Metabolism of chylomicron phosphatidylinositol in the rat: fate in vivo and hydrolysis with lipoprotein lipase and hepatic lipase in vitro

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Abstract Phosphatidylinositol (PI), mainly stearoyl-arachidonyl PI, occurs as a minor phospholipid constituent in both chyle and plasma lipoproteins. The kinetics and the pathways by which plasma and chyle PI is metabolized have not been investigated. The role of lipoprotein PI in the supply of arachidonic acid (20:4) and inositol lipid components to different tissues is thus unknown. In this study we examined the fate of chyle PI in vivo and its hydrolysis by lipoprotein lipase (LPL), hepatic lipase (HL), and postheparin plasma in vitro. Chyle and chylomicrons were labeled in the PI portion by feeding [^{3}H]myo-inositol and in the phosphatidylcholine (PC) portion by feeding [^{14}C]choline in a linoleate-rich fat meal (Intralipid) to mesenteric duct-cannulated rats. After intravenous injection of doubly labeled chyle into normal rats, [^{3}H]PI disappeared from plasma at a slower rate than [^{14}C]PC; after 60 min 41.6 ± 2.7% ^{3}H and 24.3 ± 1.8% ^{14}C (means ± SEM, n = 4, $P < 0.01$) remained in plasma lipids. About 15% of both isotopes were in liver lipids after 60 min. Previous injection of a blocking antiserum against rat HL did not significantly influence the serum and liver radioactivity after 60 min. Radioactive PI was rapidly transferred to high density lipoproteins (HDL) during the metabolism of chylomicrons. Analysis of ^{3}H and ^{14}C in different molecular species of PI and PC in chyle and in serum indicated that there was no significant difference in disappearance rates between various species, stearoyl-arachidonyl PI thus disappearing at the same rate as total [^{3}H]PI. Both lipoprotein lipase (LPL) and HL catalyzed formation of lyso-PI in vitro, the rate being increased by the addition of serum. About 60% of the lyso-PI formation catalyzed by postheparin plasma in 60 min could be blocked by antiserum to HL, which almost completely blocked the hydrolysis occurring after the first 10 min. The study thus shows that both LPL and HL hydrolyze chylomicron PI in vitro. LPL and HL may, however, be of limited importance for the clearance of chyle PI in vivo, most of the chylomicron PI being transferred to HDL, and thereafter eliminated from plasma at a slow rate mainly by other mechanisms.—**Nilsson, Å., Q. Chen, and E. Dahlman.** Metabolism of chylomicron phosphatidylinositol in the rat: fate in vivo and hydrolysis with lipoprotein lipase and hepatic lipase in vitro. *J. Lipid Res.* 1994. **35:** 2151–2160.

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Although phosphatidylcholine (PC) accounts for most of the phospholipids in both the serum and chyle, 8–12% of the chyle phospholipids is phosphatidylethanolamine (PE) (1–3) and about 2.5% is phosphatidylinositol (PI) (3). The phospholipids transport a large part of the arachidonic acid (20:4, n–6) that is present in chyle lipoproteins (2). As the proportion of 20:4 in chyle PI is particularly high (3), the quantitative role of this phospholipid class in the transport of 20:4 may be larger than is apparent from its low concentration in chyle. Although the presence of bile phospholipids is essential for the maximal rate of lipoprotein triacylglycerol (TG) output in chyle (4 and references in 5), an increase in the de novo synthesis of all glycerophospholipid classes occurs during fat absorption (6, 7). In the female gerbil, inositol deficiency causes TG accumulation in the intestinal mucosa, which can be reversed by feeding inositol (8, 9). An active biosynthesis of PI in the mucosa may thus be necessary for efficient lipoprotein secretion. These findings suggested that an efficient labeling of the PI-portion of chyle lipoproteins might be achieved by oral feeding of [^{3}H]inositol in a lipid meal.

In vitro lipoprotein lipase (LPL) hydrolyzes the *sn*-1 ester bond of chylomicron PC and PE (10, 11). In vivo most of these phospholipids are, however, transferred to high density lipoproteins (HDL) during the metabolism of the chylomicrons (12, 13), and are then metabolized by hepatic lipase (HL) (14–17) and by lecithin:cholesterol acyltransferase (LCAT) (18), or taken up by tissues as in-

Abbreviations: PI, phosphatidylinositol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; TG, triglyceride; LPL, lipoprotein lipase; HL, hepatic lipase; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; FFA, free fatty acid; HPLC, high performance liquid chromatography.

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tact phospholipids. Chyle PE is cleared from plasma much faster than PC (17), mainly because it is a preferred substrate for HL (17, 19, 20). This explains why PE accounts for only about 1.3% of the plasma phospholipids in the rat (21), i.e., a much lower proportion than of the phospholipids in chyle (1–3) and in nascent hepatic VLDL (22). PI, in contrast, accounts for a somewhat larger proportion (4.3%) of the rat plasma phospholipids (21) than of the phospholipids in chylomicrons (3) and in nascent hepatic VLDL (22). The fate of the PI that is secreted with the intestinal and hepatic lipoproteins is unknown.

In this study we compared the plasma disappearance and tissue uptake of chyle [^{3}H]PI and chyle [^{14}C]choline-labeled PC both in normal rats and in rats that had been injected with a blocking antiserum against HL. The plasma disappearance of different molecular species of [^{3}H]PI and [^{14}C]PC was examined. Furthermore, we examined the hydrolysis of chylomicron [^{3}H]PI in vitro with pure bovine milk LPL and human HL, and with postheparin plasma with and without the addition of a blocking antiserum against HL.

If chyle PI is efficiently hydrolyzed by LPL and HL, like PE (17, 19, 20), this would provide a pathway by which chyle 20:4 and inositol may be utilized both by the liver and other tissues by uptake as lyso-PI, without any intravascular release of unesterified 20:4. If, on the other hand, the chyle PI is resistant to the lipases, a slow disappearance of PI may explain why PI accounts for a larger proportion of the phospholipids in plasma (21) than in chyle (3). One must then also postulate that PI is taken up by the tissues as the intact molecular either by a particulate uptake of lipoproteins or by transfer of glycerophospholipids from lipoproteins to cells, and then degraded by enzymes participating in the metabolism of intracellular pools of PI (see references in reference 9).

MATERIALS AND METHODS

Preparation of radioactive chylomicrons

Male white Sprague-Dawley rats, 200–280 g, were obtained from Mollegaard HB, Denmark. The mesenteric duct was cannulated (23); a gastric fistula was inserted with the tip placed in the duodenum; and the rats were treated postoperatively as described earlier (2). Myo-[2-^{3}H(N)]inositol (18.30 Ci/mmol, NET 114A) and [methyl-^{14}C]choline chloride (53.00 mCi/mmol, NEC-141) were obtained from New England Nuclear. [^{3}H]inositol (250 μCi) was mixed with 2 ml Intralipid (20% w/v), and the emulsion was infused through the gastric fistula over 1 h about 24 h after the operation. For the preparation of doubly labeled ([^{3}H]PI and [^{14}C]PC) chyle 100 μCi [^{14}C]choline (ethanol solution) was taken to dryness with nitrogen together with a chloroform solution of 0.5 mg egg PC, and then dispersed and mixed with the Intralipid emulsion together with 250 μCi [^{3}H]inositol.

After feeding the radioactive emulsion to the rats, chyle was collected on ice for 3 h in the presence of Na_2 EDTA (final concentration 2 mM) and was stored at 4°C after defibrination by centrifugation at 3000 rpm for 5 min. It was then dialyzed overnight against 1.1% saline containing 2 mM EDTA and 1 mM dithiothreitol, to remove radioactive water-soluble compounds. The total incorporation of ^{3}H in chyle lipids was about 5% of the administered dose.

Chylomicrons were isolated by ultracentrifugation at 25,000 rpm for 2 h using a Beckman SW 41 swinging-bucket rotor, as described earlier. Ninety-seven percent of the ^{3}H radioactivity of the chylomicrons migrated with PI during separation on Merck silica gel 60 thin-layer plates, developed in chloroform–methanol–acetic acid–water 50:40:6:0.6. In the in vivo experiments, doubly labeled chyle was used, except in the series where the transfer of [^{3}H]PI to denser lipoprotein classes was examined. Of the ^{14}C radioactivity, 98.4% was in PC in the doubly labeled chyle used. In this chyle, 71% of the ^{3}H and 69% of the ^{14}C was in chylomicrons and the remainder was in denser lipoproteins.

The triacylglycerol content of chyle and chylomicrons was determined by an enzymatic kit method (Boehringer-Mannheim, Germany).

Incubations with lipases in vitro

Postheparin plasma was obtained from rats by aortic puncture 2 min after intravenous injection of 100 units of heparin. Antiserum against rat HL (anti-HL), prepared as previously described (24), and which has been shown not to react with LPL, was used. The antiserum was a gift from Professor Michael C. Schotz, Los Angeles, CA. Sixty μl of this antiserum was able to inactivate all the HL from 1 g liver tissue: approximately 1 unit of lipase activity. A unit of enzyme activity is defined as the release of 1 μmol FFA/min from emulsified triglyceride substrate (24). As only the fraction of HL on the cell surfaces would be inactivated by circulating antibody, the doses of antiserum used in vitro (25 μl) and in vivo (200 μl) were calculated to be in considerable excess. The pure bovine milk LPL was prepared and characterized as described by Bengtsson and Olivecrona (25). Human HL was prepared according to (26). LPL, HL, and purified human apolipoprotein C-II were gifts from Dr. Gunilla Bengtsson-Olivecrona, Dept. of Physiological Chemistry, University of Umeå, Sweden.

Incubations were performed in 10 mM Tris-HCl buffer, pH 7.4, containing 2 mM $CaCl_2$ and 0.15 M NaCl. Enzymes, serum, and postheparin plasma were added as indicated in the legends to figures and tables. The total incubation volume was 5 ml. After 5, 10, 20, 40, and 60 min 1-ml aliquots were taken and immediately extracted with

8 ml chloroform–methanol 1:1 (v/v) containing 0.005% butylated hydroxytoluene. The protein precipitate was then removed by centrifugation, and the samples were subjected to two-phase distribution by adding chloroform and water to make the proportions chloroform–methanol–water equal to 2:1:1. In this two-phase distribution, PI is partitioned quantitatively in the lower (chloroform) phase. In preliminary experiments the chloroform phase was dried with nitrogen, redissolved in chloroform, and the lipids were separated by thin-layer chromatography on silica gel G plates that were developed in chloroform–methanol–acetic acid–water 50:40:6:0.6. Using this method, rather small amounts of radioactivity were, however, found in the lyso-PI spot after incubation with LPL, HL, or postheparin plasma. When the ^{3}H radioactivity of the upper water–methanol phase was determined, a significant increase in the radioactivity with time was observed during incubation with the enzymes. After evaporation of the upper phase with nitrogen, reextraction of the residue with chloroform–methanol 1:1 and thin-layer chromatography, this upper phase radioactivity migrated as lyso-PI. When the chloroform–methanol extract was evaporated without two-phase distribution, and the residue then reextracted with chloroform–methanol 1:1 (v/v) and subjected to thin-layer chromatography, much higher values for lyso-PI formation were thus obtained. A comparison of these values with those of the upper phase ^{3}H radioactivity indicated that 84% of the lyso-PI was distributed to the upper phase. Lyso-PI formation was then estimated as percent ^{3}H in the upper phase divided by 0.84. Radioactivity was determined by liquid scintillation counting as described below.

In vivo experiments

Male white Sprague-Dawley rats, 120–137 g, were lightly anesthetized with diethyl ether and 0.5 ml chyle labeled with [^{3}H]inositol in the PI portion and with [^{14}C]choline in the PC portion was injected into the jugular vein. The animals were again anesthetized and killed by aortic puncture after 5, 20, 60, or 240 min (n = 4 or 5 at each time point). The liver, heart, lungs, spleen, and kidneys were removed and frozen in liquid nitrogen. The small intestine was rinsed thoroughly with cold saline before being frozen. One group of animals was injected with 200 μl anti-HL 5 min before the injection of chyle. These rats were killed after 60 min.

In one series of experiments, chylomicrons labeled with [^{3}H]PI only were injected, and the distribution of radioactivity in plasma lipoproteins with $d < 1.006$, $1.0006 < d < 1.063$, and $1.063 < d < 1.21$ g/ml was examined after 5, 20, and 60 min using ultracentrifugation as described earlier (17).

Lipids were extracted from the tissues with chloroform–methanol 1:1 (v/v) containing 0.005% butylated hydroxytoluene. After removing the precipitate by filtration, the proportions were adjusted to chloroform–methanol–water 2:1:1 (v/v) by adding chloroform and 0.1 M KH_2PO_4. The lower phase was taken to dryness with nitrogen and redissolved in a small amount of chloroform. Aliquots were taken for determination of radioactivity by liquid scintillation counting in 1 ml methanol–water 1:1 plus 10 ml Instagel–toluene 1:1. Samples were counted in a Packard 460 CD liquid scintillation system using automatic external standard for quench correction. Aliquots for radioactivity determination were also taken from the upper phase.

Aliquots of the lipid extract were also separated on Merck silica gel 60 plates that were developed in chloroform–methanol–acetic acid–water 50:40:6:0.6 (v/v). The spots were identified by staining with iodine vapor and scraped into counting vials. One ml methanol–water 1:1 and 10 ml Instagel–toluene 1:1 were added and the samples were counted as above. Phospholipid classes were isolated from the lipid extracts of injected chyle and serum, and from some of the liver extracts by high pressure liquid chromatography (HPLC) (27) using Shimadzu LC 6A HPLC equipment and a Nucleosil Si50 200 × 4.6 mm column. The mobile phase was hexane–isopropanol–ethanol–25 mM potassium phosphate (pH 7.0)–acetic acid 376:485:100:56:0.6 (v/v) (27, 28), and the flow rate was 1.0 ml/min. The PI and PC fractions were collected and were then subjected to separation of individual molecular species on a 4.6 × 250 mm Nucleosil C_{18} column that was eluted with methanol–water–acetonitrile 90.5:7:2.5 (v/v), containing 20 mmol choline chloride, at a flow rate of 1 ml/min (27). Radioactivity of phospholipid classes and of individual molecular species in chyle and serum samples was determined by continuous flow measurement of ^{14}C and ^{3}H using a Radiomatic A-200 Flo-One radioactivity detector.

RESULTS

In vivo experiments

Disappearance from plasma of chyle [^{3}H]PI and [^{14}C]PC. In the first series of experiments, the disappearance rates of the major chyle phospholipids, PC and PI, were compared. Chyle labeled with [^{3}H]PI and [^{14}C]PC was injected intravenously, and the animals were killed after 5, 20, 60, and 240 min. **Figure 1** shows the time course for the disappearance of radioactivity from blood. Five minutes after injection of labeled chyle the percentages of ^{3}H and ^{14}C remaining in serum were similar. After 20, 60, and 240 min the amounts of remaining ^{3}H-labeled lipid exceeded that of ^{14}C-labeled lipid, e.g., after 60 min 41.6% of the ^{3}H and 24.3% of the ^{14}C remained in serum lipids. After 240 min, 9.5% of the ^{3}H and 3.1% of the ^{14}C remained in serum lipids. Injection of antiserum to HL did not significantly decrease the clearance or tissue up-

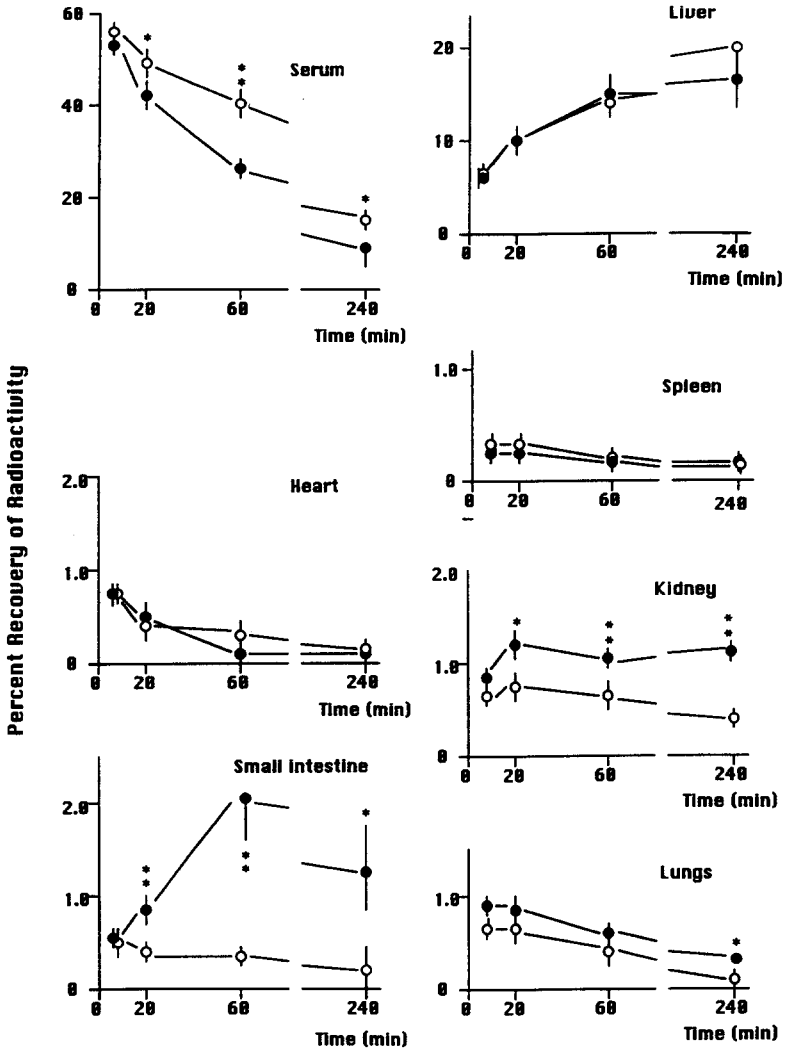

Fig. 1. Percent recovery of lipid ^{3}H (○) and ^{14}C (●) after injection of [^{3}H]PI- and [^{14}C]PC-labeled chyle. Rats weighing 120–137 g were injected intravenously with 0.5 ml chyle containing 8.9 mg triacylglycerol, 9×10^{5} dpm ^{3}H and 9×10^{5} dpm ^{14}C. Values are given as percent recovery of injected dose in lipids of the respective tissue and are means ± SEM of five (5 and 20 min), four (60 min), or three (240 min) observations. The calculation of plasma clearance is based on the assumption that the plasma volume amounts to 5% of the body weight.

take of ^{3}H or ^{14}C (**Table 1**). In the next series of experiments, chylomicrons labeled with [^{3}H]PI were injected intravenously, and blood was drawn after 5, 20, and 60 min. The time course for the disappearance of [^{3}H]PI from plasma was similar to that seen in the experiments with whole chyle. Seventy percent remained in plasma after 5 min, 57% after 20 min, and 40% after 60 min. Plasma lipoproteins were separated into $d < 1.006$, $1.006 < d < 1.063$, and $1.063 < d < 1.21$ g/ml fractions. The data indicated a rapid transfer of chylomicron PI to denser lipoprotein classes, mainly high density lipoproteins (HDL). Forty-one percent of the serum lipoprotein ^{3}H was in HDL after 5 min, 60% after 20 min, and 72% after 60 min (means of two observations: data not shown).

The ^{3}H-labeled lipid in serum was present almost entirely in PI. On thin-layer chromatography in chloroform–methanol–acetic acid–water 50:40:6:0.6 of serum lipid extracts (5–60 min, analyses not performed on 240-min samples) more than 95% of the ^{3}H migrated with PI and phosphatidylserine (PS). Although these two spots were visually separated, the PS spot always contained some (< 15%) of the ^{3}H radioactivity. On HPLC ^{3}H appeared only in the PI and not in PS (data not shown). The reason for this discrepancy was not investigated further. One possibility is that some of the labeled molecular species of PI (see below) are not as well separated from PS as 18:0–20:4 PI on thin-layer chromatography. Of the ^{14}C in serum lipids, more than 95% was in the choline glycerophospholipids (> 80%) PC and lyso-

TABLE 1. Effects of antiserum towards HL on serum and tissue lipid-soluble and water-soluble radioactivity

Tissue	Lipid-Soluble Radioactivity		Water-Soluble Radioactivity	
	^{3}H	^{14}C	^{3}H	^{14}C
Serum				
Controls	56.0 ± 5.5	33.3 ± 5.5	0.9 ± 0.0	0.5 ± 0.1
Anti-HL	63.0 ± 6.9	37.0 ± 4.9	0.6 ± 0.1	0.5 ± 0.1
Liver				
Controls	32.2 ± 3.3	36.2 ± 3.7	2.0 ± 0.3	8.7 ± 2.0
Anti-HL	31.2 ± 0.6	37.6 ± 2.6	1.4 ± 0.1	7.7 ± 0.3
Heart				
Controls	5.0 ± 0.8	5.1 ± 1.0	–	–
Anti-HL	6.4 ± 0.4	5.4 ± 0.5	–	–
Lungs				
Controls	5.2 ± 0.6	6.7 ± 0.6	1.5 ± 0.2	4.6 ± 0.3
Anti-HL	5.6 ± 0.8	6.7 ± 1.0	1.9 ± 0.2	4.8 ± 0.3
Spleen				
Controls	5.1 ± 1.6	5.3 ± 0.7	1.6 ± 0.2	10.2 ± 0.8
Anti-HL	6.6 ± 0.8	6.2 ± 0.8	1.6 ± 0.2	8.3 ± 0.7
Kidney				
Controls	5.0 ± 1.2	8.2 ± 1.3	3.9 ± 0.6	6.0 ± 0.9
Anti-HL	5.4 ± 1.7	8.0 ± 1.8	3.1 ± 0.4	4.6 ± 0.2
Small intestine				
Controls	0.6 ± 0.2	3.4 ± 0.9	–	–
Anti-HL	0.9 ± 0.4	2.9 ± 1.1	–	–

Rats weighing 120–137 g were injected intravenously with 0.5 ml [^{3}H]inositol- and [^{14}C]choline-labeled chyle containing 8.9 mg triacylglycerol, 9 × 10^{5} dpm ^{3}H and 9 × 10^{5} dpm ^{14}C. The anti-HL-treated group was injected with 200 μl antiserum 1 min before the injection of the chyle. All animals were killed after 60 min. Values (dpm × 10^{-3}/g tissue) are means ± SEM of four observations in each group. None of the differences between anti-HL-treated and control groups were statistically significant as estimated by the Student's unpaired *t*-test; (-) not analyzed.

PC (data not shown).

Disappearance rates of different molecular species of chyle PI and PC. The method for labeling chyle by feeding [^{3}H]inositol and [^{14}C]choline in a lipid emulsion would be expected to label mainly the molecular species of PI and PC which are synthesized de novo during fat absorption. The composition of these species would, in turn, be expected to reflect the fatty acid composition of the lipid meal to a larger extent than the mass distribution of different chyle PI and PC species (3). In order to examine whether the observed elimination rate of total chylomicron [^{3}H]PI was the same as that of 18:0–20:4 PI, i.e., the major PI species in chyle, we separated different molecular species of chyle and serum PI (5- and 60-min samples) using HPLC with continuous flow radioactivity measurement.

The radioactive PI of both the injected chyle and the serum samples was separated into six peaks, of which peak 2 contained only small amounts of radioactivity and was combined with fraction 3 in the presentation of the data. Peak 5 comigrated with the major mass peak and with a standard of 18:0–20:4 PI, and contained 19% of the chyle PI ^{3}H radioactivity. Using a standard of liver PI, a pattern very similar to that described by Patton, Fasulo, and Robins (27) was seen in which peak 5 corresponded to 18:0–20:4 and peak 6 to 18:0–18:2 (**Table 2**). Peaks 1, 2, 3, 4, and 6 did not contain sufficient mass for identification of fatty acids by gas chromatography. A comparison of the relative retention times with those published by Patton et al. (27) revealed that the relative retention time for peak 1 corresponded to 18:2–18:2, that of peak 2 to 16:0–20:4, peak 3 to 16:0–18:2, and peak 4 to 18:1–18:2. The analysis of radioactive PI species in serum after 5 and 60 min did not reveal any significant differences in the distribution of ^{3}H, indicating that the different species were cleared at similar rates. The disappearance rate for the predominant PI species, i.e., 18:0–20:4 PI, was thus the same as for total [^{3}H]PI. The data obtained at 60 min in animals that had been injected with a blocking antiserum towards HL did not differ from these of the control animals (Table 2).

The distribution of ^{14}C between different molecular species of serum PC also remained similar to that of the injected chyle after 5 and 60 min. In this case most of the chyle [^{14}C]PC radioactivity appeared in four molecular species with migration rates corresponding to 18:2–18:2, 16:0–18:2, 18:1–18:2, and 18:0–18:2, whereas very little radioactivity appeared in the 20:4 species (**Table 3**). The

TABLE 2. Molecular species of rat chyle and serum [^{3}H]PI

	Percent Radioactivity in Different Molecular Species				
	Peak 1 18:2–18:2	Peaks 2 + 3 16:0–20:4 + 16:0–18:2	Peak 4 18:1–18:2	Peak 5 18:0–20:4	Peak 6 18:0–18:2
Chyle	6.2	24.4	14.1	19.2	36.1
Serum, 5 min	4.0 ± 0.5	22.2 ± 2.5	16.2 ± 2.1	20.8 ± 0.5	36.9 ± 0.6
Serum, 60 min	4.9 ± 0.8	22.6 ± 1.8	13.4 ± 1.9	21.8 ± 1.5	37.4 ± 1.3
Serum + anti-HL, 60 min	4.6 ± 0.6	22.8 ± 0.9	14.9 ± 1.0	22.8 ± 1.1	35.0 ± 1.1

Molecular species of PI from injected chyle and from serum obtained 5 and 60 min after injection were separated by HPLC according to Patton et al. (27). Other experimental details are given in the text and in the legend to Fig. 1. Values are percent of total radioactivity recovered and are means ± SEM of three observations. Anti-HL indicates that this group was injected with 200 μl anti-HL 5 min before the chyle injection.

TABLE 3. Molecular species of chyle and rat serum [^{14}C]PC

	Percent Radioactivity in Different Molecular Species			
	Peak 1 18:2-18:2	Peak 2 16:0-18:2	Peak 3 18:1-18:2	Peak 4 18:0-18:2
Chyle	22.2	28.0	16.0	20.9
Serum, 5 min	22.7 ± 0.6	26.7 ± 1.6	18.6 ± 1.3	20.8 ± 0.7
Serum, 60 min	17.8 ± 0.4	23.1 ± 2.7	22.3 ± 3.6	24.7 ± 1.0
Serum min + anti-HL, 60	20.3 ± 1.2	25.6 ± 1.8	19.4 ± 3.5	23.0 ± 0.2

Molecular species of PC from injected chyle and from serum obtained 5 and 60 min after-injection were separated by HPLC according to Patton et al. (27). Other experimental details are given in the text and in the legend to Fig. 1. The radioactivity not accounted for by the 18:2 species (11.2-12.9%) was mainly in oleoyl and arachidonyl species. Values are means ± SEM of three observations. Anti-HL indicates that this group was injected with 200 μl anti-HL 5 min before the chyle injection.

clearance rate for total [^{14}C]PC observed was thus representative for the major 18:2 species, whereas the clearance rates for the 20:4 species could not be examined due to the low radioactivity in these species.

Uptake by different tissues. The time courses for the ^{3}H and ^{14}C radioactivity in lipids of liver, heart, spleen, small intestine, and kidney are shown in Fig. 1. In the liver the ^{3}H and the ^{14}C radioactivity were similar and continued to increase up to 240 min. In heart and spleen the radioactivity was highest after 5 or 20 min and then decreased, the percent ^{3}H exceeding that of ^{14}C in the heart at 20 min. In small intestine and kidney the recovery of ^{14}C exceeded that of ^{3}H at all time intervals except 5 min, the difference being 5-fold for intestine after 60 min. Expressed per g tissue weight, both the ^{3}H and the ^{14}C radioactivity values were about 6-fold higher in the liver than in heart, lungs, kidneys, and spleen. No statistically significant difference in any tissue radioactivity was seen between the group that had been injected with anti and the controls (Table 1).

Of the ^{3}H radioactivity in liver and serum, only small portions were present in water-soluble form (**Fig. 2,** Table 1). In other tissues, the proportions of water-soluble ^{3}H and ^{14}C were higher (Table 1). In kidneys, the proportion of water-soluble ^{3}H increased with time and after 4 h clearly exceeded the amount of lipid-soluble ^{3}H (Figs. 1 and 2, Table 1).

In one experiment rats were injected with [^{3}H]inositol-labeled chylomicrons and killed after 5, 20, and 60 min (n = 2 at each time point), and the uptake in pancreas and liver was determined. The average recovery of ^{3}H in pancreas lipids was 0.35%/g tissue after 5 min, 0.15%/g after 20 min, and 0.09%/g after 60 min. This means that ^{3}H radioactivity per gram was 2-fold lower than in the liver after 5 min, and 20-fold lower after 60 min. The uptake of ^{3}H by the liver was the same as in the experiments with whole chyle (7% after 5, 12% after 20, and 17% after 60 min).

The total recovery of ^{3}H in lipids in the tissues examined was 58% and that of ^{3}H as water-soluble compounds (in liver, serum, and kidneys) was 2% after 1 h.

Of the liver ^{14}C radioactivity, more than 95% was in PC and lyso-PC. On thin-layer chromatography of liver extracts, no significant ^{3}H radioactivity was found outside the PI-PS area. The presence of small amounts of ^{3}H

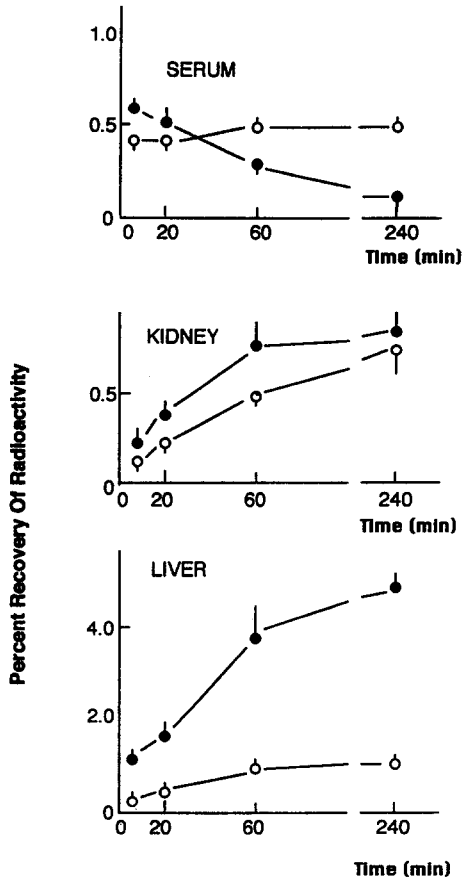

Fig. 2. Recovery of ^{3}H (○) and ^{14}C (●) as water-soluble radioactivity after injection of [^{3}H]PI- and [^{14}C]PC-labeled chyle. Values are expressed as percent of injected dose radioactivity and are means ± SEM from the experiment described in the legend to Fig. 1. The injected dose was 9 × 10^{5} dpm ^{3}H, of which 97% was in PI, and 9 × 10^{5} dpm ^{14}C, of which 98% was in PC. Other experimental conditions are given in the text and in the legend to Fig. 1.

migrating as PC could not be excluded due to the high ^{14}C radioactivity in PC (data not shown).

In vitro incubations

In the first series of experiments [^{3}H]inositol-labeled chylomicrons were incubated with postheparin plasma containing both LPL and HL (**Fig. 3**). A significant formation of lyso-PI was observed. The rate was highest during the first 10 min, although some lyso-PI formation continued during the whole 60-min period studied. When antiserum towards rat HL was added to the incubation, it inhibited about 60% of the hydrolysis that occurred in 60 min. The hydrolysis after 10–20 min was almost completely inhibited, whereas during the first 10 min the degree of inhibition was lower. If LCAT utilizes radioactive PI, this might be a source of lyso-PI during the incubation with postheparin plasma. No lyso-PI formation was, however, observed, when postheparin plasma was replaced by fresh serum (Fig. 3).

In the second series of experiments, the hydrolysis of chylomicron [^{3}H]PI with pure LPL and HL and with the two enzymes in combination was examined with and without serum present (**Fig. 4**). In the absence of serum, the hydrolysis with LPL continued with an almost linear time course for 60 min, but the rate of hydrolysis was low. With serum present there was a faster hydrolysis during the first 10 min, but then only slow hydrolysis occurred. Also, when chylomicrons were incubated with HL alone, the rate of hydrolysis was very low but was stimulated by the addition of serum. When HL and LPL were combined, the rate of hydrolysis exceeded the sum of the hydrolysis rates seen with either enzyme alone, both with and without serum present (Fig. 4).

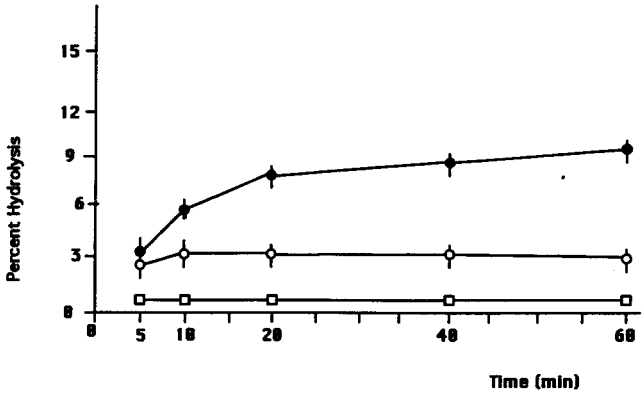

Fig. 3. Hydrolysis of chylomicron [^{3}H]PI with postheparin plasma. Chylomicrons containing 0.5 mg TG and 118,000 dpm ^{3}H, of which 97% was in PI, were incubated with 10% postheparin plasma in the presence of either 25 μl control rabbit serum (●) or 25 μl anti-HL (○). Control incubations with 10% rat serum were also performed (□). The total incubation volume was 5 ml. Other incubation conditions are given in the text. Values are means of duplicates from one of two similar experiments.

DISCUSSION

The in vivo part of this study shows, that the disappearance of chyle PI from plasma is slower than that of the major chyle phospholipid, i.e., PC (Fig. 1). After 60 min the proportion of injected [^{3}H]PI remaining in plasma (41.6%) was about 1.5 times that of [^{14}C]PC (24.6%). The disappearance rate for PI thus differs drastically from that of chyle PE, which was almost completely eliminated from blood within 60 min, due to the preferential action of HL on PE, primarily after the transfer of chylomicron phospholipids to HDL (17).

18:0–20:4 PI is the predominant molecular species of PI in tissues, including chylomicrons and serum lipoproteins, as a result of continuous deacylation–reacylation reactions (29–34). The fatty acid composition of newly synthesized PI reflects, however, mainly the composition of available acyl-CoA pools (30–32). In this study separation of different molecular species of chyle PI revealed that only 19.2% of the [^{3}H]PI was in 18:0–20:4 PI, the main part of the ^{3}H being in linoleate-containing species (Table 2) as expected from the high 18:2 content in the Intralipid meal. There were, however, no major differences in the relative distribution of ^{3}H between the different molecular species in chyle and in serum after 5 and 60 min. It is therefore concluded that the time courses for the disappearance of total chyle 3H PI from plasma and for the main chyle PI species, i.e., 18:0–20:4 PI, were the same (Table 2).

Also, in case of the [^{14}C]choline-labeled chyle PC, the main amount of the radioactivity was in 18:2-containing species, with 16:0:18:2 PC containing the most radioactivity of any individual species (28.0%). This was also an expected finding, as the fatty acid composition of PC formed by de novo synthesis is highly dependent on the fatty acids supplied (3, 35, 36). The relative distribution of ^{14}C between the different 18:2 species did not differ between the injected chyle and serum samples obtained after 5 and 60 min. The disappearance of total chyle [^{14}C]PC was thus the same as that of the major 18:2 PC species. The incorporation of ^{14}C into 20:4 PC species was low, probably because the chyle 20:4 PC species are formed mainly by acylation of absorbed 1-lyso-PC (6, 37–39) or derived from preexisting pools of mucosal phospholipids (35, 36). The rate of disappearance of the 20:4 PC species that are preferred substrates for LCAT in the rat (40, 41) could therefore not be examined. The main conclusion from the species analyses is thus that the major PI species, i.e., 18:0–20:4 PI, disappears from plasma at a slower rate than the major chyle 18:2 PC species.

In order to elucidate whether HL contributes significantly to the metabolism of chyle [^{3}H]PI in vivo, the effects of an antiserum towards HL, which was shown to block chyle PE removal in an earlier study (17), was examined. The injection of antiserum against HL did not

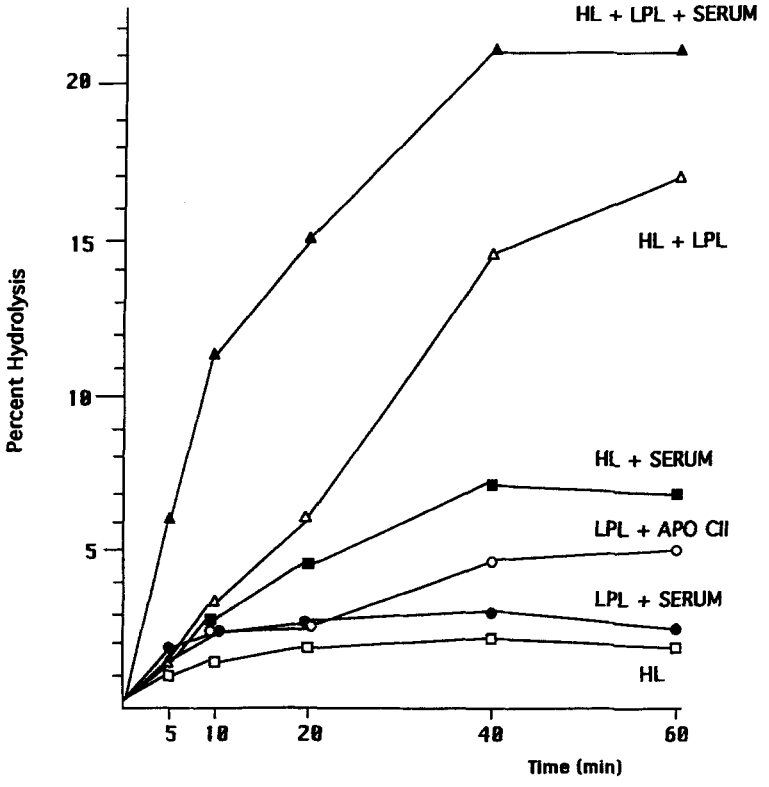

Fig. 4. Hydrolysis of chylomicron [^{3}H]PI with LPL and HL. Chylomicrons containing 0.5 mg TG and 118,000 dpm ^{3}H, of which 97% was in PI, were incubated with 1.45 units of LPL plus 2 μg apoC-II (○) or 1.50 units HL (□) with (solid symbols) or without (open symbols) 10% serum present. The same amounts of the enzymes were also combined in the presence of 2 μg apoC-II (△) or 10% serum (▲). Values are means of duplicates and were also from one of two similar experiments.

significantly delay the clearance of either [^{3}H]- or [^{14}C]PC. Nor did the antiserum influence the relative distribution among different PI species of ^{3}H remaining in plasma after 60 min (Table 2). Although a delay of chyle PI and PC removal in vivo by blocking of HL may be difficult to demonstrate in vivo, due to the relatively slow clearance of both these phospholipids, the data thus indicate that pathways other than hydrolysis by HL are quantitatively more important for the plasma disappearance of chyle PI. There is thus an obvious difference between the rapid HL-mediated elimination of PE in the earlier study (17), and the slow clearance of PI primarily by other pathways observed in this study.

The ^{3}H and the ^{14}C radioactivity per g tissue was about 6-fold higher in the liver than in the heart, lungs, spleen, and kidney. Both the lipid-soluble and water-soluble [^{3}H]PI radioactivity in the liver were higher than in other tissues, and increased with time up to 240 min. There was thus a net degradation of PI, the mechanism of which is not known. The release of arachidonic acid after cellular uptake of intact PI could occur by phospholipase A_2 or by phospholipase A_1, followed by subsequent hydrolyses of the respective lysocompounds. Cellular PI can also be degraded via phospholipase C forming intermediary 1,2-diacylglycerol that can be further hydrolyzed by diacylglycerol lipase with the formation of 2-monoacylglycerol (for references, see ref. 9). In the case of the kidneys, the water-soluble radioactivity increased with time, whereas the lipid-soluble radioactivity decreased (Fig. 1). This may be due either to a more extensive degradation of PI taken up by the kidneys or to a transport of [^{3}H]inositol from other tissues to the kidneys, which have a major role in the degradation of inositol (42, 43). The amount of ^{14}C in lipids of the small intestine exceeded that of ^{3}H after 1 and 4 h. This increase of ^{14}C in the intestine with time is probably due to the recirculation of [^{14}C]choline in bile PC, succeeded by reincorporation of absorbed lyso-PC into mucosal PC.

The in vitro study shows that chylomicron PI may act as a substrate for both LPL and HL yielding lyso PI as a product. In analogy with the known ability of these lipases to hydrolyze the *sn*-1 ester bond of PC and PE (10, 11, 14–16, 19, 20) the enzymes are thus able to hydrolyze PI, although we did not examine the positional specificity in this study. HL alone hydrolyzed chylomicron PI rather slowly. When both LPL and HL were added simultaneously, the effect of HL was more pronounced, particularly when serum was present. This suggests that HL may act

mainly on polar surface material that is released or transferred to HDL during lipolysis of chylomicron TG with LPL, and that chylomicron PI may be metabolized similarly to PC and PE (10, 12, 13, 17) in this respect. Other possible effects of serum such as the content of apolipoproteins and of albumin acting as acceptor of released free fatty acids must, however, be considered. As PI is rich in 20:4 (29, 33, 34), and LCAT in the rat has a preference for this fatty acid when acting on PC (40, 41), we also examined whether serum alone catalyzed the formation of lyso-PI. This was, however, not the case (Fig. 1). The stimulatory effect of serum on lyso-PI formation in the incubations with HL and/or LPL (Fig. 1) was thus not due to the simultaneous action of LCAT.

In summary, chylomicron PI may act as substrate for both LPL and HL. In vivo most of the PI is transferred to denser lipoprotein classes, primarily HDL, during the clearance of chylomicron triacylglycerol, and is then cleared from plasma at a slow rate, the liver being the major site of uptake. The quantitative contribution of LPL and HL to the clearance of PI may thus be limited. The possible role of other pathways, such as exchange and net transfer of intact PI from HDL to the liver, and particulate uptake of HDL have to be considered. The slow rate of clearance may explain the fact that the proportion of PI in rat plasma phospholipids (4.3%) (21) exceeds that in chylomicron phospholipids (2.5–2.7%) (3).

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