Human cecal bile acids: concentration and spectrum


1 Division of Gastroenterology and Hepatology, Department of Medicine, VA Maryland Health Care System and University of Maryland School of Medicine, Baltimore, MD.
2 Office of the Chief Medical Examiner, State of Maryland, Baltimore, MD
3 Department of Chemistry, San Diego State University, San Diego, CA
4 Division of Gastroenterology, Department of Medicine, University of California, San Diego, CA

RUNNING HEAD: Human cecal bile acids

Correspondence regarding general aspects of the study should be addressed to:

Jean-Pierre Raufman, MD University of Maryland School of Medicine
22 S. Greene St., N3W62 Baltimore, MD 21201
E-mail jraufman@medicine.umaryland.edu Phone: 410-328-8728

Correspondence regarding bile acid metabolism should be addressed to:

Alan F. Hofmann, M.D. University of California, San Diego
San Diego, La Jolla, CA 92093-0813
E-mail: ahofmann@ucsd.edu Phone: 858-459-1754

† Current address: Division of Gastroenterology, Johns Hopkins University School of Medicine, Baltimore, MD.
ABSTRACT

To obtain information on the concentration and spectrum of bile acids in human cecal content, samples were obtained from 19 persons dying an unnatural death, such as caused by trauma, homicide, suicide, or drug overdose. Bile acid concentration was measured using an enzymatic assay for 3α-hydroxy bile acids; bile acid classes were determined by electrospray ionization mass spectrometry (ESI-MS); and individual bile acids by gas-chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC-MS). The 3α-hydroxy bile acid concentration (µmol bile acid/ml cecal content) was 0.4 ± 0.2 mM (mean ± SD); the total 3-hydroxy bile acid concentration was 0.6 ± 0.3 mM. The aqueous concentration of bile acids (supernatant after centrifugation) was identical, indicating that most bile acids were in solution. By LC-MS, bile acids were mostly in unconjugated form (90 ± 9%, mean ± SD); sulfated, non-amidated bile acids were 7 ± 5%, and non-sulfated amidated bile acids (glycine or taurine conjugates), were 3 ± 7%. By GC-MS, ten bile acids were identified: deoxycholic (34 ± 16%), lithocholic (26 ± 10%), ursodeoxycholic, (6±9) as well as their primary bile acid precursors cholic (6 ± 9%) and chenodeoxycholic acid (7 ± 8%). In addition, 3β-hydroxy derivatives of some or all of these bile acids were present and averaged 27 ± 18% of total bile acids, indicating that 3β-hydroxy bile acids are normal constituents of cecal content. In the human cecum, deconjugation and dehydroxylation of bile acids are nearly complete, resulting in most bile acids being in unconjugated form at submicellar and subsecretory concentrations.

KEYWORDS: bile acid deconjugation, bile acid dehydroxylation, intestinal bile acids, mass spectrometry of bile acids
INTRODUCTION

Bile acids are secreted from the hepatocyte into bile in predominantly conjugated form (28) and undergo efficient enterohepatic circulation (18). A small fraction of bile acids is not absorbed by the small intestine and passes into the large intestine. These bile acids are absorbed in part from the colon; the remaining bile acids are eliminated in feces. In the large intestine, before excretion from the body, bile acids undergo complex bacterial biotransformations. The first major pathway is deconjugation resulting in the formation of unconjugated bile acids, a process that begins in the distal small intestine (33). The second major pathway in the large intestine is dehydroxylation at C-7 resulting in the formation of 7-deoxy acids. In addition, hydroxy groups may undergo dehydrogenation to form hydroxy, oxo bile acids. The oxo group may in turn be reduced to either an α- or a β- hydroxy group (9,38,43). Thus, cecal bile acids should include a variety of primary bile acids (synthesized in the hepatocyte) and secondary bile acids (derived from primary bile acids by the action of bacterial enzymes on the hydroxy groups of the steroid nucleus), and bile acids should be present in both unconjugated and conjugated form.

The apparent concentration of bile acids in the cecum, i.e. the mass of bile acids per ml of water, is determined by the input of bile acids into the cecum, loss from absorption of bile acids by the cecal epithelium, and water movements. The aqueous concentration of bile acids in the cecal lumen, i.e. the true monomeric concentration of bile acids, might be less than the apparent concentration for two reasons. First, bile acids may adsorb to food residue. Second, cecal pH is considerably more acidic than ileal pH (10), and the aqueous solubility of bile acids decreases logarithmically as pH decreases (19).
Based on several lines of evidence, the aqueous concentration of bile acids in the large intestine may well have physiological significance. Perfusion of the colon of human subjects (30) or that of experimental animals (2,4,13) with deoxycholic acid (DCA) or chenodeoxycholic acid (CDCA) in both unconjugated and conjugated forms induces colonic secretion. Secretion is also induced by these dihydroxy bile acids *in vitro* using mucosal preparations of colon (11,29,32,37), colonic crypts (29), or polarized colonic epithelial cells (8,21-23). Bile acid malabsorption may induce diarrhea that is responsive to bile acid sequestrants (20). In subjects without bile acid malabsorption, ingestion of cholestyramine, a bile acid sequestrant, induces constipation (23).

Little information is available on the concentration and spectrum of cecal bile acids in the human cecum, presumably because of the difficulty of obtaining samples from subjects that have not had colonic cleansing procedures for colonoscopy. In the present work, we determined the apparent and aqueous concentration as well as the spectrum of cecal bile acids in persons dying an unnatural death.
EXPERIMENTAL PROCEDURES

Cecal samples. Cecal samples (N=30) were obtained during autopsy from persons who had died an unnatural death. Such persons, who had expired fewer than 24 hours before autopsy, were identified by a forensic pathologist (S.H.) in the Office of the Chief Medical Examiner for the State of Maryland in Baltimore, Maryland. The causes of death were trauma (vehicular accident, fall, or crush injury), N=10; homicide or suicide, or drug overdose, N=15; stroke/myocardial infarction, N=2; and other, N=3.

Total apparent bile acid concentration. Cecal content, 1 ml, was aspirated from the cecum of 19 cadavers and mixed with 4 ml of reagent grade isopropanol. Samples were stored at 4°C. They were thawed, shaken thoroughly, and centrifuged. One aliquot of the supernatant was used for enzymatic determination. A second aliquot was used for determination of bile acid classes by electrospray-ionization mass spectrometry (ESI-MS) and gas chromatography-mass spectrometry (GC-MS) in the Department of Chemistry, San Diego State University. Cecal content, 1 ml, from an additional 11 newly deceased persons was collected and stored at -80°C. These 11 samples were used to determine the concentration of bile acids in aqueous solution.

Bile acid standards. Unconjugated bile acids were high-grade commercial samples that were further purified by adsorption column chromatography on silica gel (7). N-acylamidated (glycine- or taurine-conjugated) bile acids were synthesized in the laboratory of A.F.H. as described by Tserng et al. (48), and purified by column chromatography as described (7). Sulfated bile acid standards were either synthesized in this laboratory or generously donated by T. Iida, Department of Chemistry, Nihon University, Tokyo, Japan (14). Internal standards for liquid-chromatography-mass spectrometry (LC-MS) 2,2’,4,4’-²H-cholic acid (CA) and 2,2’,4,4’-²H-deoxycholic acid
(DCA) were kindly provided by Dr. Ulrich Beuers, Ludwig-Maximilian University, Munich, Germany, and Dr. Franz Stellaard, University of Groningen, Netherlands.

**Apparent and aqueous concentration of 3α-hydroxy bile acids.** The apparent concentration of 3α-hydroxy bile acids was determined enzymatically using the method described by Porter *et al.* (36) for fecal bile acids. Briefly, 100 mg of lyophilized stool was refluxed for 120 min in ethylene glycol – 0.7 M potassium hydroxide for bile acid desorption as well as deconjugation. After cooling, the solution was acidified, and bile acids were extracted with diethyl ether. The ether extract was evaporated, the residue redissolved in methanol, and its bile acid content determined by the enzymatic procedure. Based on recovery of 14C radioactivity by combustion in freeze dried feces of subjects ingesting 14C-cholic acid, the alkaline hydrolysis - ether extraction procedure used in this method gives complete recovery of fecal bile acids (36). The same is likely to be true for bile acids in cecal content.

In the second group of 11 samples, 1 ml of cecal contents was freeze-dried and reconstituted with an equal volume of distilled water. After homogenization, aqueous bile acid concentration was determined by the enzymatic procedure in the supernatant solution generated by centrifuging samples at 15,000 x g for 10 min, as described by Lapré *et al.* (24).

**Determination of bile acid classes by Electrospray ionization mass spectrometry (ESI-MS) and ESI-MS-tandem mass spectrometry (ESI-MS-MS).** ESI-SIM-MS was performed at San Diego State University (SDSU) on a Thermo TSQ Quantum quadrupole mass spectrometer operating in the negative mode. An aliquot of the isopropanol extract of cecal samples was diluted with 0.1 M sodium bicarbonate. Bile acids were then adsorbed to a C-8 reversed phase column (Isolute C8 SPE;
International Sorbent Technology, Mid Glamorgan, UK), rinsed with water, and desorbed with methanol.

Spectra were quantified using the detector response from samples injected into a flow of methanol/water, 95:5, v:v, and calibration factors obtained from model mixtures, after background subtraction. The sheath gas flow was 70 and the SID 30V. The following m/z values were measured: 373, 3-oxo-cholanoic acid; 375, monohydroxy bile acids (presumably lithocholic acid or isolithocholic acid); 391, dihydroxy bile acids (DCA, CDCA, and epimers); 407, trihydroxy bile acids (cholic acid and epimers); 448, glycine conjugates of dihydroxy bile acids; 455, monosulfate of monohydroxy bile acids; 464, glycine conjugates of trihydroxy bile acids; 471, monosulfates of dihydroxy bile acids; 482, taurine conjugates of monohydroxy bile acids; 498, taurine conjugates of dihydroxy bile acids; and 514, taurine conjugates of trihydroxy bile acids.

ESI-MS-MS was performed at the University of California, San Diego to confirm the identity of assigned m/z values using a Perkin Elmer Sciex API-III instrument (Perkin-Elmer, Alberta, Canada) modified with a nanoelectrospray source (Protana A/S, Odense, Denmark). Palladium-coated borosilicate glass capillaries (Protana) were used for sample injection. The instrument was operated in the negative mode with Q1 ion spray voltage set to 600 volts. The interface voltage was set to 100 volts, and the orifice voltage to 90 volts. A curtain gas of ultrapure nitrogen was pumped into the interface at 0.6 L/min to aid evaporation of solvent droplets and prevent particulate matter from entering the analyzer. Chemical identity of peaks was confirmed by the fragmentation pattern of selected ions (Q3 mode) using argon gas, as well as by comparison with known standards. In particular, conjugates giving rise to sulfate (m/z 97), taurine (m/z 124) or glycine (m/z 74) were identified. Results by the two MS
procedures for the 14 samples having satisfactory spectra agreed quite well for unconjugated bile acids, the major constituents identified \(r = 0.97\).

Identification of individual bile acids by GC-MS and LC-MS. Bile acids were determined by GC-MS of the methyl ester per-acetates (40). Bile acids in the unconjugated fraction were determined by derivatizing an aliquot of the isopropanol extract without a solvolysis or deconjugation step \((N=6)\). The acetylation procedure was shown to hydrolyze the ester bond of sulfolithocholate, suggesting that any bile acids present as 3-sulfated bile acids would likely be measured by the GC-MS procedure. Therefore only amidated bile acids would not be determined in this subset of samples. Total bile acids \((N=13)\), i.e. bile acids in both conjugated and unconjugated forms, were determined by performing GC-MS on the diethyl ether extract of unconjugated bile acids that are generated by the deconjugation step in the enzymatic procedure of Porter et al. (36). As most bile acids were present in unconjugated form (see Results), GC-MS results of the sets of analyses did not differ statistically and were pooled, giving GC-MS results for 19 samples.

Methyl esterification was performed using ethereal diazomethane and per-acetates were prepared as described by Roovers et al. (40). Chromatography was performed on a capillary column 30 meters x 0.25 internal diameter with a stationery phase of methyl polysiloxane (65%)–phenylpolysiloxane (35%) coated at 0.25 µ thickness (J & W Scientific, Folsom CA). The gas chromatogram was a Hewlett-Packard HP-5890 coupled to a mass selective detector Hewlett Packard HP-5970. Compounds were identified by their retention times and fragmentation pattern based on a MS library of some 40 bile acids.
LC-MS/MS analysis of the samples was also performed on the TSQ Quantum at SDSU. Samples prepared as described above were evaporated to dryness, then re-dissolved in methanol containing five internal standards [tauroursocholic acid (3α, 7β,12α-trihydroxy-5β-cholan-24-oyltaurine), glycolagodeoxycholic acid (3α,12β-dihydroxy-5β-cholan-24-oylglycine), 2,2',4,4'-2H-CA, 2,2',4,4'-2H-DCA and 7α-hydroxy-5β-cholan-24-oic acid]; these compounds acted as retention time standards. The samples were separated on a Hypersil-Keystone BetaBasic C18 column and eluted with an ammonium acetate buffer/methanol gradient. Detection was by Selected Reaction Monitoring (SRM) and quantification performed against a five-point standard curve prepared for each compound; the detector response for the reference compounds varied widely. Samples were analyzed for unconjugated bile acids (ursodeoxycholic acid, cholic acid, chenodeoxycholic acid (CDCA), deoxycholic acid and lithocholic acid as well as their corresponding taurine N-acyl amidates, glycine N-acylamidates, and sulfate conjugates. For CDCA, the 3- and 7-sulfates were measured individually. In addition, the 3β-hydroxy epimers of the unconjugated bile acids were measured. The taurine, glycine, and sulfate conjugates of these 3β-hydroxy epimers not measured as standards were not available. In addition, 3β,7α-dihydroxy- and 3β,12α-dihydroxy (isoCDCA and isoDCA) had identical retention times on the C18 columns and, as the detector response for the two compounds differed, it was not possible to include these two bile acids in the analyses. Proportions of 23 analytes were determined.

Statistical analysis. Results are expressed as mean ± standard deviation. Differences between groups were tested by Wilcoxon rank sum test. Correlation coefficients were determined using SigmaPlot Software, Systat Software Inc., San Jose.
CA. Bile acids conjugated with glycine or taurine are termed amidates although a more proper name would be N-acyl amidates.
RESULTS

Concentration of total bile acids

The apparent bile acid concentration of 3α-hydroxy bile acids in 19 subjects as determined by the enzymatic procedure was $0.43 \pm 0.19 \text{ mM}$. The median concentration was $0.39 \text{ mM}$ and the range was from $0.14$ to $0.93 \text{ mM}$. The mean aqueous concentration in 11 different samples was $0.40 \pm 0.16 \text{ mM}$. The median value was $0.37 \text{ mM}$, and the range was from $0.12 \text{ mM}$ to $0.69 \text{ mM}$. The difference in apparent concentration and aqueous concentration was not significant indicating that most bile acids were in solution in cecal content.

Measurement of 3α-hydroxy bile acids by using 3α-hydroxysteroid dehydrogenase gives an underestimate of total bile acids for three reasons. The first is that 3β-hydroxy bile acids were present, and such bile acids are not dehydrogenated by the enzyme which acts only on 3α-hydroxy bile acids. The second is that a small fraction of bile acids is present in the form of C-3 sulfates, precluding their measurement by the enzyme. Sulfated, nonamidated bile acids averaged 7% of total bile acids determined using LC-MS, but four of fifteen subjects had values exceeding 10% of bile acids. The third is that the enzymatic method does not measure 3-oxo bile acids. However, compounds having an m/z value of the 3-oxo derivatives of mono-, di- and trihydroxy bile acids comprised <5% of bile acids by ESI-MS.

Using the value obtained by GC-MS for 3β-hydroxy bile acids, we calculated the total 3-hydroxy bile acid concentration using the equation: 

$$[BA]_{3\text{-hydroxy}} = \frac{[BA]_{\text{enzymatic}}}{(1 - \text{fraction of 3β-hydroxy bile acids in total bile acids})}.$$
The total 3-hydroxy bile acid concentration was 0.6 ± 3 mM. Figure 1 shows 3α-hydroxy (enzymatic method) and total 3-hydroxy (enzymatic plus 3β-hydroxy bile acids) apparent bile acid concentrations. The true total bile acid concentration is still slightly higher because of the small fraction of 3-sulfated bile acids that was present.

**Bile acid classes.**

By ESI-MS, ESI-MS, and LC-MS, most bile acids were in unconjugated form. Data are summarized in Tables 1 and 2. The median value by ESI-MS was 92% for unconjugated bile acids, 8% for sulfated nonamidated bile acids and 1% for amidated, non-sulfated bile acids. Individual values for the three major bile acid classes are illustrated in Figure 2 using triangular coordinates. Similar values were obtained by LC-MS-MS (Table 2).

The distribution of individual bile acid classes is also presented in Table 1. The most abundant class was dihydroxy bile acids (60%). Monohydroxy bile acids (15%) and trihydroxy bile acids (14%) were the second most prevalent classes. Sulfated, non-amidated bile acids were present in all samples, averaging 9± 8%. However, there was much between-subject variation using LC-MS; some samples had little sulfation, whereas three of 15 subjects had 13% of bile acids in sulfated form, and in one subject 40% of bile acids were sulfated. As shown in Figure 3, the proportion of CDCA that was sulfated exceeded that of DCA (p=0.02). By LC-MS, sulfation of CDCA was exclusively at carbon 3, i.e. no 7-sulfates of CDCA were present. The proportion of bile acids in sulfated form determined by ESI-MS correlated highly with that obtained by LC-MS (r = 0.90). The mass spectrum of the subject with 13% dihydroxy sulfates is shown in Figure 4, which also shows a spectrum from a sample lacking sulfated bile acids. Monosulfates of trihydroxy bile acids were not present. C27 bile acids also were not
present in appreciable proportions. Based on m/z values for the parent compounds as well as the MS/MS chromatograms for the precursors of sulfate, sulfated amidates of LC were not present. However, the m/z value corresponding to the doubly charged anion of sulfolithocholyglycine (m/z = 256) and m/z value of sulfolithocholoryltaurine (m/z = 256) could not be quantified because of interference by fatty acid anions. The m/z value for the monosulfate of dihydroxy taurine amidates (m/z = 289) was present in only one sample and was 7% of total bile acids.

**Individual bile acids by GC-MS.**

Results of GC-MS of the unconjugated bile acid fraction of cecal content are summarized in Table 3 and typical gas chromatograms are shown in Figure 5. The dominant bile acids were DCA, the 7-deoxy derivative of cholic acid and lithocholic acid (LCA), the 7-deoxy derivative of CDCA. CDCA was present in 11 of 19 samples and CA was present in 9 of 19 samples. UDCA, the 7β-hydroxy epimer of CDCA was present in 7 of 19 samples. In addition, the 3β-hydroxy derivatives ("iso" bile acids) of lithocholic acid, CDCA, UDCA, and CA were present in 18 of 19 samples. Such 3β-hydroxy ("iso") bile acids constituted 26 ± 32% of bile acids (median, 18%; range 0-58%). The 3β-hydroxy epimers of LCA DCA, and CDCA were greater in proportion than those of UDCA and CA. Thus, isoLCA was present in 13 of 19 samples, isoDCA was present in 14 of 19 samples, and in 10 of 19 samples in which CDCA was present, 9 contained isoCDCA. In contrast, only 2 of 7 UDCA samples contained isoUDCA, and only 1 of 9 samples containing CA had isoCA.

No other bile acids were identified except for one sample that contained <5% of the 12 oxo (keto) derivative of isodeoxycholic acid.
DISCUSSION

To the best of our knowledge, the data presented in this paper are the first describing the concentration and spectrum of bile acids in the human cecum. In the present study, GC-MS analyses of cecal samples indicated that 3β-hydroxy bile acids were usually present. These averaged 26% of bile acids and varied in proportion from 0 to 56%. In addition, in a few subjects, a considerable proportion of bile acids was present in the sulfated form. Thus, the enzymatic measurement of 3α-hydroxy bile acids gives a variable underestimate of the total apparent bile acid concentration in cecal contents.

Postprandial bile acid concentration in the human distal ileum averages 2 mM (33), whereas postprandial bile acid concentration in the proximal small intestine is considerably higher, averaging 10 mM (35). Most bile acids secreted into the small intestine during digestion are absorbed in the distal ileum. Therefore, the decrease in the luminal concentration of bile acids in the distal ileum by a factor of five indicates that here bile acid absorption is more efficient than water absorption. The same appears to be true in the cecum, as bile acid concentration here decreases by a factor of three.

The analyses showed that most cecal bile acids are present in unconjugated form. Deconjugation of bile acids begins in the distal small intestine (32), and the state of conjugation of bile acids crossing the ileocecal valve is not known. LCA, which is secreted into bile in part in sulfated, amidated form (41), was present in cecal samples largely in unconjugated form.

Although 3β-hydroxy bile acids have been identified previously in fecal bile acids (9,43), they are not generally considered in discussions of the enterohepatic circulation of bile acids. From their physicochemical properties, i.e. number of hydrogen bonding
sites, isolithocholic, isoDCA, isoCDCA, and isoUDCA are likely to be rapidly absorbed from the colon (1,16,39,42). In agreement with this view, studies by Shefer et al. (44) showed that isoCDCA and isoUDCA are rapidly and efficiently absorbed from the intestine. Animal studies (6,25,44) and recent human studies (26,27) indicate that 3β-hydroxy bile acids are efficiently re-epimerized to the physiologically occurring 3α-hydroxy bile acids during hepatocyte transport. Therefore, our results suggest strongly that 3β-hydroxy bile acids are continuously absorbed from the colon and re-epimerized during hepatocyte transport. This hidden pathway in the enterohepatic circulation of bile acids is analogous to the deconjugation-reconjugation pathway for conjugated bile acids, another hidden pathway known to occur in animals and man (18). The 3-sulfate of isoDCA has very recently been shown to be a major urinary bile acid in man (15), indicating that 3β-hydroxy bile acids can also be sulfated and eliminated via the kidney. We did not quantify this bile acid sulfate, as a standard was not available to us when this work was performed.

Four subjects had a relatively high proportion of sulfated bile acids by LC-MS. As noted above, the sulfate of CDCA was predominantly 3-sulfo-CDCA. As the 3-sulfo-conjugates of DCA and CDCA are not present in bile in appreciable proportions (45,46), it seems likely that these sulfated bile acids were formed in the intestine. We speculate that DCA and CDCA are formed by bacterial deconjugation in the distal small intestine. They are then absorbed by enterocytes, and sulfated. The sulfated bile acid was then effluxed into the intestinal lumen. The efflux pump MRP2 is known to transport sulfated bile acids (31) and has been shown to be present in the human intestinal epithelium (12,49). Both the small intestine and large intestine possess sulfotransferase activity, although there is considerable variability in the site of expression in the intestine as well.
as between individual subjects (5). Most studies of sulfated bile acids in feces have given values less than 10% (3, 47).

As noted in the Introduction, the concentration and spectrum of cecal bile acids can be influenced by multiple factors and the analyses reported here represent the momentary composition of one sample of cecal content. A major limitation of our sample source is that postmortem changes may have occurred, and that some subjects may have been ingesting antibiotics or other drugs that could have influenced the cecal bile acid profile. We elected not to use brain-dead tissue donors, as such patients commonly receive multiple antibiotics and antibiotic exposure could alter the cecal flora. Presumably the bile acid spectrum and concentration is altered by increased entry of bile acids across the ileocecal valve during digestion. Whether subjects were eating or fasting in our study is not known. Nor is it known whether cecal content is well mixed.

In this study, we used three complementary analytical methods. ESI-MS measures the apparent amount of compounds having m/z values corresponding to those of expected bile acids and their corresponding conjugates. It is a highly sensitive method, but is not specific as a given m/z value could contain compounds other than bile acids. GC-MS provides information on 3β-hydroxy bile acids which cannot be determined by ESI-MS or LC-MS, as the 3β-hydroxy epimers of DCA and CDCA have the same m/z values as their 3α-hydroxy epimers, and the same retention time by LC. LC-MS permits individual compounds such as sulfated bile acids to be identified by both their retention time and their m/z values.

Despite these limitations, we believe that some meaningful conclusions can be drawn from these data. The first is that cecal bile acids are largely in unconjugated and 7-dehydroxylated form. Deconjugation and dehydroxylation render bile acids
membrane permeable, and their concentration falls as a result of absorption from the cecum. The second is that most cecal bile acids are in solution despite the acidic pH of the cecum (10) and the low aqueous solubility of protonated mono- and dihydroxy- bile acids (19). The third is that cecal bile acids, in contrast to small intestinal bile acids, are present at a concentration below their critical micellization concentration (CMC). The CMC of bile acids during lipid digestion has been estimated to be 1-2 mM (17). If micelles are not present, absorption of insoluble lipids from cecal content, for example toxic bacterial lipids, cannot occur. The fourth is that CDCA and DCA are present at a concentration well below that inducing secretion in the perfused human colon (30). In health, the colon absorbs about 90% of the fluid that enters it from the terminal ileum (34). Therefore, it is quite reasonable that bile acids are present at concentrations that do not induce secretion. Fifth, in some patients, an appreciable fraction of dihydroxy bile acids (DCA and CDCA) is present as the non-amidated 3-sulfate and is likely to be formed in distal intestinal enterocytes. Lastly, 3β-hydroxy bile acids are present in cecal contents and are likely to be absorbed, returned to the liver, and re-epimerized during hepatocyte transport.

The present data also provide values for previously healthy subjects and may be useful for comparison with the bile acid spectrum present in disease states such as bile acid malabsorption, inflammatory bowel disease or colon cancer.

GRANT SUPPORT

Work at the University of Maryland was supported by NIH grants T32 DK067872 (JPH and GX) and CA 107345 (JPR), and by the Office of Research and Development,
Department of Veterans Affairs. Work at the University of California, San Diego and San Diego State University was supported in part by NIH grant DK 64891 (AFH).
REFERENCES


22. **Keely S, Scharl M, Bertelsen LS, Hagey LR, Barrett KE, and Hofmann AF.**


47. **Tanida N, Hikasa Y, Hosomi M, Satomi M, Oohama I, and Shimoyama T.**


Table 1. *Bile acid class and group composition (percent total) of human cecal contents by ESI-MS and ESI-MS-MS (N=15)*

<table>
<thead>
<tr>
<th>I. Major bile acid groups</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unconjugated bile acids</td>
<td>88.7 ± 11.3</td>
<td>92.0</td>
<td>53.5-98.6</td>
</tr>
<tr>
<td>Sulfated, non-amidated bile acids</td>
<td>9.0 ± 21.0</td>
<td>8.0</td>
<td>1.4-40.3</td>
</tr>
<tr>
<td>Non-sulfated, amidated bile acids</td>
<td>2.3 ± 3.3</td>
<td>0.8</td>
<td>0-11.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. Bile acid classes</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Monohydroxy bile acids</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monohydroxy-</td>
<td>15.4 ± 9.6</td>
<td>21.2</td>
<td>0-29.3</td>
</tr>
<tr>
<td>Sulfo-monohydroxy-</td>
<td>3.9 ± 5.9</td>
<td>1.8</td>
<td>0-21.9</td>
</tr>
<tr>
<td>Monohydroxy-taurine amidates</td>
<td>0.6 ± 1.2</td>
<td>0</td>
<td>0-3.5</td>
</tr>
</tbody>
</table>

| *Dihydroxy bile acids*                        |           |        |        |
| Dihydroxy-                                    | 59.7 ± 14.1| 65.8   | 27.4-78.7|
| Sulfo-dihydroxy-                              | 5.2 ± 5.1  | 3.3    | 0-20.0  |
| Dihydroxy-taurine and glycine amidates        | 0.4 ± 1.0  | 0      |         |

<p>| <em>Trihydroxy bile acids</em>                       |           |        |        |
| Trihydroxy-                                   | 13.8 ± 11.0| 11.8   | 0-43.4 |
| Trihydroxy-taurine and glycine amidates       | 0.5 ± 1.4  | 0      | 0-6.3  |</p>
<table>
<thead>
<tr>
<th>Bile acid group</th>
<th>M±SD, %</th>
<th>Median, %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unconjugated</td>
<td>89.9 ± 9.3</td>
<td>95.0</td>
<td>57.5-99.6</td>
</tr>
<tr>
<td>Amidated, non-sulfated</td>
<td>2.8 ± 6.6</td>
<td>0.3</td>
<td>0-26.2</td>
</tr>
<tr>
<td>Sulfated, non-amidated</td>
<td>7.2 ± 5.4</td>
<td>3.7</td>
<td>0-40.1</td>
</tr>
<tr>
<td><strong>Cholic acid family</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taurocholate + Glycocholate</td>
<td>1.3 ± 3.5</td>
<td>0.1</td>
<td>0-13.4</td>
</tr>
<tr>
<td>3-sulfo-cholic</td>
<td>Trace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholic</td>
<td>14.8 ± 11.1</td>
<td>12.6</td>
<td>0-41.5</td>
</tr>
<tr>
<td>Isocholic</td>
<td>0.7 ± 0.7</td>
<td>0.5</td>
<td>0-2.6</td>
</tr>
<tr>
<td><strong>Deoxycholic acid family</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taurodeoxycholate + Glycodeoxycholate</td>
<td>0.1 ± 0.1</td>
<td>0.1</td>
<td>0-3.6</td>
</tr>
<tr>
<td>3-sulfo-deoxycholic</td>
<td>1.2 ± 0.7</td>
<td>0.0</td>
<td>0-10.8</td>
</tr>
<tr>
<td>Deoxycholic</td>
<td>29.5 ± 18.2</td>
<td>23.0</td>
<td>7.1-67.4</td>
</tr>
<tr>
<td><strong>Chenodeoxycholic acid family</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taurochenodeoxycholate + Glycochenodeoxycholate</td>
<td>1.3 ± 3.3</td>
<td>0.1</td>
<td>0-12.6</td>
</tr>
<tr>
<td>3-sulfo-chenodeoxycholic</td>
<td>3.9 ± 0.5</td>
<td>1.0</td>
<td>0-16.9</td>
</tr>
<tr>
<td>7-sulfo-chenodeoxycholic</td>
<td>Trace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chenodeoxycholic</td>
<td>20.1 ± 13.6</td>
<td>21.0</td>
<td>0.1-44.7</td>
</tr>
<tr>
<td><strong>Ursodeoxycholic acid family</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tauroursodeoxycholate + Glycoursodeoxycholate</td>
<td>Trace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-sulfo-ursodeoxycholic</td>
<td>0.6 ± 0.4</td>
<td>0.1</td>
<td>0-7.0</td>
</tr>
<tr>
<td>Ursodeoxycholic</td>
<td>3.5 ± 4.5</td>
<td>2.0</td>
<td>0-17.2</td>
</tr>
<tr>
<td><strong>Lithocholic acid family</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taurolithocholate + Glycolithocholate</td>
<td>Trace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-sulfo-lithocholic</td>
<td>1.4 ± 1.0</td>
<td>0.5</td>
<td>0-10.6</td>
</tr>
<tr>
<td>Lithocholic</td>
<td>17.5 ± 13.1</td>
<td>19.2</td>
<td>1.2-52.7</td>
</tr>
<tr>
<td>Isolithocholic</td>
<td>4.0 ± 5.5</td>
<td>3.0</td>
<td>0-21.1</td>
</tr>
</tbody>
</table>
*Isochenodeoxycholic and isodeoxycholic acids have identical retention times by LC but quite different detector responses by MS. Therefore, their proportion could not be calculated by LC-MS. Consequently, the percent unconjugated bile acids represents a slight underestimate of the true value.
Table 3. *Bile acid composition (percent total) of human cecal contents by GC-MS*

(*N=19*)

<table>
<thead>
<tr>
<th>Bile acid</th>
<th>Abbreviation</th>
<th>Nuclear Substituents</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithocholic</td>
<td>LCA</td>
<td>3α-hydroxy</td>
<td>25.8 ± 10.4</td>
<td>28.8</td>
<td>0.6 - 45.2</td>
</tr>
<tr>
<td>Isolithocholic</td>
<td>IsoLCA</td>
<td>3β-hydroxy</td>
<td>8.1 ± 9.2</td>
<td>5.1</td>
<td>0 - 25.8</td>
</tr>
<tr>
<td>Deoxycholic</td>
<td>DCA</td>
<td>3α,12α-dihydroxy</td>
<td>33.8 ± 16.4</td>
<td>26.8</td>
<td>14.9 - 69.4</td>
</tr>
<tr>
<td>Isodeoxycholic</td>
<td>IsoDCA</td>
<td>3β,12α-dihydroxy</td>
<td>7.7 ± 9.5</td>
<td>4.6</td>
<td>0 - 38.6</td>
</tr>
<tr>
<td>Chenodeoxycholic</td>
<td>CDCA</td>
<td>3α,7α-dihydroxy</td>
<td>7.3 ± 7.9</td>
<td>2.4</td>
<td>0 - 45.8</td>
</tr>
<tr>
<td>Isochenodeoxychol</td>
<td>IsoCDCA</td>
<td>3β,7α-dihydroxy</td>
<td>5.1 ± 9.6</td>
<td>1.0</td>
<td>0 - 24.6</td>
</tr>
<tr>
<td>Ursodeoxycholic</td>
<td>UDCA</td>
<td>3α,7β-dihydroxy</td>
<td>5.5 ± 9.3</td>
<td>0</td>
<td>0 - 30.2</td>
</tr>
<tr>
<td>Isoursodeoxycholic</td>
<td>IsoUDCA</td>
<td>3β,7β-dihydroxy</td>
<td>0.6 ± 1.0</td>
<td>0</td>
<td>0 - 2.9</td>
</tr>
<tr>
<td>Cholic</td>
<td>CA</td>
<td>3α,7α,12α-trihydroxy</td>
<td>6.2 ± 9.5</td>
<td>0</td>
<td>0 - 25.4</td>
</tr>
<tr>
<td>Isocholic</td>
<td>IsoCA</td>
<td>3β,7α,12α-trihydroxy</td>
<td>0.2 ± 0.7</td>
<td>0</td>
<td>0 - 2.4</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. Comparison of 3α-hydroxy bile acid concentration as determined by the enzymatic method (left) and total (unsulfated) bile acid concentration (3α-hydroxy bile acids plus 3β-hydroxy bile acids) as determined by GC-MS (right).

Figure 2. Relative proportions of bile acids groups for individual samples of cecal content as determined by ESI-MS and ESI-MS-MS. Most bile acids are in unconjugated form.

Figure 3. Relative proportions of bile acids as determined by LC-MS. Left, percentage of DCA (unconjugated) and 3-sulfo-DCA. Right, percentage of CDCA (unconjugated) and 3-sulfo-CDCA.

Figure 4. Two representative mass spectra. M/z values for peaks corresponding to bile acids are shown: m/z 375, monohydroxy bile acids; 391, dihydroxy bile acids; 407, trihydroxy bile acids, and 471, monosulfated dihydroxy bile acids. The peak at 453 has an m/z value corresponding to the sulfate of an unsaturated monohydroxy bile acid, but by MS-MS, at least two non-bile acids are present in the peak. One of these is a sulfate. In most samples, a peak at m/z 453 was present in only trace proportions.
Figure 5. Representative gas chromatograms of cecal bile acids, chromatographed as their methyl ester per-acetate derivatives. Labeled peaks indicate bile acids identified by their mass spectra. Unlabeled peaks are not bile acids. Left panel: Peak A, isolithocholic acid (isoLCA); peak B, LCA; peak C, isodeoxycholic acid (isoDCA) plus non-bile acid contaminant; peak D, DCA; peak E, isochenodeoxycholic acid (isoCDCA); peak F, CDCA; peak G, isocholeic acid (isoCA) (second half of peak); peak H, CA. Right panel. Peak A, IsoLCA, peak B, LCA; peak C, isoDCA; peak D, DCA; peak E, isoCDCA; peak F, CDCA; peak G, isoCA; peak H, CA; peak I, UDCA; and peak J, hyocholic acid (internal standard).
Figure 1

![Graph showing bile acid concentration vs. 3α-hydroxy bile acids and total 3-hydroxy bile acids]
Figure 3
Figure 5