Biliary lipid secretion in chronic cholestatic liver disease*

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SUMMARY Biliary lipid secretion rates, faecal steroids, and serum lipids were studied in patients with chronic cholestatic liver disease mainly primary biliary cirrhosis. The biliary secretion of cholesterol, bile acids, and phospholipids was markedly decreased as compared with those in the control group and in general correlated negatively with the serum cholesterol and triglyceride values. The molar percentage of cholesterol was increased in the hepatic bile. This suggests that, in cholestatic liver disease, in contrast with the normal state, the hepatic bile may be supersaturated postprandially. Faecal bile acids and neutral sterols of cholesterol origin were decreased proportionately to the corresponding biliary lipid secretion rates. In fact, both biliary and faecal steroid outputs were only about a half or less than those in the controls, indicating that the fractional absorption was not changed but absolute absorption and faecal steroid excretion were low in patients with chronic cholestatic liver disease. Thus, despite low cholesterol and bile acid absorption, cholesterol and bile acid synthesis is low. A negative correlation between faecal steroids and serum cholesterol suggests that the high serum cholesterol level contributed to regulation of cholesterol synthesis.

Marked abnormalities in cholesterol and bile acid metabolism have been reported in patients with severe chronic liver diseases. In non-cholestatic cirrhosis the production of cholesterol holding capacity of the bile is higher than in control subjects.² In liver failure in general the biological half-life of bile acids is prolonged, the pool size diminished, and the synthesis of bile acids is markedly reduced.³⁻⁵ Our previous studies on biliary lipid composition and faecal steroid excretion suggested that the patients with severe forms of chronic active hepatitis and primary biliary cirrhosis have a low biliary lipid secretion, resulting in decreased faecal steroid output.^{6,7} With this background the present study was designed to obtain more detailed information on the hourly biliary secretion rates of cholesterol, bile acids and phospholipids in patients with chronic cholestatic liver disease. This is of particular interest as the prevalence of gall stones is increased in patients with cirrhosis of the liver.⁸

Methods

PATIENTS

The series included eight patients with variable severity of primary biliary cirrhosis, one patient with a cholestatic form of chronic active hepatitis, and one patient with sclerosing cholangitis. Six of the patients (nos. 1, 2, 4, 5, 6, and 9, Table 1) were the same as reported in our previous study.⁷ Seven healthy medical students served as controls. Informed consent was obtained before the studies both from patients and controls. The diagnosis of primary biliary cirrhosis was based on typical clinical, biochemical, and histological findings as published in detail before^{9,10} and the diagnostic criteria were similar to those reported by Sherlock and Scheuer.¹¹ The patient with chronic active hepatitis was Hb_sAg negative. His diagnosis was based on the accepted histological criteria.¹² The case represented, however, the cholestatic form of the disease⁹ characterised by the presence of orcein positive copper protein accumulations in histological liver specimens.¹³ The diagnosis of sclerosing cholangitis was confirmed by endoscopic retrograde cholangiography. The symptoms had lasted for over a year in every case. The summary of the clinical and

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Table 1 Clinical data on patients with chronic cholestatic liver disease and control subjects

Subject no.	Sex	Age (yr)	Body weight		Alb* g/l (38-51)	Alat U/l (8-45)	P+P (75-100)	AFOS U/l (60-240)	LAP U/l (18-48)	γGT U/l (6-20)	Bil μmol/l (2-20)	BSP-Tm T½ (>6.8 mg/min)	Chol mmol/l (4.4-7.2)	TG mmol/l (0.4-1.7)
			kg	Relative										
1	M	22	91.0	1.19	34.0	130	82	790	138	485	11	4.1	7.0	2.77
2	F	67	60.2	1.19	29.5	46	91	852	104	83	23	2.7	6.1	1.03
3	F	31	58.3	1.08	31.4	97	96	1055	236	505	16	2.6	9.0	1.16
4	F	55	65.0	1.20	25.3	92	107	1152	143	140	39	2.4	10.7	1.21
5	F	51	65.0	1.18	24.5	96	59	626	107	33	17	2.3	6.1	0.50
6	M	66	55.3	0.95	28.1	132	87	517	79	107	63	2.3	4.8	1.33
7	F	51	53.0	1.00	23.5	64	87	1201	98	50	330	1.4	10.6	2.42
8	F	41	58.2	1.08	18.0	338	114	2656	316	320	56	0.89	9.9	0.82
9	F	35	54.7	0.94	30.7	333	93	1758	373	246	67	0.74	15.4	2.04
10	F	37	64.6	1.09	29.4	165	99	1477	248	360	19	0.56	8.7	1.11
Mean	8/2	45.6†	62.5	1.09	27.4	149.3	91.5	1208.4	184.2	232.9	64.1	2.0	8.8†	1.44†
±SE		±4.7	±3.5	±0.03	±1.5	±32.8	±4.7	±200.4	±32.4	±56.0	±30.3	±0.3	±1.0	±0.23
Controls (7)	3/4	25.3	62.4	1.00	—	—	—	—	—	—	—	—	5.2	0.65
		±1.3	±3.8	±0.04	—	—	—	—	—	—	—	—	±0.2	±0.06

The diagnosis of the patients: nos 2-5 and 7-10 primary biliary cirrhosis, no. 1 sclerosing cholangitis, no. 6 cholestatic form of chronic active hepatitis.

* Alb = serum albumin, Alat = serum alanine aminotransferase, P+P = plasma prothrombin, AFOS = serum alkaline phosphatase, LAP = serum leucine aminopeptidase, γGT = serum gamma-glutamyl transferase, Bil = serum bilirubin, BSP-Tm = transport maximum for bromosulphophthalein, Chol = serum cholesterol, TG = serum triglycerides. The normal ranges of laboratory data are given in parentheses.

† Statistically significant difference ($p < 0.01$ or less) from controls.

laboratory data of the liver patients and controls is shown in Table 1.

PROCEDURES

All the patients and controls were hospitalised and placed on a standard diet. This low-cholesterol (1.5 mg/kg/day) solid-food diet contained 40% of energy as fat, 45% as carbohydrate, and 15% as protein. The daily energy content of the diet was 30-35 kcal/kg of body weight, and was adjusted to maintain a constant body weight during the studies. The fat content of the standard diet consisted of meat, lard, soya oil, and margarine. As unabsorbable markers 600 mg of Cr_2O_3 and beta-sitosterol were each divided into three daily doses so that corrections could be made respectively for faecal flow and degradation of cholesterol during intestinal transit.^{14 15} After the patients had been on the diet and the markers for five to seven days a single two to three day faecal collection was made.

Thereafter the hepatic lipid secretion into the duodenum was determined according to Grundy and Metzger.¹⁶ After an overnight fast a two lumen radio-opaque tube (type AN 20, HW Andersen Products, Ltd, England) was positioned under fluoroscopic control into the second part of the duodenum. To make the gall bladder contract each patient received cholecystokinin (pancreozymin, Boots Company, Ltd, England) 2 units per kg of body weight, up to a maximum dose of 100 units per patient, in 20 ml of physiological saline intravenously. The liquid formula used in the study

was prepared by sonication from 20 ml olive oil, 20 g fat-free milk powder, 4.5 g glycerol-1-mono-oleate (Fluka SG, Switzerland) and 200 ml water. The energy content of the formula was 1.3 kcal/ml. The formula contained 4 g polyethylene glycol 4000 (Fluka AG, Switzerland) as a non-absorbable marker. The formula with markers was infused at a rate of 30 ml/h into the duodenum via the proximal outlet of the tube with an infusion pump. Samples of 5-10 ml were aspirated hourly through outlets, which were located 15-20 cm more distally in the duodenum. Collections were performed up to six to eight hours. The samples obtained were placed in a water bath at 70°C for five minutes to destroy the lipase activity and stored at -20°C until analysed. The biliary secretion rates of cholesterol, bile acids, and phospholipids into the duodenum were calculated according to Grundy and Metzger from the samples collected at four to eight hours of perfusion.¹⁶

ANALYTICAL METHODS

The automated methods of our hospital laboratory were used to estimate serum concentrations of albumin, alanine aminotransferase, plasma prothrombin, alkaline phosphatase, bilirubin, cholesterol, and triglycerides. Transport maximum for bromosulphophthalein was measured according to Wheeler *et al.*¹⁷ Hepatic cholesterol from duodenal fluid was analysed as described by Miettinen *et al.*¹⁸ An aliquot of petroleum ether extract of saponified hepatic lipids, containing 5-α-

Biliary lipid secretion

cholestane as an internal standard, was taken for the gas liquid chromatography analysis of cholesterol and β -sitosterol. Hepatic bile acids were determined with an enzymatic method.¹⁹ Hepatic phospholipids were measured by the method of Bartlett²⁰ using the Fiske-Subbarow reagent.²¹ Faecal bile acids were measured by gas liquid chromatography as described by Grundy *et al.*²² except that the thin layer chromatography step was omitted. Faecal neutral steroids derived from cholesterol and dietary plant sterols and their bacterial conversion products were measured by gas liquid chromatography after thin layer chromatography separation according to Miettinen *et al.*¹⁸ Chromic oxide (Cr_2O_3) was determined as reported by Bolin *et al.*²³ Polyethylene glycol was determined according to Hyden.²⁴ Faecal fat was measured according to the method of van de Kamer *et al.*²⁵

STATISTICS

The results are given as means and standard error of mean (SE). The regression analyses were carried out by the method of least squares. The statistical significance of the difference was estimated by Student's *t* test.

Results

SERUM LIPIDS

The mean serum cholesterol and triglyceride levels

were significantly raised in the patients with chronic cholestatic liver disease (Table 1). Only a few patients, however, had a marked hypercholesterolaemia and only three patients were actually hypertriglyceridaemic.

BILIARY LIPIDS

The mean biliary secretion rates of cholesterol, bile acids and phospholipids were in the patients only 52, 38, 35%, respectively, of those in the controls (Table 2). The hourly secretion rates of bile acids correlated significantly with those of cholesterol and phospholipids in both groups (Fig. 1). The mean molar percentage of cholesterol was markedly raised in the hepatic bile of the patients. The molar percentage plotted against the respective hourly output of bile acids (Fig. 2) showed a negative correlation in both groups and demonstrates the high molar percentage of cholesterol (supersaturation) at the low bile acid output in the patients. At higher bile acid secretion rates, however, around $10 \mu\text{mol kg}^{-1}\text{h}^{-1}$, for instance, the molar percentage of cholesterol tends to be even lower in the patients than in the controls.

The plot of the biliary lipid secretion rates against the liver function tests of Table 1 revealed a significant correlation between the phospholipid secretion and the maximal BSP transport ($r=0.78$; $p<0.01$, $n=10$). In addition, serum bilirubin was positively correlated with the molar percentage of

Table 2 Biliary and faecal lipids in patients with chronic cholestatic liver disease and controls

Subject no.	Biliary secretion rates (mg/kg/h)			Molar percentage of biliary lipids			Faecal fat g/day	Faecal steroids (mg/kg/day)		
	Chol	BA	PL	Chol	BA	PL		NS	BA	Total
Liver patients										
1	0.27	1.53	0.07	10.3	56.4	33.3	4.5	5.8	4.6	10.4
2	0.32	3.14	0.05	8.1	76.3	15.6	4.3	7.9	1.3	9.2
3	0.76	2.30	0.07	19.7	57.7	22.7	3.7	8.4	2.3	10.7
4	0.22	4.18	0.03	4.8	87.2	8.1	5.7	5.9	1.4	7.3
5	0.41	3.50	0.05	9.3	76.6	14.1	4.0	6.1	1.0	7.1
6	0.22	2.34	0.02	8.1	82.7	9.2	12.8	6.2	1.0	7.3
7	0.19	0.32	0.02	25.3	41.2	33.5	8.7	6.5	1.0	7.5
8	0.12	0.75	0.02	10.9	66.1	22.9	6.7	6.4	0.4	6.8
9	0.19	1.24	0.03	10.7	68.0	21.3	12.7	4.2	2.3	6.5
10	0.24	0.87	0.02	18.0	63.1	18.9	3.6	6.0	1.2 7.2	
Mean±SE	0.29±0.06*	2.02±0.41*	0.038±0.006*	12.5±2.0*	67.5±4.4	20.0±2.8	6.7±1.1*	6.3±0.4*	1.7±0.4*	8.0±0.5*
Controls										
1	0.71	4.90	0.11	10.4	69.8	19.9	3.6	12.1	4.8	16.9
2	0.46	6.95	0.10	5.5	79.9	14.7	3.9	9.1	2.6	11.7
3	0.47	5.13	0.09	7.2	75.7	17.1	3.9	11.4	5.7	17.1
4	0.85	5.29	0.12	11.4	68.4	20.2	4.6	10.7	4.6	15.3
5	0.37	4.24	0.05	7.3	80.5	12.2	2.4	12.2	3.5	15.7
6	0.38	3.77	0.07	7.7	74.1	18.1	3.4	9.0	3.9	12.9
7	0.66	7.95	0.20	6.1	70.8	23.1	4.5	13.7	4.0	17.7
Mean±SE	0.56±0.07	5.46±0.56	0.11±0.02	7.9±0.8	74.2±1.8	17.9±1.4	3.8±0.3	11.2±0.65	4.2±0.38	15.3±0.85

Chol = cholesterol, BA = bile acids, PL = phospholipids, NS = neutral sterols, BA = bile acids.

* Statistically significant difference ($p<0.05$ or less) from controls.

Fig. 1 Relationship between biliary outputs of cholesterol (left) and phospholipids (right) for patients with chronic cholestatic liver disease (above) and normal controls (below). Each circle represents an individual hourly output of the biliary lipids.

biliary cholesterol ($r=0.70$; $p<0.02$; $n=10$) and negatively with that of biliary bile acids ($r=-0.67$; $p<0.02$; $n=10$).

The biliary lipid secretion rates in general correlated negatively with serum cholesterol and triglyceride values (Table 3). In addition, the molar percentages of biliary bile acids correlated negatively ($r=0.61$; $p<0.01$; $n=17$) and those of biliary cholesterol ($r=0.55$; $p<0.05$; $n=17$) and

phospholipids ($r=0.63$; $p<0.01$; $n=17$) positively with serum triglyceride levels. For serum cholesterol these correlations were not significant.

FAECAL FAT AND STEROLS

The mean faecal fat was higher in the patient group than in the controls. An increased faecal fat (7 g/day) was found, however, in three patients only (Table 2).

Fig. 2 Relationship between molar percentage of biliary cholesterol and biliary bile acid secretion in patients with chronic cholestatic liver disease (●) and in controls (○). Each circle represents an individual hourly value of the biliary lipids.

Table 3 Correlation coefficients (*r*) of biliary and faecal lipids with some serum parameters in controls and in patients with chronic cholestatic liver disease

	Faecal bile acids	Faecal neutral steroids	Faecal total steroids	Serum cholesterol	Serum triglycerides	Serum albumin	Bromosulph- ophthalein transport maximum
Biliary cholesterol secretion	0.23† 0.60‡*	0.66* 0.63*	0.68* 0.75*	-0.23 -0.47	-0.25 -0.49*	0.41 —	0.39 —
Biliary bile acid secretion	-0.05 0.55*	0.23 0.78*	0.13 0.75*	-0.36 -0.61*	-0.49 -0.65*	0.10 —	0.50 —
Biliary phospholipid secretion	0.70* 0.70*	0.42 0.80*	0.86* 0.82*	-0.29 -0.51*	0.12 0.39	0.60 —	0.78* —
Faecal bile acids	—	—	—	-0.04 -0.45	0.68* -0.17	0.78* —	0.63* —
Faecal neutral sterols	—	—	—	-0.51 -0.65*	-0.35 -0.62*	0.01 —	0.55 —
Total faecal steroids	—	—	—	-0.36 -0.63*	0.27 -0.49*	0.62 —	0.76* —

† The upper number gives the *r*-value for the patients only.
‡ The lower number gives the *r*-value for the pooled material of controls and patients.
* Statistically significant (*p* < 0.05 at least) correlation.

The daily output of faecal bile acids, neutral steroids, and total steroids was significantly lower in the patients than in the controls. The mean respective values were only 56, 40, and 52% of those in the controls.

The correlations in Table 3 and Fig. 3 indicate that, in general, the lower the faecal steroid excretion the lower also was the biliary lipid secretion rate and the higher were the serum cholesterol and triglyceride levels. Thus, hypercholesterolaemia and hypertriglyceridaemia were

associated with impaired cholesterol elimination and reduced cholesterol synthesis (sterol balance values). Furthermore, the maximal BSP transport correlated positively with faecal steroids and bile acids (Table 3).

Discussion

Findings on the biliary lipid composition and the faecal steroid excretion in patients with primary biliary cirrhosis and chronic active hepatitis have

Fig. 3 Relationship between biliary cholesterol secretion and faecal neutral sterol elimination (left) and between biliary bile acid secretion and faecal bile acids (right) in patients with chronic cholestatic liver disease (●) and in controls (○).

suggested low biliary lipid secretion rates in the severe forms of these diseases.^{6,7} Direct measurements of the biliary lipid secretion rate in the present study on patients with varying severity of mainly primary biliary cirrhosis show this suggestion to be true. In contrast, however, with the observations on the low molar percentage of cholesterol in the fasting gall bladder bile^{2,6,7} the hepatic bile obtained during the hepatic secretion exhibited a high molar percentage (12.5% vs. 7.9% in the controls) in the present patients. In addition, fasting gall bladder bile samples were previously analysed and reported (7) for six of the patients of the present study (patients nos 1, 2, 4, 5, 6, and 9). The molar percentage of cholesterol for these six patients was significantly higher in the hepatic bile of the present study ($8.6\% \pm 0.9$ (SEM)) than in the fasting gall bladder bile of our previous study ($5.6\% \pm 0.9$) (7) ($p < 0.025$, paired t test). Thus, in severe cholestatic liver disease the hepatic bile appears to be strongly supersaturated during active enterohepatic circulation of bile acids mainly because bile acids are retained in the circulation. During night time and during the fasting state in general the hepatic bile of these patients is apparently less saturated with cholesterol, resulting in the low molar percentage in the gall bladder bile. In cholestatic liver disease the situation may thus be inverse to the normal state in which the hepatic bile is supersaturated in fasting state and undersaturated postprandially. Therefore, the situation differs from that in the patients with moderately advanced alcoholic cirrhosis²⁶ in which the biliary lipid secretion rates are not disturbed and the molar percentages of cholesterol are normal both in the gall bladder bile and in hepatic bile obtained during biliary secretion measurements. In severe alcoholic cirrhosis, in which the biliary lipid secretion is low, the cholesterol saturation of the gall bladder bile is low but appears to be normal (molar % cholesterol 5.2 vs 4.3 in the controls) during biliary lipid secretion measurements.²⁶

The finding of low fasting and high postprandial cholesterol saturation of bile in patients with chronic liver disease may be clinically important. The prevalence of gall stones is increased in patients with cirrhosis of the liver.⁸ In contrast, however, with the general population where the majority of gall stones are of the cholesterol type²⁸ cirrhotic patients have principally pigment stones.^{29,30} Therefore, it is possible that the inverse situation of supersaturated postprandial and undersaturated fasting bile observed in the patients of the present study is actually beneficial in the prevention of cholesterol gall stone formation in patients with liver disease and low biliary lipid secretion.

Ordinary liver function tests are weakly associated with biliary lipid secretion in patients with chronic cholestatic liver disease. Significant correlations were observed, however, between the serum cholesterol and triglyceride values on the one hand and the biliary and faecal steroid outputs on the other hand. Thus, in general, the development of hyperlipidaemias appears to be associated with impaired biliary and faecal steroid output in cholestatic liver disease.

Faecal bile acids and neutral sterols of cholesterol origin have been reported to be decreased in patients with chronic cholestatic liver disease^{1,6,7} and these findings were confirmed in the present study. Urinary bile acids are frequently raised,^{1,31} but this route of bile salt elimination is minor compared with faecal excretion.¹ This indicates that bile acid and cholesterol synthesis are decreased in patients with cholestatic liver disease, a finding supported by earlier observations.^{1,7} The positive correlation of faecal steroid outputs with the biliary lipid secretion rates suggests that low biliary secretion of cholesterol and bile acids determined mainly the low faecal excretion and not the increased fractional absorption of these steroids. In fact, as compared with the controls the biliary secretion rates and faecal steroid excretion were decreased by about a factor of two, as if the relative absorption had been unchanged. Calculations of the relative absorption of both bile acids (96.5%) and cholesterol (25.4%) (from the dietary, biliary, and faecal steroid data) of the patients with chronic cholestatic liver disease actually revealed similar values to those of the controls (bile acids: 96.8%; cholesterol 24.9%). Thus, the mean absolute absorption of both cholesterol (2.1 mg/kg/day for the patients vs 3.2 mg/kg/day for controls) and bile acids (47 mg/kg/day) for the patients vs 127 mg/kg/day for controls) had decreased proportionately to the decrease in biliary secretion. Low absorption of cholesterol and bile acids should have stimulated cholesterol synthesis. That the synthesis was actually low may be due to the liver damage or to an inhibitory effect of increased serum cholesterol level on hepatic cholesterol synthesis. Thus, patients with chronic cholestatic liver disease are characterised by impaired biliary lipid output frequently associated with high serum lipid values, normal fractional absorption but clearly decreased absolute absorption of cholesterol and bile acids, and decreased cholesterol and bile acid synthesis.

The absence of steatorrhoea in the present series, also shown in our previous studies,⁷ might indicate that, despite the clear-cut decrease of the biliary bile acid secretion, it was sufficient to maintain the intestinal bile acid level above the critical micellar

concentration during fat digestion. Fatty acids are, however, absorbed to some extent along the small intestine also without micellar solubilisation.^{32 33} Thus faecal fat can be only modestly increased in patients with liver disease and severe disturbance of micellar formation.¹ Micellar solubilisation is a prerequisite of cholesterol absorption.^{34 35} The normal percentage of cholesterol absorption in the patients with cholestatic liver disease suggests that, for the small intestinal cholesterol pool of these patients, the bile acid secretion was sufficient to facilitate micellar solubilisation of cholesterol. It can be speculated that on a higher cholesterol intake the relative cholesterol absorption would have been reduced.

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