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Biliary lipid metabolism in chronic pancreatitis: influence of steatorrhoea

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SUMMARY Kinetics of [24-^{14}C] cholic acid and [24-^{14}C] chenodeoxycholic acid and biliary lipid composition were determined in patients with chronic alcoholic pancreatitis with (n=8) and without (n=8) steatorrhoea. Pool sizes, syntheses and fractional catabolic rates of the two bile acids were not significantly different from corresponding values in healthy controls. Biliary lipid composition was normal in patients without steatorrhoea. Patients with steatorrhoea, on the other hand, had lower cholesterol saturation of bile than corresponding controls. This is probably because of malabsorption of cholesterol.

Patients with severe exocrine pancreatic insufficiency display steatorrhoea – that is, malabsorption of fat. The steatorrhoea has been considered to be the result of impaired lipolytic activity in the duodenal juice. Regan *et al*,[1] however, have suggested that reduced intraluminal bile acid concentration and impaired micellar solubilisation postprandially may also contribute to the fat malabsorption. These authors have shown that patients with advanced pancreatic insufficiency have a normal biliary secretion of bile acids but a low micellar concentration of bile acids because of precipitation of bile salts in the abnormally acidic contents in the duodenum. According to previous studies,[2–4] the faecal excretion of bile acids is increased in adults with chronic pancreatitis and steatorrhoea. Nothing is known about the kinetics of individual bile acids, however, or the lipid composition and cholesterol saturation of bile in patients with chronic pancreatitis.

The aim of the present study was to determine the kinetics of the two primary bile acids, cholic acid and chenodeoxycholic acid, and the lipid composition and cholesterol saturation of bile in a series of patients with chronic pancreatitis because of alcohol abuse, with and without steatorrhoea. Great care was taken to exclude patients with coexisting diabetes mellitus and/or liver damage, because it is well known that both these clinical conditions may influence bile acid metabolism and biliary lipid composition.[5–8]

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Methods

PATIENTS

The present study comprised 16 non-obese, normolipidemic patients with chronic alcoholic pancreatitis. The diagnosis was based on pathological findings at ERCP and/or abnormal exocrine pancreatic function test, except in one case in whom a peroperative biopsy of pancreas was obtained, see Table 1. Half of the patients had steatorrhoea (defined as faecal fat exretion >5 g/day). Four patients had previously undergone cholecystectomy and one had gall stones. None of the patients showed evidence of diabetes (defined as fasting blood sugar >6·0 mmol/l), hepatic, or intestinal disease. They were not receiving drugs known to affect lipid metabolism. Informed consent was obtained from each patient.

[24-^{14}C] cholic acid (specific radioactivity, 138 μCi/mg) and [24-^{14}C] chenodeoxycholic acid (specific radioactivity, 138 μCi/mg) were obtained from New England Nuclear Corp, Boston, Mass. 3 Alpha-hydroxysteroid dehydrogenase (Sterognost) and cholesterol oxidase (Nycotest Kolesterol) were purchased from Nyegaard A/S, Oslo, Norway.

EXPERIMENTAL PROCEDURE

The patients were hospitalised during the study period and fed the regular hospital diet containing

Table 1 *Clinical data of the patients*

Patient number	Sex	Age (years)	Body weight		Diagnosis based on			Faecal fat (g/day)	Gall stone disease
			kg	% of ideal*	ERCP	Pancreatic secretion test	Operation		
Without steatorrhoea									
1	M	71	73	85	+	+	−	<5	−
2	M	67	49	70	−	+	−	<5	−
3	M	52	66	94	+	+	−	<5	Cholecystectomy
4	M	50	72	87	+	−	−	<5	Cholecystectomy
5	M	62	72	100	+	+	−	<5	−
6	F	48	40	63	+	−	−	<5	−
7	F	53	62	91	+	+	−	<5	−
8	F	55	57	85	+	+	−	<5	−
Mean±SEM		57±3	61±4	84±4					
With steatorrhoea									
9	M	34	55	89	−	+	+	7·6	−
10	M	54	52	69	−	−	+	5·9	−
11	M	43	64	76	+	+	−	11·5	−
12	M	45	54	72	+	−	−	12	Cholecystectomy
13	M	50	89	93	+	−	−	31	−
14	M	62	80	91	+	+	−	30	Cholecystectomy
15	M	48	51	72	+	+	−	18	−
16	M	68	69	82	−	+	+	5·0	Gall stones
Mean±SEM		51±4	64±5	81±3					

*Calculated as $\frac{\text{body weight (kg)}}{\text{height (cm)} - 100} \times 100\%$.

about 0·5 mmol cholesterol per day. They received orally the sodium salts of (^{14}C) cholic acid (4 μCi) and (^{14}C) chenodeoxycholic acid (4 μCi) dissolved in water in the morning before breakfast. Samples of fasting duodenal bile (about 5 ml) were obtained after intravenous injection of cholecystokinin each morning for four days. The pool size, synthesis and fractional catabolic rate (FCR) of the two primary bile acids were determined. Also, the composition of biliary lipids and the cholesterol saturation of concentrated fasting duodenal bile (obtained after cholecystokinin administration) were determined. The ethical aspects of the study were approved by the Ethical Committee at Huddinge University Hospital.

DETERMINATION OF EXOCRINE PANCREATIC FUNCTION

The exocrine pancreatic function was determined by a meal test according to Lundh[9] modified to include the infusion of a duodenal dilution marker to correct for incomplete sampling of intestinal contents.[10] The corrected mean outputs of amylase, trypsin and lipase during 5×20 minutes after the test meal were expressed as percentages of outputs in healthy controls and the average enzyme output calculated. Faecal fat was determined according to van de Kamer *et al.*[11]

MEASUREMENT OF BILE ACID KINETICS

The duodenal bile samples were hydrolysed with 1 M KOH in closed steel tubes for 12 hours at 110°C. The deconjugated bile acids were extracted with ethyl ether after acidification with 6 M HCl to pH 1. The bile acids were methylated and separated by thin layer chromatography. One aliquot was then quantified with gas liquid chromatography after preparation of the trimethylsilyl ether derivatives. A 1% HiEff BP8 column was used. Another aliquot from this extract was analysed for radioactivity by liquid scintillation counting. On the basis of the specific radioactivity curves, the pool size, daily synthesis and FCR for cholic acid and chenodeoxycholic acid were calculated as described by Lindstedt.[12]

BILIARY LIPID COMPOSITION

For determination of cholesterol and phospholipids, a portion of the bile sample was immediately extracted with 20 vol of chloroform – methanol, 2:1 (vol/vol). Cholesterol was determined by an enzymatic method[13] and phospholipids by the method of Rouser *et al.*[14] The total bile acid concentration in one aliquot of the bile sample was determined using a 3 alpha-hydroxysteroid dehydrogenase assay.[15]

CALCULATION OF CHOLESTEROL SATURATION OF DUODENAL BILE

Cholesterol solubility was calculated as a percentage of the predicted cholesterol solubility at the respective biliary lipid composition, as described by Carey.[16] The total lipid concentration was assumed to

Table 2 *Bile acid kinetics in patients with chronic pancreatitis*

	Cholic acid			*Chenodeoxycholic acid*			*Total bile acid synthesis*	
Patient number	*Pool size mmol*	*Synthesis mmol/day*	*FCR/ day*	*Pool size mmol*	*Synthesis mmol/day*	*FCR/ day*	*mmol/ day*	*μmol/kg bw/day*
Without steatorrhoea								
1	3·74	0·46	0·12	1·60	0·23	0·14	0·69	9·4
2	1·26	0·33	0·26	1·07	0·28	0·26	0·61	12·4
3	1·86	1·29	0·69	1·76	0·86	0·49	2·15	32·6
4	0·50	0·83	1·65	0·41	0·49	1·20	1·32	18·3
5	1·38	1·05	0·76	1·19	0·85	0·71	1·90	26·4
6	0·67	0·53	0·79	0·96	0·50	0·52	1·03	25·8
7	1·16	0·52	0·45	0·53	0·53	1·00	1·05	16·9
8	1·26	0·26	0·21	1·32	0·49	0·37	0·75	13·2
Mean±SEM								
Men (n=5)	1·75±0·54	0·79±0·18	0·70±0·27	1·21±0·24	0·54±0·13	0·56±0·19	1·33±0·31	19·8±4·3
Women (n=3)	1·03±0·18	0·44±0·09	0·48±0·17	0·94±0·23	0·51±0·01	0·63±0·19	0·94±0·10	18·6±3·7
With steatorrhoea								
9	3·48	0·52	0·15	2·20	0·36	0·16	0·88	16·0
10	2·31	0·18	0·08	—	—	—	—	—
11	5·05	0·66	0·13	1·99	0·55	0·28	1·21	18·9
12	0·30	0·19	0·63	1·97	1·13	0·57	1·32	24·4
13	1·39	1·03	0·74	2·52	0·82	0·32	1·85	20·8
14	1·61	0·90	0·56	2·45	0·49	0·20	1·39	17·4
15	1·58	0·47	0·30	3·57	1·17	0·33	1·64	32·2
16	3·20	3·41	1·07	3·10	1·16	0·38	4·57	66·2
Mean±SEM	2·37±0·53	0·92±0·37	0·46±0·12	2·54±0·22	0·81±0·13	0·32±0·05	1·84±0·47	28·0±6·7
Control subjects* (Mean±SEM)								
Men (n=11)	2·37±0·28	0·91±0·12	0·41±0·04	1·94±0·22	0·56±0·06	0·30±0·03	1·46±0·17	18·8±2·1
Women (n=7)	1·89±0·34	0·53±0·08	0·30±0·03	1·86±0·25	0·47±0·05	0·29±0·04	1·00±0·10	16·6±1·6

*Data from reference 18.

be 10 g/dl in concentrated fasting duodenal (gall bladder) bile.

STATISTICAL ANALYSIS

Data are presented as means±SEM. The statistical significance of differences was evaluated by Wilcoxon's rank-sum test.[17]

Results

Bile acid kinetic data are presented in Table 2. Patients with steatorrhoea had normal pool sizes and fractional catabolic rates (FCR) of both cholic acid and chenodeoxycholic acid. Also the synthesis rate of cholic acid was normal. The mean values of chenodeoxycholic acid formation and total bile acid synthesis tended to be higher than corresponding values of healthy controls, but no significant differences were obtained.

Patients without steatorrhoea had normal synthesis rates of both cholic and chenodeoxycholic acid. The mean values of the pool sizes, especially that of chenodeoxycholic acid, tended to be lower than corresponding control values but the differences were not statistically significant. The mean values of FCR for the two bile acids tended to be increased, which probably reflects the fact that two of the patients were cholecystectomised.

Data on biliary lipid composition and cholesterol saturation, obtained from the gall stone free patients (four without and seven with steatorrhoea), are presented in Table 3. As cholesterol saturation of bile increases with age,[18] it was necessary to relate the

Table 3 *Biliary lipid composition and cholesterol saturation in patients with chronic pancreatitis*

Patient number	*Cholesterol molar %*	*Bile acids molar %*	*Phospholipids molar %*	*Cholesterol saturation %*
Without steatorrhoea				
1	6·9	79·3	13·8	131
3	6·4	73·7	19·9	95
5	7·2	79·2	13·6	140
8	7·8	68·8	23·4	103
Mean±SEM	7·1±0·3	75·3±2·5	17·7±2·4	118±11
With steatorrhoea				
9	7·2	71·6	21·2	102
11	2·3	79·5	18·2	38
12	2·6	84·7	12·7	54
13	4·4	67·7	27·9	56
14	4·4	76·6	18·8	69
15	3·3	79·0	17·7	54
16	5·2	73·2	21·6	74
Mean±SEM	4·2±0·6	76·0±2·1	19·7±1·8	64±8
Control subjects* (Mean±SEM)				
Men (n=31)	5·6±0·3	73·9±0·9	20·5±0·7	84±4
Women (n=29)	5·6±0·3	73·1±0·9	21·0±0·7	88±5

*Data from reference 18.

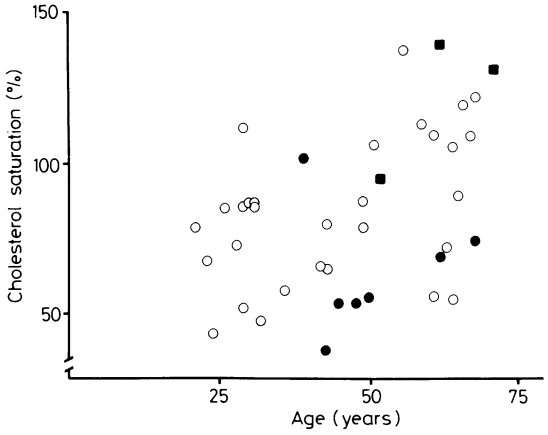

Figure *Relation between age and cholesterol saturation of bile in healthy males (○) (data from ref 18) and male patients with chronic pancreatitis without (■) and with steatorrhoea (●).*

data of our patients to data from controls of corresponding age (Figure). The cholesterol saturation of the patients without steatorrhoea was within the normal limits of the controls of corresponding age. On the other hand, all patients but one with steatorrhoea had cholesterol saturation of bile below normal. The patients with steatorrhoea had significantly lower cholesterol saturation than the male controls aged 30–70 years ($p<0.05$).

The total concentrations of lipids in the duodenal samples of patients without (50·7±14·7 μmol/ml) as well as with steatorrhoea (52·0±15·3 μmol/ml) were not different from those seen in control subjects (men 67·5±9·9 μmol/ml and women 65·2±10·7 μmol/ml).[18]

Discussion

The results of the present study indicate that patients with acquired pancreatic insufficiency have an essentially normal bile acid synthesis. This was true both for patients with and without steatorrhoea. These data are at variance with those of three previous studies,[2-4] in which faecal bile acid excretion was reported to be increased in patients with acquired pancreatic insufficiency and steatorrhoea. The apparent discrepancy between our results and those of the other studies may have several explanations, the most likely of which is the selection of patients. We selected only patients with alcoholic pancreatitis. None of them had evidence of concomitant diabetes mellitus and/or liver disease since it is well known today that such disorders may affect cholesterol and bile acid metabolism.[5-8] In contrast, six of nine patients studied by Pasanen *et al*[3] had diabetes and three of their patients had exocrine pancreatic insufficiency of unknown aetiology. Roller and Kern[2] studied nine patients with acquired pancreatic insufficiency which in only three cases was caused by excessive alcohol intake. Although Dutta *et al*[4] stated that there was no difference between insulin treated and non-insulin treated patients in their study, it is not clear how many of their subjects actually had diabetes (defined as raised blood sugar).

Regan *et al*[1] have previously shown that patients with severe pancreatic insufficiency, caused by alcohol abuse, have normal postprandial bile acid secretion rates but reduced micellar concentrations of bile acids in duodenum. Treatment with cimetidine, which reduces both gastric acid and volume outputs, increased total bile acid and micellar bile acid concentrations and also improved micellar solubilisation of dietary lipids. These results were interpreted by the authors to indicate that bile acids precipitate in the abnormally acidic duodenal contents often seen in chronic pancreatitis because of reduced pancreatic secretion of bicarbonate. If so, it is possible that precipitation of bile acids in the upper small intestine may cause malabsorption of chenodeoxycholic acid, which is partly reabsorbed in the proximal small intestine by passive diffusion.[19] This may explain the slightly increased formation of chenodeoxycholic acid found in some of our patients. Using an enzymatic assay for faecal bile acids, Dutta *et al* reported high excretion rates in their patients, with a reduction of steatorrhoea but not of bile acid malabsorption during cimetidine therapy.[4]

To our knowledge, this is the first report on the lipid composition and cholesterol saturation of bile in chronic pancreatitis. A major finding was that patients with pancreatic insufficiency and steatorrhoea have subnormal cholesterol saturation. In fact, all but one of our patients with steatorrhoea had unsaturated bile, well below the range seen in the healthy controls. A low relative concentration of biliary cholesterol may be caused by a decreased secretion rate of cholesterol and/or increased secretion rates of bile acids and/or phospholipids.[18] Biliary lipid secretion rates were not determined in the present study. As discussed above, patients with advanced chronic pancreatitis probably have normal secretion rates of bile acids. It is therefore likely that the low cholesterol content of bile observed in our patients was the result of decreased cholesterol secretion. This may be the consequence of a reduced cholesterol synthesis or be related to a decreased absorption of cholesterol. As only patients with chronic pancreatitis and concomitant fat malabsorption had low cholesterol saturation of bile, it is likely that these patients also had malabsorption of cholesterol. Some experimental evidence for

cholesterol malabsorption in patients with exocrine pancreatic insufficiency has previously been given by Pasanen *et al*.[3]

In conclusion, this study has shown that chronic pancreatitis is associated with essentially normal bile acid metabolism. Patients with severe exocrine pancreatic insufficiency (fat malabsorption) display reduced cholesterol saturation of bile, probably because of decreased secretion of cholesterol.

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