Bile Metabolism and Lithogenesis

Kathleen O'Connell, MD[a], Karen Brasel, MD, MPH[b,*]

KEYWORDS

• Gallstones • Gallbladder • Cholelithiasis • Bile • Bile acids

KEY POINTS

- Bile acids play a prominent role in expression of genes involved in their own uptake and secretion within the enterohepatic circulation.
- Bile acids serve as ligands for the nuclear receptor farnesoid X receptor and transmembrane protein TGR5, through which they exert their regulatory effects in hepatic and extrahepatic tissues.
- Cholelithiasis is a disease associated with the metabolic syndrome and is the result of both modifiable and nonmodifiable risk factors.
- Different types of gallstones (cholesterol, black pigment, brown pigment stones) are associated with separate risk factors and disease processes.
- Disease states and therapies can alter bile metabolism, leading to an increased risk of gallstone formation.

INTRODUCTION

Gallstone disease affects 20 to 25 million adults in the United States.[1,2] Although most patients remain asymptomatic, some patients do eventually progress to symptomatic or complicated disease, leading to more than 750,000 cholecystectomies in the United States every year.[3] Of all hospital admissions in 2009, cholelithiasis and cholecystitis were the second most common discharge diagnoses among patients admitted with gastrointestinal illnesses.[4] Cholelithiasis poses a significant economic burden in this country, with direct and indirect costs totaling $6.2 billion annually.[2,5] As our population continues to age and the obesity epidemic persists, the incidence of gallstone disease is increasing. A thorough understanding of the underlying physiology of bile metabolism and lithogenesis is necessary to provide optimal management of these patients and for developing new strategies to prevent gallstone formation.

BILE METABOLISM

The synthesis of bile acids, the formation of bile, the enterohepatic circulation, and the modifications of bile acids throughout their lifespan all contribute in the metabolism of

[a] Department of Surgery, Medical College of Wisconsin, 9200 West Wisconsin Avenue, Milwaukee, WI 53226, USA; [b] Surgery, Bioethics and Medical Humanities, Medical College of Wisconsin, 9200 West Wisconsin Avenue, Milwaukee, WI 53226, USA
* Corresponding author.
E-mail address: kbrasel@mcw.edu

Surg Clin N Am 94 (2014) 361–375
http://dx.doi.org/10.1016/j.suc.2014.01.004 **surgical.theclinics.com**
0039-6109/14/$ – see front matter

bile. In recent years, many advances have been made in the understanding of the widespread activities of bile acids on a cellular and molecular level.

Function of Bile Acids

Bile acids function as the detergent component of bile, emulsifying dietary fats, fat-soluble vitamins, and drugs to allow intestinal absorption. Bile acids and phosphatidylcholine maintain the solubility of cholesterol in bile; the excretion of bile acids is the primary pathway for cholesterol catabolism, accounting for 50% of the daily turnover.[6] Recently, many studies have shown that bile acids have other important physiologic activities beyond fat digestion, including regulation of their own synthesis, endocrine and paracrine functions.[7–9] Bile acids serve as ligands for nuclear receptors, mainly farnesoid X receptor (FXR) and pregnane X receptor, which are involved in carbohydrate, triglyceride, and sterol metabolism.[10–13] Bile acids have also been shown to interact with cell surface receptors, namely TGR5, and be involved in energy expenditure, lipid metabolism, glucose homeostasis, and inflammatory/immune responses.[14] TGR5 receptors are located in brown adipose tissue, skeletal muscle, nervous system tissue, immune tissue, and colonic tissue, demonstrating the widespread effects of bile acids beyond the biliary system.[15,16] The molecular structure of TGR5 consists of a unique ligand-binding pocket for bile acids, making this receptor a potential drug target for new pharmaceuticals developed to treat metabolic syndrome.[17]

Bile Composition

The liver produces 600 to 750 mL of bile daily. The major lipid components of bile include bile acids (72%), phospholipids (24%), and cholesterol (4%).[18] The bile acid pool consists of primary bile acids (cholic acid and chenodeoxycholic acid) and secondary bile acids (deoxycholic acid and lithocholic acid). Primary bile acids are those that are produced de novo by the liver, and secondary bile acids are primary bile acids that have undergone deconjugation by intestinal bacteria. Phospholipids in healthy individuals consist mainly of phosphatidylcholine (>95%). These proportions are altered in chronic cholestatic conditions, such as primary sclerosis cholangitis or primary biliary cirrhosis.

Bile Acid Synthesis

Bile acids, the main lipid component of bile, are formed in the perivenous hepatocytes. Formation of all bile acids begins with a steroid nucleus, to which subsequent modifications are made. The amphipathic nature of bile salts is caused by the combination of hydrophilic hydroxyl groups and hydrophobic methyl groups, which are oriented opposite each other around the steroid nucleus.[16]

There are 2 pathways by which bile acid biosynthesis occurs: the classic or neutral pathway and the alternative or acidic pathway. The classic pathway begins with hydroxylation of the steroid nucleus, which is the rate-limiting step controlled by cholesterol 7α-hydroxylase (CYP7A1).[19] This enzyme is only found in hepatocytes; thus, the classic pathway only takes place in the liver. This pathway is regulated by a negative feedback loop with bile acids inhibiting CYP7A1 activity and expression.[7]

The alternative pathway is controlled by oxysterol 7α-hydroxylases (CYP7B1), which are constitutively expressed in extrahepatic tissue, such as macrophages, kidney, and vascular endothelium. These enzymes oxidize cholesterol to oxysterols in the peripheral tissues, which are then transported to the liver for final modification to form primary bile acids.[20] Normally, the alternative pathway contributes approximately 10% to overall daily bile acid synthesis; however, this pathway may become more prominent in patients with liver disease.[16]

Bile acid intermediates from both the classic and alternative pathways are then hydroxylated by sterol 12-α-hydroxylase (CYP8B1), which determines the ratio of primary bile acids, cholic acid versus chenodeoxycholic acid.[19] The primary bile acids are then conjugated with glycine (75%) or taurine (25%), increasing the hydrophilicity of the molecules.[21,22] At this point, the conjugated bile acids are then ready for transport into the bile canalicular lumen.

ENTEROHEPATIC CIRCULATION

Bile acids follow a circular pathway passing through the liver, biliary tree, intestine, and portal blood. The purpose of this enterohepatic circulation (EHC) is to recover and recycle bile acids. Ninety-five percent of the bile acid pool is recovered by the EHC, and 5% is excreted in stool. The rate-limiting step of EHC is bile acid secretion from hepatocytes, an ATP-dependent process governed by the bile salt export pump (BSEP).[23]

Interorgan Transport

When stimulated by a meal, the gallbladder contracts, releasing the stored bile containing conjugated bile acids, into the duodenum. Here they form mixed micelles with other biliary lipids and dietary lipids. Once in the ileum, a small amount of bile acids are deconjugated and subsequently passively reabsorbed in the terminal ileum. Most of the bile acids remain conjugated in the small intestine and are reabsorbed by an active transporter in the ileal enterocytes, the apical sodium-dependent bile acid transporter (ASBT).[21] The bile acids that escape reabsorption in the terminal ileum enter the colon where they are subjected to modifications introduced by the gut microflora. After reabsorption by either the ileum or colon, the bile acids enter the portal blood and are transported back to the liver. Conjugated and unconjugated bile acids are extracted from portal blood at the sinusoidal membrane of hepatocytes via the sodium/taurocholate cotransporting polypeptide and via the organic anion–transporting polypeptides (OATP1B1, OATP1B3), respectively.[21] Recycled unconjugated bile acids are then reconjugated to glycine or taurine, and re-secreted into the bile canaliculi along with newly synthesized bile acids by the BSEP. The cycle then repeats itself, with the bile acids completing multiple cycles before they undergo excretion in the stool. A small amount of bile acids spill into the systemic circulation and are transported in the blood via serum albumin.[24] In the peripheral tissues, bile acids interact with TGR5 receptors to regulate gene expression outside of the biliary system as discussed previously. Bile acids have been detected in brain, heart, kidney, thyroid, and ovarian tissues.[25–27] Details of the molecular modifications that take place throughout various points in the EHC are found later.

Intracellular Transport

As depicted in **Fig. 1**, bile acids enter the apical pole of the ileal enterocytes through the ASBT, an energy-dependent process. Once inside the cell, transport to the opposite pole is mediated by proteins including fatty acid–binding protein (FABP1) and ileal lipid–binding protein (ILBP).[28,29] FABP1 is abundant in the liver and small bowel and preferentially binds fatty acids as opposed to bile acids. ILBP is predominantly located in enterocytes in the distal ileum and has greater affinity for conjugated bile acids. Once the bile acids are transported to the basal membrane, they exit the enterocyte via the basolateral heterodimeric organic solute transporter (OSTα/OSTβ) into the portal blood.[30]

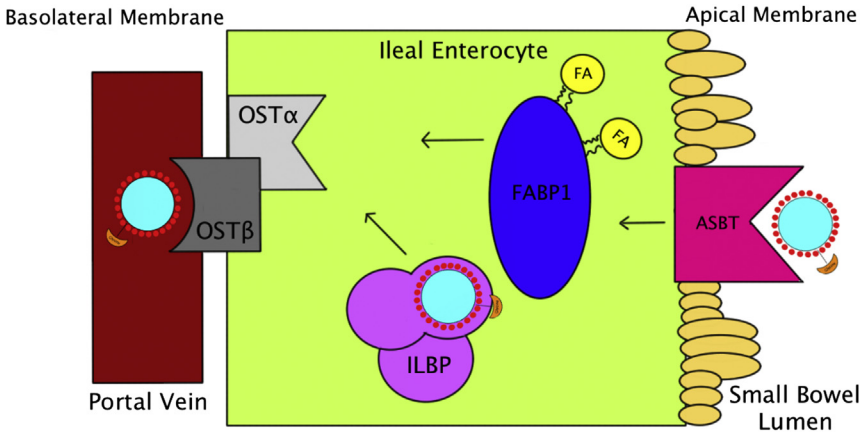

Fig. 1. Intracellular transport of bile acids. Conjugated bile acids enter the apical pole of the ileal enterocytes through ASBT. Inside the cell, bile acid transport is mediated by the ileal lipid–binding protein (ILBP), while the fatty acid–binding protein (FABP1) transports fatty acids. Exit at the basolateral membrane into the portal system is mediated by the organic solute transporter (OSTα/OSTβ).

Modifications by Gut Bacteria

Bile acids have multiple mechanisms by which they exert bactericidal activity. First, antibacterial effects are inherent in the detergent molecular structure of bile acids. Second, conjugated bile acids bind to FXR receptors in the ileum, inducing gene expression of angiogenin, nitric oxide synthase, and interleukin 18, all enzymes known to have antimicrobial effects.[31] Through this mechanism, it is thought that conjugated bile acids assist in controlling bacterial overgrowth in the gut. Colonic bacteria metabolize bile acids and, thus, decrease the bactericidal activity.

As stated previously, the small amount of conjugated bile acids that escape to the colon are deconjugated by bile acid hydrolases, enzymes produced by colonic bacteria (**Fig. 2**). The process of deconjugation includes enzymatic hydrolysis of the C-24 N-acyl amide bond linking bile acids to their amino acid conjugates (glycine and taurine).[16] Deconjugation and subsequent oxidation converts the primary bile acids, cholic acid and chenodeoxycholic acid, to secondary bile acids, deoxycholic and lithocholic acids, respectively. This biotransformation decreases the toxicity of the bile acids to the bacteria while increasing the toxicity to enterocytes and hepatocytes. The carcinogenic effects of secondary bile acids on the cells of the colon and liver are mediated by various cell signaling pathways resulting in the inhibition of DNA repair enzymes, interference of tumor suppressor genes, and stimulation of growth promoters.[32] Bile acids have been well established in the carcinogenesis of colorectal cancer.[33,34]

FXR

FXR is a nuclear receptor that is highly expressed in the liver, intestine, and kidney. In hepatocytes, bile acids activate FXR to suppress their own de novo synthesis, producing a negative feedback loop.[20,35] There are 2 known bile acid/FXR–dependent pathways that inhibit CYP7A1 (rate-limiting enzyme in bile acid synthesis in the neutral pathway) and CYP8B1 (enzyme responsible for production of cholic acid). First, bile acids activate FXR, leading to upregulation of small heterodimer partner (SHP). SHP interacts with multiple transcription factors that, in turn, bind to bile acid response elements located in the promoter region of CYP7A1 and CYP8B1, leading to the

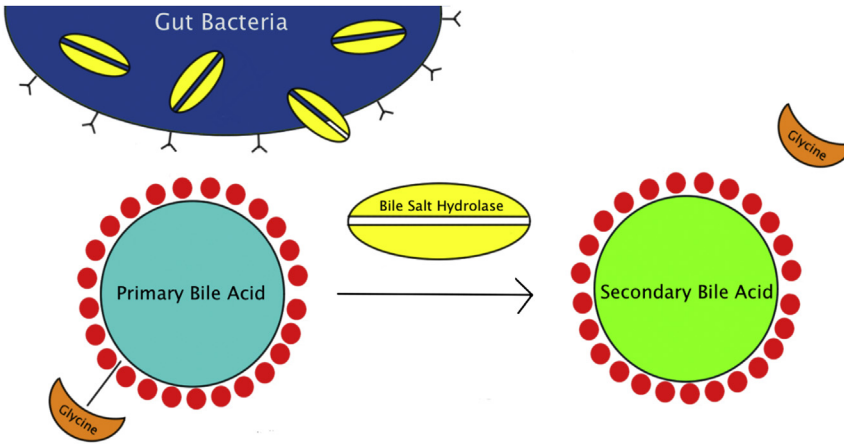

Fig. 2. Deconjugation of bile acids. Colonic bacteria produce bile acid hydrolyses, which lyse the bond to glycine or taurine in primary bile acids, resulting in the formation of secondary bile acids.

inhibition of bile acid synthesis.[36–38] Second, binding of bile acids to FXR causes increased expression of fibroblast growth factor 19, which binds to fibroblast growth factor receptor 4 (FGFR4) receptors on hepatocytes. This activity initiates a cascade of events leading to the activation of the JNK (c-Jun N-terminal Kinase) pathway, ultimately repressing CYP7A1 transcription.[39]

The activation of FXR in hepatocytes also enhances the conjugation and detoxification of bile acids in addition to increasing bile acid efflux to prevent hepatic accumulation. FXR is involved in the conjugation of bile acids via the regulation of bile acid coenzyme A synthetase and bile acid coenzyme A amino acid N-acetyltransferase.[40] Bile acids promote their own excretion from hepatocytes into the canalicular lumen through FXR-activated expression of the BSEP in the hepatocyte membrane, thus, preventing hepatocyte toxicity.

GALLBLADDER DISEASE

Gallstone disease affects 10% to 20% of American adults within their lifetime and is one of the leading indications for surgery in the United States.[41] Eighty percent of people with gallstones remain asymptomatic, with the diagnosis usually made by ultrasound or computed tomography for an unrelated cause. However, 2% to 3% of patients progress to symptomatic disease per year, with 10% of patients considered symptomatic at 5 years.[42] A total of 1% to 2% of patients with gallstones develop complicated disease (ie, cholecystitis, pancreatitis, cholangitis) annually.[43]

Lithogenesis

Gallstones form in the gallbladder and in the bile ducts. The gallbladder is the site of the formation of most gallstones and its functions are essential to the process of lithogenesis. Filling of the gallbladder occurs with tonic contraction of the ampullary sphincter at 10 to 15 mm Hg. Bile flow is increased during periods of partial emptying (in between meals) and involves the activity of the migrating motor complex and motilin. After a meal, cholecystokinin (CCK) is released from the duodenum, stimulating contraction of the gallbladder. The contraction empties 50% to 70% of the volume into the duodenum and refills within 60 to 90 minutes.[44]

The mucosa of the gallbladder functions in absorption of electrolytes and water, concentration of bile, and secretion of proteins. The volume of the gallbladder is 40 to 50 mL; however, it is capable of storing up to 750 mL of bile because it has the greatest absorptive capacity per unit of any bodily tissue. Concentration of bile occurs by active sodium chloride transport, resulting in passive water absorption and concentration of the bile to 5 to 10 times its native form. Hydrogen ions are secreted by the gallbladder to decrease the pH of the bile from approximately 7.5 to 7.8 to 7.1 to 7.3, leading to increased calcium solubility.[44] The secretion of mucous glycoproteins provides a resistant barrier to the concentrated bile salts as well as a nidus for cholesterol nucleation.

Cholesterol stones

Eighty percent of all gallstones are classified as cholesterol stones. The formation of cholesterol gallstones is multifactorial and involves cholesterol supersaturation in bile, crystal nucleation, gallbladder dysmotility, and gallbladder absorption and secretion. First, as bile is concentrated in the gallbladder, there is a transfer of cholesterol and phospholipids from vesicles to micelles. The phospholipids preferentially transfer to the micelles, leaving behind cholesterol-rich vesicles, which then precipitate into crystals. Second, crystallization is accelerated by pronucleating factors in the gallbladder, including mucin glycoproteins, immunoglobulins, and transferrin. Third, incomplete emptying or dysmotility of the gallbladder allows concentrated bile to stagnate, increasing the residence time within the gallbladder. Dismotility of the gallbladder also permits an increase in the size of existing gallstones, thus, increasing the probability of transforming into symptomatic disease. Lastly, alteration in the concentrations of sodium, chloride, and bicarbonate changes the saturation of cholesterol leading to precipitation of calcium and crystal formation (**Fig. 3**).[44]

Risk factors There are various well-established risk factors leading to the development of cholesterol gallstones. Modifiable risk factors include diet, sedentary lifestyle, rapid weight loss, and obesity. Nonmodifiable risk factors include ethnicity, genetics, advancing age, and female sex (**Table 1**).[45]

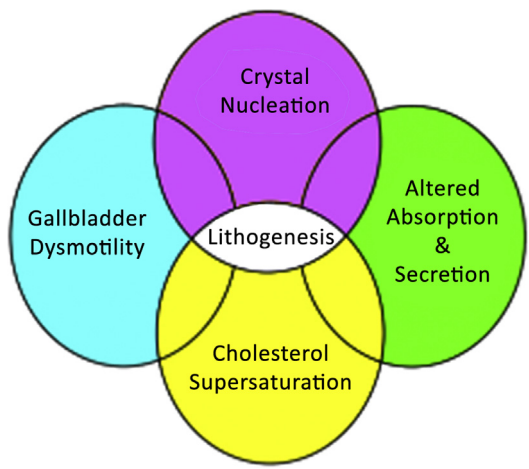

Fig. 3. Lithogenesis. Gallstone formation consists of 4 components: cholesterol supersaturation, crystal nucleation, gallbladder dysmotility, and abnormal gallbladder absorption/secretion.

Table 1
Modifiable and nonmodifiable risk factors for cholesterol gallstone formation

Cholesterol Gallstone Risk Factors	
Modifiable	**Nonmodifiable**
Diet	Genetics
Physical activity	Ethnicity
Rapid weight loss	Advancing age
Obesity	Female sex
Dyslipidemia	Chronic disease states

Ethnicity/geography Cholesterol gallstones are most prevalent in developed countries, and this is attributed to the popularization of the Western diet. The Third National Health and Nutrition Examination Survey, a large cross-sectional ultrasonographic study, established the prevalence of gallstone disease in the United States.[46] This study demonstrated that North American Indians have the highest rates of cholelithiasis, with women at 64.1% and men at 29.5% prevalence rates. Mexican Americans have a higher prevalence of gallstones compared with non-Hispanic groups in the United States, with rates equal to 8.9% in men and 26.7% in women. Caucasian woman have a prevalence of 16.6% and men at 8.6%, which is higher than African American women at 13.9% and men at 5.0%. On a global scale, Asians have an overall prevalence of 3% to 15%, with multiple recent studies reporting a significant increase in cholesterol gallstone disease over the past few decades, largely attributed to adoption of a Westernized diet.[45] The lowest rates of gallstones are in sub-Saharan Africans at less than 5%.[41]

Genetics The contribution of hereditary versus environmental factors to the development of gallstones has yet to be determined. Familial studies have demonstrated that people with first-degree relatives with gallstones are 2 to 4 times more likely to develop gallstones compared with controls.[47,48] The Swedish twin study, published in 2005, included 43,141 pairs of monozygotic and dizygotic twins. This study revealed significantly higher rates of gallstone disease in monozygotic twins compared with dizygotic twins, especially in the younger cohort. The researchers concluded that heritability accounts for 25%, shared environmental influences for 13%, and unique environmental influences for 62% of gallstone disease variance among the twins.[49] Another study of 358 Midwestern families found the heritability of symptomatic gallstones to be at least 30%, similar to what was found in the Swedish study.[50] These studies demonstrated that, although family members share common environmental exposures, including dietary habits, genetic predisposition is a major risk factor to the development of stones.

Sex Women are twice as likely as men to form gallstones, a process related to female sex hormones, birth control medications, parity, and hormone replacement therapies. Estrogens increase cholesterol secretion and decrease bile salt secretion, and progesterone also reduces bile salt secretion and impairs gallbladder emptying. The combination of these 2 hormones creates a high cholesterol-cholestatic environment, optimal for lithogenesis.

Additionally, women who are pregnant are more likely to suffer from symptomatic gallstones. The increased levels of estrogen and progesterone lead to cholesterol hypersecretion and gallbladder stasis. During pregnancy, it is estimated that 30% of women develop biliary sludge and 2% develop gallstones. In the postpartum period

when gallbladder motility and bile composition return to normal, there is complete resolution of sludge in 61% of women and in 28% of women with gallstones.[51]

Age The risk of developing gallstones increases markedly with advancing age. After 40 years of age, the incidence of gallstones increases by 1% to 3% per year.[52] With increasing age, hepatic cholesterol secretion is increased, cholesterol saturation increases, and bile acid synthesis is decreased. These alterations are attributed to decreased activity of the rate-limiting enzyme for bile acid synthesis CYP7A1.[53] There is also a higher prevalence of black pigment stones compared with cholesterol stones in people of increasing age.

Dietary factors Multiple large global epidemiologic studies have identified that diets high in refined carbohydrates, high in triglycerides, and low in fiber are associated with the development of gallstones. Diets high in protein and fiber have been shown to have the opposite effect, actually protecting against gallstone development.[54,55] Fiber, by increasing bulk, accelerates intestinal transit time, leading to decreased formation of secondary bile acids. Because secondary bile acids are associated with an increased cholesterol saturation index in bile, increased consumption of dietary fiber hinders the development of gallstones.[45] Other dietary factors with an inverse association to gallstone development include alcohol and caffeine.[56,57] Moderate amounts of alcohol, 1 to 2 drinks daily, is protective in that the conversion of cholesterol to bile acids is increased. Caffeine, on the other hand, stimulates CCK release from the duodenum, effectively increasing gallbladder motility.

Total parenteral nutrition (TPN) is a well-known risk factor for the development of sludge and gallstones. A possible explanation for this strong correlation is that the loss of enteric stimulation of gallbladder contraction leads to gallbladder stasis. One study demonstrated that after 4 weeks of TPN, 50% of patients developed gallbladder sludge, which progressed to 100% with sludge at 6 weeks.[58] Most of these patients are asymptomatic, and there is resolution with discontinuation of TPN.

Obesity Obesity is an epidemic in developed nations and is a strong risk factor for gallstone disease. This factor may be, in part, caused by the increased activity of 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis, leading to increased cholesterol synthesis in the liver and secretion into the bile.[59] Although obese individuals hypersecrete bile salts and phospholipids in addition to cholesterol, the rate of cholesterol hypersecretion exceeds that of the other factors, leading to supersaturation of the bile with increased lithogenicity.

Abdominal adiposity, especially in women, has been identified as a major risk factor for gallstone development. Waist circumference and waist-to-hip ratio have been shown to be better predictors of gallstone development compared with body mass index or overall total body fat.[60] Individuals with obesity in their late teenage years carry the greatest risk of developing gallstone disease throughout their lifetime. The prevalence of gallstone disease among obese children and adolescents may be as high as 2%, whereas the prevalence among unselected pediatric groups is between 0.1% and 1.0%.[61,62]

Rapid weight loss Bariatric surgery patients with rapid postoperative weight loss develop gallstones in 30% to 71% of cases. The incidence of gallstones is highest within the first 2 years after surgery. Most of these stones are asymptomatic, but weight loss exceeding 25% of total body weight is predictive of symptomatic disease.[63] The mechanism for increased lithogenesis in bariatric patients is

unclear; however, one hypothesis implicates increased secretion of mucin and calcium from the gallbladder as contributing factors to the formation of gallstones.[64]

Patients with a history of low-caloric dieting and extreme fluctuations in weight are also at risk for stone formation. Weight reduction leads to mobilization of hepatic stores of cholesterol. In combination with decreased gallbladder emptying and reduced bile acid synthesis, supersaturation of bile and rapid stone formation takes place.

Dyslipidemia Cholesterol gallstone formation is a metabolic issue that is correlated with lipid abnormalities. Hypertriglyceridemia and low high-density lipoprotein concentration are associated with increased cholesterol saturation of bile and increased risk for cholesterol stone formation.[64–66] Both of these dyslipidemias have been shown to be independent risk factors for cholelithiasis. Hypercholesterolemia is not strongly associated with cholesterol stone disease.

Drugs Certain drugs interfere with cholesterol and bile acid synthesis. Clofibrate, used to treat hypertriglyceridemia, is an inhibitor of hepatic acyl coenzyme A: cholesterol acyltransferase (ACAT). The inhibition of ACAT increases the concentration of free cholesterol in bile, thus, lowering the threshold for gallstone formation. Statins, also used in patients with dyslipidemia, inhibit HMG-CoA reductase, decreasing cholesterol synthesis in the liver. Statins seemingly protect against gallstone formation by decreasing the amount of cholesterol in the bile.[67] Ceftriaxone, a third-generation cephalosporin, is secreted unmetabolized into bile. Especially in children, ceftriaxone leads to sludge and pseudolithiasis, which spontaneously resolves on discontinuation of the antibiotic. Octreotide, used clinically for its global gastrointestinal inhibitory properties, also inhibits contraction of the gallbladder resulting in stasis. The inhibition of CCK by octreotide leads to decreased small bowel motility and enhancement of secondary bile acid formation. An increased ratio of secondary bile acids results in enhanced cholesterol secretion and gallbladder mucin production. Lastly, octreotide stimulates calcium release into bile, creating a more lithogenic environment.

Diabetes mellitus Diabetes mellitus (DM) has frequently been associated with cholelithiasis, although the relationship has not been fully delineated. There is evidence that bile acid composition and size of the pool are altered in humans with type 1 and type 2 DM.[68,69] In large series of patients with diabetes, gallstone disease is significantly higher than observed in the general population. Independent risk factors for gallstone formation in patients with diabetes include advancing age, higher body mass index, and positive family history.

On a molecular level, bile acids regulate gluconeogenesis, glycogen synthesis, and insulin sensitivity via FXR gene expression. CYP7A1 is negatively regulated by FXR. With the dysregulation of FXR expression as is seen in patients with diabetes, CYP7A1 activity is increased and the size of the bile acid pool increases. Additionally, evidence suggests glucose and insulin are involved in the modulation of bile acid synthesis. Glucose has been shown to enhance FXR gene expression, whereas insulin represses FXR gene expression.[70]

Pigment stones

Pigment stones account for 20% of all gallstones. This group consists of brown and black pigment stones.

Brown stones Brown pigment gallstones are associated with biliary dysmotility and bacterial infection of the bile. They are composed of calcium bilirubinate, fatty acid soaps (calcium palmitate and calcium stearate), cholesterol, and mucinous

glycoproteins (products of bacterial biofilms). The most common risk factor is bile duct stasis, which is encountered in the following disease states: sclerosing cholangitis, congenital cysts, strictures, chronic pancreatitis, and duodenal diverticula. Unlike cholesterol stones, brown pigment stones are mostly identified as primary ductal stones, with patients suffering from choledocholithiasis and hepatolithiasis.[41]

Brown pigment stones also result from bacterial infections and biliary parasites. East Asians traditionally have higher rates of brown pigment stones secondary to recurrent pyogenic cholangitis, also known as *Oriental cholangiohepatitis*, characterized by biliary obstruction with recurrent cholangitis. The bacteria produce a film containing glucuronidase, which hydrolyses conjugated bilirubin to free bilirubin. Free bilirubin then precipitates when mixed with calcium. Recently there has been an increase in cholesterol stones in East Asia, likely attributable to improved hygiene with reduced biliary infections and consumption of a westernized diet.

Black stones Black pigment stones account for 2% of all gallstones and are associated with chronic liver disease and hemolytic conditions.[71] These stones develop exclusively in the gallbladder and consist of calcium bilirubinate with mucin glycoproteins. The prevalence of gallstones in patients with chronic liver disease is near 30%.[72] Cholelithiasis associated with liver disease is secondary to altered pigment secretion, abnormal gallbladder motility, and increased estrogen levels.[2] Sickle cell disease results in excessive unconjugated bilirubin excretion, which precipitates with calcium. Prophylactic cholecystectomy may be considered in this patient population because differentiating the cause of abdominal pain in this group can be difficult. Although infrequent, black pigment stones are also identified in children born prematurely and those that require TPN.[41]

Complications of Cholelithiasis

Most gallstones remain clinically silent, with a risk of progression of 2% to 3% per year, including 1% to 2% of patients who develop major complications.[42,73] Within 5 years of diagnosis, 10% of patients with gallstones will become symptomatic, which increases to 20% at 20 years.[43] The longer gallstones remain silent, the less likely patients are to develop symptoms. At one end of the disease spectrum is biliary colic, of which 10% to 20% of patients will go on to develop cholecystitis. At the other end of the spectrum is cholangitis, which occurs with biliary obstruction and bactibilia. Bile is normally sterile, but bacteria can reflux from the duodenum into the biliary system, enter the hepatic veins and perihepatic lymphatics causing systemic bacteremia. Other disease processes secondary to gallstone complications include choledocholithiasis, gallstone pancreatitis, and gallstone ileus.

Gallstone disease is a known risk factor for gallbladder carcinoma, with a relative risk of 4.9.[74] Most people with gallbladder cancer have gallstones, suggesting stones may function as a cofactor in the development of cancer. One hypothesis is that the presence of gallstones creates chronic inflammation of the mucosa, which over time leads to dysplasia. Increased risk of gallbladder carcinoma is associated with stones larger than 3 cm, and increased number, volume, and weight of the stones.[75] Patients with stones greater than 3 cm should be considered for a prophylactic cholecystectomy because the risk of cancer is 4% over 20 years.

Disease States Causing Altered Bile Acid Metabolism

Crohn's disease with ileocecal involvement or surgical resection of the terminal ileum leads to diminished ileal reabsorption of bile acids. This diminished reabsorption allows more bile acids to escape to the colon, where they solubilize unconjugated

bilirubin, facilitating passive absorption and transport back to the liver. The liver then secretes excess pigment (bilirubin) into the bile, leading to a higher proportion of black pigment stones in patients with Crohn's disease. Reduced hepatic secretion of bile acids, supersaturation with cholesterol, and increased enterohepatic cycling of bile pigment results in gallstone formation in 13% to 34% of all patients with Crohn's disease. The prevalence of gallstones is lower in patients with colonic Crohn's disease and is highest in patients with terminal ileal disease.[76–78]

Patients with cystic fibrosis (CF) are known to have excessive loss of bile acids through fecal excretion. The mechanism by which mutations in CF transmembrane conductase regulator (CFTR) affect EHC have not been fully delineated. Dysfunction of CFTR in the apical plasma membranes of cholangiocytes impairs chloride/bicarbonate exchange, thus, altering the composition of bile. Studies in CF mice have demonstrated significantly decreased pH in the ileum, leading to increased loss of bile salts in the feces. This increased loss, in turn, results in hyperbilirubinbilia (elevated secretion of conjugated bilirubin into bile) and increased proportion of hydrophobic bile acids. Additionally these mice had higher concentrations of biliary cholesterol, although the gallstones that form are considered more of a black pigment stone. The overall prevalence of gallstones in patients with CF is between 10% and 30%.[79]

Individuals with spinal cord injury (SCI) have an increased risk of developing gallstone disease, with a prevalence of 30%.[80,81] The gallbladder receives sympathetic innervation via the splanchnic nerves originating from T7-T10, which allow relaxation of the gallbladder smooth muscle. Interruption of this pathway, especially in high SCI, is thought to cause gallbladder dysfunction. In addition, patients with SCI also frequently have neurogenic bowel resulting in slow intestinal transit times, which results in an increase in the formation of secondary bile acids, thus, promoting the formation of gallstones.

SUMMARY

Cholelithiasis is a disease process affecting a significant number of American adults, accounting for a considerable burden on the health care system. The development of gallstones is largely multifactorial, consisting of both modifiable and nonmodifiable risk factors. There have been major advances in uncovering the molecular basis of bile metabolism and lithogenesis in recent years; however, because of the complexity of the pathways involved, investigations are ongoing. Understanding the pathogenesis of gallstone formation will direct the development of targeted therapies for the prevention and treatment of this disease.

REFERENCES

1. Tazuma S. Gallstone disease: epidemiology, pathogenesis, and classification of biliary stones (common bile duct and intrahepatic). Best Pract Res Clin Gastroenterol 2006;20:1075–83.
2. Shaffer EA. Epidemiology and risk factors for gallstone disease: has the paradigm changed in the 21st century? Curr Gastroenterol Rep 2005;7:132–40.
3. Graves EJ, Owings MF. 1995 summary: National Hospital Discharge Survey. Adv Data 1997;(291):1–10.
4. Peery E, Dellon S, Lund J, et al. Burden of gastrointestinal disease in the United States: 2012 update. Gastroenterology 2012;143:1179–87.
5. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States part I: overall and upper gastrointestinal diseases. Gastroenterology 2009;136:376–86.

6. Insull W Jr. Clinical utility of bile acid sequestrants in the treatment of dyslipidemia: a scientific review. South Med J 2006;99:257–73.
7. Keitel V, Kubitz R, Häussinger D. Endocrine and paracrine role of bile acids. World J Gastroenterol 2008;14:5620–9.
8. Nguyen A, Bouscarel B. Bile acids and signal transduction: role in glucose homeostasis. Cell Signal 2008;20:2180–97.
9. Trauner M, Claudel T, Fickert P, et al. Bile acids as regulators of hepatic lipid and glucose metabolism. Dig Dis 2010;28:220–4.
10. Wang H, Chen J, Hollister K, et al. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 1999;3:543–53.
11. Makishima M, Okamoto AY, Repa JJ, et al. Identification of a nuclear receptor for bile acids. Science 1999;284:1362–5.
12. Staudinger JL, Goodwin B, Jones SA, et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc Natl Acad Sci U S A 2001;98:3369–74.
13. Halilbasic E, Claudel T, Trauner M. Bile acid transporters and regulatory nuclear receptors in the liver and beyond. J Hepatol 2013;58:155–68.
14. Pols TW, Noriega LG, Nomura M, et al. The bile acid membrane receptor TGR%: a valuable metabolic target. Dig Dis 2011;29:37–44.
15. Kawamata Y, Fujii R, Hosoya M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem 2003;278:9435–40.
16. Monte MJ, Marin JJ, Antelo A, et al. Bile acids: chemistry physiology, and pathophysiology. World J Gastroenterol 2009;15:804–16.
17. Macchiarulo A, Gioiello A, Thomas C, et al. Molecular field analysis and 3D-quantitative structure-activity relationship study (MFA3D-QSAR) unveil novel features of bile acid recognition at TGR5. J Chem Inf Model 2008;48:1792–801.
18. Hay DW, Carey MC. Chemical species of lipids in bile. Hepatology 1990;12:6S–14S.
19. Staels B, Fonseca VA. Bile acids and metabolic regulation: mechanisms and clinical responses to bile acid sequestration. Diabetes Care 2009;32:S237–45.
20. Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. Annu Rev Biochem 2003;72:137–74.
21. Zwicker BL, Agellon LB. Transport and biological activities of bile acids. Int J Biochem Cell Biol 2013;45:1389–98.
22. Warren DB, Chalmers DK, Hutchison K, et al. Molecular dynamics simulations of spontaneous bile salt aggregation. Colloids Surf A Physicochem Eng Asp 2006;280:182–93.
23. Gerloff T, Stieger B, Hagenbuch B, et al. The sister of P-glycoprotein represents the canalicular bile salt export pump of mammalian liver. J Biol Chem 1998;273:10046–50.
24. Rudman D, Kendall FE. Bile acid content of human serum. The binding of cholanic acids by human plasma proteins. J Clin Invest 1957;36:538–42.
25. Smith LP, Nierstenhoefer M, Yoo SW, et al. The bile acid synthesis pathway is present and functional in the human ovary. PLoS One 2009;4:e7333.
26. Khurana S, Raufman JP, Pallone TL. Bile acids regulate cardiovascular function. Clin Transl Sci 2011;4:210–8.
27. Mukaisho K, Araki Y, Sugihara H, et al. High serum bile acids cause hyperthyroidism and goiter. Dig Dis Sci 2008;53:1411–6.
28. Sacchettini JC, Hauft SM, Van Camp SL, et al. Developmental and structural studies of an intracellular lipid binding protein expressed in the ileal epithelium. J Biol Chem 1990;265:19199–207.

29. Levy E, Menard D, Delvin E, et al. Localization, function and regulation of the two intestinal fatty acid-binding protein types. Histochem Cell Biol 2009;132:351–67.
30. Dawson PA, Hubbert M, Haywood J, et al. The heteromeric organic solute transporter alpha-beta, Ostalpha-Ostbeta, is an ileal basolateral bile acid transporter. J Biol Chem 2005;280:6960–8.
31. Inagaki T, Maschetta A, Lee YK, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci U S A 2006; 103:3920–5.
32. McGarr SE, Ridlong JM, Hylemon PB. Diet, anaerobic bacterial metabolism, and colon cancer: a review of the literature. J Clin Gastroenterol 2005;39:98–109.
33. Roberton A. Roles of endogenous substances and bacteria in colorectal cancer. Mutat Res 1993;290:71–8.
34. Bernstein C, Bernstein H, Garewal H, et al. A bile acid-induced apoptosis assay for colon cancer risk and associated quality control studies. Cancer Res 1999; 59:2353–7.
35. Heuman DM, Hylemon PB, Vlahcevic ZR. Regulation of bile acid synthesis. III. Correlation between biliary bile salt hydrophobicity index and the activities of enzymes regulating cholesterol and bile acid synthesis in the rat. J Lipid Res 1989;30:1161–71.
36. Stroup D, Crestani M, Chiang JY. Identification of a bile acid response element in the cholesterol 7 alpha-hydroxylase gene CYP7A. Am J Physiol 1997;273: G508–17.
37. Yang Y, Zhang M, Eggertsen G, et al. On the mechanism of bile acid inhibition of rat sterol 12alpha-hydroxylase gene (CYP8B1) transcription: role of alpha-fetoprotein transcription factor and hepatocyte nuclear factor 4alpha. Biochim Biophys Acta 2002;1583:63–73.
38. Goodwin B, Jones SA, Price RR, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol Cell 2000; 6:517–26.
39. Holt JA, Luo G, Billin AN, et al. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. Genes Dev 2003;17:1581–91.
40. Pircher PC, Kitto JL, Petrowski ML, et al. Farnesoid X receptor regulates bile acid-amino acid conjugation. J Biol Chem 2003;278:27703–11.
41. Stinton LM, Shaffer EA. Epidemiology of gallbladder disease: cholelithiasis and cancer. Gut Liver 2012;6:172–87.
42. Ransohoff DF, Gracie WA, Wolfenson LB, et al. Prophylactic cholecystectomy or expectant management for silent gallstones. A decision analysis to assess survival. Ann Intern Med 1983;99:199–204.
43. Thistle JL, Cleary PA, Lachin JM, et al. The natural history of cholelithiasis: the National Cooperative Gallstone Study. Ann Intern Med 1984;101:171–5.
44. Pitt HA, Ahrendt SA, Nakeeb A. Calculous biliary disease. In: Mulholland MW, editor. Greenfield's surgery: scientific principles and practice. Philadelphia: Lippincott Williams & Wilkins; 2011. Chapter 60.
45. Yoo EH, Lee SY. The prevalence and risk factors for gallstone disease. Clin Chem Lab Med 2009;47:795–807.
46. National Center for Health Statistics, Plan and operation of the Third National Health and Nutrition Examination Survey 1988-94. Vital Health Stat 1 1994;(32): 1–407.
47. Gilat T, Feldman C, Halpem Z, et al. An increased familial frequency of gallstones. Gastroenterology 1983;84:242–6.

48. Sarin SK, Negi VS, Dewan R. High familial prevalence of gallstones in the first-degree relatives of gallstone patients. Hepatology 1995;22:138–41.
49. Katsika D, Grijibovski A, Einarsson C, et al. Genetic and environmental influences on symptomatic gallstone disease: a Swedish study of 43,141 twin pairs. Hepatology 2005;41:1138–43.
50. Nakeeb A, Comuzzie AG, Martin L, et al. Gallstones: genetics versus environment. Ann Surg 2002;235:842–9.
51. Maringhini A, Ciambra M, Baccelliere P, et al. Biliary sludge and gallstones in pregnancy: incidence, risk factors, and natural history. Ann Intern Med 1993; 119:116–20.
52. Einarsson K, Nilsell K, Leijd B, et al. Influence of age on secretion of cholesterol and synthesis of bile acids by the liver. N Engl J Med 1985;313:277–82.
53. Bertolotti M, Gabbi C, Anzivino C, et al. Age-related changes in bile acid synthesis and hepatic nuclear receptor expression. Eur J Clin Invest 2007;37: 501–8.
54. Kritchevsky D, Dlurfeld DM. Gallstone formation in hamsters: effect of varying animal and vegetable protein levels. Am J Clin Nutr 1983;37:802–4.
55. Attili AF, Scafato E, Marchioli R, et al. Diet and gallstones in Italy: the cross-sectional MICOL results. Hepatology 1998;27:1492–8.
56. Nestel PJ, Simons LA, Homma Y. Effects of ethanol on bile acid and cholesterol metabolism. Am J Clin Nutr 1976;29:1007–15.
57. Leitzmann MF, Willett WC, Rimm EB. A prospective study of coffee consumption and the risk of symptomatic gallstone disease in men. JAMA 1999;22:2106–12.
58. Messing B, Bories C, Kunstlinger F, et al. Does total parenteral nutrition induce gallbladder sludge formation and lithiasis? Gastroenterology 1983; 84:1012–9.
59. Erlinger S. Gallstones in obesity and weight loss. Eur J Gastroenterol Hepatol 2000;12:1347–52.
60. Tsai CJ, Leitzmann MF, Willett WC, et al. Central adiposity, regional fat distribution, and the risk of cholecystectomy in women. Gut 2006;55:708–14.
61. Kaechele V, Wabitsch M, Thiere D, et al. Prevalence of gallbladder stone disease in obese children and adolescents: influence of the degree of obesity, sex, and pubertal development. J Pediatr Gastroenterol Nutr 2006;42:66–70.
62. Kratzer W, Walcher T, Arnold F, et al. Gallstone prevalence and risk factors for gallstone disease in an urban population of children and adolescents. Z Gastroenterol 2010;48:683–7.
63. Li VK, Pulido N, Fajnwaks P, et al. Predictors of gallstone formation after bariatric surgery: a multivariate analysis of risk factors comparing gastric bypass, gastric banding, and sleeve gastrectomy. Surg Endosc 2009;23:1640–4.
64. Shiffman ML, Shamburek RD, Schwartz CC, et al. Gallbladder mucin, arachidonic acid, and bile lipids in patients who develop gallstones during weight reduction. Gastroenterology 1993;105:1200–8.
65. Petitti DB, Friedman GD, Klatsky AL. Association of a history of gallbladder disease with a reduced concentration of high-density-lipoprotein cholesterol. N Engl J Med 1981;304:1396–8.
66. Thijs C, Knipschild P, Brombacher P. Serum lipids and gallstones: a case-control study. Gastroenterology 1990;99:843–9.
67. Tsai CJ, Leitzmann MF, Willet WC, et al. Statin use and the risk of cholecystectomy in women. Gastroenterology 2009;136:1593–600.
68. Andersen E, Karlaganis G, Sjovalll J. Altered bile acid profiles in duodenal bile and urine in diabetic subjects. Eur J Clin Invest 1988;18:166–72.

69. Brufau G, Stellaard F, Prado K, et al. Improved glycemic control with colesevelam treatment in patients with type 2 diabetes is not directly associated with changes in bile acid metabolism. Hepatology 2010;52:1455–64.
70. Duran-Sandoval D, Mautino G, Martin G, et al. Glucose regulates the expression of the farnesoid X receptor in liver. Diabetes 2004;53:890–8.
71. Lammert F, Miquel JF. Gallstone disease: from genes to evidence-based therapy. J Hepatol 2008;48:S124–35.
72. Acalovschi M, Badea R, Dumitraçcu D, et al. Prevalence of gallstones in liver cirrhosis: a sonographic survey. Am J Gastroenterol 1988;83:954–6.
73. Friedman GD. Natural history of asymptomatic and symptomatic gallstones. Am J Surg 1993;165:399–404.
74. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Cancer 2006;118:1591–602.
75. Shrikhande SV, Barreto SG, Singh S, et al. Cholelithiasis in gallbladder cancer: coincidence, cofactor, or cause! Eur J Surg Oncol 2010;36:514–9.
76. Cohen S, Kaplan M, Gottlieb L, et al. Liver disease and gallstones in regional enteritis. Gastroenterology 1971;60:237–45.
77. Whorwell PJ, Hawkins R, Dewbury K, et al. Ultrasound survey of gallstones and other hepatobiliary disorders in patients with Crohn's disease. Dig Dis Sci 1984;29:930–3.
78. Hutchinson R, Tyrrell PN, Kumar D, et al. Pathogenesis of gallstones in Crohn's disease: an alternative explanation. Gut 1994;35:94–7.
79. Angelico M, Gandin C, Canuzzi P, et al. Gallstones in cystic fibrosis: a critical reappraisal. Hepatology 1991;14:768–75.
80. Moonka R, Stiens SA, Resnick WJ, et al. The prevalence and natural history of gallstones in spinal cord injured patients. J Am Coll Surg 1999;189:274–81.
81. Apstein MD, Dalecki-Chipperfield K. Spinal cord injury is a risk factor for gallstone disease. Gastroenterology 1987;92:966–8.