Effects of Cholecystectomy on the Kinetics of Primary and Secondary Bile Acids

Frieder Berr, Frans Stellaard, Ekkehard Pratschke,* and Gustav Paumgartner
*Departments of Medicine II and *Surgery, Klinikum Grosshadern, University of Munich, Federal Republic of Germany*

Abstract

Removal of the gallbladder is thought to increase formation and pool size of secondary bile acids, mainly deoxycholic acid (DCA), by increased exposure of primary bile acids (cholic acid [CA], chenodeoxycholic acid [CDCA]) to bacterial dehydroxylation in the intestine. We have tested this hypothesis by simultaneous determination of pool size and turnover of DCA, CA, and CDCA in nine women before and at various intervals after removal of a functioning gallbladder. An isotope dilution technique using marker bile acids labeled with stable isotopes ($^2H_4$-DCA, ^{13}C-CA, ^{13}C-CDCA) was used. After cholecystectomy, concentration and output of bile acids relative to bilirubin increased ($P < 0.02$) in fasting duodenal bile and cholesterol saturation decreased by 27% ($P < 0.05$) consistent with enhanced enterohepatic cycling of bile acids. Three months after removal of the gallbladder bile acid kinetics were in a new steady state: pool size and turnover of CDCA were unchanged. Synthesis of CA, the precursor of DCA, was diminished by 37% ($P = 0.05$), probably resulting from feedback inhibition by continuous transhepatic flux of bile acids. The fraction of CA transferred after 7α-dehydroxylation to the DCA pool increased from 46±16 to 66±32% ($P < 0.05$). However, this enhanced transfer did not lead to increased input or size of the DCA pool, because synthesis of the precursor CA had decreased.

Introduction

The effects of cholecystectomy on biliary physiology have recently received new attention, as alternative conservative-treatment strategies for cholesterol gallstones have been developed (1, 2). Cholecystectomy offers an excellent chance of curing gallstone disease permanently. Concern, however, has risen about the hypothesis, that cholecystectomy might increase formation and pool size of secondary bile acids, mainly DCA (3–5). Several studies have reported an increased incidence of colonic neoplasia 6–15 yr after cholecystectomy (6–8). Even though not confirmed by others (9, 10), this observation has been linked to the cocarcinogenic role of excess DCA observed in animal experiments (11, 12).

The literature on the effects of cholecystectomy on hepatobiliary physiology is still controversial. After removal of the gallbladder, cycling of primary bile acids is enhanced (13). Turnover of primary bile acids and deoxycholic acid (DCA)[1] content of bile were increased in one followup study (3), but DCA did not increase in bile in another larger followup study (14). Studies relying on group comparison of patients with and without gallbladder have also reported controversial findings on DCA content of bile (4, 5, 15); but the effect of cholecystectomy on size and turnover of the DCA pool has not yet been studied in the same subjects before and after surgery.

We, therefore, investigated the effects of cholecystectomy on biliary lipid composition and on the kinetics of the major three bile acids (cholic acid [CA], chenodeoxycholic acid [CDCA], DCA) in the same patients before and at various intervals after cholecystectomy. Patients with well-functioning gallbladders were selected for this objective.

Methods

Patients. All patients had been referred to this hospital for elective cholecystectomy. They had suffered from occasional episodes of biliary pain, but were otherwise healthy. The protocol had been approved by the Ethics Committee of this hospital. All participants gave their written informed consent before the study. Only women < 50 yr of age (16) were studied. Criteria for exclusion from the study were obesity (> 125% ideal body weight), hyperlipidemia (serum cholesterol > 250 mg/dl; serum triglycerides > 200 mg/dl), diabetes mellitus, cholangitis, diseases of the intestine or the liver (elevated alanine aminotransferase or aspartate aminotransferase, prolonged prothrombine time, clinical or sonographic evidence) as well as administration of antibiotics, lipid-lowering drugs, or steroid hormones within the last 4 wk. Patients were selected who had a functioning gallbladder, documented by visualization and by contraction on oral cholecystography or ultrasonography during cholecystokinin (CCK) infusion, and a rather low ratio (< 0.33) of stone size to gallbladder size. Except for subject A.E., all had radiolucent stones. Histology yielded no (E.G., K.F.) or minor-to-moderate grade chronic cholecystitis. Patient characteristics are given in Table I. All patients were studied as outpatients while on their customary diet. Cholesterol intake was moderate and not different before and after cholecystectomy (Table I). Body weight and stool frequency did not change. Patients were classified according to cholesterol content of gallstones: group A ($n = 6$) had cholesterol gallstones as defined by a cholesterol content of > 60% on a dry weight basis. Patients in group B ($n = 3$) had either mixed gallstones (A.E.) or no stones. None had common duct stones.

Study protocol. All patients were studied within 10 d before operation and again at 6 wk and 3 mo after cholecystectomy. Six of the nine

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Dr. Frans Stellaard's present address is Free University Hospital, Department of Pediatrics, De Boelelaan 1117, NL 1007 MB Amsterdam. Address reprint requests to Dr. Frieder Berr, Medizinsche Klinik II, Klinikum Grosshadern, Marchioninstrasse 15, D-8000 Muenchen 70, Federal Republic of Germany.

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1. *Abbreviations used in this paper:* CA, cholic acid; CDCA, chenodeoxyxholic acid; DCA, deoxycholic acid; FTR, fractional turnover rate.

Table I. Patient Characteristics

	Age	Body weight: Before operation	Body weight: 3 mo after operation	Diagnosis	Gallstones: Volume			Gallstones: Cholesterol content	Dietary cholesterol intake: Before operation	Dietary cholesterol intake: 3 mo after operation	Serum lipids: Cholesterol	Serum lipids: Triglycerides	Stool frequency: Before operation	Stool frequency: 3 mo after operation
	yr	*% IBW**	*kg*	*kg*	*n*	*ml*	*%‡*	*% dry wt*	*mg/d*		*mg/dl*		*d^{-1}*	
Group A														
W.W.	39	125	71	69	GB stones	2	3.0	15.8	79	627	500	227	151	0.6	1.0
H.A.	44	103	67	69	GB stones	2	7.5	23.4	65	267	466	160	56	1.0	1.0
A.N.	38	119	70	72	GB stones	8	3.5	17.5	83	350	401	233	184	0.6	1.0
C.S.	44	109	71	68.5	GB stones	2	1.5	3.9	78	537	442	239	129	1.0	1.0
E.B.	38	102	55	53	GB stones§	4	0.5	1.1	73	349	323	189	130	1.0	0.6
C.V.	35	118	76	73	GB stones	40	1.1	6.9	70	207	343	156	90	1.0	1.0
x	39.7	112.7	68.3	67.4			2.8	11.4	75	390	413	193	122	0.87	0.93
SD	±3.6	±9.4	±7.2	±7.3			±2.6	±8.8	±7	±161	±70	±36	±50	0.21	0.19
Group B															
E.G.	44	101	52	53	GB polyp	0	—	—	—	196	256	218	71	1.0	1.0
K.F.	47	116	66	64	Polycystic liver disease	0	—	—	—	253	272	232	110	1.0	1.0
A.E.	36	96	48	48.5	Mixed gallstones, cystic duct stone	15	0.5	2.0	19	213	348	208	87	0.75	1.0
x	42.3	104.3	55.3	55.2						221	292	219	89	0.92	1.0
SD	±5.7	±10.4	±9.5	±8.0						±29	±49	±12	±20	± 0.14	—

GB, gallbladder. * Ideal body weight according to Statistical Bulletin #40, Metropolitan Life Insurance Co., Nov.–Dec., 1959. ‡ Total gallstone volume as percentage of fasting gallbladder volume (by ultrasound). § E.B. had an aortic bioprosthesis for 1 yr and took 50 mg chlorthalidone daily.

patients were available for restudy at 9–12 mo after operation. Each time dietary cholesterol intake, stool frequency (over a period of 6 d), and bile acid kinetics were assessed. Lipid composition of duodenal bile was checked the day before cholecystectomy and again after 7 wk. During cholecystectomy all gallbladder bile was collected for lipid analysis and the gallstones for cholesterol analysis.

Dietary protocols. During the study period, the patients kept a detailed daily record of food and beverage intake for 7 d (17). The records were evaluated by a dietitian for cholesterol intake using food tables (18). All patients were on their mixed diet with moderate cholesterol intake (< 700 mg/d).

Bile acid kinetics. Pool sizes and turnover rates of CA, CDCA, and DCA were simultaneously determined from serum samples obtained over 4 d after oral intake of the marker bile acids, which were labeled with stable isotopes (^{13}C; ^{2}H). In the first three patients, only kinetics of CDCA and DCA were performed using ^{13}C-labeled marker bile acids. Marker bile acids labeled with stable isotopes were purchased from Merck Sharp & Dohme, Montreal, Canada with the following isotopic purities: 24-^{13}C-DCA 91.5% ^{13}C; 2,2,4,4-$^{2}H_4$-DCA 99.4% $^{2}H_4$; 24-^{13}C-CA 90% ^{13}C; 24-^{13}C-CDCA 91.9% ^{13}C.

Materials, sample preparation, measurement of isotope ratios of individual bile acids using capillary gas liquid chromatography mass spectrometry, selected ion monitoring, and the calculation of bile acid kinetics from the decay curves of their isotopic enrichment have previously been validated and described in detail (19, 20). The mass ions *m/z* 255 and 259 were used for measurement of $^{2}H_4/^{2}H_0$ isotope ratios of DCA, *m/z* 370 and 371 for measurement of $^{13}C/^{12}C$ isotope ratios of DCA and CDCA, and *m/z* 458 and 459 for the $^{13}C/^{12}C$ isotope ratio measurements of CA (20). An example of a single kinetic study (A.N., before operation) is shown in Fig. 1.

Before oral intake of label a blood sample (15 ml) was drawn to measure the isotope ratios of endogenous bile acids (i.e., the natural abundance of these isotopes). Between 6:00 and 10:00 p.m., 50 mg of each marker bile acid (^{13}C-CA, ^{13}C-CDCA, $^{2}H_4$-DCA) dissolved in 200 ml 0.25% sodium bicarbonate solution were simultaneously taken orally. On the next 4 d, blood specimens for analysis were drawn once daily 1.5–3 h after the evening meal.

Calculation of bile acid kinetics. Natural abundance (R_0) of $^{13}C/^{12}C$ isotope ratio in serum bile acids before administration of marker bile acids was 0.3691±0.0067 for CA and 0.3319±0.0072 for CDCA; natural abundance of $^{2}H_4/^{2}H_0$ isotope ratio for DCA was 0.0054±0.0020. Isotopic enrichment (R) over natural abundance was converted to atoms percent excess (APE).

$$\text{APE} = \frac{R - R_0}{1 + (R - R_0)} \times 100\%. \quad (1)$$

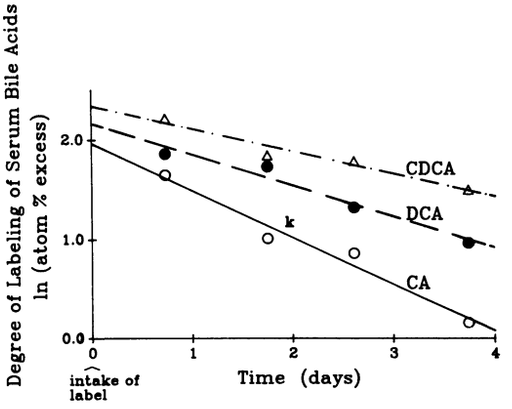

Figure 1. Simultaneous decay curves of 2,2,4,4-$^{2}H_4$-DCA, 24-^{13}C-CA, and 24-^{13}C-CDCA measured in serum of a patient with cholesterol gallstones (A.N., before cholecystectomy) after oral administration of 50 mg of each label. Isotope ratios for the three bile acids were determined using gas liquid chromatography, mass spectrometry, and selected ion monitoring. The kinetics were calculated according to these formulas: pool size = (dose: $APE_0 \cdot 100$) − dose (in micromoles per kilogram); fractional turnover rate (FTR) = k (in $days^{-1}$); synthesis rate = pool size · FTR (in micromoles per kilogram per day).

The ln APE vs. time curve corresponded to monoexponential first-order decay (19) with excellent fit (regression coefficient $r > 0.90$ by linear regression analysis). Kinetics were calculated with the following equations (19):

$$\text{Pool size} = \frac{\text{dose} \times b \times 100}{e^a} - \text{dose} \quad (2)$$

$$\text{Synthesis rate} = \text{Pool size} \times \text{FTR}, \quad (3)$$

where a is the y intercept of the APE decay curve, b the degree of labeling (range 0.900–0.994) of the administered marker bile acid, and FTR (fractional turnover rate), the slope of the regression line. Pool sizes are reported as $\mu mol \cdot kg^{-1}$, FTR as d^{-1}, and synthesis rates of primary bile acids or input rate of DCA as $\mu mol \cdot kg^{-1} \cdot d^{-1}$.

To follow the transfer of ^{13}C label from administered CA marker to the DCA pool, the amount of excess ^{13}C-DCA in the enterohepatic bile acid pool was estimated:

$$\text{Excess } ^{13}\text{C-DCA}_t = \frac{\text{APE}_{^{13}\text{C-DCA},t} \times \text{pool size}_{\text{DCA}} \times \text{body weight}}{100}, \quad (4)$$

where APE_t was the respective APE in the sample drawn at time t after intake of 50 mg of ^{13}C-CA, and DCA pool size was estimated from decay of $^2H_4$-DCA.

Gallbladder motor function. On the day before cholecystectomy, the fasting volume of the gallbladder and its emptying in response to a CCK infusion (1.2 Ivy dog units $kg^{-1} \cdot h^{-1}$ for 50 min) (21) was documented every 5 minutes for 50 min by ultrasound (Sonolayer B, Toshiba Corp., Tokyo). Serial volumes and emptying of the gallbladder were quantitated from longitudinal linear array images of the gallbladder (22).

Sampling of duodenal bile. After overnight fasting (12 h), the patients swallowed a thin Teflon duodenal tube equipped with a guiding wire for positioning. Correct positioning of the aspiration port in the proximal duodenum was initially checked by fluoroscopy and in later studies by aspiration of bile-rich duodenal fluid of pH > 7.0, provided the tube had been advanced 70–80 cm. Unstimulated duodenal bile (3 ml) was collected on ice. Subsequently CCK (Kabi-Vitrum, Stockholm) was infused intravenously and bile collected in 10-min intervals for 50 min. The two darkest bile specimens, which were collected at 10–30 min of the infusion, were combined and a specimen (3 ml) was taken. The remaining bile was returned to the duodenum at the end of the procedure. All bile specimens had been collected on ice, and stored in chloroform/methanol solution (1:2 vol/vol) (0.5 ml bile per 7.5 ml solvent) for analysis.

Intraoperative specimens. All gallbladder bile was aspirated from the excised gallbladder into a sterile syringe, its volume measured and a specimen stored in chloroform/methanol solution. The volume of the gallstones was estimated by water displacement in a graded cylinder. Cholesterol content of the stones (percent dry weight) was determined by colorimetric analysis after grinding and isopropanol extraction of the debris (23).

Analysis of bile samples. Specimens in chloroform/methanol solution (corresponding to 50 or 100 μl of bile) were analyzed by capillary gas liquid chromatography for individual bile acids (19). Bilirubin (24), cholesterol (25), and lipid phosphorus (26) in bile were assayed by colorimetric methods. The molar percentage of each lipid and the cholesterol saturation index were calculated (27–30). Bilirubin due to

Table II. Gallbladder Function

	Oral cholecystography		Sonographic test of motor function*				Gallbladder bile at operation					
								Lipid concentrations				
	Contrast	Contraction	Fasting volume‡	Volume emptied	Fraction emptied	k of emptying	Volume	Bile acid	Phospholipids	Cholesterol	CSI	Fraction of BA pool§ stored
	(0–++)	(0–++)	ml		%	%/min	ml		mM			%
Group A												
W.W.	++	++	16.0	8	50	−5.8	22.5	81.8	20.5	12.5	185	53‖
H.A.	++	++	24.5	16	65	−5.9	20.5	128.4	31.0	15.0	138	85‖
A.N.	++	+	16.5	7	42	−1.3	17.5	140.0	36.0	16.7	132	78
C.S.	Not performed		36.5	16	44	−2.4	35.5	76.9	20.3	12.9	195	59
E.B.	Not performed		18.0	17	94	−10.0	15.5	120.7	62.5	24.0	136	81
C.V.	++	++	14.5	13.5	93	−9.0	18.0	156.5	72.5	30.3	135	73
x			21.0	12.9	64.7	−5.7	22.7	117.4	40.6	16.9	139	72
SD			±8.4	±4.4	±23.8	±3.5	±8.6	±31.9	±22.0	±9.0	±52	±13
Group B												
E.G.	++	++	13	11.5	88	−10.0	15	259.9	77.0	27.0	94	89‖
K.F.	++	++	15	13.5	90	−8.4	16	132.8	40.2	15.0	112	76
A.E.	++	++	24.5	12	49¶	−2.6¶	20	58.8¶	18.8	5.8	111	45¶
x			17.5	12.3	75.7	−7.0	17.3	149.9	45.3	15.9	106	67
SD			±6.1	±1.0	±23.1	±3.9	±3.2	±102.5	±29.4	±10.6	±10	±22
Groups A and B ($n = 9$)												
x			19.8	12.7	68.3	−6.2	20.6	128.4	42.1	17.7	138	70
SD			7.5	3.5	22.7	±3.4	±6.3	59.1	22.9	±7.8	±33	±16

* Sonographic determination of gallbladder volumes (22) during infusion of CCK (1.2 U $kg^{-1} \cdot h^{-1} \times 50$ min). ‡ Corrected for stone volume (see Table I). § Bile acid pool calculated as sum of the pools of CA, CDCA, and DCA. ‖ Calculated for the sum of CDCA and DCA pools and CDCA and DCA content of gallbladder bile. ¶ Stone impaction in the cystic duct at ~ 30 min during the sonographic test, 24 h before surgery. Initial k −7.3 min^{-1}. CSI, cholesterol saturation index according to Carey and Small (29).

Table III. Lipid Concentrations of Fasting Duodenal Bile

Subjects	Before cholecystectomy					7 wk after cholecystectomy				
	Bilirubin*	Bile salts	Phospholipids	Cholesterol	CSI‡	Bilirubin*	Bile salts	Phospholipids	Cholesterol	CSI‡
			mM		%			*mM*		%
Group A										
W.W.	0.038	4.81	3.3	1.15	151	0.009	12.33	6.1	1.30	80
H.A.	0.021	4.78	1.5	0.38	79	0.014	8.31	2.4	0.50	64
A.N.	0.005	2.62	1.4	0.45	122	0.012	16.23	7.0	2.30	111
C.S.	—	—	—	—	—	—	—	—	—	—
E.B.	0.005	2.94	1.2	0.40	111	0.007	6.69	2.8	0.56	70
C.V.	0.051	5.25	2.6	0.82	114	0.092	23.72	6.3	1.75	83
(n = 5)										
x	0.024	4.08	2.0	0.64	115	0.027	13.46	4.9	1.28	82
SD	±0.020	1.21	0.9	0.38	41	±0.092	±6.38§	±2.2§	±0.77§	±18§
Group B										
E.G.	0.037	10.16	2.7	0.70	78	0.039	20.90	5.8	0.80	43
K.F.	0.027	2.58	1.0	0.20	67	0.025	11.69	3.0	0.65	65
A.E.	0.031	13.01	6.0	1.30	78	0.015	17.90	6.9	1.35	66
(n = 3)										
x	0.032	8.58	3.2	0.73	74	0.026	16.83	5.2	0.93	58
SD	±0.005	±5.39	±2.5	±0.55	±6	±0.012	±4.70	±2.0	±0.37	±13
Groups A and B (n = 8)										
x	0.027	5.77	2.46	0.68	100.0	0.027	14.7	5.0	1.15	72.8
SD	±0.016	±3.82	±1.65	±0.39	±29.0	±0.028	±6.0‖	±2.0‖	0.64‖	19.6‖

* Molar ratios of bile salts/bilirubin were before vs. after surgery: 314±227 vs. 906±483 in group A ($P < 0.05$), 260±167 vs. 732±400 in group B, and 294±195 vs. 841±433 in groups A and B ($P < 0.02$). ‡ Cholesterol saturation (27, 28) decreased 27±22% (n = 8) in groups A and B ($P < 0.02$). § $P < 0.05$ as compared with preoperative value. ‖ $P < 0.02$ as compared with preoperative value.

its constant rate of hepatic secretion was used as endogenous marker of bile secretion (31).

Statistical analysis. For paired comparison, the paired t test and the Wilcoxon matched-pair signed-rank test were used (32).

Results

Gallbladder function

Gallbladder motor function during CCK infusion at a physiologic rate (21, 33) was impaired in two patients in group A (A.N. and C.S.; Table II) according to the criteria of Pomeranz and Shaffer for this CCK test (21) who defined the lower limit of normal as 19.1 min $t_{1/2}$ of emptying (fractional rate of 3.6% per min) and a fraction of 45% emptied. Gallbladder contraction of patient A.N. was impaired to the same degree also in response to a liquid meal (400 kcal, 40% fat; Biosorb formula; Pfrimmer & Co., Erlangen, FRG). Motor function of the gallbladder was normal in the other four patients of group A.

Two patients of group B had well-functioning gallbladders without stones. The third patient (A.E.) contracted the gallbladder at a normal rate k_e −7.3% per min during the first 20 min, but then delayed and finally stopped emptying at 35 min. 24 h later at operation, her cystic duct was obstructed by a gallstone. Because the occlusion of the cystic duct had occurred after study of bile acid kinetics, we classified her gallbladder function as normal. She had pigment gallstones with small amounts of cholesterol (Table II).

The fraction of the bile acid pool stored during fasting and the concentrative function as estimated from the intraoperative gallbladder bile specimen were not impaired in groups A and B (Table II). The reduced bile acid concentration and bile acid storage in the gallbladder of patient A.E. is explained by gallbladder hydrops lasting 24 h before operation.

Biliary lipid composition

In fasting duodenal bile, total bile acid concentration and bile acid/bilirubin molar ratio increased ($P < 0.02$) on the average

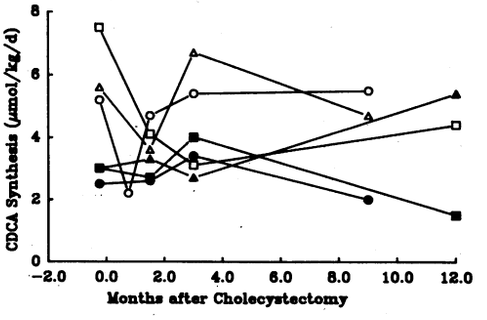

Figure 2. Time-related changes in CDCA synthesis of six patients after cholecystectomy. CDCA synthesis has returned to steady rates at 3 mo after surgery. Synthesis rates were described in the legend to Fig. 1.

threefold at comparable bilirubin concentration after removal of the gallbladder (Table III). The increase in molar percent bile acids lowered cholesterol saturation (27, 28) of unstimulated duodenal bile by 27% ($P < 0.02$).

Bile acid kinetics

Time-related changes. Transient decreases in synthesis rate and pool size of CDCA were observed 6 wk after cholecystectomy in some of the early studies, but at 3 and 9–12 mo after operation, synthesis or input rates and pool sizes of CDCA and DCA were at steady levels (see Fig. 2).

Primary bile acids (Table IV). CDCA kinetics were not altered by removal of the gallbladder. The kinetics of CA, however, changed. 3 mo after cholecystectomy, synthesis rate of CA had decreased on the average by 37% ($n = 6$; $P = 0.05$) with concomitant reductions in average pool size (−19%; NS) and fractional turnover rate (−28%; NS). Hepatic synthesis of primary bile acids declined slightly from 14.0±4.8 to 10.7±3.2 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ (−18%; NS) ($n = 6$) and total bile salt pool (sum of the pools of CA, CDCA, and DCA) from 52.2±9.6 to 43.9±11.3 $\mu mol \cdot kg^{-1}$ (−16%; $P < 0.05$).

Increased levels of unconjugated primary bile acids or an increased ratio of unconjugated to conjugated bile acids (data not given) in postprandial serum, a common finding with bacterial overgrowth of the small bowel (34, 35), were not observed after cholecystectomy. The unconjugated fraction ac-

Table IV. Kinetics of Primary Bile Acids

Cholic acid	Pool size				Synthesis				Rate constant			
mo after operation . . .	–	1.5	3	9–12	–	1.5	3	9–12	–	1.5	3	9–12
	$\mu mol \cdot kg^{-1}$				$\mu mol \cdot kg^{-1} \cdot d^{-1}$				d^{-1}			
Group A												
W.W.	—				—				—			
H.A.	—				—				—			
A.N.	16.9	10.2	13.5	15.9	8.3	5.9	5.4	5.1	0.49	0.58	0.40	0.32
C.S.	31.5	26.8	16.6	—	7.2	5.1	5.8	—	0.23	0.19	0.35	—
E.B.	12.9	10.6	16.4	10.0	14.3	8.8	4.4	7.9	1.11	0.83	0.27	0.79
C.V.	17.9	30.4	13.3	—	8.1	14.3	7.9	—	0.45	0.47	0.59	—
Group B												
E.G.	—				—				—			
K.F.	20.3	20.1	22.4	22.3	5.9	5.2	3.6	6.6	0.29	0.26	0.16	0.3
A.E.	26.9	10.7	20.5	—	12.1	6.2	8.2	—	0.45	0.58	0.40	—
Groups A and B ($n = 6$)												
x	21.0	18.1	17.1		9.3	7.6	5.9		0.50	0.49	0.36	
SD	6.9	9.0	3.7		3.2	3.4	1.9		0.31	0.23	0.14	
Chenodeoxycholic acid												
Group A												
W.W.	19.4	15.3	21.6	18.5	5.2	4.7	5.4	5.5	0.27	0.31	0.25	0.30
H.A.	14.8	16.3	12.2	11.8	2.5	2.6	3.4	2.0	0.17	0.15	0.28	0.17
A.N.	13.4	16.4	16.6	14.2	3.0	3.3	2.7	5.4	0.22	0.2	0.16	0.38
C.S.	18.1	19.9	13.6	—	3.8	2.8	4.1	—	0.21	0.14	0.31	—
E.B.	14.5	14.2	10.4	12.7	7.5	4.1	3.1	4.4	0.52	0.29	0.30	0.35
C.V.	13.6	12.3	6.5	—	5.4	4.8	4.0	—	0.40	0.39	0.58	—
x	15.6	15.8	13.5	14.3	4.6	3.7	3.8	4.3	0.30	0.25	0.31	0.30
SD	2.5	2.6	5.2	3.0	1.8	1.0	1.0	1.6	0.13	0.10	0.14	0.09
Group B												
E.G.	37.6	23.9	32.0	24.8	5.6	3.6	6.7	4.7	0.15	0.15	0.21	0.19
K.F.	16.8	16.8	19.0	21.2	3.0	2.7	4.0	1.5	0.18	0.16	0.21	0.07
A.E.	21.5	20.6	22.0	—	5.6	6.8	8.1	—	0.26	0.33	0.37	—
x	25.3	20.4	24.3	—	4.7	4.4	6.3	—	0.20	0.21	0.26	—
SD	10.9	3.6	6.8	—	1.5	2.2	2.1	—	0.06	0.10	0.09	—
Groups A and B ($n = 9$)												
x	18.9	17.3	17.1	17.2	4.6	3.9	4.6	3.9	0.26	0.24	0.30	0.24
SD	±7.6	±3.6	±7.6	±5.2	1.6	1.4	1.8	1.7	0.12	±0.09	±0.12	0.12

Table V. Kinetics of Deoxycholic Acid

	Pool size				Input rate				Rate constant			
mo after operation	0	1.5	3	9–12	0	1.5	3	9–12	0	1.5	3	9–12
	$\mu mol \cdot kg^{-1}$				$\mu mol \cdot kg^{-1} \cdot d^{-1}$				d^{-1}			
Group A												
W.W.	13.3	9.4	10.5	5.2	2.6	2.4	2.0	2.4	0.20	0.25	0.19	0.46
H.A.	9.2	11.8	8.5	8.2	1.8	1.3	2.0	1.5	0.28	0.11	0.24	0.18
A.N.	14.6	10.9	14.2	13.7	4.5	4.2	4.8	4.6	0.31	0.38	0.34	0.25
C.S.	15.9	9.8	21.2	—	3.5	3.6	5.1	—	0.22	0.37	0.24	—
E.B.	14.4	20.9	4.8	24.4	5.7	5.0	1.6	7.8	0.40	0.25	0.34	0.32
C.V.	19.0	12.0	16.5	—	5.4	5.3	7.9	—	0.28	0.44	0.48	—
x	14.4	12.5	12.6	12.9	3.9	3.6	3.9	4.1	0.28	0.30	0.31	0.30
SD	±3.2	±4.3	±5.0	±8.5	±1.6	±1.5	±2.5	±2.8	±0.07	±0.12	±0.10	±0.12
Group B												
E.G.	4.7	6.4	3.2	8.2	1.4	1.7	1.3	3.5	0.24	0.27	0.41	0.42
K.F.	10.8	2.0	3.8	2.0	1.1	0.3	0.8	0.6	0.10	0.15	0.20	0.31
A.E.	13.9	13.1	20.9	—	5.4	4.2	5.2	—	0.39	0.32	0.25	—
x	9.8	7.2	9.3		2.6	2.1	2.4		0.24	0.25	0.29	
SD	±4.7	±5.6	±10.1		±2.4	±2.0	±2.4		±0.15	±0.09	±0.11	
Groups A and B (n = 9)												
x	12.9	10.7	11.5	10.3	3.5	3.1	3.4	3.4	0.27	0.28	0.30	0.32
SD	±4.2	±5.1	7.1	7.9	1.8	1.8	2.4	2.6	±0.09	±0.11	±0.10	±0.10

counted for 44±24% of CA in postprandial serum after cholecystectomy vs. 59±27% before surgery, and for 36±27% vs. 45±22% of CDCA, respectively (n = 9; groups A and B).

Secondary bile acids. Pool size, FTR, and input rate of DCA were not altered by removal of the gallbladder (Table V; groups A and B). DCA pool size was correlated to DCA input rate in the same positive linear fashion before and after cholecystectomy ($P < 0.01$). In accordance with the individual bile acid pools, the fraction of DCA in unstimulated duodenal bile did not change, whereas the contribution of CA decreased by 6.1% (Table VI). Also 9 mo after surgery, as studied in the first three patients (W.W., H.A., E.G.), there was no increase in the fraction of DCA (11.8±6.6 vs. 13.8±7.3%, preoperatively), or of secondary bile acids (11.8±6.6 vs. 17.3±10.0%), nor in the molar ratio of DCA/CA (0.27±0.21 vs. 0.40±0.38, preoperatively).

Transfer of CA to the DCA pool. The fraction of CA serving as precursor for the DCA pool (DCA input/CA synthesis) increased by 30% ($P < 0.05$) from 46±16 to 66±32% (Table VII). This could be directly shown by more rapid and increased transfer of ^{13}C label from ^{13}C-CA to the DCA pool (Fig. 3). After removal of the gallbladder, ^{13}C label administered orally as 24-^{13}C-CA appeared as ^{13}C-DCA more rapidly and peaked there already 2 d after oral intake. By comparison, this peak occurred at ~ 4 d when the gallbladder was intact. After cholecystectomy, positive correlations existed between CA synthe-

Table VI. Bile Acid Pattern in Fasting Duodenal Bile

		% of total bile acids					
	n	CDCA	CA	DCA	UDCA	LCA	DCA/CA
Before cholecystectomy							
Group A	5	29.2±9.2	47.1±10.7	21.8±8.0	1.7±2.9	0.3±0.7	0.54±0.31
Group B	3	38.0±3.7	42.5±7.2	16.3±8.9	2.6±2.3	0.7±1.3	0.42±0.31
Groups A and B	8	32.5±8.6	45.3±9.7	19.8±8.2	2.0±2.6	0.5±0.9	0.50±0.29
After cholecystectomy*							
Group A	5	37.2±5.0	38.1±9.7‡	22.3±6.9	2.8±2.2	1.1±1.2	0.60±0.28
Group B	3	37.3±5.5	41.0±8.6	14.9±13.6	6.7±3.4	0	0.42±0.44
Groups A and B	8	37.3±4.8	39.2±8.8	18.5±9.2	4.3±3.2	0.7±1.1	0.53±0.33

‡ $P < 0.05$ as compared with preoperative percentage. * 7 wk after surgery. LCA, lithocholic acid; UDCA, ursodeoxycholic acid.

*Table VII. Fraction of Cholic Acid Transferred to the Deoxycholic Acid Pool**

Subject	Before	Mo after cholecystectomy 1.5	3	12
			%	
A.N.	54	71	89	90
C.S.	49	71	88	—
E.B.	40	57	36	99
C.V.	67	37	100	—
K.F.	19	6	22	9
A.E.	45	68	63	—
x	46	52	66‡	—
SD	±16	±26	±32	—

* Calculated from bile acid kinetics as DCA input rate divided by CA synthesis rate times 100.
‡ $P < 0.05$ as compared with preoperative percentage (paired t test).

sis rate and DCA input rate ($P < 0.02$) and between CA synthesis rate and DCA pool size ($P < 0.01$). Before cholecystectomy, these parameters were not significantly correlated.

Discussion

To investigate the role of the gallbladder in bile acid metabolism, we have simultaneously studied pool size and synthesis of the three major bile acids before cholecystectomy and repeatedly in the first year after operation. Bile acids increased in fasting duodenal bile in absolute concentration and relative to bilirubin, consistent with their increased biliary output and enterohepatic cycling after removal of the gallbladder (13). Accordingly, cholesterol saturation of fasting bile was decreased, as previously summarized by Palmer (36). Increased enterohepatic cycling had not altered size or turnover of the CDCA pool. CA synthesis, however, had declined resulting in decreased pool size and FTR. Contrary to the initial hypothesis, size and input of the DCA pool did not increase, although its precursor CA was transferred to the DCA pool at a higher fractional rate.

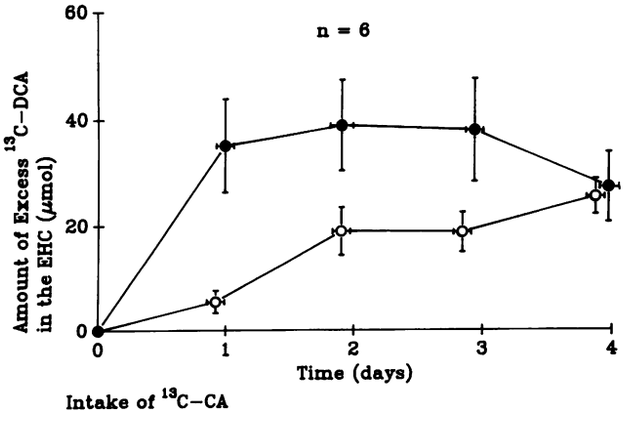

Figure 3. Time course of the amount of excess ^{13}C-labeled DCA in the DCA pool after oral intake of 50 mg ^{13}C-cholic acid in six subjects before (o) and after (•) cholecystectomy (X±SEM). Enhanced formation and increased input of ^{13}C-DCA after cholecystectomy. For calculation see Methods.

All our patients had functioning gallbladders before surgery (Table II). Factors other than removal of the gallbladder were balanced. Thus, the intraindividual comparison (3, 14) was more reliable than a group comparison between cholecystectomized gallstone patients and healthy controls (4), controls with other diseases (5, 37), or even disease controls (15, 38, 39). In contrast to some of the previous studies (4, 5, 15, 37–39), we had excluded other factors known to influence bile acid metabolism such as older age, hyperlipidemias, obesity, cholangitis, diseases of the liver and small bowel, and intake of contraceptive steroids. All patients had comparable body weight, cholesterol intake, and bowel habits before and after cholecystectomy (Table I). The technique to estimate simultaneously the kinetics of all three major bile acids had been validated (20); in both the presence and absence of the gallbladder it yielded monoexponential isotopic decay curves. Because we had administered unconjugated marker bile acids, the true turnover of the respective bile acid pools was estimated (40); some other studies had used conjugated marker bile acids (4, 5), which do not completely equilibrate with the endogenous pool (40). In our study, the daily serum samples for measurement of isotope ratios were obtained after the evening meal, when the marker bile acids were well mixed with the endogenous bile acids. This appears superior to previous kinetic studies (41) that used CCK-stimulated gallbladder bile before surgery, but unstimulated duodenal bile after surgery (3–5, 15). In unstimulated duodenal bile, specific activity of primary bile acids probably is not in isotopic equilibrium with that of the pool during morning hours, when daily synthesis of primary bile acids peaks (42).

After cholecystectomy, the total bile acid pool shrank by 16% ($P < 0.05$). This was caused by a reduction of the CA pool. Similar observations have been made in other studies (4, 15, 38), but this had always been associated with increased fractional turnover of the bile acid pool (3–5, 15). Accordingly, the reduction in bile acid pool size has been explained by enhanced loss of primary bile acids from the enterohepatic circulation (15, 38). By contrast, using advanced techniques, we observed reduced fractional turnover and synthesis of CA.

Previous studies based on group comparison (4, 5, 15) do not allow definitive conclusions on the effects of cholecystectomy on bile acid metabolism: Pomare and Heaton (4) and Hepner et al. (5) had used conjugated marker bile acids that were turned over more rapidly after cholecystectomy due to increased bacterial deconjugation of bile acids (43). Roda et al. (15) had observed a small CA pool with enhanced fractional turnover rate in cholecystectomized patients, but the FTR was enhanced in a subgroup of cholecystectomized patients only, who had bile acid malabsorption.

Only Almond et al. (3) had studied the kinetics of CA and CDCA in 10 patients before and after cholecystectomy. They reported increased fractional turnover of CA and CDCA, but no change in pool sizes or synthesis of the two primary bile acids. Further analysis of their data reveals two subgroups of five patients each that differed in their CA metabolism before surgery. The first group (subjects 1, 2, 5, 6, and 7) with very low CA synthesis (141±7 mg/70 kg/d) doubled its synthesis rate (274±77 mg/70 kg/d; $P < 0.05$) increasing the size (+64%) and the fractional turnover rate (+37%) of the CA pool and the estimated pool of secondary bile acids (0.24±0.12 vs. 0.36±0.15 mg/70 kg). Unfortunately, we do not have additional characteristics of these patients who had repressed CA

Table VIII. Studies of Bile Acid Kinetics with and without Gallbladder (Mean Values)

	Marker	Comparison	Pool	FTR	Turnover	Bile acid pattern (%) in bile				DCA/CA ratio
						CA	CDCA	DCA	Others	
		n	*g*	d^{-1}	*g/d*					
Almond (1973)	24-^{14}C-CA	Intraindividual								
		Before CCX (10)	0.37	0.63	0.208	35	43	20	3	0.57
		After CCX	0.34	0.81*	0.255	28.5	38.5	30*	3	1.05
	24-^{14}C-CDCA	Before CCX (10)	0.39	0.39	0.143					
		After CCX	0.33	0.50*	0.155					
Pomare‡ (1973)	Tauro 24-^{14}C-CA	Interindividual								
		Healthy (10)	0.36	0.42	0.149	38	35	27	0	0.71
		CCX XGS (10)	0.19	0.72	0.142	32.5	23	45	0	1.38
Hepner (1974)	1-^{14}C-Glyco-24-^{3}H-CA	Interindividual								
		Healthy (12)§	1.11	0.35	0.347	48	30	21	1	0.44
		XGS (13)§	0.67*	0.43	0.275	42	28	29	1	0.69
		CCX XGS (10)§	0.75*	0.44	0.333	24.5*	29	37	10	1.51
Roda (1978)	24-^{14}C-CA	Interindividual								
		Healthy (10)	0.80	0.28	0.239	40	40.5	18	1.5	0.45
		XGS (10)	0.50	0.38	0.117	24	36	35	5	1.46
		CCX‖ XGS (10)	0.40*	0.56*	0.233	20.5	40	33	6.5	1.61
This study (groups A and B)	24-^{13}C-CA	Intraindividual								
		Before CCX (6)	0.60§	0.50	0.266	45	33	20	2	0.50
		After CCX	0.49§	0.36	0.169*	39	37	19	5	0.53
	24-^{13}C-CDCA	Before CCX (9)	0.52§	0.26	0.128					
		After CCX	0.47§	0.30	0.126					
	2,4-$^{2}H_4$-DCA	Before CCX (9)	0.34§	0.27	0.096					
		After CCX	0.32§	0.30	0.093					

* Significantly different from control. ‡ Only one of four additional patients studied before and after cholecystectomy had clearly decreased pool size and FTR, whereas changes in the other three were minor. § Converted to average body weight of 70 kg. ‖ 5 of the 10 patients probably had bile acid malabsorption (increased fecal output of ^{14}C). Abbreviations: CCX, cholecystectomy; XGS, cholesterol gallstones; BA, bile acid.

synthesis before surgery. The other subgroup had higher CA synthesis (340±119 mg/70 kg/d; $P < 0.005$), which was in a similar range as that of our patients (see Table VIII). They responded to cholecystectomy in the same way as our patients: synthesis of CA decreased by 38% (to 169±59 mg/70 kg/d; $P < 0.05$) and pool size by 47% without significant increase in fractional turnover rate (0.64±0.32 vs. 0.78±0.38 d^{-1}); the estimated pool of secondary bile acids remained unaltered (0.26±0.14 vs. 0.24±0.09 mg/70 kg after cholecystectomy). Thus, even Almond's data suggest that cholecystectomy leads to suppression of CA synthesis, if it is not repressed a priori.

The widely accepted hypothesis that cholecystectomy increases formation and pool size of secondary bile acids, mainly DCA, originated from studies of bile acid pattern of duodenal bile (3–5) (Table VIII). Two of these studies (4, 5) using an inappropriate control group reported a higher DCA fraction in bile of cholecystectomized patients. Roda et al. (15), who had studied an appropriate disease control group, found the DCA content of bile elevated to the same extent in the presence as in the absence of the gallbladder. Three studies, including our own, (3, 14) have compared biliary bile acids in the same patients before and after cholecystectomy. In Van der Linden's (14) and our patients, cholecystectomy did not increase DCA in bile (Table VIII). As already mentioned, one subgroup of Almond's (3) patients increased the synthesis of CA and the pool of secondary bile acids after cholecystectomy, whereas the other subgroup, like our patients, responded to cholecystectomy with a decrease in CA synthesis at unaltered secondary bile acids.

We have directly measured the kinetics of DCA before and after cholecystectomy. Despite reduced synthesis and pool size of CA, input, and size of the DCA pool did not change, because the fraction of CA transferred to the DCA pool increased (Table VII). This has also been shown directly by more rapid and increased influx of ^{13}C label into the DCA pool after intake of ^{13}C-CA (Fig. 3). Because under normal conditions practically all CA (≥ 95%) is dehydroxylated to DCA in the colon (44), increased fractional transfer must have resulted from enhanced fractional absorption of newly formed DCA.

Two mechanisms are possible: increased fractional absorption of DCA from the colon or formation of DCA in the ileum. Fractional absorption of DCA from the large bowel is mainly determined by colonic transit time (45). However, increased absorption of DCA from the colon, the usual site of DCA formation (46), could not sufficiently explain the more rapid transfer of CA to the DCA pool (Fig. 3), because the synthesis of CA (reduction of both pool size and fractional turnover) and consequently the loss of CA into the colon were reduced. Our findings could be well explained by the assumption that after cholecystectomy a larger fraction of DCA is formed in the

ileum, where it is very efficiently absorbed by the active transport mechanism for bile acids (47). By contrast, in normal man only very little DCA (< 5%) is formed in the ileum (45). Continuous presence of CA in the lower ileum and prolonged small bowel transit after cholecystectomy (48) could facilitate formation of DCA in the ileum by 7α-dehydroxylating species of anaerobic bacteria (49) that are found in substantial concentrations in the distal ileum (50).

In conclusion, the following hypothesis is consistent with our data as well as that of most previous studies (3, 4, 13, 14): cholecystectomy enhances enterohepatic cycling of bile acids in the fasting state. This does not increase their loss from the enterohepatic circulation, but reduces the synthesis of CA, consistent with the concept that synthesis of CA is under control of the transhepatic flux of bile acids. Prolonged exposure of CA to anaerobic bacteria, presumably in the distal ileum, leads to earlier 7α-dehydroxylation of CA and increased fractional absorption of newly formed DCA. Together, these changes result in a slight reduction in the CA and in the total bile acid pool, whereas turnover and size of the DCA pool remain constant.

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