

Concentrations of cholestenic acids in plasma from patients with liver disease

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Abstract The concentrations of 3β -hydroxy-5-cholestenic acid, $3\beta,7\alpha$ -dihydroxy-5-cholestenic acid, and 7α -hydroxy-3-oxo-4-cholestenic acid were determined in plasma from patients with different liver diseases and compared with those of unconjugated and conjugated C_{24} bile acids. The levels of the cholestenic acids were similar in patients with extrahepatic cholestasis and in controls (median concentration 153 and 162 ng/ml, respectively), whereas significantly elevated levels were found in plasma from patients with primary biliary cirrhosis (median concentration 298 ng/ml) and alcoholic liver cirrhosis (median concentration 262 ng/ml). As expected, conjugated C_{24} bile acids were elevated in most patients whereas the corresponding unconjugated compounds were low in cholestasis and elevated in alcoholic liver cirrhosis. The levels of the individual C_{27} acids were usually positively correlated to each other and also to the levels of conjugated C_{24} bile acids in plasma from patients with liver cirrhosis. In contrast, there was no correlation between the levels of C_{27} acids and conjugated bile acids in patients with extrahepatic cholestasis. The levels of unconjugated C_{24} bile acids were not correlated to C_{27} acids or conjugated bile acids in any of the groups. ■ The results indicate that there is a close metabolic relationship between the individual C_{27} acids, that they do not participate in an enterohepatic circulation, and that the liver is important for their elimination/metabolism. —Axelson, M., B. Mörk, A. Aly, O. Wisén, and J. Sjövall. Concentrations of cholestenic acids in plasma from patients with liver disease. *J. Lipid Res.* 1989. 30: 1877–1882.

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Cholesterol is the precursor of many biologically important steroids (1). Recently, we reported the occurrence of three cholestenic acids, 3β -hydroxy-5-cholestenic acid, $3\beta,7\alpha$ -dihydroxy-5-cholestenic acid, and 7α -hydroxy-3-oxo-4-cholestenic acid as normal constituents in human blood (2). The origin and biological role of these compounds are not yet known. Their structures indicate that they may be intermediates or side products formed in the biosynthesis of bile acids. In an attempt to obtain more information about the possible relationship between the C_{27} acids and bile acid production, we have now determined the concentrations of C_{27} acids in plasma from patients with liver diseases known to be associated with

abnormal bile acid production and metabolism. The results are described in this report.

MATERIALS AND METHODS

Subjects and samples

Patients. Blood was obtained from patients with different types of liver diseases. The following groups of patients were selected: Group A: Three men and eight women 49–87 years old (median 68), with large bile duct obstruction secondary to carcinoma or gallstones. Group B: Four men and eight women, 40–75 years old (median 62), with primary biliary cirrhosis. Group C: Nine men and three women, 51–75 years old (median 57), with moderate or severe forms of alcoholic liver cirrhosis. The diagnosis of patients in groups B and C was made on the basis of full clinical and chemical investigation including liver biopsy in most cases.

Control subjects. Blood from 20 apparently healthy men and women, 21–48 years old (median 37) was collected in tubes with or without heparin. After centrifugation, plasma/serum was separated and stored at -20°C until analyzed. All subjects were not fasting, but most samples were collected in the morning.

Analytical procedure

Chemicals, column packing materials, and reference compounds were the same as those used in previous studies (2). Details of the analytical procedure have also been described. Briefly, plasma/serum (2 ml) was diluted with one volume of 0.5 M aqueous triethylamine sulfate, pH 7, followed by extraction on a small column of octadecylsilane-bonded silica at 64°C . After washing the sorbent with water and 10% aqueous methanol, bile acids were

Abbreviations: GLC, gas-liquid chromatography; TMS, trimethylsilyl.

eluted with 95% aqueous methanol. An aliquot of this extract (1/5) was enzymatically hydrolyzed (3) for determination of total bile acids. The remaining extract was passed through a column (6 × 0.4 cm) of the lipophilic anion exchanger, triethylaminohydroxypropyl-Sephadex LH-20 (TEAP-LH-20) in bicarbonate form. After washing with 95% aqueous methanol and methanol-chloroform 1:1 (v/v) to remove sterols and other neutral lipids, unconjugated steroids with one carboxyl group were eluted from the anion exchanger with 0.15 M acetic acid in 95% aqueous methanol. Following addition of a known amount of hexatriacontane as internal standard, methylation with diazomethane and formation of trimethylsilyl

(TMS) ethers, the acids were analyzed by gas-liquid chromatography (GLC) and gas-liquid chromatography-mass spectrometry using fused-silica capillary columns (25 m × 0.32 mm) coated with a 0.25- μ m layer of cross-linked methyl silicone. Amounts were calculated from peak areas given by the bile acid derivatives and the internal standard in the GLC analyses. The areas given by the TMS ethers of methyl 3 β -hydroxy-5-cholestenoate, 3 β ,7 α -dihydroxy-5-cholestenoate, and 7 α -hydroxy-3-oxo-4-cholestenoate were multiplied by 1.2, 1.2, and 1.5, respectively, to correct for differences in response factors. Conjugated bile acids were calculated as the difference between total and unconjugated bile acids.

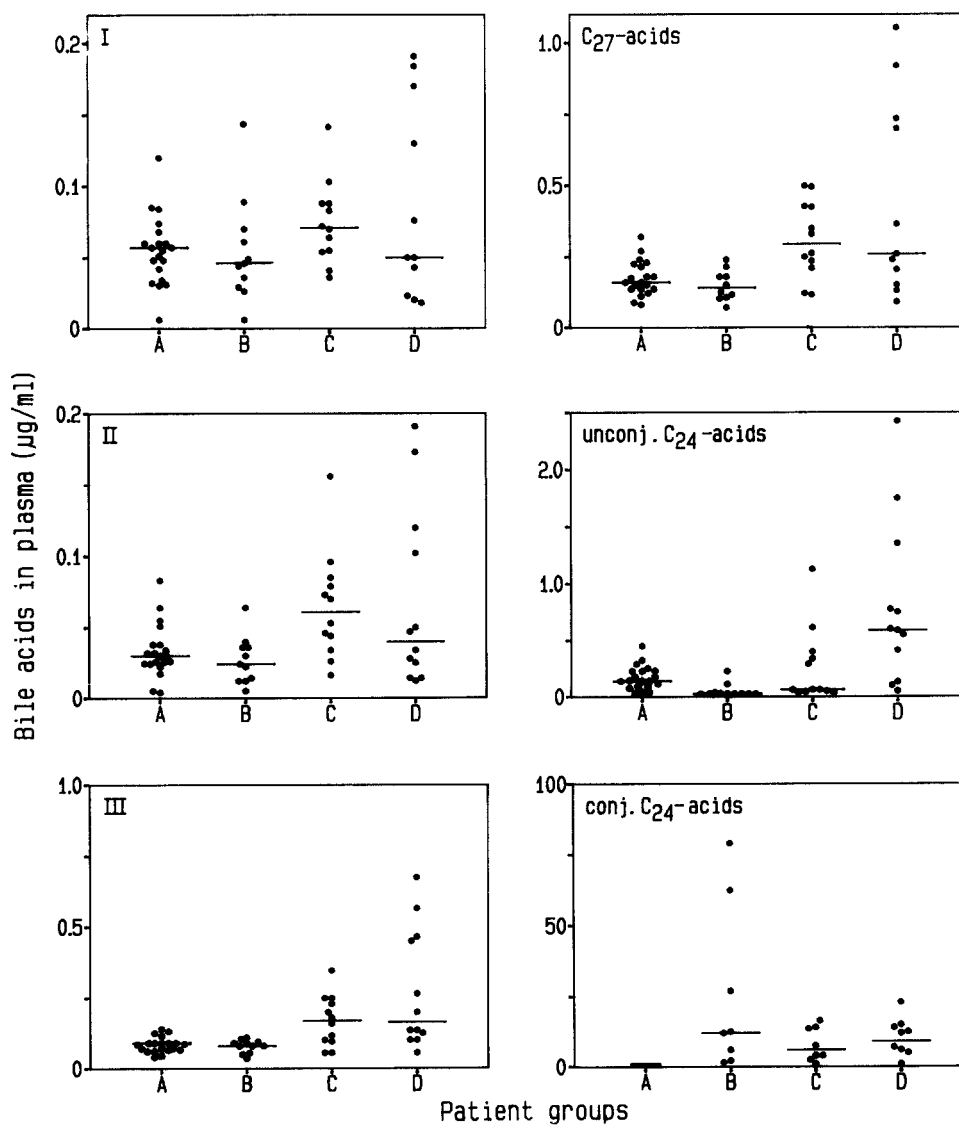


Fig. 1. Concentrations of individual and total C₂₇ bile acids and of total unconjugated and conjugated C₂₄ bile acids in plasma from control subjects (A) and patients with extrahepatic cholestasis (B), primary biliary cirrhosis (C), and alcoholic liver cirrhosis (D). Median values are indicated by horizontal lines. I, 3 β -hydroxy-5-cholestenoic acid; II, 3 β ,7 α -dihydroxy-5-cholestenoic acid; III, 7 α -hydroxy-3-oxo-4-cholestenoic acid.

Statistical analysis

Statistical evaluation of data included calculation 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 C₂₇ and C₂₄ acids in plasma

The concentrations of the three cholestenic acids and the major unconjugated and conjugated C₂₄ bile acids were determined in plasma from patients with common bile duct obstruction, primary biliary cirrhosis, and alcoholic liver cirrhosis. The unconjugated bile acids were also analyzed in 20 apparently healthy subjects. The results are shown in Fig. 1 and Table 1.

The concentrations of the C₂₇ and C₂₄ acids in the control group agree well with previously reported values (2, 5). The order of increasing concentration of the C₂₇ acids was usually 3β,7α-dihydroxy-5-cholestenic acid < 3β-hydroxy-5-cholestenic acid < 7α-hydroxy-3-oxo-4-cholestenic acid both in the controls and in the patients (Table 1). The concentrations of C₂₇ acids in patients with extrahepatic cholestasis were similar to those in the control group and definitely not higher. In contrast, many patients with primary biliary cirrhosis and alcoholic liver

cirrhosis had elevated levels of C₂₇ acids, particularly of 7α-hydroxy-3-oxo-4-cholestenic acid. The concentration of the latter was significantly higher ($P < 0.005$) in these patient groups than in the controls.

The concentrations of unconjugated C₂₄ acids were low in patients with extrahepatic cholestasis and in the majority of patients with primary biliary cirrhosis. This is to be expected if the unconjugated bile acids in blood originate from the intestine following bacterial deconjugation. Primary biliary cirrhosis is associated with intrahepatic cholestasis (6) while cholestasis is less prominent in alcoholic liver cirrhosis. The levels of free C₂₄ bile acids in the latter patients were, in fact, elevated. This is most likely due to a reduced hepatic clearance of these compounds from the portal blood, due to portal-systemic shunting and cellular dysfunction (7).

As expected, the levels of conjugated bile acids were elevated in most of the patients with cholestasis and cirrhosis. Ratios of cholic acid to chenodeoxycholic acid were higher in cholestasis than in alcoholic liver cirrhosis which is in agreement with previous studies (8).

Correlations of plasma levels

Statistical evaluation of the concentrations of C₂₇ and C₂₄ bile acids revealed a number of positive correlations. Correlations between levels of 3β-hydroxy-5-cholestenic acid and 3β,7α-dihydroxy-5-cholestenic acid (Fig. 2) were significant ($r = 0.76-0.88$, $P < 0.001-0.05$) for controls and within each patient group. As shown in Fig. 2, the correlation was also highly significant for the entire

TABLE 1. Concentrations of C₂₇ and C₂₄ bile acids in plasma/serum of healthy subjects and patients with extrahepatic cholestasis, primary biliary cirrhosis, and alcoholic liver cirrhosis

Bile Acids	Subjects ^a			
	Healthy	Extrahepatic Cholestasis	Primary Biliary Cirrhosis	Alcoholic Liver Cirrhosis
	<i>ng/ml^b</i>			
Unconjugated				
3β-Hydroxy-5-cholestenic acid	57:39-64	46:29-71	71:55-89	50:23-170
3β,7α-Dihydroxy-5-cholestenic acid	30:25-38	24:13-37	62:39-82	41:20-111
7α-Hydroxy-3-oxo-4-cholestenic acid	85:67-93	84:56-95	170:99-242	171:113-457
Chenodeoxycholic acid	34:16-73	< 10: < 10-10	28: < 10-189	410:110-545
Cholic acid	25:15-70	< 10: < 10-14	30:20-195	136:28-326
Deoxycholic acid	66:46-79	< 10: < 10-10	12: < 10-24	18: < 10-165
Conjugated ^c				
Chenodeoxycholic acid	ND ^d	5530:1993-20400	3100:1540-7440	7370:2610-7940
Cholic acid	ND	7250:2400-24200	2800:1610-5870	4110:3120-4980
Deoxycholic acid	ND	< 20: < 20- 20	123:79-199	390:181-443

^aDetails on patients are given in Materials and Methods.

^bConcentration expressed as median: lower quartile-upper quartile.

^cExpressed as ng unconjugated acid.

^dND, not determined.

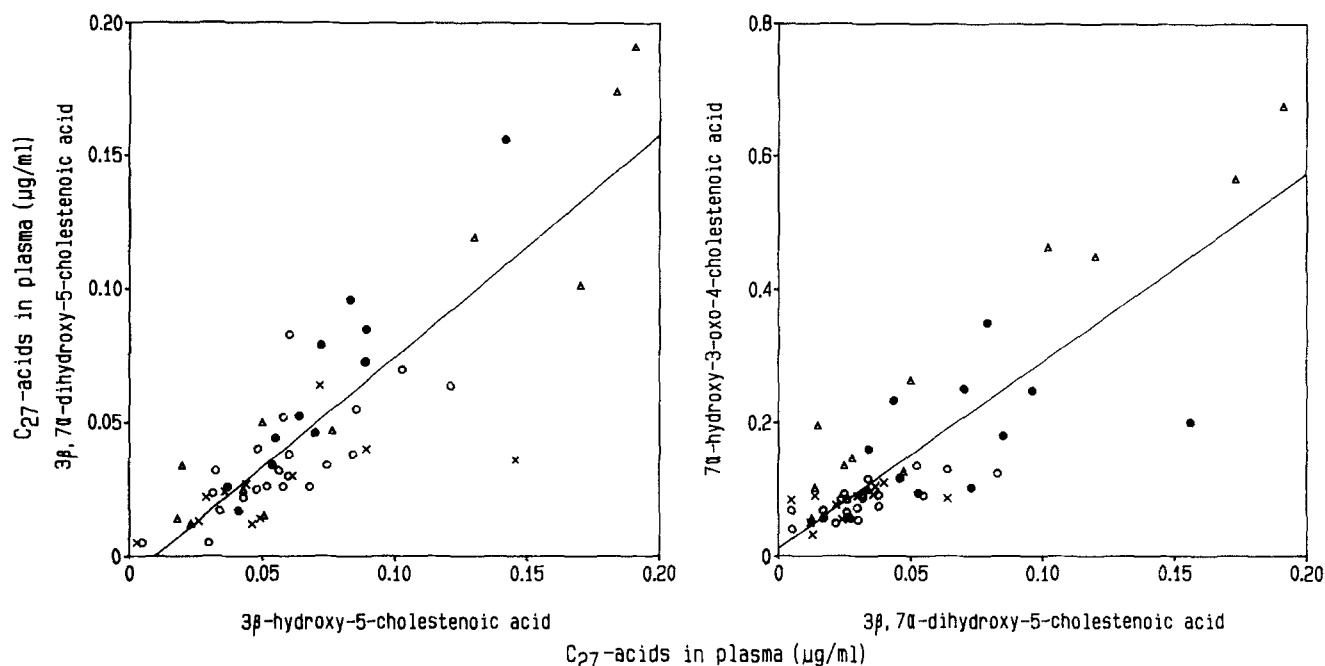


Fig. 2. Correlations between the concentrations of 3β -hydroxy-5-cholestenoic, $3\beta,7\alpha$ -dihydroxy-5-cholestenoic, and 7α -hydroxy-3-oxo-4-cholestenoic acids in plasma from healthy subjects (O) and patients with extrahepatic cholestasis (x), primary biliary cirrhosis (●), and alcoholic liver cirrhosis (Δ).

material ($r = 0.85$, $P < 0.0001$). The correlation between levels of $3\beta,7\alpha$ -dihydroxy-5-cholestenoic acid and 7α -hydroxy-3-oxo-4-cholestenoic acid was significant for the controls and patients with liver cirrhosis ($r = 0.61$ – 0.88 , $P < 0.001$ – 0.05) and for the entire material ($r = 0.77$, $P < 0.0001$) (Fig. 2). These results suggest a close metabolic relationship between the three cholestenic acids.

The levels of unconjugated cholic and chenodeoxycholic acids were usually positively correlated. This might be explained by their common formation by bacterial hydrolysis. The levels of cholestenic acids were not related to those of unconjugated C_{24} bile acids. However, with the exclusion of one patient (Fig. 3), significant positive correlations were observed between the levels of cholestenic acids and conjugated bile acids in blood from patients with alcoholic liver cirrhosis ($r = 0.93$, $P < 0.05$) or primary biliary cirrhosis ($r = 0.83$, $P < 0.05$) (Fig. 3). This might be due to a reduced hepatic clearance of all these acids. In contrast, there was no such correlation in patients with extrahepatic cholestasis.

DISCUSSION

The biosynthesis of bile acids from cholesterol may involve multiple pathways (9). In the major pathway, metabolic changes of the steroid nucleus are usually believed to be completed before the oxidation of the side chain. However, 26-hydroxylation may occur at an earlier stage.

In any case, the rate-limiting step in bile acid biosynthesis is thought to be the 7α -hydroxylation of cholesterol.

The structures of the cholestenic acids in plasma indicate that these compounds are either intermediates in a pathway from cholesterol to bile acids or side products formed from such intermediates. We expected that analyses of the C_{27} acids in plasma from patients with liver diseases might give some information about the metabolic position of these acids in humans. In the diseases studied, bile acid production is known to be reduced (10–15), due at least partly to a reduced amount or activity of cholesterol 7α -hydroxylase (13, 14, 16).

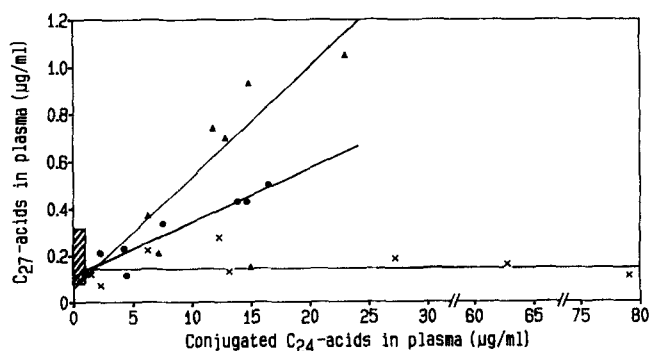


Fig. 3. Relationships between the concentrations of total cholestenic acids and total conjugated C_{24} bile acids in plasma of patients with extrahepatic cholestasis (x), primary biliary cirrhosis (●), and alcoholic liver cirrhosis (Δ). Values for control subjects lie in the hatched area.

The levels of cholestenic acids in patients with extrahepatic cholestasis did not differ significantly from those in the controls. This is in marked contrast to the grossly elevated levels of common conjugated bile acids caused by the biliary obstruction. Because of the interruption of the enterohepatic circulation, the levels of unconjugated C₂₄ bile acids were decreased. Thus, there was no correlation between the levels of C₂₇ and C₂₄ acids. This is compatible with the absence of significant amounts of the cholestenic acids in bile (2) and indicates that these acids do not participate in an enterohepatic circulation. Furthermore, their levels in plasma did not seem to be influenced by the decrease of cholesterol 7 α -hydroxylase activity expected in patients with extrahepatic cholestasis (14). These results show that different factors regulate the formation and secretion of conventional bile acids and the cholestenic acids.

The levels of cholestenic acids were increased in several cirrhotic patients. This could be due to an increased production/secretion or a decreased clearance/metabolism of the acids. Bile acid production is reduced in alcoholic cirrhosis (10–13) and the half-life times of bile acids in blood are increased due to a decreased hepatic clearance (17). The latter is the most likely reason for the high levels of both conjugated and unconjugated bile acids in plasma. The positive correlation between the cholestenic acids and conjugated C₂₄ bile acids in cirrhotic patients indicates that the same mechanism but not cholestasis is responsible for the elevated levels of the cholestenic acids in this disease. Intrahepatic cholestasis is usually more pronounced in primary biliary cirrhosis than in alcoholic liver cirrhosis (6). Correspondingly, patients with biliary cirrhosis had lower levels of cholestenic acids, in relation to conjugated bile acids, than patients with alcoholic cirrhosis and higher levels than patients with extrahepatic cholestasis (Fig. 3). Although a reduced hepatic clearance should also affect the levels of unconjugated bile acids, the poor correlation between these and the other groups of bile acids can probably be explained by the additional involvement of an intestinal microflora in their formation.

The site(s) of formation of the cholestenic acids cannot be deduced from the present results. The 7 α -hydroxylation may not be limited to the hepatocyte (18) and 26-hydroxylation can occur in several cell types, e.g., in skin fibroblasts (19). The subsequent oxidation to C₂₇ acids has only been demonstrated with hepatic enzymes (9, 20) but involvement of extrahepatic oxidoreductases cannot be excluded. Irrespective of the location of these biosynthetic reactions, this study indicates that the liver is of importance for the removal of the cholestenic acids from blood. Studies with labeled compounds will be required to show if this can occur by an expected conversion to common biliary bile acids. Following the submission of this study it was reported that 26-hydroxycholesterol but not 3 β -hydroxy-5-cholestenic acid is an efficient precursor of

cholic acid in the rabbit (21). Thus, this C₂₇ acid seems to be a side product of bile acid biosynthesis, at least in the rabbit. ■

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