Feeding natural hydrophilic bile acids inhibits intestinal cholesterol absorption: studies in the gallstone-susceptible mouse

David Q.-H. Wang,1,2 Susumu Tazuma,3 David E. Cohen,4 and Martin C. Carey2
1Division of Gastroenterology, Department of Medicine, Beth Israel Deaconess Medical Center, and 2Division of Gastroenterology, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School and Harvard Digestive Diseases Center, Boston, Massachusetts 02215; 3First Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734-8551, Japan; and 4Marion Bessin Liver Research Center, Albert Einstein College of Medicine, Bronx, New York 10461

Submitted 2 April 2003; accepted in final form 7 May 2003

Wang, David Q.-H., Susumu Tazuma, David E. Cohen, and Martin C. Carey. Feeding natural hydrophilic bile acids inhibits intestinal cholesterol absorption: studies in the gallstone-susceptible mouse. Am J Physiol Gastrointest Liver Physiol 285: G494–G502, 2003. First published May 15, 2003; 10.1152/ajpgi.00156.2003.—We explored the influence of the hydrophilic-hydrophobic balance of secreted bile salts in the bile salt pool on the efficiency of intestinal cholesterol absorption in mice. Male C57L/J mice were fed standard chow or chow supplemented with 0.5% cholic; chenodeoxycholic; deoxycholic; dehydrocholic; hyocholic; hyodeoxycholic; orursodiol acid for 7 days. Biliary bile acids were measured by reverse-phase HPLC, and hydrophobicity indices were estimated by Hulten’s method. Cholesterol absorption efficiency was determined by a plasma dual-isotope ratio method. In mice fed chow, natural proportions of tauro-β-muricholate (42 ± 6%) and taurocholate (50 ± 7%) with a hydrophobicity index of −0.35 ± 0.04 produced cholesterol absorption of 37 ± 5%. Because bacterial and especially hepatic biotransformations of specific bile acids occur, hydrophobicity indices of the resultant bile salt pools differed from fed bile acids. We observed a significant positive correlation between hydrophobicity indices of the bile salt pool and percent cholesterol absorption. The principal mechanism whereby hydrophilic bile acids inhibit cholesterol absorption appears to be diminution of intraluminal micellar cholesterol solubilization. Gene expression of intestinal sterol efflux transporters Abcg5 and Abcg8 was upregulated by feeding cholic acid but not by hydrophilic β-muricholic acid nor by hydrophobic deoxycholic acid. We conclude that the hydrophobicity of the bile salt pool predicts the effects of individual fed bile acids on intestinal cholesterol absorption. Natural α- and β-muricholic acids are the most powerful inhibitors of cholesterol absorption in mice and might act as potent cholesterol-lowering agents for prevention of cholesterol deposition diseases in humans.

ATP-binding cassette transporters; bile flow; bile salt pool size; biliary secretion; mixed micelles

HIGH EFFICIENCY OF INTESTINAL cholesterol absorption is an independent risk factor for cholesterol gallstones in mice (45), and more hydrophilic bile acids prevent cholesterol gallstone formation in several animal models (12, 31, 32, 37, 47) in part by inhibiting intestinal cholesterol absorption (47). The detergency of bile acids is obligatory for intestinal cholesterol uptake through micellar solubilization of the intraluminal sterol (33). In comparisons of the effectiveness of cholic acid (CA), ursodeoxycholic acid (UDCA), and chenodeoxycholic acid (CDCA) on cholesterol absorption, the influence of these bile acids has been studied in humans (13, 16, 19, 20, 22–24) and several animal species (4, 7, 11, 14, 27, 30, 38, 42, 47, 49). From these studies, it is apparent that the variable effects of individual bile acids on cholesterol absorption may be attributed to their hydrophilic-hydrophobic balance (6), which is known to predict their sterol solubilization efficiency (6). For example, the hydrophilic properties of UDCA preclude efficient sterol solubilization, and as a result cholesterol absorption from the intestine is reduced when UDCA is fed chronically to mice through formation of tauroursodeoxycholate (TUDC)-rich bile. However, dehydrocholic acid (DHA), a more hydrophilic bile acid than UDCA, does not exert a greater effect (9), most likely due to its hepatic and bacterial biotransformations with increases in biliary taurodeoxycholate (TDC) and taurochenodeoxycholate (TCDC) and a decrease in tauro-β-muricholate (T-β-MC) levels (9). These limited observations suggest that the hydrophilic-hydrophobic balance of secreted bile salts in the bile salt pool may have a close relationship to the efficiency of intestinal cholesterol absorption.

Nonetheless, several studies in the literature suggest that effects of different bile acids on cholesterol absorption may not be related simply to their solubilization capacity. In two animal studies (42, 49), the investigators noted that feeding CA promotes intestinal cholesterol absorption more efficiently than CDCA, yet in vitro the conjugates of the former solubilize less cholesterol at equilibrium than do the latter (40). Furthermore, other workers (11, 27) have suggested that neither CA nor its taurine conjugate (taurocholate; TC) added to chow or to cholesterol-enriched diets influence cholesterol absorption in the mouse.

Address for reprint requests and other correspondence: D. Q.-H. Wang, Dept. of Medicine, Gastroenterology Division, Beth Israel Deaconess Medical Center, 330 Brookline Ave., DA 601, Boston MA 02215 (E-mail: dqwang@caregroup.harvard.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Together, these divergent studies indicate that the influence of individual bile acids on cholesterol absorption require systematic evaluation in a well-characterized animal model.

In the current study, we used gallstone-susceptible C57L mice carrying several Lith alleles (44) to investigate how variations in the hydrophilic-hydrophobic balance of bile acids influence intestinal cholesterol absorption. Our results demonstrate that the overall hydrophilic-hydrophobic balance of the bile salt pool, and not the fed bile acids per se, is the critical determinant of cholesterol absorption from the small intestine. As inferred from gene expression studies, the intestinal sterol efflux transporters Abcg5 and Abcg8 were upregulated by feeding CA but not by the more hydrophilic β-muricholic acid (β-MCA) nor by the more hydrophobic deoxycholic acid (DCA). Most likely this finding is related to the augmented mass of cholesterol and oxysterols that were absorbed by being solubilized in a bile salt pool composed principally of TC. Overall, our study establishes that natural hydrophilic bile acids are powerful inhibitors of intestinal cholesterol absorption in the mouse.

MATERIALS AND METHODS

Chemicals. CA was purchased from Sigma (St. Louis, MO). UDCA, CDCA, and DCA were from Falk (Freiburg, Germany). DHCA, hyocholic acid (HCA), hyodeoxycholic acid (HDCA), and ursodeoxycholic acid (3α,7β,12α-trihydroxy-5β-cholan-24-oic; UCA) were purchased from Calbiochem-Behring (San Diego, CA), and α-, β-, and ω-MCAs were from Tokyo Tanabe (Tokyo, Japan). Purity of individual bile acids was >98% as determined by HPLC and thin layer chromatographic analyses (40). Intralipid (20% w/vol) was purchased from Pharmacia (Clayton, NC), and medium-chain triglyceride was from Mead Johnson (Evansville, IN). [1,2-3H]cholesterol and [4,4-14C]cholesterol were purchased from New England Nuclear Life Science Products (Boston, MA), and [5,6-3H]sitosterol was from American Radiolabeled Chemicals (St. Louis, MO). Radiochemical purity of all isotopes was >98% as determined by HPLC or thin layer chromatographic analyses.

Animals and diets. Inbred C57L/J male mice, 6–8 wk old, were purchased from Jackson Laboratory (Bar Harbor, ME) and were maintained in a temperature-controlled room (22 ± 1°C) with a 12:12-h light-dark cycle (lights on 6 AM–6 PM). Mice were allowed to adapt to the environment for at least 2 wk before bile acid feeding and were provided free access to water and rodent chow (Harlan Teklad Laboratory, Madison, WI) containing trace (≤0.02%) cholesterol (42). Once mice reached 10 wk of age, they were fed chow or chow supplemented with 0.5% (by weight) each of CA, CDCA, DHCA, DCA, HCA, HDCA, α-MCA, β-MCA, ω-MCA, UDCA, or UCA. Bile acids were added in ethanol to the powdered chow and dried for 48 h on trays under reduced pressure in a 60°C oven. All experiments were executed according to accepted criteria for the care and experimental use of laboratory animals, and euthanasia was consistent with recommendations of the American Veterinary Medical Association. Protocols were approved by the Institutional Animal Care and Use Committees of Harvard University.

Cannulation of the common bile duct and collection of hepatic bile. For biliary lipid secretion studies (44), 12 groups of mice (n = 5/group) fed chow or chow supplemented with 0.5% of each bile acid (see Animals and diets) for 7 days were used. In brief, nonfasted mice were anesthetized with intraperitoneal injections of 35 mg/kg pentobarbital. The cystic duct was doubly ligated, and a cholecystectomy was performed. The common bile duct was then cannulated via a PE-10 polyethylene catheter. Hepatic bile was collected by gravity during the first hour of acute fistulation for measurement of biliary lipid secretion rates and molecular compositions of the bile salt pool. The circulating bile salt pool sizes were determined by employing 8-h biliary “washout” techniques (44). During surgery and bile collection, mouse body temperature was maintained at 37 ± 0.5°C with a heating lamp and monitored with a thermometer. After hepatic bile volumes were determined by weighing and employing a specific density of 1, bile samples were frozen and stored at −20°C for later lipid analyses (see Lipid analyses).

Determination of intestinal cholesterol absorption. For measurement of intestinal cholesterol absorption, 12 groups of mice (n = 10–22/group) were fed either chow or chow supplemented with 0.5% bile acid (see Animals and diets) for 7 days. We validated earlier that this feeding period was long enough to reach a steady state of intestinal tissue was removed in bile (44). Cholesterol absorption was determined by a plasma dual-isotope ratio method (41, 42, 46). In brief, nonfasted mice were anesthetized with pentobarbital. Exactly 2.5 μCi of [3H]cholesterol in 100 μl of Intralipid was injected intravenously via the jugular vein. Following this procedure, each animal was given, by gavage, an intragastric dose of 1 μCi of [14C]cholesterol in 150 μl of medium-chain triglyceride. After being dosed, mice were transferred to individual cages with wire-mesh bottoms, where they were free to eat chow or the appropriate bile acid and diet for an additional 3 days. Mice were then anesthetized and bled from the heart into heparinized microtubes. Ten milliliters of EcoLite were mixed with 100 μl of plasma and 100 μl of the original dosing mixture. Plasma ratios of the two radioisotopes were determined by liquid scintillation spectrometry, and the percent cholesterol absorption was calculated (41, 42, 46).

Measurement of small intestinal transit times. Small intestinal transit was measured according to previous methods (46). In brief, mice (n = 7/group) were housed individually in cages with wire-mesh bottoms and fed chow or chow supplemented with 0.5% CA or β-MCA until being fasted 18 h before study. Exactly 2 μCi of [3H]sitosterol in 100 μl of medium-chain triglyceride was instilled into the duodenum via a transabdominal catheter implanted surgically 24 h earlier. Exactly 30 min later, the mice were anesthetized with pentobarbital, the abdomen was opened quickly, and the stomach, small, and large intestines, and cecum were removed. The small intestine was frozen with liquid nitrogen and cut into 20 equal segments with a scalpel blade. The radioisotope was isolated from individual intestinal segments, and radioactivity was determined by liquid scintillation counting. Samples of stomach, cecum, and large intestine were also analyzed, but none showed appreciable radioactivity. Intestinal transit time was evaluated by a geometric center method as described elsewhere (46).

Quantitative real-time PCR assays of Abcg5/g8, the sterol efflux transporters. Freshly harvested small intestines were flushed with ice-cold saline and cut into three segments with duodenum/jejunum ileum length ratios of 1:3:2. In the middle of each segment, 1.5 cm of intestinal tissue was removed, and tissues from four mice per strain were pooled. Total RNA was extracted using RNeasy Midi (Qiagen, Valencia, CA), and reverse-transcription reaction was performed using the SuperScript II first-strand synthesis system (Invitrogen, Carlsbad, CA) with 5 μg of total RNA and random hexamers

G495
to generate cDNA. Primer Express software (Applied Biosystems, Foster City, CA) was used to design the following primers: mouse Abcg5 (AF312713), forward 5’-TGGATGCCTGACCTTGCGAT-3’; probe 5’-CAAGCTGTCGTTCCTCCGGTGGTG-3’; mouse Abcg8 (AF324495), forward 5’-TGGATGTCGCCTGTGACATC-3’; reverse 5’-AATTTGAGATCTGCAOACC-3’; probe 5’-GACCTCTTCGCTGCTGG-3’. Real-time PCR assays were performed in triplicate on a GeneAmp 5700 sequence detection system (Applied Biosystems). The real-time PCR reaction contained, in a final volume of 50 μl, 1 μl of cDNA, optimized concentration (100–300 μM) of each primer, 200 μM of probe, and 25 μl of 2X TaqMan Universal PCR Master Mix (Applied Biosystems). Relative mRNA levels were calculated by using the threshold cycle of an unknown sample against a standard curve with known copy numbers. To obtain a normalized target value, the target amount was divided by the endogenous reference quantity of rodent Gapdh as control (Applied Biosystems).

Lipid analyses. Total and individual bile salt concentrations were measured by HPLC according to the method of Rho et al. Biliary phospholipids were hydrolyzed in inorganic phosphorus by the method of Bartlett (2). Cholesterol content in chow and biliary cholesterol levels were determined by HPLC (42). Hydrophobicity indices of individual bile acids as well as bile salts in hepatic biles were calculated according to Heuman’s method (18).

Statistical methods. All data are expressed as means ± SD. Statistically significant differences among groups of mice fed chow or different bile acids were assessed by Student’s t-test or by Mann-Whitney U-test. Analyses were performed with SuperANOVA software (Abacus Concepts, Berkeley, CA). Employing linear regression analyses, parameters associated significantly with the hydrophobicity index and percent cholesterol absorption were further assessed by a stepwise multiple regression analysis to identify independence of the associations. Statistical significance was defined as a two-tailed probability of <0.05.

RESULTS

Molecular compositions and hydrophobicity of the bile salt pool. Table 1 summarizes bile salt species and hydrophobicity indices of the biliary bile salt pools. HPLC analyses were carried out during the first hour of biliary secretion in individual hepatic biles of C57L mice fed bile acids for 7 days. This revealed that all bile salts were taurine conjugated. The predominant bile salt species in chow-fed mice were TC (49.7 ± 6.7%) and T-ß-MC (42.3 ± 5.7%), whereas T-ω-MC (1.4 ± 0.6), TUDC (2.6 ± 1.6%), TCDC (0.6 ± 0.3%), and TDC (3.4 ± 1.3%) were present in small concentrations. In mice fed UDCA, UCA, CA, ß-MCA, ω-MCA, α-MCA, HCA, or HDCA, the conjugates of the fed bile acids became the predominant (50–86%) bile salts in bile (Table 1). This indicates that the fed bile acids were absorbed from the intestine, delivered in portal blood to the liver, conjugated with taurine, and incorporated efficiently into the bile salt pool. Furthermore, feeding UDCA, UCA, ω-MCA, α-MCA, HCA, and HDCA reduced TC and T-ß-MC levels in bile significantly (Table 1). Mice fed CA display a marked increase in TC (83.3 ± 4.7%) and a strong decrease in T-MC concentrations (3.9 ± 1.6%). In certain instances, bile acid biotransformation occurred consequent to bile acid feeding: for example, during CDCA administration, T-ß-MC (64.2 ± 1.8%) became the major constituent of the bile salt pool, followed by TCDC (24.8 ± 8.3%), with TC showing a significant decrease (5.2 ± 1.5%). When mice were fed DCA, the formation of TC (66.2 ± 8.8%) from rodent hepatic 7α-hydroxylation was appreciably larger than that of TDC (26.5 ± 7.9%), and both were accompanied by a significant decrease in T-ß-MC (4.7 ± 2.2%). Feeding DHCA significantly increased TDC (18.0 ± 3.4%), TCDC (9.1 ± 2.3%), and TUDC (7.1 ± 3.7%), but TC remained unchanged and T-ß-MC (12.2 ± 3.0%) decreased. These biotransformations occurred as a result of the rich capacity of the mouse liver to hydroxylate the steroid nucleus of both endogenous and exogenous bile acids in the 6β and 7α positions. Because of hepatic metabolism of specific bile acids, hydrophobicity indices of biliary bile salt pools (Table 1) differed markedly from the individually fed bile acids (Fig. 1).

Table 1. Percent bile salt species in hepatic bile acids after biliary feeding

<table>
<thead>
<tr>
<th>Bile Acid</th>
<th>TC</th>
<th>T-ß-MC</th>
<th>TCDC</th>
<th>T-ω-MC</th>
<th>TUDC</th>
<th>TDC</th>
<th>T-α-MC</th>
<th>TUC</th>
<th>THC</th>
<th>THDC</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow</td>
<td>49.7±6.7</td>
<td>42.3±5.7</td>
<td>0.6±0.3</td>
<td>1.4±0.6</td>
<td>2.6±1.6</td>
<td>3.4±1.3</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>-0.33±0.04</td>
</tr>
<tr>
<td>CA</td>
<td>83.3±4.7</td>
<td>3.9±1.6</td>
<td>2.0±1.1</td>
<td>0.9±0.7</td>
<td>1.5±1.2</td>
<td>8.4±2.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>ß-MCA</td>
<td>13.7±3.4</td>
<td>84.4±3.3</td>
<td>0.2±0.1</td>
<td>0.0±0.0</td>
<td>1.7±0.1</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>CDCA</td>
<td>5.2±1.5</td>
<td>64.2±1.8</td>
<td>24.8±8.3</td>
<td>0.0±0.0</td>
<td>5.1±1.7</td>
<td>0.7±0.5</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>ω-MCA</td>
<td>11.7±1.2</td>
<td>30.1±2.9</td>
<td>1.4±0.5</td>
<td>53.0±2.1</td>
<td>2.2±0.2</td>
<td>1.5±0.8</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>UDCA</td>
<td>0.8±0.2</td>
<td>9.3±3.0</td>
<td>2.2±2.1</td>
<td>1.7±0.8</td>
<td>85.7±4.4</td>
<td>0.2±0.1</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>-0.48±0.04</td>
</tr>
<tr>
<td>DCA</td>
<td>66.2±8.8</td>
<td>4.7±2.2</td>
<td>0.7±0.3</td>
<td>0.0±0.1</td>
<td>1.5±0.8</td>
<td>26.6±7.9</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.11±0.04</td>
</tr>
<tr>
<td>α-MCA</td>
<td>13.7±5.1</td>
<td>30.4±7.8</td>
<td>6.5±0.3</td>
<td>0.0±0.0</td>
<td>2.8±2.3</td>
<td>1.2±1.6</td>
<td>51.4±6.9</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>UCA</td>
<td>15.7±3.4</td>
<td>11.5±4.1</td>
<td>3.8±3.2</td>
<td>0.0±0.0</td>
<td>5.2±2.3</td>
<td>2.4±1.3</td>
<td>0.0±0.0</td>
<td>61.4±7.1</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>-0.66±0.07</td>
</tr>
<tr>
<td>HCA</td>
<td>11.9±2.8</td>
<td>17.8±3.2</td>
<td>2.6±2.8</td>
<td>0.0±0.0</td>
<td>4.8±1.6</td>
<td>0.6±0.1</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>62.3±3.4</td>
<td>0.0±0.0</td>
<td>-0.39±0.05</td>
</tr>
<tr>
<td>HDCA</td>
<td>7.1±3.1</td>
<td>16.4±4.0</td>
<td>4.1±2.0</td>
<td>12.5±5.5</td>
<td>11.0±4.5</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>49.1±17.0</td>
<td>0.0±0.0</td>
<td>-0.01±0.02</td>
</tr>
</tbody>
</table>

Values are mean ± SD determined from 5 hepatic biles (first hour of biliary secretion) per group. TC, taurocholate; T-ß-MC, tauro-ß-muricholate; TCDC, taurochenodeoxycholate; T-ω-MC, tauro-ω-muricholate; TUDC, tauroursodeoxycholate; TDC, taurodeoxycholate; T-α-MC, tauro-α-muricholate; TUC, taurourscholate; THC, taurocholate; THDC, taurodeoxycholate; HL, hydrophobicity index; UDCA, ursodeoxycholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; MCA, muricholic acid; UCA, ursodeoxycholic acid; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; DHCA, dehydroxycholic acid.
Influence of bile salt hydrophobicity on bile flow, biliary lipid secretion, and bile salt pool sizes. Table 2 lists mean bile flow rates at 7 days during acute (first-hour) interruption of the enterohepatic circulation in mice fed chow or chow supplemented with 0.5% bile acid. Bile flow rates demonstrated a great degree of variability (87–119 μl·min⁻¹·kg⁻¹) but approximated the results observed with chow (P = not significant (NS)). Table 2 also summarizes biliary cholesterol, phospholipid, and bile salt secretion rates. Compared with lipid secretion rates of chow-fed mice (cholesterol = 10.2 ± 3.5 and phospholipid = 25.6 ± 4.5 μmol·h⁻¹·kg⁻¹), biliary cholesterol outputs were markedly decreased (8.1–8.9 μmol·h⁻¹·kg⁻¹) with unaltered phospholipid outputs (26.2–31.9 μmol·h⁻¹·kg⁻¹) in mice fed hydrophilic bile acids (UDCA, UCA, α-, β-, and ω-MCA, and HCA). In contrast, administration of more hydrophobic bile acids (CA and DCA) increased both biliary cholesterol (11.2–12.6 μmol·h⁻¹·kg⁻¹) and phospholipid outputs (29.1–31.2 μmol·h⁻¹·kg⁻¹). We noted that dietary supplementation with the more hydrophilic DHCA markedly augmented biliary cholesterol secretion by 40% (14.2 ± 5.6 μmol·h⁻¹·kg⁻¹) but decreased biliary phospholipid secretion by 10% (23.4 ± 4.9 μmol·h⁻¹·kg⁻¹). Because its biotransformation produced T-β-MC, CDCA reduced biliary cholesterol secretion (9.0 ± 3.3 μmol·h⁻¹·kg⁻¹) without altering biliary phospholipid output (25.6 ± 4.5 μmol·h⁻¹·kg⁻¹). Figure 2A shows that there are significant and positive linear correlations between the hydrophobicity indices of the biliary bile salt pools and biliary cholesterol outputs (P < 0.0001, r = 0.91). Figure 2B shows a similar positive correlation for cholesterol/phospholipid molar ratios in hepatic bile and hydrophobicity indices (P < 0.001, r = 0.85). This suggests that decreasing hydrophobicity of the bile salt pool reduces biliary cholesterol secretion significantly in mice (5). No correlations were found between the hydrophobicity indices of the bile salt pools and the biliary outputs of either phospholipids or bile salts. The range of bile salt outputs (Table 2) in mice fed various bile acids (159.3–200.7 μmol·h⁻¹·kg⁻¹) was comparable with those on chow (157.9 ± 56.8 μmol·h⁻¹·kg⁻¹) and showed no statistically significant differences. Furthermore, compared with chow, we did not find any significant differences in sizes of circulating (3.2–3.9 μmol) or total (4.7–5.3 μmol) bile salt pools in mice fed bile acids.

**Table 2. Bile flow and biliary lipid outputs during bile acid feeding**

<table>
<thead>
<tr>
<th>Bile Acid</th>
<th>Flow Rate, μl·h⁻¹·kg⁻¹</th>
<th>Secretion Rate of Biliary Lipids, μmol·h⁻¹·kg⁻¹</th>
<th>Ch/PL</th>
<th>PL/BS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow</td>
<td>95 ± 14</td>
<td>10.2 ± 3.5 to 25.6 ± 4.5</td>
<td>157.9 ± 56.8</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td>CA</td>
<td>119 ± 23</td>
<td>12.6 ± 4.2 to 29.1 ± 5.6</td>
<td>200.7 ± 62.3</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>α-MCA</td>
<td>107 ± 30</td>
<td>7.9 ± 3.1 to 31.9 ± 3.8</td>
<td>171.2 ± 58.9</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>CDCA</td>
<td>97 ± 13</td>
<td>9.0 ± 3.3 to 25.4 ± 4.9</td>
<td>180.1 ± 60.5</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>ω-MCA</td>
<td>97 ± 19</td>
<td>8.2 ± 3.4 to 30.2 ± 3.9</td>
<td>182.0 ± 52.7</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>UDCA</td>
<td>107 ± 16</td>
<td>8.8 ± 2.8 to 26.2 ± 5.3</td>
<td>179.2 ± 64.3</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>DCA</td>
<td>87 ± 12</td>
<td>11.2 ± 4.3 to 31.2 ± 6.1</td>
<td>199.6 ± 63.3</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>ω-MCA</td>
<td>100 ± 14</td>
<td>8.1 ± 3.5 to 31.2 ± 4.0</td>
<td>179.4 ± 64.3</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>UCA</td>
<td>96 ± 16</td>
<td>9.7 ± 3.7 to 23.2 ± 3.8</td>
<td>159.3 ± 49.8</td>
<td>0.42 ± 0.05</td>
</tr>
<tr>
<td>HCA</td>
<td>102 ± 21</td>
<td>9.8 ± 3.9 to 24.7 ± 3.9</td>
<td>167.2 ± 52.1</td>
<td>0.36 ± 0.04</td>
</tr>
<tr>
<td>HDCA</td>
<td>117 ± 13</td>
<td>11.2 ± 4.3 to 28.1 ± 4.2</td>
<td>169.2 ± 59.8</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>DHCA</td>
<td>108 ± 15</td>
<td>14.2 ± 5.6 to 23.6 ± 4.5</td>
<td>187.5 ± 63.4</td>
<td>0.59 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD determined from 5 hepatic biles (first hour of biliary secretion) per group. Ch, cholesterol; PL, phospholipid; BS, bile salt.
Influence of bile acid species on intestinal cholesterol absorption. Figure 3 displays percent cholesterol absorption for each of the dietary bile acids tested as well as the chow control. Compared with cholesterol absorption on the chow diet (37 ± 5%), percent cholesterol absorption was inhibited significantly in mice fed the seven hydrophilic bile acids (Fig. 3) increasing in the rank order: β-MCA (11 ± 2%), α-MCA (12 ± 2%), HCA (14 ± 3%), ω-MCA (15 ± 3%), UCA (17 ± 4%), UDCA (19 ± 5%), and HDCA (30 ± 4%). In contrast, feeding DCA and CA, two hydrophobic bile acids, increased cholesterol absorption significantly (51 ± 6 and 63 ± 7%, respectively) from that with chow. Because of its hepatic biotransformation to principally T-β-MCA, CDCA, a hydrophobic bile acid, decreased cholesterol absorption to 25 ± 3%. In contrast, DHCA, a hydrophilic bile acid, increased cholesterol absorption to 45 ± 6% since it was biotransformed principally to the more hydrophobic taurocholate and taurodeoxycholate.

Fig. 3. Influence of the congener series of bile acids on percent cholesterol absorption when fed chronically to C57L mice. Cholesterol absorption was determined by the plasma dual-isotope ratio method (41, 42, 46) in 12 groups of male C57L mice (n = 10–22/group) fed chow or chow supplemented with 0.5% (by weight) of each bile acid. Compared with chow (37 ± 5%), cholesterol absorption was lowered significantly by β-MCA (11 ± 2%), α-MCA (12 ± 2%), HCA (14 ± 3%), ω-MCA (15 ± 3%), UCA (17 ± 4%), UDCA (19 ± 5%), and HDCA (30 ± 4%). In contrast, feeding DCA and CA, two hydrophobic bile acids, increased cholesterol absorption significantly (51 ± 6 and 63 ± 7%, respectively) from that with chow. Because of its hepatic biotransformation to principally T-β-MCA, CDCA, a hydrophobic bile acid, decreased cholesterol absorption to 25 ± 3%. In contrast, DHCA, a hydrophilic bile acid, increased cholesterol absorption to 45 ± 6% since it was biotransformed principally to the more hydrophilic taurocholate and taurodeoxycholate.

Fig. 4. Relationship between percent cholesterol absorption and hydrophilic-hydrophobic balance of the biliary bile salt pools. These results display a significant (P < 0.0001), positive (r = 0.95) correlation between the percent cholesterol absorption and hydrophobicity indices of increasing hydrophobicity of the bile salt pool (Table 1).
the bile salt pool. As a corollary, the efficiency of intestinal cholesterol absorption is curtailed significantly by decreasing hydrophobicity of the bile salt pool.

**Small intestinal transit times.** We found that small intestinal transit, as inferred from the distributions of radioactivity in the small intestine, were essentially unchanged in mice fed the relatively hydrophobic CA or hydrophilic β-MCA compared with chow (data not shown). Mean values for the geometric centers of the profiles were 10.5 ± 1.1, 11.0 ± 0.9, and 10.7 ± 1.0 for CA and β-MCA (P = NS). These results demonstrate that feeding neither hydrophobic nor hydrophilic bile acids alters small intestinal transit time in the mouse.

**Gene expression of intestinal sterol efflux transporters.** Figure 5 shows relative mRNA levels of Abcg5 and Abcg8, two genes that encode the half-transporters for efflux of sterols from enterocytes in duodenum, jejunum, and ileum of C57L mice fed chow and chow supplemented with 0.5% β-MCA, CA, or DCA for 7 days. Gene expression levels of Abcg5 and Abcg8 in the duodenum were lower than in jejunum or ileum but were essentially identical among the four groups of mice. In the jejunum and ileum, gene expression levels were similar in mice fed β-MCA and DCA compared with chow feeding but were increased significantly by CA feeding.

**DISCUSSION**

Long-term administration of two hydrophilic bile acids, UDCA in the human (35) and β-MCA in the mouse (47), has been shown to promote the dissolution of cholesterol gallstones. Although decreasing intestinal cholesterol absorption is one of the most important therapeutic mechanisms whereby UDCA and β-MCA elicit this salutary response, the effectiveness of other individual bile acids on cholesterol absorption has not been systematically evaluated. In the present study, we used a wide hydrophilic-hydrophobic range of unconjugated bile acids to investigate the relationship between the efficiency of intestinal cholesterol absorption and the physical-chemical properties of individual bile acids, specifically hydrophobicity of the biliary bile salt pools. We also documented biliary bile salt metabolism, biliary lipid secretion, and small intestinal transit times during these feeding studies. The most important findings were: 1) the overall hydrophilic-hydrophobic balance of the bile salts in bile plays an important role in regulating intestinal cholesterol absorption; 2) natural hydrophilic bile acids are powerful inhibitors of intestinal cholesterol absorption; and 3) gene expression levels of the intestinal sterol efflux transporters Abcg5 and Abcg8 are upregulated by feeding CA but not by the hydrophilic β-MCA nor by the hydrophobic DCA.

When an individual bile acid is fully ionized, its hydrophobicity index (18) is determined by the number, position, and orientation of hydroxyl groups, which are crucial factors in determining their physical-chemical and physiological functions. Bile salts, together with ionized and nonionized fatty acids, monoacylglycerides, lysophospholipids, and unesterified cholesterol, form intestinal mixed micelles (17, 34). These micelles function as a concentrated reservoir and transport vehicle for cholesterol across the unstirred water layer toward the brush border of the small intestine to facilitate uptake of monomeric cholesterol by the enterocyte. As shown in the present study, the efficiency of intestinal cholesterol absorption was inhibited significantly by feeding hydrophilic bile acids (Figs. 3 and 4). Within the intestinal lumen, the presence of hydrophilic bile salts may reduce solubility of cholesterol by inducing phase separation of the sterol from mixed micelles to a coexisting liquid crystalline vesicle phase. Most likely, hydrophilic bile salts facilitate incorporation of cholesterol molecules into a stable liquid crystalline-vesicle phase (17, 34) from which they are poorly absorbed by small intestinal enterocytes (8). In contrast, feeding CA increased hydrophobicity of bile salts in bile, which markedly increased micellar cho-
Bile acids and intestinal cholesterol absorption

G500

BIL ET ACIDS AND INTESTINAL CHOLESTEROL ABSORPTION

lesterol solubility (40) and thereby augmented choles-
terol absorption (Ref. 42 and Fig. 3). This suggests that
hydrophobic bile acids are more effective promoters of
cholesterol absorption than hydrophilic bile acids.
However, CDCA, a more hydrophobic bile acid than
CA, paradoxically caused a reduction of intestinal cho-
lesterol absorption since it was extensively biotrans-
formed in the liver to the hydrophilic T-β-CA, paradoxically caused a reduction of intestinal cho-
terol absorption (Ref. 42 and Fig. 3). This suggests that
lesterol solubility (40) and thereby augmented choles-
terol absorption markedly because of its bio-
transformations into TDC and TCDC, both hydrophobic
partners in the bile salt pool. Because of their novel
biotransformations in this rodent model, the hydropho-
bicity indices of individual bile acids fed cannot invari-
ably forecast their cholesterol absorption efficiency. It
is clear that a knowledge of the hydrophobicity index
of the bile salt pool is necessary to predict the influence
of individual bile acids on intestinal cholesterol absorp-
tion.

Biliary secretion rates of bile salts can also demon-
strate marked effects on intestinal cholesterol absorp-
tion (29, 39, 43, 47), most likely related to the increased
micellar solubilization capacity of an augmented flow
of bile salts within the upper small intestine. We (44,
46) have observed that gallstone-susceptible C57L
mice display significantly higher biliary bile salt out-
puts and intestinal cholesterol absorption compared
with gallstone-resistant AKR mice. However, we found
in this study that feeding various bile acids did not
alter bile salt pool sizes or biliary outputs significantly
(Table 2). This is consistent with an adaptive response
to alterations in the bile salt pool sizes and outputs at
the level of the ileal apical sodium-dependent bile acid
transporter (1). In addition, we observed a significant
positive correlation between hydrophobicity of the bili-
yre bile salts and biliary outputs of cholesterol (Fig.
2A), suggesting that, compared with hydrophobic bile
acids, not only do hydrophilic bile acids curtail intesti-
nal cholesterol absorption, but they are also less effi-
cient in promoting biliary cholesterol secretion (Table
2). These hydrophilic bile acids, as might be expected,
are significantly associated with decreases in chole-
terol/phospholipid ratios in bile (Fig. 2B) and conse-
quently in the upper small intestine, where during
digestion they may reduce transfer of cholesterol mol-
cules from intermediate liquid crystalline/vesicle
phases to mixed micellar phases (9, 17, 34). Overall,
enrichment of bile with hydrophilic bile acids may
reduce both concentration and bioavailability of in-
traluminal cholesterol molecules for absorption by en-
terocytes.

The newly identified intestinal ATP-binding cassette
transporters ABCG5 and ABCG8 (3, 21) in humans are
apical cholesterol export pumps that efflux cholesterol
(and most phytosterols) from enterocytes back to the
intestinal lumen and reduce their fractional absorption
(50). We observed that feeding the hydrophilic β-MCA
did not influence expression of the genes for these two
transporters. Nonetheless, feeding CA, and to a lesser
extent DCA (Fig. 5), did so significantly by upregulat-
ing mRNA expression of the jejunal and ileal Abcg5
and Abcg8. This response is most likely an indirect
effect because expression of these ABC transporters is
highly sensitive to the mass of absorbed cholesterol (3,
10), which is promoted principally by feeding CA (Fig. 3).
Another possible explanation is that CA feeding may
markedly increase intestinal absorption of biliary (and
dietary) oxysterols (15) that bind and activate the ox-
ysterol receptor LXR, a transcriptional regulator of
these transporter genes (26). Consistent with our
results, Watt and Simmonds (48) observed many years
ago that TC infused intraduodenally in bile-diverted
rats produced significantly higher uptake and trans-
port of intestinal cholesterol compared with TDC or
UDC. This may explain why intestinal Abcg5 and
Abcg8 expression increased significantly only during
CA feeding as a result of increased sterol uptake. Our
data do not exclude the possibility that CA per se may
have a specific enhancing effect on the expression of
the genes for these sterol efflux transporters. We took
note that in the chow-fed mouse, the mRNA levels of
Abcg5/g8 are slightly higher in the jejunum and duo-
denum compared with the ileum as assayed by North-
ern blot analysis (3, 26, 50). In the present study, the
expression levels of Abcg5/g8 are somewhat greater in
the jejunum and ileum than in the duodenum as mea-
sured by our highly precise quantitative real-time PCR
techniques. A possible reason for these differences is
that we cut the small intestines into three segments with
length ratios of 1:3:2 (duodenum/jejunum/ileum). Total
RNA was extracted from the middle 1.5 cm of
intestinal tissue in each segment. Other investigators
(3, 26, 50) extracted total RNA from the entire intesti-
nal mucosa of three segments with length ratios of
1:1:1 (duodenum/jejunum/ileum). Another possible ex-
planation is suggested by the different genetic back-
grounds of the murine strains employed in previous
and present studies.

The importance of small intestinal transit rates as a
determining factor of cholesterol absorption has been
validated in human (25) and animal studies (Ref. 36
and Wang DQ-H, Kopin AS, and Carey MC, unpub-
blished observations). Reynier et al. (27) concluded that
intestinal transit time is similar in mice whether CA,
CDCA, or UDCA is fed, as evidenced by the rates of
fecal excretion of unabsorbed cholesterol and its deriv-
atives together with a carmine red marker. In the
present study, our new and validated methodology (46)
adapted to luminal lipid transit in the mouse allowed
us to observe that feeding bile acids with different
hydrophobicities did not influence small intestinal
transit times in C57L mice.

In summary, the overall results of our study estab-
lish that modulating the hydrophilic-hydrophobic bal-
ance of the bile salt pool profoundly influences intesti-
nal cholesterol absorption. We speculate that the
mechanisms whereby hydrophilic bile acids inhibit in-
testinal cholesterol absorption is via the uptake step by
curtailing micellar cholesterol solubilization intralu-
minally. Hence, decreasing the hydrophobicity index
of the biliary bile salt pool reduces cholesterol’s bioavail-
ability for absorption by enterocytes. Although the size
and composition of the intestinal bile salt pool exert major influences on the amount of chylomicron cholesterol reaching the liver from the intestine, the pharmacological inhibition of various proteins involved in other steps in the absorption process, e.g., the putative sterol transporter in the brush-border membrane, acyl-CoA:cholesterol acyltransferase isozyme 2, or microsomal triglyceride transfer protein in the enterocyte, can also affect a dramatic change in cholesterol absorption without there being any change in the amount or species of bile salts in the intestinal pool. Nonetheless, our current study suggests that natural hydrophilic bile acids efficiently suppress cholesterol absorption and may act as potent plasma and biliary cholesterollowering agents, even more so than UDCA, for prevention of cholesterol deposition diseases in humans. An example of the latter is our recent study (47) showing that β-MCA prevents cholesterol gallstone formation in gallstone-susceptible C57L mice by chronically inhibiting intestinal cholesterol absorption.

We are greatly indebted to Helen H.-F. Wang (Beth Israel Deaconess Medical Center) for excellent technical assistance.

D. Q.-H. Wang is a recipient of a New Scholar Award from the Ellison Medical Foundation (1998–2003). This paper was presented in part at the Annual Meeting of the American Association of the Study for Liver Diseases, Dallas, TX, in 1999 and published as an abstract in Hepatology (30: 395A, 1999).

DISCLOSURES

This work was supported in part by the Ellison Medical Foundation (D. Q.-H. Wang) as well as research and center grants DK-54012 (D. Q.-H. Wang), DK-48873 and DK-56626 (D. E. Cohen), and DK-36588, DK-34854, and DK-52911 (M. C. Carey), all from the National Institute of Diabetes and Digestive and Kidney Diseases (US Public Health Service).

REFERENCES


27. Reynier MO, Montet JC, Gerolami A, Marteau C, Crotte C, Montet AM, and Mathieu S. Comparative effects of cholic, chenodeoxycholic, and ursodeoxycholic acids on micellar solubili-