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Transport, utilization and biliary secretion of lysophosphatidylcholine in the rat liver

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The hepatic uptake, transport and utilization of plasma lysophosphatidylcholine (lysoPC) and its contribution to biliary lipid secretion have been investigated in bile-fistula rats. The animals were given a single intravenous dose of *sn*-1-[1-^{14}C]palmitoyl-lysoPC, under constant intravenous sodium taurocholate infusion (1 μmol/min), and the fate of the label was followed in blood, bile and liver for up to 3 h. The livers were excised at given timepoints, extracted and/or homogenized to determine the lipid distribution and subcellular location of radioactivity. LysoPC was rapidly cleared from plasma, though a consistent fraction of the label persisted in plasma over the experimental time-period in the form of either lysoPC or PC. Recovery of radioactivity in the liver varied from 15.6% after 5 min to 19.5% after 3 h. Hepatic lysoPC underwent rapid microsomal acylation to form specific PC molecular species (mainly 16:0–20:4 and, to a lesser extent, 16:0–18:2 and 16:0–16:1). Ultrafiltration, dialysis and gel-chromatographic analyses of cytosolic fractions (post 105000 × *g* supernatants) indicated that lysoPC is transported to the site of acylation mostly as a macromolecular aggregate with an approx. M_r of 14400. Small amounts of radioactivity were secreted into bile over 3 h (20% in the form of lysoPC and the remainder as 16:0–18:2 and 16:0–20:4 PC species). Plasma lysoPC, taken up by the liver, is mostly transported by a cytosolic carrier with a molecular weight close to fatty-acid-binding proteins; it then enters a distinct acylation pathway, selective for some polyunsaturated-PC species and does not contribute significantly to biliary secretion, either directly, or through its products.

Introduction

Plasma lysophosphatidylcholine (lysoPC) is predominantly a non-lipoprotein-bound fraction of plasma lipids, which makes up 15% of total plasma phospholipids in the rat [1,2]. LysoPC is a mixture of molecular species deriving from enzymatic deacylations of phosphatidylcholine (PC). These reactions are a major turnover mechanism of membrane PC and occur at both extra and intracellular levels. The two major sources of lysoPC are the activity of circulating lecithin–cholesterol acyltransferase on plasma lipoprotein PC [3], and the effect of phospholipase A_2 on cellular membrane PC [4]. Both these enzymes release the *sn*-1-acyl isomer of lysoPC, the predominant and most stable form at equilibrium [5]. Little information is available on the formation of *sn*-2-lysoPC, despite the fact that phos-

Abbreviation: lysoPC, lysophosphatidylcholine.

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pholipase A_1 activity is present both in plasma and cells.

Several reports [6–8] have described the membranolytic potential of *sn*-1-lysoPC. This property led to the hypothesis of a pathogenic role of lysoPC in a variety of diseases [9]. LysoPC partially accumulates in plasma [1,2], but membrane toxicity is prevented by albumin binding [10]. As with other amphiphatic molecules [11], albumin binding may also facilitate lysoPC removal from plasma. Even though it has been recognized that the liver plays a major role in the removal of plasma lysoPC [12], little is known about the transport, biotransformation and utilization of lysoPC in the liver cell. In this study, we employed an in vivo bile-fistula rat model to address the following questions: (1) how a typical product of lecithin–cholesterol acyltransferase activity, i.e., plasma *sn*-1-palmitoyl-lysoPC, is taken up by the liver; (2) how it is metabolized; (3) where it is distributed at the subcellular level; (4) in which physical form it is present and how it is transported in the liver cell; and (5) whether lysoPC and its metabolic products are secreted into bile. At present, little is known about all these processes.

Materials and Methods

Chemicals

sn-1-[*palmitoyl*-1-^{14}C]Palmitoyl-lysoPC (spec. act. 115 mCi/mg) was obtained from New England Nuclear (Boston, MA) and was pure at TLC analysis. Sodium taurocholate (Calbiochem-Behring Corp., La Jolla, CA) was recrystallized twice from ethanol before use. Bovine albumin, fraction V, was purchased from Serva (Heidelberg, F.R.G.) and 5,5′-dithiobis(2-nitrobenzoic) acid from Sigma Chemicals (St. Louis, MO). Soluene and the liquid scintillation cocktail (Insta-gel) were obtained from Packard Instruments (Downers Grove, IL). *sn*-1-Palmitoyl-lysoPC, diacylglycerol, egg-yolk PC and egg-yolk phosphatidylethanolamine were supplied by Avanti (Birmingham, AL). All these standards gave a single spot at TLC [13] after iodine visualization. The molecular-weight standards for gel-chromatography were obtained from Fluka AG (Buchs, Switzerland). Analytical grade A solvents (Merck, Darmstadt, F.R.G. and Carlo Erba, Milan, Italy) were used throughout the study.

Dialysis and filtration devices

A separation cell for flow dialysis with half-volume capacity of 2.5 ml and dialysis discs (M_r 12000–14000 cut-off) were obtained from Spectrum Medical Industries (Los Angeles, CA). Millipore filters (0.22 μm pore size) were purchased from Millipore (Bedford, MA) and Centrifree micropartition systems from Amicon (Lexington, MA).

Animal model

Male Sprague-Dawley rats (275–325 g, Charles River, Calco, Como, Italy) were housed in common cages and given free access to food (Purina Rat Chow, St. Louis, MO) and water for at least 2 weeks prior to the study. Between 9 and 11 a.m., the animals were operated on under ether anesthesia. In brief, after opening the abdomen, the bile duct was cannulated (PE-20, Clay Adams, Parsippany, NJ) and a catheter (PE-20) was placed in the left jugular vein. Silk sutures were used throughout. The animals were restrained in individual cages and allowed to recover for 20–22 h. During this period, isotonic saline was intravenously infused (0.8 ml/h) using a Vario Perpex Peristaltic Pump mod. 1200 (LKB, Broma, Sweden) and bile was externally drained to washout the endogenous bile salt pool. The animals were given free access to food and water. To obtain a constant bile flow, an intravenous infusion of 1 μmol/min of sodium taurocholate (2 ml/h of a 30 mM taurocholate solution in saline) was started the morning after the operation and basal samples of bile were collected every 15 min.

Experimental protocol

Once stable biliary secretion rates had been achieved (typical secretory data after 1 h of continous taurocholate infusion were as follows: bile flow, 21 μl/min; bile salt secretion, 33 μmol/h; PC secretion, 3.1 μmol/h), 2 μCi of *sn*-1-[1-^{14}C]palmitoyl-lysoPC were injected intravenously in the form of a lysoPC-albumin complex. The latter was prepared by dissolving the nitrogen-dried [^{14}C]lysoPC in 0.5 ml of 1% albumin in saline and vortexing it for 2 min. Blood and bile samples were then taken at regular intervals and processed as follows: blood from the cut end of the tail was immediately divided into two 25 μl

portions. One was used for measuring total radioactivity after digestion with Soluene and the other was diluted with 1 ml of 150 mM NaCl containing 0.1% of lecithin–cholesterol acyltransferase inhibitor (5,-5′-dithiobis(2-nitrobenzoic) acid, 0.6 mg/ml, in 0.2 M $NaHCO_3$), extracted with chloroform/methanol (2 : 1, v/v) and used for chemistry as specified below. Bile was quantitatively collected and rapidly extracted with 9 vol. of isopropanol. Aliquots of this extract were used to measure total radioactivity and chemistry. Blood and bile extracts were stored at −30 °C.

At intervals of 5, 20, 45 and 180 min after the injection of radioactive lysoPC, the animals were killed by intravenous injection of 0.5 ml of ice-cold KCl solution (1 M). The abdomen was opened and the liver was excised, weighed and extracted according to Folch et al. [14]. In selected cases, aliquots of liver homogenate were used for fractionation of subcellular organelles. Blood was drawn from the heart cavity into heparinized tubes and lipid extracts were prepared as above.

Isolation of cellular organelles

All the following operations were carried out at temperatures between 2 and 4 °C. Aliquots (1–3 g) of fresh liver were homogenized using a gentle Potter hand homogenizer with 4 vol. of 0.25 M sucrose/1 mM EDTA and centrifuged at 1000 × *g* for 10 min. The resulting pellet was discarded. Mitochondria were prepared by centrifugation of the combined supernatants for 20 min at 10 000 × *g* (L8-70 M ultracentrifuge, 50.3 Ti rotor, Beckman, Fullerton, CA). Microsomes were prepared by recentrifugation of the post-mitochondrial supernatant for 60 min at 105 000 × *g*. The pellets were resuspended in 0.25 M sucrose/1 mM EDTA and used for radioactivity counting, extraction in chloroform/methanol (2 : 1, v/v), and protein assay. The post-microsomal supernatant (cytosol) was submitted to the same analysis and also to dialysis, ultrafiltration and fractionation by gel-chromatography.

Gel chromatography of the cytosolic fraction

Aliquots of the cytosol were applied to a Sephadex G-150 column (70 × 1.5 cm), equilibrated with 0.1 M Tris-HCl/1 M NaCl buffer (pH 7.8) and eluted at room temperature with the same buffer (7.0 ml/h). The column was calibrated with a standard mixture containing Dextran Blue, bovine albumin and egg white lysozime. 50 elution fractions (3.2 ml each) were collected and used for liquid-scintillation counting, protein assay and lipid analysis after solvent extraction.

Analytical procedures

Chloroform/methanol extracts were washed according to Folch et al. [14]. Isopropanol extracts of bile were centrifuged for 10 min at 2000 × *g* before use. Aliquots of the extracts were dried and used for radioactivity counting after the addition of Insta-gel scintillant. Neutral lipids and phospholipid classes were separated by thin-layer chromatography on silica gel plates (Merck, Darmstadt, F.R.G.), according to the method of Christie et al. [13], modified as previously described [15]. Each sample was run in double, using the same plate. The individual spots visualized with iodine were identified by comparison with authentic standards. The bands corresponding to lysoPC sphingomyelin, PC, phosphatidylethanolamine, diacylglycerol, triacylglycerol, free fatty acids and cholesterol esters were scraped off the plate, suspended in 3 ml of water, and 10 ml of Insta-gel were added for radioactivity counting. The average recovery of phospholipids using this procedure

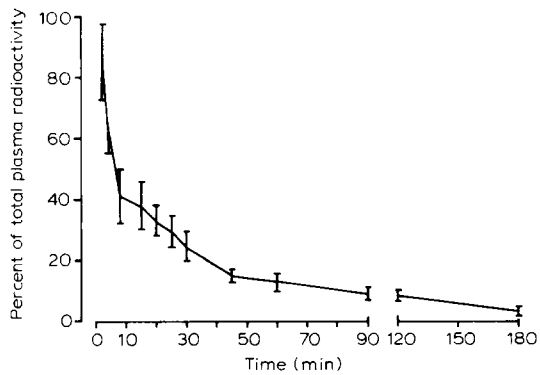

Fig. 1. Percent decrease of total plasma radioactivity after intravenous injection of [^{14}C]lysophosphatidylcholine (2 μCi in 0.5 ml of saline with 1% albumin) in bile-fistula rats. Blood samples obtained from the tail were analyzed for total radioactivity and for its distribution in serum lipids after fractionation by TLC. Values at any given time point are means ± S.D. of 3–5 experiments. The value at time point 0 (100%) was calculated by approximating blood volume to 8% body wt. [37].

was 90% [16]. The radioactive spots were scraped off from the second spotting and eluted with chloroform/methanol (2:1, v/v). The latter was dried under nitrogen and used for quantification of lipids and identification of PC molecular species by reversed-phase HPLC as described previously [17]. The fractions eluted from HPLC runs were individually collected and analyzed for radioactivity and lipid concentration.

The total biliary bile salt concentration was determined by the 3α-hydroxysteroid dehydrogenase method [18]. Total PC concentration was measured by the choline oxidase method [19] and by phosphorus assay [20]. Protein concentration in subcellular fractions was determined with the Bio-Rad (Munchen, F.R.G.) protein assay [21], using bovine albumin as the reference standard. Radioactivity was measured using a mod. 1210 Ultrobeta liquid scintillation counter (LKB, Broma, Sweden).

Results

Variations of [^{14}C]lysophosphatidylcholine and products in plasma

The time-dependent variations of total plasma radioactivity are shown in Fig. 1. A two-component disappearance curve was observed: the first, rapid ($T_{1/2}$ = 4–6 min), the second, slower ($T_{1/2}$ = 30–60 min). This is compatible with rapid and efficient tissue extraction of the lysoPC, followed by lysoPC recirculation and/or secretion of labeled products. To elucidate this, we studied the lipid distribution of plasma radioactivity, as a function of time. At all time points, most of the label was present in lysoPC. An increasing percent fraction of plasma radioactivity was found in PC (15.5 ± 3.4(S.D.)% after 8 min, 16.0 ± 3.9% after 20 min, and 38.6 ± 5.2% after 180 min) No plasma radioactivity was present in lipids other than lysoPC or PC.

Recovery and lipid distribution of radioactivity in the liver

The percent liver radioactivity and its lipid distribution as a function of time are reported in Table I. The total recovery in the liver ranged from 15.6% of the injected dose at 5 min to 19.5% at 180 min. More than 85% of the isotope was located in lysoPC and PC at any given time-point. There was a progressive decrease in radioactivity associated with lysoPC and a correspondent increase in that associated with PC over the experimental period. The specific activity of liver PC (Fig. 2) increased from 5 to 180 min and was inversely correlated ($r = -0.815$, $P < 0.001$) with that of lysoPC. Small fractions of liver radioactivity were associated with phosphatidylethanolamine, diacylglycerols and free fatty acids and traces with cholesterol esters and triacylglycerols.

Subcellular lipid distribution of liver radioactivity

20 min after the injection of the label, 69.2 ±

TABLE I

TIME-DEPENDENT VARIATIONS OF TOTAL RADIOACTIVITY IN THE LIVER AND OF PERCENT LIPID DISTRIBUTION AFTER INTRAVENOUS INJECTION OF [^{14}C]LYSOPHOSPHATIDYLCHOLINE IN BILE-FISTULA RATS

2 μCi of [^{14}C]lysophosphatidylcholine were injected intravenously in bile-fistula rats under constant infusion of 1 μmol/min sodium taurocholate. Livers were homogenized, extracted and analyzed for total radioactivity and for its distribution after lipid fractionation by TLC. [a] Total liver radioactivity was expressed as the percent of the injected amount recovered in the liver extracts. Each value is the mean ± S.D. from three to five experiments. LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; DG, diacylglycerol; CE, cholesterol esters; FFA, free fatty acids; TG, triacylglycerol.

Time (min)	Total liver [^{14}C]radioactivity (%) [a]	Percent lipid distribution of radioactivity						
		LPC	PC	PE	DG	CE	FFA	TG
5	15.6 ± 1.9	77.6 ± 13.6	19.1 ± 5.2	0.3 ± 0.08	0.8 ± 0.3	–	1.6 ± 0.3	0.1 ± 0.02
20	15.5 ± 1.5	58.4 ± 14.7	33.1 ± 8.5	1.4 ± 0.4	2.0 ± 0.9	0.8 ± 0.01	1.9 ± 0.3	0.2 ± 0.03
45	16.2 ± 1.1	5.4 ± 0.5	81.2 ± 4.5	3.9 ± 0.8	8.9 ± 1.5	0.3 ± 0.01	0.7 ± 0.1	0.3 ± 0.01
180	19.5 ± 3.9	3.6 ± 0.9	81.6 ± 3.5	7.5 ± 1.6	5.9 ± 0.8	0.5 ± 0.1	0.8 ± 0.02	0.3 ± 0.02

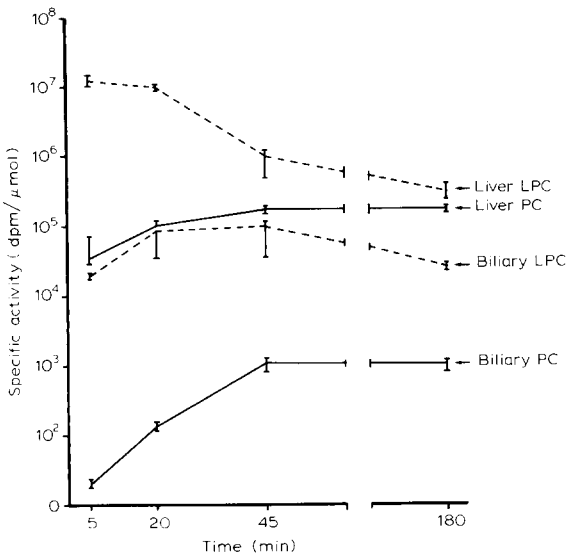

Fig. 2. Specific activity (DPM/μmol) of phosphatidylcholine (————) and lysophosphatidylcholine (- - - - -) in bile and liver after intravenous injection of [^{14}C]lysophosphatidylcholine in bile-fistula rats. 2 μCi of [^{14}C]lysophosphatidylcholine (in 0.5 ml of saline with 1% albumin) were injected intravenously and hepatic and biliary lipids, fractionated by TLC, were analyzed for radioactivity. Each value represents the mean ± S.D. from three to five experiments. At any given time point, the specific activities of lysophosphatidylcholine and phosphatidylcholine were significantly higher in the liver than in bile ($P < 0.01$). Both in the liver and in bile the specific activity of lysophosphatidylcholine was significantly higher than that of phosphatidylcholine.

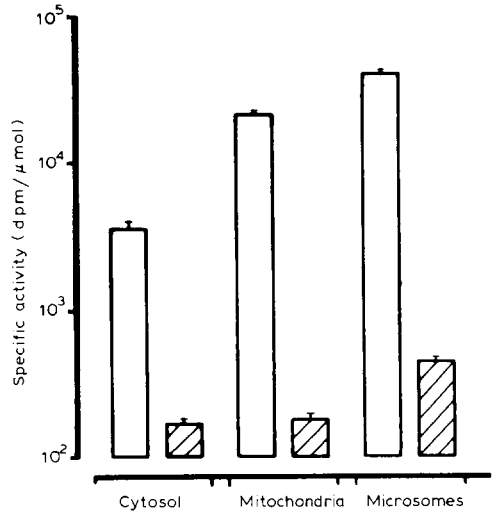

Fig. 3. Specific activity (dpm/μmol) of lysophosphatidylcholine (open bars) and phosphatidylcholine (hatched bars) in liver subcellular fractions, 20 min after intravenous injection of [^{14}C]lysophosphatidylcholine (2 μCi) in bile-fistula rats under constant infusion of sodium taurocholate (1 μmol/min). Livers were homogenized and ultracentrifuged: cytosolic, mitochondrial and microsomal lipids were fractionated by TLC and analyzed for radioactivity and mass. Lysophosphatidylcholine and phosphatidylcholine showed the highest specific activity in microsomes. Each value is the mean ± S.D. from five experiments.

9.1% of the total liver radioactivity was present in mithochondria and microsomes, where PC and lysoPC were virtually the only labeled chemical forms. A fraction of liver radioactivity (7.0 ± 1.1%) was recovered in the cytosol. In this fraction, the label was distributed almost equally among PC, lysoPC and free fatty acids. The specific activities of lysoPC and PC were higher in microsomes than in mitochondria or cytosol (Fig. 3).

Physical state of cytosolic radioactivity

Assuming an average liver water content of 68% [22], we estimated that the concentration of unlabeled lysoPC in the cytosol ranged from 36 to 130 μM. These values are above the critical micellar concentration. To determine the physical form of cytosolic lysoPC, we submitted the cytosol to ultrafiltration using Amicon Centrifree. No radioactivity was ultrafiltered after 20 min of centrifugation of the cones at 2000 rpm. We next submitted aliquots of the cytosol to flow dialysis (2 ml/min at room temperature) against normal saline using a Spectrum flow-cell equipped with 12000–14000 M_r cut-off dialysis discs. We found that 4.3% of the radioactivity was dialyzed within 24 h. We next fractionated the cytosol by gel-chromatography on Sephadex G-150. As shown in Fig. 4, the cytosolic radioactivity was distributed in three peaks, which were collected and analyzed. Peak 1, eluted at the void volume, contained mainly PC as the labeled compound; peak 2 (approx. M_r 67000) contained predominantly labeled free fatty acids; peak 3 (approx. M_r 14400) contained both labeled lysoPC and fatty acids. This peak composition was not significantly changed by running the column at 5° C.

Secretion of [^{14}C]lysophosphatidylcholine and products into bile

The percent cumulative secretion and lipid distribution of radioactivity into bile is shown in Fig.

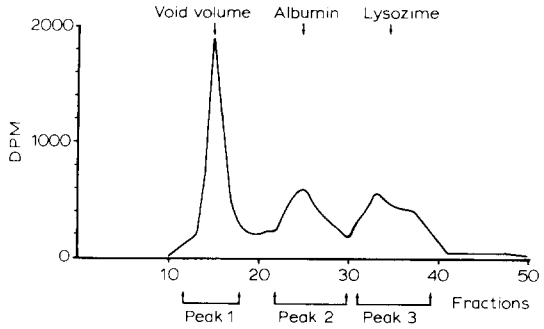

Fig. 4. Sephadex G-150 gel-chromatography of liver cytosol (post-microsomal 105000 × *g* supernatant) 20 min after intravenous injection of [^{14}C]lysophosphatidylcholine. 2 μCi of [^{14}C]lysophosphatidylcholine were injected in bile-fistula rats under constant infusion of sodium taurocholate (1 μmol/min). The animals were killed 20 min after the injection of the label. Liver homogenates were submitted to ultracentrifugation. Aliquots of the cytosol were submitted to gel chromatography. Three radioactive peaks were found: peak 1 (corresponding to the void volume); peak 2 (approx. M_r 67000); peak 3 (approx. M_r 14400). Each peak was collected, extracted for lipids and the radioactivity was measured after fractionation by TLC. In peak 1 the label was distributed in PC (69.4 ± 10.2%), FFA (22 ± 3%) and lysoPC (8.6 ± 1.5). In peak 2 it was present in FFA (89 ± 15), PC (9 ± 2%) and lysoPC (2 ± 0.8%). The radioactivity of peak 3 was distributed in lysoPC (47 ± 9%), FFA (40 ± 9%) and PC (13 ± 2.5%). FFA, free fatty acid.

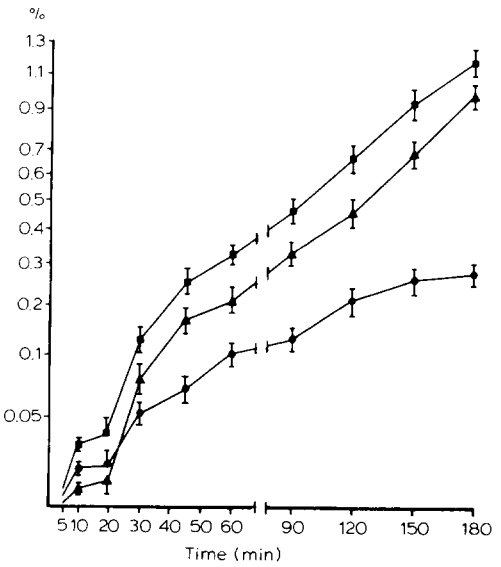

Fig. 5. Cumulative biliary secretion of total radioactivity (■) and of labeled lysophosphatidylcholine (●) and phosphatidylcholine (▲) following intravenous injection of [^{14}C]lysophosphatidylcholine in bile-fistula rats. Data are means ± S.D. of three to five experiments.

5. A total of 1.2 ± 0.2% of the injected radioactivity was recovered in bile over the experimental period. From 0 to 20 min, lysoPC was the predominant labeled component of bile, then PC became prevalent. By the end of the study period, lysoPC accounted for 22.4 ± 2.5% of the radioactivity secreted into bile.

The specific activities of lysoPC and PC in liver and bile are given in Fig. 2. The values obtained in bile were always significantly ($P < 0.01$) lower than the correspondent values in the liver. This indicates that plasma lysoPC and PC synthesized in the liver via lysoPC acylation contribute little to biliary secretion. In bile, the specific activity of lysoPC was constantly higher than that of PC, indicating that part of lysoPC is secreted without any apparent metabolic modification.

Molecular selectivity of liver metabolism and biliary secretion of lysophosphatidylcholine

Liver and biliary extracts were analyzed by combined reversed-phase HPLC-liquid scintillation to identify the molecular species of PC synthesized via the acylation of [^{14}C]lysoPC and secreted into bile. As indicated by the specific activities of the individual molecular species given in Table II, both in liver and bile the label was incorporated mainly in 16:0–20:4 and, to a lower extent, in 16:0–16:1 and 16:0–18:2 PC. This selective pattern of acylation was virtually identical in liver microsomes and mithochondria and was independent of the time elapsed after [^{14}C]lysoPC injection. No radioactivity was present in the other PC molecular species detected in liver and bile.

Discussion

Previous studies have indicated the important role of the liver in the removal of plasma lysoPC [12]. We have examined this role further and, for the first time, explored the mechanisms of intracellular transport, the hepatic biotransformation and the contribution of plasma lysoPC to biliary lipid secretion. Our data refer to a typical end-product of lecithin–cholesterol acyltransferase activity, i.e., *sn*-1-palmitoyl-lysoPC and the results shown do not necessarily apply to other forms of

TABLE II

MOLAR PERCENTAGES AND RESPECTIVE SPECIFIC ACTIVITIES (±S.D.) [a] OF HEPATIC AND BILIARY MOLECULAR SPECIES OF PHOSPHATIDYLCHOLINE 180 MIN AFTER INTRAVENOUS INJECTION OF [^{14}C]LYSOPHOSPHATIDYLCHOLINE IN BILE-FISTULA RATS

2 μCi of [^{14}C]*sn*-1-palmitoyllysophosphatidylcholine were injected in bile-fistula rats under constant intravenous infusion of 1 μmol/min sodium taurocholate. After 180 min liver and biliary extracts were analyzed for PC molecular species and radioactivity by a combined HPLC-liquid scintillation method.

	Liver		Bile	
	(%)	spec. act. (dpm/μmol × 10^3)	(%)	spec. act. (dpm/μmol × 10^3)
16:0–16:1	6.9 ± 0.3	468 ± 55	5.1 ± 1.4	1.79 ± 0.60
16:1–18:1	6.8 ± 1.0		4.5 ± 1.4	
16:0–20:4	15.8 ± 1.1	1424 ± 391 [b]	18.5 ± 5.8	3.41 ± 0.86 [c]
16:0–18:2	23.3 ± 3.1	248 ± 135	64.3 ± 8.2	0.57 ± 0.33
16:0–20:3	1.3 ± 0.4		0.4 ± 0.1	
16:0–18:1	3.5 ± 0.8		0.8 ± 0.2	
18:1–18:1	10.7 ± 0.9		1.7 ± 0.9	
18:0–20:4	23.7 ± 2.9		2.7 ± 0.8	
18:0–18:2	6.2 ± 2.1		3.1 ± 2.2	

[a] Mean values ± S.D. from five experiments.
[b] $P < 0.01$ vs. 16:0–18:2 and 16:0–16:1.
[c] $P < 0.05$ vs. 16:0–18:2 and 16:0–16:1.

plasma lysoPC, such as, for example, the *sn*-2-acyl species.

In keeping with previous studies [23], we found a fast, two-component shaped, clearance of lysoPC from plasma. This is compatible with rapid and efficient tissue extraction of the lipid, followed by recirculation and/or secretion of metabolic products. The long-standing persistence in plasma of an aliquot of lysoPC is unexplained. Although not specifically studied, we hypothesize that it might be due to delayed clearance of non-albumin-bound lysoPC. It has been shown, in this respect, that an aliquot of lysoPC is carried by plasma lipoproteins [24].

Approx. one-fifth of the total radioactivity injected as lysoPC was recovered in the liver. Microsomal acylation of lysoPC to PC was by far the predominant biotransformation pathway of hepatic lysoPC. Although it is conceivable that the liver takes up a fraction of labeled PC formed in extrahepatic tissues (which might mimic very rapid hepatic acylation), the acylation process was already evident 5 min after [^{14}C]lysoPC injection and almost completed after 45 min. There was only marginal incorporation of lysoPC in other lipid classes or metabolic intermediates, with the exception of phosphatidylethanolamine and diacylglycerol. In contrast to what we found [15] by infusing perfused rat livers with [^{14}C]palmitoyl-PC, in this study, virtually no radioactivity given as *sn*-1-[^{14}C]palmitoyl-lysoPC was incorporated in triacylglycerol. This suggests that exogenous PC and lysoPC are taken up and/or transported by different mechanisms and do not share the same metabolic pathway.

It is not known how lysoPC moves across the liver cell. In theory, due to its relatively high hydrophilicity [25,26], lysoPC released by circulating and endocellular phospholipases should easily desorb from membranes and diffuse through the aqueous phase to be redistributed (and re-acylated) in other membranes. Thus, rapid intermembrane exchange of lysoPC may occur without any special transport system.

We estimated that the cytosolic concentration of unlabeled lysoPC was between 36 and 130 μM. Both these values are well above the CMC reported for palmitoyl lysoPC [27]. In contrast to PC, in model systems, lysoPC does not assume a bilayer arrangement, but forms micelles. Thus, it is possible that lysoPC molecules self-aggregate in the cytosol to form micelles. A recent in vitro

study has, in fact, proposed that lysoPC micelles are involved in the intracellular transport of cytochrome b_5 [28]. As, in this study very high lysoPC concentrations were tested, we believe that there is not yet enough evidence for the existence of intracellular lysoPC micelles.

Our preliminary dialysis experiments suggested that cytosolic lysoPC is mostly in the form of high-molecular weight aggregates. This was confirmed by the gel-chromatographic isolation of a cytosolic fraction, with an approx. M_r of 14400, which comprises the bulk of lysoPC. Because of the similarity between this molecular weight and that of the fatty-acid binding protein [29], the question arises whether lysoPC and fatty acids share the same intracellular carrier, as they do in plasma. A recent study [30] has shown that in vitro lysoPC binds to a rat liver fatty-acid-binding protein, with a M_r of 13500, which is consistent with our in vivo results. A minor fraction of the cytosolic radioactivity present as lysoPC was eluted from the gel-chromatographic column at the void volume. Hence, it could have been present in the form of either a micelles or a vesicle. Taken together, these data support the view that most of the lysoPC is transported in the cytosol by a $M_r \approx 14400$ carrier protein and that other carriers give limited contribution. Binding to a cytosolic protein may also be a way to protect from cellular lysoPC toxicity.

We next considered the possibility that lysoPC is involved in the export of PC from liver into bile. The origin of biliary phospholipids is not known. Robins et al. [31] have shown that the neosynthesis of PC in the liver is insufficient to compensate for bile secretion, suggesting that a substantial contribution may derive from plasma phospholipids. As lysoPC is an important fraction of plasma phospholipids, it might well be a precursor of biliary lipids. This possibility is based on the fact that lysoPC is formed continuously in plasma; it is largely cleared by the liver; it is bound to albumin, like many other cholephiles [11]; it is much more water-soluble than PC [26]; there is evidence of secretion of lysoPC by the liver into plasma [32]. Despite this, our study shows that: (1) the direct contribution of lysoPC to biliary secretion is small and of short duration; (2) lysoPC is not a preferential precursor of biliary PCs.

With respect to the first point, the early appearance of lysoPC in bile, and the apparent short duration of its secretion, suggest that part of lysoPC, when available in the cytosol, rapidly moves into bile (and likely into plasma), possibly with the aid of the vectorial carrier discussed above.

With respect to the second point, it is possible that the small contribution of lysoPC as precursor of biliary PCs is due to its preferential utilization in the synthesis of 16:0–20:4 PC [33–35] (a molecular species not typical of bile [36]). This species is an important component of lipoprotein PC, which indirectly suggests its preferential secretion into plasma rather than bile.

In conclusion, this study suggests that plasma lysoPC is transported in the liver by an intracellular carrier, possibly a fatty acid-binding protein, and partly as a micellar or vesicular aggregate. A small fraction of hepatic lysoPC is directly secreted into bile. The rest is efficiently and rapidly reacylated in microsomes, to form almost selectively *sn*-2-arachidonoyl-PC. Neosynthesized PCs are distributed among cellular membranes and do not contribute much to biliary secretion.

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