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Relation of Gallbladder Motility to Viscosity and Composition of Gallbladder Bile in Patients with Cholesterol Gallstones

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Key Words
Biliary lipids · Cholecystolithiasis · Ejection fraction of the gallbladder · Newtonian and non-Newtonian fluid · Gallstones, pathogenesis

Abstract
Background/Aims: Increased viscosity and supersaturation of cholesterol in gallbladder bile, as well as an impaired motility of the gallbladder, are considered to be important factors in the pathogenesis of cholesterol gallstones. However, the relation of these parameters has not yet been determined. ***Material and Methods:*** Bile viscosity (mPa·s) was measured by rotation viscosimetry and the composition of gallbladder bile was determined using standard methodology. Gallbladder motility was calculated as ejection fraction in percent of total volume 45 min after a test meal using ultrasonography in patients with gallstones prior to elective cholecystectomy. ***Results:*** The study included 35 patients with cholesterol gallstones. Viscosity of gallbladder bile ranged between 0.9 and 12.5 mPa·s (median 2.2 mPa·s) and an ejection fraction of the gallbladder of 55.4 ± 18.3% (mean ± SD) was determined. No significant correlation ($r = 0.19$, $p < 0.2$) between the 2 parameters could be calculated. Analysis of the composition of gallbladder bile revealed a positive correlation of all components to biliary viscosity but not to the motility of the gallbladder, with the exceptions of a negative correlation ($r = 0.39$, $p < 0.02$) between mucin concentration and the ejection fraction at 45 min after the test meal. ***Conclusions:*** The motility of the gallbladder appears to be unrelated to the viscosity of gallbladder bile or gallbladder bile composition. The negative correlation between the ejection fraction of the gallbladder and mucin concentration of gallbladder bile suggests that chronic inflammation of the gallbladder wall is associated with both an impaired motility of the gallbladder and increased mucin release into gallbladder bile.

The formation of cholesterol monohydrate crystals is believed to be crucial in the pathogenesis of cholesterol gallstones [1]. Although virtually insoluble in water, cholesterol is made soluble in bile through carriers which include bile salts and phospholipids. In unsaturated bile, cholesterol is primarily transported in simple and mixed micelles. As cholesterol saturation increases in bile, more cholesterol is carried in larger phospholipid cholesterol vesicles. Unilamellar vesicles can coalesce into multilamellar vesicles, which tend to be less stable and allow the

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growth of cholesterol crystals on the surface [2–4]. These vesicles may interact with soluble mucin which acts as an annealing agent, favoring the further nucleation and agglomeration of cholesterol monohydrate crystals [5–7]. These crystals are entrapped in the soluble or gel form of mucin which makes up biliary sludge [8].

An association between hexosamine concentrations in bile, which are mostly derived from soluble mucin, and the viscosity of bile has already been described by Bouchier et al. [9] and was confirmed more recently by Shoda et al. [10]. Bile behaves as a Newtonian fluid with a constant viscosity only at high shear rates, whereas a non-Newtonian fluid shows a disproportionate increase in viscosity at a low-flow velocity. We used the Contraves LS-30 coaxial rotation viscosimeter at different high shear rates (65.6–152.7 s^{-1}), thus obtaining a Newtonian flow of gallbladder bile.

Impaired motility of the gallbladder is a well-known risk factor of cholesterol gallstone disease and its pathogenetic role has been extensively reviewed [11]. In a recent study, we found that the concentration of mucin is the major determinant of biliary viscosity. We concluded that an increased secretion of mucin by the gallbladder epithelium might inhibit the emptying of the gallbladder, thereby favoring the formation of gallstones [12].

However, the relation of the composition of gallbladder bile, e.g. mucin concentration and viscosity, to gallbladder motility has not yet been investigated. For this reason, we made it the aim of our study.

Methods

Patients, Determination of Gallbladder Motility and Collection of Bile

Forty-two patients who underwent laparoscopic surgery for symptomatic gallstone disease were included in the study. Gallstones were visualized by ultrasonography and gallbladder motility was determined by comparing the fasting volume to volumes measured after a liquid test meal [13, 14]. The sum of the cylinder method with 3D-ultrasound technology was used for the determination of gallbladder volume in the fasting state (12 h) and every 5 min after the application of a liquid test meal for 90 min (250 ml Nutrodip® energy drink, Wander, Ostholm, Germany). The drink consisted of 14.3 g protein, 15.5 g fat, 50.3 g carbohydrates and 188 g water. As a quantitative parameter for gallbladder motility, we calculated the ejection fraction 45 min after the test meal (fasting volume – volume at 45 min)/fasting volume × 100 = %. All patients gave informed consent after a detailed explanation of the procedure required for intraoperative bile collection. During laparoscopic cholecystectomy, the gallbladder was punctured and bile was aspirated as completely as possible on account of the known stratification of human bile [15]. The procedures were in accordance with the ethical standards of the responsible committee (No. 108/01; August 21, 2001) and with the Helsinki Declaration of 1975, as revised in 1983. After stone analysis, 7 patients with gallstones containing less than 50% cholesterol were excluded from the study. The remaining 35 patients, 22 women and 13 men with a mean age of 66 years (range 25–82; BMI 26.4 ± 2.9), were analyzed. The exact amount of the gallstone burden was not calculated, but 26 patients had multiple stones and 9 patients had solitary stones. Moreover, in 20 healthy controls, 14 women and 6 men with a mean age of 48 years (range 22–76; BMI 23.4 ± 2.5), only gallbladder motility was assessed.

Stone Analysis and Microscopy of Bile

Stones were removed, washed with distilled water, dried, weighed and ground to a powder. The cholesterol content of the stones was measured chemically after extraction with an organic solvent and expressed as a percentage of dry weight [16].

Analysis of Bile Composition

For the analysis of bile composition, duplicate aliquots were stored at –30°C prior to determination. Cholesterol was determined colorimetrically with the Liebermann-Burchard reaction after the double extraction of 1 ml of a methanolic bile sample with petroleum ether [17]. Phospholipids were measured as total biliary phosphate after hydrolysis at a temperature of 150°C with sulfuric acid, using the colorimetric assay of Fiske-Subbarow, and total bile salts were determined by a modified 3-α-hydroxysteroid dehydrogenase method [18, 19]. The saturation index of each sample was calculated in native bile by dividing the cholesterol concentration with the maximum cholesterol solubility according to Carey and was corrected for the total lipid content of individual bile [20]. Total protein content was analyzed using the Lowry assay after the purification of biliary proteins [21], and biliary mucin concentration was determined according to an assay first described by Miquel et al. [22].

Determination of Viscosity

The rheologic measurements were carried out on a calibrated Contraves Low Shear-30 rotation viscosimeter, using a coaxial cylinder system with a gap width of 0.5 mm (LS 2T-2T, Contraves AG, Zürich, Switzerland). The rotation viscosimeter allows accurate measurements of viscosity for both Newtonian and non-Newtonian fluids. Programming of measurements and the processing of the measured data were performed utilizing the Contraves Rheoscan 30. To obtain a rapid standardized measurement within the applied high shear rates (65.6–152.7 s^{-1}), a computer program was used to enable measurements in one sample within 5 min [12]. These were repeated twice after intervals of 60 s. Thus, the final result of biliary viscosity represented the mean value from a total of 30 determinations. One milliliter of the bile sample was taken for all viscosity assays and all measurements were performed at 37°C. All samples were centrifuged for 2 min at 12,000 *g*, to eliminate sediment that could interfere with the determinations.

Statistical Analysis

Values of parametric data are expressed as the mean ± SD and nonparametric data are expressed as median and range. Spearman's correlation coefficients were calculated between variables and $p < 0.05$ was considered to be significant.

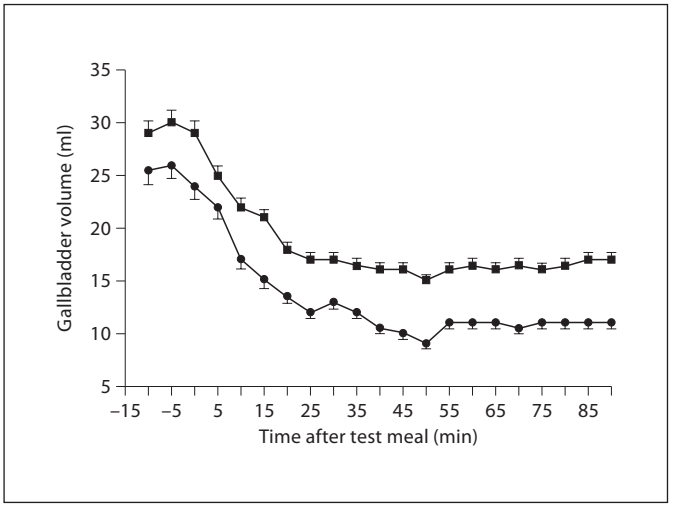

Fig. 1. Kinetics of gallbladder volume between the fasting state and after a liquid test meal in 20 healthy controls (●) and 35 gallstone patients (■).

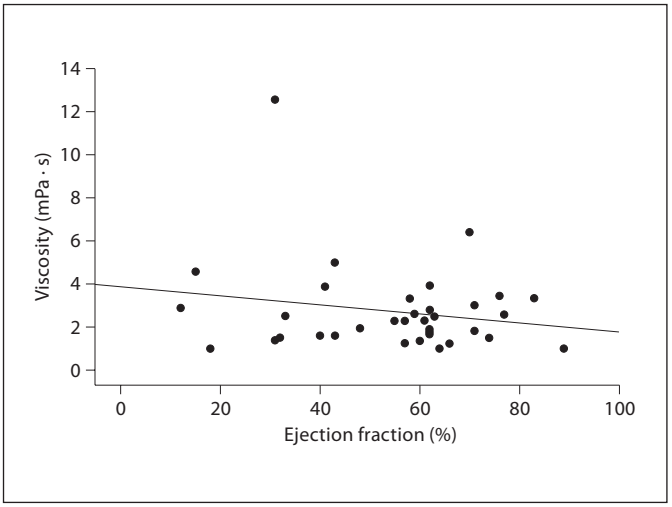

Fig. 2. Relation of viscosity of gallbladder bile and the ejection fraction of the gallbladder after 45 min of a liquid test meal in 35 patients with cholesterol gallstones ($r = 0.19$, $p < 0.2$).

Results

Kinetics of Gallbladder Volume

Gallbladder volume in the fasting state and after a liquid test meal in 20 healthy controls and 35 cholesterol gallstone patients is illustrated in figure 1. The minimal residual gallbladder volume was reached in nearly all controls and patients within 45 min after the test meal. Therefore, we used this fixed time-point in order to assess the extent of gallbladder emptying and the calculation of the ejection fraction.

Ejection Fraction and Viscosity

The ejection fraction 45 min after the test meal (Nutrodip) ranged between 12 and 89% of the fasting volume of the gallbladder (55.4 ± 18.3%, mean ± SD). At different high shear rates (65.6–152.7 s^{-1}), viscosities between 0.85 and 12.55 mPa·s were determined (median 2.28 mPa·s). Both parameters were correlated in figure 2 and no significant correlation was visible ($r = 0.19$, $p < 0.2$).

Composition of Gallbladder Bile

Biochemical analysis of gallbladder bile revealed concentrations of proteins (9.9 ± 6.1 mg/ml), mucin (0.15 ± 0.09 mg/ml), bile acids (113.3 ± 47.7 mmol/l), phospholipids (52.1 ± 25.7 mmol/l), cholesterol (21.0 ± 12.4 mmol/l) and total lipids (11.1 ± 4.2 g/dl). Based on the lipid composition, a Cholesterol Saturation Index of 1.6 ± 1.1 was calculated.

Correlation of Viscosity and Composition of Gallbladder Bile

Further evaluation of individual values showed a positive statistical correlation of viscosity to all components of gallbladder bile, yielding the correlation coefficients $r = 0.59$, $p < 0.001$ vs. protein, $r = 0.49$, $p < 0.01$ vs. mucin, $r = 0.55$, $p < 0.001$ vs. bile acids, $r = 0.47$, $p < 0.01$ vs. phospholipids, $r = 0.66$, $p < 0.001$ vs. cholesterol and $r = 0.63$, $p < 0.001$ vs. total lipids (fig. 3a–f).

Correlation of Ejection Fraction to BMI and Composition of Gallbladder Bile

No correlation between BMI or any of the components of gallbladder bile, including cholesterol saturation and the ejection fraction, was obtained with the exception of an inverse correlation to mucin concentration ($r = -0.39$, $p < 0.02$) as illustrated in figure 4.

Discussion

No discussion of cholesterol gallstone formation would be complete without a Venn diagram illustrating the 3 components of the 'triple defect' which is (1) supersaturated bile, (2) abnormal nucleation and (3) stasis within the gallbladder [23]. Stasis may be due to the impaired gallbladder emptying, or to excess mucus-glycoprotein synthesis and secretion by the gallbladder mucosa. The cycle of gallbladder filling and emptying controls the

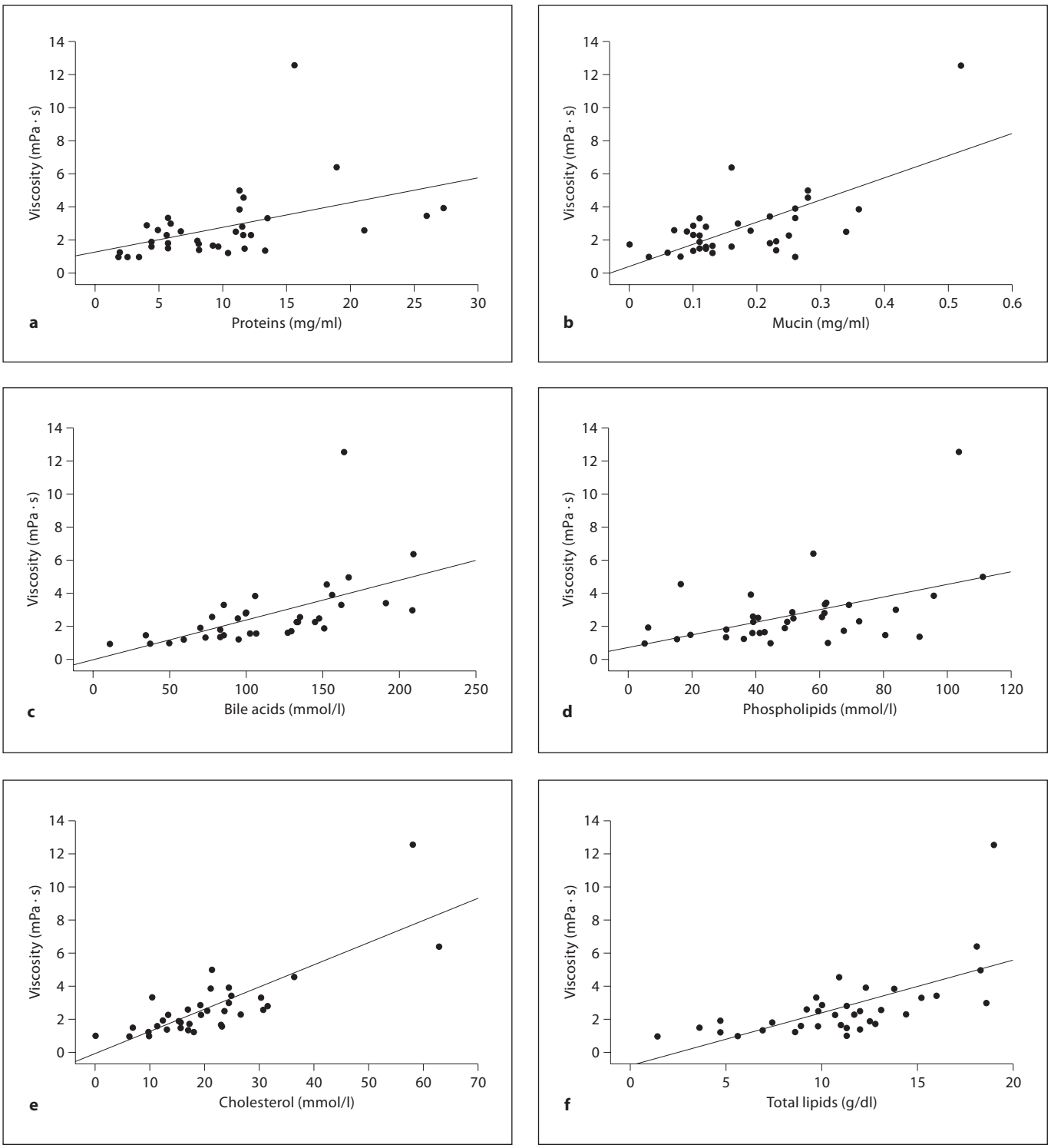

Fig. 3. Relation of viscosity to the concentration of proteins, mucin, bile acids, phospholipids, cholesterol and total lipids in gallbladder bile of 35 patients with cholesterol stones.

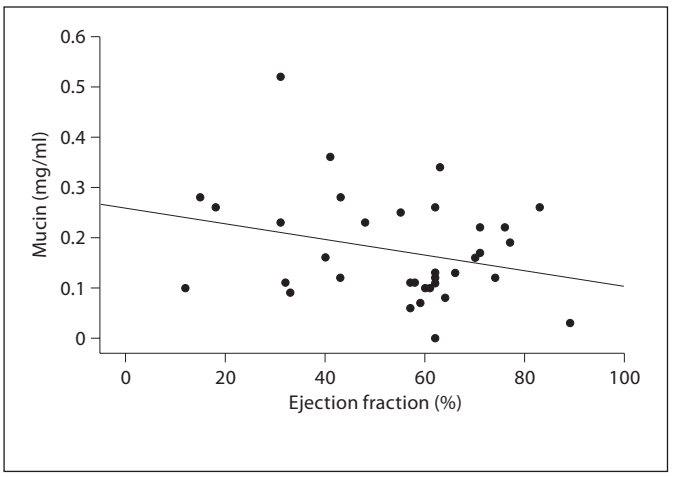

Fig. 4. Relation of mucin concentration of gallbladder bile and the ejection fraction of the gallbladder after 45 min of a liquid test meal in 35 patients with cholesterol gallstones ($r = 0.39$, $p < 0.02$).

flow of bile into the intestines for digestion. It has recently been shown that fibroblast growth factor 15, a hormone made by the distal small intestine in response to bile acids, is required for gallbladder filling [24]. Obviously, gallbladder filling is actively regulated by an endocrine pathway and suggests the presence of a postprandial timing mechanism that controls gallbladder motility.

In patients with cholesterol gallstones, gallbladder dysmotility is principally characterized by increased fasting and residual volumes [25, 26]. In studies of large numbers of gallstone patients, contraction is impaired only in about 50% and many have normal emptying in response to a fat-containing meal. The impairment of gallbladder emptying is not the result of the physical presence of gallstones, as the defect does not correlate with the size or number of stones, and stone clearance with lithotripsy does not reverse the motility defect [27, 28]. It has been shown that the degree of impairment of gallbladder emptying increases in proportion to the cholesterol content of gallbladder bile even in healthy subjects without stones [29]. Apparently, hypomotility is not found in pigment-stone gallbladders as inferred from ex vivo studies, but is a feature of cholesterolosis of the gallbladder [30].

The intracellular mechanisms for smooth muscle contraction in human gallbladder muscle cells in gallbladders with cholesterol gallstones appear to be intact [26]. This supports the hypothesis that the absorption of molecular cholesterol from the gallbladder lumen is associated with muscle dysfunction.

Indeed, following epithelial absorption, cholesterol is incorporated into the sarcolemma of gallbladder smooth muscle cells causing contraction defects and gallbladder relaxation. The impairment of gallbladder motor function delays gallbladder emptying. This alteration increases the contact time of precipitated cholesterol with the mucosa, leading to additional cholesterol absorption and more extensive muscular damage [11].

In our study, however, no relation between the ejection fraction as a parameter of gallbladder motility and the cholesterol concentration or saturation of gallbladder bile could be obtained.

Moreover, we did not find a relation between the ejection fraction of the gallbladder 45 min after the test meal and any other component of gallbladder bile, with the exception of an inverse relation to mucin concentration. Although it has been shown repeatedly, and also confirmed in this study, that mucin concentration and viscosity of gallbladder bile are positively correlated, it is unlikely that mucin in bile directly influences gallbladder motility [10, 12].

No relation between viscosity in bile and the ejection fraction of the gallbladder after the test meal could be demonstrated. Therefore, we assume that the increased concentration of mucin in gallbladder bile is a marker of chronic inflammation of the gallbladder wall caused by an increased release of mucin from gallbladder epithelial cells into the lumen. Recent results supporting a promoter effect of gallbladder mucin hypersecretion by lipid peroxidation and the roles of infection, inflammation and the immune system in cholesterol gallstone formation have been extensively reviewed [31, 32]. Substances involved in inflammation processes can strongly influence gallbladder contractility. An increased proportion of arachidonyl-phosphatidylcholine species, a marker of gallbladder-mucosa inflammation and mucin concentration, are found in the animal model fed on a lithogenic diet [11].

Furthermore, in a large previous study including more than 100 patients with cholesterol stones, histological features of the inflammation of the gallbladder wall were quantitated and related to the composition of gallbladder bile. A higher grade of inflammation did not affect the concentration of biliary lipids, but was clearly associated with an increased concentration of total protein and mucin levels in gallbladder bile [33]. Although we cannot provide a quantitative assessment of the histological gallbladder inflammation in this study, it seems to justify our final conclusion that chronic inflammation of the gallbladder wall is associated with both an impaired motility

of the gallbladder and increased mucin release into gallbladder bile.

Thus, chronic inflammation of the gallbladder wall may well be a factor responsible for impaired gallbladder contraction and increased mucin concentration in bile, favoring the formation of cholesterol gallstones by both mechanisms.

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