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Serum bile acids in patients with hyperlipidaemia

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SUMMARY Individual serum bile acids were analysed by an improved gas liquid chromatography method in 12 patients with primary hyperlipidaemia. Total serum bile acid concentrations were raised in 10 subjects. Ursodeoxycholic acid was found in all 12 patients. It was present in significantly greater concentrations, accounted for a greater proportion of the total serum bile acids, and occurred more frequently than in patients with various forms of hepatobiliary disease. Patients with hyperlipidaemia had proportionately less deoxycholic acid than controls but more than patients with liver disease. There was proportionately less chenodeoxycholic acid in patients with hypercholesterolaemia, in whom the primary bile acid ratio was raised.

That an increased concentration of total serum bile acids accompanies hepatobiliary disease has been known for a long time (Sherlock and Walshe, 1948; Rudman and Kendall, 1957; Makino *et al.*, 1969) and their measurement has been advocated in the detection of hepatobiliary disease (Kaplowitz *et al.*, 1973; Barnes *et al.*, 1975; Fausa and Gjone, 1976). We have confirmed the sensitivity of total serum bile acid estimation as an indicator of hepatobiliary disease and provided evidence that the cholic:chenodeoxycholic acid ratio may be useful in clinical diagnosis (Pennington *et al.*, 1977).

Implicit in these studies is the assumption that serum bile acids have the advantage of specificity over conventional liver function tests. It is possible, however, that factors other than liver dysfunction might influence serum bile acid concentrations. Einarsson *et al.* (1974) reported changes in bile acid synthesis in patients with hyperlipidaemia. We report here a study of the serum bile acids in patients with hyperlipidaemia.

Patients and methods

Details of the patients studied are given in Table 1. The control subjects were not receiving drug therapy and had no symptoms, signs, or history of liver disease. They had normal serum lipids and conventional liver function tests. All gave informed consent to the study. The patients with primary hyperlipidaemia consisted of eight subjects with type IIa and four subjects with type IV hyperlipidaemia. Patients with type IIa and type IV disorder had serum cholesterols of > 7·9 mmol/l and < 7·9 mmol/l, and triglycerides of < 2·5 mmol/l and > 2·5 mmol/l, respectively. These subjects also had no clinical features of liver disease, normal liver function tests, and negative cholecystograms. They were studied before treatment was started. The diagnoses in patients with hepatobiliary disorders were established by liver histology or laparotomy with the exception of patients with infectious mononucleosis and some patients with viral hepatitis, in whom the diagnosis was made on clinical and serological grounds.

Table 1 *The patients studied*

Group	*Diagnosis*	*Number*
Control	Normal subjects	14
Cirrhosis	Chronic active hepatitis	5
	Primary biliary cirrhosis	1
	Alcoholic	10
	Haemochromatosis	1
Common bile duct obstruction	Gallstone	8
	Carcinoma of pancreas	6
	Carcinoma of ampulla	1
Viral hepatitis	Type A	12
	Type B	6
Infectious mononucleosis	Infectious mononucleosis	7
Neoplasia	Carcinoma of bronchus	3
	Miscellaneous secondary carcinomas	5
Hyperlipidaemia	Hypercholesterolaemia	8
	Hypertriglyceridaemia	4

After a 12-hour fast 2 ml of blood was withdrawn. Serum was obtained from 10 ml of blood and frozen until analysed for serum bile acids by a sensitive gas liquid chromatography method (Ross *et al.*, 1977).

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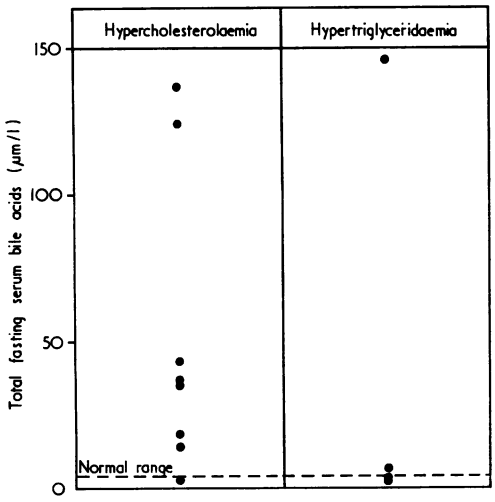

Fig. 1 *Total serum bile acids in hyperlipidaemic patients (normal range < 4·5 μmol/l).*

The remainder of the blood was used for liver function tests and lipid measurement. The liver function tests included bilirubin, alkaline phosphatase (Vickers M300 multichannel analyser), aspartate aminotransferase, and gamma glutamyl transpeptidase (Boehringer automated kits, LKB reaction-rate analyser). Serum lipids were analysed on the Technicon analyser using the methods described by Kessler and Lederer (1965) for cholesterol and by Rush *et al.* (1970) for triglycerides.

The significance of differences in the frequency of detection of ursodeoxycholic acid in different diagnostic groups was established by χ^2 test. The Student *t* test was used to compare differences in the amount and percentage composition of ursodeoxycholic acid.

Results

The serum bile acid values in most of the patients with hepatobiliary disease have been given elsewhere (Pennington *et al.*, 1977).

TOTAL SERUM BILE ACIDS

Total serum bile acids were found to be moderately raised in most patients with hyperlipidaemia, as shown in Figure 1. The mean value (47·8 μmol/l) was similar to that previously found in patients with cirrhosis (37·5 μmol/l) and neoplasia (44·2 μmol/l). The upper limit of normal in our laboratory is 4·5 μmol/l.

Table 2 *Detection of ursodeoxycholic acid and lithocholic acid in the fasting serum of control subjects and patients with hepatobiliary disease and hyperlipidaemia*

Diagnostic group	*No. of patients*		
	Total	*Ursodeoxycholic acid detected*	*Lithocholic acid detected*
Controls	14	2	5
Cirrhosis	17	10	7
Common bile duct obstruction	15	5	9
Viral hepatitis	18	7	8
Infectious mononucleosis	7	1	1
Neoplasia	8	4	4
Hyperlipidaemia	12	12	1

INDIVIDUAL BILE ACIDS

Ursodeoxycholic acid

Ursodeoxycholic acid was detected in all 12 hyperlipidaemic patients and was found significantly more frequently ($\chi^2 = 25·4$, $P < 0·0005$) in patients with hyperlipidaemia than in other diagnostic groups, as shown in Table 2. The ursodeoxycholic acid could be quantified in 35 of the 39 patients in whom it was detected. The total amount of ursodeoxycholic acid found and the ursodeoxycholic acid expressed as a percentage of the total bile acids, as well as the ursodeoxycholic:chenodeoxycholic acid ratios are shown in Table 3. Significantly more ursodeoxycholic acid was found in hyperlipidaemic patients than in the cirrhotics ($P < 0·025$) and other patients ($P < 0·01$). Furthermore, the percentage of total bile acids found as ursodeoxycholic acid was very much greater ($P < 0·001$) in patients with hyperlipidaemia than in the cirrhotic and other patients.

Other secondary bile acids

The percentage composition of deoxycholic acid was significantly smaller ($P < 0·005$) than in the control subjects but greater than in other patients with hepatobiliary disease, as shown in Figure 2. No differences were observed in the frequency or quantity of lithocholic acid between each group (Table 2).

Primary bile acids

Cholic acid predominated in all eight hypercholesterolaemic patients, and chenodeoxycholic acid predominated in three out of four patients with hypertriglyceridaemia. The proportions of cholic acid and chenodeoxycholic acid expressed as a percentage of the total serum bile acids in the hypercholesterolaemic patients are shown in Figure 3. A significantly smaller percentage of chenodeoxycholic acid was observed than in all other groups.

Table 3 *Ursodeoxycholic acid (μmol/l), percentage of total serum bile acids, and ursodeoxycholic:chenodeoxycholic acid ratio in different patient groups*

Diagnostic group	*Total ursodeoxycholic acid (μmol/l)*	*% of ursodeoxycholic acid*	*Ursodeoxycholic : chenodeoxycholic acid ratio*
Hyperlipidaemia	0·85	29	1·05
	2·81	21	1·06
	7·25	21	1·03
	5·42	31	2·18
	25·9	21	1·46
	8·89	24	1·62
	11·9	9	0·32
	1·26	33	0·96
	1·15	27	0·74
	28·8	20	1·15
Cirrhosis	1·81	3	0·03
	0·13	1	0·11
	0·38	2	0·03
	0·36	10	0·16
	0·7	8	0·15
	0·82	1	0·02
	1·35	6	0·12
	0·56	3	0·06
	0·74	3	0·03
Common bile duct obstruction	5·5	6	0·12
	4·22	2	0·05
	0·73	1	0·03
	0·61	1	0·03
Viral hepatitis	2·6	2	0·05
	3·27	1	0·01
	2·81	1	0·04
	1·28	1	0·01
	2·29	2	0·02
	0·66	1	0·01
	1·4	1	0·01
Infectious mononucleosis	0·02	1	0·01
	0·53	2	0·01
	2·21	5	0·1
	0·46	3	0·05
	2·87	4	0·05

BILE ACID RATIOS

Ursodeoxycholic: chenodeoxycholic acid ratio

The hyperlipidaemic patients fell into two groups: eight, in whom cholic acid was the predominant primary bile acid, had ratios above 1 (1·03-2·18); the other two, in whom chenodeoxycholic acid predominated, had values of 0·32, 0·74, and 0·96. The values in hyperlipidaemic patients (1·16 ± 0·51) differed significantly ($P < 0·001$) from those in patients with cirrhosis (0·079 ± 0·056) and in the other patients from whom ursodeoxycholic acid was detected (0·037 ± 0·03). No overlap was observed.

Cholic: chenodeoxycholic acid ratio

This exceeded 1 in all patients with hypercholesterolaemia and in one patient with hypertriglyceridaemia. Values ranged from 1·25 to 3·7. In the remaining patients the values were 0·38, 0·56, and 0·59 (normal range 0·5-1·0).

Discussion

The relatively large amount of ursodeoxycholic acid in the sera of patients with hyperlipidaemia was an unexpected finding. Ursodeoxycholic acid is considered to be formed from the hepatic epimerisation of 7 ketolithocholic acid that has been produced in the gut by bacterial dehydrogenation of chenodeoxycholic acid (Hofmann, 1977). Serum ursodeoxycholic acid might, therefore, be expected to increase in the presence of an unusual bowel flora which has been responsible for the increased conversion of chenodeoxycholic acid to 7 ketolithocholic acid. In such an event, however, the serum ursodeoxycholic acid: chenodeoxycholic acid ratio should be less than unity, unless either all the 7 ketolithocholic acid was converted to ursodeoxycholic acid in the liver, instead of being randomly epimerised to chenodeoxycholic acid or 7 ketolithocholic acid, or the cycling frequency of ursodeoxycholic acid was very much greater than that of chenodeoxycholic acid. Neither of these two possibilities seems likely, particularly as chenodeoxycholic acid is known to undergo rapid and early absorption (Angelin *et al.*, 1976). The alternative possibilities which need to be considered are that in hyperlipidaemic subjects ursodeoxycholic acid is synthesised either directly in the liver or possibly in the bowel.

Cholic acid synthesis has been reported previously as being reduced in patients with hypercholesterolaemia with compensatory increase of chenodeoxycholic acid (Einarsson *et al.*, 1974). Our observation of a high serum cholic:chenodeoxycholic acid ratio is, therefore, also unexpected. A reduced bile acid recycling frequency and the failure to absorb chenodeoxycholic acid appear unlikely explanations, especially as the total serum bile acid values are normally determined by intestinal absorption (LaRusso *et al.*, 1974). It is possible that the abnormal primary bile acid ratios and high total serum bile acids result from binding of bile acids to the lipoproteins. However, no correlation was observed between bile acids, cholesterol, and triglycerides. An alternative explanation is that the patients had liver disease because of the known association between alcohol consumption and blood lipid abnormalities. This seems unlikely in our patients because all had normal liver function tests. We have previously shown that the total serum bile acid estimation in fasting serum is equal to but not better than the AST and γGTP estimations in the detection of liver disease. Furthermore, cirrhotic patients all had low

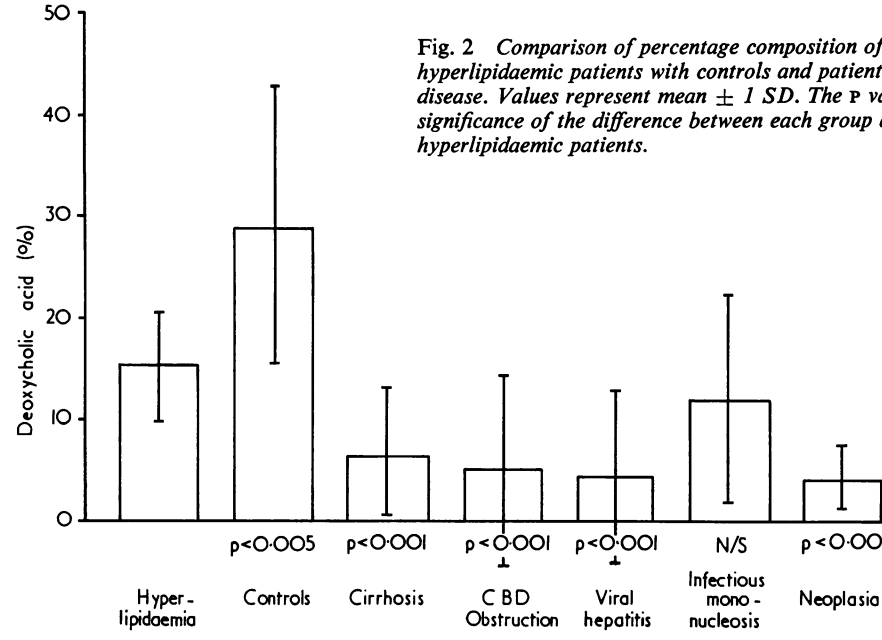

Fig. 2 *Comparison of percentage composition of deoxycholic acid in hyperlipidaemic patients with controls and patients with hepatobiliary disease. Values represent mean ± 1 SD. The* P *value represents the significance of the difference between each group and the hyperlipidaemic patients.*

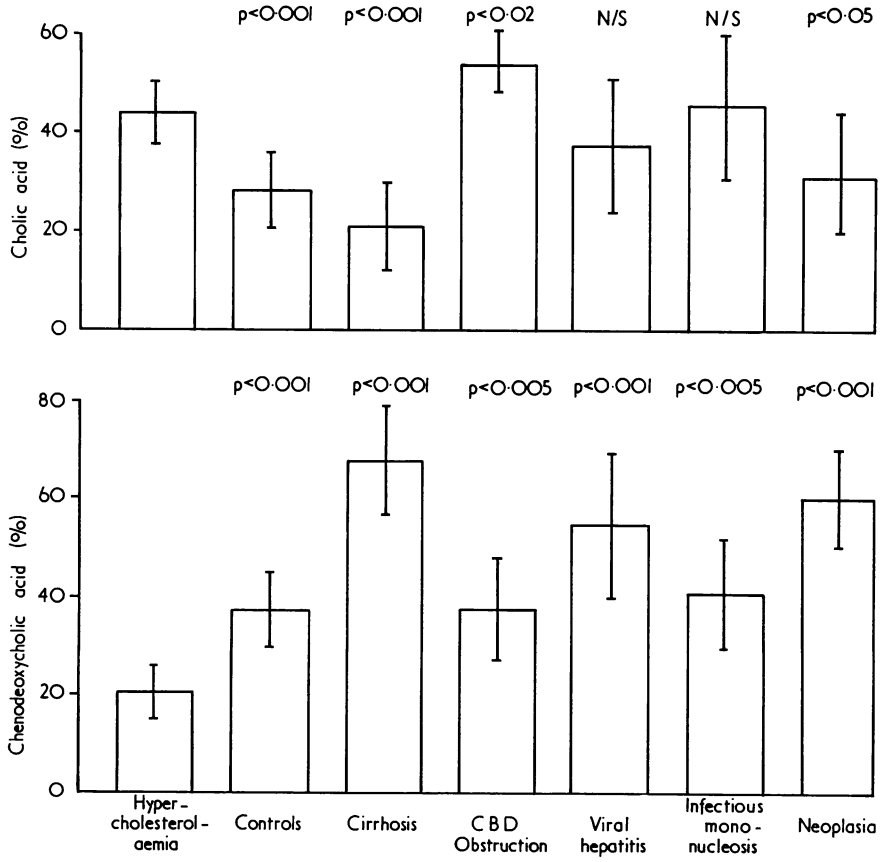

Fig. 3 *Comparison of percentage composition of cholic acid and chenodeoxycholic acid in hypercholesterolaemic patients with controls and patients with hepatobiliary disease. The values represent mean ± 1 SD. The* P *values represent the significance of the difference between each group and the hypercholesterolaemic patients.*

primary bile acid ratios < 0·5 (Pennington *et al.*, 1977).

In conclusion, our study showed that most patients with hyperlipidaemia had raised serum bile acid concentrations. Ursodeoxycholic acid was detected more frequently and accounted for a greater proportion of the total bile acids than in patients with hepatic disease. Patients with hypercholesterolaemia had raised cholic:chenodeoxycholic acid ratios. These observations have not been described previously. The cause and significance of these findings remain to be established.

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References

Angelin, B., Einarsson, K., and Hellström, K. (1976). Evidence for the absorption of bile acids in the proximal small intestine of normo- and hyperlipidaemic subjects. *Gut*, **17**, 420-425.

Barnes, S., Gallo, G. A., Trash, D. B., and Morris, J. S. (1975). Diagnostic value of serum bile acid estimations in liver disease. *Journal of Clinical Pathology*, **28**, 506-509.

Einarsson, K., Hellström, K., and Kallner, M. (1974). Bile acid kinetics in relation to sex, serum lipids, body weights, and gallbladder disease in patients with various types of hyperlipoproteinemia. *Journal of Clinical Investigation*, **54**, 1301-1311.

Fausa, O., and Gjone, E. (1976). Serum bile acid concentrations in patients with liver disease. *Scandinavian Journal of Gastroenterology*, **11**, 537-543.

Hofmann, A. F. (1977). The enterohepatic circulation of bile acids in man. *Clinics in Gastroenterology*, **6**, 3-24.

Kaplowitz, N., Kok, E., and Javitt, N. B. (1973). Postprandial serum bile acid for the detection of hepatobiliary disease. *Journal of the American Medical Association*, **225**, 292-293.

Kessler, G., and Lederer, H. (1965). In *Automation in Analytical Chemistry* (Technicon), pp. 345-347. Mediad, New York.

LaRusso, N. F., Korman, M. G., Hoffman, N. E., and Hofmann, A. F. (1974). Dynamics of the enterohepatic circulation of bile acids. *New England Journal of Medicine*, **291**, 689-692.

Makino, I., Nakagawa, S., and Mashimo, K. (1969). Conjugated and unconjugated serum bile acid levels in patients with hepatobiliary diseases. *Gastroenterology*, **56**, 1033-1039.

Pennington, C. R., Ross, P. E., and Bouchier, I. A. D. (1977). Serum bile acids in hepatobiliary disease. *Gut* (In press).

Ross, P. E., Pennington, C. R., and Bouchier, I. A. D. (1977). A GLC method for serum bile acid analysis. *Analytical Biochemistry* **80**, 458-465.

Rudman, D., and Kendall, F. E. (1957). Bile acid content of human serum. 1. Serum bile acids in patients with hepatic disease. *Journal of Clinical Investigation*, **36**, 530-537.

Rush, R. L., Leon, L., and Turrell, J. (1970). In *Advances in Automated Analysis* (Technicon), pp. 503-507. Mediad, New York.

Sherlock, S., and Walshe, V. (1948). Blood cholates in normal subjects and in liver disease. *Clinical Science*, **6**, 223-234.