Ursodeoxycholic acid treatment in cholesterol gallstone disease: effects on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, biliary lipid composition, and plasma lipid levels

Bo Angelin, Staffan Ewerth, and Kurt Einarsson

Departments of Medicine and Surgery, Karolinska Institutet at Huddinge University Hospital, Stockholm, Sweden

Abstract The present study was undertaken to characterize the effects of ursodeoxycholic acid on biliary lipid metabolism in man. Fifteen gallstone patients were treated with ursodeoxycholic acid at a daily dosage of 15 mg per kg body weight for about 4 weeks before cholecystectomy. At operation a liver biopsy, together with gallbladder and hepatic bile, were obtained. Eighteen untreated gallstone patients undergoing cholecystectomy served as controls. During treatment with ursodeoxycholic acid, hepatic bile became unsaturated with cholesterol in all patients investigated. The total biliary lipid concentration remained unchanged. The hepatic cholesterol concentration decreased by about 20%. No significant change in the microsomal HMG CoA reductase activity was observed (38.5 ± 6.7 pmol · min^{-1} · mg protein^{-1} vs 38.3 ± 4.7 pmol · min^{-1} · mg protein^{-1} in the controls; means ± SEM). Plasma concentrations of total cholesterol were reduced by about 10%, and those of high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol by about 15%. Plasma triglyceride levels remained essentially unchanged during treatment. We conclude that, similar to chenodeoxycholic acid therapy, ursodeoxycholic acid treatment results in unsaturation of fasting hepatic bile. In contrast to the changes seen during chenodeoxycholic acid feeding, however, the unsaturation of hepatic bile during ursodeoxycholic acid treatment is not primarily related to a decreased hepatic HMG CoA reductase activity. Furthermore, while chenodeoxycholic acid tends to increase plasma LDL levels, such changes are not seen during ursodeoxycholic acid treatment.—**Angelin, B., S. Ewerth, and K. Einarsson.** Ursodeoxycholic acid treatment in cholesterol gallstone disease: effects on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, biliary lipid composition, and plasma lipid levels. *J. Lipid Res.* 1983. **24:** 461–468.

Supplementary key words bile acid • cholesterol • phospholipids • lipoproteins

Supersaturation of gallbladder bile with cholesterol precedes and predisposes to formation of cholesterol gallstones in man. Accordingly, unsaturation of bile is a prerequisite for dissolution of cholesterol gallstones. Administration of chenodeoxycholic acid or ursodeoxycholic acid to patients with gallstones decreases cholesterol saturation of gallbladder bile and may induce gallstone dissolution (1–4). The exact mechanisms of action of the two bile acids in man have not been completely elucidated.

According to several investigations chenodeoxycholic acid reduces the biliary secretion of cholesterol both in the meal-stimulated and in the fasted state (5–7). Of particular importance may be the fact that chenodeoxycholic acid treatment results in unsaturation of fasting hepatic bile (8). This effect is apparently linked to a decreased hepatic cholesterol synthesis, since the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (mevalonate:NADP oxidoreductase, E.C. 1.1.1.34), the rate-limiting step in cholesterol biosynthesis, is suppressed (8–11).

Ursodeoxycholic acid has recently been reported to also reduce the stimulated biliary secretion of cholesterol (12). There is presently no information on the effect of ursodeoxycholic acid on the saturation of fasting hepatic bile. Furthermore, conflicting reports have appeared about the influence of ursodeoxycholic acid on hepatic HMG CoA reductase activity in gallstone patients (10, 11, 13).

Since ursodeoxycholic acid seems to be more effective at lower dosage than chenodeoxycholic acid, and without producing side effects as diarrhea and hypertransaminasemia (3, 4) it may be anticipated to become an important alternative as a gallstone dissolving agent in the future. A more detailed knowledge of the effects of ursodeoxycholic acid treatment on biliary lipid metabolism therefore seems warranted. The present study

Abbreviations: HDL, high density lipoproteins; HMG, 3-hydroxy-3-methylglutaryl; HMG CoA reductase, mevalonate:NADP oxidoreductase, E.C. 1.1.1.34; LDL, low density lipoproteins; VLDL, very low density lipoproteins.

TABLE 1. Clinical data on patients (individual data and means ± SEM)

Patient Number	Sex	Age	Relative Body Weight[a]	Plasma Cholesterol Concentration		Plasma Triglyceride Concentration	
				Before	During	Before	During
		yr		*mmol · l⁻¹*		*mmol · l⁻¹*	
Patients with cholesterol gallstones, treated with ursodeoxycholic acid							
1. GA	F	41	116	4.0	3.4	1.2	0.9
2. LC	F	63	107	6.2	6.0	1.5	1.3
3. RC	F	48	121	5.9	7.3	1.7	2.4
4. AE	F	66	90	5.2	4.2	1.2	0.8
5. IE	F	60	81	8.5[b]	7.3	0.6	0.6
6. M-L.H	F	31	93	5.0	4.4	0.5	0.6
7. UH	F	35	80	5.2	5.2	0.7	0.5
8. EN	F	58	107	5.4	5.8	1.5	1.6
9. R-M.O	F	40	90	4.8	4.6	0.6	0.5
10. UO	F	28	113	4.4	4.2	0.7	1.1
11. LK	F	62	106	6.2	6.0	1.5	1.3
12. IH	F	60	91	7.2	6.9	1.4	1.5
13. TS	M	63	90	6.6	5.0	1.5	1.1
14. EC	M	76	106	5.1	3.4	1.1	1.3
15. RN	M	60	106	5.4	3.6	1.4	1.2
Total (15)		53 ± 4	100 ± 3	5.7 ± 0.3	5.2 ± 0.3[c]	1.1 ± 0.1	1.1 ± 0.1
Patients with cholesterol gallstones, untreated							
Female (10)		54 ± 4	100 ± 3	6.1 ± 0.6		1.5 ± 0.2	
Male (8)		55 ± 5	103 ± 5	4.8 ± 0.6		1.5 ± 0.2	
Total (18)		54 ± 3	101 ± 3	5.5 ± 0.4		1.5 ± 0.2	

[a] Calculated as $\frac{\text{body weight (kg)}}{\text{height (cm)} - 100} \times 100\%$.
[b] This patient had normal serum cholesterol concentration at the initial screening.
[c] Significantly different from before treatment, $P < 0.02$.

was undertaken to investigate the influence of ursodeoxycholic acid on hepatic HMG CoA reductase activity, hepatic cholesterol concentration, biliary lipid concentration and composition, as well as on cholesterol saturation of hepatic and gallbladder bile, and plasma lipids in patients with cholesterol gallstones.

MATERIALS AND METHODS

Subjects

The present study comprised 33 nonobese patients, 22 females and 11 males, undergoing elective cholecystectomy because of cholesterol gallstones. They had all been admitted to the outpatient clinic of the Department of Surgery, where they had passed a health screening. Initially, patients with clinical or laboratory evidence of diabetes mellitus, ethanol overconsumption, hyperlipidemia, or diseases affecting liver, kidney, or thyroid function had been excluded. Fifteen of the patients were randomly chosen to be treated with ursodeoxycholic acid prior to operation. Clinical data on the patients are given in **Table 1.**

Experimental procedure

Fifteen patients were given ursodeoxycholic acid in a daily dose of 15 mg per kg body weight for about 4 weeks before operation. They tolerated the medication well and none developed diarrhea. Hepatic function tests remained unchanged during the treatment period as did body weights.

The patients were hospitalized in the surgical ward 2–3 days prior to operation. They were fed the regular hospital diet, in which 35, 20, and 45% of calories were supplied as fat, protein, and carbohydrate, respectively. The daily intake of cholesterol was about 0.5 mmol per day.

All operations were performed between 8 and 9 AM after a 12-hr fast to avoid a possible diurnal variation of enzyme activity. Standardized anesthesia was given (cf. 8, 14). After opening of the abdomen, a 2–4-g liver biopsy was obtained from the left lobe of the liver, immediately placed in ice-cold buffer, and transported to the laboratory within 10 min. A specimen of the biopsy was sent for histological examination. Slight fatty infiltration was seen in four of the untreated gallstone patients.

The cystic duct was identified and clamped, and bile from the gallbladder and from the common duct was obtained by aspiration and kept on ice. Aliquots were extracted immediately with 20 vol of chloroform–methanol 2:1 (v/v). A regular cholecystectomy was then performed. Analysis of the stones showed them to consist in all cases of more than 70% cholesterol. None of the patients had stones in the common duct as judged by operative cholangiography. No complications were encountered during the operation.

The ethical aspects of the study were approved by the Ethical Committee of Karolinska Institutet, Stockholm. Informed consent was obtained from each patient before the study.

Materials

[3-^{14}C] HMG CoA (sp act 20 μCi/mg) and DL [5-^{3}H(N)]mevalonic acid (sp act 25 μCi/mg) were obtained from New England Nuclear Corp., Boston, MA. The radioactive HMG CoA was diluted with unlabeled material obtained from P-L Biochemicals, Inc., Milwaukee, WI, to yield a final specific radioactivity of 1.45 μCi/mg. Unlabeled DL-mevalonic acid lactone, NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from Sigma Chemical Co., St. Louis, MO. 3α-Hydroxysteroid dehydrogenase (Sterognost^{R}) and cholesterol oxidase [Nyco-test^{R} kolesterol) were purchased from Nyegaard A/S, Oslo, Norway.

Ursodeoxycholic acid was provided by Tokyo Tanabe Co., Tokyo, Japan, and was shown to be more than 98% pure by gas–liquid chromatography. It was administered to the patients in 125-mg (0.32 mmol) capsules twice a day.

Preparation of liver microsomes

About 1 g of the liver biopsy was cut into pieces and homogenized with a Potter-Elvehjem homogenizer equipped with a loosely fitting Teflon pestle in a medium containing 0.3 M sucrose, 0.075 M nicotinamide, 0.002 M EDTA, and 0.02 M mercaptoethanol. The homogenate (10%, w/v) was centrifuged at 20,000 *g* for 15 min. The microsomal fraction was obtained by centrifugation of the 20,000 *g* supernatant fluid at 100,000 *g* for 60 min. This was then washed once with the same buffer and recentrifuged at 100,000 *g* for 30 min. The microsomal fraction obtained was resuspended in 0.17 M phosphate buffer, pH 7.4, containing 0.034 M mercaptoethanol.

The cholesterol concentration of the homogenate was determined by the method of Hanel and Dam (15) and the microsomal protein concentration was determined by the method of Lowry et al. (16).

Assay of HMG CoA reductase activity

The assay system has recently been described in detail (14). The microsomal fraction (0.15–0.5 mg of protein) was preincubated for 10 min at 37°C in a total volume of 0.80 ml containing 100 mM phosphate buffer (pH 7.2), 3 mM NADP, 10 mM glucose-6-phosphate, 5 units of glucose-6-phosphate dehydrogenase, and 20 mM mercaptoethanol. Then, 0.5 μCi (0.4 mM) of [3-^{14}C]HMG CoA, dissolved in 50 μl of distilled water, was added and the incubation was run for 15 min. The incubation was stopped by the addition of 0.1 ml of 5 M HCl. The analysis of mevalonate formation and calculation of HMG CoA reductase activity were the same as described (14).

Analysis of biliary lipids

The cholesterol content of the chloroform extract was determined by an enzymatic method (17). The phospholipid content was determined by the method of Rouser, Sidney, and Akira (18). Biliary bile acid concentration was determined using a 3α-hydroxysteroid dehydrogenase assay (19). The coefficients of variation of the methods for analyzing cholesterol, phospholipids, and bile acids as determined from duplicate determinations were 4, 3, and 3%, respectively.

The individual bile acid composition of bile was analyzed after hydrolysis in 1 M KOH at 110°C for 12 hr. After acidification, the bile acids were extracted with ethyl ether, and the methyl trimethylsilyl ethers were prepared and analyzed by gas–liquid chromatography using a 1% Hi-Eff BP 8 column.

Calculations of biliary lipid composition and cholesterol saturation

The relative lipid composition of bile was calculated as moles of cholesterol, bile acids, and phospholipids per 100 moles of total lipids (20). The total lipid concentration was expressed in g/dl. Cholesterol saturation was calculated as a percentage of the predicted cholesterol solubility at the respective biliary lipid concentration and composition as recently described by Carey (21). In ursodeoxycholic acid-treated patients, Carey has suggested the use of a correction factor that takes into consideration the percentage of ursodeoxycholic acid in bile (21). However, since the correction factor reported is valid only for a total lipid concentration of 10 g/dl, it was not used in the present calculations.

Analysis of plasma lipids

Plasma cholesterol and triglycerides were determined with automated enzymatic techniques (Boehringer Mannheim Test Combination Cholesterol and Boehringer Mannheim Test Combination Triglycerides, respec-

TABLE 2. Effects of treatment with ursodeoxycholic acid on hepatic microsomal HMG CoA reductase activity and hepatic cholesterol concentration in patients with cholesterol gallstones (individual data and means ± SEM)

Patient Number	Sex	Hepatic Microsomal HMG CoA Reductase Activity	Hepatic Cholesterol Concentration
		pmol · min⁻¹ · mg protein⁻¹	*μmol · g liver⁻¹*
Patients with cholesterol gallstones, treated with ursodeoxycholic acid			
1.	F	21.8	3.9
3.	F	34.9	5.2
4.	F	26.4	5.0
5.	F	20.7	3.3
6.	F		4.3
7.	F	63.2	3.2
8.	F	36.0	4.6
10.	F	16.3	4.7
11.	F	88.3	5.2
13.	M	27.4	7.5
14.	M	59.2	5.7
15.	M	29.3	5.3
Total (12)		38.5 ± 6.7	4.8 ± 0.3
Patients with cholesterol gallstones, untreated[a]			
Female (10)		38.4 ± 7.3	6.2 ± 0.5[b]
Male (8)		38.2 ± 5.9	6.2 ± 0.5
Total (18)		38.3 ± 4.7	6.2 ± 0.3[b]

[a] Data on HMG CoA reductase activity in these patients have been included in a previous paper (14).
[b] Significantly different from corresponding group of treated patients, $P < 0.02$.

tively). Plasma very low density lipoproteins (VLDL) were separated by ultracentrifugation, and high density lipoproteins (HDL) were isolated from the infranatant by heparin-manganese chloride precipitation of low density lipoproteins (LDL) (22). Cholesterol concentration was determined in each lipoprotein fraction.

Statistical analysis

Means are given ± SEM. The statistical significance of differences was evaluated with Wilcoxon's rank sum test and Wilcoxon's test for pair differences. Correlations were tested with Spearman's method (23).

RESULTS

HMG CoA reductase activity and hepatic cholesterol concentration

Table 2 summarizes the results. There was a wide range of HMG CoA reductase activities in the control group (14.4–76.7 pmol · min^{-1} · mg protein^{-1}). There were no differences between females and males. No relationship was seen between enzyme activity and age, body weight, or serum lipids. Neither was there any correlation between enzyme activity and hepatic cholesterol concentration.

Administration of ursodeoxycholic acid was not associated with any significant change of HMG CoA reductase activity (Table 2, **Fig. 1**). The hepatic cholesterol concentration was about 20% lower ($P < 0.02$) in the ursodeoxycholic acid-treated patients compared with the controls.

Biliary lipid composition

Table 3 summarizes the results. Hepatic bile was supersaturated in all controls and gallbladder bile was supersaturated in all but one of the control subjects.

Ursodeoxycholic acid treatment resulted in reduction of the relative concentration of cholesterol in hepatic as well as gallbladder bile. The relative concentration of bile acids increased in hepatic bile whereas the phospholipid fraction remained essentially unchanged. Hepatic bile became unsaturated in all cases and gallbladder bile became unsaturated in eight out of nine patients studied. The total concentration of biliary lipids in hepatic bile and gallbladder bile was not significantly changed by ursodeoxycholic acid treatment. There was no correlation between hepatic bile saturation and HMG CoA reductase activity.

The ratios between the biliary lipid molar concentrations in hepatic bile reflect the ratios between their secretion rates in the fasting state. The cholesterol–bile acid ratio averaged 0.156 ± 0.012 in the control patients and was significantly lower in the ursodeoxycholic acid-treated patients (0.044 ± 0.005, $P < 0.01$). Similarly the cholesterol–phospholipid ratio was decreased

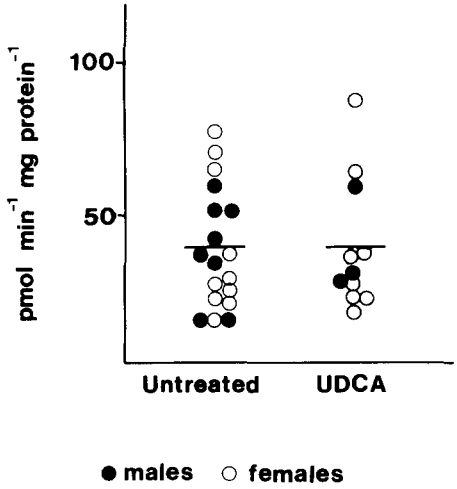

Fig. 1. Activity of hepatic microsomal HMG CoA reductase in untreated gallstone patients and in gallstone patients treated with ursodeoxycholic acid, 15 mg · kg^{-1} · day^{-1}, for 4 weeks. Horizontal bars indicate means within each group.

in the treated patients (0.183 ± 0.010) compared with the untreated patients (0.495 ± 0.021, $P < 0.01$). The phospholipid–bile acid ratio was not significantly different in ursodeoxycholic acid-treated patients (0.242 ± 0.026) compared to controls (0.316 ± 0.020).

Biliary bile acid composition

Ursodeoxycholic acid amounted to 52.3 ± 3.1% of the biliary bile acids in the ursodeoxycholic acid-treated patients and to 2.2 ± 0.9% in the control patients ($P < 0.01$). The other three major bile acids, cholic acid, chenodeoxycholic acid, and deoxycholic acid, were about 50% lower ($P < 0.01$) in the ursodeoxycholic acid-treated patients (14.7 ± 1.8%, 17.2 ± 0.6%, and 14.2 ± 2.7%, respectively) compared with the control patients (33.8 ± 2.8%, 31.0 ± 2.6%, and 31.9 ± 3.0%, respectively). The percentage of lithocholic acid was about the same in the ursodeoxycholic acid-treated patients (1.6 ± 0.7%) and the control patients (1.1 ± 0.6%).

Plasma lipids and lipoprotein cholesterol

The plasma concentration of total cholesterol decreased in 12 of the 15 patients during treatment with ursodeoxycholic acid ($P < 0.02$, Table 1). The LDL cholesterol concentration decreased in 10 out of 12 patients investigated from 3.82 ± 0.30 mmol · liter^{-1} to 3.18 ± 0.30 mmol · liter^{-1} ($P < 0.02$). Also the HDL cholesterol concentration was decreased (1.60 ± 0.15 mmol · liter^{-1} before and 1.32 ± 0.14 mmol · liter^{-1} during treatment, $P < 0.02$). Thus, the ratio LDL/HDL cholesterol was similar before (2.5 ± 0.3) and during (2.6 ± 0.2) treatment. The plasma concentration of triglycerides was not changed during the treatment period.

DISCUSSION

The present study demonstrates that ursodeoxycholic acid treatment is associated with distinct changes in fasting biliary lipid composition. In agreement with previous reports (3, 4, 24), we found that ursodeoxycholic acid feeding reduces the relative concentration of cholesterol in gallbladder bile. Furthermore, the observation that this treatment did not influence the total concentration of biliary lipids, which is an important factor in determining the cholesterol-holding capacity of bile (20, 21), permits the conclusion that ursodeoxycholic acid unsaturates gallbladder bile.

For the first time, data on lipid concentration and cholesterol saturation in hepatic bile during treatment with ursodeoxycholic acid were obtained. The finding that ursodeoxycholic acid made fasting hepatic bile unsaturated in all patients studied is probably of great importance in explaining why this bile acid is effective in dissolving gallstones. The change seen with ursodeoxycholic acid was even more impressive than that previously demonstrated for chenodeoxycholic acid (8). Similar to the findings seen with chenodeoxycholic acid therapy (7, 8), the ratios between cholesterol and bile acids, and between cholesterol and phospholipids were clearly reduced in fasting bile during ursodeoxycholic acid treatment. It is thus reasonable to speculate that ursodeoxycholic acid, like chenodeoxycholic acid (7), leads to a reduction of cholesterol secretion in the fasting state. A diminished biliary cholesterol output during stimulated bile secretion has recently been demonstrated during treatment with both bile acids (5–7, 12, 25).

Thus, the present study indicates that treatment with ursodeoxycholic acid produces changes in biliary lipid composition similar to those seen with chenodeoxycholic acid therapy of comparable duration and dosage (8). However, the mechanism behind these effects may not be the same for the two bile acids. The reduction in cholesterol saturation of hepatic bile during chenodeoxycholic acid treatment appears to be linked to a reduced activity of hepatic microsomal HMG CoA reductase (8–11). Under the highly standardized conditions employed (for discussion see ref. 14), no significant influence on HMG CoA reductase activity was observed during ursodeoxycholic acid feeding. This result is at variance with data obtained by some other authors (10, 11, 13). Thus, Maton et al. (10) reported that feeding ursodeoxycholic acid for 1–6 months resulted in a 40% decrease in HMG CoA reductase activity. Their doses were lower (4.5–7.2 mg kg^{-1}day^{-1}) and their patients were generally somewhat overweight (115% of ideal) compared to those in the present study. Furthermore, the untreated gallstone patients in their report had increased HMG CoA reductase activities compared to controls, so treatment with ursodeoxycholic acid actually only "normalized" enzyme activity in these patients. We have been unable to find an increased HMG CoA reductase activity in nonobese, normolipidemic patients with cholesterol gallstones (8), and the patients studied by Maton et al. (10) may thus not be fully comparable to those investigated in the present report. Salen et al. (13) recently reported a reduction of hepatic HMG CoA reductase activity in two gallstone patients treated with ursodeoxycholic acid for 1 year. On the other hand, Carulli et al. (11) found that 1 week of ursodeoxycholic acid treatment stimulated HMG CoA reductase activity in gallstone patients. In the study of Carulli et al. (11), there was no change in the composition of biliary lipids, and it is therefore doubtful if the patients had reached a metabolic steady state. However,

TABLE 3. Lipid concentration, lipid composition, and cholesterol saturation of hepatic bile and gallbladder bile (Individual data and means ± SEM)

Patient Number	Sex	Hepatic Bile				
		Lipid Concentration	Cholesterol	Bile Acids	Phospholipids	Cholesterol Saturation
		$g \cdot dl^{-1}$	*mol%*	*mol%*	*mol%*	*%*
Patients with cholesterol gallstones, treated with ursodeoxycholic acid						
2.	F	2.3	3.0	81.3	15.7	74
3.	F	4.4	4.6	72.2	23.2	72
4.	F	2.9	2.6	82.4	15.0	62
5.	F	7.4	3.8	77.2	19.0	62
6.	F					
7.	F	4.5	4.1	78.6	17.3	79
8.	F	4.8	3.2	76.4	20.4	55
9.	F	3.6	1.9	86.0	12.1	49
10.	F	3.3	3.9	70.5	25.6	62
14.	M	2.9	3.2	81.6	15.2	76
15.	M					
Total (9)		4.0 ± 0.5	3.4 ± 0.3	78.4 ± 1.7	18.2 ± 1.4	66 ± 3
Patients with cholesterol gallstones, untreated						
Female (7)		3.5 ± 0.6	9.7 ± 0.8[a]	69.2 ± 2.1[a]	21.1 ± 1.3	168 ± 11[a]
Male (6)		3.8 ± 0.7	11.4 ± 0.4	67.2 ± 1.7	21.4 ± 1.3	196 ± 16
Total (13)		3.6 ± 0.4	10.5 ± 0.6[a]	68.3 ± 1.4[a]	21.2 ± 0.9	181 ± 10[a]

[a] Significantly different from treated patients, $P < 0.01$.

under similar circumstances, these authors did observe a decrease of reductase activity during chenodeoxycholic acid feeding (11).

Thus, in the nonobese, normolipidemic patient with cholesterol gallstones, the induced unsaturation of hepatic and gallbladder bile seen during ursodeoxycholic acid does not appear to be linked to a reduced hepatic cholesterol synthesis. Therefore, although we cannot exclude the possibility of a relatively small decrease of reductase activity in some patients during ursodeoxycholic acid treatment, the present results are in contrast to the clear reduction of HMG CoA reductase activity seen during chenodeoxycholic acid therapy under similar experimental conditions (8). Further support for this concept is gained by the fact that during chenodeoxycholic acid treatment (8), but not during ursodeoxycholic acid treatment (present study), there was a positive correlation between hepatic bile saturation and HMG CoA reductase activity.

If suppression of cholesterol biosynthesis in the liver is not a major mechanism of action of ursodeoxycholic acid, how can one explain its remarkable effects on biliary lipids? First, the inflow of cholesterol from the intestine may be inhibited. The poor cholesterol-solubilizing capacity of ursodeoxycholic acid may, when it becomes the major bile acid secreted, result in a reduced absorption and a diminished transport of cholesterol to the liver. Some evidence for this mechanism has been presented (26), but it is not yet clear whether a reduced cholesterol uptake may reduce cholesterol secretion. Thus, beta-sitosterol and neomycin, which block cholesterol uptake, did not change biliary lipid composition significantly (27, 28).

Second, ursodeoxycholic acid treatment could lead to a reduced degradation of endogenous, cholesterol-containing lipoproteins. We did not, however, find any increase in total plasma cholesterol or triglyceride, nor in plasma LDL or HDL cholesterol. In contrast to chenodeoxycholic acid treatment, there was no reduction of plasma (VLDL) triglycerides (29, 30). Recent data also give some evidence for an increase of LDL cholesterol and LDL/HDL cholesterol ratio during chenodeoxycholic acid therapy (31–33). As such changes are definitely unwanted with regard to long-term risk for development of ischemic heart disease (34), the present finding that ursodeoxycholic acid treatment lowered plasma LDL and HDL cholesterol levels slightly without affecting the LDL/HDL ratio may have some clinical implications in the choice between these two bile acids. However, further controlled studies are needed to confirm and expand these observations.

A third possibility is that ursodeoxycholic acid does not suppress endogenous bile acid synthesis to the same extent as other bile acids. Whereas chenodeoxycholic

TABLE 3. (*Continued*)

Patient Number	Sex	Gallbladder Bile: Lipid Concentration	Cholesterol	Bile Acids	Phospholipids	Cholesterol Saturation
		$g \cdot dl^{-1}$	*mol%*	*mol%*	*mol%*	*%*
Patients with cholesterol gallstones, treated with ursodeoxycholic acid						
2.	F					
3.	F	5.3	5.8	77.4	16.8	109
4.	F	6.5	2.2	84.5	13.3	48
5.	F	11.9	3.3	80.2	16.5	55
6.	F	17.2	5.0	66.2	28.8	57
7.	F	4.1	4.7	75.5	19.9	83
8.	F	4.7	4.5	75.0	20.5	76
9.	F					
10.	F	7.8	3.7	80.9	15.5	70
14.	M	3.0	3.2	81.4	15.4	74
15.	M	8.4	3.6	79.1	17.3	62
Total (9)		7.7 ± 1.5	4.0 ± 0.4	77.8 ± 1.8	18.2 ± 1.5	70 ± 7
Patients with cholesterol gallstones, untreated						
Female (7)		5.8 ± 1.4	7.8 ± 0.8[a]	73.9 ± 2.5	18.3 ± 1.9	142 ± 13[a]
Male (4)		10.5 ± 2.4	9.6 ± 1.7	71.5 ± 5.6	19.0 ± 4.0	148 ± 10
Total (11)		7.5 ± 1.4	8.4 ± 0.8[a]	73.0 ± 2.4	18.5 ± 1.7	144 ± 9[a]

acid treatment inhibits cholic acid synthesis in man by about 50% (35, 36), data from our laboratory indicate that this is not seen during ursodeoxycholic acid feeding (25). This may also explain why the percentage of cholic acid in the bile is never reduced to the same extent during treatment with ursodeoxycholic acid as during treatment with chenodeoxycholic acid (1–4, 8, 12, present study). If ursodeoxycholic acid in itself does not have a suppressive effect on cholesterol breakdown to bile acids, feeding with this bile acid would thus result in *i*) expansion of the bile acid pool, *ii*) saturation of the intestinal uptake system for bile acids, and *iii*) a less efficient feedback inhibition of bile acid synthesis. In this situation, cholesterol breakdown would be stimulated at the same time as bile acid secretion was unchanged or increased (cf. 25). This interesting possibility merits further study.

The skillful technical assistance of Ms. Ann-Christine Andersson, Ms. Lisbet Benthin, Ms. Ruth Detlofsson, Ms. Gerd Ledin, Ms. Helene Saranius, Ms. Anna-Maja Suontaka, and Ms. Margret Wahlström, and the secretarial help of Ms. Lena Ericsson are gratefully acknowledged. This investigation was supported by grants from the Swedish Medical Research Council (03X-04793), Karolinska Institutets Fonder, the Loo and Hans Osterman Foundation, and the Swedish Society of Medical Sciences. Dr. Angelin was the recipient of a fellowship from the Ernst Klenk Foundation.

Manuscript received 29 July 1982 and in revised form 22 December 1982.

REFERENCES

1. Thistle, J. L., A. F. Hofmann, B. J. Ott, and D. H. Stephens. 1978. Chenotherapy for gallstone dissolution: I. Efficacy and safety. *J. Am. Med. Assoc.* **239:** 1041–1046.
2. Hofmann, A. F., J. L. Thistle, P. D. Klein, P. A. Szczepanik, and P. Y. S. Yu. 1978. Chenotherapy for gallstone dissolution: II. Induced changes in bile composition and gallstone response. *J. Am. Med. Assoc.* **239:** 1138–1144.
3. Workshop on Ursodeoxycholic Acid. 1978. R. H. Dowling, A. F. Hofmann, and L. Barbara, editors. MTP Press, Ltd., London.
4. Tokyo Cooperative Gallstone Study Group. 1980. Efficacy and indications of ursodeoxycholic acid treatment for dissolving gallstones. A multicenter doubleblind trial. *Gastroenterology.* **78:** 542–548.
5. Adler, R. D., L. J. Bennion, W. C. Duane, and S. M. Grundy. 1975. Effects of low dose chenodeoxycholic acid feeding on biliary lipid metabolism. *Gastroenterology.* **68:** 326–334.
6. La Russo, N. F., N. E. Hoffman, A. F. Hofmann, T. C. Northfield, and J. L. Thistle. 1975. Effect of primary bile acid ingestion on bile acid metabolism and biliary lipid secretion in gallstone patients. *Gastroenterology.* **68:** 1301–1314.
7. Einarsson, K., and S. M. Grundy. 1980. Effects of feeding cholic acid and chenodeoxycholic acid on cholesterol absorption and hepatic secretion of biliary lipids in man. *J. Lipid Res.* **21:** 23–34.
8. Ahlberg, J., B. Angelin, and K. Einarsson. 1981. Hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and biliary lipid composition in man: relation to cholesterol gallstone disease and effects of cholic acid and

chenodeoxycholic acid treatment. *J. Lipid Res.* **22:** 410–422.
9. Coyne, M. J., G. G. Bonorris, L. I. Goldstein, and L. J. Schoenfield. 1976. Effects of chenodeoxycholic acid and phenobarbital on the rate-limiting enzymes of hepatic cholesterol and bile acid synthesis in patients with gallstones. *J. Lab. Clin. Med.* **87:** 281–291.
10. Maton, P. N., H. J. Ellis, M. J. P. Higgins, and R. H. Dowling. 1980. Hepatic HMG CoA reductase in human cholelithiasis: effects of chenodeoxycholic and ursodeoxycholic acids. *Eur. J. Clin. Invest.* **10:** 325–332.
11. Carulli, N., M. Ponz De Leon, F. Zironi, A. Pinetti, A. Smerieri, R. Iori, and P. Loria. 1980. Hepatic cholesterol and bile acid metabolism in subjects with gallstones: comparative effects of short term feeding of chenodeoxycholic and ursodeoxycholic acid. *J. Lipid Res.* **21:** 35–43.
12. Von Bergmann, K., M. Gutsfeld, K. Schulze-Hagen, and G. von Unruh. 1979. Effects of ursodeoxycholic acid on biliary lipid secretion in patients with radiolucent gallstones. *In* Biological Effects of Bile Acids. G. Paumgartner, W. Gerok, and A. Stiehl, editors. MTP Press, Ltd., London. 61–66.
13. Salen, G., A. Colalillo, D. Verga, E. Bagan, G. S. Tint, and S. Shefer. 1980. Effect of high and low doses of ursodeoxycholic acid on gallstone dissolution in humans. *Gastroenterology.* **78:** 1412–1418.
14. Angelin, B., L. Backman, K. Einarsson, L. Eriksson, and S. Ewerth. 1982. Hepatic cholesterol metabolism in obesity: activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Lipid Res.* **23:** 770–773.
15. Hanel, H. K., and H. Dam. 1955. Determination of small amounts of total cholesterol by the Tschugaeff reaction with a note on the determination of lathosterol. *Acta Chem. Scand.* **9:** 677–682.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
17. Roda, A., D. Festa, C. Sama, G. Mazzelli, R. Aldini, E. Roda, and L. Barbara. 1975. Enzymatic determination of cholesterol in bile. *Clin. Chim. Acta.* **64:** 337–341.
18. Rouser, G., F. Sidney, and Y. Akira. 1970. Two-dimensional thin-layer chromatographic separation of polar lipids and determinations of phosphorus analysis of spots. *Lipids.* **5:** 494–496.
19. Fausa, O., and B. A. Skålhegg. 1974. Quantitative determination of bile acids and their conjugates using thin-layer chromatography and purified 3α-hydroxysteroid dehydrogenase. *Scand. J. Gastroenterol.* **9:** 249–254.
20. Carey, M. C., and D. M. Small. 1978. The physical chemistry of cholesterol solubility in bile. Relationship to gallstone formation and dissolution in man. *J. Clin. Invest.* **61:** 998–1026.
21. Carey, M. C. 1978. Critical tables for calculating the cholesterol saturation of native bile. *J. Lipid Res.* **19:** 945–955.
22. Carlson, K. 1973. Lipoprotein fractionation. *J. Clin. Pathol.* **26:** Suppl. 5, 32–37.
23. Snedecor, G. W., and W. G. Cochran. 1974. Statistical Methods. 6th edition. Iowa State University Press, Ames, IA.
24. Stiehl, A., P. Czygan, B. Kommerell, H. J. Weis, and K. H. Holtermüller, 1978. Ursodeoxycholic acid versus chenodeoxycholic acid: comparison of their effects on bile acid and bile lipid composition in patients with cholesterol gallstones. *Gastroenterology.* **75:** 1016–1020.
25. Einarsson, K., K. Nilsell, B. Angelin, and B. Leijd. 1983. Effects of ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) on bile acid kinetics and secretion rates of biliary lipids in man (Abstract). *In* Bile Acids and Cholesterol in Health and Disease. G. Paumgartner, A. Stiehl, and W. Gerok. editors. MTP Press, Ltd., London. In press.
26. Ponz De Leon, M., N. Carulli, P. Loria, R. Iori, and F. Zironi. 1980. Cholesterol absorption during bile acid feeding. *Gastroenterology.* **78:** 214–219.
27. Tangedahl, T. N., J. L. Thistle, A. F. Hofmann, and J. W. Matseshe. 1979. Effect of beta-sitosterol alone or in combination with chenic acid on cholesterol saturation of bile and cholesterol absorption in gallstone patients. *Gastroenterology.* **76:** 1341–1346.
28. Mok, H. Y. I., K. von Bergmann, J. R. Crouse, and S. M. Grundy. 1979. Biliary lipid metabolism in obesity: effects of bile acid feeding before and during weight reduction. *Gastroenterology.* **76:** 556–567.
29. Angelin, B., K. Einarsson, and B. Leijd. 1978. Effect of chenodeoxycholic acid on serum and biliary lipids in patients with hyperlipoproteinaemia. *Clin. Sci. Mol. Med.* **54:** 451–455.
30. Angelin, B., K. Einarsson, K. Hellström, and B. Leijd. 1978. Effects of cholestyramine and chenodeoxycholic acid on the metabolism of endogenous triglyceride in hyperlipoproteinemia. *J. Lipid Res.* **19:** 1017–1024.
31. Angelin, B., K. Einarsson, and B. Leijd. 1981. Clofibrate treatment and bile cholesterol saturation: short-term and long-term effects and influence of combination with chenodeoxycholic acid. *Eur. J. Clin. Invest.* **11:** 185–189.
32. Albers, J. J., S. M. Grundy, P. A. Cleary, D. M. Small, J. M. Lachin, L. J. Schoenfield, and the National Cooperative Gallstone Study Group. 1982. National Cooperative Gallstone Study. The effect of chenodeoxycholic acid on lipoproteins and apolipoproteins. *Gastroenterology.* **82:** 638–646.
33. Leijd, B., and B. Angelin. 1983. Effects of chenodeoxycholic acid and cholic acid feeding on plasma lipoproteins and cholesterol absorption in hyperlipoproteinemia: a cross-over study. *In* Bile Acids and Cholesterol in Health and Disease. G. Paumgartner, A. Stiehl, and W. Gerok, editors. MTP Press, Ltd., London. In press.
34. Steinberg, D. 1978. The rediscovery of high density lipoprotein: a negative risk factor in atherosclerosis (Editorial). *Eur. J. Clin. Invest.* **8:** 107–109.
35. Danzinger, R. G., A. F. Hofmann, J. L. Thistle, and L. J. Schoenfield. 1973. Effect of oral chenodeoxycholic acid on bile acid kinetics and biliary lipid composition in women with cholelithiasis. *J. Clin. Invest.* **52:** 2809–2821.
36. Kallner, M. 1975. The effect of chenodeoxycholic acid feeding on bile acid kinetics and fecal neutral steroid excretion in patients with hyperlipoproteinemia types II and IV. *J. Lab. Clin. Med.* **86:** 595–604.