Absorption and distribution of arachidonate in rats receiving lysophospholipids by oral route

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Abstract Absorption and distribution of polyunsaturated fatty acids was investigated in rats receiving lysophospholipids per os (30 mg kg^{-1}). Lysophosphatidylcholine (lysoPC) increased [^{3}H]arachidonate absorption and its incorporation into mucosal phosphatidylcholine. Transport of [^{3}H]arachidonate by the phospholipid fraction of lymph lipoproteins and the level of [^{3}H]arachidonate in plasma and liver lipids was also increased by lyso PC. Lysophosphatidylserine also increased [^{3}H]arachidonate absorption but channeled the fatty acids into the aminophospholipid fraction of mucosal phospholipids, thus decreasing its efflux in lymph lipoproteins. As a consequence, lysophosphatidylserine caused [^{3}H]arachidonate accumulation in mucosa. As similar results were obtained with [^{14}C]linoleate, the data suggest that the addition of an appropriate lysophospholipid to the diet may direct absorption and distribution of polyunsaturated fatty acids.—**Viola, G., L. Mietto, F. E. Secchi, L. Ping, and A. Bruni.** Absorption and distribution of arachidonate in rats receiving lysophospholipids by oral route. *J. Lipid Res.* 1993. **34:** 1843-1852.

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Studies of absorption and distribution of dietary polyunsaturated fatty acids in plasma and tissue lipoproteins are of physiological and therapeutic interest. The major focus of research is on the distribution of n-6 and n-3 fatty acids as they include essential diet constituents and originate lipid chemical mediators active in the regulation of hemostasis and inflammatory and immune reactions. Arachidonic acid (20:4, n-6) and its precursor linoleic acid (18:2, n-6) promote the synthesis of most active prostanoids and leukotrienes. In contrast, n-3 fatty acids are precursors of leukotrienes of the 5-series and prostanoids of the 3-series which are less active (1). Disposition of dietary fatty acids largely depends on whether they are included in the triacylglycerol (TG) or phospholipid (PL) fraction of intestinal lipoproteins (chylomicrons and VLDL). When these lipoproteins reach the general circulation, TG and PL follow a different route (2). TG are hydrolyzed by lipoprotein lipase and the majority of released fatty acids are used for storage or energy source. On the other hand, PL are either incorporated in HDL to generate cholesteryl esters or transported to the liver as components of remnant particles. We have investigated the possibility of influencing the distribution of polyunsaturated fatty acids between TG and PL by the oral administration of 1-acyl lysophospholipids (lysoPL). The fraction of these compounds escaping degradation in the intestinal lumen is estimated to be at least 50% according to previous investigations of phosphatidylcholine (PC) absorption (3). This residual amount is acylated in the absorptive cell to produce PL for lipoprotein assembly. Unlike dietary diacyl-PL, unsaturated fatty acids are not released from lysoPL upon digestion, but rather, lysoPL may function as an acceptor system for the unsaturated fatty acids of other dietary constituents. By selecting the appropriate lysoPL, a PL is obtained that is more or less abundant in lipoproteins. Hence, lysoPL may or may not enhance fatty acid retention in the mucosa. Finally, lysoPL may direct fatty acids in individual PL with different affinity for lipid-metabolizing enzymes as shown by the preference of hepatic lipase (4) or LCAT (5) for phosphatidylethanolamine (PE). The major focus of this study has been on the absorption and distribution of labeled arachidonate given together with 1-acyl lysophosphatidylcholine (lysoPC) or 1-acyl lysophosphatidylserine (lysoPS). These lysoPL generate phosphatidylcholine (PC) and phosphatidylserine (PS), respectively, that

Abbreviations: PL, phospholipids; lysoPL, lysophospholipids; lysoPC, lysophosphatidylcholine; lysoPS, lysophosphatidylserine; DAG, diacylglycerols; TG, triacylglycerols; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; VLDL, very low density lipoproteins.

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represent the extremes in the utilization of PL for lipoprotein assembly as PC forms the largest PL fraction whereas PS is excluded. Comparison experiments were also performed with labeled linoleate. The data indicate that lysoPC enhances fatty acid absorption and delivery to lymph whereas lysoPS favors their retention in mucosa.

MATERIALS AND METHODS

Reagents and lysophospholipid dispersion

Bovine brain PS was prepared and purified as described (6) using a column of Q-Sepharose (Pharmacia) instead of DEAE cellulose. LysoPS was then obtained by the action of phospholipase A_2 from porcine pancreas (Boehringer). After digestion, lysoPS was recovered by differential solvent extraction (7). In the final product, 86% of acyl chains consisted of stearic acid. LysoPC from egg PC (containing mainly palmitic and stearic acid) was purchased from Sigma. The two lysophospholipids gave a single spot on two-dimensional thin-layer chromatography. [^{3}H]glycerol (1 Ci mmol^{-1}) and [^{3}H]arachidonic acid (76 Ci mmol^{-1}) were from New England Nuclear; [^{14}C]linoleic acid (55 mCi mmol^{-1}) was from Amersham.

Aliquots of lysophospholipids in chloroform–methanol 2:1 (v/v) were dried under a stream of nitrogen and then under vacuum overnight. Complete removal of solvents was essential as it was found that a residual amount of solvent inhibited fatty acid absorption. In a first series of experiments, a saline buffer (BSS-BSA) containing 4 mM Na_2HPO_4, 2.7 mM KH_2PO_4, 2.7 mM KCl, 150 mM NaCl, 10 mg ml^{-1} of bovine serum albumin (pH 7.4) was used as a vehicle. In a second series bovine milk was substituted for the saline buffer. The TG in milk was 27 mg ml^{-1}, corresponding to 30 mM of triolein. Essentially the same results were obtained with the two vehicles. The lysoPL dispersion (15–30 mg ml^{-1}) was briefly sonicated (four bursts of 30 sec each) and used for bolus administration or duodenal infusion in fasted rats. Radiolabeled fatty acids were mixed with lysophospholipids in organic solvent and [^{3}H]glycerol was added with the vehicle. Lysophospholipids were used at a single dose of 30 mg kg^{-1} corresponding to 18 μmol in a rat weighing 300 g. If all lysophospholipids were completely hydrolyzed this would yield a maximum of 18 μmol of saturated fatty acids, sufficient for the synthesis of 6 μmol TG. When milk was used (1–2 ml kg^{-1}), the rats also received 9–18 μmol TG.

Administration by intragastric bolus

Male Sprague-Dawley rats (250–350 g) were fasted overnight. The lysophospholipid dispersion was given by a stomach tube at a volume of 2 ml kg^{-1}. At the selected time, blood was sampled by heart puncture while the rats were under ether anesthesia which was then prolonged until the animal was dead. The upper 35–40 cm of small intestine, active in lipid absorption (8–10), was removed, flushed with water and then with 5 mM taurocholate at neutral pH. After rinsing, the intestine was opened, blotted, and the mucosa was scraped off for extraction of lipids. In some experiments the small intestine was divided into small segments (15 cm each); in others a specimen of liver was also taken.

Administration by duodenal infusion

The day before the experiment, cannulae were inserted into the main mesenteric lymph duct and into the duodenum of rats under pentobarbital anesthesia. After the operation the rats were kept in restraining cages and infused overnight with 2 ml h^{-1} of 150 mM NaCl and 5 mM KCl through the duodenal cannula. The next morning the duodenal infusion was changed into the lysophospholipid dispersion. Lysophospholipids were slowly infused for 1 h. During this period rats received 1 ml kg^{-1} of vehicle and 30 mg kg^{-1} of lysoPL. Then, the infusion was continued with the saline solution at a rate of 2 ml h^{-1} to support lymph secretion. Lymph was collected at 0°C for 6 h in hourly aliquots in tubes containing EDTA (final concentration, 2 mM). Before lymph extraction the tubes were centrifuged to remove cells. The lymph flow, which was not changed by the lysophospholipid administration, averaged 1 ml h^{-1} in the 45 rats used in these experiments.

Analytical procedures

As described by Folch, Lees, and Sloane Stanley (11), lymph and tissues were extracted with 20 vol chloroform–methanol 2:1 (v/v). The lipid extract was washed with 0.2 vol of 10 mM $CaCl_2$ (mucosa) or 0.05 M HCl (lymph). Neutral lipids and fatty acids were separated by one-dimensional thin-layer chromatography using a solvent mixture of hexane–ethyl ether–acetic acid 60:40:4 (v/v). Phospholipids were resolved by the two-dimensional system described previously (12). Triacylglycerols were determined as described (13); phospholipid phosphorus was determined according to Bartlett (14).

The distribution of [^{3}H]arachidonate in plasma lipoproteins was determined as previously described (15). Briefly, plasma was first centrifuged at 2.5 × 10^6 *g*-min to remove chylomicrons. The subnatant was supplemented with 0.1 M of $MgCl_2$ and 1 mg ml^{-1} of dextran sulfate 500. After 5 min at 0°C the samples were centrifuged to collect a sediment (VLDL + LDL) and a supernatant (HDL). In fasted rats HDL forms the major class of plasma lipoproteins (100–250 mg dl^{-1} of serum), 3–4 times more abundant than VLDL and LDL (16).

Statistics

The difference between untreated and lysophospholipid-treated rats was analyzed using one-way analysis of variance and the Student-Newman-Keuls test. *P* values lower

than 0.05 were considered significant. Unless otherwise indicated, results were expressed as arithmetic means ± SEM.

RESULTS

General observations

Examination at light microscopy of small intestine, fixed in formalin and stained with hematoxylin-eosin, showed normal mucosa 1 h after the administration of 30 mg kg^{-1} of lysoPL (18 μmol/rat). The mucosal protein content (46.8 ± 1.9 mg g^{-1} wet wt) and the PL/protein ratio (0.23 ± 0.01 μmol mg^{-1}) were the same in control and treated rats. Lack of cytotoxic effect was expected as 440 μmol lysoPL (predominantly lysoPC) is normally generated per day in rat intestinal lumen from diet and bile PL (17). As we used fasted rats in which bile PL were the only source of exogenous lipids, the dose of lysoPL given in these experiments corresponded to the amount of bile PC secreted in 7-10 h (18). LysoPL did not alter the mucosal lipid content except for a slight increase of phosphatidylinositol (PI) (**Table 1**). The low TG content and the unchanged PL/TG ratio in the mucosa indicated that lysoPL did not influence TG secretion. Consistently, duodenal infusion of lysoPL minimally affected the steady output of TG and PL that occurred in fasted rats (**Fig. 1**). LysoPC infusion caused a small and not significant TG peak at 2 h (increase of 24%) and a small increase in PL efflux (0.15 μmol h^{-1}, $P < 0.05$). The lymph PL/TG ratio varied between 0.3 and 0.4 during the experiment indicating that formation of chylomicrons did not occur (PL/TG ratio in chylomicrons < 0.1, ref. 19). If cells were removed from the samples (see Materials and Methods), PS and lysoPS were not detected in lymph either with or without lysoPS administration. To investigate the contribution of the glycerol-3-phosphate pathway to the synthesis of lymph lipids under our conditions, [^{3}H]glycerol was infused together with lysoPL (**Table 2**). At 0-2 h (the time of maximal efflux of labeled lipids) [^{3}H]glycerol was incorporated mainly into TG whereas PL formed a small fraction in agreement with previous data (20). LysoPS did not change this pattern, whereas lysoPC increased TG labeling (91%, $P < 0.05$). As lysoPC induced only a small increase in TG mass in the same rats, this resulted in a substantial increment in TG specific activity. Analysis of mucosa within the time of maximal labeling was consistent with the observations in lymph (**Table 3**). LysoPC induced a significant shift in [^{3}H]glycerol distribution increasing the fraction associated with neutral lipids (85% formed by TG). The decrease in PL labeling caused by lysoPC and the lack of an effect by lysoPS indicated that the lysoPC-induced increase in TG labeling was not the consequence of enhanced [^{3}H]glycerol influx due to nonspecific alteration of membrane permeability. Among individual PL, lysoPC decreased label distribution in PC in agreement with previous data demonstrating negative regulation of de novo PC synthesis by the lumenal supply of PC (20) or by addition of lysoPC to cultured hepatocytes (21). In contrast, lysoPC enhanced the fraction of [^{3}H]glycerol incorporated into PI confirming the positive influence of lysoPC on the synthesis of this PL observed in mass determinations (see Table 1).

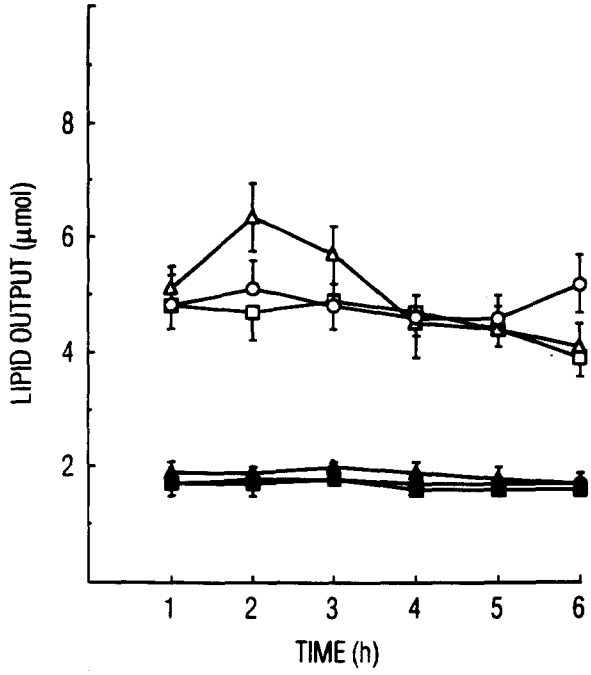

Fig. 1. Effect of a duodenal infusion of lysophospholipids on the triacylglycerol (open symbols) and phospholipid (closed symbols) output in mesenteric lymph. Thirty mg kg^{-1} of lysophospholipids dispersed in BSS-BSA or milk were infused in the duodenum in the first hour. At the indicated time lymph was collected and extracted. (○, ●) vehicle, (△, ▲) lyso PC; (□, ■) lyso PS. Lymph flow (0.95 ± 0.14 ml h^{-1}) was not changed by the lysophospholipid infusion. Means ± SEM for 12 experiments.

TABLE 1. Lipid content of intestinal mucosa

	Vehicle n = 9	LysoPC n = 7	LysoPS n = 11
		μmol g^{-1} (wet wt)	
Triacylglycerols	2.3 ± 0.2	2.7 ± 0.3	3.0 ± 0.4
Phospholipids	11.3 ± 0.4	12.1 ± 0.6	12.2 ± 0.7
PL/TG ratio	4.9	4.5	4.1
PC	5.2 ± 0.3	5.7 ± 0.3	5.4 ± 0.3
PE	2.6 ± 0.1	2.7 ± 0.3	3.0 ± 0.2
PI	0.6 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
PS	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1

Determinations were made 1 h after the oral administration of lysophospholipids (30 mg kg^{-1}). Values are given as means ± SEM.

TABLE 2. Lipids derived from infused [^{3}H]glycerol in mesenteric lymph

	Vehicle n = 6	LysoPC n = 5	LysoPS n = 6
Triacylglycerols			
dpm/2 h (× 10^{-3})	391.0 ± 107	749.0 ± 73[a]	340.0 ± 54.0
Percent of total labeled lipids	93.2 ± 0.8	94.4 ± 1.8	94.2 ± 0.8
Efflux (μmol/2 h)	8.7 ± 1.6	10.7 ± 0.9	10.0 ± 1.3
dpm μmol^{-1}	45.9 ± 7.3	71.0 ± 6.5[a]	36.0 ± 5.1
Phospholipids			
dpm/2 h (× 10^{-3})	7.0 ± 1.8	6.7 ± 1.2	5.3 ± 0.8
Percent of total labeled lipids	1.7 ± 0.1	0.8 ± 0.1[a]	1.6 ± 0.3
Efflux (μmol/2 h)	3.2 ± 0.4	3.2 ± 0.2	3.2 ± 0.3
dpm μmol^{-1}	2.2 ± 0.5	2.1 ± 0.3	1.7 ± 0.3

Five × 10^6 dpm of [^{3}H]glycerol dissolved in milk (1 ml kg^{-1}) was infused for 1 h in the duodenum. Lysophospholipids (30 mg kg^{-1}) were added as indicated. The data are means ± SEM of determinations performed on the lymph sample collected in the first 2 h (including the hour of infusion). Lymph flow was 2.5 ± 0.3 ml/2 h.
[a] $P < 0.05$.

Distribution of [^{3}H]arachidonate

As shown previously (22, 23) the duodenal infusion of [^{3}H]arachidonate was followed by the appearance of radioactivity in lymph, peaking 1 h after the end of infusion (**Fig. 2**). LysoPC enhanced incorporation of the labeled arachidonate into PC and therefore increased the efflux of labeled PL. In contrast, lysoPS decreased and delayed the peak of labeled TG, PL, and PC. Although increased, the peak of labeled PE was also delayed. Inspection of quantitative data (**Table 4**) showed that lysoPC did not change the dose recovery because the increase in the efflux of labeled PL (both PC and PI) corresponded to a slight decrease in the efflux of labeled TG, sufficient to significantly reduce their specific activity. The lysoPC-induced diversion of [^{3}H]arachidonate from TG to PL was clearly manifest by calculating the percent contribution to the total lymph radioactivity. In contrast, lysoPS caused a general decrease of [^{3}H]arachidonate appearance in lymph. The only exception was that labeled PE increased its contribution to the PL pool from 8.7 ± 0.5% to 15.9 ± 1 ($P < 0.05$).

To investigate whether lysoPS affected the site or the extent of [^{3}H]arachidonate absorption, label distribution was examined in different intestinal segments (**Table 5**). It can be seen that lysoPS actually increased [^{3}H]arachidonate absorption, this effect being predominant in the intestinal tract near the pylorus which collected 80% of radioactivity found in all segments. [^{3}H]arachidonate incorporation into the aminophospholipids generated by lysoPS reached 54% of label found in the PL fraction. Like lysoPS, lysoPC also acted in the upper tract of small intestine where high arachidonate absorption is normally detected. In a comparative analysis of [^{3}H]arachidonate distribution in the intestinal mucosa, liver, and plasma, it was found that lysoPC increased label incorporation in all compartments (**Table 6**). The lysoPC-induced increase was similar in plasma and liver confirming that circulating lipoproteins are mainly derived from hepatic secretion under fasting conditions (24). In contrast, lysoPS did not change the plasma and liver [^{3}H]arachidonate content in spite of increased label incorporation in the intestinal mucosa. The ratio between lipid-soluble radioactivity per gram mucosa and per ml plasma was 167 ± 23 and 198

TABLE 3. Distribution of [^{3}H]glycerol in the lipids of rat intestinal mucosa

	Vehicle n = 4	LysoPC n = 4	LysoPS n = 4
Total lipids (dpm g^{-1} × 10^{-3})	311.8 ± 57.5	435.2 ± 87.7	271.8 ± 72.8
Percent in neutral lipids	48.0 ± 5.3	68.5 ± 5.0[a]	57.5 ± 4.0
Percent in PL	51.9 ± 5.4	31.4 ± 5.0[a]	42.4 ± 4.0
Distribution (%) in mucosal PL			
PC	34.8 ± 2.9	21.0 ± 0.6[a]	38.6 ± 1.5
PE	16.1 ± 0.9	12.9 ± 1.2	13.9 ± 0.9
PI	34.0 ± 1.8	51.7 ± 2.3[a]	33.0 ± 1.9
PS	4.0 ± 1.2	1.9 ± 0.8	1.5 ± 0.6

[^{3}H]glycerol (4 × 10^7 dpm kg^{-1}) and the indicated lysophospholipid (30 mg kg^{-1}) were dispersed in bovine milk and given to rats as an intragastric bolus. After 60 min the intestinal mucosa was extracted. Values are given as means ± SEM.
[a] $P < 0.05$.

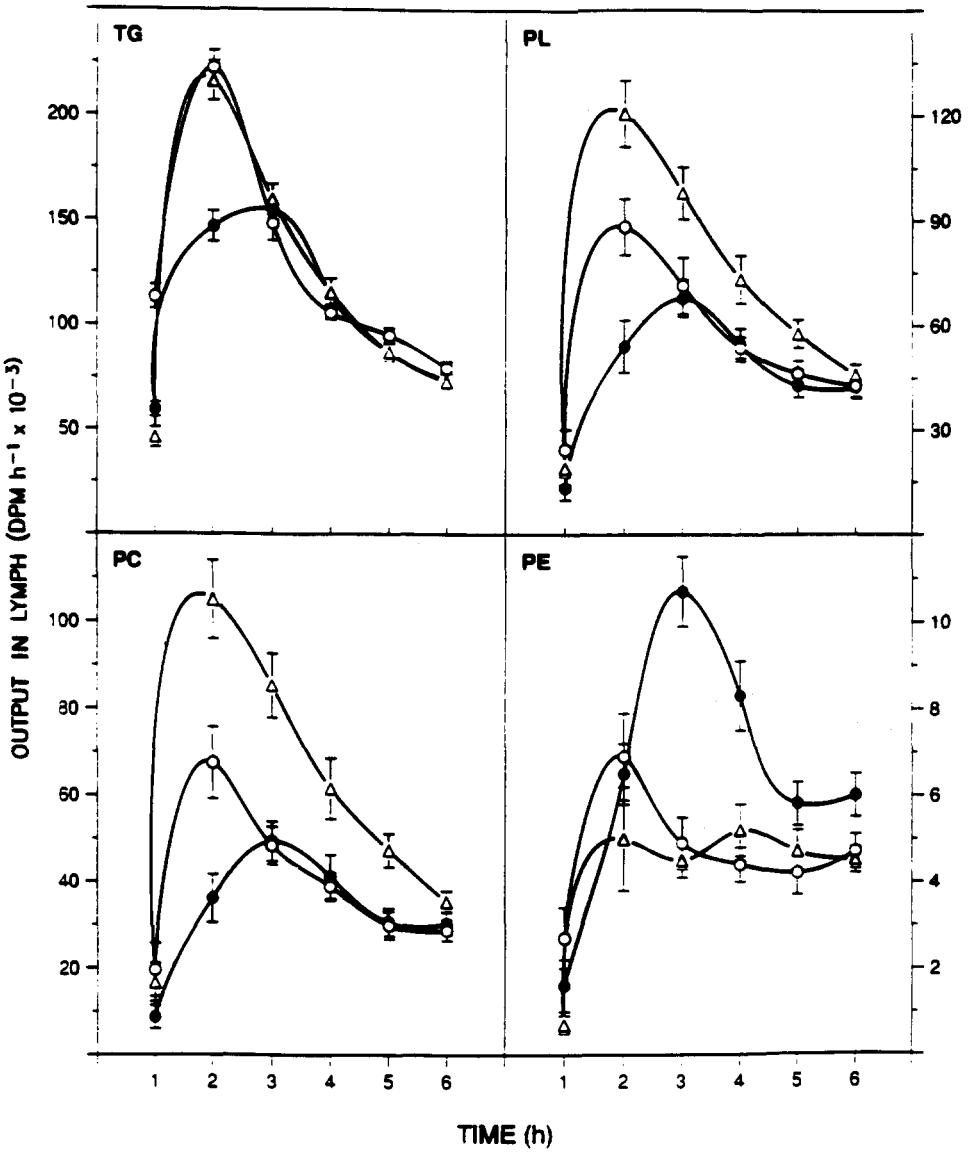

Fig. 2. Effect of lysoPC and lysoPS on the lymphatic efflux of [^{3}H]arachidonate-labeled triacylglycerols (TG), total phospholipids (PL), PC and PE. [^{3}H]arachidonate (3×10^6 dpm) and lysophospholipids (30 mg kg^{-1}) were infused in the first hour dispersed in BSS-BSA or milk; (○), vehicle; (△), lysoPC; (●) lysoPS. Each point is the means ± SEM for 9-18 rats. The action of lysophospholipids, when manifest, was significant.

± 56 in control and lysoPC-treated rats, respectively, indicating that the absorbed [^{3}H]arachidonate was normally delivered to plasma under these conditions. The value in lysoPS-treated rats was 294 ± 44, suggesting arachidonate retention in the mucosa. Similar experiments 2 h after the [^{3}H]arachidonate administration showed that the effect of lysoPC was still manifest in plasma lipoproteins known to be rich in PL and cholesteryl esters (**Fig. 3**). Unexpectedly, the lysoPS values in plasma and liver remained at the level of untreated rats in spite of reduced lymphatic transport.

Distribution of [^{14}C]linoleate

When compared to [^{3}H]arachidonate, oral [^{14}C]linoleate is known to have a greater distribution in the TG fraction of intestinal mucosa (10). Our results (**Table 7**) confirmed this pattern in plasma and intestinal mucosa. Due to the preference of [^{14}C]linoleate for TG, the lysoPL-induced redistribution in favor of PL fraction was more manifest than with [^{3}H]arachidonate. In plasma, lysoPC increased total lipoprotein labeling whereas lysoPS produced a slight decrease. In the intestinal

TABLE 4. Lymphatic transport of [^{3}H]arachidonate

	Vehicle n = 10–12	LysoPC n = 16–18	LysoPS n = 16–18
Recovery of administered dose (% in 6 h)	42.4 ± 2.6	41.8 ± 1.8	33.0 ± 1.8[a]
dpm efflux in 6 h ($\times 10^{-3}$)			
TG fraction	794.6 ± 55.6	714.8 ± 31.4	646.2 ± 31.8[a]
PL fraction	337.1 ± 19.9	424.6 ± 22.5[a]	279.8 ± 19.3
PC	242.4 ± 16.7	356.3 ± 20.0[a]	202.0 ± 16.9
PE	28.7 ± 2.9	25.8 ± 2.5	39.6 ± 2.3[a]
PI	8.2 ± 0.8	11.2 ± 0.8[a]	7.4 ± 0.8
Percent distribution			
TG	65.7 ± 0.8	60.1 ± 1.0[a]	67.1 ± 0.6
PL	29.3 ± 0.8	35.2 ± 1.0[a]	28.0 ± 0.7
Specific activity at peak time (dpm μmol^{-1} $\times 10^{-3}$)			
TG	44.8 ± 4.3	34.0 ± 2.9[a]	31.7 ± 1.9[a]
PL	49.3 ± 4.6	63.8 ± 5.0[a]	37.2 ± 2.9

The experimental conditions are those described in Fig. 2. The peak time for vehicle and lysoPC was at 2 h, for lysoPS at 3 h. Values are given as means ± SEM.
[a] $P < 0.05$.

mucosa, lysoPC enhanced PC labeling and lysoPS enhanced the labeling of aminophospholipids. Both lysoPL increased [^{14}C]linoleate absorption. The ratio between the radioactivity found in mucosa (dpm g^{-1}) and plasma (dpm ml^{-1}) was 72.1 ± 19.2 in untreated rats and 91.3 ± 21.4 in rats fed lysoPC. In lysoPS-treated rats the ratio was 178.7 ± 17.9 ($P < 0.05$). This difference was higher than that calculated with [^{3}H]arachidonate and confirmed the property of lysoPS to induce retention of polyunsaturated fatty acid in the intestinal mucosa. As lysoPS decreased the plasma level of [^{14}C]linoleate, it seemed likely that less fatty acid reached the liver in these rats. Consistent with this determination of labeled lipids in the liver of two rats under the conditions described in Table 7 gave a mean value of 11.9 dpm g^{-1} $\times 10^{-3}$ (range 10.2–13.6), 59.7% in TG and 19.4% in PL. Values in three rats treated with lysoPS were 8.7 dpm g^{-1} $\times 10^{-3}$ (range 8.2–9.5), 50.8% in TG and 33.4% in PL. The lysoPS-induced decrease in TG fraction (36%) was close to the reduction observed in plasma level (40%).

DISCUSSION

In this study we address the possibility of changing the disposition of dietary polyunsaturated fatty acids by the oral administration of 1-acyl lysoPL. Arachidonate has been more extensively investigated considering its biological functions and high activity in acylation and transacylation reactions (25). However, comparative experiments have been performed with linoleate, the essential fatty acid precursor of arachidonate. Among lysophospholipids,

TABLE 5. [^{3}H]arachidonate absorption in segments of rat small intestine

	Vehicle n = 2	LysoPC n = 2	LysoPS n = 2
Label distribution in first segment			
Total lipids (dpm g^{-1} $\times 10^{-3}$)	232.5 ± 82.5	1425.2 ± 46.5	1001.7 ± 96.7
Percent in neutral lipids	16.4 ± 1.4	11.4 ± 0.3	10.5 ± 2.4
Percent in PL	74.9 ± 3.9	82.2 ± 2.2	80.7 ± 8.7
PC in PL fraction (%)	65.0 ± 0.6	77.6 ± 0.5	40.0 ± 1.1
PE plus PS in PL fraction (%)	20.5 ± 0.8	10.0 ± 0.0	53.6 ± 0.1
Label distribution in second segment			
Total lipids (dpm g^{-1} $\times 10^{-3}$)	151.8 ± 94.2	353.7 ± 120.2	179.0 ± 84.5
Label distribution in third segment			
Total lipids (dpm g^{-1} $\times 10^{-3}$)	62.8 ± 41.8	154.7 ± 44.9	72.9 ± 12.2

[^{3}H]arachidonate (6×10^6 dpm kg^{-1}) and lysophospholipids (30 mg kg^{-1}) dispersed in BSS-BSA were given to fasted rats by a stomach tube. After 60 min the small intestine was divided in segments of 15 cm, beginning from the pylorus, and the mucosa was extracted. The data show the distribution of [^{3}H]arachidonate in the first three proximal segments. The others showed negligible radioactivity. Values are given as means ± the range between the two experiments.

TABLE 6. Distribution of orally administered [^{3}H]arachidonate in rats

	Vehicle	LysoPC	LysoPS
Plasma			
Total lipids (dpm $ml^{-1} \times 10^{-3}$)	1.8 ± 0.4	5.7 ± 1.4[a]	2.1 ± 0.3
Liver			
Total lipids (dpm $ml^{-1} \times 10^{-3}$)	4.3 ± 0.8	11.0 ± 2.7[a]	4.5 ± 0.2
Intestinal mucosa			
Total lipids (dpm $g^{-1} \times 10^{-3}$)	373.4 ± 75.9	660.3 ± 55.6[a]	569.4 ± 82.0
Distribution (%) in mucosal PL			
PC	64.1 ± 1.3	79.0 ± 1.0[a]	48.4 ± 1.9[a]
PE	18.3 ± 0.4	7.1 ± 0.5[a]	27.4 ± 1.3[a]
PS	1.5 ± 0.1	1.2 ± 0.2	10.9 ± 1.1[a]
PI	9.7 ± 1.0	7.2 ± 0.6[a]	6.8 ± 0.5[a]

[^{3}H]arachidonate (8-9 $\times$ 10^6 dpm kg^{-1}) and lysophospholipids (30 mg kg^{-1}) dispersed in BSS-BSA were given to fasted rats by a stomach tube. Plasma, liver, and the mucosa of small intestine (proximal 35 cm) were extracted after 60 min. Values are given as means ± SEM for four rats (liver) and for six to eight rats (plasma and mucosa). In plasma, 39 ± 5% of label was in TG, 36 ± 6% in PL, and 11 ± 1% in CE. In liver 28 ± 2% of label was in TG, 62 ± 2% in PL. In mucosa 15 ± 2% of label was in total neutral lipids, 80 ± 2% in PL. These values of distribution were not significantly changed by lysophospholipids.

[a] $P < 0.05$.

lysoPC and lysoPS have been selected as they generate diacylphospholipids (PC and PS) that differ widely in their utilization for the assembly of plasma and lymph lipoproteins. To avoid interference by other exogenous fats, in this first study we used fasted rats in which lipid absorption was restricted to biliary PL. Efflux of PL in rat bile amounts to 1.8–2.6 μmol h^{-1}, 90% formed by PC (18). In bile PC, arachidonate comprises 20% and linoleate 60% of the acyl chains esterified in the 2-position of glycerol (18). To probe the absorption and the distribution of these fatty acids we administered a radiolabeled tracer together with the lysoPL dispersion. Absorption of fatty acids and their delivery to plasma in fasted rats occur mainly through a continuous VLDL secretion (26). In order not to alter this pattern, we administered a lysoPL dose insufficient to substantially increase lymphatic lipid efflux and therefore unable to activate the process of chylomicron assembly which includes the segregation of substrates used for TG and PL synthesis (9, 26, 27). The data show that lysoPC enhances [^{3}H]arachidonate incorporation into mucosal PC, thereby increasing the transport of this fatty acid by the PL fraction of lymph, 70% of which is comprised by PC. Labeling of lipoproteins in plasma and liver is also increased. As the contribution of intestinal VLDL to the plasma lipoproteins is small under fasting conditions (24), the action of lysoPC in plasma lipids reflects the transfer of PL to circulating HDL and the greater amount of label reaching the liver as a component of the PL fraction of remnant particles. The lysoPC-induced redistribution in the PL fraction has been confirmed with [^{14}C]linoleate. Due to a preference of [^{14}C]linoleate for the TG fraction of mucosa and plasma lipids, the action of lysoPC on this fatty acid has been even more evident. We also find that lysoPC decreases [^{3}H]glycerol incorporation into mucosal PC and increases the inclusion of this label in TG of both intestinal mucosa and lymph, confirming that lysoPC inhibits the CDP-choline pathway of PC synthesis (20) and promotes de novo TG synthesis (19, 26). Furthermore, we find that

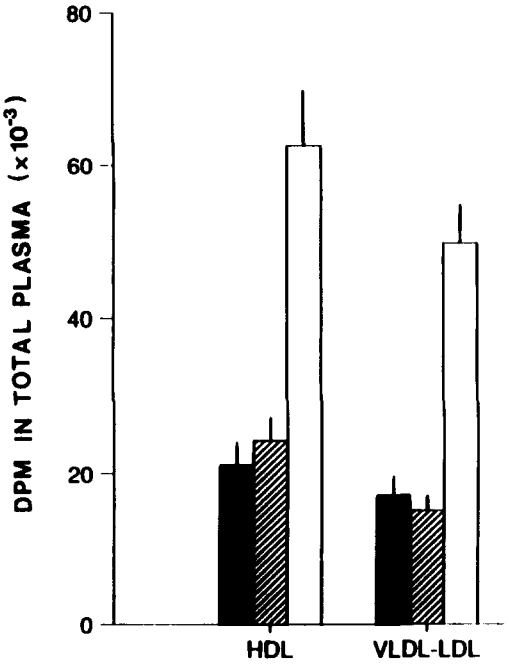

Fig. 3. Distribution of [^{3}H]arachidonate in plasma lipoproteins. [^{3}H]arachidonate (10^7 dpm kg^{-1}) and lysophospholipids (30 mg kg^{-1}) were given to rats as an intragastric bolus. After 2 h, plasma was collected and the lipoprotein classes were separated as described in Materials and Methods. The data are the dpm detected in total plasma (4% of body weight). Means ± SEM for 7 rats. Black bars, vehicle; hatched bars, lysoPS; white bars, lysoPC ($P < 0.05$ for the effect of lysoPC).

TABLE 7. Distribution of orally administered [^{14}C]linoleate in rats

	Vehicle n = 5	LysoPC n = 4	LysoPS n = 5
Plasma			
Total lipids (dpm ml^{-1} × 10^{-3})	6.3 ± 1.0	11.5 ± 1.0[a]	4.4 ± 0.4
Distribution (%) in TG	74.6 ± 4.5	60.4 ± 2.2	63.2 ± 3.3
PL	10.2 ± 1.6	30.7 ± 2.4[a]	22.2 ± 3.9[a]
CE	7.3 ± 2.0	4.2 ± 0.4	7.5 ± 0.6
Mucosa			
Total lipids (dpm g^{-1} × 10^{-3})	381.2 ± 36.3	1033.2 ± 161.6[a]	802.5 ± 129.7[a]
Distribution (%) in neutral lipids	32.2 ± 2.7	20.0 ± 2.5[a]	16.5 ± 2.5[a]
PL	63.0 ± 3.4	77.6 ± 3.0[a]	82.5 ± 2.9[a]
Distribution (%) in mucosal PL			
PC	83.3 ± 1.1	90.9 ± 1.0[a]	66.3 ± 1.8[a]
PE	8.5 ± 0.6	3.3 ± 0.4[a]	13.7 ± 0.9[a]
PS	1.3 ± 0.0	1.5 ± 0.1	15.8 ± 1.1[a]
PI	2.9 ± 0.1	1.5 ± 0.2[a]	1.0 ± 0.1[a]

[^{14}C]linoleate (10^{7} dpm kg^{-1}) and lysophospholipids (30 mg kg^{-1}) dispersed in BSS-BSA were given to fasted rats as an intragastric bolus. Plasma and the mucosa of small intestine (proximal 35 cm) were extracted after 60 min. Values are given as means ± SEM.

[a] $P < 0.05$.

lysoPC increases mucosal PI content and the labeling of this phospholipid by [^{3}H]glycerol. Efflux in lymph of PI containing labeled arachidonate is also increased. Previous observations of enhanced TG synthesis by infused PC have been attributed to increased availability of fatty acids either released in the lumen by the action of phospholipase A_2 or in the mucosa by lysoPC hydrolysis (19, 26). Our data further define this effect, suggesting that the lysoPC-induced inhibition of de novo PC synthesis makes more DAG available for both TG and PI synthesis. Within this possibility it is interesting to note that lysoPC fails to enhance [^{3}H]arachidonate incorporation into lymph TG as it would be expected. Nor does lysoPC induce accumulation of arachidonoyl-PI in the enterocyte. The data suggest that the fraction of arachidonoyl-DAG accumulating under the influence of lysoPC is preferentially channelled into a PI species that is promptly secreted. Low utilization of arachidonoyl-DAG for TG synthesis in the intestinal mucosa has been previously detected (10). In addition, the preference of arachidonoyl-DAG for PI synthesis (28) and the prompt mobilization of newly synthesized PL for lipoprotein secretion (29) have been observed in other tissues.

Unlike lysoPC, lysoPS promotes [^{3}H]arachidonate incorporation into PS which is then decarboxylated to PE (30). Under the influence of lysoPS, the secretion of [^{3}H]arachidonate-labeled TG and PC in lymph is delayed and reduced. Although increased, the secretion of arachidonoyl-PE is also delayed. As lysoPS does not change the TG and PL content of lymph, retarded arachidonate secretion is apparently due to delayed transfer of fatty acid to lipids that are to be secreted. In accord, both TG and PL specific activity is reduced in lymph by lysoPS. Determination of lipids in the intestinal mucosa shows unchanged TG content, indicating that lysoPS does not inhibit lipoprotein secretion. Furthermore, experiments on [^{3}H]arachidonate incorporation in sequential segments of small intestine show that the reduced transport in lymph is not due to impaired arachidonate absorption, or to a shift of arachidonate absorption into more distal and less efficient absorptive sites. One possible explanation of the lysoPS effect is that the incorporation of arachidonate into the aminophospholipids reduces its availability for the generation of arachidonoyl-PC and TG. The requirement for a transacylation reaction moving [^{3}H]arachidonate from PS or PE to PC would explain the delay in the secretion of label associated with this lipid. Alternatively, a transient increase of PE concentration immediately after lysoPS absorption results in the inhibition of acyltransferase reactions. Early studies of rats receiving PE intraduodenally for 48 h have shown a significant decrease in the specific activity of mucosal acyl-CoA synthetase and monoglycerol-acyl-transferase (31). In accord with lymph data, the level of radiolabeled arachidonate detected in plasma and liver lipids has been lower in lysoPS-treated rats with respect to animals receiving lysoPC. However, we also note that the lysoPS values are comparable to those of untreated rats in spite of reduced lymphatic transport. When these experiments were repeated with [^{14}C]linoleate, lysoPS induced the same changes in distribution observed with [^{3}H]arachidonate in the intestinal mucosa. However, with this fatty acid the labeling of liver and plasma lipoproteins was lower than the level found in untreated rats, as would be expected. These data suggest that under the influence of

lysoPS the two fatty acids differ in being able to reach the liver through the portal circulation, i.e., the route alternative to lymphatic transport (32, 33). Alternatively, in the case of [^{3}H]arachidonate, tritium exchange with water in the intestinal tract is responsible for the transport of radioactivity to liver (3). Additional experiments are required to clarify this point. As with [^{3}H]arachidonate, lysoPS increases [^{14}C]linoleate incorporation into mucosal PL without increasing plasma concentration. This is at variance with the lysoPC-induced regulatory effect and indicates that lysoPS causes retention of these polyunsaturated fatty acids in the intestinal mucosa. Whether the lysoPS-induced accumulation of polyunsaturated fatty acids affects the function of absorptive cells remains to be established. Indeed, it is interesting to note that short-term feeding with diets enriched in polyunsaturated fatty acids changes the intestinal uptake of nutrients (34) and that polyunsaturated fatty acids concur with PS in the activation of protein kinase C (35).

In conclusion, the results of this study show the possibility of changing the absorption and the distribution of polyunsaturated fatty acids by the oral administration of lysoPL. Further investigations are now warranted to examine whether appropriate doses of these compounds may affect the mass movement of these fatty acids under ordinary dietary conditions.

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