Review

Gallstones: an intestinal disease?

During the first half of this century, cholelithiasis was regarded as a disease of the gall bladder, with inflammation of the wall as the primary defect and exfoliated cells as the source of biliary cholesterol.[1 2] During the 1960s, Small and Rapo elucidated the physical–chemical basis of biliary cholesterol supersaturation and demonstrated that excessive hepatic cholesterol secretion is the underlying defect.[3] Cholesterol gallstones were then considered to be the consequence of a metabolic defect of the liver. The importance of the gall bladder during gallstone formation was resurrected during the 1980s, when impaired gall bladder emptying was identified as a factor contributing to gallstone formation.[4 5] During the past 10 years, a third organ has been proposed as an important player in gallstone pathogenesis—the intestine. Several recent observations indicate slow intestinal transit in gallstone patients and animal models. Impaired intestinal transit might promote gallstone formation by increasing the amounts of the hydrophobic bile salt deoxycholate or by other mechanisms. In this review, we shall evaluate critically the pros and cons of the "intestinal hypothesis".

Cholesterol supersaturation and crystal formation

Cholesterol is a relatively insoluble amphiphile. Its solubility in water is only ~20 $\times 10^{-9}$ M, but in gall bladder bile, ~20 $\times 10^{-3}$ M of the sterol can be kept in solution.[6] This millionfold increase in solubility is explained by incorporation of cholesterol into micelles, together with bile salts and phospholipids (mainly phosphatidylcholine). Whereas cholesterol is soluble only to a limited extent in micelles that contain exclusively bile salts ("simple micelles"), addition of phosphatidylcholine leads to strongly enhanced solubility in "mixed" micelles, provided that this phospholipid contains unsaturated acyl chains at the sn-2 position.[7] Supersaturation occurs when either too much cholesterol or not enough bile salts and phosphatidylcholine are secreted to allow complete micellar solubilisation of all cholesterol in bile. Current thought is that increased biliary cholesterol secretion rather than decreased bile salt or phospholipid secretion is the primary cause of biliary cholesterol supersaturation.[8] Excessive cholesterol may be solubilised in vesicles (spherical bilayers of cholesterol and phospholipids, without bile salts). As these vesicles are often cholesterol-rich, they may—possibly after aggregation and fusion—nucleate cholesterol crystals, an essential step in gallstone formation.[9 10] A large number of proteins are secreted in bile, among them apolipoproteins A1 and A2, anionic polypeptide fraction, aminopeptidase N, α1 acid glycoprotein, mucin, haptoglobin, immune globulins, phospholipases A2 and C, and others. Based on in vitro studies, several of these proteins may either promote or inhibit crystallisation by stabilising or destabilising cholesterol-rich vesicles.

Postprandial gall bladder motility

Ingestion of a meal induces considerable gall bladder emptying, up to 70–80% of fasting gall bladder volume, by releasing the hormone cholecystokinin (CCK) from the duodenal wall. Apart from biliary cholesterol supersaturation and proteins, gall bladder motility may also influence gallstone formation. The basic concept is relatively simple: impaired gall bladder emptying prolongs residence of bile in the gall bladder, thus allowing more time for nucleation of cholesterol crystals from supersaturated bile. Furthermore, in the case of adequate gall bladder emptying, cholesterol crystals that have nucleated are ejected to the duodenum, whereas in the case of impaired gall bladder emptying, these crystals aggregate into macroscopic gallstones. Indeed, studies in patients with gallstones by either scintigraphic or ultrasonographic methods have identified a subgroup with impaired emptying, as defined by a low percentage decrease in fasting volumes or low amounts of ejected bile after consumption of a meal.[4] Even in patients with normal gall bladder emptying according to these criteria, there are often increased fasting and residual gall bladder volumes, despite normal amounts of bile ejected after feeding.[11 12] Traditionally, it has been assumed that after a meal, the gall bladder empties in a steady, progressive fashion and that gall bladder refill occurs only in the fasting state, between meals. This assumption has been challenged by studies using combined duodenal perfusion and scintigraphy[13] or minute by minute ultrasound[14]: there seems to be very frequent, short periods of gall bladder emtying and refilling ("bellow" concept), the balance between them determining whether net emptying—such as in the immediate postprandial period—or net refilling occurs. One should realise that, strictly speaking, ultrasound measures only gall bladder volumes (which are influenced by refilling), and HIDA scintigraphy measures the reduction in total gall bladder isotope counts (which are independent of refilling). Jazrawi and colleagues[15] recently introduced a method of combined scintigraphy and ultrasound that enables simultaneous, accurate quantitation of gall bladder emptying and refilling, as well as bile turnover. Interestingly, postprandial gall bladder emptying, refilling and bile turnover were all strongly depressed in patients with gallstones, thus suggesting a reduced postprandial washout effect. Nevertheless, one may argue that abnormal gall bladder motility in these patients could be secondary to the presence of stones or supersaturated bile in the gall bladder, with no relevance for gallstone formation. Indeed, studies in gallstone forming animal models have shown that, after its absorption by the gall bladder wall from supersaturated bile, excess cholesterol is incorporated within the sarcolemnal plasma membrane of the gall bladder muscle cell, with decreased membrane fluidity, impaired contractility and impaired relaxation as a result.[16–18] Although impaired motility could be in many cases secondary to biliary cholesterol supersaturation, it may still facilitate the process of gallstone formation: in the prairie dog model[19] and in humans,[20] impaired gall bladder motility occurs in the earliest stage of gallstone formation, before stones have formed. Gall bladder motility is also often impaired in many high risk situations for gallstone formation, such as pregnancy, obesity, diabetes mellitus, treatment with the somatostatin analogue octreotide, very low calory diets, and total parenteral nutrition. Prospective studies indicate that impaired gall bladder motility is also an independent risk factor for gallstone recurrence after extracorporeal shockwave lithotripsy.[21] Daily CCK injection during total parenteral nutrition[22] or

Abbreviations used in this paper: CCK, cholecystokinin; MMC, migrating motor complex.

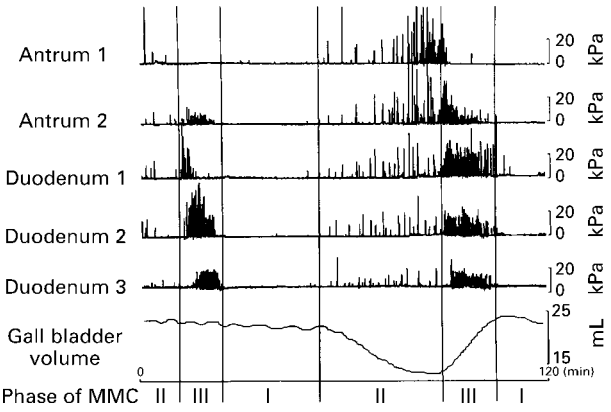

Figure 1 The intestinal migrating motor complex occurs periodically (1–2 hour cycle) during the fasting state and is characterised by three phases: contractile activity is absent during phase I; irregular activity occurs during phase II; and there are intense, regular coordinated contractions during phase III. Significant periodic gall bladder contraction occurs before phase III and is associated with a rise in plasma motilin concentrations.

inclusion of small amounts of fat in the diet during rapid weight loss[23] restore normal gall bladder motility and avoid the risk of gallstone formation. All these data suggest a role for gall bladder motility in gallstone formation.

The intestine: its relation to bile composition and gall bladder motility in the fasting (interdigestive) state

Recent data indicate that, along with the liver and the gall bladder, the intestine might also be involved in gallstone formation. The gall bladder and the small intestine determine enterohepatic cycling of bile salts and their flux through the liver. In patients with gallstones, both small intestinal[24] and whole gut[25] transit times are prolonged. What explanations could be offered for the association between prolonged intestinal transit and gallstone disease? One should remember one potential caveat: gallstones are rather frequent in the western world and the symptoms often attributed to them, such as right upper quadrant pain—with or without radiation to the back—or nausea, are not specific to gallstones.[26] These symptoms can also be caused by constipation as they often disappear upon changes in diet or pharmacological intervention. Aspecific abdominal symptoms may have led to ultrasonographic detection of gallstones and clinicians with special interest in gallstone research may have measured intestinal transit and included these patients in their case-control studies. Nevertheless, one study explicitly states that patients were unaware of the presence of stones.[25] One could also hypothesise that impaired intestinal motility is somehow causally related to gallstone formation. Indeed, the hydrophobic bile salt deoxycholate could be the link between impaired intestinal motility and lithogenic bile. The main bile salts in humans are cholate and chenodeoxycholate, both "primary" bile salts synthesised from cholesterol in the liver, and the "secondary" bile salt deoxycholate formed in the distal small intestine and colon by bacterial 7α-dehydroxylation of cholate. Deoxycholate is partly absorbed from the intestinal lumen and joins the enterohepatic circulation of bile salts after taurine or glycine conjugation in the liver. Prolonged intestinal transit time probably enhances formation of deoxycholate by increasing the intestinal residence time of bile salts, whereas decreasing intestinal transit time may have the opposite effect. As a result amounts of deoxycholate vary considerably in humans, between 10 and 30% of the total bile salt pool. Preliminary data from Dowling and coworkers[27] have revealed that patients with gallstones have increased amounts of Gram positive anaerobic bacteria and increased 7α-dehydroxylating activity in their caecum compared with controls. In these patients, slowness of colonic transit correlates with increased caecal pH and water solubility of deoxycholate (factors promoting intestinal absorption of the bile salt). What effects do increased amounts of biliary deoxycholate in bile have on lithogenicity? Several studies in patients with gallstones have found a positive correlation between the amount of biliary deoxycholate on the one hand and the cholesterol saturation index[24 28] or the speed of cholesterol crystallisation[28] on the other. In acromegalics, octreotide treatment induces a similar combination of increased intestinal transit times, more biliary deoxycholate, increased biliary cholesterol saturation index and fast crystallisation.[29 30] In contrast, treatment with ampicillin leads to decreased faecal 7α-dehydroxylation activity, decreased amounts of biliary deoxycholate and a lower biliary cholesterol saturation index.[31] In the ground squirrel[32] and in the C57L inbred mouse,[33] a cholesterol-rich diet induces a significant increase in the proportion of biliary deoxycholate, cholesterol supersaturation and gallstone formation. Furthermore, in the ground squirrel model, small intestinal transit is prolonged.[32] Thus, data from patients with gallstones and acromegalics on octreotide as well as animal models suggest an association between slow intestinal transit, high deoxycholate, cholesterol supersaturation and gallstone formation. One should realise, however, that this association is not universal: in humans on oral contraceptives (another risk factor for gallstone formation), cholesterol supersaturation and slow intestinal transit are associated with a decreased rather than increased deoxycholic acid pool size.[34] Furthermore, in both the ground squirrel and the C57L inbred mouse models on lithogenic diets, there is an increased proportion, not only of biliary deoxycholate, but also of the primary bile salt chenodeoxycholate. This might be explained by negative feedback suppression through the hydrophobic deoxycholate on hepatic cholesterol 7α-hydroxylase (the rate controlling enzyme in the classic pathway of bile salt synthesis) and a higher contribution to bile salt synthesis of the alternative or acidic pathway through the mitochondrial enzyme sterol 27α-hydroxylase as the alternative pathway has a preference for chenodeoxycholate over cholate synthesis.[35 36]

Increased deoxycholate could promote lithogenic bile and gallstone formation through several mechanisms. Firstly, deoxycholate itself can slow down intestinal transit,[37] thereby allowing more time for intestinal cholesterol absorption and exerting a positive feedback on its own formation. The mechanism for this effect is not entirely clear: although in in vitro studies, bile salts seem to exert a depressant effect on intestinal muscle contractility,[38] increased maximal contractile responses of intestinal circular and longitudinal smooth muscle strips on bethanechol stimulation have been reported in ground squirrels on a lithogenic diet, associated with slow small intestinal transit and biliary deoxycholate enrichment.[32] More deoxycholate could theoretically also increase intestinal cholesterol absorption by means of enhanced micellar solubilisation of the sterol. This effect, however, is probably more relevant in animal models with rather hydrophilic endogenous bile salts[32 33] than in humans with a relatively hydrophobic endogenous bile salt pool. Secondly, deoxycholate could enhance biliary cholesterol secretion by an effect on the hepatocytic canalicular membrane. The outer leaflet of the membrane contains large amounts of cholesterol and the phospholipid sphingomyelin (associated in laterally separated domains), which confers resistance to the detergent action of bile salts by decreasing membrane

fluidity. Particularly hydrophobic bile salts such as deoxycholate can release cholesterol from the sphingomyelin domains (most likely by decreasing the activation energy necessary for desorption of the sterol from the membrane), thus allowing its secretion into bile.[7] Thirdly, in vitro studies have revealed that deoxycholate enhances biliary cholesterol crystallisation by destabilising cholesterol-rich vesicles.[39] This effect (paradoxical if one would only consider the enhanced micellar cholesterol solubilisation in deoxycholate-rich biles) can be nicely explained by the elegant model studies performed by Wang and Carey[40]: deoxycholate leads to a rightward shift in the ternary (cholesterol-phospholipid-bile salt) phase diagram, with inherent faster cholesterol crystallisation.

Apart from the effects on biliary deoxycholate another, possibly complementary, link between the intestine and gallstone formation could be the relation between intestinal and gall bladder motility in the fasting (also called "interdigestive") state. As fig 1 shows, significant periodic gall bladder emptying occurs during this period (20–30% emptying in the fasting state compared with 70–80% emptying after a meal) at one to two hour intervals, associated with the cycle of the intestinal migrating motor complex (MMC) and with a rise in plasma motilin concentrations.[41 42] The MMC, a pattern of cyclic contractile activity displayed in the fasting state by the upper intestinal tract, is characterised by three phases: during phase I, contractile activity is absent; irregular activity occurs during phase II; and during phase III there are intense, regular coordinated contractions. In healthy people, significant gall bladder emptying and high plasma motilin concentrations are observed before phase III.[42–44] We found that patients with gallstones have less frequent MMC cycles, absent interdigestive gall bladder emptying and an altered pattern of motilin release compared with controls.[45] A similarly prolonged MMC cycle has been found in the ground squirrel model of gallstone formation.[46] The fasting state, namely the night, would seem to be the most vulnerable period for gallstone formation. During this period, biliary cholesterol saturation is highest as a result of relatively low bile salt secretion and relatively high cholesterol secretion. There is also a progressive concentration of gall bladder bile during this period, which is partially counteracted by periodic interdigestive gall bladder contractions in association with phase III of the MMC.[47] Bile concentration has important consequences: it leads to higher micellar cholesterol (and phospholipid) solubilisation by increasing the micellar phase boundary.[40] As phospholipids are much more easily solubilised in micelles than cholesterol, there is a much larger shift of phospholipid than cholesterol from vesicles to micelles. The remaining vesicles should therefore be enriched in cholesterol and prone to nucleate cholesterol crystals.[48 49] According to this concept, the reduced frequency of phase III and absent interdigestive gall bladder contractility, as found in our patients with gallstones, could promote bile concentration, crystallisation and gallstone formation. During the earliest stages of gallstone formation in the prairie dog model there is also excessive concentration of bile within the gall bladder[50] and prevention of excessive bile concentration with the aid of the drug amiloride prevents gallstone formation.[51] Interestingly, cholesterol absorption by the gall bladder wall seems to function as a protective mechanism against cholesterol crystallisation under these circumstances, because it leads to a lower biliary cholesterol saturation. Using the isolated pig gall bladder model, Corradini and colleagues[52] have confirmed earlier reports[53 54] that significant cholesterol absorption as well as some phospholipid absorption occurs from supersaturated biles. In contrast, there is virtually no bile salt absorption. This defence mechanism may be operating particularly in the case of impaired gall bladder emptying.[55] Based on indirect evidence (comparison of protein concentrations in hepatic and associated gall bladder biles), Groen and coworkers[56] have postulated a similar absorption of pronucleating proteins by the gall bladder wall as an additional defence mechanism against crystallisation.

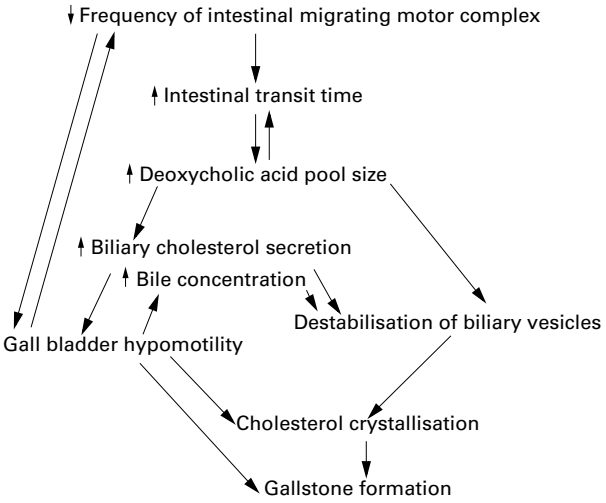

Figure 2 Potential mechanisms of cholesterol gallstone formation.

Summary

Current evidence suggests that impaired intestinal motility may facilitate gallstone formation by influencing biliary deoxycholate levels or by modulating interdigestive gall bladder motility (fig 2), although a primary intestinal defect in gallstone pathogenesis has not yet been demonstrated. In the cold war period, most interesting events, from a political point of view, occurred at the border between capitalist and communist systems, near the iron curtain. Similarly, the gall bladder and biliary tract can be viewed as the border between liver and intestinal tract, where many interesting things occur with profound impact on both systems. Combined efforts by researchers in the field of hepatology and gastrointestinal motility should brake down the Berlin wall of ignorance of one of the most common diseases in the Western world.

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