Utilization of different fatty acids for hepatic and biliary phosphatidylcholine formation and the effect of changes in phosphatidylcholine molecular species on biliary lipid secretion

Sander J. Robins, Joan M. Fasulo, Valerie F. Robins, and George M. Patton

Lipid Metabolism Laboratory and Department of Medicine, Veterans Administration Medical Center, Boston, MA 02130

Abstract Biliary cholesterol secretion is ordinarily tightly coupled to phosphatidylcholine (PC) secretion. Bile PCs are distinct in composition and predominantly composed of molecular species with 16:0 in the *sn*-1 position and 18:2 and 18:1 in the *sn*-2 position. In an attempt to acutely change the composition of biliary PCs and to assess the effect of a change in PCs on biliary cholesterol secretion, isolated livers were perfused with a variety of single free fatty acids. Rat livers with bile duct cannulas were perfused with a recirculating medium, taurocholate (40 μmol/h), and albumin-bound 16:1, 17:1, 18:1, 20:1, 18:2, 20:4, or 20:5 fatty acids (90 μmol/h) for 2 h. Biliary lipid secretion was measured and bile and liver PC compositions were compared at the start and end of perfusion. Results showed *1*) greater utilization of shorter chain than longer chain fatty acids for bile PC formation (16:1 > 17:1 > 18:2 or 18:1 > 20:5, 20:4 or 20:1); *2*) no similar pattern of FA utilization for liver PC formation; *3*) preferentially greater incorporation of fatty acids into bile PCs compared to liver PCs when perfused fatty acids were used for esterification at both *sn*-1 and *sn*-2 positions of PC (to form diunsaturated PCs); and *4*) increased biliary secretion of cholesterol relative to PC only when the population of PCs that was newly formed included more hydrophilic molecular species of PC than are present in native bile (that was observed only with perfusion of 16:1). Changes in biliary PC secretion or cholesterol/PC secretion occurred independently of any change in bile salt secretion. These data show that bile PC composition can be acutely and selectively changed and suggest that different molecular species of PC may solubilize different amounts of cholesterol for transport into bile. —**Robins, S. J., J. M. Fasulo, V. F. Robins, and G. M. Patton.** Utilization of different fatty acids for hepatic and biliary phosphatidylcholine formation and the effect of changes in phosphatidylcholine molecular species on biliary lipid secretion. *J. Lipid Res.* 1991. **32:** 985–992.

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Bile provides the major route for cholesterol elimination from the body and biliary cholesterol secretion is dependent on the phospholipid, phosphatidylcholine (PC). In a variety of experimental settings biliary PC and cholesterol secretion appear to be tightly coupled and increase in parallel in response to a bile salt infusion (1, 2), cholesterol feeding (3), choline feeding (4), or estrogen administration (5) and decrease in parallel with dietary choline restriction (4), colchicine administration (6), or with an infusion of a number of organic anions (7–10).

Compared to the liver, bile is especially rich in molecular species of PC with 16:0 in the *sn*-1 position and 18:2 and 18:1 in the *sn*-2 position (11, 12). Although there is no explanation for what appears to be selective hepatic secretion of these particular molecular species of PC in bile, we have previously reported (13) that it is possible under certain circumstances to change the composition of bile PCs. We found that when rats were chronically fed fatty acids that are more hydrophilic than the fatty acids that are ordinarily prevalent in bile PCs, bile became highly enriched in new molecular species of PC that contained the particular fatty acid that was fed. In contrast, when a fatty acid was fed that was less hydrophilic than those that ordinarily comprise biliary PCs, the composition of biliary PCs was not changed. We undertook the present study to determine whether the composition of biliary PCs could be acutely changed by perfusing the rat liver with single free fatty acids; to further characterize the changes in bile PC composition that occur in response to the administration of a range of fatty acids that differ in chain length and unsaturation; and to determine whether short-term changes in the molecular species composition of PCs can affect the rate of biliary PC and cholesterol secretion.

Abbreviations: PC(s), phosphatidylcholine(s); HPLC, high performance liquid chromatography; GC, gas chromatography; 16:1, *cis*-9-hexadecenoic; 17:1, *cis*-10-heptadecenoic; 18:1, *cis*-9-octadecenoic; 20:1, *cis*-11-eicosenoic; 18:2, *cis*-9,12-octadecadienoic; 20:4, *cis*-5,8,11,14-eicosatetraenoic; 20:5, *cis*-5,8,11,14,17-eicosapentaenoic.

MATERIALS AND METHODS

Materials for liver perfusion

Sodium taurocholate, A grade, was obtained from Calbiochem (La Jolla, CA). Bovine serum albumin, fraction V, was obtained from Sigma Chemical Co. (St. Louis, MO); by direct analysis it contained 0.374 μmol free fatty acids/g of albumin. The following fatty acids were obtained from Nu Chek Prep, Inc (Elysian, MN) as greater than 99% pure: *cis*-9-hexadecenoic (16:1); *cis*-10-heptadecenoic (17:1); *cis*-9-octadecenoic (18:1); *cis*-11-eicosenoic (20:1); *cis*-9,12-octadecadienoic (18:2); *cis*-5,8,11,14-eicosatetraenoic (20:4); and *cis*-5,8,11,14,17-eicosapentaenoic (20:5). The triacylglycerols, trihexadecenoin and trioctadecenoin, were also obtained from Nu Chek Prep.

Liver perfusion

Perfusions were performed using livers from male Sprague-Dawley rats (Taconic Animal Farms, Germantown, NY) that had been fed Purina Chow ad libitium. Rats were maintained in a 12-h light-cycled room and weighed 250–300 g at the time study. The bile duct was cannulated and livers were perfused in situ at 37°C with an oxygenated medium of ~100 ml of Krebs-Ringer bicarbonate buffer (pH 7.4), 3% bovine serum albumin, and 25 mM glucose (14), maintaining portal flow on average at 3.4 $ml \cdot min^{-1} \cdot g\ liver^{-1}$. The liver was first perfused with an open system for 25 min and the perfusate was discarded. Perfusions were then continued with a fresh recirculating system for the next 2 h. Throughout the perfusion, sodium taurocholate was directly instilled into the portal vein cannula at a constant rate of 40 μmol/h (23.5 mM taurocholate at 0.028 ml/min). Individual fatty acids as potassium salts complexed to 3% albumin were added to the perfusion at the beginning of the 2-h period of recirculation as described by Ide and Ontko (15) and Fukuda, Azain, and Ontko (16): a bolus of 100 μmol (in 5.3 ml) as a priming dose was added to the perfusate reservoir followed by a constant infusion of 90 μmol/h (at 4.8 ml/h). The hepatic uptake of fatty acids was calculated as the difference between the amount of a fatty acid that was added (as a bolus and then infused for 2 h) and the amount of this fatty acid that remained in the perfusate at the end of perfusions. Addition of fatty acids by bolus and constant infusion resulted in similar rates of uptake for 16:1, 18:2, 20:1, and 20:4 (10.0, 10.4, 11.8, and 13.0 $\mu mol \cdot h^{-1} \cdot g\ liver^{-1}$, respectively) and was the same as the rate of 18:1 uptake that has been reported by Ontko and his associates (15–17) of 10–13 $\mu mol \cdot h^{-1} \cdot g\ liver^{-1}$ for chow-fed rats. Liver viability was assessed by bile flow (which for all perfusions averaged 60.3 ± 7.3 (SD) $\mu l \cdot h^{-1} \cdot g\ liver^{-1}$), perfusate oxygen consumption (which averaged 101.5 ± 16.6 (SD) $\mu mol \cdot h^{-1} \cdot g\ liver^{-1}$), and the general appearance of the liver.

Bile was collected throughout the 2-h period of recirculating perfusion. The liver was sampled at the start of perfusion, by excising an anterior lobe (~0.3 g), and at the conclusion of the perfusion by excising an adjacent anterior lobe (after manually perfusing the liver with 30 ml of cold saline). The perfusate (in 2-ml aliquots) was sampled at the outset and at the end of perfusion.

Studies were also performed in rats that were fed for 3 successive days prior to the day of liver perfusion a triacylglycerol with fatty acids that were the same as the fatty acid that was perfused. Perfusions were performed 24–26 h after the last triacylglycerol feeding. Triacylglycerols were given as a supplement to the chow diet by gastric tube in a 0.5 g daily dose.

Analytical procedures

The rate of biliary secretion of bile salts, PCs, and cholesterol was determined as previously reported (14). PCs were isolated by HPLC (18) and the molecular species of PCs in bile and liver were determined as benzoylated diacylglycerols by a reverse phase HPLC procedure that we have previously described (19). The fatty acids of individual PCs were identified and quantitated as methyl esters by capillary column GC (20). Statistical differences between groups were determined by Student's *t*-test for unpaired variables (see Fig. 1 and Tables 1, 2 and 4) or by one-way analysis of variance (see Figs. 2 and 3 and Table 3). Pairwise comparisons, subsequent to analysis of variance, were carried out by the Fisher procedure at a significance of 0.05.

RESULTS

Effect of perfusion with different fatty acids on PC composition in bile and liver

Changes in individual molecular species of PC. Without addition of a fatty acid to the perfusate, changes in the major molecular species of PCs in the liver and bile after 2 h of liver perfusion were small and confined to just two molecular species (**Table 1**). Compared to the liver, bile contained appreciably more *sn*-1-16:0 PCs (especially 16:0–18:2 PC) and less *sn*-1-18:0 PCs. Perfusion of a single fatty acid resulted in an increase of PCs that contained the fatty acid that was administered (**Table 2**). In every case, the PC molecular species that increased the most increased more in bile than in the liver. The PC molecular species that increased the most in bile either contained a 16:0 acyl group (16:0–18:1, 16:0–20:4, or 16:0–20:5) or contained in both of its acyl group positions the fatty acid that was perfused (16:1–16:1, 17:1–17:1 or 18:2–18:2). Moreover, all of the fatty acids that were perfused were unsaturated and virtually all of the other PCs ("All other PCs", Table 2) that were increased in both bile and liver in response to the perfusion of each of these fatty acids

TABLE 1. Effect of isolated liver perfusion on the composition of PCs in liver and bile

PC Molecular Species	Liver		Bile	
	Pre-Perfusion	Post-Perfusion	Initial	Final
	mol %		*mol %*	
sn-1-16:0 PCs				
16:0-18:1	8.4 ± 0.4	10.0 ± 0.2[a]	13.3 ± 1.8	15.7 ± 1.8
16:0-18:2	18.1 ± 0.7	17.6 ± 0.9	38.9 ± 2.3	38.1 ± 2.8
16:0-20:4	12.1 ± 1.0	12.0 ± 0.7	11.1 ± 0.6	11.2 ± 0.3
16:0-22:6	5.6 ± 0.5	5.9 ± 0.5	3.9 ± 0.2	4.1 ± 0.4
sn-1-18:0 PCs				
18:0-18:2	12.9 ± 1.7	12.7 ± 0.8	6.8 ± 0.9	6.9 ± 1.2
18:0-20:4	13.1 ± 0.8	13.2 ± 1.2	2.4 ± 0.6	2.5 ± 0.8
Diunsaturated PCs				
18:1-18:2	4.2 ± 0.3	3.7 ± 0.3	4.0 ± 0.4	3.3 ± 0.2[a]
18:1-20:4	3.2 ± 0.7	3.0 ± 0.5	1.7 ± 0.4	1.4 ± 0.1
18:1-22:6	1.3 ± 0.3	1.2 ± 0.3	2.4 ± 1.3	2.6 ± 1.5
18:2-18:2	1.2 ± 0.1	0.8 ± 0.1	1.9 ± 0.4	1.2 ± 0.5
Total PCs	80.1 ± 0.8	80.1 ± 0.9	86.3 ± 1.2	87.0 ± 1.5

Data is shown as mean ± SD for three perfusions, performed for 120 min without addition of a fatty acid to perfusate (see Methods). The initial bile was obtained from 0 to 5 min after beginning the perfusion and the final bile from 90 to 120 min. Individual PCs are listed when present as > 1.0 mol % in the initial bile collection.

[a]Pre- and post-infusion values were significantly different ($P < 0.05$).

contained unsaturated fatty acids in both acyl group positions. The extent of increase in individual molecular species of PC in bile in response to perfusion with different fatty acids was highly variable: the largest single increase in a PC occurred with perfusion of 16:1 (with the formation of 16:1-16:1 PC) and the least with the perfusion of 20:1. The perfusion of fatty acids to form new PCs in bile was accompanied by a reduction in other PC molecular species that was proportional to the extent that new PCs were formed. With the exception of perfusion with 18:2, the reduction in single PCs in bile was clearly most prominent for 16:0-18:2 PC, ordinarily the most prevalent PC (Table 1), and for other single PCs was generally proportional to their initial concentrations in bile (data not shown).

Changes in the fatty acid composition of PCs. In addition to an analysis of the molecular species of PC, the total fatty acid composition of PCs was determined to more readily quantitate changes in overall PC composition. The increase of each of the perfused fatty acids in the total PC mass of the bile and liver is shown in **Fig. 1A**. Although there were differences in the extent of utilization of each fatty acid for PC formation in the liver, there appeared to be no particular pattern of utilization that could be

TABLE 2. Effect of perfusion of different fatty acids on the composition of PCs in bile and liver

Fatty Acid	Individual PCs that Increased[a]		Increase of PCs in Bile		Increase of PCs in Liver	
Perfused	Major PC[b]	All Other PCs	Major PC	Other PCs	Major PC	Other PCs
			mol %			
16:1	16:1-16:1	16:1-18:2, 16:1-20:4, 16:1-18:1, 16:0-16:1	13.0 ± 3.5	16.2 ± 1.4	6.2 ± 1.3	5.9 ± 0.6
17:1	17:1-17:1	17:1-18:2, 17:1-18:1, 17:1-22:6, 16:0-17:1	7.4 ± 1.0	15.7 ± 1.4	5.6 ± 1.6	9.0 ± 2.6
18:1	16:0-18:1	18:1-18:1, 18:1-20:4, 18:1-18:2	4.6 ± 1.6	6.9 ± 0.4	2.3 ± 0.3	4.7 ± 0.2
18:2	18:2-18:2	none	9.2 ± 1.8		3.6 ± 0.9	
20:1	16:0-18:1	20:1-18:2, 20:1-20:4, 20:1-18:1, 18:1-20:4	3.0 ± 1.1*	4.2 ± 0.3	0.5 ± 0.1*	6.1 ± 1.2
20:4	16:0-20:4	18:0-20:4, 20:4-18:1, 20:4-18:2, 20:4-20:4	9.6 ± 0.3	3.2 ± 0.6	4.9 ± 0.9	7.4 ± 0.5
20:5	16:0-20:5	20:5-18:1, 20:5-18:2, 20:5-20:4, 20:5-20:5	6.2 ± 1.3	4.6 ± 2.3	4.0 ± 0.6	4.8 ± 0.4

Data are shown as a mol % increase (mean ± SD) in individual molecular species of PC for three or four perfusions for each fatty acid. All increases were significant (at $P < 0.01$), comparing pre-perfusion to post-perfusion values, except where indicated by an asterisk.

[a]Individual molecular species of PCs are listed that increased by at least 0.5 mol % from the initial bile, collected from 0-5 min, to the final bile, collected from 90-120 min. The PC that is listed as "major" increased the most. Samples of liver were obtained for analysis just before each fatty acid was perfused and at the end of perfusions (at 120 min).

[b]The acyl group position of fatty acids in newly formed PCs was not directly determined. Thus, the fatty acids in diunsaturated PCs may be interchanged in *sn*-1 and *sn*-2 positions. PCs with a saturated and unsaturated fatty acid are written, as usually found in animal tissues, with the saturated fatty acid in the *sn*-1 acyl group position.

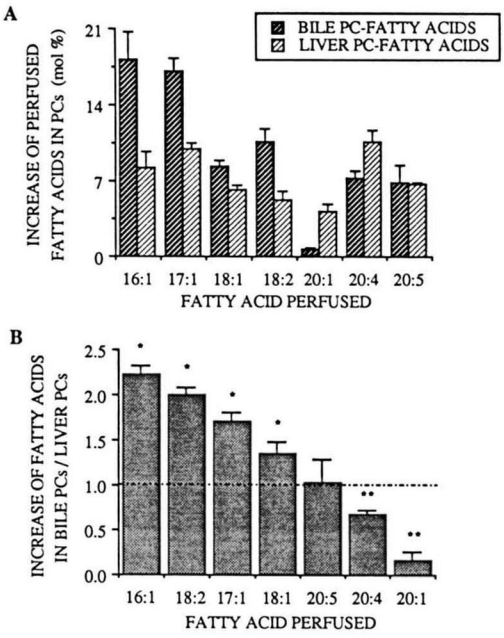

Fig. 1. Effect of perfusion of different fatty acids on the composition of PCs in bile and liver. Data in the upper panel (A) represent the increase (± SE) of perfused fatty acids in the PCs of bile and liver after liver perfusion for 120 min. Increases were determined by subtracting PC-fatty acids in bile collected from 0–5 min from bile collected from 90–120 min after fatty acid perfusions were begun and by subtracting PC-fatty acids in the liver before fatty acid perfusions were begun from the liver after perfusions were completed. Fatty acids that were perfused are arranged in order of increasing chain length and unsaturation. Increases in PC-fatty acids in the bile relative to the liver are shown in the lower panel (B) for each of the perfused fatty acids by dividing the increase of fatty acids in the PCs of bile by the increase in the PCs of the liver. Fatty acids are arranged in order of their utilization for bile PC formation relative to liver PC formation. Fatty acids that were incorporated to a greater extent in bile than liver PCs have values >1.0. A single asterisk marks perfusion groups where bile values were significantly greater than liver values ($P < 0.02$ for all groups) and a double asterisk marks groups where liver values were significantly greater than bile values ($P < 0.05$ for 20:4 perfusions and $P < 0.01$ for 20:1 perfusions).

related to the chain length or degree of unsaturation of the different fatty acids that were perfused. In contrast, there did appear to be a pattern of utilization of fatty acids that were incorporated into biliary PCs with shorter chain fatty acids preferentially utilized. As shown in Fig. 1B, there were significantly greater amounts of perfused fatty acids in the PCs of the bile than the liver when 16:1, 17:1, 18:1, and 18:2 were administered ($P < 0.02$ for each fatty acid), the same amounts of perfused fatty acids in bile and liver PCs when 20:5 was administered, and greater amounts of perfused fatty acids in the liver than in bile when 20:4 ($P < 0.05$) and 20:1 were administered ($P < 0.01$).

The preferential secretion in bile of PCs with shorter chain fatty acids could not simply be attributed to the relatively greater hydrophilic strength of these PCs. As assessed by the relative retention times of PCs with different fatty acids on a reverse phase column, the hydrophilic strength of a PC with 20:5 or 20:4 is greater or at least as great as the corresponding PC with 16:1, 17:1 or 18:2, yet, as shown, biliary secretion of PCs with the shorter chain fatty acids was clearly greater (**Fig. 2**).

Effect of perfusion with different fatty acids on biliary lipid secretion

Compared to perfusion without a fatty acid, perfusions with fatty acids resulted in no significant changes in the biliary secretion rate of bile salts, PCs or cholesterol, with the single exception of higher bile salt secretion with 20:4 (**Table 3**). PC secretion was more variable than bile salt secretion and was greater with perfusions of 18:1, 17:1, and 20:1 than with 18:2 and 20:5 ($P < 0.05$). Cholesterol secretion was also more variable than bile salt secretion but was not significantly different for any of the different fatty acids that were perfused. Ratios of PCs/bile salts and cholesterol/PCs are also shown in Table 3. The PC to bile salt ratio for any of the perfusions performed with fatty acid was not significantly different than for perfusions without a fatty acid. However, because of greater PC secretion, the ratio of PC to bile salt was significantly greater for perfusions with 18:1 and 20:1 than for 18:2, 20:4, and 20:5 ($P < 0.05$). Although perfusion with 16:1

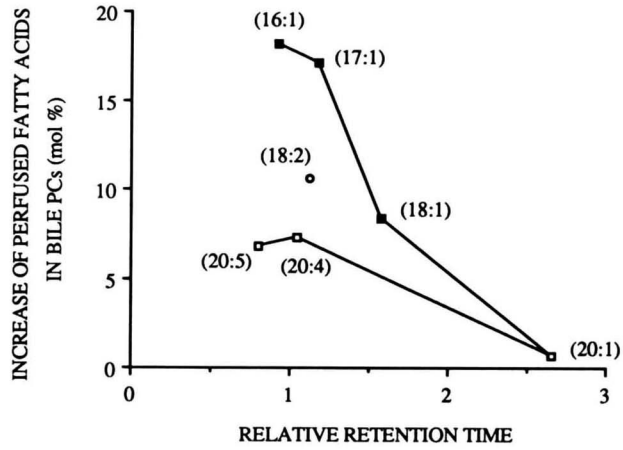

Fig. 2. Relation of the hydrophilic-hydrophobic balance of PCs to the secretion of PCs in bile. Data are shown as the increase of different perfused fatty acids in bile PCs (from Fig. 1A) as a function of PC hydrophilic strength. Hydrophilic strength can be functionally estimated by the relative retention time of PCs by reverse phase HPLC. Relative retention times were calculated be previously published methods (18) for, in this case, individual PC molecular species that contained 16:0 in the *sn*-1 acyl group position and each of the perfused fatty acids in the *sn*-2 acyl group position (e.g., 16:0–16:1 PC, 16:0–18:1 PC, 16:0–20:5 PC, etc). 16:0–22:6 PC was used for a reference and given a retention time of 1.0. Fatty acids in parentheses were the fatty acids perfused. Lines connect the values obtained after perfusion of the four monounsaturated fatty acids and the values obtained after perfusion of the three fatty acids with 20-carbon chains. Bile PC-fatty acid secretion (in mol %) was significantly different ($P < 0.05$) for the following: 16:1 and 17:1 differed from all other fatty acids; 18:2 differed from 20:5, 20:4, and 20:1; 18:1 differed from 20:1; 20:5 differed from 20:1; and 20:4 differed from 20:1.

TABLE 3. Effect of fatty acid perfusion on the secretion of biliary lipids by the isolated rat liver

Fatty Acid Perfused	Bile Salt Secretion	PC Secretion	Cholesterol Secretion	PC/Bile Salt	Cholesterol/PC
	μmol/h	*μmol/h*	*μmol/h*		
None	37.2 ± 1.6	3.60 ± 0.62	0.540 ± 0.132	0.097 ± 0.018	0.151 ± 0.029
16:1	39.7 ± 4.2	3.39 ± 0.97	0.655 ± 0.139	0.085 ± 0.020	0.197 ± 0.023[a]
17:1	40.1 ± 0.8	4.42 ± 1.15	0.645 ± 0.152	0.110 ± 0.029	0.147 ± 0.004
18:1	39.1 ± 0.8	4.45 ± 1.40	0.665 ± 0.135	0.114 ± 0.036	0.155 ± 0.031
18:2	39.6 ± 1.7	2.98 ± 1.18	0.560 ± 0.207	0.075 ± 0.027	0.190 ± 0.031
20:1	39.3 ± 1.5	4.49 ± 0.40	0.578 ± 0.134	0.114 ± 0.009	0.128 ± 0.021
20:4	41.9 ± 1.2[a]	3.26 ± 0.14	0.450 ± 0.078	0.078 ± 0.005	0.138 ± 0.019
20:5	36.8 ± 2.2	2.98 ± 0.26	0.463 ± 0.085	0.081 ± 0.004	0.158 ± 0.039

Data are the mean ± SD for three or four perfusions with each fatty acid. Bile was obtained from livers perfused for 120 min with taurocholate and with fatty acids or no fatty acids (Methods).
[a]Different (at $P < 0.05$) from control perfusions (without fatty acid).

was not associated with any change in bile salt, PC, or cholesterol secretion, only with this fatty acid was there a significant change ($P < 0.05$) in the cholesterol to PC ratio compared to perfusions performed without fatty acid or with any other fatty acid.

Effect of perfusion with 16:1 compared to 18:1, after feeding these fatty acids, on biliary lipid secretion

To contrast the effect on biliary lipid secretion of more prolonged administration of fatty acids that were utilized for biliary PC formation to a different extent, rats were fed for 3 successive days either 16:1 or 18:1 before liver perfusions were performed. Compared to the perfusions without prior fat feeding, perfusion with either 16:1 or 18:1 after feeding these same fatty acids resulted in no substantial change in the molecular species composition of PCs in the liver or bile that were sampled at the start and conclusion of the perfusion (data not shown). Furthermore, as shown in **Table 4**, perfusion with 18:1 after feeding 18:1 resulted in no significant changes in bile salt, PC, or cholesterol secretion in bile, compared to perfusions that were performed without prior fat feeding (Table 3). After feeding 16:1, however, perfusion with 16:1 resulted in an increase in biliary cholesterol secretion and a much greater different in the ratio of biliary cholesterol to PC compared to perfusions with 18:1 (0.251 ± 0.073 with 16:1 compared to 0.156 ± 0.033 with 18:1, $P < 0.015$).

DISCUSSION

We have perfused the isolated rat liver with fatty acids and demonstrated that fatty acids of different chain length and unsaturation are incorporated to a different extent into hepatic and biliary PCs. We found, comparing seven unsaturated fatty acids, *1*) that the extent of incorporation of a fatty acid into liver PCs was unrelated to fatty acid chain length, degree of unsaturation, or overall hydrophilic strength; *2*) that certain shorter chain fatty acids (16:1, 17:1, 18:1, and 18:2) were incorporated into bile PCs to a greater extent than longer chain fatty acids (20:1, 20:4, and 20:5); *3*) that these shorter chain fatty acids were present in greater amounts in bile than in the whole liver (i.e., were preferentially secreted in bile); and *4*) that changing biliary PC composition can alter the secretion in bile of cholesterol relative to PC.

We reported in an earlier study in which rats were fed different unsaturated fatty acids for several days (13), that biliary PCs can be changed with the introduction of new hydrophilic fatty acids in the diet. This previous study was performed with a limited number of fatty acids and, consequently, did not allow us to be more specific with regard to the characteristics of the fatty acids that were incorporated into biliary PCs. From the present study, we would conclude that although the relative hydrophilic strength of fatty acids or individual PC molecular species may influence biliary PC secretion, the effect of fatty acid (or acyl group) chain length has a greater influence. Thus, PCs with 16:1 and 17:1 that have approximately the same

TABLE 4. Effect of fatty acid perfusion after fat feeding on the secretion of biliary lipids by the isolated rat liver

	Free Fatty Acid Perfused	
Biliary Lipid	16:1	18:1
Bile salt (μmol/h)	41.1 ± 1.9	40.2 ± 3.1
PC (μmol/h)	3.58 ± 0.99	4.15 ± 0.64
Cholesterol (μmol/h)	0.871 ± 0.280	0.644 ± 0.158
PC/Bile Salt	0.087 ± 0.021	0.104 ± 0.018
Cholesterol/PC	0.251 ± 0.073[a]	0.156 ± 0.033

Data shown as mean ± SD for five perfusions with each fatty acid. Rats were fed for 3 successive days either 16:1 or 18:1 (in the form of triacylglycerols) and then used for liver perfusion on the fourth day. The liver was perfused with the same fatty acid that was previously fed (Methods). There were no significant differences between 16:1 and 18:1 groups, except as indicated.
[a]$P < 0.02$.

hydrophilic strength as PCs with 20:5 and 20:4, respectively (Fig. 2), are to a greater extent secreted in bile. A similar pattern is observed in native bile where, compared to the liver, PCs with 16:0 in the *sn*-1 position are far in excess of PCs with 18:0 and where PCs with 18:2 and 18:1 in the *sn*-2 position are in excess of PCs with 20:4 and 22:6.

The mechanism by which PCs are formed and secreted in bile in conjunction with cholesterol is poorly understood. Although it is likely that intrahepatic cholesterol is solubilized within a PC vesicle for transport to the biliary canaliculus (21, 22), the source and site of origin of this PC has not been determined. More particularly, there has been no explanation for a process that is triggered by bile salts and that results in the selection of certain PC molecular species for secretion into bile. Bile salts could conceivably promote selective PC secretion by either selectively solubilizing PCs or stimulating the synthesis of selective PCs. The possibility that bile salts selectively solubilize PCs has been examined by determining the phospholipid composition of a variety of hepatic membranes before and after a bile salt infusion (23) or by mixing bile salts with isolated membranes (24) and with vesicles composed of PCs and cholesterol (25). These studies show that bile salts will preferentially solubilize molecular species of PC based on hydrophilic strength (25). However, the studies with natural membranes also show that bile salts will solubilize different kinds of phospholipids that are ordinarily not present in bile (23, 24). Thus, convincing evidence is not available that selective solubilization of PCs is responsible for the distinct phospholipid composition of bile. This may be attributed to the difficulty in fashioning an in vitro model that will adequately simulate the conditions of bile salt passing through an intact liver with an intact biliary secretory apparatus.

The solubilization experiments conducted with bile salt and PC vesicles (25) are highly reminiscent of work that we and others have previously reported regarding the mobility of individual PCs between native lipoproteins (26), lipid-protein recombinants (27, 28), and lipid particles and cell membranes (29, 30). All of these studies have found that PCs that are transferred the most readily through an aqueous phase are those PCs that can be generally characterized as the most hydrophilic molecular species of PC that were available for transfer.

The possibility that bile salts promote the selective synthesis of biliary PCs has also been explored, in studies with a variety of radiolabeled precursors of PC synthesis. In studies in which PC molecular species have been identified (12, 31, 32), bile salts have been shown to increase the specific activities of hepatic PCs with 16:0 in the *sn*-1 position and 18:2 in the *sn*-2 position, i.e., those PCs that are preferentially secreted in bile. However, we have previously reported (33) that, even with bile salt administration, hepatic PC synthesis is quantitatively small and the contribution of newly synthesized PCs to biliary secretion is only about 3% of the total PC output. These previous results now appear to be in conflict with our present study that clearly shows, when the liver is perfused with certain unsaturated fatty acids, that a number of new diunsaturated PCs may be formed in relatively large amounts and are preferentially secreted in bile (Table 2). However, in our earlier study which was also performed using the isolated rat liver, fatty acids were not perfused and PC synthesis was not stimulated. We therefore suggest that the most likely mechanism to explain selective PC secretion is one that is ordinarily not regulated by PC synthesis but is principally dependent on the physical characteristics of the particular hepatic PCs that are available for biliary secretion when bile salt is perfused. In both the present experimental setting, when new PCs were synthesized, and in an unperturbed state, PCs with relatively short acyl chains are decidedly favored for secretion in bile.

We found that perfusion with 16:1 was associated with an increase in the output of cholesterol relative to PCs in bile compared to perfusions performed without fatty acids or perfusions with equimolar amounts of other fatty acids (Tables 3 and 4). There appears to be no previous study similar to ours that has assessed the effect of the hepatic uptake of different, single fatty acids on biliary lipid secretion. Although in a number of instances biliary cholesterol secretion has been determined in response to changes in the composition of dietary fat in laboratory animals (34) and humans (35, 36), the changes that have been reported may be largely indirect and predominantly reflect changes in plasma lipoproteins and differences in the hepatic uptake of cholesterol from the plasma. Experiments that might be more readily compared with our own are those that have been performed in isolated systems to assess the effect of PCs of different composition on the transport of cholesterol between vesicles (37, 38) and between a variety of lipid particles and cells (39–45). In these studies, cholesterol transport has clearly been shown to be a function of PC structure and, in general, is greater in the presence of more unsaturated PCs and PCs with shorter acyl chains.

The complexity and diversity of PCs in a natural system was considered when we sought to explain the effect of perfusion with 16:1 on the secretion in bile of cholesterol relative to PC. Enhanced secretion in bile of cholesterol relative to PC with perfusion of 16:1 but not other fatty acids can be explained by the distinctly different effect of 16:1 on the overall hydrophilic-hydrophobic balance of PCs in bile. Thus, although perfusion with both 16:1 and 17:1 resulted in a marked increase in the formation and secretion in bile of new molecular species of PCs (Table 2), only with perfusion of 16:1 was the overall hydrophilic-hydrophobic balance of the total bile PC population shifted to be decidedly more hydrophilic than the population of PCs in native bile,

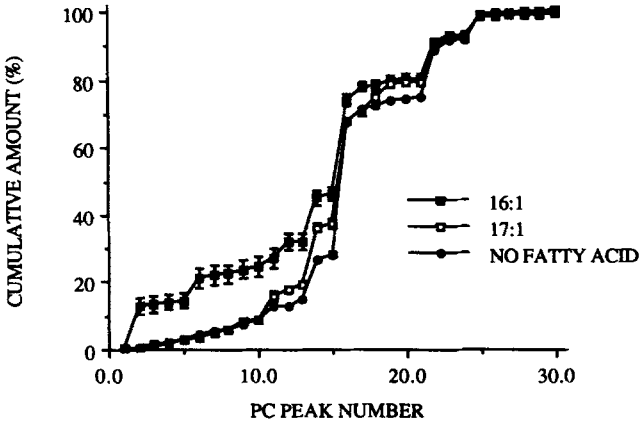

Fig. 3. Effect of liver perfusion with different fatty acids on the hydrophilic-hydrophobic balance of the total population of PCs in bile. Bile PCs were numbered from 1 to 30 in order of their elution from a reverse phase column. PCs with the shortest retention times (smallest peak numbers) are the most hydrophilic. The peak number is plotted against the percent distribution (± SE) for the entire population of PCs in bile, obtained from three perfusions performed with 16:1, 17:1 and no added fatty acid. The percent distribution of PCs was significantly different ($P < 0.05$) for the following: peaks 2–13 and 16, 17, 16:1 differed from 17:1 and No Fatty Acid; peaks 18–21, 16:1 and 17:1 differed from No Fatty Acid; peaks 14, 15, 16:1, 17:1, and No Fatty Acid groups were all different.

already highly enriched in hydrophilic PCs (i.e., containing 16:0 and 18:2 acyl groups). As shown by a plot of the effect of 16:1 and 17:1 perfusion on the retention time of all the PCs in bile relative to the PCs in native bile (**Fig. 3**), 16:1 perfusion produced a population of biliary PCs with substantially increased hydrophilic strength (measured as shorter retention times) while perfusion with 17:1 did not. Changes in the PC population in bile with perfusion of other fatty acids were smaller than with 16:1 and 17:1 and, like 17:1, did not result in a change in biliary cholesterol/PC secretion.

With techniques now available to isolate intact molecular species of PCs, it should be possible in future studies to relate the cholesterol content of hepatic membranes to their PCs composition, which might be acutely changed by the perfusion of different fatty acids. It should also be possible to examine cholesterol transport out of isolated hepatic membranes in relation to the overall PC composition of these membranes and the dependence of this process on the presence of bile salts.

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