Individual serum bile acid concentrations in normo- and hyperlipoproteinemia as determined by mass fragmentography: relation to bile acid pool size

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Abstract Combined gas-liquid chromatography-mass spectrometry with specific ion monitoring (mass fragmentography) has been used for assay of cholic acid (C), chenodeoxycholic acid (CD), and deoxycholic acid (D) in human serum. Deuterium-labeled C and D were used as internal standards. The relative standard deviation of duplicate samples was 3, 4, and 7% for C, CD, and D, respectively. The variation within the same individual in the fasting state was small, while the day-to-day variation was greater, especially for the dihydroxy bile acids. In normal control subjects (n = 24), the fasting serum concentration of C averaged 184 ± 24 ng/ml (mean ± SEM), and that of CD and D 526 ± 62 and 407 ± 44 ng/ml, respectively. Patients with type IIa hyperlipoproteinemia (n = 32) displayed low values of serum bile acids, with a C concentration of 121 ± 11 ng/ml (P < 0.01 vs. controls). A similar pattern was seen in patients with a type IIb lipoprotein pattern (n = 10). Subjects with type IV hyperlipoproteinemia (n = 32) showed serum bile acid levels within the normal limits. No relationship to age, sex, or body weight was seen in any of the patient subgroups. Bile acid kinetics were determined with an isotope dilution technique using 14C-labeled C and CD under steady state conditions in control subjects and patients with type IIa and type IV hyperlipoproteinemia. The serum concentration of C correlated significantly to its pool size in control subjects and in patients with type IIa hyperlipoproteinemia but not in patients with type IV. The serum level of CD was not related to CD pool size in any of the subgroups. The data obtained are discussed in relation to present concepts of the enterohepatic circulation. It is suggested that the intestinal content of C in the fasting state is proportional to the total C pool size. The possibility of a defective intestinal uptake of C in some patients with type IV hyperlipoproteinemia is raised.

Supplementary key words gas-liquid chromatography-mass spectrometry · deuterium-labeled bile acids · cholic acid · chenodeoxycholic acid · deoxycholic acid

Bile acids are formed from cholesterol in the liver, secreted in the bile, and stored in the gallbladder, ready to be released into the duodenum in response to a meal. Thus, in the fasting state the major part of the bile acid pool is located in the gallbladder and the intestine (for a review, see ref. 1). Due to efficient clearance by the normal liver (2), only a minute part of the total bile acid content is present in the peripheral circulation. Consequently, the fasting serum levels of bile acids are very low in health, and they have been difficult to determine accurately (3). At the same time, the accessibility of serum bile acids has made them a subject of much interest. Recently, a radioimmunoassay technique was developed for the determination of conjugated choly bile acids in serum (4) and a preliminary report has appeared on a radioimmunoassay for conjugated chenodeoxycholic acid (5). The importance of intestinal absorption for serum bile acid levels in health has been demonstrated (6, 7), but previous work does not show whether or not a relationship exists between the serum concentration and the pool size of bile acids in the fasting state.

In the present work a newly developed assay for serum bile acids is presented. Based on combined gas-liquid chromatography and mass spectrometry, using deuterium-labeled internal standards, it has a high specificity as compared to previous methods. In an effort to evaluate the possible relevance of serum bile acid determinations, analyses have been made of pool sizes and serum concentrations of cholic acid (C) and chenodeoxycholic acid (CD) in a series of normo- and hyperlipoproteinemic subjects, covering a wide range of pool sizes (8). Furthermore, fasting levels of C, CD, and deoxycholic acid (D) are reported for

Abbreviations: C, cholic acid (3α,7α,12α-trihydroxy-5β-cholanoic acid); CD, chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid); D, deoxycholic acid (3α,12α-dihydroxy-5β-cholanoic acid); GBD, gallbladder disease (cholelithiasis, cholecystitis, cholecystectomy).
a series of normo- and hyperlipoproteinemic subjects, and the possible influence of sex, body weight, and gallbladder disease has been analyzed.

METHODS

The subjects

Altogether 98 subjects were included in the present study, 24 normolipidemic controls and 74 patients with various types of hyperlipoproteinemia. The control subjects (9 males and 15 females) were healthy volunteers, and the hyperlipidemic patients were those consecutively admitted during the period of study to the Department of Medicine because of hyperlipoproteinemia. Informed consent was obtained from all individuals. According to the lipoprotein analysis (see below), 32 of the patients had hyperlipoproteinemia type IIa, 10 had type IIb, and 32 had type IV. Not included were a few patients with hyperlipoproteinemia types III and V, as well as patients who showed evidence of intestinal, liver, or kidney disease, hypo- or hyperthyroidism, or addiction to alcohol or narcotics. No drugs or diets known to affect lipid metabolism had been given in the month preceding the study. A routine physical and laboratory investigation, including determinations of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin, was performed on all subjects, and bromsulphalein clearance was assessed in most of the patients. An oral cholecystogram was obtained in all noncholecystectomized subjects. Basal data on the subjects are listed in Table 1. The type IV patients were somewhat older and heavier than the other groups; otherwise the groups were similar.

Eight control subjects, 18 patients with type IIa, and 19 with type IV lipoprotein pattern were included in the part of the investigation that deals with bile acid pool size determinations (see below). Basal data on these subjects are shown in Table 2.

Experimental procedure

In the first part of the study, fasting serum samples were obtained from all subjects and analyzed for serum bile acids, lipids, and lipoprotein pattern together with routine laboratory tests. To evaluate the constancy of the fasting serum bile acid levels, repeated serum samples were drawn at intervals of 15–30 min in six subjects. Furthermore, in 11 subjects a repeated determination of serum bile acid con-

TABLE 1. Basal data of subjects

<table>
<thead>
<tr>
<th>Lipoprotein Pattern</th>
<th>Male</th>
<th>Female</th>
<th>Age</th>
<th>Body Weight</th>
<th>Relative Body Weight</th>
<th>Serum Cholesterol</th>
<th>Serum Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yr</td>
<td>kg</td>
<td>% of ideal</td>
<td>mmol/l</td>
<td>mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>9a</td>
<td>15a</td>
<td>46 ± 3b</td>
<td>71 ± 4</td>
<td>101 ± 5</td>
<td>5.8 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>IIa</td>
<td>15</td>
<td>17</td>
<td>46 ± 2</td>
<td>66 ± 2</td>
<td>100 ± 3</td>
<td>9.2 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>IIb</td>
<td>6</td>
<td>4</td>
<td>54 ± 3</td>
<td>75 ± 4</td>
<td>107 ± 4</td>
<td>8.3 ± 0.2</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>IV</td>
<td>25</td>
<td>7</td>
<td>54 ± 2</td>
<td>76 ± 4</td>
<td>110 ± 3</td>
<td>6.4 ± 0.2</td>
<td>4.1 ± 0.3</td>
</tr>
</tbody>
</table>

* Number of subjects.

† Mean ± SEM.

' Calculated as weight (kg) / height (cm) - 100.

" Significantly different from normolipidemic controls, P < 0.001.

^ Significantly different from normolipidemic controls, P < 0.05.

TABLE 2. Basal data of subjects investigated with respect to bile acid pool size

<table>
<thead>
<tr>
<th>Lipoprotein Pattern</th>
<th>Male</th>
<th>Female</th>
<th>Age</th>
<th>Body Weight</th>
<th>Relative Body Weight</th>
<th>Serum Cholesterol</th>
<th>Serum Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yr</td>
<td>kg</td>
<td>% of ideal</td>
<td>mmol/l</td>
<td>mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>5a</td>
<td>3a</td>
<td>44 ± 4b</td>
<td>71 ± 4</td>
<td>99 ± 6</td>
<td>5.6 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>IIa</td>
<td>10</td>
<td>8</td>
<td>41 ± 3</td>
<td>68 ± 2</td>
<td>100 ± 4</td>
<td>9.2 ± 0.2</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>IV</td>
<td>13</td>
<td>6</td>
<td>54 ± 2</td>
<td>74 ± 3</td>
<td>107 ± 3</td>
<td>6.5 ± 0.3</td>
<td>4.1 ± 0.3</td>
</tr>
</tbody>
</table>

* Number of subjects.

† Mean ± SEM.

' Calculated as weight (kg) / height (cm) - 100.

" Significantly different from normolipidemic controls, P < 0.001.

^ Significantly different from normolipidemic controls, P < 0.05.
centration in the fasting state was made with an interval of 1 week to 3 months.

In the second part of the investigation, 45 subjects were hospitalized in a metabolic ward and fed a standardized diet of natural type for 4–7 days before and during the experimental period. Constant body weight was maintained, and the intake of cholesterol was about 200 mg/day in each subject. Forty, 21, and 39% of the total energy intake was supplied as fat, protein, and carbohydrate, respectively. 14C-Labeled cholic acid and 14C-labeled chenodeoxycholic acid as sodium salts dissolved in water were taken orally by the subjects in the morning before breakfast. Four samples of duodenal bile were collected from each subject at intervals of 2–4 days. Cholecystokinin was administered intravenously, and 5–10 ml of concentrated duodenal bile was obtained through a thin polyvinyl tube. Venous blood samples were drawn several times during the period of study and analyzed for cholesterol, triglyceride, and lipoprotein pattern. Fasting serum bile acid concentration was analyzed in a sample obtained from the middle part of the experimental period.

Materials

[24-14C]Cholic acid (138 μCi/mg) and [24-14C]-chenodeoxycholic acid (138 μCi/mg) were obtained from New England Nuclear Corp., Boston, MA. The radiochemical purity was checked by radioautography of thin-layer chromatograms. The deuterium-labeled internal standards, [2,2,3,4,4-2H5]cholic acid and [11,11,12-2H3]deoxycholic acid were prepared by enolizing methyl 3-oxo-7α,12α-dihydroxy-5β-cholan-3β-enoate and methyl 12-oxo-3α-hydroxy-5β-cholan-3α-ol in 2 M potassium hydroxide in deuterated water (99.8%, w/v, Merck, Darmstadt, Germany), followed by reduction with sodium borodeuteride and preparative thin-layer chromatography (9). The ion corresponding to M – 3 × 90 of [2,2,3,4,4-2H5]cholic acid contained 0% unlabeled molecules, 8% monodeuterated molecules, 8% dideuterated molecules, 19% trideuterated molecules, 28% tetradeuterated molecules, and 32% pentadeuterated molecules. The ion corresponding to M – 2 × 90 of [11,11,12-2H3]deoxycholic acid contained 0% unlabeled molecules, 0% monodeuterated molecules, 25% dideuterated molecules, and 75% trideuterated molecules. The calculations were performed from the mass spectra of methylated trimethylsilyl derivatives (10). The composition of the ions with respect to deuterium was not influenced by the alkaline hydrolysis (cf. below). Unlabeled bile acids were all obtained from Icapharm (Ramat-Gan, Israel). Cholecystokinin was obtained from the Gastrointestinal Hormone Research Group, Department of Chemistry, Karolinska Institutet, Stockholm.

Serum analysis

After clotting of the blood at room temperature, serum was obtained by centrifugation and frozen at −20°C for later analysis. To exactly 1 ml of serum, 2.5 μg of [2,2,3,4,4-2H5]cholic acid (dissolved in 25 μl of acetone) and 5.0 μg of [11,11,12-2H3]deoxycholic acid (dissolved in 50 μl of acetone) were added. The serum, together with the internal standards, was then subjected to hydrolysis with 1 M KOH at 110°C for 12 hr. The alkaline solution was extracted three times with ethyl ether. The bile acids were then extracted from the acidified water phase with ethyl ether. Recovery experiments showed that the losses of C in the extraction procedure were about 10% greater than those of CD and D, which were identical. The residue of the ether extract was methylated with diazomethane and converted into trimethylsilyl ether (11). The derivatives were analyzed by gas–liquid chromatography–mass spectrometry, using an LKB 9000 instrument equipped with an MID-unit (multiple ion detector). A 1.5% SE-30 column was used at a temperature of 210–220°C. Three of the channels of the MID-unit were focused on the ions at m/e 368, 370, and 373 (cf. Results). Under the chromatographic conditions used, the derivatives of hyodeoxycholic acid, ursodeoxycholic acid, allodeoxycholic acid, and hyocholic acid were completely separated from the derivatives of C, D, and CD. There was an incomplete separation, however, between derivatives of CD and allocholic acid and between derivatives of D and allochenodeoxycholic acid. The electron multiplier sensitivity was set to 180. The amplification of the channels was in general 300×. The electron energy was set to 20 eV and the trap current to 60 μA. The peak height of the MID-recordings was measured, as this was found to give more reproducible results than measurement of peak area. The temperature of the flash heater and ion source was set to at least 40°C above the temperature of the column.

Serum cholesterol and triglyceride were measured using a Technicon Auto-Analyzer (Technicon Instruments Corp., Tarrytown, NY). Lipoprotein phenotyping was performed according to WHO recommendations (12) as described earlier (8). The normal upper limit was set at 7.2 mmol/l for cholesterol, 2.0 mmol/l for triglyceride, and 5.2 mmol/l for β-lipoprotein cholesterol.

Bile analysis

The duodenal bile samples were hydrolyzed with 1 M KOH in closed steel containers for 12 hr at
110°C. The deconjugated bile acids were extracted from the acidified water phase with ethyl ether. After preparation of the methyl esters, the derivatives were separated by thin-layer chromatography (solvent system, trimethylpentane–ethyl acetate–acetic acid 10:10:2, v/v/v, cf. 13). C and CD were extracted from the scraped-off gel bands, and the trimethylsilyl ethers were prepared. One aliquot was used for determination of radioactivity in a Packard Tri-Carb liquid scintillation counter, and one aliquot was quantitated for mass by gas–liquid chromatography, using a 1.5% SE-30 column. Further details of the procedure are given in a previous paper (8). The pool size and turnover of C and CD were determined as outlined by Lindstedt (14).

Statistical analysis

Data are presented as means ± SEM (standard error of the mean). The significance of differences was evaluated by Student’s t test, or by Student’s paired t test where appropriate. Linear regressions have been calculated by the method of least squares, and their significances have been tested by estimating the correlation coefficient, r (15).

RESULTS

Mass fragmentography of individual bile acids

Figs. 1 and 2 show the partial mass spectra of unlabeled and deuterium-labeled methyl trimethylsilyl ethers of C and D, respectively. Fig. 3 shows the partial mass spectrum of unlabeled CD. The complete mass spectra of methyl trimethylsilyl ethers of C and CD have been published previously (16). An intense ion is seen at m/e 368 (Fig. 1) in the mass spectrum of the derivative of unlabeled C (corresponding to M – 3 × 90) and at m/e 370 in the mass spectrum of the derivative of unlabeled D (Fig. 2) or CD (Fig. 3) (corresponding to M – 2 × 90). The absolute intensity of the ion at m/e 370 was considerably lower in the mass spectrum of the derivative of D than in that of CD. An intense ion at m/e 373 was found in the mass spectrum of both [2,2,3,4,4-2H₅]cholic acid and [11,11,12-2H₃]deoxycholic acid.

Fig. 4A shows a mass fragmentogram of a mixture of [2,2,3,4,4-2H₅]cholic acid and [11,11,12-2H₃]deoxycholic acid obtained by following the ions at m/e 368, 370, and 373 through the gas–liquid chroma-
Fig. 4. A, Mass fragmentogram of methyl trimethylsilyl derivative of a mixture of [2,2,3,4,4-2H5]cholic acid and [11,11,12-2H3]deoxycholic acid (cf. Methods). B, Mass fragmentogram of derivative of [2,2,3,4,4-2H5]cholic acid and [11,11,12-2H3]deoxycholic acid to which unlabeled cholic acid, chenodeoxycholic acid, and deoxycholic acid had been added.

Quantitation of C was performed by using the ratio between the tracings at m/e 368 and m/e 373. Quantitations of D and CD were performed with the ratios between the tracing at m/e 370 for the respective acid and the tracing at m/e 373 for the derivative of [11,11,12-2H3]deoxycholic acid. The latter thus served as internal standard for both CD and D. In Fig. 5 the ratios between the different peak heights, m/e 368/m/e 370 and m/e 373/m/e 373, are plotted against the concentration of the respective bile acid. The plot was obtained from analysis of standard mixtures of a fixed amount of the two internal standards together with different amounts of the three bile acids (cf. Methods). It is shown in Fig. 5 that ratios were linear with the bile acid concentrations up to 1500 ng/ml. In fact, under the conditions employed, linearity was observed up to about 8000 ng/ml for all three bile acids.

Fig. 6A shows a mass fragmentographic analysis of a preparation of serum to which the two internal standards had been added. This serum had a relatively high concentration of each bile acid. In Fig. 6B a corresponding analysis is shown of a serum with a low concentration of bile acids.

The accuracy of the method was tested by adding different amounts of different bile acids to a specific serum with a known concentration of bile acids. As shown by the results in Table 3, the error in the determinations of C and CD within the range tested was less than 10%, while the error in the determina-
concentration of bile acids respectively. In contrast, the corresponding coefficients of variation were weight, serum cholesterol, or triglyceride. A significant correlation was found between the serum levels of between the sexes, and no significant correlation was obtained between bile acid concentrations and body

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Concentration</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholic acid in serum</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>+ 200 ng cholic acid</td>
<td>244</td>
<td>-8</td>
</tr>
<tr>
<td>+ 600 ng cholic acid</td>
<td>694</td>
<td>+4</td>
</tr>
<tr>
<td>Cholic acid in serum</td>
<td>265</td>
<td></td>
</tr>
<tr>
<td>+ 618 ng Na-glycocholic acid</td>
<td>766</td>
<td></td>
</tr>
<tr>
<td>(corresponding to 500 ng cholic acid)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Chenodeoxycholic acid in serum</td>
<td>172</td>
<td></td>
</tr>
<tr>
<td>+ 200 ng chenodeoxycholic acid</td>
<td>360</td>
<td>-3</td>
</tr>
<tr>
<td>+ 600 ng chenodeoxycholic acid</td>
<td>816</td>
<td>+6</td>
</tr>
<tr>
<td>Deoxycholic acid in serum</td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>+ 200 ng deoxycholic acid</td>
<td>350</td>
<td>-4</td>
</tr>
<tr>
<td>+ 600 ng deoxycholic acid</td>
<td>670</td>
<td>-12</td>
</tr>
</tbody>
</table>

Intra-individual variation in the serum concentration of bile acids

In six subjects, the coefficient of variation

\[
\left( \text{calculated as} \frac{100}{\mu} \sqrt{\frac{\sum d^2}{n}} \right)
\]

for samples drawn in the fasting state at intervals of 15–30 min was 4, 3, and 3% for C, CD, and D, respectively. In contrast, the corresponding coefficients of variation were 13, 21, and 33%, respectively, when fasting serum samples were obtained in 11 subjects on two occasions one week to three months apart.

Individual serum bile acids in hyperlipoproteinemic patients

Patients with type IIa displayed a slightly lower total concentration of the bile acids determined, mainly due to a reduced amount of C in serum (121 ± 11 ng/ml vs. 184 ± 24 ng/ml, P < 0.01) (Table 5, Fig. 7) but also to a tendency for decreased levels of CD and D (Table 5).

The type IIb lipoprotein pattern was associated with a similar reduction of the serum levels of C and also of CD, resulting in a subnormal total serum bile acid concentration (Table 5, Fig. 7). The patients with type IV hyperlipoproteinemia did not show any significant differences from the normolipidemic control subjects with regard to total or individual serum bile acids, but their concentration of C was significantly higher than that found in hyperlipoproteinemia type IIa or IIb (Table 5, Fig. 7).

In all three groups of hyperlipoproteinemic subjects the serum concentration of C was clearly lower than that of CD or D (Table 5). The serum bile acid pattern did not differ significantly between male and female patients in any of the subgroups, and there was no correlation with absolute or relative bodyweight or with serum lipids.

Serum bile acids in gallbladder disease

Gallbladder disease (GBD, cholelithiasis, cholecystitis, or cholecystectomy) was present in 9 normolipidemic controls, 3 patients with type IIa, 4 with type IIb, and 10 with type IV lipoprotein pattern. No differences between subjects with and without GBD with regard to total serum bile acid level or serum concentrations of C and CD were found in any of the subgroups or in the total series. The concentration of D was higher in patients with GBD in type IIa (793 ± 337 ng/ml vs. 325 ± 34 ng/ml, P < 0.005) and

<table>
<thead>
<tr>
<th>Sex, Number of Subjects</th>
<th>Cholic Acid (ng/ml)</th>
<th>Chenodeoxycholic Acid (ng/ml)</th>
<th>Deoxycholic Acid (ng/ml)</th>
<th>Total Bile Acids (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (9)</td>
<td>166 ± 36</td>
<td>494 ± 65*</td>
<td>539 ± 69*</td>
<td>999 ± 133</td>
</tr>
<tr>
<td>F (15)</td>
<td>195 ± 33</td>
<td>545 ± 92*</td>
<td>448 ± 56*</td>
<td>1187 ± 164</td>
</tr>
<tr>
<td>Total (24)</td>
<td>184 ± 24</td>
<td>526 ± 62*</td>
<td>407 ± 44*</td>
<td>1117 ± 114</td>
</tr>
</tbody>
</table>

* Significantly different from cholic acid, P < 0.001.

* Significantly different from cholic acid, P < 0.005.

* Significantly different from Chenodeoxycholic acid, P < 0.005.
TABLE 5. Serum concentrations (mean ± SEM) of individual bile acids in various types of hyperlipoproteinemia

<table>
<thead>
<tr>
<th>Lipoprotein Pattern, (number of subjects)</th>
<th>Cholic Acid</th>
<th>Chenodeoxycholic Acid</th>
<th>Deoxycholic Acid</th>
<th>Total Bile Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (24)</td>
<td>184 ± 24</td>
<td>526 ± 62e</td>
<td>407 ± 44e,b</td>
<td>1117 ± 114</td>
</tr>
<tr>
<td>IIa (32)</td>
<td>121 ± 11f</td>
<td>418 ± 44e</td>
<td>369 ± 47e</td>
<td>914 ± 83</td>
</tr>
<tr>
<td>IIb (10)</td>
<td>91 ± 19d</td>
<td>309 ± 45e,d</td>
<td>323 ± 33e</td>
<td>723 ± 79d</td>
</tr>
<tr>
<td>IV (32)</td>
<td>218 ± 31f,j</td>
<td>452 ± 56e</td>
<td>298 ± 38</td>
<td>968 ± 88</td>
</tr>
</tbody>
</table>

a Significantly different from cholic acid, \( P < 0.05 \).
b Significantly different from chenodeoxycholic acid, \( P < 0.05 \).
c Significantly different from normolipidemic controls, \( P < 0.01 \).
d Significantly different from normolipidemic controls, \( P < 0.02 \) or 0.05.
e Significantly different from patients with type IIa hyperlipoproteinemia, \( P < 0.005 \).
f Significantly different from patients with type IIb hyperlipoproteinemia, \( P < 0.05 \).

type IV (428 ± 76 ng/ml vs. 238 ± 38 ng/ml, \( P < 0.02 \)) hyperlipoproteinemia, as well as in the total series (442 ± 57 vs. 317 ± 23 ng/ml, \( P < 0.01 \)).

Serum bile acid levels in relation to bile acid pool size determinations

The serum bile acid levels in the subjects undergoing pool size determinations were similar to those seen in the total series of patients. The pool size of C was 732 ± 164, 511 ± 62, and 1058 ± 110 mg, and that of CD was 621 ± 112, 466 ± 54, and 649 ± 64 mg in controls (n = 8), type IIa (n = 18), and type IV (n = 19) hyperlipoproteinemia, respectively. The C pool size of the type IV hyperlipoproteinemic subjects was significantly larger (\( P < 0.001 \)) than that of the patients with type IIa.

The plot of pool size against serum concentration is shown for C in Fig. 8. Although the individual values display a considerable scatter, a positive correlation is evident between the two parameters both in the small group of normolipidemic controls and in the type IIa hyperlipoproteinemic patients. Combining the two groups of subjects gives the equation of the regression line \( y = 0.190x + 31.8 \) (SD of the regression coefficient is 0.032, n = 26) with \( r = +0.77 \) (\( P < 0.001 \)). In contrast, the plot of the patients with type IV hyperlipoproteinemia gives no significant correlation. However, eight of the patients do fall within the 95% confidence limits of the regression line calculated for controls + type IIa hyperlipoproteinemic patients, while the rest of the patients with type IV lipoprotein pattern clearly show no correlation between the parameters analyzed (Fig. 8).

For CD, there was no significant correlation between pool size and serum concentration, as evidenced by the \( r \) values +0.30, +0.18, and −0.08 for controls, type IIa, and type IV hyperlipoproteinemic patients, respectively (Fig. 9).

No significant correlation was obtained between production of C or CD and corresponding serum concentration (\( r = -0.31, +0.39, \) and +0.32 for C and -0.30, +0.24, and +0.10 for CD in controls, type IIa, and type IV hyperlipoproteinemic subjects, respectively).

DISCUSSION

Mass fragmentographic or mass chromatographic techniques for the determination of bile acids have
been published previously (16, 17). With the exception of some recent work from our laboratory (7, 18), these techniques have not been used for quantitation of the small amounts of bile acids present in serum. Miyazaki et al. (16) described a technique, similar to the one used in the present study, in which multideuterium-labeled bile acids were used as internal standards for the three major bile acids in bile. Methyl trimethylsilyl derivatives were used and quantitations were performed with several different ions. The procedure involved repetitive scanning through gas-liquid chromatography, with storage of complete mass spectra on a magnetic tape memory followed by computerized reconstruction of ion chromatograms (cf. 19). Such a method is less sensitive than the technique used here, but it has the advantage that several different ions can be followed. In a mass fragmentographic or mass chromatographic procedure it would be preferable to use ions that are highly specific for the compound to be determined. From this point of view, the ion at m/e 255 might be preferred in the quantitation of D and the ion at m/e 623 in the quantitation of C. These ions, however, cannot be followed simultaneously in a mass fragmentographic analysis with our instrument as the difference in mass number is too great.

In our procedure, deuterium-labeled deoxycholic acid served as internal standard for both deoxycholic and chenodeoxycholic acids as recovery experiments showed no difference in yield of the two acids. There is, however, a difference in recovery between cholic acid and the two dihydroxy bile acids in the extraction and consequently it is not possible to use a single deuterium-labeled internal standard for all three bile acids. The internal standard used in our procedure was not conjugated with glycine or taurine, as is the major part of the bile acids present in serum. If there is some destruction of unlabeled bile acids during the hydrolysis, however, this should probably take place equally in all three compounds, and as a result the recovery of the internal standard should be the same for all three acids. The method described here should be applicable to other bile acids as well. The complete mass spectra for the ions used as internal standards are shown in Fig. 3. A complete list of the ions detected in the different bile acids is presented in Table 1.
The concentrations of cholic, chenodeoxycholic, and deoxycholic acids found in healthy subjects in the present study (Table 4) are in reasonable agreement with those reported earlier by various authors using gas–liquid chromatography (3, 21–24). The data from these studies are summarized in Table 6.

TABLE 6. Mean serum bile acid concentrations in healthy subjects obtained in previous studies using gas–liquid chromatography

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cholic Acid</th>
<th>Chenodeoxycholic Acid</th>
<th>Deoxycholic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandberg et al. (3)</td>
<td>200</td>
<td>340</td>
<td>240</td>
</tr>
<tr>
<td>Erb et al. (22)</td>
<td>65</td>
<td>250</td>
<td>170</td>
</tr>
<tr>
<td>van Berge Henegouwen et al. (23)</td>
<td>200</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>Makino et al. (21)</td>
<td>90</td>
<td>360</td>
<td>205</td>
</tr>
<tr>
<td>Laatikainen and Hesso (24)</td>
<td>230</td>
<td>390</td>
<td>230</td>
</tr>
<tr>
<td>Present study</td>
<td>185</td>
<td>525</td>
<td>405</td>
</tr>
</tbody>
</table>

In spite of these minor limitations, the present mass fragmentographic technique for determination of bile acids should be considerably more specific than previous gas–liquid chromatographic techniques (3) and techniques based on the use of antibodies towards conjugated bile acids (4, 5). The deuterium-labeled bile acids are ideal as internal standards and, in order to interfere with a determination, a compound must have both the same retention time in the gas–liquid chromatogram and contain the same specific ion in its mass spectrum.
that of C in the fasting state. The lower hepatic clear-
gallbladder contraction and in bile acid secretion
to its pool size. The intra-individual variation was
also greater for CD than for C. As mentioned, there
importance for the instability of the CD level in serum.
may thus affect the serum level of CD more than
is more predominant. Small variations in the state of
circulation by means of passive jejunal diffusion
of such a process makes the serum level of CD more
unstable than that of C, for which active ileal transport
seems to be a substantial short-cut of the enterohepatic
transport was revealed between pool size and serum concentra-
tion in patients with normal
hepatic function would then mainly depend on varia-
tions in the intestinal input, as suggested by LaRusso
et al. (27). If so, the results of the present study imply that the intestinal C content is directly related to
the pool size of this bile acid. It has been observed
that the molar ratio between C and CD in fasting duodenal bile is rather constant, while the ratio between
the pool sizes of the two bile acids varies (8). It may
be concluded that the larger the pool size of C, the
more of it is present outside the gallbladder, presumably in the intestine.
The serum concentration of CD was not correlated
to its pool size. The intra-individual variation was
also greater for CD than for C. As mentioned, there
seems to be a substantial short-cut of the enterohepatic
circulation by means of passive jejunal diffusion
for CD and D (7, 18, 25). It is possible that the presence
of such a process makes the serum level of CD more
unstable than that of C, for which active ileal transport
is more predominant. Small variations in the state of
gallbladder contraction and in bile acid secretion
may thus affect the serum level of CD more than
that of C in the fasting state. The lower hepatic clear-
ance of CD compared to C (2, 18) may also be of im-
portance for the instability of the CD level in serum.

Type IIa hyperlipoproteinemia is often associated
with a reduced formation and pool size of C (8). Ac-
cordingly, these patients displayed low serum concentra-
tions of C in the fasting state. In agreement with
our results, Korman, Ellefson, and Hofmann (28),
using a radioimmunoassay technique, recently re-
ported low serum levels of conjugated C in this type of
hyperlipoproteinemia. These authors found normal
levels of C conjugates in fasting serum in patients with
the type IIb lipoprotein pattern, while the present
investigation revealed a low serum C concentration.
We have previously described a similarity in C synthe-
sis and pool size between type IIb and type IIa
hyperlipoproteinemia (8), and the present results
agree with this view. The possibility of differences
between the type IIb patients of the present work and
those investigated by Korman et al. (28) must of course
be considered, too.

In type IV hyperlipoproteinemia, the serum con-
centration of C was within normal limits, in keeping
with the report mentioned above (28). In these pa-
tients, no correlation was found between C pool size and serum concentration. There appear, however, to
be two subgroups of patients, one showing a normal
relationship between pool size and serum concentra-
tion, and one displaying low-normal serum levels
and large pools of C (Fig. 8). Disregarding the remote
possibility of an elevated hepatic clearance of C in the
latter subgroup, it is tempting to speculate that these
patients have a defective intestinal uptake of C. Indirect support for a defective intestinal uptake of C
in some patients with type IV hyperlipoproteinemia
has been gained previously. Thus, some of these pa-
tients have an increased synthesis and fractional turn-
over of C (8), a large C pool mainly located to the
distal intestine (25), and a decreased capability to
retain orally administered C (29).

The serum level of D was not compared to pool
size in the present study. The observed increase of
serum D in patients with GBD may be of some interest,
however, as such patients often have an elevated rela-
tive concentration of this bile acid in duodenal bile
(30, 31).

To summarize, the serum concentration of C to
some extent reflects its pool size, while that of CD does
not. Further studies in which the serum levels of the
bile acids are followed during various perturbations of
the enterohepatic circulation are needed, however,
before the possible clinical significance of their deter-
mination in subjects without hepatic disease can be
fully elucidated.46

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