Distribution of phosphatidylcholine molecular species between mixed micelles and phospholipid-cholesterol vesicles in human gallbladder bile: dependence on acyl chain length and unsaturation

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Abstract The partitioning of phosphatidylcholine (PC) molecular species between mixed micelles and vesicles was studied in each of seven human gallbladder biles. Biles were fractionated by Sephacryl S-300 SF gel filtration chromatography, and PC species in the micellar and vesicular fractions were quantitated by high performance liquid chromatography. Micelles were enriched in species containing unsaturated acyl groups (e.g., 16:1-18:2, 18:1-18:2, and 18:1-18:3); vesicles were enriched in more highly saturated species (e.g., 16:0-16:1, 16:0-18:1, and 18:0-18:1). Separate multivariate analyses for each bile demonstrated that the distribution of PC species between vesicles and micelles was related to the degree of *sn*-1 and *sn*-2 unsaturation, and *sn*-1, but not *sn*-2, chain length. In addition, the tendency to partition into the micellar phase was particularly marked when unsaturation was present at both the *sn*-1 and *sn*-2 positions. When this interaction was included in the multivariate analyses, the regression models accounted for virtually all of the variation in PC partitioning (for each of the seven patients $r^2 = 0.92$–0.98, $P < 0.03$). These results suggest that the partitioning of PC species between micelles and vesicles is strictly determined by *sn*-1 chain length and the degree of unsaturation at both the *sn*-1 and *sn*-2 positions. In light of recent reports that fatty acyl composition influences the cholesterol content of vesicles and micelles in model biles, these results raise the possibility that diet-induced alterations in the phospholipid species and the relative proportions of biliary lipid particles may influence the cholesterol-carrying capacity of bile.—**Booker, M. L., W. W. LaMorte, S. A. Ahrendt, K. D. Lillemoe, and H. A. Pitt.** Distribution of phosphatidylcholine molecular species between mixed micelles and phospholipid-cholesterol vesicles in human gallbladder bile: dependence on acyl chain length and unsaturation. *J. Lipid Res.* 1992. **33:** 1485–1492.

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Phospholipids (PL) and bile salts (BS) are essential molecules for the solubilization of cholesterol (CH) in bile (1, 2). PLs comprise roughly 15% of total biliary lipids on a molar basis, with approximately 95% of biliary PL consisting of phosphatidylcholine (PC) (3). Within the PC class is a number of molecular species containing unique pairs of fatty acids (FAs) of varying carbon chain lengths and degrees of unsaturation (4). The *sn*-1 and *sn*-2 positions on the glycerol backbone of the PC molecule are generally occupied by a saturated and an unsaturated FA, respectively. Recent studies have indicated that the molecular species of biliary PC may be altered by diet (4, 5) or disease (6, 7), and such changes may have consequences for CH solubilization.

Cholesterol in bile was believed to be carried entirely in mixed micelles of BS and PL (8). However, subsequent studies demonstrated that CH can also be solubilized and transported in PL-CH vesicles (9), particularly when the concentration of CH exceeds its micellar solubility (10). The importance of vesicles has become even more apparent with the discovery that the nucleation of CH monohydrate crystals that precedes gallstone formation occurs as a result of vesicle aggregation and fusion within the gallbladder (11). Furthermore, subsequent crystal growth may occur by direct deposition of CH from unilamellar vesicles (12).

Recent studies have indicated that the molecular species of PC may be distributed asymmetrically between vesicles and micelles. Vesicles isolated from cholesterol-supersaturated model biles were relatively enriched in PC

Abbreviations: PL, phospholipid; PC, phosphatidylcholine; BS, bile salt; CH, cholesterol; HPLC, high performance liquid chromatography; C_1, C_2: *sn*-1, *sn*-2 carbon chain lengths; B_1, B_2: double bonds at *sn*-1, *sn*-2; RT, retention time; VMR, vesicle/micelle mole % ratio; CSI, cholesterol saturation index; FA, fatty acid.

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species containing saturated acyl chains, in contrast to mixed micelles, which were enriched in unsaturated species (13). It has also been established that differences in PC acyl chain length and the degree of unsaturation at the *sn*-1 and *sn*-2 positions can account for differences in hydrophobicity (14), but the relationship between the hydrophobicity of a specific PC and its tendency to partition into micelles or vesicles remains unclear. The specific goals of this study were, first, to determine whether the asymmetric distribution of PC species reported by Cohen and Carey (13) in model biles exists in native human gallbladder biles, and, second, to delineate the parameters that determine PC partitioning between biliary vesicles and micelles.

METHODS

Sample collection and biliary lipid analysis

Gallbladder biles were obtained at cholecystectomy from a group of 22 patients undergoing elective surgery. All 7 patients whose bile contained both biliary vesicles and mixed micelles were chosen for inclusion in this study. All bile samples were checked for bacterial contamination by culture with EMB agar, sheep blood agar, and thioglycollate broth. Biles were centrifuged for 5 min at 2000 rpm and an aliquot of each supernatant was immediately used for gel filtration chromatography. The remainder of each supernatant was stored at −20°C for later lipid assay. Phospholipids were quantitated by the method of Dryer, Tammes, and Routh (15) and cholesterol concentration was determined by the method of Röschlau, Bernt, and Gruber (16). Total bile acids were measured by the method of Talalay (17). The cholesterol saturation index (CSI) was calculated using Carey's critical tables (18).

Assay of phospholipase activity in bile

Biliary phospholipases were assayed by incubating 200 μl of bile with 0.3 μCi [^{3}H-methylcholine]dipalmitoyl PC (31.0 Ci/mmol, New England Nuclear) and 0.05 μCi 1-palmitoyl-2-[^{14}C-linoleoyl]PC (50.0 mCi/mmol, New England Nuclear) at 37°C with shaking for 3 h. A model bile with a CSI of 0.6 and a total lipid concentration of 10 g/dl was used as a control. The incubations were terminated by the addition of 50 μl 2 N HCl and extraction by the method of Folch, Lees, and Sloane Stanley (19). An aliquot of the upper aqueous phase was counted for the presence of [^{3}H-methyl]phosphocholine. The organic extracts were fractionated on 4.0 × 0.6 cm columns of Bio-Sil HA 325 mesh (Bio-Rad, Richmond, CA) into neutral lipid (10 ml chloroform) and polar lipid (6 ml methanol, 4 ml 1% water in methanol) fractions. The neutral lipids were further fractionated by thin-layer chromatography on silica gel G using the solvent system hexane–diethyl ether–acetic acid 70:30:1, and spots corresponding to monoglycerides, diglycerides, and fatty acids were scraped and counted. The polar lipids were fractionated by thin-layer chromatography on silica gel G using the solvent system chloroform–methanol–water–acetic acid 65:25:4:1, and spots corresponding to PC, lysoPC, phosphatidic acid, and glycerophosphocholine were scraped and counted.

Isolation of vesicles and micelles

Biliary vesicles and micelles were isolated by gel filtration chromatography after radioactive labeling as described by Ahrendt et al. (20). Fresh, isotropic gallbladder bile samples (500 μl) were incubated with 0.1 μCi [1a,2a(n)-^{3}H]cholesterol (44.3 Ci/mmol, Amersham, Arlington Heights, IL) and 0.025 μCi 1-palmitoyl-2-[1-^{14}C]oleoyl phosphatidylcholine (55.2 mCi/mmol, Amersham) for 1 h at 37°C. Bile samples were applied to a Pharmacia LKB C10/40 gel filtration column (Pharmacia LKB, Piscataway, NJ) packed with Sephacryl S-300 SF (Pharmacia) to a gel bed of 1.0 × 38 cm. The column was eluted with buffer (0.15 mM NaCl, 50 mM Tris HCl, 1.5 mM EDTA, pH 8.0) containing 10 mM Na cholate. A peristaltic pump was calibrated to deliver a flow rate of 0.5 ml/min, and 0.5-ml fractions were collected. Blue dextran and [^{3}H]leucine were used as markers for void volume and total volume, respectively. Distinct vesicular (void volume) and micellar (included volume) peaks were located by monitoring the appearance of radiolabel (**Fig. 1**), and the appropriate fractions were pooled prior to analysis.

Phospholipid high performance liquid chromatography (HPLC)

HPLC was performed with a Shimadzu (Columbia, MD) system consisting of an LC-6A solvent delivery system, an SPD-6A variable wavelength detector, and a C-R3A Chromatopac integrator.

The biliary PC class was isolated by the HPLC method of Patton, Fasulo, and Robins (14). Lipid extracts were evaporated under nitrogen and redissolved in hexane–isopropanol–water 40:54:6 (by vol.) prior to injection. A 250 × 4.6 mm Hibar column packed with 10 μm LiChrospher Si-100 (Alltech Associates, Deerfield, IL) was used for the separation of PL classes with detection at 205 nm. The elution solvent (isopropanol–hexane–25 mM phosphate buffer, pH 7.0–ethanol–acetic acid 490:367:62:100:1.25 (by vol.) was delivered at a rate of 1.5 ml/min.

The PC fraction was perbenzoylated by the method of Ullman and McCluer (21). The resulting benzoyl diacylglycerides were isolated by a modification of the HPLC method of Nakagawa and Horrocks (22). Samples were evaporated and redissolved in hexane prior to chromatography on a 250 × 4.6 mm Hibar column packed with 10 μm LiChrospher Si-100 (Alltech). The elution solvent consisted of cyclohexane–hexane–methyl-*t*-butyl ether–

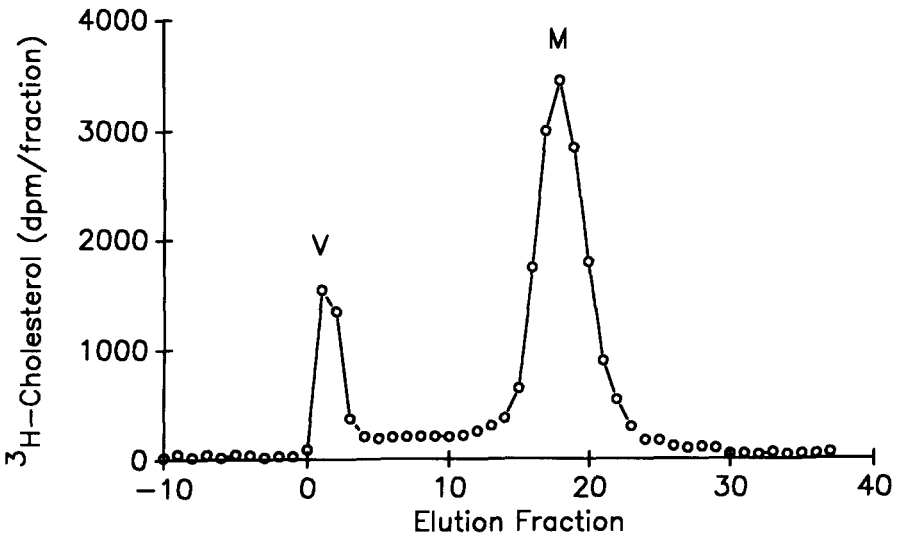

Fig. 1. Separation of vesicles and mixed micelles from human gallbladder bile by gel filtration chromatography. Bile was radiolabeled and applied to a column of Sephacryl S-300 as described in Methods. Vesicles (V) and micelles (M) were located by monitoring the appearance of radiolabeled fractions.

acetic acid 375:125:10:0.1 (by vol.) delivered at a rate of 1.0 ml/min.

Benzoyl diacylglyceride molecular species derived from PC were separated and quantitated by reverse phase HPLC according to Patton and Robins (23). The separations were carried out on a 250 × 4.6 mm Ultrasphere ODS column (Beckman, San Ramon, CA), and the solvent system consisted of methanol–water–acetonitrile 950:35:15 (by vol.) delivered at a rate of 2.0 ml/min. Molecular species identifications were confirmed by capillary gas–liquid chromatography (GLC) of their fatty acid methyl esters. GLC was performed on a Shimadzu GC-Mini 2 gas chromatograph equipped with a 60 m × 0.2 μm SP-2340 capillary column (Supelco, Bellefonte, PA) and flame ionization detector.

Data analysis

Relationships between PC fatty acyl chain length and unsaturation and *a)* reverse phase HPLC retention time and *b)* partitioning between micelles and vesicles were examined by multivariate analysis using the general linear models procedure in the Statistical Analysis System (SAS).

RESULTS

Patient population

The seven patients chosen for this study included six females and one male ranging in age from 31 to 69 years, with weights ranging from 60 to 93 kg. Five patients had a cholecystectomy performed for chronic cholecystitis and cholelithiasis, while one patient underwent cholecystectomy for acute cholecystitis. The final patient's gallbladder was aspirated at the time of hepatic resection for metastatic cancer. All patients had normal liver function tests. All patients were eating a standard diet at the time of surgery; one patient had ingested fish oil (Promega®, 3 g/day) during the 2 weeks prior to surgery (Patient #6). Five patients had cholesterol stones, one patient had a pigment stone, and no stones were apparent in the patient whose gallbladder was aspirated. Microscopic evaluation of whole bile was available in six patients revealing cholesterol crystals in five and calcium bilirubinate granules in one. All bile samples were demonstrated to be free from bacterial contamination by standard culture techniques. In addition, all biles were devoid of phospholipase activity as compared to a model bile control (data not shown). The bile salt, phospholipid, cholesterol, and total lipid concentrations, as well as the cholesterol saturation indices for the individual bile samples are presented in **Table 1.** The percentages of total PC carried in vesicles as calculated from the vesicular and micellar reverse phase HPLC total peak areas are also shown in Table 1. For this study, only the 12 PC molecular species that were completely separated by reverse phase HPLC with no cross-contamination from adjacent peaks were included in the analyses. The relative mole % composition for these species in each patient is presented in **Table 2.** As shown, these species accounted for approximately 80% of the total PC contained within the vesicular and micellar fractions.

Influence of fatty acyl composition on partitioning of PC between vesicles and micelles

Cohen and Carey (13) demonstrated that, in model biles, the distribution of PC species between vesicles and

TABLE 1. Lipid composition of human gallbladder bile

Patient	Bile Salt	Phospholipid	Cholesterol	Total Lipid	CSI[a]	PC in Vesicles[b]
	mM	*mM*	*mM*	*g/dl*		%
1	41.7	30.2	3.7	4.5	0.7	4.9
2	133.5	80.6	15.1	13.4	0.8	2.3
3	116.2	44.6	15.8	9.8	1.2	2.8
4	133.2	49.4	19.6	11.1	1.2	0.4
5	130.8	53.7	16.4	11.2	1.0	3.6
6	55.1	21.8	5.8	4.6	1.0	4.7
7	21.1	13.3	3.1	2.2	1.3	7.0

[a]Cholesterol saturation index.
[b]Percent of total PC carried in vesicles.

micelles depends upon the degree of unsaturation and the chain length of the fatty acyl components. We investigated the relationship between PC fatty acyl composition and partitioning in human gallbladder bile. The mean vesicle/micelle mole % ratios (mean VMR) for the 12 PC species as calculated from the ratios in the seven individual patients are shown in **Table 3.** Two-factor analysis of variance showed highly significant differences in VMR between PC species ($P = 0.0001$) with no evidence of patient to patient variation ($P = 0.99$). In general, the PC species that partitioned preferentially into micelles were those with a greater degree of acyl unsaturation. An exception

TABLE 2. Mole % composition of gallbladder bile PC from seven patients

		Patient							
		1	2	3	4	5	6[a]	7	
Fatty Acids		Vesicles							Mean ± SD
sn-1	*sn*-2								
16:1	18:2	1.22	0.52	0.43	0.40	1.00	1.40	0.46	0.78 ± 0.42
16:0	20:5	0.85	1.24	0.74	0.49	0.98	2.14	0.91	1.05 ± 0.53
18:1	18:2	3.25	1.70	2.21	2.80	3.31	2.80	1.70	2.54 ± 0.68
14:0	18:2	0.37	0.41	0.21	0.40	0.70	0.49	0.27	0.41 ± 0.16
18:1	18:3	0.64	0.53	0.33	0.28	0.33	0.58	0.51	0.46 ± 0.14
16:0	22:6	2.39	2.89	1.58	1.62	1.87	2.64	2.74	2.25 ± 0.55
16:0	18:2	36.47	44.86	32.91	48.47	43.27	45.02	43.20	42.03 ± 5.41
18:0	20:3	0.59	0.44	0.53	ND[b]	0.39	0.26	0.64	0.41 ± 0.22
18:0	18:2	6.07	7.30	8.74	5.12	7.52	6.25	7.73	6.96 ± 1.22
16:0	16:1	3.92	2.91	3.60	3.12	4.12	3.01	3.30	3.43 ± 0.47
16:0	18:1	21.30	19.86	27.77	23.66	20.08	16.18	20.28	21.30 ± 3.61
18:0	18:1	1.23	0.87	2.16	0.70	1.00	0.64	0.96	1.08 ± 0.52
Total		78.30	83.53	81.21	87.06	84.57	81.41	82.70	82.68 ± 2.78
Fatty Acids		Micelles							
sn-1	*sn*-2								
16:1	18:2	1.24	1.31	1.47	1.77	2.41	2.56	1.54	1.76 ± 0.53
16:0	20:5	1.83	2.28	1.59	1.56	1.62	2.90	1.62	1.91 ± 0.50
18:1	18:2	4.51	3.29	3.90	4.80	4.21	4.82	3.60	4.16 ± 0.59
14:0	18:2	0.69	0.58	0.43	0.45	0.54	0.55	0.46	0.53 ± 0.09
18:1	18:3	0.77	0.57	0.58	0.50	0.39	0.75	0.50	0.58 ± 0.14
16:0	22:6	1.95	3.34	2.26	2.17	2.04	2.65	2.19	2.37 ± 0.48
16:0	18:2	34.35	41.68	34.08	47.56	41.86	42.66	42.78	40.71 ± 4.86
18:0	20:3	0.53	0.36	0.62	0.20	0.44	ND[b]	0.40	0.36 ± 0.21
18:0	18:2	5.39	6.47	6.10	3.54	6.08	4.73	6.31	5.52 ± 1.06
16:0	16:1	3.41	1.91	3.70	2.09	3.50	2.00	2.41	2.72 ± 0.79
16:0	18:1	15.38	12.97	18.30	15.65	14.42	12.19	13.84	14.68 ± 2.02
18:0	18:1	0.79	0.50	0.63	0.26	0.60	0.28	0.57	0.52 ± 0.19
Total		70.84	75.26	73.66	80.55	78.11	76.09	76.22	75.81 ± 3.10

[a]This patient's diet was supplemented with fish oil (Promega® 3 g/day) for 2 weeks prior to surgery.
[b]ND, not detectable.

TABLE 3. Distribution of PC species between vesicles and micelles

Fatty Acids		
sn-1	*sn*-2	Vesicle/Micelle Ratio
		mole %
16:1	18:2	0.45 ± 0.26
16:0	20:5	0.53 ± 0.13
18:1	18:2	0.60 ± 0.11
14:0	18:2	0.77 ± 0.28
18:1	18:3	0.79 ± 0.17
16:0	22:6	0.96 ± 0.22
16:0	18:2	1.03 ± 0.04
18:0	20:3	1.13 ± 0.30
18:0	18:2	1.28 ± 0.13
16:0	16:1	1.31 ± 0.21
16:0	18:1	1.45 ± 0.08
18:0	18:1	2.15 ± 0.70

was 16:0–22:6, which despite a highly unsaturated *sn*-2 fatty acid was evenly distributed between vesicles and micelles. In contrast to micelles, vesicles tended to be enriched in species containing primarily saturated or monounsaturated acyl chains. The overall distribution of total biliary PC molecular species in patients whose bile contained both lipid fractions was similar to that of the 15 patients whose bile contained only micelles (data not shown).

Influence of fatty acyl composition on hydrophobicity as estimated by HPLC retention time

As the hydrophobicity of a given PC molecule is a major determinant of its behavior in an aqueous system, the relationship between fatty acyl composition and hydrophobicity was investigated. For this purpose, the reverse phase HPLC retention times were used as approximate indicators of the relative PC hydrophobicities. Patton et al. (14) demonstrated that, for underivatized PC, the logarithm of the reverse phase HPLC retention time was strictly a function of fatty acyl chain length and unsaturation. In order to develop a multivariate model to describe this relationship for benzoyl diacylglycerides derived from human gallbladder bile PC, the natural logarithm of the retention time (ln RT) was expressed as a function of chain length at the *sn*-1 and *sn*-2 positions (C_1 and C_2, respectively) and the number of double bonds in each acyl chain (B_1 and B_2, respectively) for the 12 PC species shown in Table 2. As the chain length and number of double bonds within a given fatty acid are co-dependent, interaction terms were included in the regression model to account for this interaction ($C_1 \times B_1$ and $C_2 \times B_2$). Ln RT was positively related to C_1 ($P = 0.0001$), C_2 ($P = 0.0001$), and $C_2 \times B_2$ ($P = 0.0671$), and negatively related to B_1 ($P = 0.0001$) and B_2 ($P = 0.0010$). The $C_1 \times B_1$ term did not contribute significantly to the model, probably due to the limited variability in chain length and unsaturation at the *sn*-1 position. This regression model accounted for virtually all of the variability in HPLC retention time ($r^2 = 0.99$, $P = 0.0001$).

Relationship between hydrophobicity and partitioning of PC between vesicles and micelles

As PC fatty acyl unsaturation appears to be a determinant of both hydrophobicity and vesicle/micelle partitioning, a relationship between HPLC retention time and PC distribution might be expected. **Fig. 2** expresses VMR as a function of HPLC retention time for each of

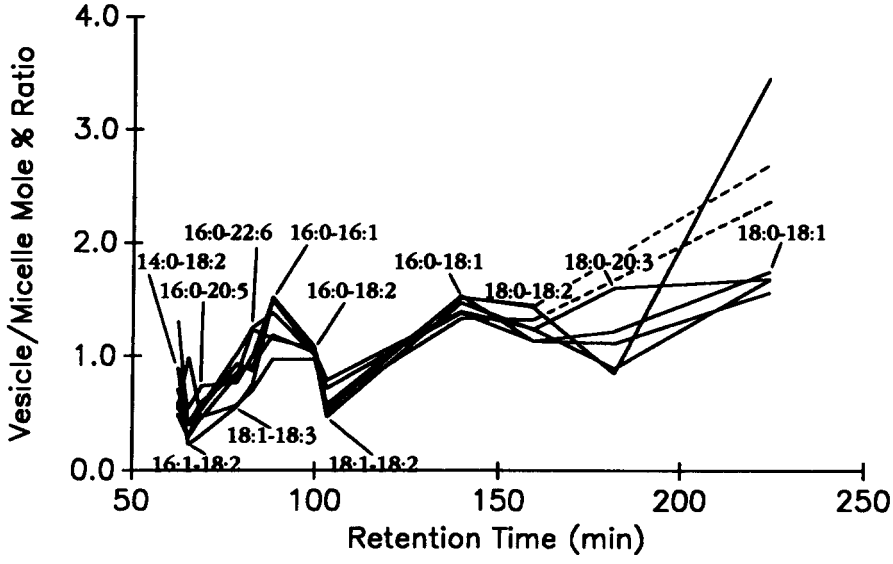

Fig. 2. Relationship between vesicle/micelle mole % ratio and reverse phase HPLC retention time for human gallbladder bile phosphatidylcholine species. Results are presented for the 12 species completely separated by reverse phase HPLC for seven individual patients. The dashed lines signify the absence of data points for 18:0–20:3 in two patients in whom this species was below the limits of detection.

the seven patients. As is shown, the relationship was not linear. The PC species with the most preferential distribution into vesicles contained a saturated fatty acid at the *sn*-1 position (16:0–16:1, 16:0–18:1, 18:0–18:1). Conversely, species containing unsaturated fatty acids at both the *sn*-1 and *sn*-2 positions (16:1–18:2, 18:1–18:2) partitioned most preferentially into micelles.

Regression models for PC partitioning

To investigate the relationship between PC acyl composition and vesicle/micelle partitioning, a multivariate model was developed for each patient, expressing the natural logarithm of the VMR (ln VMR) as a function of C_1, C_2, B_1, B_2, $C_1 \times B_1$, and $C_2 \times B_2$. These parameters were significantly related to ln VMR (for each of the seven patients $r^2 = 0.80$–0.95 and $P < 0.10$), but they could not account for all of the variability as they did for ln RT. Fig. 2 suggested that the influence of *sn*-2 unsaturation was modified by the presence of *sn*-1 double bonds, so the multivariate models were reconstructed with an additional interaction term, $B_1 \times B_2$. Furthermore, C_2 was removed from the regression models since the contribution of this term was found to be insignificant for each of the seven patients ($P > 0.32$). The other independent variables (C_1, B_1, B_2, $C_1 \times B_1$, $C_2 \times B_2$, and $B_1 \times B_2$) consistently made significant contributions to the multivariate regression models. These revised models accounted for virtually all of the variability in ln VMR for each of the seven patients ($r^2 = 0.92$–0.98 and $P < 0.03$), including Patient 6 ($r^2 = 0.97$, $P = 0.006$), whose relative percentage of 16:0–20:5 was elevated as a result of fish oil ingestion (Table 2).

DISCUSSION

This study demonstrates that the molecular species of PC are asymmetrically distributed between vesicles and micelles in human gallbladder bile. Micelles were enriched in species containing a greater number of double bonds, while vesicles contained a greater proportion of more highly saturated species. Partitioning of PC species in human gallbladder bile was also found to depend upon the fatty acyl chain length at the *sn*-1 position. Cohen and Carey (13) reported similar findings in model biles constructed from egg yolk and soybean lecithins. However, to our knowledge, the present study provides the first evidence of a relationship between fatty acyl composition and the VMR of PC species in native bile. The use of multivariate regression analysis is a novel approach for exploring the determinants of the physical chemistry of biliary lipids, and these findings illustrate the utility of this approach.

Patton et al. (14) demonstrated that the retention time of a given PC species is a function of the fatty acyl chain length and degree of unsaturation. The present study demonstrated that the retention time of a PC species is also related to the relative distribution of that molecule between vesicles and micelles. As shown in Fig. 2, the general trend was for the more hydrophobic species of PC, i.e., those with a greater retention time on HPLC, to preferentially partition into vesicles. Conversely, micelles appeared to be enriched with species that eluted earlier and therefore were more hydrophilic. However, the relationship between vesicle/micelle partitioning and HPLC retention time was clearly not linear. Specifically, it was demonstrated in Fig. 2 that the species with the greatest relative micellar distribution were those with unsaturated fatty acids at both the *sn*-1 and *sn*-2 positions (16:1–18:2, 18:1–18:2). As the parameters used in the initial regression model for retention time did not sufficiently account for all of the variability in VMR, an additional term was introduced into the multivariate models to reflect the effect modification which occurred when double bonds were present in both acyl chains. In addition, the chain length at the *sn*-2 position did not significantly contribute to the overall models, so the term for this parameter was eliminated. For each of the seven bile specimens, a regression that included C_1, B_1, B_2, $C_1 \times B_1$, $C_2 \times B_2$, and $B_1 \times B_2$ as dependent variables, accounted for virtually all of the variability in VMR.

While these studies demonstrate interaction between the two fatty acyl chains, the nature of this interaction and how it influences PC partitioning is unclear. Applegate and Glomset (24) reported that the conformation of model diacylglycerols was dependent upon the number and location of double bonds within the fatty acyl chains. In addition, the various conformations adopted by these diacylglycerols promoted distinct packing arrangements within simulated monolayers (25). In studies on PC monolayers at an air–water interface, Demel, Geurts van Kessel, and van Deenen (26) and Ghosh, Williams, and Tinoco (27) demonstrated that the area of a PC molecule decreased as the degree of fatty acyl saturation increased. This resulted in a greater intermolecular cohesiveness, presumably due to attractive Van der Waals forces that increase as the saturated portions of acyl chains increase in length (28). When cholesterol was added to the PC monolayers, films composed of species containing both a saturated and an unsaturated fatty acid were condensed, while those containing unsaturated chains at both positions were not (26). Furthermore, Joos and Demel (29) demonstrated that the degree of condensation with cholesterol was proportional to the length of saturated chain present within the PC molecule. All of these observations may have consequences for the tendency of a PC species to partition into highly ordered biliary vesicles, in which PC molecules must closely associate not only with each other but with cholesterol as well.

While the cholesterol-carrying capacity of bile is largely

dependent on the relative and total concentrations of biliary lipids, it is interesting to note that despite large variations in biliary lipid concentrations among the seven patients in this study (Table 1), the relative distributions of the 12 PC species shown in Fig. 2 demonstrated virtually no patient-to-patient variation. Furthermore, an examination of the distribution of PC species in whole bile revealed no differences between patients whose bile contained micelles and vesicles and those whose bile contained micelles only, suggesting that differences in the PC species secreted by the liver were not responsible for determining the presence or absence of vesicles. While the intermicellar concentration of BS almost certainly differed somewhat from patient to patient, a sodium cholate concentration of 10 mM was chosen for all chromatographic separations of lipid particles. While this may have resulted in slight alterations in the relative numbers of vesicles and micelles as compared to the original native biles, the ln VMR for each patient was still highly dependent upon fatty acyl chain length and saturation despite wide variations in the % of PC carried in vesicles.

Previous studies have suggested that the fatty acyl composition of biliary PC may be of primary importance to the cholesterol-carrying capacity of bile. Cohen and Carey (13) reported that model biles which contained a high proportion of unsaturated species had higher micellar CH solubilities. However, these biles also demonstrated higher vesicular CH/PC molar ratios, presumably because PC species were preferentially distributed into micelles with less PC available for vesicles (13). It is clear from recent reports that the fatty acyl composition of biliary PC can be altered by dietary triglycerides, and these changes may influence the cholesterol-carrying capacity of bile. Dietary supplementation with fish oil, for example, has been shown to increase the relative proportions of biliary PC species containing highly unsaturated n-3 fatty acids in the prairie dog (5) and the African green monkey (30). Extrapolating from the findings of Cohen and Carey (13) in model biles, this increase in polyunsaturated PC species might be expected to decrease the cholesterol-carrying capacity of bile and to increase gallstone formation. However, fish oil supplementation has also been shown to increase total hepatic PC secretion, resulting in an increased mole fraction of total PC and a decreased lithogenicity in gallbladder bile. This effect probably accounted for the decreased gallstone formation observed in these animal models (5, 30, 31). Such findings demonstrate that the effects of dietary triglyceride on biliary lipids are complex and will require further elucidation.

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