatic circulation awaited studies in the biliary fistula dog in 1870 by Moritz Schiff in Geneva. (Schiff had been forced to abandon his laboratory in Florence, Italy and flee to Switzerland after being put on trial by antivivisectionists; at his trial, he made an eloquent defense of the need and moral justification for animal experimentation.)

The view that conjugated bile acids were functional detergent molecules that solubilized lipids, thereby promoting their absorption, was well accepted by physiologists a half-century ago even if mixed micelle formation had not yet been described. The enterohepatic circulation was considered an extraordinary physiological adaptation that made a large flux of detergent molecules available for digestion with minimal biosynthesis requirements. However, this view that the efficient enterohepatic circulation was “good” for the organism has been modified in the past decade, largely as a result of advances in understanding cholesterol and lipoprotein metabolism. It now seems increasingly likely that efficient intestinal conservation of bile acids also has a “bad” aspect. Brown and Goldstein, in their seminal work on the low-density lipoprotein (LDL) receptor, pointed out that impaired function of this receptor caused hypercholesterolemia. Upregulation of this receptor could be achieved by increasing the demands of the hepatocyte for cholesterol, because the hepatocyte appears to defend cholesterol homeostasis at all costs. They noted that bile acid sequestrants induced bile acid and cholesterol biosynthesis, which in turn upregulated LDL-receptor activity. Thus, in the adult, efficient intestinal conservation of bile acids may be considered bad in that it downregulates cholesterol biosynthesis and LDL-receptor activity, which in turn leads to higher plasma LDL cholesterol levels.

Even before the elegant depiction of Brown and Goldstein, pharmaceutical companies had identified the active transport of bile acids by the ileum as a physiological process that should be targeted for treating hypercholesterolemia. The first agent, the bile acid sequestrant cholestyramine, caused a threefold increase in cholesterol and bile acid biosynthesis and lowered plasma LDL cholesterol levels. Although cholestyramine has been shown to be safe and effective for the prevention of coronary events in patients with familial hypercholesterolemia, it is not widely used because of undesirable side effects such as constipation. More potent sequestrants with fewer side effects are being developed. A second approach is the synthesis of inhibitors of the Na+-bile acid cotransporter present on the apical membrane of the ileal enterocyte. Such agents have been shown to inhibit bile acid absorption, to lower plasma cholesterol, and to decrease atherosclerosis in the cholesterol-fed Watanabe rabbit that is known to have defective LDL receptors (13).

The ugly

Bile acids as cytotoxic agents. The amphipathic properties of bile acids that cause them to be such powerful solubilizers of membrane lipids are also responsible for bile acids being cytotoxic when present at abnormally high concentrations either intracellularly or extracellularly. The cytotoxic effects of bile acids may cause distressing symptoms or even death. This is the “ugly” side of bile acids.

Cytotoxicity caused by increased intracellular concentrations of bile acids. In the healthy hepatocyte, uptake of bile acids across the basolateral membrane and export via the canalicular export pump are tightly coupled. This efficient coupling, together with the presence of binding proteins in the cytosol, keeps the monomeric concentrations of bile acids within the hepatocyte at extremely low concentrations, <3 μM. When canalicular export is defective either because of an inborn or acquired defect in canalicular transport or when there is a physical obstruction to bile flow, bile acids accumulate within the hepatocyte. When their concentration exceeds the binding capacity of the binding proteins, bile acids induce apoptosis and necrosis, probably by damage to mitochondria (14). In patients with cholestatic liver disease, the extent of hepatocyte damage caused by intracellular accumulation of bile acids can be decreased by ingesting a nontoxic bile acid (ursodiol), which accumulates in the circulating bile acid pool and decreases the cytotoxicity of the bile acid mixture circulating through the hepatocyte (12).

Certain inborn errors of bile acid biosynthesis lead to increased synthesis of bile acid precursors. These accumulate within the hepatocyte because they are not substrates for the canalicular export pump and cause hepatocyte death. Infants with these extremely rare conditions develop progressive jaundice after birth. The diagnosis is usually made by mass spectrometric identification of the intermediates in urine or plasma. Treatment with natural bile acids suppresses the synthesis of the toxic intermediates, induces normal bile flow, restores the concentration of bile acids in the small intestine, and is life saving.

In principle, bile acids should accumulate in the ileal enterocyte when basolateral transport is...
impaired, and such accumulation should cause enterocyte death. However, this situation has not as yet been induced experimentally or identified clinically.

Cytotoxicity caused by increased extracellular concentrations of bile acids. In health, efficient ileal conservation, together with rapid bacterial modification of bile acids entering the colon, results in the aqueous concentration of bile acids in colonic contents being quite low, <1 mM. When ileal transport of bile acids is defective, either because of a congenital absence of the ileal bile acid transporter or because of ileal resection or disease, a compensatory increase in hepatic biosynthesis occurs, and a greatly increased amount of bile acids passes into the colon. The elevated intraluminal concentration induces secretion of electrolytes and water, manifested clinically as diarrhea. Administration of bile acid sequestrants lowers the elevated intraluminal concentration of bile acids and provides symptomatic benefit (6).

Bile acid deficiency states. A bile acid deficiency in the intestine occurs when the enterohepatic circulation of bile acids is obstructed or when it is interrupted by impaired intestinal conservation. If bile acid malabsorption is sufficiently severe, the compensatory increase in bile acid biosynthesis is insufficient to restore bile acid secretion into the intestine. (In humans, the liver can increase its synthesis of bile acids only ~15-fold. The maximal rate of bile acid biosynthesis is ~6 g/day, which is less than one-half of the normal daily secretion rate of bile acids.) Decreased bile acid secretion leads to defective micellar solubilization of dietary lipid, and this contributes to lipid malabsorption in these patients. If conjugated bile acids are fed orally, micellar solubilization is restored and improved lipid absorption results. The clinical utility of such conjugated bile acid replacement therapy is being tested (3).

**Conclusion**

Elucidation of the metabolism, enterohepatic circulation, and functions of bile acids as well as disturbances of these processes in disease has involved a multidisciplinary effort that has ranged from physical biochemistry to clinical trials. In my opinion, these efforts have been in the best traditions of integrative physiology.

Helpful suggestions were made by Dr. Carolina Cerrè and Dr. Lee R. Hagey. Some of the material in this article was presented in the Horace W. Davenport Lecture in April, 1996. The author's work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-21506 as well as a grant-in-aid from the Falk Foundation e.V., Freiburg, Germany.

References


“The elevated intraluminal concentration induces secretion…”

“…”The elevated intraluminal concentration induces secretion…”