Effects of hydrophobicity on turnover of plasma high density lipoproteins labeled with phosphatidylcholine ethers in the rat

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Abstract Rat high density lipoproteins (HDL) were labeled with a series of phosphatidylcholines and ether analogs of phosphatidylcholine. The rates of turnover of the phosphatidylcholine ethers in the rat decreased as a function of increasing hydrophobicity and were more than five times faster than those of apolipoprotein A-I turnover and spontaneous lipid transfer. The major tissue sites for uptake were the liver, adrenals, and ovaries. The rate of turnover of a phosphatidylcholine was faster than that of the corresponding ether analog due to the action of lecithin:cholesterol acyltransferase, although this activity was slow compared to the turnover of high density lipoprotein-phosphatidylcholine. Injection of a purified human phosphatidylcholine transfer protein increased the turnover rate of a phosphatidylcholine and its ether analog. We conclude that a major route for the turnover of plasma high density lipoprotein-phosphatidylcholine in the rat is independent of spontaneous lipid transfer, hydrolysis, and HDL particle uptake, and that it involves the activity of a plasma phosphatidylcholine transfer protein. —**Pownall, H. J., D. Hickson-Bick, and J. B. Massey.** Effects of hydrophobicity on turnover of plasma high density lipoproteins labeled with phosphatidylcholine ethers in the rat. *J. Lipid Res.* 1991. **32:** 793–800.

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Plasma levels of human HDL-cholesterol correlate negatively with coronary artery disease (1), and it has been suggested that this lipoprotein plays a pivotal role in transporting peripheral tissue cholesterol to the liver, where it is catabolized (2, 3). HDL is composed of free and esterified cholesterol, phospholipids, small amounts of triglyceride, and several apolipoproteins; apoA-I, the LCAT-activating factor, is the most abundant apolipoprotein (2, 3). PCs represent the major phospholipid species of HDL and are important because they form the surface lipid phase of HDL to which free cholesterol and apoA-I bind (4–6), and because they are the acyl donor in the formation of cholesteryl esters that is catalyzed by LCAT (7). PCs may be transferred between HDL or other lipoproteins and the extravascular compartment by endocytosis (8), spontaneous transfer (9), protein-mediated transfer (10), or hydrolysis (11). The hydrophobicity of the transferring species is the most important in vitro determinant of spontaneous lipid transfer; each additional methylene unit in the fatty acyl chain of a phospholipid increases the transfer time in a predictable way (12). Although in vitro studies have shown that increased unsaturation of symmetrically substituted PCs decreases its reactivity in the LCAT-catalyzed reaction (13), the in vivo effects of chain length on lipid transfer and hydrolysis are less clearly defined. In particular, less is known about the effects of acyl chain length on the turnover of HDL-PCs, which are required for the binding and esterification of plasma cholesterol.

Diether analogs of PCs (PC ethers) share many properties with PCs. Their miscibility with other PCs is similar (14); they exhibit thermal transitions in the same temperature range as their ester analogs (14); like PCs, they associate with cholesterol; and additionally, they are competitive inhibitors of LCAT (13). Moreover, the rates of transfer mediated by the plasma PC exchange protein are comparable for PCs and PC ethers (15). We compared the turnover of a rat HDL containing a series of PCs and PC ethers having different acyl chain compositions to determine the relative importance of various catabolic routes; from these data we infer the identity of the tissue sites at which the HDL-PCs are catabolized and correlate the various catabolic pathways with the structures of the PCs and PC ethers.

Abbreviations: PC, phosphatidylcholine; HDL, high density lipoprotein; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; R-HDL, reassembled high density lipoprotein; lysoPC, 2-lysophosphatidylcholine; PCTP, PC transfer protein; LCAT, lecithin:cholesterol acyltransferase; DTNB, 5,5'-dithiobis-2-nitrobenzoic acid; apoA-I, apolipoprotein A-I.

METHODS

Materials

ApoA-I and HDL_3 were isolated from human plasma (16) and iodinated as described previously (17). Unlabeled PCs were obtained from Avanti Polar Lipids (Birmingham, AL) and used as received. POPC ether and DMPC ether were synthesized from *sn*-3-benzyl glycerol (13, 18). Other ether lipids were from Bachem (Bubendorf, Switzerland). PCs and PC ethers containing ^{14}C and ^{3}H, respectively, in the methyl moiety of the polar headgroup were prepared from the corresponding N,N-dimethyl-phosphatidyl-ethanol-amine (19). All PCs and PC ethers were derivatives of *sn*-glycero-3-phosphocholine, which is the naturally occurring stereoisomer. Reassembled HDL (R-HDL) having the molar composition [^{14}C]PC-[^{3}H[PC ether-POPC-apoA-I 5:5:90:1 were prepared by a cholate removal technique (20). The specific activities of the PCs and PC ethers, respectively, were 0.2 and 1.0 Ci/mole.

PC transfer protein (PCTP) was purified from the $d > 1.063$ g/ml fraction of human plasma by successive chromatography over phenyl-Sepharose, DEAE Sepharose, CM-Sepharose, and chromatofocusing (21). The purified protein was dialyzed and stored in 150 mM NaCl and 10 mM phosphate, pH 7.4.

Turnover studies

After ether anesthesia, a cannula for sample injection was inserted into the femoral veins of female Sprague-Dawley rats (200-225 g), which were used throughout; a second cannula for sample withdrawal was inserted into the opposite femoral vein. After recovery from anesthesia, the rats received no food or water. Typically, a bolus of R-HDL containing 2.5 μmol of phospholipid in 75 mM NaCl and 75 mM phosphate, pH 7.4, was injected via the cannula. Blood was withdrawn periodically and the plasma radioactivity was determined. The turnover halftimes were determined from a plot of plasma radioactivity as a function of time. A portion of the plasma was delipidated (22), the extracted lipids were chromatographed on thin-layer plates, and the phosphorus-positive spots were collected and counted. The specific activity of a given PC in plasma was calculated as follows: PC (dpm/ml) = total plasma dpm/ml × PC(dpm)/[PC(dpm) + lysoPC(dpm)]. The turnover of ^{125}I-labeled apoA-I was measured as previously described (23). Corrections for radioactive spillover were made.

The effect of a purified PCTP on the turnover of POPC and POPC ether was determined as follows. Rats (6) were prepared for injection as described above. R-HDL containing human apoA-I (150 μg), [^{14}C]POPC (5 μg, 0.08 μCi), and [^{3}H]POPC ether (305 μg, 1.8 μCi) were injected into each animal. Five min later, PCTP was injected in 0.5 ml of phosphate-buffered saline. Based on a comparison of the activity of the injected samples with that of rat plasma and assuming that 4% of the weight of the animal represented its plasma volume, the amount of injected transfer activity was three times the measured endogenous activity. In the three control animals, 0.5 ml of phosphate-buffered saline was injected without the transfer factor. The plasma radioactivity was monitored and analyzed as described above.

Plasma distribution of POPC and POPC ether in vitro and in vivo

R-HDL (2.5 μmol phospholipid, [^{14}C]POPC ether, [^{3}H]POPC, and apoA-I, 5:95:1) were mixed with 2 ml rat plasma at 37°C. After 1 and 6 h, 1 ml was applied to a column of Sepharose CL-4B (1.6 cm × 30 cm) and the absorbance (280 nm) and radioactivity of each fraction (1.5 ml) were recorded. A bolus of the same R-HDL sample was injected into rats (4) that were exsanguinated at 1 and 6 h. Plasma (1 ml) was analyzed by chromatography over Sepharose as described above.

Tissue sites of PC and PC ether localization

The tissue distributions of [^{3}H]POPC ether and [^{14}C]POPC were measured 2 h after injection of reassembled HDL ([^{3}H]POPC ether-[^{14}C]POPC-human apoA-I 5:95:1 (molar ratios)). The single bolus was 2.5 μmol phospholipid having 1.0 Ci/mole [^{3}H] and 0.2 Ci/mole [^{14}C]. After 110 min, a trace of ^{125}I-labeled human serum albumin was injected (0.25 μCi ^{125}I). Ten minutes later, the animal was anesthetized with ether and the abdomen was opened; blood was aspirated from the abdominal aorta and the animal was flushed via the aorta with saline solution. After this, the organs were removed, rinsed with cold 0.9% NaCl, blotted, and weighed. Aliquots (100-200 mg) were counted for ^{125}I and then digested with Protosol (New England Nuclear) at 55°C, decolorized with benzoyl peroxide, and the ^{3}H and ^{14}C radioactivities were measured. The ^{125}I radioactivity was used to correct the tissue radioactivity of the PC and PC ether for residual trapped plasma.

RESULTS

Plasma distribution of POPC and POPC ether

R-HDL containing both [^{14}C]POPC and [^{3}H]POPC ether were mixed with plasma and analyzed by gel filtration chromatography after 1 and 6 h (**Fig. 1**). Initially, most of the radioactivity eluted with HDL, but after 6 h a portion of the ^{14}C-label eluted much later and at a retention time that corresponded to that of serum albumin. In contrast, the elution profile of [^{3}H]POPC ether was virtually the same at both 1 and 6 h. Similar experiments were conducted in vivo. After injection of R-HDL into rats, a 1-ml portion of plasma was collected and chro-

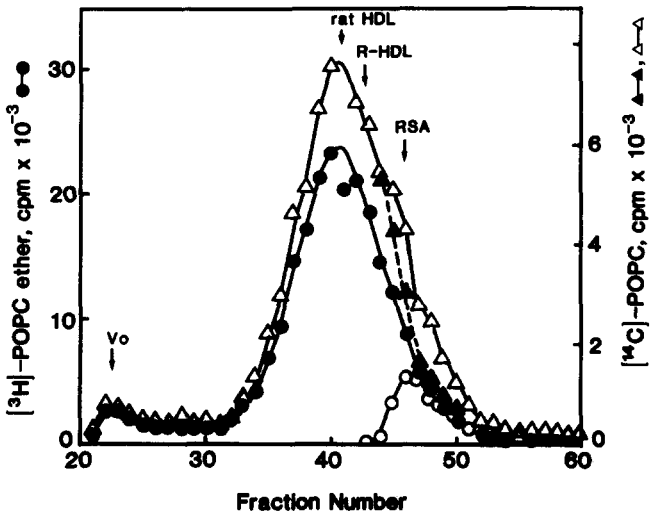

Fig. 1. Gel permeation chromatography of rat plasma and R-HDL after in vitro incubation for 1 and 6 h at 37°C. R-HDL were composed of [^{14}C]POPC-[^{3}H]POPC ether-apoA-I 5:95:1. R-HDL (60 μg) were mixed with rat plasma (1 ml) for 1 and 6 h at 37°C. When the data obtained with POPC ether (●) after 1-h and 6-h incubations were normalized to give the same value at fraction 42, the other points were superimposable. Normalization of the [^{14}C]POPC profiles at fraction 42 gave identical profiles except between fractions 46 and 51, where the 6-h sample (△) contained more radioactivity than the 1-h sample (▲). The difference (○) between the normalized 1- and 6-h profiles of the [^{14}C]POPC gives a maximum at an elution volume that correponds to that of rat serum albumin (RSA). V_o designates the void volume.

matographed at 1 and 6 h (**Fig. 2**). After 1 h, nearly all of the labeled lipid coeluted with HDL. After 6 h, all of the [^{3}H]POPC ether still eluted in the range of HDL, but some of the ^{14}C-label was found with retention times that corresponded to serum albumin and the salt peak. Thin-layer chromatographic analysis of the lipid that eluted with the albumin showed it was composed entirely of lysoPC; a similar analysis showed that the only labeled lipids that eluted with the HDL were POPC and POPC ether. These data are consistent with the conversion of POPC to lysoPC, which has a measurable aqueous solubility and which also binds to serum albumin.

The relative rates of in vivo hydrolysis of PCs were evaluated by injecting R-HDL containing a series of [^{14}C]PCs into separate sets of rats and determining the fraction of radiolabeled lipid that appeared as lysoPC as a function of time. From the initial slopes of the plots of percent lysoPC versus time (**Fig. 3**), we found that the relative amounts of lysoPC formed from the labeled PCs were in the ratio, DMPC-DPPC-POPC-DSPC 100:38:16:7.

In vivo turnover of PC and PC ethers

The plasma halftimes of various components of native and R-HDL were measured in several groups of rats. The in vivo halftime for human ^{125}I-labeled apoA-I in R-HDL composed of apoA-I and POPC (**Table 1**) was similar to that reported previously (23, 24). Similar turnover studies were conducted with R-HDL in which the alkyl and acyl chain lengths of a homologous series of labeled PC ethers and PCs, respectively, were the same. These data, which are shown in **Fig. 4,** reveal several common features that are summarized as follows. *1*) The halftime of a PC (Table 1) was always shorter than that of its corresponding PC ether. *2*) The halftimes of both PCs and PC ethers increased as the acyl and alkyl chain lengths, respectively, were increased. *3*) The halftimes for both POPC and POPC ether were longer than those of any of the other PCs and PC ethers, respectively. *4*) The turnover times of [^{14}C]POPC and [^{3}H]POPC ether were not significantly different when the major phospholipid species that formed the lipid matrix of the R-HDL was changed from POPC to POPC ether. *5*) Although the halftimes of the PC ethers were different, each kinetic curve plateaued at a value that represented approximately 20% of the injected counts; in contrast, the amount of labeled PC remaining after 4 h increased as a function of increasing acyl chain length.

If the PC ethers are reliable nonhydrolyzable analogs, the hypothetical difference between the decay curves for a PC with a given acyl chain and a PC ether that has the same number of carbons in its alkyl chain should represent the formation and subsequent disappearance of lysoPC. In Fig. 4, the curves for the disappearance of PC radioactivity pass through the maximum in the calculated curve for lysoPC. This relationship between the PC ether

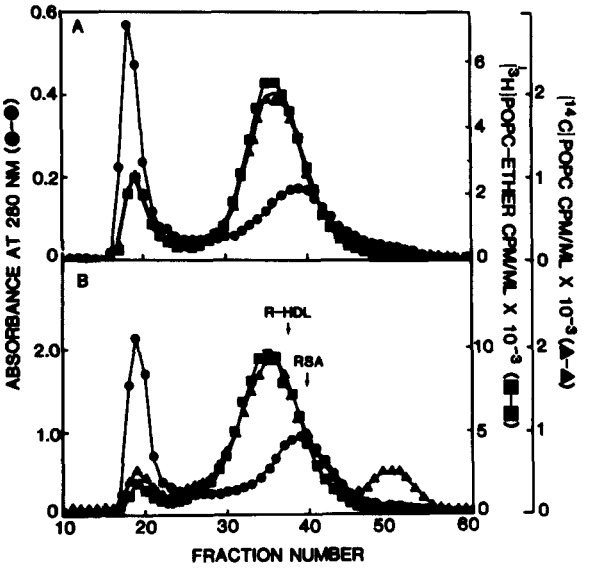

Fig. 2. Gel permeation chromatography of rat plasma at 1 h (A) and 6 h (B) after in vivo injection of R-HDL. The R-HDL (2.5 μmol phospholipid composed of [^{3}H]POPC ether-[^{14}C]POPC-apoA-I, 5:95:1) was intravenously injected into female Sprague-Dawley rats. After an appropriate time interval, the animals were anesthetized and their blood was aspirated from the abdominal aorta. In A and B, respectively, 100 and 500 μl plasma in 1 ml were applied to a Sepharose CL 4B column (not the same column as Fig. 1).

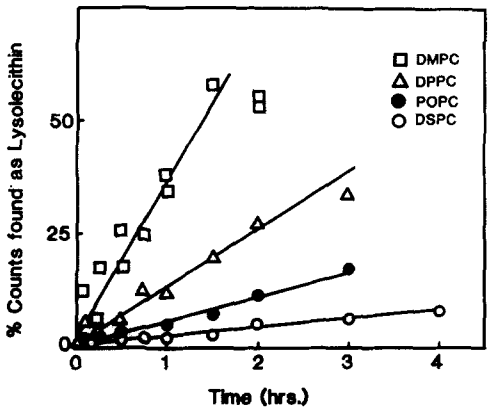

Fig. 3. Determination of the rates of lysoPC formation from R-HDL in vivo. R-HDL contained [^{3}H]PC-POPC-apoA-I 10:90:1. (○) DSPC; (●) POPC; (△) DPPC; (□) DMPC. The respective rates were 2.5%/h, 6.0%/h, 14%/h, and 37%/h. R > 0.94.

decay curves and the difference curves was observed despite a twofold difference in the halftimes of the longest- and shortest-lived PC ethers. This behavior is consistent with a precursor-product relationship (25) and provides additional evidence that PC ethers are reliable nonhydrolyzable analogs of PC in vivo.

When a purified human PCTP was injected immediately prior to injection of an R-HDL containing [^{3}H]POPC ether, a 50% decrease in the halftime of the plasma radioactivity was observed (**Fig. 5**). The plateau of about 25% of injected dose that was observed after 4 h was identical to that observed without the injection of exogenous PCTP.

Tissue localization of PCs and PC ethers

Two h after injection of R-HDL, the liver was the major site of deposition of POPC and POPC ether (**Table 2**). Thin-layer chromatography of the extracted phospholipids from liver showed that the [^{14}C]PC coincided with the retention times of endogenous PC and lysoPC; in contrast >90% of the [^{3}H]PC ether coeluted with endogenous PC. When expressed in terms of specific binding, the major site of binding was the adrenals. However, because of its much greater size, the liver is the site where the majority of POPC and POPC ether is removed from plasma. While no POPC ether was found in kidneys, a small amount of POPC was found in kidneys, ovaries, and lungs.

DISCUSSION

Plasma distribution of PCs and PC ethers

Although LCAT action can be inhibited by DTNB (11), this reagent inhibits only its acyltransferase activity and not its phospholipase activity (26). Therefore, we concluded that an alternative strategy that uses PC ethers to infer HDL-PC turnover in the absence of hydrolysis should be validated. Previous studies suggest that PC ethers should be excellent candidates for nonhydrolyzable analogs of PCs. The structure and behavior of PCs and PC ethers are similar on the basis of monolayer measurements (27, 28), nuclear magnetic resonance (29), permeability (30), and calorimetry (14). The predicted rates of

TABLE 1. In vivo plasma halftimes for the disappearance of R-HDL in the rat[a]

Radiolabeled Tracer	Major Lipid in R-HDL	$t_{1/2}$	k_{obs}	$t_{1/2}$ Spontaneous Transfer[b]	k_{sp}[b]
		min ± AD	h^{-1}	*min*	h^{-1}
DMPC	POPC	28 ± 6	1.5	23	1.8
DMPC ether	POPC	51 ± 5	0.82		
DPPC	POPC	39 ± 5	1.1	800	0.05
DPPC ether	POPC	63 ± 8	0.67		
DSPC	POPC	46 ± 3	0.91	>800	<0.05
DSPC ether	POPC	85 ± 6	0.49		
POPC	POPC	53 ± 5	0.79	800	0.05
POPC ether	POPC	110 ± 8	0.38		
POPC	POPC ether	49 ± 5	0.86		
POPC ether	POPC ether	90 ± 5	0.47		
POPC + PCTP	POPC ether	44 ± 5	0.95		
POPC ether + PCTP	POPC ether	65 ± 5	0.65		
Human apoA-I	POPC	563 ± 60	0.075		
HDL particle lifetime[c]	POPC		0.058		

[a]Average of at least four animals using a double-labeled R-HDL. The average deviations (AD) listed represent the differences between animals in each group. For each curve, the calculated error based on an exponential decay was never more than 2%. The R-HDL contain [^{3}H]PC ether and a [^{14}C]PC as tracers and POPC or POPC ether in a 100 to 1 molar ratio to apoA-I.

[b]Measured or calculated for transfer between R-HDL; k_{sp} = rate constant for spontaneous transfer.

[c]Reference 30.

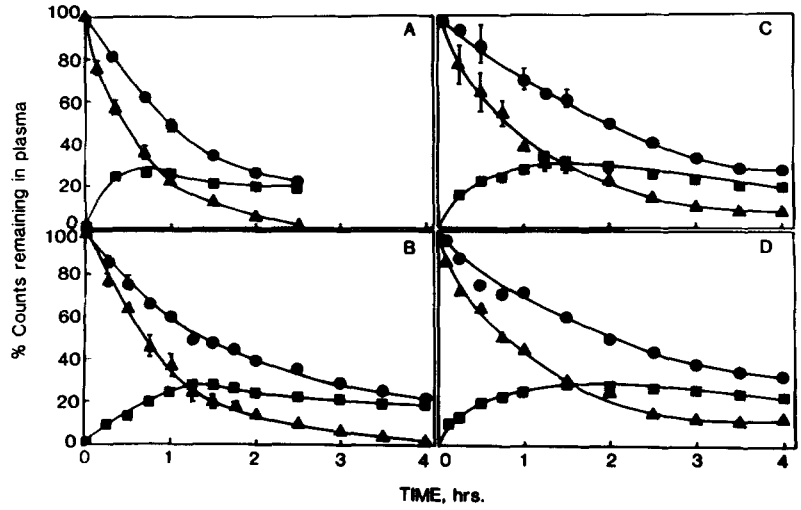

Fig. 4. Disappearance from rat plasma of A) [^{3}H]DMPC ether and [^{14}C]DMPC; B) [^{3}H]DPPC ether and [^{14}C]DPPC; C) [^{3}H]DSPC ether and [^{14}C]DSPC; D) [^{3}H]POPC ether and [^{14}C]POPC in R-HDL ([^{3}H]PC ether-[^{14}C]PC-POPC-apoA-I 5:5:90:1 (molar ratios). Rats were injected intravenously with a sample containing 2.5 μmol phospholipid (1.0 Ci [^{3}H]/mol phospholipid and 0.2 Ci [^{14}C]/mol phospholipid) in 1 ml of phosphate-buffered saline. For each decay curve, n = 4; error bars represent average deviations. PC, ▲-▲; PC ether, ●-●, difference ■-■.

spontaneous transfer of PCs and PC ethers are similar due to the strong structural homology (15). A number of bioassays suggest that lipid-associating proteins in plasma recognize PCs and PC ethers in a similar way. First, PC ethers competitively inhibit LCAT, suggesting that the plasma enzyme that degrades most of the HDL-PC does not discriminate between these two phospholipid classes (13). Second, the structures of complexes formed by PCs and PC ethers are similar (14). Finally, the transfer rates of PCs and PC ethers that are mediated by a rat plasma PCTP are similar (15). According to our gel filtration data, both PCs and PC ethers of R-HDL associate with rat HDL very soon after they are mixed with rat plasma. More recent measurements have shown that the halftime for the association of R-HDL composed of POPC and DMPC with rat HDL in vitro at 37°C is less than 5 min (G. Ponsin, T. Pulcini, J. T. Sparrow, A. M. Gotto, Jr., and H. J. Pownall, unpublished data), a time interval much shorter than that of HDL turnover.

Our data show that the association of PCs and PC ethers with rat plasma lipoproteins in vitro and in vivo are nearly the same; the major difference being that some of the radioactivity that corresponded to PC was converted to lysoPC. Since the addition of exogenous PC or PC ether to rat plasma in vivo does not have any measurable effect on the lifetime of apoA-I, we conclude that the incorporation of these tracers into the HDL does not affect its metabolism as a particle. Therefore, we assume in the remainder of the discussion that the PC ethers incorporated into rat HDL by this method are valid markers for the transport of native HDL-PCs in the absence of hydrolysis.

PC hydrolysis

Over a period of 6 h we observed significant hydrolysis of PCs with the attendant shift of the radioactivity corresponding to the lysoPC to the albumin fraction or the aqueous phase (Figs. 1 and 2). This hydrolysis is probably due to the activity of plasma LCAT because the order of the reactivity, DMPC > DPPC > POPC > DSPC, is nearly the same as that previously observed in vitro using R-HDL (13, 31) and native HDL (32) as substrates. We note that the rate of hydrolysis is small when compared to the rate of HDL-PC turnover, suggesting that a route that is independent of LCAT action and HDL particle uptake is responsible for HDL-PC turnover.

Tissue sites of PC and PC ether localization

The in vivo distribution of PCs and PC ethers into various tissues is in many ways consistent with the degradation sites that have been reported for HDL-apoA-I (33). In the rat, the major sites for the degradation of HDL-protein and -cholesteryl esters and ethers are the steroidogenic tissues, liver, adrenals, and ovaries (23, 32). Our data (Table 2) are consistent with these findings in that

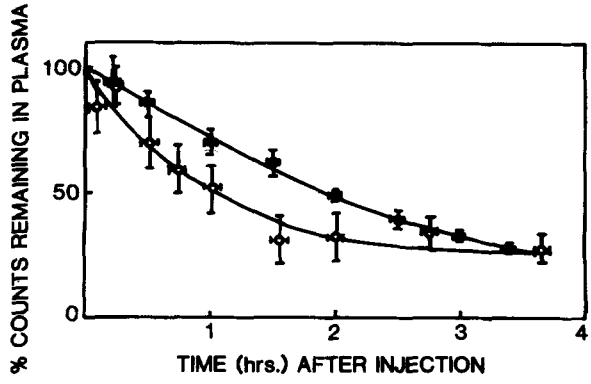

Fig. 5. Effect of PCTP on phospholipid turnover. Disappearance of [^{3}H]POPC ether from rat plasma after injection of a phospholipid transfer protein from human plasma (○-○). The disappearance of [^{3}H]POPC ether from control rats injected with phosphate-buffered saline ●-●; n = 3 and 6, respectively, for the test and control animals.

TABLE 2. Tissue distribution of [^{3}H]POPC ether and [^{14}C]POPC 2 h after intravenous injection as R-HDL

Tissue	[^{3}H]POPC Ether			[^{14}C]POPC		
	dpm/mg of Tissue	% Injected dpm in Tissue	Tissue Space, μl/g	dpm/mg of Tissue	% Injected dpm in Tissue	Tissue Space, μl/g
Liver	85	12.2	500 ± 40	90	28.7	2000 ± 300
Spleen	36	0.4	120 ± 60	27	0.6	300 ± 50
Kidney	27	0.8	0	21	1.4	300 ± 40
Adrenals	106	0.1	800 ± 275	61	0.1	1800 ± 450
Ovaries	79	0.2	200 ± 150	29	0.2	400 ± 200
Heart	31	0.4	150 ± 130	5	0.2	90 ± 60
Lungs	24	0.6	70 ± 40	23	1.4	450 ± 224

most of the labeled PCs and PC ethers were found at the same sites. In addition, a small but significant fraction of the label that was injected as PC was also found in lungs and kidney. The latter is a site of selective uptake of water-soluble monomeric compounds, including apoA-I (33), and in both cases it is probably in the form of lysoPC since none of the PC ethers transferred to either of these tissues.

HDL-PC turnover in the rat

Analysis of HDL turnover is complex because HDL contains lipids and proteins that transfer to cells or other lipoproteins at different rates. Given the exchangeable nature and preferential uptake of some components of HDL (23, 32), it is likely that most reports underestimate HDL particle turnover. With a few assumptions, one can estimate the fractions of each of the HDL-PCs that are catabolized by *1*) HDL particle uptake, *2*) hydrolysis via LCAT, *3*) spontaneous transfer, and *4*) PCTP-mediated transfer. According to data obtained with a nontransferable peptide, HDL particles have a plasma halftime of about 12 h (30), which corresponds to a rate constant of 0.058 h^{-1}. When ^{125}I-labeled apoA-I is used as marker (Table 1) the rate constant is slightly faster. Both of these are much slower than the rates of disappearance of PCs and PC ethers due to other competing processes, which include hydrolysis by LCAT and PCTP-mediated transfer.

The rate constants for the disappearance of the long-chain PC ethers are the least complex because they are nonhydrolyzable and have spontaneous transfer times much longer than those of HDL turnover. Therefore, the faster turnover times must be due to another factor, which may be preferential uptake by specific tissues mediated by a PCTP or some other cell surface activity that is associated with those tissues. Comparison of the rate constants for the disappearance of POPC ether with that for total HDL-particle uptake, shows that only 15% (0.058/0.38) can be explained by the uptake of the entire particle. The results are similar when one compares DPPC ether (8.7%) and DSPC ether (12%) turnover rates with that of the total particle. Even using apoA-I as a marker for the turnover of HDL particles does not alter the conclusion that a large fraction of the PC ethers are removed from plasma by a mechanism that does not involve hydrolysis and particle uptake. In fact, for all of the PC ethers and some of the PCs, which are LCAT substrates, another route that is independent of hydrolysis or particle uptake must be involved. For example, according to the data in Fig. 3, within the approximately 2 h required for half of the plasma PC radioactivity to disappear there is very little conversion of POPC or DSPC to lysoPC, so it is likely that the major route for their removal from the plasma compartment is similar to poorly hydrolyzable PCs (34) and does not depend on hydrolysis or particle uptake. The addition of a purified PCTP increases the disappearance rate of plasma POPC ether suggesting that this activity is quantitatively important in the turnover of HDL-PC in rat. From this observation we infer that a major route for the removal of PCs and PC ethers from plasma involves this activity.

DMPC ether disappears at a faster rate than the other PC ethers through the combined effects of spontaneous transfer, which has a halftime of 20 min for the transfer from human HDL to human low density lipoproteins (H. J. Pownall, unpublished results) and its rapid conversion to lysoPC via LCAT (Fig. 3) (13). This finding is consistent with the report that the more water-soluble PCs disappear from the plasma at a faster rate that is independent of LCAT activity (34). In fact, there is an inverse correlation between the plasma lifetimes of PCs and PC ethers and their hydrophobicity as determined by their respective acyl chain lengths. This is probably due to the large decrease in the rates of spontaneous lipid transfer that occurs when the hydrocarbon content of a lipid is increased (9, 12, 21, 35).

All of the PC ethers exhibited a long-lived component that represented nearly 25% of the injected dose. This finding is similar to observations with poorly hydrolyzable PCs and single bilayer vesicles of a PC ether, which seem to persist in plasma longer than many of the other PCs (36); this long-lived component is still observed when exogenous PCTP is injected. None of the other known

plasma lipoproteins have lifetimes as long as that of HDL, and gel filtration data never showed the coelution of PC ethers with any other plasma component. The long-lived component could be due to a separate HDL-PC pool in which the PCs are not accessible to hydrolysis and from which the spontaneous and PCTP-mediated transfer rates are very slow. This pool might be formed during the remodeling of HDL that is associated with some of its interconversions but additional studies are required to clarify this point.

Some of these data are relevant to human studies. PCs are important because they are the substrate that irreversibly commits free cholesterol to cholesteryl esters in the overall process of reverse cholesterol transport. It seems probable that PCTP activity, which is similar in rat and humans (15), is of some importance in the turnover of human HDL. LCAT activity and the rates of spontaneous and PCTP-mediated PC transfer are similar in rat and humans (13, 15, 31). However, the plasma lifetime of human plasma HDL is nearly an order of magnitude longer than that of rat. For this reason, spontaneous and protein-mediated transfers and LCAT activity are relatively fast on the time scale for HDL turnove. Thus, it is likely that all three of these processes contribute to the distinct differences in the structures of rat and human plasma HDL (37).

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