Hepatic cholesterol metabolism in normo- and hyperlipidemic patients with cholesterol gallstones

Jon Ahlberg, Bo Angelin,[1] Ingemar Björkhem, Kurt Einarsson, and Babro Leijd

Departments of Medicine and Surgery, Serafimerlasarettet, and Department of Clinical Chemistry, Huddinge Sjukhus, Karolinska Institutet, Stockholm, Sweden

Abstract In vivo studies have shown abnormalities in cholesterol and bile acid metabolism in primary hyperlipoproteinemia (HLP). The aim of the present investigation was to determine if the increased production of cholesterol in HLP type IV can be attributed to a correspondingly high level of the hepatic 3-hydroxy-3-methylglutaryl (HMG) CoA reductase activity and if the low cholic acid: chenodeoxycholic acid synthesis ratio in HLP type II is due to some hydroxylase deficiency. Liver biopsies from 26 normolipidemic and 25 hyperlipidemic (10 type IIa, 6 type IIb, and 9 type IV) patients undergoing elective cholecystectomy were assayed for HMG CoA reductase activity, 12α-hydroxylase activity, and 25-hydroxylase activity. The HMG CoA reductase activity was normal in HLP type IIa and type IIb and was increased about twice in HLP type IV ($P < 0.001$). The 12α- and 25-hydroxylase activities were normal in all groups of patients. The results are compatible with a normal cholesterol synthesis in the liver in HLP type II. A reduced 12α- or 25-hydroxylase activity cannot explain the low production of cholic acid relative to chenodeoxycholic acid in this type of HLP. The elevated HMG CoA reductase activity found in the liver of type IV patients may, however, be part of the explanation for the elevated synthesis of cholesterol often seen in these patients.

Supplementary key words HMG CoA reductase · cholic acid · chenodeoxycholic acid · bile acid synthesis

Several in vivo studies have indicated abnormalities in cholesterol and bile acid metabolism in primary hyperlipoproteinemia (HLP) (for a review, see 1). Patients with types IIa and IIb HLP have a normal total production of bile acids and cholesterol (2, 3); however, the ratio between the synthesis of the two primary bile acids, cholic acid and chenodeoxycholic acid, is usually reduced (2). Furthermore, patients with type IIa HLP have a decreased formation and pool size of cholic acid (2). HLP type IV, on the other hand, is often associated with an abnormally high synthesis of cholesterol and bile acids, especially cholic acid (2, 3).

The liver might be a key organ in the pathogenesis of HLP. Thus, the liver is considered to be one of the main sites for the endogenous formation of cholesterol in man (4, 5). The rate-determining step in the biosynthesis of cholesterol is the microsomal formation of mevalonic acid from HMG CoA (3-hydroxy-3-methylglutaryl CoA), catalyzed by the enzyme HMG CoA reductase (mevalonate: NADP oxidoreductase, EC 1.1.1. 34) (6). A high HMG CoA reductase activity may explain the high formation of cholesterol in HLP type IV. The liver is also the site for degradation of cholesterol into cholic acid and chenodeoxycholic acid. The first and rate-limiting step in this degradation is a 7α-hydroxylation (7, 8). In the following transformations, 7α-hydroxy-4-cholesten-3-one is a key intermediate which is further 12α-hydroxylated to yield 7α,12α-dihydroxy-4-cholesten-3-one in the synthesis of cholic acid (9, 10). It is possible that patients with type IIa and type IIb HLP have a relative deficiency of the 12α-hydroxylase. The initial reaction in the degradation of the side chain in cholic acid formation is a 26-hydroxylation or possibly a 25-hydroxylation (11–15). The quantitative importance of these two hydroxylases has not yet been evaluated. If the 25-hydroxylase is of importance in cholic acid synthesis in human liver, the low synthesis of cholic acid relative to chenodeoxycholic acid in these patients might also be attributed to a decreased activity of this enzyme.

To further characterize the abnormalities of cholesterol and bile acid metabolism in HLP, we have now determined 12α- and 25-hydroxylase activities as well as HMG CoA reductase activity in liver biopsies taken from normo- and hyperlipidemic patients during surgery for uncomplicated gallstone disease.

[1] To whom all correspondence should be addressed at the Department of Medicine, Serafimerlasarettet, S-112 83 Stockholm, Sweden.

Abbreviations: HLP, hyperlipoproteinemia; HMG, 3-hydroxy-3-methylglutaryl; HMG-CoA reductase, mevalonate:NADP oxidoreductase, EC 1.1.1.34; cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

TABLE 1. Basal data on patients

Patient Number	Sex[a]	Age	Relative Body Weight[b]	Serum Cholesterol Concentration[c]	Serum Triglyceride Concentration[c]
		yr		*mmol · l⁻¹ (mean)*	*mmol · l⁻¹ (mean)*
Normolipidemic controls					
1. VT	F	72	91	6.9	1.9
2. EH	F	70	92	4.9	1.6
3. MS	F	67	110	6.3	1.0
4. EG	F	66	111	6.0	1.1
5. SJ	F	65	101	6.7	1.0
6. MN	F	62	85	5.6	1.3
7. IR	F	61	106	5.8	1.7
8. KN	F	57	88	6.0	1.3
9. BL	F	52	120	5.0	1.0
10. ME	F	52	145	6.8	1.6
11. GB	F	51	87	5.7	1.1
12. MA	F	49	157	4.4	1.4
13. RA	F	49	116	5.6	1.3
14. GF	F	38	74	5.5	1.5
15. GN	F	36	129	3.6	0.4
16. GM	F	32	80	4.4	1.0
17. VB	F	30	86	5.0	1.2
18. UH	F	27	98	6.2	0.7
19. EL	M	64	93	3.6	0.8
20. EM	M	64	99	4.2	0.9
21. BA	M	57	93	4.8	1.5
22. HH	M	52	109	6.2	1.3
23. SA	M	43	102	6.8	0.9
24. VN	M	40	118	6.9	1.9
25. KL	M	31	84	3.1	1.2
26. PL	M	28	94	4.6	1.6
Hyperlipoproteinemia type IIa					
27. AH	F	72	112	9.5	2.0
28. GJ	F	69	109	8.7	1.8
29. MW	F	66	131	10.6	1.6
30. MN	F	63	96	7.7	1.2
31. MB	F	59	99	7.6	1.6
32. MH	F	47	94	8.4	1.4
33. ZP	F	46	111	7.4	1.5
34. SJ	M	66	108	7.7	1.3
35. DJ	M	66	106	8.9	2.0
36. BH	M	41	123	8.0	1.8
Hyperlipoproteinemia type IIb					
37. EL	F	60	105	8.0	2.3
38. SS	F	59	110	9.8	4.2
39. EF	F	56	168	8.5	3.7
40. DA	F	53	108	7.8	2.6
41. BM	M	63	83	8.3	2.9
42. AS	M	62	106	7.9	2.8
Hyperlipoproteinemia type IV					
43. MS	F	71	165	6.7	2.5
44. EK	F	65	93	6.0	4.7
45. RH	F	60	94	4.8	4.1
46. HS	F	58	119	5.4	5.1
47. UW	F	53	110	7.0	2.3
48. KS	F	27	115	5.1	3.2
49. KG	M	65	103	6.6	2.9
50. RK	M	53	109	5.7	2.9
51. GW	M	43	95	6.0	2.9

TABLE 1. (*Continued*)

Patient Group	Age	Relative Body Weight[b]	Serum Cholesterol Concentration[c]	Serum Triglyceride Concentration[c]
	yr		*mmol·l⁻¹ (mean)*	*mmol·l⁻¹ (mean)*
Normolipidemic females (18)	52 ± 3[d]	104 ± 5[d]	5.6 ± 0.2[d]	1.2 ± 0.1[d]
Normolipidemic males (8)	47 ± 5	99 ± 4	5.0 ± 0.5	1.3 ± 0.1
Normolipidemic total (26)	51 ± 3	103 ± 4	5.4 ± 0.2	1.2 ± 0.1
HLP IIa (10; 7F, 3M)	60 ± 3	110 ± 4	8.5 ± 0.3[e]	1.6 ± 0.1
HLP IIb (6; 4F, 2M)	59 ± 2	113 ± 12	8.4 ± 0.3[e]	3.1 ± 0.3[e]
HLP IV (9; 6F, 3M)	55 ± 4	111 ± 7	5.9 ± 0.2	3.4 ± 0.3[e]

[a] F, female; M, male.
[b] Calculated as [weight(kg)/(height(cm) − 100)] × 100.
[c] To convert mmol/l to mg/dl multiply cholesterol concentrations by 38.7 and triglyceride concentrations by 0.1 × mol wt of triglyceride (e.g., 885 for triolein).
[d] Mean ± SEM.
[e] Significantly different from normolipidemic group ($P < 0.001$).

MATERIALS AND METHODS

Subjects

The present study comprised 26 normolipidemic and 25 hyperlipidemic patients undergoing elective cholecystectomy (**Table 1**). They had all been admitted to the outpatient clinic of the Department of Surgery, Serafimerlasarettet, where they were offered a routine laboratory health screening including determination of serum lipid levels. Initially, all patients with clinical or laboratory evidence of diabetes mellitus, ethanol overconsumption, or diseases affecting liver, kidney, or thyroid function (altogether five subjects) were excluded from the study. None of the patients had overt cardiovascular disease and none was on treatment with antihypertensive drugs. The patients were not informed of their serum lipid levels until hospitalization (see below), and thus they had not previously been on any specific dietary or drug treatment because of hyperlipidemia. Furthermore, they had not taken any other drugs affecting liver function or plasma lipids from the time of the outpatient visit, which was at least 2 months before hospitalization. None of the patients had taken oral contraceptives previously.

The lipoprotein patterns of the patients were determined from repeated blood samples after overnight fast during hospitalization. The agreement of repeated samples was in all cases within 10%, but in three cases patients with initial hyperlipidemia were excluded because the repeated set of lipid values was within normal limits. The serum lipoprotein pattern was obtained by determination of serum triglyceride, total serum cholesterol, β-lipoprotein cholesterol, and by lipoprotein electrophoresis on agarose gel (2). The upper limit of normal was set at 7.2 mmol/l for cholesterol, 5.2 mmol/l for β-lipoprotein cholesterol, and 2.0 mmol/l for triglycerides. Patients with abnormal plasma lipids were classified according to WHO recommendations (16).

Ten patients were classified as type IIa HLP, six as type IIb HLP, and nine as type IV HLP. The age distribution in the different groups of subjects was similar (Table 1). The normolipidemic men were close to ideal weight, whereas all the other groups of patients were slightly overweight. The differences between the groups were not statistically significant.

One important consideration is whether a population of type II HLP patients with low cholic acid synthesis was actually studied. A proper evaluation of this question would call for direct measurements of bile acid kinetics preoperatively. Such a study could not be performed; however, two indirect implications of differences in cholic acid metabolism between the type II HLP patients and the normolipidemic controls were found. First, the fasting concentration of cholic acid in serum,[2] which appears to be related to cholic acid pool size in type II HLP and in normolipidemia (17), was lower in the type II HLP patients (0.45 ± 0.08 μmol/l, mean ± SEM) than in the controls (0.74 ± 0.26 μmol/l, $P < 0.05$). Second, the proportion of cholic acid of total bile acids[3] in gall bladder bile of the type II HLP patients was lower than that seen in the controls (32 ± 4% vs. 38 ± 2%, $P < 0.05$).

[2] This was determined preoperatively in 9 of the patients with type II HLP and 12 of the control subjects by using a slight modification of the mass fragmentographic technique described previously (17).
[3] This was determined preoperatively in 8 of the patients with type II HLP and in 10 of the controls by using gas–liquid chromatography as described previously (18).

Experimental procedure

The patients were hospitalized in the surgical ward, where laboratory tests and a clinical examination were performed. They were fed the regular diet for 2–3 days.[4] All operations were performed between 8 and 9 AM after a 12-hr fast to avoid a possible diurnal variation of enzyme activity (cf. 6). Anesthesia was induced by thiopentothal and continued with nitrous oxide, diazepam, and fentanyl.

After opening the abdomen, a 2–4 g liver biopsy was obtained from the left lobe of the liver to avoid any local inflammatory changes caused by cholecystitis. The biopsy was immediately placed in ice-cold 0.1 M Tris-buffer solution, pH 7.4, and within 10 min it was transported to the laboratory. A specimen of the biopsy was sent for histologic examination. Liver morphology was normal in all but two cases (subjects 24 and 38) in which there was a slight fatty infiltration of the parenchymal cells.

The patients were then subjected to a regular cholecystectomy. In all cases analysis of the stones showed them to consist mainly of cholesterol. In agreement with this observation, the gallbladder bile was in all cases determined supersaturated with cholesterol using the solubility limits of Hegardt and Dam (19) and Holzbach et al. (20).[5] None of the patients had stones in the common duct as judged by operative cholangiography. No complications were encountered during or after the operation.

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

Materials

[3-^{14}C]HMG CoA (sp act 26 mCi per mmol) was obtained from New England Nuclear Corp., Boston, MA. 7α-[6β-^{3}H]hydroxy-4-cholesten-3-one (sp act 11.7 μCi per mg) and 5β-[7β-^{3}H]cholestane-3α,7α,12α-triol (sp act 33.3 μCi per mg) were prepared as described previously (21). Unlabeled HMG CoA was purchased from P-L Biochemicals, Inc., Milwaukee, WI. NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from Sigma Chemical Co., St. Louis, MO.

Preparation of liver microsomes

For assay of HMG CoA reductase activity, 1 g of the liver biopsy was used to form a 10% (w/v) homogenate. This was prepared with a Potter-Elvehjem homogenizer equipped with a loosely fitting Teflon pestle in a homogenizing 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.

For assay of 12α-hydroxylase and 25-hydroxylase, 1 g of the liver biopsy was homogenized in 0.1 M Tris-buffer solution, pH 7.4, to form a 20% (w/v) homogenate. The latter was centrifuged at 20,000 *g* for 15 min. The supernatant fluid was centrifuged at 100,000 *g* for 60 min. The microsomal fraction was suspended in 0.1 M Tris-buffer solution to a volume corresponding to that of the 20,000 *g* supernatant fluid.

The protein concentrations of the microsomal fractions were determined by the method of Lowry et al. (22).

Assay of HMG CoA reductase activity

The assay system was similar to that described by Nicolau et al. (23). The system contained 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 in a total volume of 0.85 ml. The incubation was for 15 min at 37°C. The reaction was stopped by the addition of 0.1 ml of 5 M HCl. Tritium-labeled mevalonic acid, 0.01 μCi, was added together with 3 mg of unlabeled mevalonic acid lactone and the mixture was shaken at 37°C for 30 min. After cooling to room temperature, 0.5 ml of absolute ethanol was added and the mixture was dried over anhydrous Na_2SO_4. The solution was extracted three times with 3 ml of ethyl ether. The ethyl ether layers were combined, dried over anhydrous Na_2SO_4, and evaporated. The residue, dissolved in acetone, was subjected to thin-layer chromatography (precoated silica gel plates; 250 μm; Merck, Darmstadt, Germany) with benzene–acetone 1:1 (v/v) as the developing solvent. The mevalonic acid lactone zone was located and scraped off into a counting vial. A Packard liquid scintillation spectrometer, Model 3003, was used for determining the radioactivity, using Instagel as scintillator liquid. Corrections for quenching were made by using suitable ^{3}H- and ^{14}C-labeled standards. Corrections for losses during extraction and thin-layer chro-

[4] In this diet 35, 20, and 45% of energy is supplied as fat, protein, and carbohydrate, respectively. The daily intake of cholesterol is about 0.5 mmol/day.

[5] Determined from the relative concentrations of cholesterol, bile acids, and phospholipids as described previously (18).

matography were made by the internal standard. The recovery of mevalonic acid lactone was about 60%. The coefficient of variation

$$\text{(calculated as } \frac{100}{\mu}\sqrt{\frac{\sum d^2}{n}}\ \%\text{)}$$

of the assay, as determined from six replicate determinations, was 11.7%.

Assay of 12α-hydroxylase activity

Tritium-labeled 7α-hydroxy-4-cholesten-3-one, 100 μg, dissolved in 50 μl of acetone, was incubated with 0.75 ml of the microsomal fraction in a total volume of 2 ml of 0.1 M Tris–buffer solution, pH 7.4. A glucose-6-phosphate-dependent NADPH-generating system was used; it contained 3 μmol of NADP, 10 μmol of glucose-6-phosphate, 5 enzyme units of glucose-6-phosphate dehydrogenase, and 15 μmol of $MgCl_2$. The incubation was for 10 min at 37°C and was terminated by the addition of 20 vol of chloroform–methanol 2:1 (v/v). After addition of 0.2 vol of 0.9% sodium chloride solution (w/v) and separation, the chloroform phase was evaporated to dryness. Unlabeled 7α-hydroxy-4-cholesten-3-one and 7α,12α-dihydroxy-4-cholesten-3-one were added as internal standards and the incubation products were separated by thin-layer chromatography using benzene–ethyl acetate 1:1 (v/v) as developing solvent. The compounds were located with iodine vapor and scraped off into counting vials. Radioactivity was determined using a Packard liquid scintillation spectrometer, Model 3003, using PPO–POPOP toluene as scintillator liquid. The coefficient of variation (see above) was 11.5%.

Assay of 25-hydroxylase activity

Tritium-labeled 5β-cholestane-3α,7α,12α-triol, 250 μg, dissolved in 50 μl of acetone, was incubated with 1.5 ml of the microsomal fraction in a total volume of 3 ml of 0.1 M Tris–buffer solution. The same NADPH-generating system was used as above. The incubation was performed at 37°C for 20 min and was stopped by the addition of 20 vol of chloroform–methanol 2:1 (v/v). After addition of 0.2 vol of 0.9% sodium chloride solution (w/v) and separation, the chloroform phase was evaporated and subjected to thin-layer chromatography using benzene–isopropanol–acetic acid 30:10:1 (v/v/v) as solvent. Radioactivity was assayed with a thin-layer scanner (Berthold, Karlsruhe, W. Germany). The coefficient of variation (see above) of the assay was 12.0%. In 33 of the 52 incubations 24-, 25-, and 26-hydroxylated products were separated using a radio–gas chromatographic technique (cf. 21). The 24- and 26-hydroxylated products constituted less than 5% of the total products and were not subjected to further investigation. For further details of the assay systems for 12α- and 25-hydroxylase see Björkhem et al. (9, 11).

Statistics

Data are presented as mean ± SEM. The significance of differences between means was determined by Student's *t* test. Linear regressions were calculated by the method of least squares and their significances were tested by estimating the correlation coefficient *r* (24).

RESULTS

Table 2 summarizes the results.

Normolipidemic patients

HMG CoA reductase activity in female liver (23.0 ± 3.0 pmol · min^{-1} · mg $prot^{-1}$) was not significantly different from that in male liver (16.8 ± 1.5 pmol · min^{-1} · mg $prot^{-1}$). There was no correlation with age or relative body weight in the group as a whole, or among females and males, nor was there a significant difference between post- and premenopausal females.

12α-hydroxylase activity was about the same in female liver (152 ± 15 pmol · min^{-1} · mg $prot^{-1}$) as in male liver (132 ± 21 pmol · min^{-1} · mg $prot^{-1}$). There was no correlation with relative body weight in females or males. Among the males there was no correlation with age. The activity in postmenopausal females (135 ± 17 pmol · min^{-1} · mg $prot^{-1}$) was not significantly different from premenopausal ones (189 ± 20 pmol · min^{-1} · mg $prot^{-1}$).

25-hydroxylase activity was not significantly different between the sexes (231 ± 21 and 223 ± 22 pmol·min^{-1} ·mg $prot^{-1}$, females and males, respectively). The activity was not correlated with age or relative body weight. There was also no correlation between this activity and the activities of the 12α-hydroxylase or the HMG CoA reductase.

Patients with type IIa HLP

The activities of HMG CoA reductase (24.0 ± 3.2 pmol·min^{-1}·mg $prot^{-1}$), 12α-hydroxylase (137 ± 17 pmol·min^{-1}·mg $prot^{-1}$), and 25 hydroxylase (213 ± 28 pmol·min^{-1}·mg $prot^{-1}$) were not different from those of the normolipidemic group.

Patients with type IIb HLP

The activities of HMG CoA reductase (26.2 ± 3.0 pmol·min^{-1}·mg $prot^{-1}$), 12α-hydroxylase (119 ± 21 pmol·min^{-1}·mg $prot^{-1}$), and 25-hydroxylase (183 ± 33

TABLE 2. Enzyme activities in normo- and hyperlipidemic patients

Patient Number Sex[a]	HMG CoA Reductase	12α-Hydroxylase	25-Hydroxylase
	pmol·min⁻¹·mg protein⁻¹		
Normolipidemic group			
1. F		213	448
2. F	10.2	104	123
3. F	16.9	71	201
4. F	54.6		210
5. F	11.2	71	180
6. F	31.7	123	224
7. F	21.7	107	362
8. F	11.9		245
9. F	15.3	145	92
10. F	19.1	239	257
11. F	27.5	78	276
12. F	19.3	163	154
13. F	22.0	173	225
14. F		235	359
15. F	16.1	181	173
16. F	30.6	188	217
17. F		119	191
18. F	36.6	221	223
19. M	19.5	30	351
20. M	12.8	232	240
21. M	13.8	157	153
22. M	20.5	175	216
23. M	10.8	92	193
24. M	15.9	121	210
25. M	23.7	128	259
26. M	17.6	119	160
Hyperlipoproteinemia type IIa			
27. F	12.7	103	212
28. F		124	217
29. F	25.2	252	230
30. F	35.8	131	360
31. F	36.9	76	
32. F	28.0	75	105
33. F	16.3	136	272
34. M	22.2	200	
35. M	28.9	108	178
36. M	10.4	160	133
Hyperlipoproteinemia type IIb			
37. F		146	303
38. F	24.4	35	67
39. F	35.0	176	208
40. F	23.6	82	229
41. M		123	154
42. M	21.9	151	138
Hyperlipoproteinemia type IV			
43. F	77.7	232	269
44. F	85.7	98	79
45. F	27.7	188	161
46. F	40.4	132	178
47. F	53.1	147	370
48. F	14.6	122	166
49. M	24.7	159	240
50. M	44.0	72	119
51. M	26.6	106	204

TABLE 2. (*Continued*)

Patient Group	HMG CoA Reductase	12α-Hydroxylase	25-Hydroxylase
	pmol·min⁻¹·mg protein⁻¹		
Normolipidemic females	23.0 ± 3.0[b] (n = 15)	152 ± 15[b] (n = 16)	231 ± 21[b] (n = 18)
Normolipidemic males	16.8 ± 1.5 (n = 8)	132 ± 21 (n = 8)	223 ± 22 (n = 8)
Normolipidemic total	20.8 ± 2.1 (n = 23)	145 ± 12 (n = 24)	229 ± 16 (n = 26)
HLP IIa	24.0 ± 3.2 (n = 9)	137 ± 17 (n = 10)	213 ± 28 (n = 8)
HLP IIb	26.2 ± 3.0 (n = 4)	119 ± 21 (n = 6)	183 ± 33 (n = 6)
HLP IV	43.8 ± 8.1[c] (n = 9)	138 ± 16 (n = 9)	198 ± 29 (n = 9)

[a] F, female; M, male.
[b] Mean ± SEM.
[c] Significantly different from normolipidemic group ($P < 0.001$).

pmol·min^{-1}·mg prot^{-1}) were not statistically different from those of the normolipidemic group.

Patients with type IV HLP

HMG CoA reductase activity was about twice as high in this group (43.8 ± 8.1 pmol·min^{-1}·mg prot^{-1}) as in the normolipidemic group. This difference was highly significant for the group as a whole ($P < 0.001$), as well as for females ($P < 0.01$) and males ($P < 0.01$) alone. The activities of 12α-hydroxylase (138 ± 16 pmol·min^{-1}·mg prot^{-1}) and of 25-hydroxylase (198 ± 29 pmol·min^{-1}·mg prot^{-1}) were not different from those of the normolipidemic group.

DISCUSSION

The present study showed a rather wide inter-individual variation of the HMG CoA reductase activities in the normolipidemic subjects. A similarly wide range of the net steroid "balance" was recently observed in a group of healthy subjects (3). There were no differences between the sexes and no correlation between age and enzyme activity. In accordance with the work of Salen et al. (25), there was no relationship between body weight and hepatic HMG CoA reductase activity. The enzyme activity levels were somewhat lower than earlier reported for gallstone patients (25, 26). There is no obvious explanation, but several possibilities may be considered. In the present study, great care was taken to standardize the timing of the operation and the preceding fast. If a diurnal variation of hepatic HMG CoA reductase activity exists in

man, similar to that in the rat (cf. 6), it is reasonable to assume that our assays were performed at the nadir of activity.

Preoperative dietary conditions may be of importance for enzyme activity. The standardized hospital diet given for 2–3 days before the operation in the present study is similar to that of the average Swedish household (27) but may differ from the average American diet. Ethnic differences may also be of importance. In a recent publication from Great Britain, HMG CoA reductase activities in close agreement with those of the present study were reported (28). To our knowledge, the effect of anesthesia on HMG CoA reductase activity has not been studied; at least in the rat, the 12α- and 26-hydroxylase activities are not affected by the type of anesthesia given in the present study (29). Disregarding the remote possibility that the different groups of patients react differently to anesthesia, there is no reason to believe that any of these possible influences should invalidate the principal aim of the present study, namely to compare enzyme activities in different types of HLP.

The HMG CoA reductase activity is subject to a sensitive feedback control, still incompletely known in its details. In several cell types, such as cultured human skin fibroblasts, the regulation of enzyme activity appears to be mediated by binding of serum lipoproteins, preferentially LDL, to receptors of the cell surface (6, 30). The synthesis of these receptors is also subject to regulatory feedback (30). Such LDL receptors have not as yet been found in liver or intestine. In the rat, liver cholesterol synthesis appears to be controlled predominantly by dietary cholesterol transported to the liver by lipoproteins of intestinal origin, preferentially chylomicron remnants (4, 31, 32).

The role of bile acids in the regulation of hepatic cholesterogenesis is more uncertain. Although some studies in the rat have suggested an inhibitory effect of bile acids on HMG CoA reductase activity in the liver (33, 34), some recent investigations indicate that this may possibly be an indirect effect mediated over effects on cholesterol absorption (35, 36). In man, feeding with chenodeoxycholic acid has been shown to be associated with a reduced hepatic HMG CoA reductase activity (26, 37).[6]

Brown and Goldstein (30) have shown that patients with familial hypercholesterolemia have defective or deficient binding sites of LDL on the surface of the fibroblast which may lead to a less suppressed HMG CoA reductase activity. In spite of that, several in vivo studies have failed to show an oversynthesis of cholesterol in patients with familial or with nonfamilial hypercholesterolemia (3, 38–43). Nicolau et al. (23) found a relatively low HMG CoA reductase activity in a liver biopsy from a patient with hereditary hypercholesterolemia. In the present study the patients with HLP type IIa and type IIb all had normal HMG CoA reductase activity. This is in accordance with our previous finding that these patients have a normal endogenous cholesterol formation (3). It may be pointed out, however, that the patients had not been genotyped according to Goldstein et al. (44).

In HLP type IV, on the other hand, the mean value of HMG CoA reductase activity exceeded that encountered for control subjects by a factor of about two. There was a marked heterogeneity, and four of the nine patients had enzyme activities within the normal range. These data agree fairly well with those obtained for the fecal steroid excretion (41) and net steroid balance (3) in HLP type IV. Thus Angelin et al. (3) found that about 50% of the patients had a steroid balance above the normal range.

The reason for the increased formation of cholesterol and bile acids in some patients with type IV HLP is unclear. This type of HLP is evidently of heterogeneous origin, but overproduction of very low density lipoprotein (VLDL) appears to be of major importance in most patients (45–48). It is tempting to speculate that the elevated HMG CoA reductase activity observed in some patients with a type IV lipoprotein pattern is linked to an increased production of VLDL cholesterol. There seems to be a tight coupling between the rate-limiting step in the biosynthesis of bile acids and HMG CoA reductase activity under most conditions (8). Thus, if there is a primary linkage between lipoprotein synthesis and HMG CoA reductase activity in the liver, this may also affect bile acid biosynthesis. The previous finding of a positive correlation between lipoprotein triglyceride synthesis and bile acid formation in patients with type IV HLP (48) is of interest in this connection. On the other hand, a primary linkage between lipoprotein synthesis and bile acid formation may affect HMG CoA reductase activity as well.

Patients with HLP type IIa and type IIb have a low ratio between the synthesis of cholic and chenodeoxycholic acids and often a subnormal production of cholic acid (2). This might be explained by a reduction or deficiency of an enzyme, selective for cholic acid formation. A low cholic acid production is also seen in portal cirrhosis of the liver (49, 50). In this disease the impairment of the formation of cholic acid correlates with the severity of the liver disease, and it has been suggested that the primary defect may reside in the 12α-hydroxylase system (51, 52). The present results

[6] Ahlberg, J., B. Angelin, and K. Einarsson. Manuscript in preparation.

give no evidence for a selective deficiency of the 12α- or the 25-hydroxylase activities in patients with HLP type IIa or type IIb.

Another possible explanation for the low ratio between the synthesis of cholic acid and chenodeoxycholic acid in patients with type II HLP is related to the possibility that the two bile acids originate from different pools of cholesterol, which are degraded at different rates. Previous investigations in the rat have given evidence for a certain compartmentation of liver cholesterol (53–55), with cholic acid being derived from newly synthesized cholesterol to a greater extent than chenodeoxycholic acid (54). The greater supply of cholesterol from the circulation in type II HLP may affect the relative rate of 7α-hydroxylation of preformed cholesterol and newly synthesized cholesterol. Thus, determination of the 7α-hydroxylase activity acting on endogenous and exogenous cholesterol (56) may be of interest in these patients. Attempts to assay these activities in human liver have, however, not been successful in our hands so far.

The skillful technical assistance of Mrs. Kerstin Hedström, Mrs. Margret Wahlström, and Mrs. Eija Varhenmaa is gratefully acknowledged. The study was supported by grants from the Swedish Medical Research Council (Project no. 19X-04793) and from the Swedish National Association against Heart and Chest Diseases.

Manuscript received 19 December 1977; accepted 31 July 1978.

REFERENCES

1. Hellström, K., and K. Einarsson. 1977. Bile acid metabolism in hyperlipoproteinaemia. *Clin. Gastroenterol.* **6:** 103–128.
2. Einarsson, K., K. Hellström, and M. Kallner. 1974. Bile acid kinetics in relation to sex, serum lipids, body weight, and gallbladder disease in patients with various types of hyperlipoproteinemia. *J. Clin. Invest.* **54:** 1301–1311.
3. Angelin, B., K. Einarsson, K. Hellström, and M. Kallner. 1976. Elimination of cholesterol in hyperlipoproteinaemia. *Clin. Sci. Mol. Med.* **51:** 393–397.
4. Dietschy, J. M., and J. D. Wilson. 1970. Regulation of cholesterol metabolism. *N. Engl. J. Med.* **282:** 1128–1138, 1179–1183, 1241–1249.
5. Sodhi, H. S. 1975. Cholesterol metabolism in man. *In* Hypolipidemic Agents. D. Kritchevsky, editor. Springer-Verlag, Berlin, Heidelberg, New York, 29–107.
6. Rodwell, V. W., J. L. Nordstrom, and J. J. Mitschelen. 1976. Regulation of HMG-CoA reductase. *Adv. Lipid Res.* **14:** 1–74.
7. Nicolau, G., S. Shefer, G. Salen, and E. H. Mosbach. 1974. Determination of hepatic cholesterol 7α-hydroxylase activity in man. *J. Lipid Res.* **15:** 146–151.
8. Myant, N. B., and K. A. Mitropoulos. 1977. Cholesterol 7α-hydroxylase. *J. Lipid Res.* **18:** 135–153.
9. Björkhem, I., H. Danielsson, K. Einarsson, and G. Johansson. 1968. Formation of bile acids in man: conversion of cholesterol into 5β-cholestane-3α,7α,12α-triol in liver homogenates. *J. Clin. Invest.* **47:** 1573–1583.
10. Björkhem, I., and H. Danielsson. 1976. Biosynthesis and metabolism of bile acids in man. *In* Progress in Liver Diseases, Vol. V. H. Popper and F. Schaffner, editors. Grune & Stratton, New York-San Francisco-London. 215–231.
11. Björkhem, I., J. Gustafsson, G. Johansson, and B. Persson. 1975. Biosynthesis of bile acids in man. Hydroxylation of the C_{27}-steroid side chain. *J. Clin. Invest.* **55:** 478–486.
12. Setoguchi, T., G. Salen, G. S. Tint, and E. H. Mosbach. 1974. A biochemical abnormality in cerebrotendinous xanthomatosis. Impairment of bile acid biosynthesis associated with incomplete degradation of the cholesterol side chain. *J. Clin. Invest.* **53:** 1393–1401.
13. Salen, G., S. Shefer, T. Setoguchi, and E. H. Mosbach. 1975. Bile alcohol metabolism in man. Conversion of 5β-cholestane-3α,7α,12α,25-tetrol to cholic acid. *J. Clin. Invest.* **56:** 226–231.
14. Schwartz, C. C., B. I. Cohen, Z. R. Vlahcevic, D. H. Gregory, L. G. Halloran, T. Kuramoto, E. H. Mosbach, and L. Swell. 1976. Quantitative aspects of the conversion of 5α-cholestane intermediates to bile acids in man. *J. Clin. Invest.* **251:** 6308–6314.
15. Shefer, S., F. W. Cheng, B. Dayal, S. Hansen, G. S. Tint, G. Salen, and E. H. Mosbach. 1976. A 25-hydroxylation pathway of cholic acid biosynthesis in man and rat. *J. Clin. Invest.* **57:** 897–903.
16. Beaumont, J. L., L. A. Carlson, G. R. Cooper, Z. Fejfar, D. S. Fredrickson, and T. Strasser. 1970. Classification of hyperlipidaemias and hyperlipoproteinaemias. *Bull. WHO.* **43:** 891–915.
17. Angelin, B., I. Björkhem, and K. Einarsson. 1978. Individual serum bile acid concentrations in normo- and hyperlipoproteinemia as determined by mass fragmentography: relation to bile acid pool size. *J. Lipid Res.* **19:** 527–537.
18. Ahlberg, J., B. Angelin, K. Einarsson, K. Hellström, and B. Leijd. 1977. Influence of deoxycholic acid on biliary lipids in man. *Clin. Sci. Mol. Med.* **53:** 249–256.
19. Hegardt, F. G., and H. Dam. 1971. The solubility of cholesterol in aqueous solutions of bile salts and lecithin. *Z. Ernährungswiss.* **10:** 223–233.
20. Holzbach, R. T., M. March, M. Olszewski, and K. Holan. 1973. Cholesterol solubility in bile. Evidence that supersaturated bile is frequent in healthy man. *J. Clin. Invest.* **52:** 1467–1479.
21. Björkhem, I., and J. Gustafsson. 1973. ω-Hydroxylation of steroid side-chain in biosynthesis of bile acids. *Eur. J. Biochem.* **36:** 201–212.
22. 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.
23. Nicolau, G., S. Shefer, G. Salen, and E. H. Mosbach. 1974. Determination of hepatic 3-hydroxy-3-methylglutaryl CoA reductase activity in man. *J. Lipid Res.* **15:** 94–98.
24. Snedecor, G. W., and W. G. Cochran. 1974. Statistical Methods. Iowa State University Press, Ames, IA. 6th edition.
25. Salen, G., G. Nicolau, S. Shefer, and E. H. Mosbach. 1975. Hepatic cholesterol metabolism in patients with gallstones. *Gastroenterology.* **69:** 676–684.
26. Coyne, M. J., G. G. Bonorris, L. I. Goldstein, and L. J.

Schoenfield. 1976. Effect 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.
27. Borgström, B., Å. Nordén, B. Åkesson, and M. Jägerstad. 1975. A study of the food consumption by the duplicate portion technique in a sample of the Dalby population. *Scand. J. Soc. Med.*, Suppl. 10.
28. Maton, P. N., G. M. Murphy, and R. H. Dowling. 1977. Ursodeoxycholic acid treatment of gallstones. *Lancet.* **2:** 1297–1301.
29. Hallén, B., and G. Johansson. 1975. Inhalation anesthetics and cytochrome P-450-dependent reactions in rat liver microsomes. *Anesthesiology.* **43:** 34–40.
30. Brown, M. S., and J. L. Goldstein. Receptor-mediated control of cholesterol metabolism. *Science.* **191:** 150–154.
31. Nervi, F. O., and J. M. Dietschy. 1975. Ability of six different lipoprotein fractions to regulate the rate of hepatic cholesterogenesis in vivo. *J. Biol. Chem.* **250:** 8704–8711.
32. Andersen, J. M., F. O. Nervi, and J. M. Dietschy. 1977. Rate constants for the uptake of cholesterol from various intestinal and serum lipoprotein fractions by the liver of the rat in vivo. *Biochim. Biophys. Acta.* **486:** 298–307.
33. Hamprecht, B., R. Roscher, G. Waltinger, and C. Nüssler. 1971. Influence of bile acids on the activity of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. 2. Effect of cholic acid in lymph fistula rats. *Eur. J. Biochem.* **18:** 15–19.
34. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1973. Regulatory effects of sterols and bile acids on hepatic HMG CoA reductase and cholesterol 7α-hydroxylase. *J. Lipid Res.* **14:** 573–580.
35. Cooper, A. D. 1976. The regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the isolated perfused rat liver. *J. Clin. Invest.* **57:** 1461–1470.
36. Nervi, F. O., and J. M. Dietschy. 1978. The mechanisms of and the interrelationship between bile acid and chylomicron-mediated regulation of hepatic cholesterol synthesis in the liver of the rat. *J. Clin. Invest.* **61:** 895–909.
37. Mosbach, E. H., and G. Salen. 1974. Bile acid biosynthesis. Pathways and regulation. *Am. J. Dig. Dis.* **19:** 920–929.
38. Nestel, P. J., H. M. Whyte, and D. S. Goodman. 1969. Distribution and turnover of cholesterol in humans. *J. Clin. Invest.* **48:** 982–991.
39. Samuel, P., and W. Perl. 1970. Long-term decay of serum cholesterol radioactivity: Body cholesterol metabolism in normals and in patients with hyperlipoproteinemia and atherosclerosis. *J. Clin. Invest.* **49:** 346–357.
40. Bolzano, K., F. Sandhofer, S. Sailer, and H. Braunsteiner 1973. Studies on cholesterol turnover in hypercholesterolemic subjects. *Horm. Metab. Res.* **5:** 444–449.
41. Miettinen, T. A. 1975. Bile acid metabolism. *In* Hypolipidemic Agents. D. Kritchevsky, editor. Springer-Verlag, Berlin, Heidelberg, New York. 109–150.
42. Bhattacharyya, A. K., W. E. Connor, and A. A. Spector. 1976. Abnormalities of cholesterol turnover in hypercholesterolemic (type II) patients. *J. Lab. Clin. Med.* **88:** 202–214.
43. Smith, F. R., R. B. Dell, R. P. Noble, and D. S. Goodman. 1976. Parameters of the three-pool model of the turnover of plasma cholesterol in normal and hyperlipidemic humans. *J. Clin. Invest.* **57:** 137–148.
44. Goldstein, J. L., H. G. Schrott, W. R. Hazzard, E. L. Bierman, and A. G. Motulsky. 1973. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J. Clin. Invest.* **52:** 1544–1568.
45. Nikkilä, E. A., and M. Kekki. 1972. Plasma endogenous triglyceride transport in hypertriglyceridaemia and effect of a hypolipidaemic drug (SU-13437). *Eur. J. Clin. Invest.* **2:** 231–238.
46. Kaye, J. P., and D. J. Galton. 1975. Triglyceride-production rates in patients with type IV hypertriglyceridaemia. *Lancet* **1:** 1005–1007.
47. Sigurdsson, G., A. Nicoll, and B. Lewis. 1976. Metabolism of very low density lipoproteins in hyperlipidaemia: Studies of apolipoprotein B kinetics in man. *Eur. J. Clin. Invest.* **6:** 167–177.
48. Angelin, B., K. Einarsson, K. Hellström, and B. Leijd. 1978. Bile acid kinetics in relation to endogenous triglyceride metabolism in various types of hyperlipoproteinemia. *J. Lipid Res.* **19:** 1004–1016.
49. Vlahcevic, Z. R., P. Juttijudata, C. C. Bell, Jr., and L. Swell. 1972. Bile acid metabolism in patients with cirrhosis. II. Cholic and chenodeoxycholic acid metabolism. *Gastroenterology.* **62:** 1174–1181.
50. Einarsson, K., K. Hellström, and T. Scherstén. 1975. The formation of bile acids in patients with portal liver cirrhosis. *Scand. J. Gastroenterol.* **10:** 299–304.
51. McCormick, W. C. III, C. C. Bell, Jr., L. Swell, and Z. R. Vlahcevic. 1973. Cholic acid synthesis as an index of the severity of liver disease in man. *Gut.* **14:** 895–902.
52. Angelin, B., K. Einarsson, and K. Hellström. 1978. Effect of cholestyramine on bile acid kinetics in patients with portal cirrhosis of the liver. *Am. J. Dig. Dis.* **23** (in press).
53. Balasubramaniam, S., K. A. Mitropoulos, and N. B. Myant. 1973. Evidence for the compartmentation of cholesterol in rat liver microsomes. *Eur. J. Biochem.* **34:** 77–83.
54. Mitropoulos, K. A., N. B. Myant, G. F. Gibbons, S. Balasubramaniam, and B. E. A. Reeves. 1974. Cholesterol precursor pools for the synthesis of cholic and chenodeoxycholic acids in rats. *J. Biol. Chem.* **249:** 6052–6056.
55. Cronholm, T., A. L. Burlingame, and J. Sjövall. 1974. Utilization of the carbon and hydrogen atoms of ethanol in the biosynthesis of steroids and bile acids. *Eur. J. Biochem.* **49:** 497–510.
56. Björkhem, I., and H. Danielsson. 1975. 7α-Hydroxylation of exogenous and endogenous cholesterol in rat-liver microsomes. *Eur. J. Biochem.* **53:** 63–70.