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[1]

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Abstract The present work was undertaken in order to study whether or not there is a relation between hepatic HMG CoA reductase, hepatic cholesterol concentration, and biliary lipid composition. In 55 patients (10 with adenomyoma of the gallbladder wall, 45 with cholesterol gallstones) a liver biopsy together with gallbladder and hepatic bile were obtained at laparotomy under standardized conditions. Of the gallstone patients, twelve had been treated with cholic acid and ten with chenodeoxycholic acid in a dose of 15 mg·kg⁻¹·d⁻¹ for 6–8 weeks prior to operation. Hepatic bile was supersaturated with cholesterol both in cholesterol gallstone patients and in patients with gallbladder adenomyoma. Treatment with cholic acid reduced the cholesterol saturation of hepatic bile, although supersaturation persisted. During chenodeoxycholic acid treatment, hepatic bile became unsaturated in most of the patients. Hepatic cholesterol concentration was about 20% higher in patients with cholesterol gallstone disease than in gallstone-free controls. During treatment with cholic acid or chenodeoxycholic acid, hepatic cholesterol concentration was normalized. Microsomal HMG CoA reductase activity was similar in males and females with cholesterol gallstone disease and not different from that seen in the gallstone-free controls. Treatment with chenodeoxycholic acid resulted in a 40% reduction of HMG CoA reductase activity. Cholic acid had no effect. In gallstone-free controls and in bile acid-treated but not in untreated gallstone patients, saturation of hepatic bile correlated with HMG CoA reductase activity. It is concluded that treatment with chenodeoxycholic acid but not with cholic acid results in unsaturated hepatic bile. This unsaturation may in part be explained by a decreased hepatic HMG CoA reductase activity.—Ahlberg, J., B. Angelin, and K. Einarsson. 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.* 1981. **22:** 410–422.

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Hepatic cholesterol can be derived from three sources: dietary cholesterol via chylomicron remnants, peripheral tissues by plasma lipoproteins, and the liver itself by de novo synthesis (1, 2). The rate-limiting step in the biosynthesis of cholesterol, the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) to mevalonate by the microsomal enzyme HMG CoA reductase (mevalonate:NADP oxidoreductase, EC 1.1.1.34) is subject to a sensitive feedback control, still incompletely known in its details (3). In the rat, hepatic cholesterogenesis appears to be predominantly influenced by the inflow of chylomicron cholesterol to the liver (4). The importance of bile acids in this respect is more uncertain (4–6), as it may be related to effects on cholesterol absorption (4).

In the liver, cholesterol may have several fates: it can be stored as cholesteryl esters, secreted into plasma in lipoproteins, secreted into the bile as free cholesterol, and it can be converted into the primary bile acids, cholic acid (C) and chenodeoxycholic acid (CD) (1, 2). Special interest has been paid to cholesterol secreted in the bile, which is rendered soluble by forming micelles with phospholipids and bile acids

(7, 8). According to current views, supersaturation of the bile with cholesterol precedes and predisposes to formation of cholesterol gallstones in man (7). Treatment with CD, but not with C, causes desaturation of gallbladder bile and sometimes dissolution of cholesterol gallstones (9–12). This effect has been ascribed to decreased hepatic HMG CoA reductase activity during CD treatment (13). However, information on enzyme activities in relation to hepatic bile composition as well as comparable data on the effects of C are not available in man.

As part of a series of investigations on the regulation of cholesterol metabolism in man, the present study was undertaken with the following specific aims: a) to determine whether or not there is a relation between hepatic HMG CoA reductase activity, hepatic cholesterol concentration, and biliary lipid composition in healthy man; b) to characterize the possible disturbances of hepatic cholesterol metabolism and biliary lipid composition in cholesterol gallstone disease; and c) to investigate the effects of feeding with C or CD on hepatic HMG CoA reductase activity, cholesterol concentration, and biliary lipid composition in patients with cholesterol gallstones.

MATERIAL AND METHODS

Subjects

Altogether, 55 normolipidemic patients were included in the present study, 40 females and 15 males. They were all admitted to the Department of Surgery, Serafimerlasarettet, for elective cholecystectomy. A total of 45 patients had cholesterol gallstone disease[3]; twelve had been treated with C and ten with CD prior to operation. In ten of the patients (eight females and two males) the indication for surgery was suspected adenomyoma or polyps of the gallbladder wall with otherwise normal function of the gallbladder and no evidence of gallstone disease. Basal data on the patients are given in **Table 1.**

An initial screening of the subjects had been performed at the outpatient clinic. All patients with evidence of diabetes mellitus, hyperlipoproteinemia, ethanol overconsumption, or diseases affecting liver, kidney, or thyroid function had thus been excluded (cf. 14, 15). No drugs affecting liver function or lipid metabolism (except the C or CD medication) had been given from the time of the outpatient visit, which was at least 2 months before hospitalization.

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[3] Data on HMG CoA reductase activity in 23 of these patients have been included in a previous report (15).

Experimental procedure

At the outpatient clinic, twelve patients were given C, 15 mg per kg body weight per day, for at least 6 weeks prior to operation, and twelve were given CD in the same dose. This medication was well tolerated without side-effects in all patients but two, treated with CD, who developed diarrhea and quit the study. Body weight and routine indexes of hepatic function were unchanged during the treatment period.

About 2–3 days before surgery, the patients were hospitalized and given the regular hospital diet, in which 35, 20, and 45% of energy was supplied as fat, protein, and carbohydrate, respectively. The daily intake of cholesterol was about 0.5 mmol/day.

All operations were performed between 8 and 9 AM after a 12-hr fast. Standardized anesthesia was given with thiopentothal induction and continuous treatment with nitrous oxide, diazepam, and fentanyl. After opening of the abdomen, a 2 to 4-g liver biopsy was obtained from the left lobe of the liver and immediately placed in ice-cold 0.1 M Tris-Buffer solution, pH 7.4. A specimen of the biopsy was sent for histological examination. Liver morphology was normal in all subjects but one (patient no. 31), where a slight fatty infiltration was seen.

The cystic duct was identified and clamped, and bile from the gallbladder and from the common duct was obtained by aspiration. Bile samples were kept in ice and immediately extracted with organic solvents (see below). A regular cholecystectomy was then performed. Analyses of the stones showed them in all cases to consist of more than 70% cholesterol. Histological examination of the gallbladder confirmed the suspected diagnosis of adenomyoma in all ten subjects. None of the patients had stones in the common duct, as judged by peroperative cholangiography which was performed in all cases. No complications were seen during or after 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 operation.

Materials

[3-^{14}C]HMG CoA (sp act 20 μCi/mg), and [mevalonic-5-[3]H (N)]DL-mevalonic acid (sp act 25 μCi/mg) were obtained from New England Nuclear Corp., Boston, MA. The radioactively-labeled HMG CoA was diluted with unlabeled material, obtained from P-L Biochemicals, Inc., Milwaukee, WI, to yield a specific radioactivity of 1.45 μCi/mg. Unlabeled DL-mevalonic acid lactone, NADP, glucose-6-phosphate, and glu-

TABLE 1.   Basal data, hepatic microsomal HMG CoA reductase activity, and hepatic cholesterol concentration. Individual data and means ± SEM.

Patient Number	Sex[a]	Age	Relative Bodyweight[b]	Gallbladder Function	Hepatic Microsomal HMG CoA Reductase Activity	Hepatic Cholesterol Concentration
		yr			$pmol \cdot min^{-1} \, mg \, prot^{-1}$	$\mu mol \cdot g \, liver^{-1}$
Patients with adenomyoma of the gallbladder						
1. TM	F	64	133	+	38.7	5.9
2. LC	F	58	96	+	33.3	7.1
3. KH	F	57	106	+	22.9	6.5
4. KO	F	53	107	+	25.1	4.9
5. AW	F	48	106	+	14.3	7.9
6. BP	F	44	105	+	15.1	5.4
7. AH	F	40	111	+	55.4	6.7
8. KS	F	23	93	+	11.8	8.5
9. BB	M	67	92	+	18.2	9.0
10. SB	M	52	92	+	18.9	7.5
Patients with cholesterol gallstones						
11. EH	F	70	92	+	10.2	7.5
12. MS	F	67	110	+	16.9	7.2
13. EG	F	66	111	−	54.6	9.6
14. SJ	F	65	101	+	11.2	7.0
15. MN	F	62	85	−	31.7	6.7
16. IR	F	61	106	−	21.7	9.3
17. KN	F	57	88	+	11.9	6.7
18. BL	F	52	120	+	15.3	13.2
19. ME	F	52	145	+	19.1	8.3
20. GB	F	51	87	−	27.5	8.8
21. MA	F	49	157	−	19.3	8.5
22. RA	F	49	116	+	22.0	8.3
23. GN	F	36	129	+	16.1	9.6
24. GM	F	32	80	+	30.6	8.3
25. UH	F	27	98	+	36.6	7.8
26. EL	M	64	93	+	19.5	7.8
27. EM	M	64	99	−	12.8	9.0
28. BA	M	57	93	+	13.8	9.6
29. HH	M	52	109	+	20.5	7.8
30. SA	M	43	102	−	10.8	6.5
31. VN	M	40	118	+	15.9	13.2
32. HL	M	31	84	−	23.7	8.0
33. PL	M	28	94	+	17.6	5.7
Patients with cholesterol gallstones, treated with cholic acid						
34. MN	F	63	115	−	23.5	10.9
35. MK	F	56	110	+	36.1	5.7
36. KMm	F	46	112	+	17.4	6.2
37. ML	F	39	88	+	11.0	8.8
38. AK	F	34	101	+	10.0	5.4
39. GR	F	33	88	+	17.6	5.9
40. IW	F	33	78	−	16.1	5.4
41. SJ	F	19	95	−	24.1	6.2
42. KMn	M	61	104	+	28.7	7.5
43. ES	M	60	113	−	22.9	7.8
44. AN	M	57	106	+	24.7	5.7
45. OJ	M	51	103	+	17.0	6.7
Patients with cholesterol gallstones, treated with chenodeoxycholic acid						
46. MNn	F	65	89	−	13.0	6.3
47. RN	F	65	103	−	—	—
48. MS	F	54	80	+	8.7	7.2
49. EJ	F	53	109	+	9.1	6.3
50. IR	F	49	97	+	7.5	7.2
51. MW	F	43	97	+	14.8	6.5
52. AS	F	42	92	−	17.6	6.1
53. AA	F	38	103	−	15.6	6.2
54. ESn	F	27	75	+	9.7	5.7
55. GJ	M	45	103	+	11.2	9.3

TABLE 1.   (Continued)

Patient Number	Sex[a]	Age	Relative Bodyweight[b]	Gallbladder Function	Hepatic Microsomal HMG CoA Reductase Activity	Hepatic Cholesterol Concentration
		yr			$pmol \cdot min^{-1}\, mg\, prot^{-1}$	$\mu mol \cdot g\, liver^{-1}$
Gallbladder adenomyoma (8 females, 2 males)		51 ± 4	104 ± 4	10	25.4 ± 4.3	6.9 ± 0.4
Cholesterol gallstones						
Female (15)		53 ± 3	108 ± 6	10	23.0 ± 3.0	8.5 ± 0.4
Male (8)		47 ± 5	99 ± 4	5	16.8 ± 1.5	8.5 ± 0.8
Total (23)		51 ± 3	105 ± 4	15	20.8 ± 2.1	8.5 ± 0.4^c
Cholic acid treatment (8 females, 4 males)		46 ± 4	101 ± 3	8	20.8 ± 2.1	6.9 ± 0.5^d
Chenodeoxycholic acid treatment (9 females, 1 male)		48 ± 4	95 ± 3	7	$11.9 \pm 1.2^{c,d,e}$	6.8 ± 0.4^d

[a] Male, female.

[b] Calculated as $\dfrac{\text{body weight (kg)}}{\text{height (cm)} - 100} \times 100\%$.

[c] Significantly different from patient with adenomyoma of the gallbladder, $P < 0.02$.

[d] Significantly different from patients with cholesterol gallstones, $P < 0.02$.

[e] Significantly different from gallstone patients treated with cholic acid, $P < 0.005$.

cose-6-phosphate dehydrogenase were obtained from Sigma Chemical Co., St. Louis, MO. 3α-Hydroxy-steroid dehydrogenase was supplied as a kit (Sterognost®) from Nyegaard A/S, Oslo, Norway.

Chenodeoxycholic acid (Chendal®) was obtained from Draco, Sweden, and was administered in 125-mg (0.32 mmol) capsules. Cholic acid was purchased from Sigma Co., St. Louis, MO, and was given in 250-mg (0.64 mmol) capsules. Both bile acids were shown to be more than 98% pure by thin-layer chromatography.

Preparation of liver microsomes

About 1 g of the liver biopsy was used to form a 10% (w/v) homogenate in a medium containing 0.3 M sucrose, 0.075 M nicotinamide, 0.002 M EDTA, and 0.02 M mercaptoethanol. The homogenate was centrifuged at $20,000\, g$ for 15 min. The supernatant fluid was centrifuged at $100,000\, g$ for 60 min to obtain the microsomal fraction, which was washed with the homogenizing medium and recentrifuged at $100,000\, g$ for 30 min. The microsomal fraction was then suspended in phosphate buffer (0.17 M, pH 7.2, and mercaptoethanol, 0.034 M) to a volume corresponding to that of the $20,000\, g$ supernatant fluid.

The cholesterol concentration of the homogenate and of the microsomal fraction was determined by the method of Hanel and Dam (16), and the protein concentration of the microsomal fraction by the method of Lowry et al. (17). The concentrations of microsomal protein were similar in the four groups of patients (23 ± 2 mg/g liver in controls, mean ± SEM, 22 ± 1 mg/g in patients with gallstones, 22 ± 2 mg/g

in C-treated, and 23 ± 1 mg/g in CD-treated). Microsomal cholesterol, expressed as nmol/mg protein, was also similar in the different groups (58.1 ± 0.7, 59.9 ± 5.9, 61.6 ± 6.3, and 61.9 ± 3.0, respectively).

Assay of HMG CoA reductase activity

Each incubation flask contained, in a total volume of 0.85 ml, 0.2 ml of microsomal fraction, 100 mM phosphate buffer, pH 7.2, 3 mM $MgCl_2$, 3 mM NADP, 10 mM glucose-6-phosphate, 5 units of glucose-6-phosphate dehydrogenase, 20 mM mercaptoethanol, and 0.5 μCi (0.4 mM) of [3-^{14}C]HMG CoA. The incubation conditions, analysis of mevalonate formation, and calculation of HMG CoA reductase activity were the same as recently described (15). The coefficient of variation of the assay was about 12%.

Analysis of biliary lipids

For determination of cholesterol and phospholipids, a portion of the common duct bile or gallbladder bile obtained was immediately extracted with 20 vol of chloroform–methanol 2:1 (v/v). Cholesterol was determined by the method of Hanel and Dam (16), and phospholipids by the method of Bartlett (18).

The total bile acid concentration was determined using a 3α-hydroxysteroid dehydrogenase assay (19). The individual bile acid composition was determined in portions of bile hydrolyzed in 1 M KOH at 110°C for 12 hr. After acidification, the deconjugated bile acids were extracted with diethyl ether, and their methyl trimethylsilyl ethers were analyzed by gas–liquid chromatography using a 1% Hi-Eff BP8

TABLE 2. Lipid composition and cholesterol saturation of

Patient Number, Sex	Hepatic Bile				
	Lipid Concentration	Cholesterol	Bile Acids	Phospholipids	Cholesterol Saturation
	g dl⁻¹	mol%	mol%	mol%	%

Patients with adenomyoma of the gallbladder

1. F	1.6	8.6	72.8	18.6	200
2. F	0.76	5.9	83.4	10.7	274
3. F	3.2	9.6	65.2	25.2	151
4. F	1.0	9.4	68.4	22.2	202
5. F					
6. F	7.3	6.6	72.2	21.2	100
7. F					
8. F	0.81	5.9	71.7	22.4	133
9. M	0.74	9.2	65.8	25.0	193
10. M	4.4	9.2	64.7	26.2	135

Patients with cholesterol gallstones

11. F	1.0	15.6	56.0	28.5	311
12. F	5.3	16.0	45.5	38.5	227
13. F	2.0	12.0	64.4	23.7	209
14. F	1.4	18.8	54.5	26.7	318
15. F	2.1	5.9	76.0	18.1	133
16. F	1.7	8.9	66.4	24.7	158
17. F	0.85	8.8	62.7	28.5	179
18. F					
19. F	1.7	8.0	71.5	20.5	164
20. F	4.5	11.6	56.6	31.8	156
21. F					
22. F	1.6	9.6	65.6	24.8	175
23. F	4.5	16.1	56.0	27.9	218
24. F	0.55	7.6	68.5	23.9	212
25. F					
26. M	2.6	12.9	61.3	25.8	197
27. M	2.2	15.2	66.4	18.5	309
28. M	1.4	7.7	67.2	25.1	141
29. M	0.49	9.5	60.8	29.6	423
30. M	3.1	12.6	57.4	30.0	186
31. M	3.6	12.8	60.4	26.9	183
32. M					
33. M					

Patients with cholesterol gallstones treated with cholic acid

34. F	3.7	8.6	67.6	23.8	133
35. F	2.4	6.4	68.1	25.6	106
36. F					
37. F	4.7	5.8	73.8	20.4	96
38. F	11.9	6.1	77.8	16.2	100
39. F	0.66	7.7	65.4	26.9	172
40. F	1.9	12.9	61.8	25.4	215
41. F	3.1	6.9	71.3	21.8	116
42. M	1.0	13.0	64.8	22.2	271
43. M	3.5	6.9	72.4	20.7	117
44. M	0.86	8.1	64.6	27.3	163
45. M					

Patients with cholesterol gallstones treated with chenodeoxycholic acid

46. F	2.6	3.5	74.6	21.9	65
47. F	5.9	3.2	75.8	21.0	53
48. F	5.9	5.5	74.7	19.8	93
49. F	1.7	6.1	67.0	26.9	105
50. F	4.0	5.2	72.4	22.4	85
51. F	0.62	5.0	72.4	22.7	121
52. F	1.2	5.5	70.1	24.4	109
53. F					
54. F					
55. M	4.7	5.3	72.7	22.0	84

hepatic bile and gallbladder bile. Individual data and means ± SEM.

Patient Number, Sex	Gallbladder Bile				
	Lipid Concentration	Cholesterol	Bile Acids	Phospholipids	Cholesterol Saturation
	$g\ dl^{-1}$	mol%	mol%	mol%	%
Patients with adenomyoma of the gallbladder					
1. F	18.0	5.6	76.9	17.5	83
2. F	16.1	9.0	69.0	22.1	116
3. F	4.3	9.1	63.8	27.1	132
4. F	14.8	5.7	68.0	26.3	68
5. F	16.9	6.3	69.2	25.6	74
6. F	14.0	6.3	71.5	22.2	82
7. F	17.8	5.4	62.8	31.8	60
8. F	8.6	4.0	78.9	17.1	71
9. M	4.3	9.2	63.7	27.1	133
10. M	14.8	7.4	70.1	22.5	95
Patients with cholesterol gallstones					
11. F	7.2	12.1	62.1	25.8	159
12. F	15.5	7.9	66.4	25.6	105
13. F	5.1	9.3	68.3	22.5	142
14. F	3.7	12.2	62.7	25.1	179
15. F					
16. F					
17. F	5.1	6.4	70.7	22.9	99
18. F	9.3	11.9	59.0	29.1	144
19. F	7.2	7.3	71.1	21.6	108
20. F					
21. F	3.9	15.6	54.4	30.1	219
22. F					
23. F	14.1	12.2	59.8	28.0	140
24. F					
25. F					
26. M					
27. M					
28. M	4.4	5.4	74.0	20.7	93
29. M	16.8	10.9	59.4	29.6	121
30. M					
31. M	8.2	10.7	65.7	23.7	140
32. M					
33. M					
Patients with cholesterol gallstones treated with cholic acid					
34. F					
35. F	11.4	5.3	75.7	19.0	78
36. F	7.2	6.9	72.7	20.4	107
37. F	12.1	5.4	73.2	21.4	74
38. F	11.2	5.9	79.4	14.7	109
39. F	9.4	7.4	61.0	31.7	89
40. F					
41. F					
42. M	11.7	10.0	65.0	25.1	124
43. M					
44. M	10.4	3.5	59.6	37.0	42
45. M	3.5	4.7	81.8	13.5	109
Patients with cholesterol gallstones treated with chenodeoxycholic acid					
46. F					
47. F					
48. F	7.6	5.3	74.0	20.6	82
49. F	4.5	5.1	70.3	24.7	76
50. F	11.8	3.3	59.7	37.0	38
51. F	6.3	6.5	53.6	40.0	84
52. F	11.3	4.1	74.0	21.9	56
53. F					
54. F	14.0	6.3	63.0	30.8	72
55. M	18.5	5.1	71.1	23.8	62

TABLE 2. *(Continued)*

Patient Group	Hepatic Bile				
	Lipid Concentration	Cholesterol	Bile Acids	Phospholipids	Cholesterol Saturation
	$g\ dl^{-1}$	$mol\%$	$mol\%$	$mol\%$	$\%$
Gallbladder adenomyoma (8)	2.5 ± 0.8^m	8.1 ± 0.6	70.5 ± 2.2	21.4 ± 1.8	174 ± 19
Cholesterol gallstones					
Female (12)	2.3 ± 0.5	11.6 ± 1.2	62.0 ± 2.5	26.5 ± 1.5	205 ± 17
Male (6)	2.3 ± 0.5	11.8 ± 1.1	62.3 ± 1.5	26.0 ± 1.7	240 ± 43
Total (18)	2.3 ± 0.3	11.6 ± 0.9^b	62.1 ± 1.7^c	26.3 ± 1.1^a	217 ± 18
Cholic acid treatment (10)	3.4 ± 1.0	8.2 ± 0.8^g	68.8 ± 1.6^h	23.0 ± 1.1	149 ± 18^f
Chenodeoxycholic acid treatment (8)	3.3 ± 0.7	$4.9 \pm 0.4^{e,j,l}$	72.5 ± 1.0^j	22.6 ± 0.8	$89 \pm 8^{d,j,k}$

[a] Significantly different from patients with adenomyoma, $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.01$; [d] $P < 0.005$; [e] $P < 0.001$.
[f] Significantly different from patients with cholesterol gallstones, $P < 0.025$; [g] $P < 0.02$; [h] $P < 0.01$; [i] $P < 0.005$; [j] $P < 0.001$.
[k] Significantly different from patients treated with cholic acid, $P < 0.02$; [l] $P < 0.005$.
[m] Mean $\pm$ SEM.

column. Responses of the individual bile acids were checked every day.

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 (bile acids + phospholipids + cholesterol) (8). The total lipid concentration was expressed in g/dl.

The saturation of bile with cholesterol was calculated as a percentage of the predicted cholesterol solubility using the fifth-degree polynomial functions given by Carey and Small (8). The appropriate total biliary lipid concentration was chosen in each case, whereas temperature was assumed to be 37°C and ionic strength 0.15 M.

Statistical analysis

Means are given $\pm$ SEM. The statistical significance of differences was evaluated with Student's t-test or Student's paired t-test where appropriate. Correlations between parameters were tested by calculating the Spearman rank correlation coefficient, R_s (20).

RESULTS

Hepatic cholesterol concentration and microsomal HMG CoA reductase activity (Table 1)

The hepatic cholesterol concentration in patients with cholesterol gallstone disease was about 20% higher than that seen in patients with adenomyoma of the gallbladder. The activity of microsomal HMG CoA reductase was not different in males and females with cholesterol gallstones, and not significantly dif-

ferent from the activity seen in patients with adenomyoma of the gallbladder. Gallstone patients with and without intact function of the gallbladder displayed similar enzyme activities.

Treatment of patients with cholesterol gallstones with cholic acid or chenodeoxycholic acid was associated with 20% decrease in hepatic cholesterol concentration. Feeding with CD resulted in a 40% reduction of HMG CoA reductase activity, whereas enzyme activity was unchanged during C therapy.

There was no correlation between hepatic cholesterol concentration and microsomal HMG CoA reductase activity in any of the patient groups or in the total series. Neither cholesterol concentration nor HMG CoA reductase activity was related to age, absolute or relative body weight, or serum lipid levels.

Lipid composition of hepatic bile (Table 2)

In the gallstone-free controls, the hepatic bile was in all cases saturated with cholesterol. In patients with cholesterol gallstone disease, hepatic bile contained significantly more cholesterol, less bile acids, and more phospholipids than that of controls. The total concentration of biliary lipids was similar in the two groups of patients (Table 2). Also in cholesterol gallstone disease, the hepatic bile was in all cases supersaturated with cholesterol, and there was a tendency for bile to be more supersaturated in patients with intact function of the gallbladder (229 $\pm$ 24%) than in those without (192 $\pm$ 26%). This difference was not statistically significant, however. There was no relation of the degree of supersaturation to age, sex, absolute or relative body weight, or serum lipid concentrations.

Although not significant on a statistical basis, there was a clear tendency for patients with adenomyoma

TABLE 2. (Continued)

| Patient Group | Gallbladder Bile | | | | |
	Lipid Concentration	Cholesterol	Bile Acids	Phospholipids	Cholesterol Saturation
	$g\ dl^{-1}$	mol%	mol%	mol%	%
Gallbladder adenomyoma (10)	13.0 ± 1.7	6.8 ± 0.6	69.4 ± 1.7	23.9 ± 1.4	91 ± 8
Cholesterol gallstones					
Female (9)	7.9 ± 1.4	10.5 ± 1.0	63.8 ± 1.9	25.6 ± 1.0	144 ± 13
Male (3)	9.8 ± 3.7	9.0 ± 1.8	66.4 ± 4.2	24.7 ± 2.6	118 ± 14
Total (12)	8.4 ± 1.3[a]	10.2 ± 0.9[c]	64.5 ± 1.7	25.4 ± 0.9	137 ± 11[d]
Cholic acid treatment (8)	9.6 ± 1.0	6.1 ± 0.7[i]	71.1 ± 2.9	22.9 ± 2.9	92 ± 9[h]
Chenodeoxycholic acid treatment (7)	10.6 ± 1.8	5.1 ± 0.4[a,j]	66.5 ± 3.0	28.4 ± 2.9	67 ± 6[j]

to have less supersaturated bile, especially when compared to gallstone patients with intact gallbladder function (174 ± 19% versus 229 ± 24%).

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 0.116 ± 0.011 in subjects with adenomyoma and was significantly higher in patients with cholesterol gallstones, 0.195 ± 0.019 ($P < 0.02$). The ratio between phospholipids and bile acids was higher in the patients with gallstones, (0.438 ± 0.032) than in those with adenomyoma (0.311 ± 0.032, $P < 0.025$), whereas the ratio between cholesterol and phospholipids was similar in both groups, 0.447 ± 0.034 and 0.389 ± 0.032, respectively.

Effects of bile acid treatment on hepatic bile (Table 2)

Treatment with C gave an increase in the relative concentration of bile acids and a decrease in that of cholesterol, whereas the phospholipid fraction remained essentially unchanged. These changes were associated with reduced cholesterol saturation of the hepatic bile, although bile was still supersaturated in all but one of the patients studied. The ratio between cholesterol and bile acids was decreased (0.122 ± 0.015, $P < 0.02$), as was that between phospholipids and bile acids (0.338 ± 0.057, $P < 0.05$). The cholesterol/phospholipid ratio was essentially unchanged (0.360 ± 0.034). The pattern observed during treatment with C was similar to that seen in the gallstone-free controls.

CD feeding resulted in a considerable reduction in the relative concentration of cholesterol and an increase in that of bile acids. Hepatic bile became unsaturated with cholesterol in five out of the eight

patients studied. Compared to gallstone patients, the ratio between cholesterol and bile acids was considerably reduced (0.068 ± 0.006, $P < 0.001$), as was that between cholesterol and phospholipids (0.217 ± 0.015, $P < 0.001$). The phospholipid/bile acid ratio was decreased (0.314 ± 0.015, $P < 0.02$). Compared to gallstone-free controls and patients treated with C, the ratios between cholesterol and bile acids and between cholesterol and phospholipids were clearly reduced.

Individual bile acids in hepatic bile (Table 3)

C, CD, and deoxycholic acid (D) were the dominating bile acids in all hepatic biles analyzed, and only trace amounts of lithocholic acid and ursodeoxycholic acid were generally found. There were no differences between the patients with cholesterol gallstones and those with adenomyoma of the gallbladder with regard to the bile acid composition. Patients without functioning gallbladder displayed a tendency to a decrease in C (28.8 ± 3.3 versus 35.6 ± 2.0%) and an increase in D (30.4 ± 7.3 versus 25.3 ± 3.6%) compared to those with intact function of the gallbladder, but the differences were not statistically significant.

Treatment with C resulted in an increase of this bile acid and its metabolite, D, together with a decrease of CD. A reversed pattern was seen during CD therapy; an increase in lithocholic acid and ursodeoxycholic acid was also seen (Table 3).

Gallbladder bile composition (Table 2)

In patients with cholesterol gallstones, the gallbladder contained less total lipids than in patients with adenomyoma of the gallbladder in the fasting state. The relative concentration of cholesterol was increased, and gallbladder bile was saturated with

TABLE 3.   Individual bile acids in hepatic bile and gallbladder bile. Means ± SEM.

Patient Group	Collection Site (Number of Subjects)	Cholic Acid	Chenodeoxy-cholic Acid	Deoxycholic Acid	Lithocholic Acid	Ursodeoxy-cholic Acid
		%				
Gallbladder adenomyoma	Hepatic (8)	35.3 ± 2.3	37.1 ± 4.0	25.2 ± 3.2	0.5 ± 0.4	2.0 ± 1.3
	Gallbladder (9)	35.8 ± 2.1	39.7 ± 3.4	23.3 ± 3.2	0.2 ± 0.1	1.3 ± 0.9
Cholesterol gallstones	Hepatic (17)	33.6 ± 1.8	37.9 ± 2.3	26.8 ± 3.2	0.8 ± 0.3	0.9 ± 0.5
	Gallbladder (11)	37.3 ± 2.1	40.0 ± 2.0	21.3 ± 2.6	0.7 ± 0.3	0.8 ± 0.5
Cholic acid-treated	Hepatic (8)	47.6 ± 6.6[b]	12.5 ± 1.9[c]	39.9 ± 7.3	0 ± 0	0 ± 0
	Gallbladder (8)	48.0 ± 6.8	12.3 ± 1.6[c]	39.7 ± 7.6[b]	0 ± 0	0 ± 0
Chenodeoxycholic acid-treated	Hepatic (8)	5.9 ± 2.0[c]	84.6 ± 3.7[c]	4.9 ± 2.1[c]	2.5 ± 0.8[a]	2.4 ± 0.8
	Gallbladder (7)	6.0 ± 1.6[c]	83.8 ± 3.5[c]	5.1 ± 1.7[c]	3.4 ± 1.1[b]	2.0 ± 0.7

[a] Significantly different from patients with cholesterol gallstones, $P < 0.05$; [b]$P < 0.02$; [c]$P < 0.001$.

cholesterol in ten of the twelve patients with cholesterol gallstones studied (mean, 137 ± 11%) compared to three of the ten patients with adenomyoma of the gallbladder (mean, 91 ± 8%).

When compared to untreated cholesterol gallstone patients, those treated with C had a cholesterol saturation of 92 ± 9% (unsaturated bile in four out of eight patients). CD feeding was associated with a highly unsaturated gallbladder bile in all cases (mean, 67 ± 6%).

The individual bile acid composition of gallbladder bile showed a pattern similar to that present in hepatic bile (Table 3).

By using pair comparisons (paired observations

Fig. 1. Relation between cholesterol saturation (%) in hepatic bile and gallbladder bile. ○ indicates patients with gallbladder adenomyoma, ● patients with cholesterol gallstones, ▲ patients treated with cholic acid, and △ patients treated with chenodeoxycholic acid.

were available in 30 subjects), it could be demonstrated that the gallbladder bile always contained more lipid ($P < 0.001$) and was less saturated with cholesterol ($P < 0.001$) compared to hepatic bile. As seen from **Fig. 1,** there was a highly significant correlation between hepatic and gallbladder bile saturation ($R_s = +0.738, P < 0.001$).

Relationship between hepatic cholesterol metabolism and hepatic bile composition

The cholesterol saturation of hepatic bile was not related to hepatic cholesterol concentration in any of the patient subgroups. As seen in **Fig. 2A,** hepatic bile cholesterol saturation was positively correlated with microsomal HMG CoA reductase in the patients with adenomyoma of the gallbladder ($R_s = +0.833, P < 0.02$). A clearly different pattern was seen in the patients with cholesterol gallstones, where the highest saturation values were seen among patients with low or normal HMG CoA reductase activities (Fig. 2B). In the combined bile acid-treated group (Fig. 2C), a positive correlation between the two variables was again observed ($R_s = +0.672, P < 0.01$).

In order to evaluate these relationships further, we plotted the ratio between cholesterol and phospholipid against HMG CoA reductase activity. This ratio should reflect the ratio between cholesterol and phospholipid secretion in the fasting state. A highly significant correlation was found between the two parameters in gallstone-free controls ($R_s = +0.952, P < 0.005$). No significant correlation between the two variables was observed in the untreated or in the bile acid-treated gallstone patients.

The ratio between cholesterol and bile acids in hepatic bile was relatively constant over a wide range of HMG CoA reductase activity in the patients with gallbladder adenomyoma. Gallstone patients with low
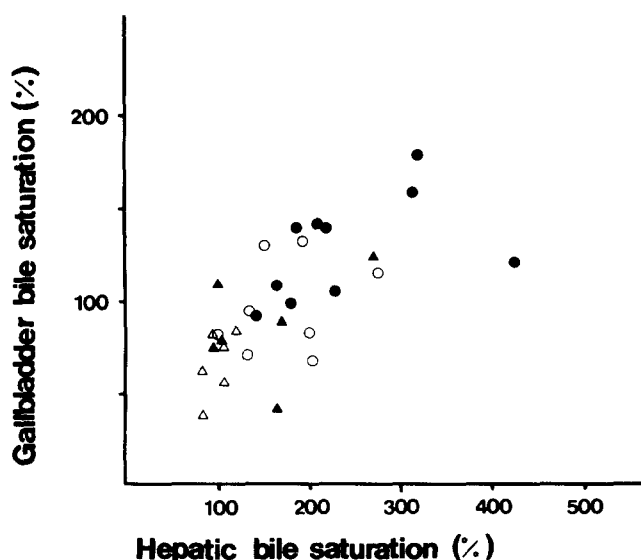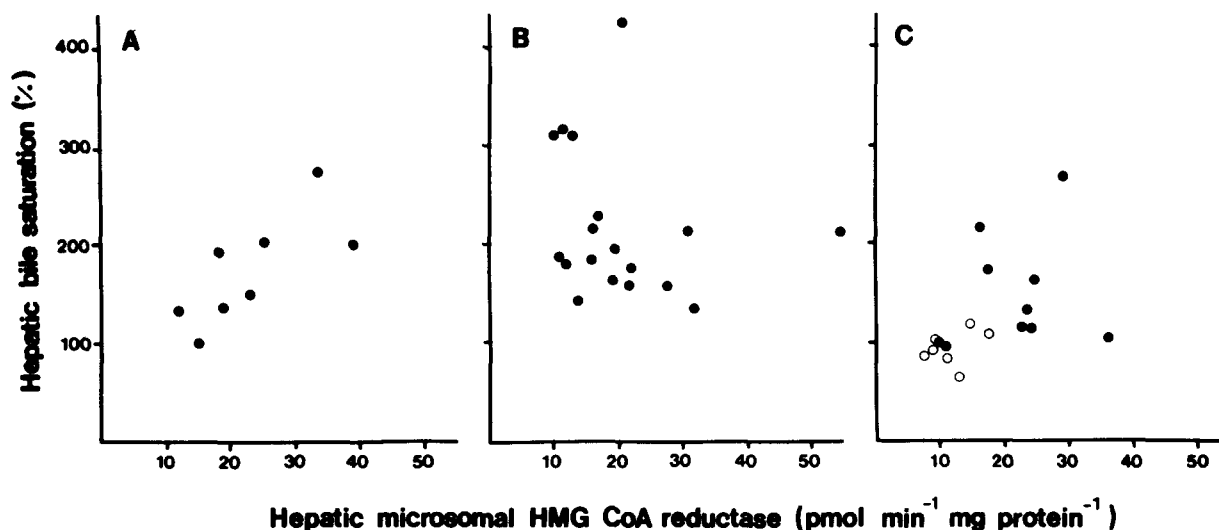
Fig. 2. Cholesterol saturation (%) of hepatic bile in relation to hepatic HMG CoA reductase activity (pmol·min^{-1}·mg protein^{-1}). A: Patients with adenomyoma of the gallbladder (R_s = +0.883, P < 0.02). B: Patients with cholesterol gallstones. C: Gallstone patients treated with bile acids (● cholic acid; ○ chenodeoxycholic acid) (R_s = +0.672, P < 0.01).

enzyme activity displayed a particularly high cholesterol/bile acid ratio resulting in a negative correlation between the two parameters (R_s = −0.556, P < 0.05). Contrarily, bile acid feeding was associated with a significant positive relationship between HMG CoA reductase and cholesterol/bile acid ratio in hepatic bile (R_s = +0.650, P < 0.01). No relationships were observed between HMG CoA reductase activity and phospholipid/bile acid ratio in hepatic bile in any of the patient groups.

DISCUSSION

In the present study, microsomal HMG CoA reductase activity was assayed in surgical liver biopsies obtained in the fasting state. It is obvious that the measurement of an enzymatic activity in vitro under optimal conditions may not be a true index of cholesterol formation within the tissue in vivo. This may be particularly important for measurements of HMG CoA reductase, as the activity of this enzyme is known from animal studies to be influenced by several regulatory factors such as stress, caloric intake, hormonal activation, and diurnal variation (3). Attention was therefore paid to standardization of preoperative conditions including diet and timing of operation, and operative as well as anesthetic procedures.

A further concern relates to the possibility of rapid regulation of enzyme activity by reversible inactivation–activation, probably through a phosphorylation–dephosphorylation mechanism (21–23). Although the importance of this phenomenon is still unclear,

its presence may influence measurement of enzyme activity, as activation of originally inactive enzyme may occur during preparation of microsomes. However, detailed studies in the rat liver suggest that measurements of maximally and minimally "activated" enzyme activity parallel those by the standard procedure (as used in the present work) (24). Furthermore, such measurements were closely related to independent determinations of hepatic cholesterogenesis (24). Thus, although there is some uncertainty of which type of assay actually reflects true "physiological" activity, there is no reason to believe that this should influence the main objectives of this study.

The first purpose of the present investigation was to determine hepatic HMG CoA reductase activity in relation to hepatic cholesterol concentration and biliary lipid composition in healthy man. Patients undergoing cholecystectomy for adenomyoma or polyps of the gallbladder wall were chosen, as they were thought to be nearly ideal as controls. Thus, they had the same operation performed as the patients with gallstones; still, their disease is not related to the formation of gallstones or any other hepatobiliary disease. They were not subject to any dietary manipulations (which is common e.g., in patients operated for peptic ulcer), they did not show any other clinical or laboratory abnormalities, and they were all nonobese.

Despite precautions for pre- and peroperative standardization, the healthy controls displayed a 4- to 5-fold range of HMG CoA reductase activity. This is not unexpected, however, as measurements of net steroid "balance" (reflecting whole-body choles-

terol synthesis) varies 3- to 4-fold between healthy individuals (25–27). We found no relationship between enzyme activity and factors such as age, sex, absolute or relative body weight, serum lipid levels, or hepatic cholesterol concentration. This should not be taken as evidence for lack of influence of these parameters, however. Thus, patients with hyperlipoproteinemia type IV (elevation of very low density lipoprotein levels) often have increased activity of hepatic HMG CoA reductase (15). Furthermore, grossly obese patients also appear to have increased activity of hepatic HMG CoA reductase.[4]

In agreement with the recent work of Carey and Small (8), the fasting hepatic bile was saturated with cholesterol in all patients without gallstone disease. The presence of supersaturated gallbladder bile in three of ten subjects is in agreement with the prevalence in a normal-weight, normolipidemic Swedish population sample without gallstones (28). The saturation of hepatic bile secreted in the fasting state was positively correlated to the activity of the hepatic HMG CoA reductase activity. Although suggestive of a direct causative effect, this finding does not necessarily prove that hepatic HMG CoA reductase activity determines the cholesterol saturation of hepatic bile. Thus, it may equally well reflect parallel responses to a common regulatory factor, such as bile acid inflow to the liver. A low portal venous inflow to the liver may result in a low secretion rate of bile with a relatively high cholesterol/phospholipid ratio. Bile acid synthesis would simultaneously be increased due to the diminished feedback inhibition, and increased biosynthesis of bile acids will increase the need for hepatic cholesterogenesis.

The second purpose of the present investigation was to look for possible disturbances in hepatic cholesterol metabolism and biliary lipid composition in cholesterol gallstone disease. We did not find any difference in hepatic HMG CoA reductase activity between patients with cholesterol gallstones and with adenomyoma of the gallbladder. The two groups were quite comparable with regards to age, body weight and serum lipids, and there was no influence of sex. There was a difference in total hepatic (but not in microsomal) cholesterol, however, with a 20% increase in gallstone patients. This latter finding is in agreement with the work of Salen et al. (29). These authors, as well as Coyne et al. (13), did report about 30% higher HMG CoA reductase activities in cholesterol patients as compared to controls (generally patients with duodenal ulcer). As mentioned above, we

feel our controls to be closer to the "healthy" normal population. The studies also differ from the present one in that percutaneous biopsies were often used; furthermore, data on serum lipid levels are not given by Coyne et al. (13).

The gallbladder bile of the patients with cholesterol gallstones was generally supersaturated with cholesterol. There was a good correlation between the saturation of gallbladder bile and that of hepatic bile, with a higher degree of saturation at the latter site. This pattern is in accordance with the concept that the liver is the source of abnormal bile in cholesterol gallstone disease (7). We found no correlations between hepatic or gallbladder bile saturation and HMG CoA reductase activity of gallstone patients. This lends further support to the view that cholesterol gallstone disease in non-obese, normolipidemic man is not due to an increased hepatic cholesterol production. Previous work has indicated that such patients often display a reduced bile acid pool size (30, 31) and a decreased stimulated bile acid secretion rate (31). The present results concerning biliary lipid secretion ratios are in accordance with this view, and may actually suggest that similar mechanisms are at work in the fasting state, when the supersaturated bile enters the gallbladder.

The third purpose of the study was to investigate the effect of feeding with C or CD on hepatic cholesterol metabolism and biliary lipid composition. The results provide ample evidence for a difference between the effects of treatment with C and CD on hepatic microsomal HMG CoA reductase activity. Thus, in agreement with previous work (13, 32), CD feeding was associated with a clear reduction of enzyme activity. Medication with C, however, did not influence HMG CoA reductase activity significantly. Several possible mechanisms for this difference may be discussed.

First, feeding with the two bile acids may have different effects on the promotion of cholesterol absorption. Also, in the fasting state, biliary cholesterol reaches the intestinal lumen and is absorbed. An enhanced cholesterol absorption during CD feeding could thus be a possible explanation for the decreased hepatic HMG CoA reductase activity. However, cholesterol absorption in man is generally similar (11, 33) or even lower (34)[5] during treatment with CD compared to C. Furthermore, the hepatic cholesterol concentration was actually *decreased* during feeding with both bile acids.

Second, CD may be more potent in suppressing conversion of cholesterol into bile acids than C. This

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[4] Angelin, B., L. Backman, K. Einarsson, and S. Ewerth, Manuscript in preparation.

[5] Angelin, B., and B. Leijd. Manuscript in preparation.

could possibly result in a more efficient product feedback inhibition of HMG CoA reductase. Feeding with C does result in a suppression of CD synthesis, however, which is generally within the same magnitude as the reduction of C synthesis during CD treatment (12, 35, 36). Furthermore, the finding of similar (lower) hepatic cholesterol concentrations after treatment with C and CD is not in accord with such a mechanism.

Third, there may be a direct effect of bile acids on hepatic cholesterogenesis. Although this possibility is in accordance with our data, there is no definite evidence. Parallel measurements of transhepatic bile acid flux and HMG CoA reductase activity may be of interest in this context. Preliminary results suggest that the fasting portal venous concentration of bile acids is higher during CD than during C treatment (37). This higher concentration may be of importance for a more efficient suppression of hepatic HMG CoA reductase activity.

The unsaturation of *gallbladder bile* with cholesterol observed during CD treatment is in good agreement with several previous reports (9–12). The present study also demonstrated a small but significant reduction of the saturation of gallbladder bile during C treatment. This is in contrast to some previous work (10–12) and may be related to the relatively high dose given in the present study. To our knowledge, this is the first report in which data on cholesterol saturation in gallbladder and hepatic bile during treatment with the two primary bile acids are presented using the solubility equations of Carey and Small (8) at appropriate biliary lipid concentrations. The fact that CD produced unsaturation of fasting *hepatic bile* in most patients, whereas C did not, may be of major importance for the different degrees of success in dissolution of gallstones using these bile acids.

If, as discussed above, the primary defect in normal-weight, normolipidemic patients with cholesterol gallstones is a diminished bile acid pool size and reduced (fasting) bile acid secretion, the finding of a "normalized" pattern during expansion of the bile acid pool is reasonable. The additional reduction of cholesterol secretion during CD treatment may be related to the decreased hepatic HMG CoA reductase. In agreement with this contention is the finding of a positive correlation between enzyme activity and cholesterol saturation of hepatic bile during bile acid treatment. CD but not C prevents a dissociation of hepatic secretions of cholesterol and phospholipids at the low outputs of bile that are present in the fasting state (33). It is tempting to speculate that this phenomenon may be related to the different effects on hepatic HMG CoA reductase activity.

To summarize, the results of the present study suggest that elevated hepatic HMG CoA reductase is not a major defect in normal-weight, normolipidemic patients with cholesterol gallstones. The supersaturated bile in these patients may instead depend mainly on a reduced bile acid secretion in the fasting state, probably related to a decreased conversion of cholesterol to bile acids. Treatment with CD but not with C results in unsaturated fasting hepatic bile which may explain the success in gallstone dissolution with the former bile acid. This effect is due to a reduced secretion of biliary cholesterol, possibly related to a decreased hepatic HMG CoA reductase activity.∎

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