Effects of feeding cholic acid and chenodeoxycholic acid on cholesterol absorption and hepatic secretion of biliary lipids in man

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Abstract Chenodeoxycholic acid (CDCA), in contrast to cholic acid (CA), reduces cholesterol saturation of bile. The mechanisms for these differences were the object of this study. Investigations were carried out in nine white men; three nonobese subjects and one obese subject were fed a weight-maintenance diet, and five obese patients had a reduced caloric intake for weight reduction. They were given a daily dose of 750–1000 mg CDCA or CA for one month after which they received the other bile acid for another month. The effects of both bile acids on bile acid pool size and hepatic secretion rates of biliary lipids were determined. Total bile acid pools were increased markedly by both CDCA and CA, but to about the same degree for each. Thus, the superior action of CDCA for lowering saturation of bile could not be explained by its effect on the pool sizes of bile acids. On the other hand, hepatic secretion of cholesterol, both during feeding and fasting, was found to be reduced to a greater extent by CDCA than by CA. A theoretical mechanism by which CDCA might lower hepatic outputs of cholesterol is the inhibition of cholesterol absorption. To examine this possibility, cholesterol absorption was estimated by use of an intestinal perfusion technique. No differences were obtained between the two treatment periods for either percentage or net absorption of cholesterol; thus it is unlikely that decreased absorption could account for the reduced cholesterol secretion. Another possibility is that CDCA might affect the interrelations of the three biliary lipids differently than CA. This was explored by measurements of hepatic secretion rates of these lipids. We observed a linear relationship between the secretion rates of bile acids and cholesterol, cholesterol and phospholipids, and bile acids during both treatment periods. However, cholesterol:phospholipid ratios were higher during CA therapy than with CDCA, and they increased still more during fasting in most CA-treated subjects, but not with CDCA. This indicated that there is a marked difference between the two bile acids in the degree of coupling of cholesterol and phospholipids in fasting. We suggest that the reduction in bile saturation on CDCA is most likely the result of changes in the interrelations of the different biliary lipids at the site of their secretion and/or inhibition of cholesterol output from the liver because of suppressed cholesterol synthesis in this organ.—**Einarsson, K., and S. M. Grundy.** Effects of feeding cholic acid and chenodeoxycholic acid on cholesterol absorption and hepatic secretion of biliary lipids in man. *J. Lipid Res.* 1980. **21:** 23–34.

Supplementary key words bile saturation · cholesterol synthesis

Oral administration of chenodeoxycholic acid (CDCA) is associated with decreased saturation of bile with cholesterol and may induce dissolution of cholesterol gallstones in man, whereas cholic acid (CA) generally has been ineffective (1–5). Previous studies have shown that the biliary excretion of cholesterol is lower during treatment with CDCA than with CA (6, 7), and this may be an important factor in decreased bile saturation on CDCA. The exact mechanisms by which CDCA lowers the cholesterol secretion have not been clarified. From animal experiments it has been suggested that CDCA is more effective in suppressing the activity of HMG-CoA reductase, the rate determining enzyme in cholesterol synthesis (8). Other studies have shown that bile acids do not directly inhibit HMG-CoA reductase but may indirectly regulate cholesterol synthesis by changing the flow of cholesterol to and/or within the liver (9, 10). Recently Lindblad, Lundholm and Schersten (11) demonstrated by acute infusion studies in man that CA and CDCA may have different effects on the secretion of bile cholesterol that are unrelated to changes in HMG-CoA reductase activity and cholesterol synthesis.

The present study was undertaken to further elucidate the mechanisms by which CDCA in contrast to CA lowers the secretion rate of bile cholesterol in man. We compared the effects of feeding CDCA and CA on the intestinal absorption of cholesterol, the bile acid pool size, and the hepatic secretion of biliary lipids. The relationships between hepatic outputs of

Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; LCA, lithocholic acid; CH, cholesterol; BA, bile acid; PL, phospholipid; HMG, 3-hydroxy-3-methylglutaryl; HMG-CoA reductase, mevalonate:NADP oxidoreductase, EC 1.1.1.34; GLC, gas–liquid chromatography.

TABLE 1. Clinical data of subjects

Patients	Age	Weight	% Ideal Weight	Plasma Cholesterol	Plasma Triglycerides	Clinical History
	yrs	*kg*		*mg/dl*	*mg/dl*	
1	54	78	114	180	138	Adult glucose intolerance
2	59	74	95	211	215	
3	50	63	93	156	118	Ischemic heart disease
4	50	111	148	173	186	Hypertension
5	51	124	165	257	201	Cholelithiasis
6	61	124	175	230	207	Hypertension
7	48	95	142	183	158	Ischemic heart disease, Hypertension
8	57	123	164	264	150	
9	57	211	264	223	283	

cholesterol, bile acids, and phospholipids were defined over a wide range of bile acid secretion rates.

MATERIALS AND METHODS

Patients

Nine white male patients were studied on the Special Diagnostic and Treatment Unit, Veterans Administration Medical Center, San Diego, CA. Informed consent was obtained from each subject. Clinical data are given in **Table 1.** One subject had a mild glucose intolerance which was adequately controlled by diet. Subjects 4 to 9 were obese. Patient 5 had radiolucent gallstones in a gallbladder that was well-visualized by oral cholecystogram. Gallstones were not present in other patients as evidenced by ultrasonography or cholecystography. None of the subjects had evidence of gastrointestinal disease and routine liver function tests were normal. None received hypolipidemic or antibiotic drugs throughout the study, but other medications, if any, remained unchanged.

Diets

Patients 1 to 4 were studied during weight maintenance on a diet of mixed solid food and formula containing 40% of calories as lard, as used previously in this laboratory (12). The remaining subjects were in the hospital primarily for weight loss. They were given a 960-kcal diet of liquid formula (Sustacal, Mead Johnson, Evansville, IN).

Experimental design

The study included two periods which were about 1 month each. During the first period patients 1 to 6 were given CDCA and the other subjects received CA. During the second period each patient received the other bile acid. Details of bile acid feeding are described below. Plasma lipids were estimated twice weekly and liver function tests (SGOT, SGPT and alkaline phosphatase) once a week. After 3 weeks treatment with each bile acid, measurements of intestinal absorption of cholesterol were performed by an intestinal perfusion technique. Seven patients completed the absorption study. At the end of both periods estimations were made of hourly hepatic secretion rates of biliary lipids, bile acid pool size and biliary lipid composition of gallbladder bile. Altogether eight subjects completed this part of the study.

Intestinal absorption of cholesterol

The procedure used has been described recently by Grundy and Mok (13). The patients were intubated with a 3-lumen polyvinyl tube in the evening before the study. After an overnight fast the tube was placed in the correct position by X-ray with the most proximal outlet adjacent to the ampulla of Vater and the second outlet 10 cm distally. The third outlet was either 50 or 100 cm beyond the second outlet. The 100-cm absorption segment was used in three patients (Nos. 1, 2, and 3), but because of some difficulty in tube placement in two patients, the segment was reduced to 50 cm in four patients (Nos. 5, 6, 8, and 9). A liquid formula was infused continuously through the most proximal lumen together with β-sitosterol as a marker. After an equilibration period of 4–6 hr, continuous aspiration was started from the second proximal and distal outlets. Hourly 10-ml aliquots were collected for the next 8 hr and analyzed for cholesterol and β-sitosterol by GLC. Since the rate of marker infusion was known, measurements of the ratio cholesterol to marker in the proximal samples gave the input of cholesterol. Net cholesterol absorption was estimated from the disappearance of cholesterol relative to β-sitosterol over the absorption segment.

Hepatic secretion rates of biliary lipids

For these measurements, the intestinal perfusion technique described by Grundy and Metzger (14) was used. Patients swallowed a three-lumen tube the

evening before the study that was positioned the next morning with two proximal outlets adjacent to ampulla of Vater and a third outlet 10 cm distally, just beyond the ligament of Treitz. Liquid formula was infused constantly together with β-sitosterol as a marker through one of the proximal lumens. This formula diet supplied the patient with the daily requirement of calories and kept the gallbladder contracted. After 4 hr, hourly 10-ml samples were obtained by continuous aspiration from the second proximal and the distal outlets. After 8 hr of sampling the infusion of formula was discontinued, and only β-sitosterol was infused during the next 12-hr sampling period. The aim of the latter part of the study was to take advantage of the partial interruption of the enterohepatic circulation during fasting to investigate biliary lipid outputs when bile acid secretion was in the lower range. The hourly secretion of cholesterol into the duodenum was determined from measurement of the ratio of cholesterol to marker at the distal outlet. The secretion rates of bile acids and phospholipids were obtained from the ratios of bile acids and phospholipids to cholesterol in the proximal samples. Cholesterol and β-sitosterol were measured on GLC as trimethylsilyl ethers. Bile acids were determined enzymatically and phospholipids were measured by the method of Rouser, Sidney, and Akira (15).

Because part of the biliary lipids is stored in the gallbladder during fasting, the amount of lipids secreted into the intestine does not represent the total amount of biliary lipids secreted by the liver. In the present study we therefore used bilirubin as an internal marker to measure the hepatic secretion rate of biliary lipids with fasting. The hourly hepatic secretion of bilirubin into bile has recently been demonstrated to be stable during day and night, showing insignificant diurnal variation (16). By measuring the amount of bilirubin in the proximal samples (17) we could calculate the secretion rate of bilirubin into the duodenum from the ratio of bilirubin to cholesterol. The mean hourly value of bilirubin secreted during the first 8 hr of formula infusion was considered to be equivalent to the hepatic secretion rate of bilirubin during the fasting period. The hepatic output of biliary lipids during fasting was then obtained by multiplying the outputs of biliary lipids into the duodenum by the ratio:

$$\frac{\text{hourly hepatic secretion of bilirubin}}{\text{hourly secretion of bilirubin into duodenum}}$$

Bile acid pool size

This was measured simultaneously with hepatic secretion rates as described recently (12). [^{14}C]Cholic acid, 5 μCi (New England Nuclear Corp., Boston, MA), was administered intraduodenally at the beginning of the formula infusion. After an equilibration period of 4 hr the mean ratio of radioactive counts to mass of bile acids ("specific activity") was determined in the hourly samples collected over the next 6 hr. The total pool size of bile acids was obtained by dividing the amount of radioactivity given by the mean specific radioactivity. The frequency of circulation of the bile acid pool was calculated as described recently (18).

Lipid composition of gallbladder bile

At the end of the fasting period, gallbladder contraction was stimulated by infusion of liquid formula and gallbladder bile was obtained. The biliary lipids, cholesterol, bile acids, and phospholipids were quantified as described above. Bile lipid composition was expressed as molar percent for each lipid component according to Admirand and Small (19). Saturation indices (percentage saturation) were calculated by the criteria of Carey and Small (20). These workers found that saturation of bile is a function of total solids present. For calculation of percent saturation, it was assumed that gallbladder bile contained 10% solids. For stimulated hepatic bile, which is more dilute, calculations were made at 3% solids, but values for 10% solids are also given to show what saturation would be if this bile had accumulated in the gallbladder.

The relative proportions of the individual bile acids in gallbladder bile were measured after solvolysis in acidic ethyl ether and enzymatic deconjugation with cholylglycine hydrolase (Schwartz-Mann, Orangeburg, NY). The trimethylsilyl ethers of the methyl esters of bile acids were determined by GLC on 1% HiEff 8 BP column.

Bile acid feeding

CA was obtained from J. T. Baker Chemical Co., Philipsburg, NJ, and CDCA from Rowell Laboratories, Baduette, MN. The bile acids were administered to the subjects in 250-mg capsules. Patients 1–4 and 7 received 750 mg of bile acid per day in three divided doses and the other patients received 1000 mg in four divided doses because of their obesity. Patients tolerated the bile acids well. Most of them noticed softer stools during the CDCA period but none of them developed diarrhea. During the day of the cholesterol absorption test, the bile acids were administered in capsules as before. During the measurements of biliary lipid secretion the bile acids were not given by mouth so as to avoid interference with measurement of bile acid secretion. Instead they were neutralized with sodium hydroxide and dissolved in

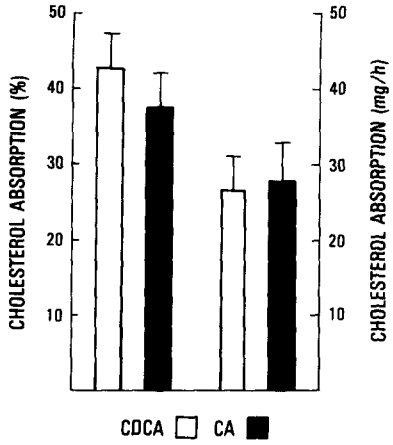

Fig. 1. Absorption of cholesterol during feeding with CDCA, □ and CA, ■.

water. Five hundred mg in this solution was given intraduodenally in the beginning of the formula infusion and the rest of the bile acid was infused after discontinuation of the formula infusion.

RESULTS

As indicated under Methods, weights of nonobese subjects 1–4 were maintained at a constant level throughout the study. The remaining five patients were obese and in the hospital primarily for weight loss, and they received 960 Kcal daily; their rate of weight loss was constant throughout both study periods. Patients 5, 8, and 9 lost at a rate of 2.5 kg/wk while patients 6 and 7 lost 1.4 kg/wk. Previously, Bennion and Grundy (21) showed that cholesterol and bile acid synthesis, and hepatic secretion rates of biliary lipids, are essentially in the normal range in obese subjects on 1000-Kcal diets; in contrast, all of these variables are elevated markedly when they are on weight maintenance diets. A review of all present data showed that lipid outputs and related factors were of similar magnitude for nonobese subjects on weight maintenance and obese patients on weight reduction, and responses to bile acid feeding were also the same; therefore, data for both groups were pooled for analysis. This pooling of data also seemed justified because each patient received both CDCA and CA and because the weight changes in the obese patients were constant throughout each study period.

Cholesterol absorption

In three patients net absorption of cholesterol was determined over a 100-cm segment of jejunum, and over a 50-cm segment in four others. The study could not be completed in two other patients who did not tolerate the tube. No differences were observed in absorption values estimated over 50-cm and 100-cm segments, and the data therefore were combined. The percentage absorption of cholesterol during CDCA treatment (42.6 ± 4.6%, mean ± SEM) was not statistically different from that during CA therapy (37.5 ± 4.6%) (**Fig. 1**). Similarly net absorption of cholesterol mass was essentially the same on the two treatment regimens, 26.6 ± 4.5 mg/hr for CDCA and 27.7 ± 4.5 mg/hr for CA. Thus, neither bile acid appeared to promote or retard cholesterol absorption more than the other. Although the absorption segment under study would seem relatively short (50–100 cm), the actual segment of bowel traversed was almost certainly much greater because of the tendency of the small intestine to gather around a fixed structure (13). Thus, much of the actively-absorbing surface for cholesterol was probably incorporated under the absorption segment. As indicated above, the present technique estimates net absorption of total cholesterol, i.e., both endogenous and exogenous cholesterol, that enters the intestine, but since the infused formula contained very small amounts of cholesterol, the present study dealt mainly with absorption of cholesterol that was secreted with bile.

Bile acid and lipid composition of gallbladder bile

CDCA was the predominant bile acid during CDCA treatment (range, 61–92%; mean, 79%) and together with UDCA constituted an average of 84.4 ± 4.1% of the total bile acids (**Table 2**). With CA therapy, DCA and CA became the major bile acids accounting for 80.3 ± 1.4% (range, 75.7–86.8%) of the pool. Only minor amounts of LCA were found. As shown in **Table 3,** all patients except one (No. 3) had significantly lower saturation of gallbladder bile during administration of CDCA. In individual cases, the cause of reduced saturation on CDCA was due either to a lesser molar percent of cholesterol or an increase in percentage phospholipids; almost never was the molar percent total bile acids greater during CDCA feeding than with CA.

Lipid composition of hepatic bile

Table 4 shows lipid composition of hepatic bile during the formula infusion and the last four hours of fasting. During the period of formula infusion, the molar percent cholesterol was somewhat lower with CDCA treatment than with CA, but the difference in percent saturation was not significant between the two bile acids. Of particular interest, on the other hand, the saturation of bile with CA therapy was significantly greater during fasting than with feeding, while the increment with CDCA administration was not significant. By the same token, fasting bile was more highly

TABLE 2. Bile acid pool size and bile acid composition in the subjects

Patients	Treatment	Bile Acid Pool Size	Bile Acid Composition				
			CA	CDCA	DCA	UDCA	LCA
		mg			*molar %*		
1	CDCA	7940	4.1	88.3	0.9	6.7	0.0
	CA	11100	45.8	21.1	30.5	2.7	0.0
2	CDCA	7830	13.3	71.0	3.4	12.3	0.0
	CA	6290	41.9	19.9	36.7	1.5	0.0
3	CDCA	7630	11.0	75.5	10.6	2.7	0.2
	CA	7050	41.1	22.3	34.6	1.9	0.0
5	CDCA	4910	4.9	84.9	1.6	5.5	3.0
	CA	4620	42.1	13.2	44.7	0.0	0.0
6	CDCA	2860	16.7	61.1	10.1	5.1	7.0
	CA	3650	37.0	18.2	42.1	2.2	0.6
7	CDCA	2770	12.2	69.4	7.1	1.6	9.8
	CA	4320	36.4	17.6	42.3	3.0	0.6
8	CDCA	6340	3.3	87.9	0.7	8.1	0.0
	CA	6050	38.6	14.1	45.1	0.3	1.8
9	CDCA	5280	4.1	92.2	0.8	2.8	0.0
	CA	4260	64.1	15.8	19.2	0.9	0.0
Mean ± SEM	CDCA	5700 ± 750	8.7 ± 1.8	78.8 ± 3.9	4.4 ± 1.5	5.6 ± 1.2	2.5 ± 1.4
	CA	5920 ± 850	43.4 ± 3.2[a]	17.8 ± 1.2[a]	36.9 ± 3.1[a]	1.6 ± 0.4[b]	0.4 ± 0.2

[a] Significantly different from CDCA period. $P < 0.001$.
[b] Significantly different from CDCA period. $P < 0.025$.

TABLE 3. Lipid composition of gallbladder bile and percent saturation

Patients	Treatment	Lipid Composition			% Saturation (10% Solids)
		Cholesterol	Bile Acids	Phospholipids	
			molar %		*%*
1	CDCA	5.4	71.8	22.3	93.7
	CA	9.4	72.4	18.7	180.4
2	CDCA	4.3	75.2	20.6	79.8
	CA	6.0	75.6	18.4	119.2
3	CDCA	5.9	76.2	18.0	119.3
	CA	5.0	80.2	14.8	116.9
5	CDCA	6.5	69.0	24.5	105.8
	CA	7.6	75.2	17.2	156.6
6	CDCA	14.4	60.1	25.5	219.6
	CA	14.8	67.4	17.7	281.4
7	CDCA	8.0	68.3	23.7	131.5
	CA	8.5	67.8	23.7	139.3
8	CDCA	4.6	72.7	22.8	79.4
	CA	7.0	70.9	22.1	121.2
9	CDCA	—	—	—	—
	CA	14.7	57.2	28.1	217.6
Mean ± SEM	CDCA	7.0 ± 1.3	70.5 ± 2.0	22.5 ± 1.0	118.4 ± 18.4
	CA	9.1 ± 1.3	70.8 ± 2.5	20.1 ± 1.5[a]	159.3 ± 22.2[a]

[a] Significantly different from CDCA period. $P < 0.025$.

TABLE 4. Lipid composition and percent saturation of hepatic bile collected during formula infusion (I) and during the last 4 hr of fasting (II)

Patients	Treatment		Lipid Composition			% Saturation	
			Cholesterol	Bile Acids	Phospholipids	10% Solids	3% Solids
			molar %			*%*	
1	CDCA	I	4.4	73.8	21.7	62.9	78.5
		II	4.5	75.8	19.6	68.6	86.3
	CA	I	6.2	78.3	15.4	110.2	139.7
		II	7.2	78.8	13.9	136.2	172.9
2	CDCA	I	3.8	76.7	19.4	58.6	73.8
		II	3.7	76.8	19.5	57.0	71.7
	CA	I	5.9	74.3	19.7	88.8	111.6
		II	5.8	73.6	20.4	85.3	107.0
3	CDCA	I	3.6	79.1	17.0	60.8	77.0
		II	3.8	79.6	16.7	65.1	82.4
	CA	I	3.9	81.8	14.1	74.8	94.9
		II	4.0	79.9	15.9	70.6	89.5
5	CDCA	I	4.8	72.7	22.4	67.1	83.6
		II	6.3	70.2	23.4	85.0	105.5
	CA	I	5.8	77.8	16.2	99.8	126.4
		II	6.9	73.8	19.2	104.9	131.9
6	CDCA	I	8.3	67.7	23.7	109.8	135.9
		II	12.3	67.7	19.9	175.7	219.6
	CA	I	8.2	69.6	22.1	113.0	140.6
		II	14.2	61.5	24.1	180.4	222.2
7	CDCA	I	5.9	82.6	11.3	128.8	163.1
		II	7.3	78.0	14.5	134.0	170.0
	CA	I	6.5	70.3	23.2	88.1	109.4
		II	7.2	74.9	17.8	115.0	145.1
8	CDCA	I	3.2	71.8	24.9	42.5	52.6
		II	3.9	72.2	23.7	53.0	65.8
	CA	I	5.0	73.6	21.2	72.2	90.3
		II	8.3	69.2	22.3	113.6	141.3
9	CDCA	I	5.3	72.2	22.3	74.0	92.2
		II	6.1	71.2	22.6	84.1	104.6
	CA	I	7.8	69.9	22.2	107.5	133.8
		II	10.7	65.1	24.1	138.6	171.1
Mean ± SEM	CDCA	I	4.9 ± 0.6	74.6 ± 1.7	20.3 ± 1.6	75.6 ± 10.2	94.6 ± 12.9
		II	6.0 ± 1.0	73.9 ± 1.5	20.0 ± 1.1	90.3 ± 15.2	113.2 ± 19
	CA	I	6.2 ± 0.5[a]	74.5 ± 1.6	19.3 ± 1.2	94.3 ± 5.6	118.3 ± 7.0
	CA	II	8.0 ± 1.1[b,c,d]	72.1 ± 2.3	19.7 ± 1.3	118.1 ± 12.1[b,c,d]	147.6 ± 14.7[b,c,d]

[a] CA I significantly greater than CDCA I at $P < 0.05$.
[b] CA II significantly greater than CDCA II at $P < 0.05$.
[c] CA II significantly greater than CA I at $P < 0.05$.
[d] CA II significantly greater than CDCA I at $P < 0.05$.

saturated with CA therapy than with CDCA. Thus, the differential effects of CDCA and CA were manifest mainly in the fasting state, when bile is entering the gallbladder.

Hepatic secretion rates of biliary lipids

As shown in **Table 5,** hepatic secretion rates for cholesterol were markedly lower after CDCA feeding than after CA therapy; this was true during both formula infusion and the fasting period. In contrast, there were no differences between secretion rates of bile acids and phospholipids with feeding either CDCA or CA. During the last 4 hours of the fasting period, secretion rates of bile acids were reduced to the same extent on both bile acids (average 47 and 48%), as compared with the formula-infusion period.

TABLE 5. Secretion rates of biliary lipids and bilirubin in subjects during formula infusion (I) and during the last 4 hr of fasting (II)

Patients	Treatment		Secretion Rates			
			Cholesterol	Bile Acids	Phospholipids	Bilirubin
				mg/hr		
1	CDCA	I	47	1029	477	10.7
		II	22	470	192	
	CA	I	52	874	241	7.5
		II	37	540	148	
2	CDCA	I	46	1211	476	8.2
		II	16	474	198	
	CA	I	79	1311	523	18.7
		II	49	855	357	
3	CDCA	I	46	1327	443	7.0
		II	16	461	151	
	CA	I	58	1611	431	8.9
		II	23	562	178	
5	CDCA	I	53	1065	500	16.2
		II	42	616	315	
	CA	I	53	921	304	10.6
		II	48	674	274	
6	CDCA	I	71	758	415	12.6
		II	41	313	142	
	CA	I	81	889	443	12.0
		II	58	381	217	
7	CDCA	I	29	512	108	21.6
		II	28	397	134	
	CA	I	55	812	411	19.5
		II	35	489	172	
8	CDCA	I	37	1102	591	12.9
		II	30	720	369	
	CA	I	48	947	419	14.2
		II	37	399	202	
9	CDCA	I	42	728	352	15.3
		II	40	608	428	
	CA	I	91	1063	520	20.5
		II	75	615	343	
Mean ± SEM	CDCA	I	46 ± 4	967 ± 97	420 ± 51	13.1 ± 1.7
		II	29 ± 4[a]	507 ± 47[e]	241 ± 40[f]	
	CA	I	65 ± 6[b]	1054 ± 97	412 ± 34	14.0 ± 1.8
		II	45 ± 6[c,d]	564 ± 54[d]	236 ± 28[d]	

[a] Significantly different from period I. $P < 0.01$.
[b] Significantly different from corresponding CDCA period. $P < 0.02$.
[c] Significantly different from corresponding CDCA period. $P < 0.01$.
[d] Significantly different from period I. $P < 0.001$.
[e] Significantly different from period I. $P < 0.005$.
[f] Significantly different from period I. $P < 0.02$.

Similarly the secretion rates of phospholipids decreased by an average of 43% on both CDCA and CA. Fasting reduced the secretion rates of cholesterol to a lesser degree than bile acids and phospholipids during feeding of both bile acids, but the fall was only 31% during CA feeding compared with 37% on CDCA therapy; the greater fall in fasting cholesterol secretion during CDCA feeding, combined with a lower baseline level during formula infusion, must have been a significant factor in the lesser saturation of fasting bile on CDCA than with CA treatment.

Relationship between bile acid and cholesterol secretion rates

In all subjects there was a linear relationship between the secretion rates of bile acids and cholesterol

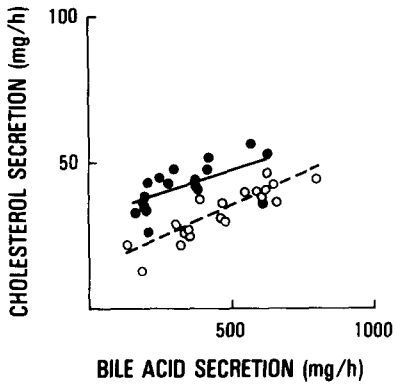

Fig. 2. Relationship between bile acid secretion and cholesterol secretion in subject No. 8 during treatment with CDCA, ○ and CA, ●. Equation of the regression line during CDCA therapy, $y = 12.74 + 0.0223x$, $r = 0.877$, $P < 0.001$ and CA therapy, $y = 31.12 + 0.0163x$, $r = 0.620$, $P < 0.01$.

during CDCA feeding as well as during CA feeding. A typical example is shown for one patient in **Fig. 2.** The *r*-values for the correlation coefficients were higher during CDCA therapy than with CA therapy in six of the subjects. As presented in **Table 6,** *r*-values averaged 0.882 ± 0.041 and 0.742 ± 0.061, respectively, for the two treatment periods. Within the range of bile acid secretion rates investigated, cholesterol: bile acid ratios were higher during CA feeding than with CDCA feeding. This was true both during formula infusion and fasting (**Table 7**).

Relationship between cholesterol and phospholipid secretion rates

There was a linear relationship between the secretion rates of phospholipids and cholesterol in all subjects during the two treatment regimens. An example is shown in **Fig. 3.** Again, Table 6 shows that the *r*-values for correlation coefficients were significantly higher during CDCA treatment (0.905 ± 0.025) than with CA treatment (0.800 ± 0.041) ($P < 0.025$). With the exception of one subject (No. 7) the cholesterol:phospholipid ratio was higher during the CA period compared with the CDCA period. The cholesterol:phospholipid ratio during fasting was significantly greater on CA than with CDCA (Table 7). Similarly the cholesterol:phospholipid ratio increased to a greater extent with decreasing outputs of bile acids when patients were on the CA limb of their study (**Fig. 4**).

Relationship between phospholipid and bile acid secretion rates

The relationship between phospholipid and bile acid was linear in all subjects. An example is demonstrated in **Fig. 5.** The *r*-values for the correlation coefficients averaged 0.950 ± 0.009 during CDCA therapy and 0.853 ± 0.065 during CA therapy (Table 6). The slope coefficients were somewhat higher in seven of the subjects during the CDCA period (mean value, 0.388 ± 0.038) compared with the CA period (mean value, 0.289 ± 0.039) indicating that CDCA can stimulate greater outputs of phospholipids from the liver with increasing outputs of bile acids than can CA.

Bile acid pool size, secretion rates, and cycling frequency

During the feeding on both bile acids, pool sizes were large in the subjects on weight maintenance diets as well as in the obese subjects on caloric restriction. No consistent differences were obtained between pool sizes during CDCA feeding (5,695 ± 746 mg) and CA feeding (5,925 ± 854 mg) (Table 2). A linear relationship was obtained between pool size and secretion rates of bile acids during CDCA feeding ($r = 0.836$, $P < 0.01$) but not during CA feeding. An inverse relationship was found between pool size and cycling frequency during both CDCA treatment ($r = 0.691$, $P < 0.05$) and CA treatment ($r = 0.813$, $P < 0.01$).

Side effects

In all our subjects liver function tests were within the normal range before treatment and bile acid therapy did not cause increases in transaminases.

TABLE 6. Slope coefficients (C) and *r*-values for relationships obtained between secretion rates of the biliary lipids. Mean ± SEM

	Cholesterol / Bile Acids		Cholesterol / Phospholipids		Phospholipids / Bile Acids	
Treatment	C	*r*	C	*r*	C	*r*
CDCA	0.0365 ± 0.0050	0.882 ± 0.041	0.1041 ± 0.0206	0.905 ± 0.025	0.388 ± 0.038	0.950 ± 0.009
CA	0.0343 ± 0.0047	0.742 ± 0.061	0.1195 ± 0.0221	0.800 ± 0.041[a]	0.289 ± 0.039	0.853 ± 0.065

[a] Significantly different from CDCA period. $P < 0.025$.

TABLE 7. Molar ratios between biliary lipids during formula infusion (I) and during the last 4 hr of fasting (II)

Patients	Treatment		Cholesterol / Bile Acids	Cholesterol / Phospholipids	Phospholipids / Bile Acids
1	CDCA	I	0.060	0.20	0.29
		II	0.059	0.23	0.26
	CA	I	0.079	0.40	0.19
		II	0.091	0.52	0.18
2	CDCA	I	0.050	0.20	0.25
		II	0.048	0.19	0.25
	CA	I	0.079	0.30	0.26
		II	0.079	0.28	0.28
3	CDCA	I	0.045	0.21	0.21
		II	0.048	0.23	0.21
	CA	I	0.048	0.28	0.17
		II	0.050	0.25	0.20
5	CDCA	I	0.066	0.21	0.31
		II	0.089	0.27	0.33
	CA	I	0.075	0.36	0.21
		II	0.093	0.36	0.26
6	CDCA	I	0.123	0.35	0.35
		II	0.182	0.62	0.29
	CA	I	0.118	0.37	0.32
		II	0.231	0.59	0.39
7	CDCA	I	0.071	0.52	0.14
		II	0.094	0.50	0.19
	CA	I	0.092	0.23	0.33
		II	0.096	0.40	0.24
8	CDCA	I	0.045	0.13	0.35
		II	0.054	0.16	0.33
	CA	I	0.068	0.24	0.29
		II	0.120	0.37	0.32
9	CDCA	I	0.073	0.24	0.31
		II	0.086	0.27	0.32
	CA	I	0.112	0.35	0.32
		II	0.164	0.44	0.37
Mean	CDCA	I	0.067	0.26	0.28
± SEM			± 0.009	± 0.04	± 0.03
		II	0.083	0.31	0.27
			± 0.016	± 0.06	± 0.02
	CA	I	0.084	0.32	0.26
			± 0.008[a]	± 0.02	± 0.02
		II	0.116	0.40	0.28
			± 0.020[a]	± 0.04[b]	± 0.03

[a] Significantly different from corresponding CDCA period. $P < 0.02$.
[b] Significantly different from period I. $P < 0.05$.

Serum cholesterol and serum triglyceride levels were about the same during CDCA and CA treatment.

DISCUSSION

The present data provide ample confirmation of previous reports that CDCA feeding is much more potent than CA for reducing saturation of bile. The purpose of this study was to elucidate further reasons for the different effects of CDCA and CA and to determine in more detail factors which regulate and increase bile saturation.

One factor that has been claimed to influence the saturation of bile is the pool size of bile acids, and reductions in pool size have been implicated in the formation of supersaturated bile (22, 23). Therefore, at the same dose, CDCA could cause a greater expansion of the bile acid pool than CA. This was found not to be the case, however; both bile acids expanded total pools to about the same extent. Furthermore, average hepatic secretion rates of total bile acids were essentially the same on the two bile acids. Thus the favorable effect of CDCA on bile lipid composition, as compared to CA, cannot be explained by expansion

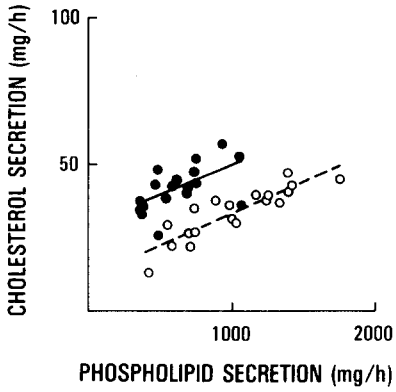

Fig. 3. Relationship between phospholipid secretion and cholesterol secretion in subject No. 8 during CDCA, ○ and CA, ● administration. Equation of the regression line during CDCA treatment, $y = 11.60 + 0.0436x$, $r = 0.876$, $P < 0.001$ and CA treatment, $y = 28.98 + 0.0412x$, $r = 0.578$, $P < 0.01$.

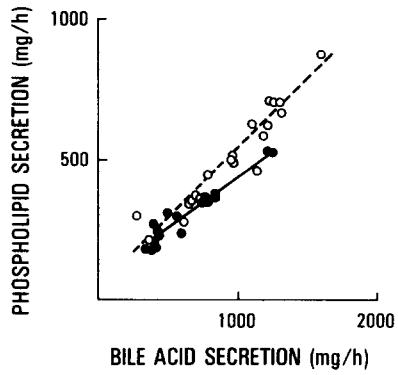

Fig. 5. Relationship between bile acid secretion and phospholipid secretion in subject No. 8 during CDCA, ○ and CA, ● therapy. Equation of the regression line during CDCA treatment, $y = 40.26 + 0.498x$, $r = 0.972$, $P < 0.001$ and CA treatment, $y = 77.07 + 0.357x$, $r = 0.970$, $P < 0.001$.

of the bile acid pool or increased secretion of bile acids. It nevertheless appears necessary to convert the pool of bile acids to at least 70% CDCA to obtain a significant reduction in saturation of gallbladder bile, as previously reported by Iser, Murphy and Dowling (24) and Thistle et al. (25).

Another mechanism whereby CDCA might reduce bile saturation is to decrease hepatic secretion of cholesterol more than CA does. This mechanism has been implicated before, but previous studies were carried out only during the feeding state (6, 7). In the present work this phenomenon has been confirmed, but we also demonstrated that the difference between CDCA and CA on cholesterol output is accentuated during fasting when the gallbladder is filling with bile.

One way in which cholesterol secretion could be reduced by CDCA is by direct inhibition of hepatic synthesis of cholesterol. Several workers (26–28) have reported that feeding CDCA to gallstone patients is associated with a decreased activity in the liver of the rate-limiting enzyme for cholesterol synthesis, HMG-CoA reductase. Furthermore, Ahlberg, Angelin, and Einarsson[1] have recently found that in gallstone patients CA feeding does not inhibit HMG-CoA reductase, while CDCA inhibits the enzyme by about 40%. Nervi and Dietschy (10) have carried out a detailed study on the role of bile acids in regulation of cholesterol synthesis in the rat liver, and their work suggests strongly that bile acids do not directly affect the enzymes involved in hepatic cholesterogenesis. However, it may be precarious to extrapolate their studies to man since rats expand their liver cholesterol pool during bile acid feeding while human subjects do not[1].

Even if CA and CDCA do not directly and differentially affect cholesterol synthesis in the liver, they might indirectly influence cholesterol production by affecting the conversion of cholesterol into bile acids. For instance, if CDCA were to inhibit synthesis of bile acids more than CA, this could lead to a temporary increase in hepatic cholesterol which itself could inhibit cholesterol synthesis. Should this occur, the reduction in cholesterol synthesis might in turn decrease cholesterol secretion into bile. However, there is no evidence from previous studies of bile acid kinetics that CDCA inhibits the conversion of cholesterol to bile acids to a greater extent than CA (32, 33). In the present study, examination of the pattern of individual bile acids in bile during the feeding of the two bile acids does not suggest that one bile acid inhibits bile acid synthesis more than the other (Table 2).

Another mechanism whereby CDCA might decrease cholesterol secretion more than CA would be through inhibition of cholesterol absorption (29). If cholesterol absorption were inhibited by CDCA, less cholesterol should return to the liver in chylomicrons and theoretically could reduce availability of cholesterol for secretion into bile. However, in the present study, using an intestinal perfusion technique (13), we could find no difference in absorption of cholesterol over a 50–100-cm segment of intestine during

[1] Ahlberg, J., B. Angelin, and K. Einarsson. Unpublished data.

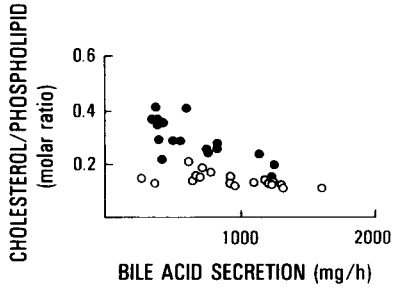

Fig. 4. Cholesterol:phospholipid molar ratios at various rates of bile acid output during treatment with CDCA, ○ and CA, ● in patient No. 8. Equation of the regression line during CDCA treatment, $y = 0.1821 - 0.00004x$, $r = 0.589$, $P < 0.01$ and during CA treatment, $y = 0.4147 - 0.00019x$, $r = 0.749$, $P < 0.001$.

CDCA and CA therapy. Thus it seems unlikely that reduced biliary cholesterol during CDCA feeding can be explained by decreased absorption of cholesterol. Even if cholesterol absorption were inhibited to a degree, which we did not find, a reduction in biliary cholesterol would probably be prevented by a compensatory *increase* in hepatic synthesis of cholesterol (30). This was shown in a recent study by Mok et al. (31) in which inhibition of cholesterol absorption by neomycin did not alter appreciably the composition of biliary lipids under an experimental design similar to that of the present study.

A final possible way in which CDCA could reduce hepatic secretion of cholesterol is to alter the coupling between cholesterol and other biliary lipids at the site of secretion into bile. In one recent study, Lindblad and Schersten (11) specifically examined the interrelations between biliary lipid secretion rates during acute infusion of CDCA and CA in patients with complete biliary drainage. Their data suggested that the secretory coupling between CDCA and cholesterol was weaker than that between CA and cholesterol. In fact they found that cholesterol output was not significantly correlated with bile acid output during CDCA infusion. In contrast, we obtained linear relationships between the secretion rates of cholesterol and bile acids during both CDCA and CA treatment. Indeed, in most of our patients, outputs of cholesterol were better correlated with CDCA than with CA. We cannot explain why our results are not in accord with those of Lindblad and Schersten (11), but long-term effects of CDCA could be different from more acute effects on biliary lipid secretion.

Another significant factor determining bile saturation is the ratio of cholesterol to phospholipids. Several studies (34–37) have demonstrated that this ratio usually remains constant over a considerable range of bile acid secretion at higher outputs of bile acids. This suggests that secretion of cholesterol and phospholipids occurs in a linked, or coupled fashion. In the present study, a linkage between cholesterol and phospholipids was revealed by a significant correlation between their secretion rates during both CA and CDCA treatment. However, the ratios of cholesterol to phospholipids were higher during CA compared with CDCA treatment in seven of eight patients investigated. This increase in the ratio on CA thus contributed significantly to the higher bile saturation on CA.

The above-mentioned studies (34–37) have also shown that at lower secretion rates of bile acids, as occurs with fasting, secretion of cholesterol and phospholipids become uncoupled; under this circumstance the ratio increases. The present investigation indicated that the relatively high ratios of cholesterol to phospholipids during CA therapy increased still more during fasting, but with CDCA, the ratio during fasting was not significantly increased (Fig. 5). The capacity of CDCA to maintain a nearly constant ratio of cholesterol to phospholipids at low bile acid outputs would thus appear to be crucial for the secretion of a relatively unsaturated bile with fasting. Although the mechanisms by which the ratio is kept from rising sharply with decreasing bile acid outputs are unclear, they would seem to us to be extremely important for the prevention of supersaturated bile.

In conclusion, our study has confirmed previous findings that CDCA reduces the saturation of bile by affecting hepatic secretion rates of cholesterol. The differential actions of CDCA and CA on cholesterol secretion is accentuated during fasting when bile is entering the gallbladder. Our data show that this difference cannot be explained by differences in availability of cholesterol derived from cholesterol absorption. The data do not exclude the possibility that availability of hepatic cholesterol for biliary secretion might be affected through differences in cholesterol synthesis on the two bile acids[1]. While this latter mechanism could be important, another mechanism of possibly equal importance is that, in contrast to CA, CDCA prevents a dissociation of hepatic secretions of cholesterol and phospholipids at low outputs of bile acids.

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