Concentrations of cholestenoic acids in plasma from patients with reduced intestinal reabsorption of bile acids

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Abstract The concentrations of 3β-hydroxy-5-cholestenoic acid, 3β,7α-dihydroxy-5-cholestenoic acid, and 7α-hydroxy-3-oxo-4-cholestenoic acid were determined in plasma from patients treated with cholestyramine or subjected to resection of the ileum or colon. The values were compared with those for conjugated and unconjugated C_{24} bile acids. Patients with an intact ileum but without colon had normal levels of cholestenoic acids. Patients treated with cholestyramine or with ileal resection had elevated levels of 7α-hydroxy-3-oxo-4-cholestenoic acid (median values 189 and 233 ng/ml, respectively, compared to 85 ng/ml in controls). The levels of the other two C_{27} acids were normal in cholestyramine-treated and low in ileoresected patients and were positively correlated to each other but not to the 3-oxo-Δ^4 acid. There were no consistent correlations between the levels of C_{27} acids and those of conjugated or unconjugated C_{24} bile acids. The results indicate an increased formation of 7α-hydroxy-3-oxo-4-cholestenoic acid in subjects having a stimulated activity of cholesterol 7α-hydroxylase.—**Axelson, M., B. Mörk, A. Aly, G. Walldius, and J. Sjövall.** Concentrations of cholestenoic acids in plasma from patients with reduced intestinal absorption of bile acids. *J. Lipid Res.* 1989. **30:** 1883–1887.

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In a preceding study, the levels of 3β-hydroxy-5-cholestenoic, 3β,7α-dihydroxy-5-cholestenoic, and 7α-hydroxy-3-oxo-4-cholestenoic acids in plasma of patients with different liver diseases were reported (1). The concentrations were similar in patients with extrahepatic cholestasis and control subjects, indicating a difference in the production or hepatic handling of C_{27} and C_{24} bile acids. However, elevated levels, particularly of 7α-hydroxy-3-oxo-4-cholestenoic acid, were found in liver cirrhosis and there was a significant correlation between these levels and those of conjugated bile acids. This was suggested to result from a reduced hepatic clearance and to indicate that the liver plays an important role for the elimination or metabolism of the C_{27} acids in blood. In order to gain more information about the relationships between bile acid biosynthesis and the formation of the cholestenoic acids, we have now determined the levels of these acids in patients who have an apparently healthy liver but an impaired bile acid absorption due to treatment with cholestyramine or intestinal resection. The results provide indirect evidence for an involvement of the liver in the biosynthesis of the cholestenoic acids.

MATERIALS AND METHODS

Subjects and samples

Patients. Blood was obtained from patients under treatment with cholestyramine or with intestinal diseases. After centrifugation, plasma/serum was separated and stored at −20°C until analyzed. The patient groups selected were: A. Eight women and one man, 39–70 years old (median 63), with hypercholesterolemia, 7.5–11.9 mmol/l, and treated with lipid-lowering diet and with 8 g of cholestyramine (Questran^R, Bristol) twice daily for 6–8 weeks prior to sampling (2). B. Five women and six men, 30–59 years old (median 40), with Crohn's disease subjected to resection of terminal ileum (>60 cm) with or without colectomy. C. Three women and three men, 28–50 years old (median 37), subjected to a complete colectomy due to polyposis of the colon. The operations on patients in groups B and C were performed more than 2 years before this investigation.

Control subjects were those described in the preceding report (1).

Analytical procedures

Chemicals, columns, reference compounds, and the analytical methods were the same as described previously (1, 3). The statistical evaluation of data included calcula-

tion of Spearman's rank correlation coefficient and Kolmogorov-Smirnov two-sample test (4). The median and interquartile range were used as measures of central tendency and variation, respectively.

RESULTS

Concentrations of C_{27} acids in plasma

The concentrations of the three cholestenoic acids and the major unconjugated and conjugated C_{24} bile acids were determined in plasma from patients treated with cholestyramine or subjected to ileal resection. Six patients in whom the colon had been removed were also studied. Values for control subjects were from the preceding study (1).

The results of the analyses are shown in **Fig. 1** and **Table 1**. As was the case in controls and patients with liver diseases, levels of 7α-hydroxy-3-oxo-4-cholestenoic acid in plasma usually exceeded those of 3β-hydroxy-5-cholestenoic and 3β,7α-dihydroxy-5-cholestenoic acids. Patients with an intact ileum but without colon had levels of C_{27} acids similar to those of the control group. In patients treated with cholestyramine or lacking the distal ileum, the most striking feature was an elevation of 7α-hydroxy-3-oxo-4-cholestenoic acid. In contrast to the results in patients with liver cirrhosis, this increase was not accompanied by an increase of 3β-hydroxy-5-cholestenoic or

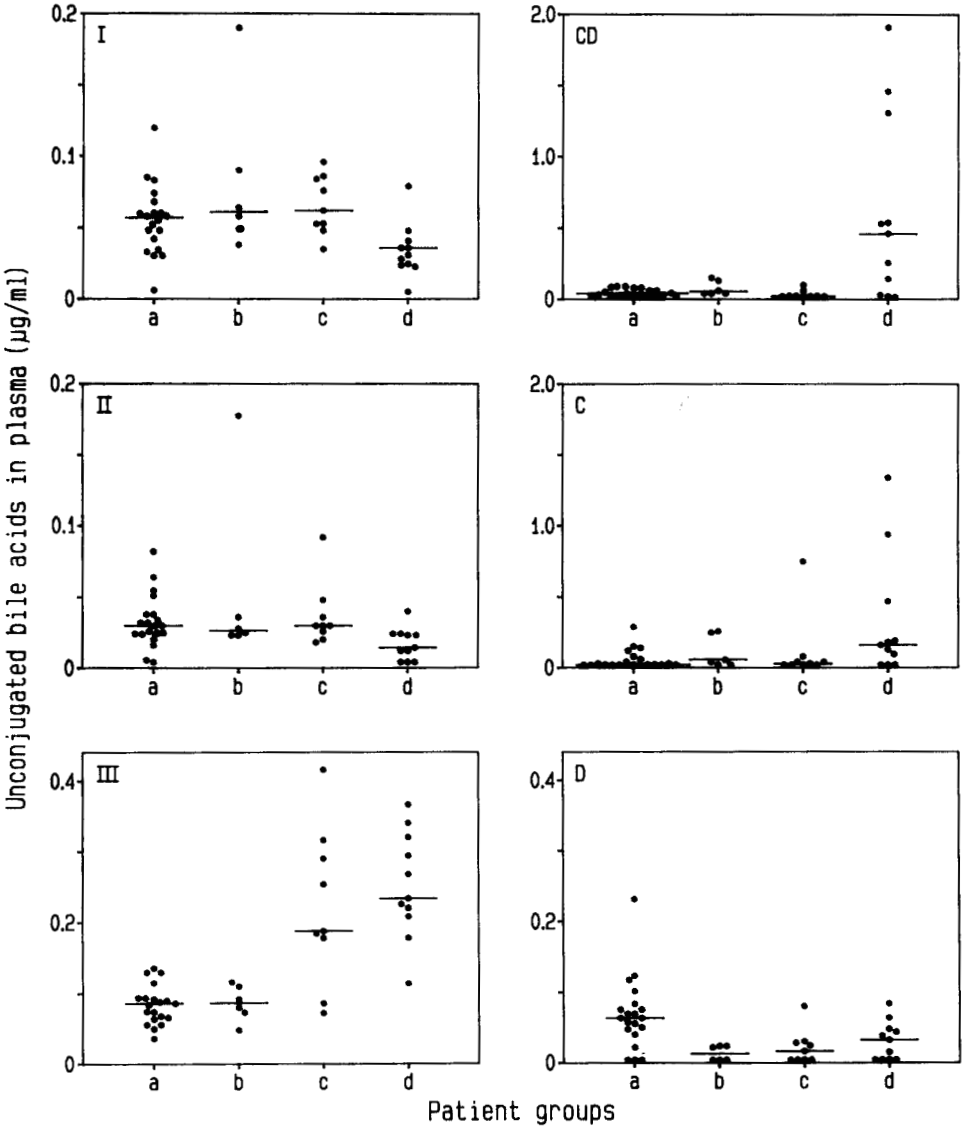

Fig. 1. Concentrations of unconjugated 3β-hydroxy-5-cholestenoic acid (I), 3β,7α-dihydroxy-5-cholestenoic acid (II), 7α-hydroxy-3-oxo-4-cholestenoic acid (III), chenodeoxycholic (CD), cholic (C), and deoxycholic (D) acids in plasma of healthy subjects (a), colectomized patients (b), patients treated with cholestyramine (c), and patients with ileal resection (d). Median values are indicated by horizontal lines.

TABLE 1. Concentrations of C_{27} and C_{24} bile acids in plasma/serum of healthy subjects, colectomized patients, patients treated with cholestyramine, and patients with ileal resection[a]

Bile Acids	Subjects[a]			
	Healthy	Colectomized	Cholestyramine-Treated	Ileal Resection
			ng/ml[b]	
Unconjugated				
3β-Hydroxy-5-cholestenoic acid	57:39-64	61:49-90	62:52-84	31:24-42
3β,7α-Dihydroxy-5-cholestenoic acid	30:25-38	27:24-36	30:26-36	14:< 10-24
7α-Hydroxy-3-oxo-4-cholestenoic acid	85:67-93	87:75-105	189:180-291	233:210-320
Chenodeoxycholic acid	34:16-73	54:34-130	17:13-23	476:23-1320
Cholic acid	25:15-70	42:19-248	28:18-39	170:13-469
Deoxycholic acid	66:46-79	16:< 10-25	17:< 10-29	33:< 10-49
Conjugated[c]				
Chenodeoxycholic acid	ND[d]	302:201-402	61:33-108	255:154-502
Cholic acid	ND	194:108-212	165:129-196	219:100-457
Deoxycholic acid	ND	< 20:< 20-< 20	< 20:< 20-< 20	< 20:< 20-< 20

[a]Details on patients are given in Materials and Methods.
[b]Concentration expressed as median: lower quartile-upper quartile.
[c]Expressed as ng unconjugated acid.
[d]ND, not determined.

3β,7α-dihydroxy-5-cholestenoic acids. The levels of the latter two acids were normal in cholestyramine-treated patients and significantly decreased ($P < 0.05$ and 0.005, respectively) in patients with ileal resection. A 2-year-old boy with ileal resection and liver cirrhosis showed a pattern of C_{27} acids similar to that seen after ileal resection alone. The positive correlation ($r = 0.82$, $P < 0.0001$) between the levels of 3β-hydroxy-5-cholestenoic and 3β,7α-dihydroxy-5-cholestenoic acids in all subjects is illustrated in **Fig. 2**. The same correlation was observed in patients with liver disease. In contrast, the levels of 3β,7α-dihydroxy-5-cholestenoic and 7α-hydroxy-3-oxo-4-cholestenoic acids were not correlated to each other in patients with reduced bile acid absorption (Fig. 2). These acids were positively correlated in controls and patients with liver disease (1).

Concentrations of C_{24} acids in plasma

Unconjugated C_{24} bile acids were low or normal in patients treated with cholestyramine, probably due to a decreased reabsorption of these acids. In contrast, patients lacking the distal ileum had elevated levels of both cholic and chenodeoxycholic acid in blood. This could be due to increased exposure to deconjugating bacteria or to changes in the routes of bile acid absorption after surgical treatment whereby a larger proportion of unconjugated bile acids might reach the systemic circulation. Low levels of deoxycholic acid indicated a decreased activity of 7α-dehydroxylating bacteria. The concentrations of unconjugated C_{24} and C_{27} acids were not correlated to each other in any group of patients or controls.

The levels of conjugated cholic and chenodeoxycholic acids were usually normal in patients with resection of the intestine, although a few of the patients with ileal resection showed moderate increases. As suggested for unconjugated C_{24} acids, this may be due to shunting of absorbed bile acids past the liver following surgery. In cholestyramine-treated patients, the levels of chenodeoxycholic acid appeared to be low. This might be related to the higher affinity of the resin for dihydroxy than for trihydroxy bile acids (5). In all patient groups, the levels of conjugated deoxycholic acid were low, consistent with the low levels of the unconjugated compound. There was no correlation between the levels of conjugated bile acids and those of the cholestenoic acids in patients treated with cholestyramine or without colon. However, there was a weak positive correlation ($r = 0.65$, $P < 0.05$) in ileoresected patients. The stronger positive correlation previously found in patients with liver cirrhosis was suggested to result from a decreased hepatic uptake of both C_{24} and C_{27} acids. A similar liver dysfunction would not be expected in patients with intestinal resections.

DISCUSSION

Structurally, the three cholestenoic acids determined in this study are potential intermediates or metabolites of intermediates in biosynthetic pathways between cholesterol

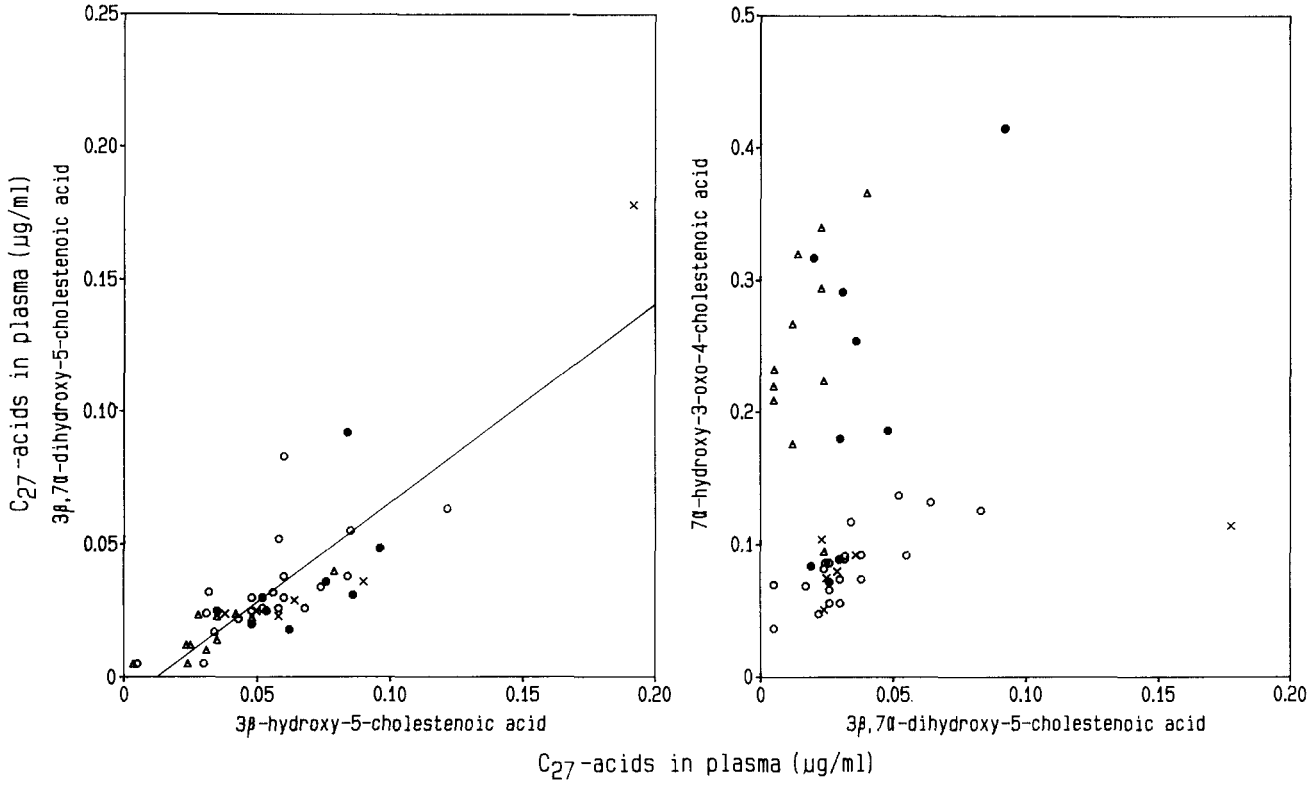

Fig. 2. Relationships between levels of 3β-hydroxy-5-cholestenoic and 3β,7α-dihydroxy-5-cholestenoic acids (left panel) and between levels of 3β,7α-dihydroxy-5-cholestenoic and 7α-hydroxy-3-oxo-4-cholestenoic acids (right panel) in plasma of control subjects (○), patients treated with cholestyramine (●), patients with colectomy (x), and patients with ileal resection (△).

and bile acids (cf. 6). The preceding study of patients with liver diseases provided evidence for the importance of the liver in their elimination (1). Since the biliary excretion of these acids is minimal or absent (3, 7), the elimination is likely to be due to metabolic conversion. However, results relating the formation of the C_{27} acids to the biosynthesis of C_{24} bile acids were not obtained. The levels of C_{27} acids in plasma were normal in patients with extrahepatic cholestasis who would be expected to have a lowered activity of cholesterol 7α-hydroxylase (8).

In the present study, groups of patients were selected which were expected to have an increased activity of cholesterol 7α-hydroxylase due to interference with the enterohepatic circulation and increased loss of bile acids (6). The most striking feature in these patients was an increase of the level of 7α-hydroxy-3-oxo-4-cholestenoic acid. This could be due to an increased production or a decreased elimination of this acid. In patients with liver cirrhosis, expected to have a defective hepatic clearance of bile acids, the elevation of 7α-hydroxy-3-oxo-4-cholestenoic acid was usually accompanied by an increase of the other two C_{27} acids (1). In contrast, the patients with a stimulated bile acid turnover showed normal or low levels of 3β-hydroxy-5-cholestenoic and 3β, 7α-dihydroxy-5-cholestenoic acids, and there was no correlation between the concentrations of these acids and that of 7α-hydroxy-3-oxo-4-cholestenoic acid. If a decreased hepatic clearance causes the elevated levels of C_{27} acids in patients with liver cirrhosis, this is unlikely to be the reason for the selective increase of 7α-hydroxy-3-oxo-4-cholestenoic acid in patients treated with cholestyramine or lacking the terminal ileum. This increase is more likely related to an increased synthesis of bile acids and it is of interest that it was not observed in patients with an intact ileum but lacking the colon.

While correlations between levels in plasma do not prove metabolic relationships, the positive correlations between the levels of 3β,7α-dihydroxy-5-cholestenoic and 7α-hydroxy-3-oxo-4-cholestenoic acids in healthy subjects and patients with liver diseases is compatible with a formation of the 3-oxo-Δ^4 from the 3β-hydroxy-Δ^5 acid catalyzed by a 3β-hydroxy-Δ^5-steroid oxidoreductase/isomerase. The complete lack of correlation between the levels of these acids in patients with an enhanced bile acid synthesis might indicate that 7α-hydroxy-3-oxo-4-cholestenoic acid is formed from another precursor in these patients. Alternatively, its rate of formation, catalyzed by 3β-hydroxy-Δ^5-steroid dehydrogenase may be increased relative to the rate of metabolism, e.g., by 5β-reduction or 12α-hydroxylation.

In conclusion, this study has shown that the levels in plasma of 7α-hydroxy-3-oxo-4-cholestenoic acid are elevated in patients with a reduced reabsorption and an ex-

pected increase in the synthesis of bile acids. This indicates a relationship between the formation of this C_{27} acid and the common C_{24} bile acids. Further studies are required to elucidate the nature of this relationship, particularly with respect to the possible formation of 7α-hydroxy-3-oxo-4-cholestenoic acid via multiple pathways.

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