Effect of different molecular species of phosphatidylcholine on the clearance of emulsion particle lipids

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Abstract Studies were performed to assess the effect of changes in the molecular species composition of phosphatidylcholine (PC) on the clearance of emulsion particles that were made to approximate chylomicrons in size and lipid composition. Emulsions were prepared with free [^{14}C]cholesterol, [^{3}H]cholesteryl oleate, triolein, and one of four single PCs that differed in hydrophilic strength (as assessed by the relative rate of elution of these PCs from a reverse phase column). Emulsions were injected as an intravenous bolus into unanesthetized rats and the clearance of lipids was determined at 2-min intervals for 10 min. All emulsion lipids were cleared from the serum in parallel and in an order that closely corresponded to the relative hydrophilic strength of the particular PC that was administered. Fractional rates of clearance, calculated from log-linear plots, were 2- to 10-fold greater for all lipids for the emulsion that was made with the most hydrophilic PC compared to the least hydrophilic PC. Although hepatectomy, performed in acutely anesthetized animals, generally slowed the clearance of lipids, hepatectomy did not abolish differences in the clearance of triolein or specific PCs from emulsions prepared with the most and least hydrophilic PCs. These results indicate that a change in the composition of emulsion particle PCs, independent of any other change in the lipid composition of these particles, can significantly change the metabolism of the whole emulsion particle in the live animal. More specifically, these studies show that the rate of clearance of all emulsion lipids closely corresponds to the hydrophilic strength of the PCs that occupy the emulsion particle surface. Results of hepatectomy suggest that the differences in clearance of emulsion triolein with different PCs are most likely the consequence of differences in the rate of formation of emulsion remnants, prior to the uptake of these particles by the liver. However, in intact animals the effect of different PCs on the clearance of emulsion cholesteryl ester was more profound than on triolein, and since the rate of cholesteryl ester clearance represents the rate of remnant uptake, it is likely that the process of remnant uptake and remnant formation are both influenced by the specific composition of PCs on the emulsion particle surface. — **Robins, S. J., J. M. Fasulo, and G. M. Patton.** Effect of different molecular species of phosphatidylcholine on the clearance of emulsion particle lipids. *J. Lipid Res.* 1988. **29:** 1195–1203.

Supplementary key words chylomicrons • lipoproteins • triglycerides • cholesterol • remnants • hydrophilic strength • hepatectomy

Phosphatidylcholines (PCs), which exist in many different molecular forms, are principal surface components of lipoproteins. In a number of different studies it has been demonstrated that the hydrolysis or transfer of lipoprotein PCs may play an integral role in lipoprotein metabolism (for reviews, see refs. 1–3) and that lipoprotein PCs of different molecular composition are hydrolyzed and transported at different rates (1, 4–6). In the complex scheme of lipoprotein metabolism, the impact of changes in PC metabolism appears to be especially relevant for the clearance of chylomicrons from the circulation. Chylomicrons, newly generated with each meal, may contain a great variety of PC molecular species that may be markedly changed by acute changes in the fatty acids of the diet (7). After entry into the circulation, chylomicrons lose PCs by transfer and/or hydrolysis when acted upon by lipoprotein lipase (LPL) during the formation of remnants (8–11), and perhaps again when acted upon by hepatic lipase during remnant uptake by the liver (12, 13). At both of these metabolic junctures, chylomicron-PCs could influence the overall rate of chylomicron clearance, especially if PCs of different molecular composition were selectively metabolized. Indeed, there is precedent for this presumption, since we have previously found (14) that individual molecular species of PC incorporated into a high density lipoprotein (HDL)-recombinant are not only cleared from the serum at different rates but that the clearance of individual PCs strongly influences the rate of clearance of free cholesterol from these particles.

Abbreviations: PC(s), phosphatidylcholine(s); LPC, lysophosphatidylcholine; FC, free cholesterol; CE, cholesteryl ester(s); LPL, lipoprotein lipase; HDL, high density lipoproteins(s); HPLC, high performance liquid chromatography; GLC, gas-liquid chromatography.

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The present study was undertaken to determine whether the metabolism of emulsion particles that resemble chylomicrons is responsive to selective changes in PC composition. Since chylomicrons contain a variety of different PC species and since diet-induced changes in chylomicron-PCs are also accompanied by changes in the composition of other chylomicron components (notably, triglycerides), the present study was conducted with sonicated emulsions that were made with single molecular species of PC but that otherwise closely approximated chylomicrons in size, lipid composition, and physiologic behavior.

MATERIALS AND METHODS

PCs were obtained from Avanti Polar Lipids (Birmingham, AL); triolein, cholesterol, and cholesteryl oleate from Nu-Chek-Prep (Elysian, MN); stigmasterol and stigmasterol acetate from Steraloids (Wilton, NH); and [1,2,6,7-^{3}H]cholesteryl oleate (65 Ci/mmol) and [4-^{14}C]cholesterol (50 mCi/mmol) from Du Pont-New England Nuclear (Boston, MA). [2-^{3}H]Glycerol-labeled tripalmitolein was a generous gift from Dr. Dharma Kodali (Boston University School of Medicine). All solvents were HPLC grade and were obtained from Fisher Scientific (Medford, MA).

Experimental design

The clearance of emulsion lipids was determined in unanesthetized, male Sprague-Dawley rats (180–230 g) that were fasted overnight. Studies were performed in animals that 16–18 hr earlier had been fitted with a femoral vein cannula for the injection of an emulsion and a femoral artery cannula for blood sampling. Emulsions were administered as a bolus in 10–12 sec. Blood samples in amounts of ~500 μl were obtained just before emulsions were administered and at 2, 4, 6, 8, and 10 min after the emulsions were injected. After the last blood sample was obtained, the animal was quickly anesthetized with Nembutal (~50 mg/kg, i.v.) and the liver was then perfused free of blood and removed. To assess the effect of hepatectomy on the clearance of emulsion lipids, this same protocol was followed except that rats were studied immediately after acute anesthesia with diethyl ether and a functional hepatectomy (15) or acute anesthesia and sham hepatectomy surgery.

Preparation of emulsions

Emulsions were made fresh for each experiment by sonicating [^{14}C]cholesterol (FC), [^{3}H]cholesteryl oleate (CE), triolein, and one of four different PCs with 20:1–20:1, 18:0–18:2, 16:0–18:2, or 16:1–16:1 acyl groups. These PCs are listed in increasing order of hydrophilic strength and were selected to represent molecular species with hydrophilic strengths over a broad range[2] (as can be demonstrated by the large differences in the rates of elution of these PCs from a C-18 reverse phase column (17)). All PCs used were in the liquid crystalline fluid chain state when hydrated at room temperature.

Lipids were first co-solubilized in $CHCl_3$, dried under N_2 and by lyophilization overnight, and then sonicated at room temperature in 8 ml of 2.78 M NaCl (d 1.10 g/ml) (18) with an Ultrasonic instrument, model 220-F, at 100% output with a 0.5 inch (dia.) probe. Sonication was continued for about 50 min and the emulsion was then ultracentrifuged in discontinuous NaCl gradients (18) to isolate by flotation particles of more uniform size. Electron microscopy of negatively stained emulsions that were made with 16:1–16:1 and 20:1–20:1 PCs (representing the extremes of the hydrophilic strengths of the PCs used) appeared identical. Particles in both cases measured 50–100 nm in diameter. About 3 mg (total) of emulsion lipids, containing ~0.05 μCi of ^{14}C and ~0.20 μCi of ^{3}H, was given each animal (in a volume of ~0.6 ml). Four to six animals were used for each emulsion that was prepared with a different PC.

In two additional experiments an attempt was made to determine the extent to which emulsion PC was hydrolyzed in the plasma to lysophosphatidylcholine (LPC) and taken up by the liver and (as an indication of reticuloendothelial tissue uptake) the spleen. This study was undertaken in intact rats using an emulsion prepared with [2-^{3}H]glycerol-labeled 16:1–16:1 PC (~1.35 mCi/mmol) that was otherwise identical in composition to the emulsions prepared with 16:1–16:1 PC described above. Each rat received ~0.72 μCi of the 16:1–16:1 PC. (The radiolabeled PC was obtained from lymph as previously described (7), after feeding [2-^{3}H]glycerol-labeled tripalmitolein to a single rat with a mesenteric lymph fistula.)

Analytical procedures

Lipids were extracted from samples by the method of Folch, Lees, and Sloane Stanley (19). Known amounts of stigmasterol, stigmasterol acetate, trieicosonoate, and either 16:1–16:1 PC or 20:1–20:1 PC were added to extracts to serve as recovery markers for FC, CE, triolein, and each of the four PCs used. Neutral lipid and PC fractions were initially separated by an isocratic HPLC system that we have described previously (17). PCs were then hydro-

[2]The hydrophilic strength (or the hydrophilic-hydrophobic balance) of lipids that contain acyl groups is a function of both the number of carbons and double bonds in these groups. The hydrophilic strength of different molecules can be assessed by HPLC and corresponds to the relative rate of elution of these molecules from a hydrophobic (octadecylsilane) reverse phase column (16). In this report, we have used this measure of hydrophilic strength to distinguish individual molecular species of PC.

lyzed and derivatized to their benzoyl esters (20) and separated into individual molecular species by reverse phase HPLC (17). The derivatized PCs were detected at 230 nm and quantitated by integration of peak areas. FC, CE, and triglycerides were separated by HPLC, using a LiChrospher Si-100 column (EM Laboratories, Elmsford, NY) and a mobile phase of hexane–tetrahydrofuran–acetic acid 500:20:0.1. Esterified sterols were saponified, and all sterol fractions were then rechromatographed, using an Ultrasphere ODS column and a mobile phase of methanol. Cholesterol was quantitated by integration in conjunction with the stigmasterol standards. Tritium and ^{14}C activities were determined by liquid scintillation counting. Triglycerides were transesterified with sodium methoxide and the amount of 18:1 in this fraction was quantitated by a previously described GLC procedure (21).

Calculations

The fractional rates of clearance from the serum of all emulsion lipids were calculated from log-linear regression plots of the percent of lipids remaining in the serum from 0 to 10 min after emulsions were injected. The amounts of triolein and each of the four PCs remaining in the serum were determined by subtracting the amounts of 18:1 in the triglyceride fraction and the molecular species of each particular PC present in the serum just before the emulsions were injected from the amounts of these lipids in the serum at each time point after emulsions were administered. The amount of FC and cholesteryl oleate remaining in the serum after emulsions were administered was determined by the recovery of radioactivity in the FC and CE fractions of serum. Statistical differences between groups were determined by one-way analysis of variance (ANOVA).

RESULTS

The composition of the four emulsions administered to rats, each prepared with a single different molecular species of PC, is shown in **Table 1.** The composition of emulsions with the three most hydrophilic PCs (i.e., with 16:1–16:1, 16:0–18:2, and 18:0–18:2 PC) was virtually identical. Emulsions with 20:1–20:1 PC, the least hydrophilic of the PCs used, contained about 10% less triolein, 9% more PC, and 1% more FC than any of the three other emulsions.

Clearance of emulsion lipids

Emulsions were injected as a bolus into awake, restrained rats. The clearance from the serum of PCs and FC, the two surface components of these emulsions, is shown in **Fig. 1** and the clearance of triolein and cholesteryl oleate, the core components, is shown in **Fig. 2.** Clearance of all emulsion lipids was closely correlated and closely corresponded to the hydrophilic strength of the particular PC species that was administered. Thus, clearance was most rapid when emulsions were prepared with 16:1–16:1 PC and least rapid when emulsions were prepared with 20:1–20:1 PC. Fractional rates of clearance of all emulsion lipids were calculated from these plots (**Table 2**). Clearance was not significantly different for emulsions that were prepared with the two most hydrophilic PCs. However, all of the lipids of one or both of the emulsions prepared with the two most hydrophilic PCs were cleared significantly faster than the lipids of the emulsions prepared with PCs of lesser hydrophilic strength. To determine whether these purely lipid emulsions, prepared with single PCs, were metabolized any differently than chylomicrons with a mixture of PCs, chylomicrons were isolated from the mesenteric lymph of a rat fed triolein and then injected as an intravenous bolus into a second rat. The lipid class composition of the chylomicrons administered was similar to the emulsions used in this study and contained 18.0% total phospholipids and 76.2% triglycerides—the fatty acids of which consisted predominantly of 18:1 (81.4%). As shown in **Fig. 3,** the clearance of 18:1-containing triglycerides from chylomicrons almost precisely mirrored the clearance of triolein from the emulsions prepared with 20:1–20:1 PC (redrawn from Fig. 2).

TABLE 1. Composition of emulsions prepared with different single phosphatidylcholines

Emulsion Component	Emulsion PC			
	20:1–20:1	18:0–18:2	16:0–18:2	16:1–16:1
	mol %[a]			
Triolein	63.5 ± 1.4	73.8 ± 0.9	73.8 ± 2.8	72.8 ± 0.8
PC	28.6 ± 1.5	19.8 ± 0.1	19.3 ± 2.8	20.4 ± 1.0
CE	3.0 ± 0.1	2.7 ± 0.5	3.1 ± 0.1	3.2 ± 0.2
FC	4.9 ± 0.2	3.9 ± 0.4	3.8 ± 0.1	3.7 ± 0.3

Data shown after preparations were sonicated and reisolated by density gradient ultracentrifugation (see Methods).
[a] Mean ± SD for two to four preparations made with each PC.

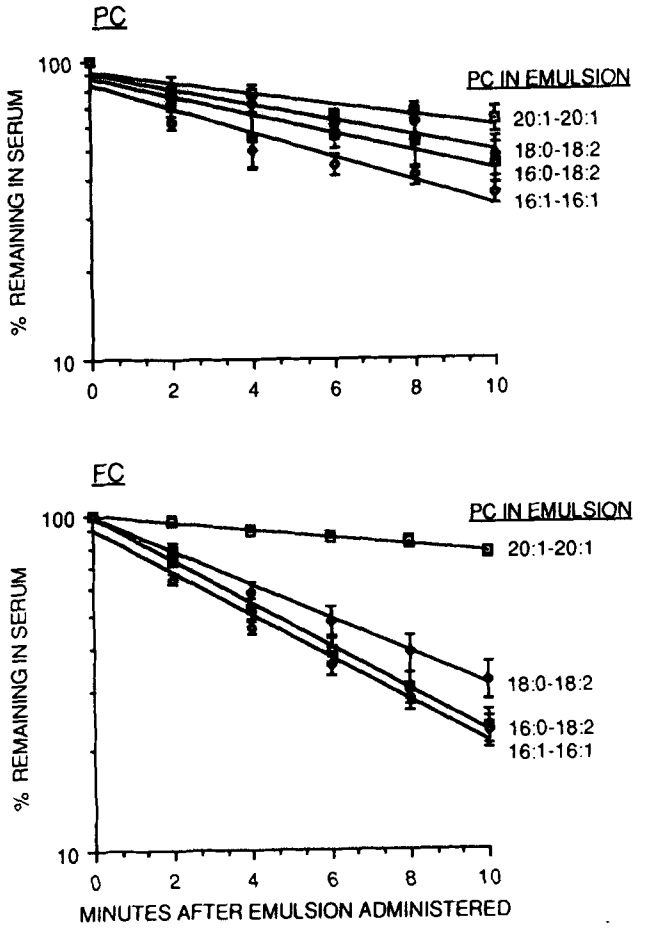

Fig. 1. Clearance of individual molecular species of PC and FC from emulsions, injected as an intravenous bolus. Emulsions were prepared with the single PCs that are shown and that are listed in increasing order of hydrophilic strength. Values are shown as % of emulsion lipid remaining in the serum ± SEM for four to six animals injected with each emulsion.

The uptake of radiolabeled FC and CE by the liver 10 min after the administration of the different emulsions ranged from 60 to 70% for emulsions prepared with 16:1-16:1, 16:0-18:2, and 18:0-18:2 PCs and from 10 to 20% for emulsions prepared with 20:1-20:1 PC (**Fig. 4**). Taken together with the amounts of radiolabeled FC and CE still present in the serum at 10 min, greater than 85% of the FC and CE that was administered was recovered in all groups at the conclusion of these studies.

The recovery of emulsion PC, as measured in the serum, liver, and spleen by the recovery of radiolabeled PC and LPC containing a 16:1 group, was far less complete than the recovery of CE and FC (**Fig. 5**). Although all of the radiolabeled PC that remained in the serum at the conclusion of this study was still present as 16:1-16:1 PC, it is clear by the presence of radiolabeled LPC in the serum as early as 2 min after the emulsion was administered that this emulsion PC was extensively hydrolyzed. Liver uptake of the radiolabeled PC was far less than that of emulsion CE and FC and was associated with the appearance of newly formed molecular species of PC containing a 16:1 acyl group. Negligible amounts of the emulsion PC were recovered in the spleen. Amounts of PC radioactivity that were administered were relatively small and no attempt was made to further trace the uptake of radioactivity into other tissues of the body or to recover all of the possible products of PC hydrolysis (some of which may have been water-soluble).

Effect of hepatectomy on the clearance of emulsion lipids

The clearance of emulsion lipids was determined in acutely hepatectomized animals and their sham-hepatectomized controls, using 16:1-16:1 PC and 20:1-20:1 PC to prepare emulsions (**Fig. 6**). As a result of anesthesia and acute surgery, the clearance of emulsion lipids, with exception of the PCs, was appreciably slowed (significance level of $P < 0.05$ or greater for each lipid, comparing sham-hepatectomized animals with intact animals). In

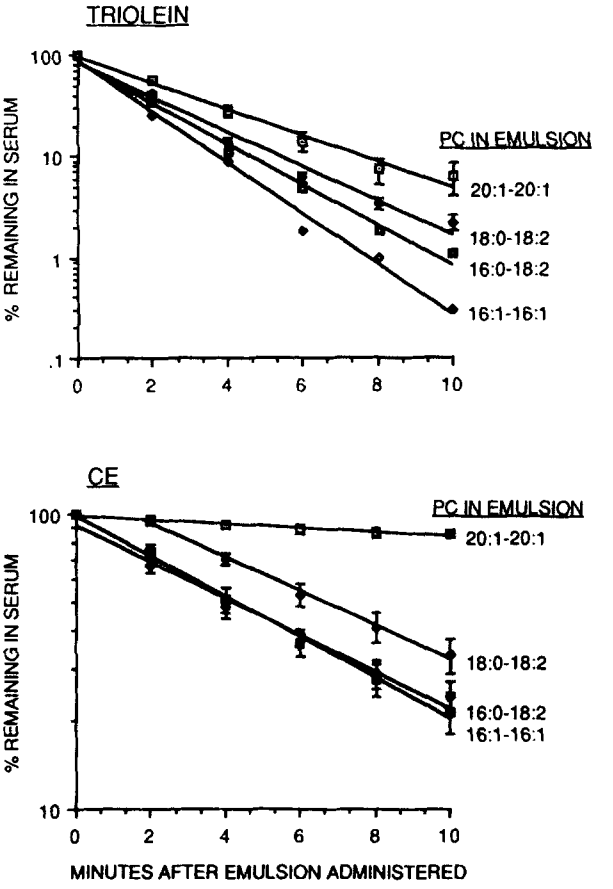

Fig. 2. Clearance of triolein and CE from emulsions, injected as an intravenous bolus. Groups are the same as shown in Fig. 1. Values are shown as % of the emulsion lipid remaining in serum ± SEM for four to six animals.

TABLE 2. Effect of different phosphatidylcholines on the rate of clearance of emulsion lipids from the serum

Emulsion PC[a] Administered	Fractional Clearance (min^{-1})[b]			
	Emulsion PC[c]	FC	CE	Triolein
1. 20:1-20:1	0.038 ± 0.014	0.020 ± 0.006	0.012 ± 0.005	0.229 ± 0.061
2. 18:0-18:2	0.058 ± 0.028	0.089 ± 0.025	0.077 ± 0.026	0.380 ± 0.046
3. 16:0-18:2	0.079 ± 0.040	0.113 ± 0.026	0.121 ± 0.032	0.467 ± 0.249
4. 16:1-16:1	0.092 ± 0.025	0.125 ± 0.013	0.124 ± 0.033	0.542 ± 0.105

[a] Listed in *increasing* order of hydrophilic strength.
[b] Calculated from the serum disappearance curves in Figs. 1 and 2 and shown as mean ± SD for four to six animals in each PC group.
[c] By ANOVA, $P < 0.05$, for emulsion PC, group 4 vs. 1 and 2; for emulsion FC, group 4 vs. 1 and 2, group 3 vs. 1 and 2, group 2 vs. 1; for emulsion CE, group 4 vs. 1 and 2, group 3 vs. 1 and 2, group 2 vs. 1; for emulsion Triolein, group 4 vs. 1 and 2.

animals injected with emulsions that contained 20:1-20:1 PC, the clearance of FC and CE was so slowed by acute surgery that there were no perceptible differences between the sham and hepatectomized groups. However, the clearance of both FC and CE was still appreciable after surgery in animals given emulsions with 16:1-16:1 PC and, in this case, was notably reduced by hepatectomy. In contrast, the clearance of triolein as well as the clearance of PCs was not decreased by hepatectomy in either the 20:1-20:1 PC or 16:1-16:1 PC group. Moreover, just as in intact animals, the clearance of both triolein and PC in animals that had undergone hepatectomy remained distinctly more rapid for emulsions prepared with 16:1-16:1 PC than with 20:1-20:1 PC ($P < 0.01$, for triolein and $P < 0.02$, for the emulsion PCs).

DISCUSSION

Phospholipid-stabilized emulsions with a high content of triglycerides acquire apolipoproteins when injected intravenously and are metabolized as chylomicrons (18, 22-25). Characteristically, this metabolism takes place in two stages in which, first, triglycerides of the emulsion (or chylomicron) particle are hydrolyzed resulting in the formation of a remnant and, second, remnants are actively taken up by the liver by a receptor-mediated process.

In the present study, we have prepared emulsions that, after intravenous injection in rats, simulated the kinetic behavior of chylomicrons. However, instead of a large array of PC molecular species that are ordinarily present in chylomicrons, these emulsions were prepared with one of four single molecular species of PC. The selection of PCs was made to include molecules that had a range of hydrophilic strengths, molecules that were all liquid at room temperature, and molecules that contained acyl groups that are either usual prominent components of rat serum PCs (16:0, 18:0, and 18:2) (6) or acyl groups which, when fed to rats for short periods (16:1 and 20:1 in the form of triglycerides), have been shown (26) to be readily incorporated into serum and tissue PCs. We found, independent of any other change in the lipid composition of these emulsions, *1*) that substitution of single PCs with different hydrophilic strengths resulted in significant changes in the metabolism of the whole emulsion particle; *2*) that the rate of clearance from the serum of emulsions closely corresponded to the hydrophilic strength of the particular PC used to prepare an emulsion; and *3*) that the differences in the clearance of emulsions made with different PCs could most probably be attributed to differences in the rate of formation of remnants (as reflected by triolein clearance) as well as to the rate of remnant uptake by the liver (as reflected by CE clearance).

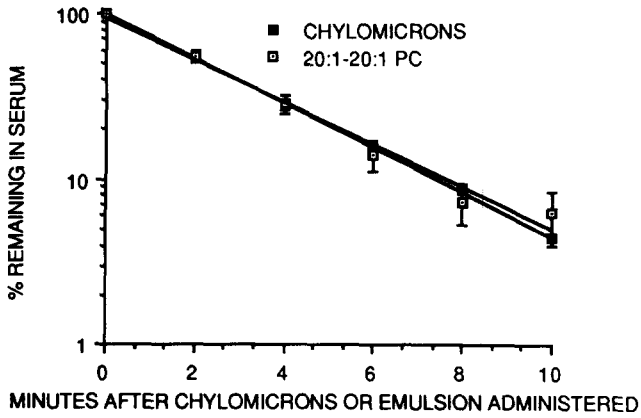

Fig. 3. Clearance of 18:1-containing triglycerides from chylomicrons and from emulsions prepared with 20:1-20:1 PC (shown in Fig. 2.). Chylomicrons, enriched in 18:1, were isolated from the mesenteric lymph of a rat fed triolein and were injected into a second rat as an intravenous bolus. After injection of chylomicrons, the amount of 18:1 remaining in serum triglycerides was determined as described for sonicated emulsion preparations.

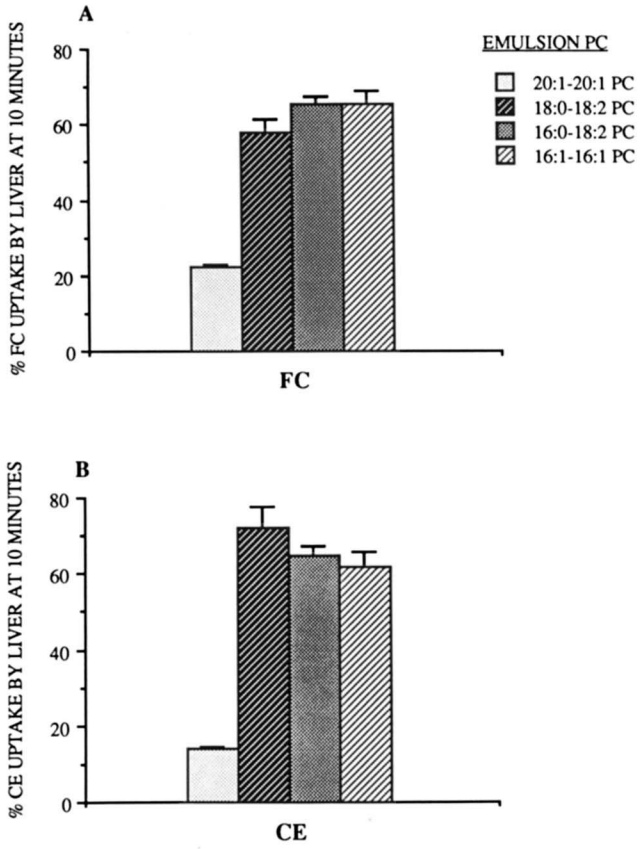

Fig. 4. Amount of emulsion [^{14}C]FC (panel A) and [^{3}H]CE (panel B) recovered in the liver 10 min after emulsions containing different single PCs were administered. Values are mean ± SEM for the groups shown in Figs. 1 and 2.

The emulsions that were made with different PCs were extremely similar in lipid composition in three of four instances (post-sonication). In only the case of emulsions prepared with the least hydrophilic PC used, 20:1-20:1 PC, were the proportions of lipids somewhat different. In spite of this disparity that might suggest that the clearance data for emulsions prepared with 20:1-20:1 PC were not comparable to the data for the other three emulsions, the clearance of triglycerides from chylomicrons (containing a mixture of naturally formed molecular species of triglycerides as well as PCs) most closely approximated the clearance of triolein from emulsions that were prepared with 20:1-20:1 PC (shown for comparison with the disappearance curve of chylomicrons in Fig. 3). The half time of triglyceride clearance in both cases was approximately 2.5 min and virtually the same as previously reported for the clearance of triglycerides from chylomicrons in the unanesthetized rat (27). It is of further note that, although by composition emulsions with 20:1-20:1 PC might not have been strictly comparable to the emulsions prepared with the other three PCs, the clearance of lipids from emulsions prepared with one or both of the two most hydrophilic PCs (16:1-16:1 PC and 16:0-18:2 PC) was still significantly different (Table 2) from emulsions prepared with the next-to-least hydrophilic PC (18:0-18:2 PC), that contained the same proportion of lipids.

In the present study, we attempted to trace the disappearance from the serum of emulsion PC, using 16:1-16:1 PC that was radiolabeled. The disappearance from the serum of this PC was appreciable at 2 min and was accompanied by the formation of LPC (Fig. 5). Since we further found that only a relatively small amount of the PC that was administered was intact in the liver at the end of the study (i.e., ~7.5% as 16:1-16:1 PC) and since hepatectomy did not slow the clearance from the serum of this PC (or 20:1-20:1 PC) (Fig. 6), it is probable that the clearance of PC from emulsions can be largely attributed to extrahepatic hydrolysis by LPL. In vitro, LPL will catalyze the hydrolysis of PCs as well as triglycerides in

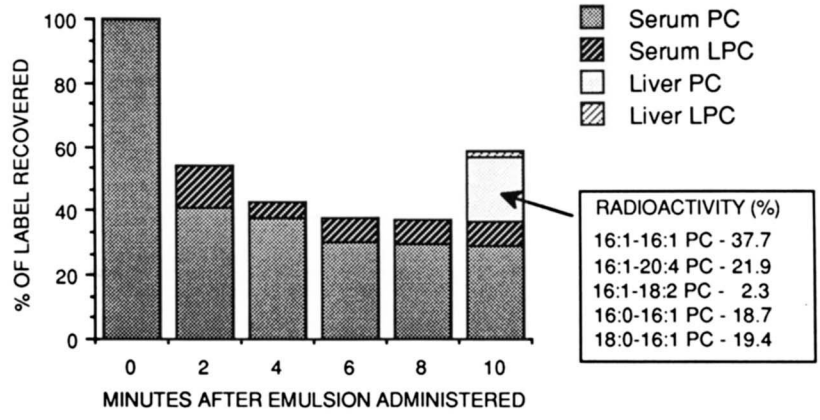

Fig. 5. Amounts of [^{3}H]PC and [^{3}H]LPC recovered in the serum, liver, and spleen after the administration of an emulsion made with [^{3}H]16:1-16:1 PC. Only 16:1-16:1 PC was detected in the serum whereas a variety of 16:1-containing PCs were measured in the liver after 10 min. Negligible amounts (~0.4%) of PC + LPC were detected in the spleen (not shown). Values are the average of results for two animals.

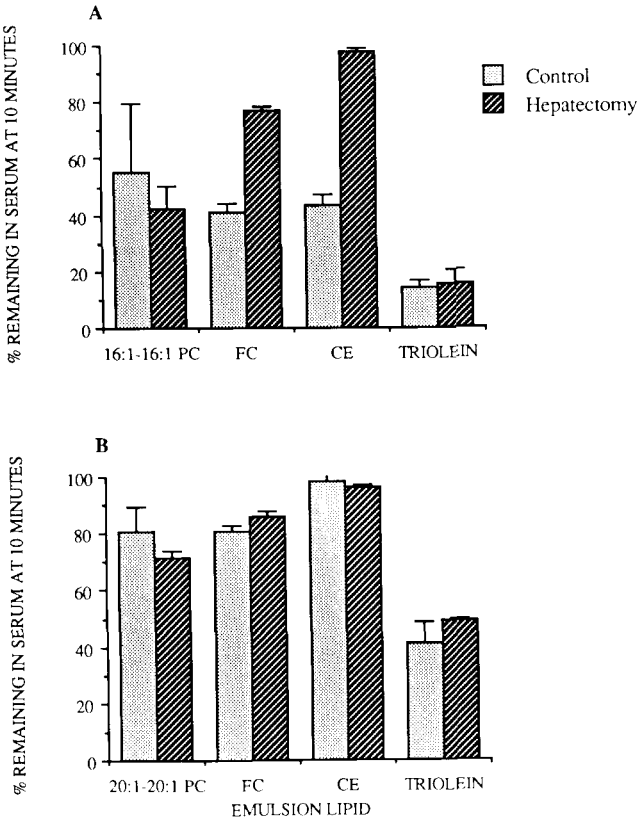

Fig. 6. Effect of (functional) hepatectomy on the clearance of emulsion lipids. The amount (± SEM) of emulsion lipids remaining in the serum 10 min after the injection of emulsions is shown for groups of three rats (hepatectomized and sham-hepatectomized controls) injected with emulsions made with either 16:1-16:1 PC or 20:1-20:1 PC.

triglyceride-rich lipoproteins (28–30), and LPL has recently been shown to selectively hydrolyze emulsion PCs with different acyl group components (31). For a number of years, it has been known that triglycerides emulsified in different PCs are hydrolyzed at different rates by LPL with the appropriate apolipoprotein cofactors (32–34). While there is no certain explanation for what has been viewed as "activation" of LDL by PCs in vitro, it seems reasonable to suggest, in view of the concordant rates of clearance of single PCs and triolein in vivo, that the hydrolysis of emulsion particle triglycerides may be predicated on the rate of hydrolysis of the PCs which occupy the emulsion particle surface and surround the triglycerides within the particle core.

Essentially all of the clearance of CE from emulsions (as from chylomicrons) can be attributed to the uptake by the liver of remnants (15, 35). In the present study, we found considerably greater differences in the clearance of CE than triolein, comparing emulsions with PCs of different hydrophilic strengths. Most notably, in contrast to the clearance of triolein, we found hardly any clearance of CE from emulsions with 20:1-20:1 PC and a decided lag in the onset of clearance of CE from emulsions with 18:0-18:2 PC (Fig. 2). In view of these differences in the clearance of triolein and CE, we think it is likely that different PCs may not only affect the peripheral metabolism of emulsions by LPL but also independently influence the uptake of emulsion remnants by the liver. It is not clear how this might occur since we obviously have found no difference in the magnitude of PC loss from the serum, comparing PC clearance in intact animals with animals that have had a hepatectomy to block remnant uptake. We presume that a loss of PCs from the surface of remnants need not necessarily occur for PCs of different composition to influence remnant uptake by the liver. However, there appears to be no study in which this possibility has been directly explored.

The clearance of FC is more difficult to interpret than the clearance of emulsion CE since, in addition to clearance by a net uptake mechanism(s), FC may undergo bidirectional exchange (for a recent review, see ref. 36). We did not attempt to distinguish these processes in the current study. However, since there was a clear dissociation in the initial rates of clearance of FC and CE in the case of emulsions prepared with 18:0-18:2 PC (Figs. 1 and 2), it seems reasonable to conclude that at least some FC clearance might be due to exchange and not depend on whole-particle (i.e., remnant) uptake by the liver.

The present study was designed to parallel previous work in which we have demonstrated, using these same four PC molecular species, that the rate of clearance of FC from an HDL recombinant was strongly correlated with the clearance of single PCs (14). In this case also, differences in PC clearance could be most readily attributed to differences in the hydrolysis of individual PC species. We believe that while a number of different changes—including changes in apoprotein (37, 38), FC (18), and phospholipid (39, 40) composition—have been shown to affect the turnover of chylomicrons (or chylomicron analogs) in vivo, the demonstration that the substitution of single molecular species of PC will, in a predictable order, markedly affect the turnover of diverse lipoproteins is a novel finding with direct implications for modifying lipoprotein metabolism, and perhaps atherosclerosis, by diet. The precise mechanism has not been established by which changes from saturated to unsaturated dietary fatty acids reduce the risk of heart disease due to atherosclerosis. However, since we have found, independent of the composition of triglycerides, that a change in PC fatty acids will in the short term markedly alter the clearance of cholesterol from lipoproteins, it is reasonable to speculate that the changes in cholesterol balance seen in association with a change in fatty acids may be attributed to a change in the metabolism of lipoprotein PCs. If, as suggested by Zilversmit (41), chylomicrons may be atherogenic, then it is possible that chylomicrons that are cleared slowly from the circulation with less hydrophilic PCs, in response to more saturated fatty acids in the diet,

may have a more prolonged opportunity to deposit cholesterol in the peripheral arterial circulation than chylomicrons with more hydrophilic PCs, in response to an unsaturated fat diet.

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