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ORIGINAL ARTICLE

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Effects of colectomy on bile composition, cholesterol saturation and cholesterol crystal formation in humans

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Abstract Total or subtotal colectomy is the surgical treatment of choice for patients with ulcerative colitis. Recently it has been reported that colectomy may lead to increased lithogenicity of bile, short nucleation time, cholesterol crystal formation, and gallstone disease. We examined whether colectomy in patients with ulcerative colitis leads to changes in bile composition that predisposes to cholesterol crystal formation and cholesterol gallstone disease. Ten consecutive patients who had previously undergone ileostomy and colectomy because of ulcerative colitis were admitted for ileal pouch surgery. At operation bile was obtained by puncture of the gallbladder. Controls were 35 patients undergoing cholecystectomy (23 for cholesterol gallstone disease and 12 for reasons other than gallstone disease). The gallbladder bile was analyzed for cholesterol crystals, bile acid, and biliary lipid composition, cholesterol saturation, and nucleation time. The colectomized patients had normal biliary lipid composition, normal cholesterol saturation, and normal nucleation time, in contrast to gallstone patients who displayed highly supersaturated bile with a short nucleation time. Thus patients with ileostomy after colectomy because of ulcerative colitis have normal cholesterol saturation and nucleation time of bile.

Keywords Bile · Colectomy · Cholelithiasis · Cholesterol saturation · Nucleation time

Introduction

The production of bile acids is a major pathway for cholesterol excretion in humans [1]. The bile acids are very efficiently reabsorbed from the intestine during their enterohepatic circulation. The absorption occurs mainly in the distal part of ileum by an active process. Primary bile acids entering the colon are deconjugated and dehydroxylated by microbial enzymes to form the secondary bile acids, deoxycholic acid, and lithocholic acid. Deoxycholic acid is partly absorbed, but lithocholic acid is mainly lost in the feces [1, 2]. Thus the colon also plays an important role in the enterohepatic circulation of bile acids.

Total or subtotal colectomy are the surgical treatments of choice for patients with ulcerative colitis. Colectomy is reported to be associated with an increased incidence of cholelithiasis [3, 4]. In agreement with this, Harvey et al. [5], Makino et al. [6], and Chijiiwa et al. [7] have shown that patients after colectomy develop an increased cholesterol saturation index and rapid nucleation time of gallbladder bile, which are two important factors for developing cholesterol gallstones. On the other hand, a study by our group [8] and a recent study by Galatola et al. [9] showed no increase in the cholesterol saturation index after colectomy. An important difference between the studies is that Harvey et al., Makino et al., and Chijiiwa et al. analyzed gallbladder bile while the analyses by Galatola et al. and our group were performed on duodenal bile samples.

The aim of the present study was to further examine whether colectomy leads to changes in bile composition

that predispose to cholesterol crystal formation and gallstone disease. To do this we determined biliary lipid composition and cholesterol nucleation time of gallbladder bile obtained from patients previously colectomized.

Methods and materials

Patients

The study comprised a group of ten consecutive patients with ileostomy (six men, four women; mean age 41 years) who were admitted for restorative proctectomy with ileal pouch formation (Table 1). They had undergone subtotal colectomy due to ulcerative colitis between 4 months and 1 year prior to the study.

Two control groups were included. One comprised 23 patients (4 men, 19 women; mean age 46 years) with cholesterol gallstone disease who were admitted for elective cholecystectomy, and the other consisted of 12 patients (3 men, 9 women; mean age 43 years) without gallstones who were also admitted for cholecystectomy. The indication for cholecystectomy in the latter group was roentenographic suspicion of polyps in the gallbladder. However, no polyps or other macroscopic abnormalities were found in the removed gallbladders, although four specimens displayed mild chronic inflammation of the gallbladder. All patients were in good health, as judged by history, clinical examination, and routine blood chemistry. None of patients was on any drug therapy.

Informed consent was obtained from each patient. The ethical aspects of the study were approved by the ethics committee of Karolinska Institute.

Experimental procedure

The patients were admitted to the hospital the day before operation and were given the hospital diet containing about 0.5 mmol cholesterol per day. To avoid any possible diurnal variation, the operations were started in the morning after a 12-h fast. Bile from the gallbladder was obtained by needle aspiration as soon as the abdomen was opened. The bile was immediately transported to the laboratory for analysis.

Analysis of biliary lipids and bile acid composition

A portion of the gallbladder bile was immediately extracted with 20 volumes of chloroform-methanol, 2:1 (vol/vol), and analyzed for cholesterol and phospholipids. Cholesterol was determined by an enzymatic method [10] and phospholipids by the method of Rouser et al. [11]. The total bile acid concentration in one aliquot of the bile sample was determined using a 3α-hydroxysteroid dehydrogenase assay [12]. The relative concentrations of cholesterol, bile acids, and phospholipids were expressed as molar percentages of the total biliary lipids. The cholesterol saturation was calculated according to Carey [13]. Bile acid composition was determined using gas-liquid chromatography [14].

Analysis of cholesterol crystals and nucleation time

Gallbladder bile samples were examined for typical rhomboid monohydrate cholesterol crystals by polarizing light microscopy on prewarmed slides. Nucleation time was determined by the method of Holan et al. [15] with minor modifications [16]. After centrifugation of about 6 ml bile at 100,000 g for 2 h, 3 ml from the middle phase was transferred into a sterile glass vial and sealed with a cap equipped with permeable silicon membrane. The vial was stored in darkness in an incubator at 37°C. About 3 µl from the top, middle, and bottom portions was aspirated each day, mixed, and placed on a prewarmed slide and viewed thoroughly using polarizing light microscopy. Nucleation time was defined as the number of days until typical rhomboid monohydrate cholesterol crystals appeared. Because of limited amounts of bile, these investigations could not be performed in two of the colectomized patients.

Statistical analysis

Data are given as mean ±SEM. The significance of differences was evaluated with Student's t test.

Results

Data on biliary lipid composition, cholesterol saturation, nucleation time and occurrence of crystals are given in Table 2. Gallstone patients had a more cholesterol, less biliary lipid concentration, and more cholesterol saturation of bile than gallstone-free patients. Most of the gallstone patients had cholesterol-saturated bile, while most of the gallstone free subjects had unsaturated bile (Fig. 1). Gallstone patients had very short nucleation time compared with gallstone-free patients (Fig. 2). About 75% of gallstone patients displayed cholesterol crystals in their bile, while only one of the gallstone free patients had crystals.

Patients with previous colectomy had normal biliary lipid composition and lipid concentration of their gallbladder bile (Table 2). The cholesterol saturation did not differ significantly from that in gallstone-free controls. Only one patient had supersaturated bile (Fig. 1). Also the nucleation time was quite normal in the patients with previous colectomy (Table 2). As can be seen in Fig. 2, only one patient had a nucleation time shorter than 5 days. None of the patients displayed cholesterol crystals in their bile (Table 2).

Bile acid composition is shown in Table 3. Cholic acid, chenodeoxycholic acid, and deoxycholic acid were

Table 1 Basal clinical data of the patients

	Colectomized		Gallstone free		Gallstone	
	Mean	Range	Mean	Range	Mean	Range
Age (years)	41	31–51	43	17–74	46	22–73
Relative body weight (%)[a]	93	80–110	97	73–122	95	64–119
Plasma cholesterol (mmol/l)	4.8	3.4–5.9	5.2	3.6–7.0	4.9	3.6–6.9
Plasma triglycerides (mmol/l)	1.3	0.4–2.3	1.3	0.6–2.1	1.1	0.3–2.3

[a] Calculated as (kg weight)/[(cm height)–100]×100

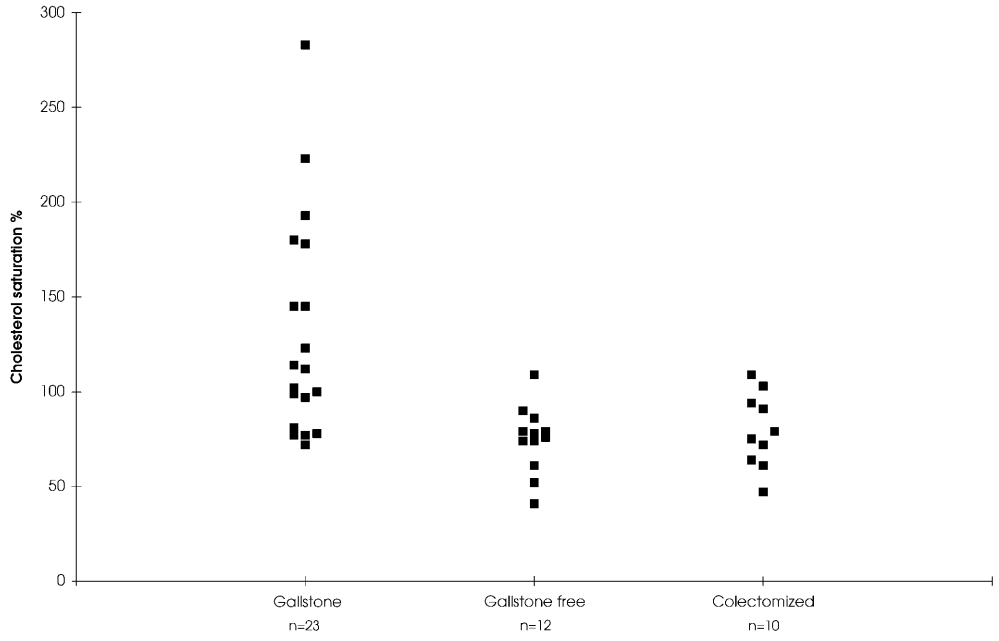

Fig. 1 Cholesterol saturation of gallbladder bile in cholesterol gallstone patients, gallstone-free subjects, and colectomized patients

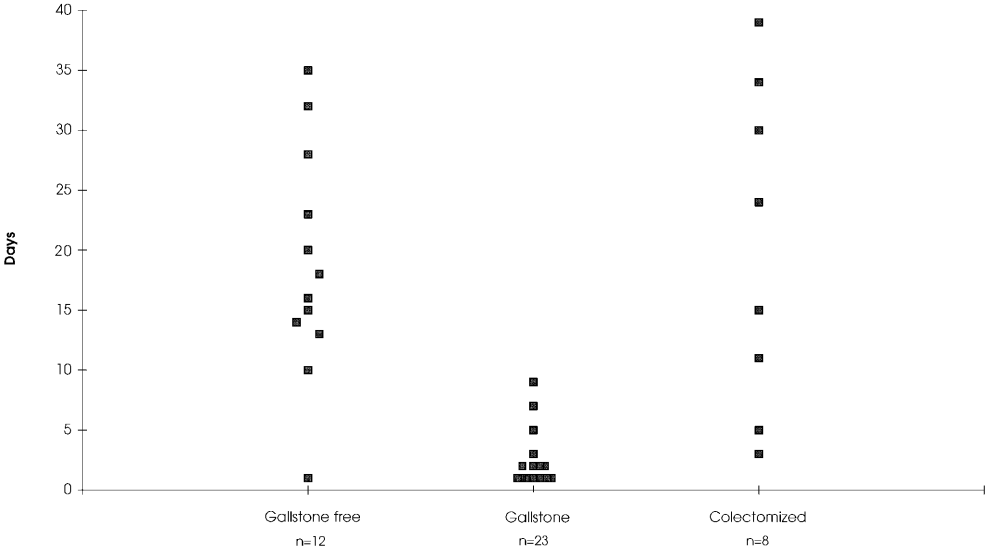

Fig. 2 Nucleation time of gallbladder bile in cholesterol gallstone patients, gallstone-free subjects, and colectomized patients

Table 2 Biliary lipid composition, cholesterol saturation, nucleation time, and occurrence of cholesterol crystals

	Colectomized	Gallstone free	Gallstone
Cholesterol (molar %)	5.8±0.4	5.4±0.5	7.8±0.6*
Bile acids (molar %)	69.6±1.1	72.7±1.2	70.2±1.1
Phospholipids (molar %)	24.5±1.3	21.7±0.8	21.9±0.8
Lipid concentration (g/dl)	13.0±1.7	10.7±1.2	5.8±0.9**
Cholesterol saturation (%)	79±6	75±5	130±13**
Nucleation time (days)	20±5	18±2	2±1***
Number of patients crystals/no crystals	0/8	1/11	17/6

*$P<0.01$, **$P<0.005$, ***$P<0.0001$ vs. gallstone free

Table 3 Bile acid composition (*ND* not detected)

	Colectomized	Gallstone free	Gallstone
Cholic acid (%)	61±3	45±4	40±3
Chenodeoxycholic acid (%)	39±3	31±2	34±2
Deoxycholic acid (%)	ND	23±4	25±3
Ursodeoxycholic acid (%)	ND	1.2±0.5	5.5±2.1
Lithocholic acid (%)	ND	0.3±0.3	1.4±0.3

the dominant bile acids both in gallstone patients and in gallstone-free subjects. Only small amounts of ursodeoxycholic acid and lithocholic acid were found. In patients with previous colectomy only cholic acid and chenodeoxycholic acid were found.

Discussion

This study represents the extension of a previous one from our group on biliary lipid composition of duodenal bile in patients with ileostomy and previous colectomy because of ulcerative colitis [8]. In the present study gallbladder bile was obtained by direct puncture of the gallbladder. The results of the two studies show that colectomy per se is associated with normal biliary lipid composition and cholesterol saturation of bile. Also the nucleation time was quite normal, and none of the patients displayed cholesterol crystals. In agreement with our results, Galatola et al. [9] have recently shown that patients with colectomy and ileoanal anastomosis also have a normal saturation index of duodenal bile. In contrast to the above studies, Harvey et al. [5], Makino et al. [6], and Chijiiwa et al. [7] have reported that patients with previous colectomy show abnormal lipid composition of their gallbladder bile. Their patients, as with gallstone patients, had supersaturated bile with short nucleation time. Most of the patients also displayed cholesterol crystals in the bile.

In our patients with previous colectomy only cholic acid and chenodeoxycholic acid were found in the bile. Secondary bile acids, such as deoxycholic and lithocholic acid, were absent. This is a well recognized phenomenon resulting from the loss of the colonic bacterial flora [17, 18, 19]. According to some authors, gallstone disease is associated with an increased proportion of deoxycholic acid in bile [20, 21]. Some authors have reported a positive correlation between the percentage of biliary deoxycholic acid and the molar percentage of biliary cholesterol and cholesterol saturation of bile in gallstone patients and gallstone-free subjects [22, 23]. However, other authors have not found any correlation between cholesterol saturation and the percentage of deoxycholic acid [24, 25] (U. Gustafsson, S. Sahlin, C. Einarsson, unpublished data). In agreement with this, lack of deoxycholic acid in the colectomy group of the present study did not lead to a reduction in cholesterol saturation.

The reason for the discrepancy between the results on cholesterol saturation and nucleation time obtained by the various research groups can only be speculated on. Makino et al. [6] and Chijiiwa et al. [7] studied a heterogeneous group of only 6 patients. Their controls were 11 patients with various types of carcinoma. Harvey et al. [5] compared colectomized patients with a group of patients admitted for elective colectomy because of ulcerative colitis. Surprisingly, almost half of the patients had short nucleation time, and several displayed crystals in their bile even before operation and can hardly be considered as healthy controls. It seems as if the two groups of patients with ulcerative colitis studied by Harvey et al., the precolectomy and postcolectomy groups, were patients prone to develop cholesterol gallstones. In fact 3 of 20 patients did develop cholesterol gallstones during a follow-up time of minimal 3 years.

Why should colectomy be associated with an enhanced risk of gallstone formation, as has been reported [3], if colectomized patients have normal lipid composition, cholesterol saturation, and nucleation time of their gallbladder bile. Could it be due to a motility defect of the gallbladder? Impaired gallbladder emptying has previously been documented in a high proportion of patients with gallstones [26]. In fact, Damiao et al. [27] have recently reported diminished gallbladder emptying with ensuing stasis in colectomized patients with ulcerative colitis. A motility defect is therefore a possible explanation for an increased prevalence of gallstones in colectomized patients. Another possible explanation is that colectomized patients have an increased risk to form pigment stones.

In conclusion, our findings show that patients colectomized because of ulcerative colitis have normal cholesterol saturation and normal nucleation time of their bile, and consequently they should have a relatively low risk of forming cholesterol gallstones.

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