ELSEVIER

# Uptake of arachidonic acid from blood 2-lyso-phosphatidylcholine by extrahepatic tissues in vivo: An experimental study in the rat

**Li Zhou and Åke Nilsson***

*Cell Biology Department 1 and *Division of Gastroenterology, Department of Medicine, University Hospital, Lund, Sweden*

*In previous work, we demonstrated that part of arachidonic acid (AA, n-6) pools in the gastrointestinal tract and blood forming tissues could be formed by local interconversion of linoleic acid taken up as free fatty acid from blood. In the rat the liver releases significant amounts of 2-arachidonyl-lysophosphatidylcholine (2-AA-LPC). In this study, we took advantage of the high hydrolytic activity of recombinant guinea pig pancreatic lipase-related protein 2 against the 1-ester bond of glycerophospholipids to conveniently prepare 2-[$^{14}$C]AA-LPC by hydrolysis of radioactive phosphatidylcholine (L-α-1-Palmitoyl-2-arachidonyl [arachidonyl-1-$^{14}$C]) and examined the rate of uptake of 2-[$^{14}$C]AA-LPC by the tissues in rats. Albumin-bound 2-[$^{14}$C]AA-LPC was injected intravenously into male rats. The clearance rate of the 2-[$^{14}$C]AA-LPC was fast, the initial half-life being 1.07 min. At 10 min after injection, the retention of $^{14}$C was 2.0–3.3%/g tissue of injected dose in the liver, 0.4 to 0.6%/g in stomach, 1.1 to 1.3%/g in small intestine, 0.4 to 0.6%/g in colon, 0.3 to 0.4%/g in bone marrow, and 0.7 to 1.0%/g in spleen. In most tissues, the proportion of radioactivity in tissue lipids was highest in PC (51 to 72%). In the heart, lung, and spleen the radioactivity in LPC was most predominant. The rate of uptake of AA as LPC by the liver was 61.0 ± 7.3 nmol/min, and by the gastrointestinal tract including stomach, small intestine and colon was 25.0 nmol/min. This study provides a quantitative estimation of the role of 2-AA-LPC in the supply of AA to the AA pools in extrahepatic tissues. It indicates that uptake of liver derived 2-AA-LPC may be one of the most important AA sources for extrahepatic tissues in the rat, particularly the gastrointestinal tract.* (J. Nutr. Biochem. 8: 641–646, 1997) © *Elsevier Science Inc. 1997*

**Keywords:** arachidonic acid; eicosanoids; gastrointestinal tract; lysophosphatidylcholine; phospholipids

## Introduction

Plasma lysophosphatidylcholine (LPC) is formed mainly by the action of lecthin-cholesterol acyltransferase (LCAT) when a fatty acid is removed from plasma phosphatidylcholine (PC) and transfered to cholesterol. Several papers have demonstrated that plasma LPC could also be generated by the hydrolysis of hepatic PC.[1,2] The LPC formed by LCAT is highly saturated, in contrast to the highly unsaturated LPC secreted by the liver.[1] LPC is often the second most prevalent phospholipid in the plasma, averaging 18% of the total plasma phospholipids[4] and its concentration remains relatively high in patients with deficiency of LCAT.[4] It is bound to albumin in the plasma and is readily taken up from the plasma by a variety of organs and reacylated to PC without any adverse effects.[1] It has been shown that LPC is a nutrient necessary for the normal growth of certain isolated cells in culture.[5]

It was recently suggested that much of the arachidonic acid (AA) in extrahepatic tissues may be formed locally by desaturation-elongation (DE) of linoleate (LA) taken up as plasma free fatty acid.[6] Plasma 2-AA-lysophosphatidylcholine (AA-LPC) may be an important alternative way to supply AA to extrahepatic tissues.[7] Because LPC in rat plasma contains large amounts of PUFA,[1,2] the unsaturated LPC being produced by the liver,[1,2,7] this pathway can

Address reprint requests to: Åke Nilsson, M.D., Ph.D., at Division of Gastroenterology, Department of Medicine, University Hospital of Lund, S-221 85 Lund, Sweden.
Received January 31, 1997; accepted July 9, 1997.

Nutritional Biochemistry 8:641–646, 1997
© Elsevier Science Inc. 1997
655 Avenue of the Americas, New York, NY 10010

0955-2863/97/$17.00
PII S0955-2863(97)00114-9

provide a transport system also for choline and other polyunsaturated fatty acids to tissues.[7]

This study provides a quantitative estimation of the role of AA-LPC in the supply of AA to the AA pools in extrahepatic tissues. The rate of uptake of AA from 2-LPC by the tissues in rats was studied by injecting 2-[14C]AA-LPC, and then measured the disappearance kinetics, plasma concentration of AA-LPC, and tissue retention of $^{14}C$.

## Methods and materials

### *Preparation of 2-arachidonyl[1-$^{14}C$]-lysophosphatidylcholine (2-[$^{14}C$]AA-LPC)*

2-[$^{14}C$]AA-LPC was prepared by hydrolysis of radioactive phosphatidylcholine (L-α-1-Palmitoyl-2-arachidonyl [arachidonyl-1-$^{14}C$]) (specific activity was 55 mCi/mmol) which was obtained from American Radiolabeled Chemicals, Inc., St. Louis, MO, USA, using recombinant guinea pig pancreatic lipase related protein 2 ($GPLRP_2$), which has a high activity toward the 1-ester bond of glycerophospholipids.[8-10] The $GPLRP_2$ was kindly supplied by Dr. Frédéric Carrière, Laboratoire de Lipolyse Enzymatique UPR 9025, Centre National de la Recherche Scientifique, 13402 Marseille cedex 20, France. In brief, 0.5 mL radioactive phosphatidylcholine was dried under $N_2$, and was redissolved in 2 mL of 10 mM Tris-Maleate buffer at pH 7.0 containing 2 mM $CaCl_2$, 0.12 M NaCl with 6.4 mM bile salt mixture.[10] The mixture was incubated with 10 μg of $GPLRP_2$ at 37°C for 30 min. After lipid extraction the 2-[$^{14}C$]AA-LPC was separated by thin layer chromatography (TLC) under $N_2$ as described below and eluted.[11] The purity of formed 2-[$^{14}C$]AA-LPC was 96.2%, and it was stored at −20°C under $N_2$ before using.

### *Intravenous injection of 2-[$^{14}C$]AA-LPC*

2-[$^{14}C$]AA-LPC was prepared as described above. It was bound to albumin in serum that was obtained from a nonfasting rat kept on the standard pellet diet, as described earlier.[12]

Male Sprague-Dawly rats were obtained from Möllegaard (Denmark). They were kept under a controlled dark/light schedule and had free access to a standard commercial pellet diet until the time of the experiment. The body weight of the rats were 200 to 250 g. One half mL radioactive serum (0.5 μCi 2-[$^{14}C$]AA-LPC) was injected into the jugular vein under a light ether anaesthesia. After 10 min the rats (5) were killed by aortic puncture. The liver, stomach, upper and lower halves of small intestine, colon, bone marrow from two femurs, tibias and humerus, pancreas, spleen, heart, lungs, brain, adipose tissues, and kidneys were immediately removed and the lipids were extracted with chloroform:methanol (2:1 v/v) containing 0.005% butylated hydroxy-toluene (BHT) as an antioxidant. For determining the clearance of 2-[$^{14}C$]AA-LPC in plasma, five rats weighing 215 to 235 g were intravenously injected with 0.5 mL of labeled serum containing $2.2 \times 10^6$ dpm 2-[$^{14}C$]AA-LPC into the jugular vein under anaesthesia. Blood samples (0.3 to 0.4 mL) were obtained by cutting the tail of the rats at 30, 60, 180, 300, and 600 sec. Sodium heparin was used as anticoagulant. Ten μL of plasma were counted in a Packard Tri-Carb 2100TR liquid scintillation analyzer using the automatic external standard for quench correction, and another 100 μL of plasma were extracted immediately with chloroform: methanol (1:1 v/v) containing 0.005% BHT for plasma phospholipids separation.

### *Determination of radioactivity in different lipid classes*

After lipid extraction and two-phase distribution as described earlier[6] aliquots of the lower phase were taken to dryness under nitrogen and redissolved in a small volume of chloroform. Aliquots were taken for thin layer chromatography (TLC). Phospholipids were separated on Merck silica gel 60 plates, which were developed in chloroform:methanol:acetic acid:water 100:80:12:1.2 (v/v). Spots were identified by staining with iodine vapor and scraped into counting vials. One mL methanol:water 1:1 (v/v) and 10 mL Instagel:toluene 1:1 (v/v) were added, and the radioactivity of the samples was determined as described above.

### *Analysis of fatty acid composition of LPC in rat plasma*

Rat (200 to 250 g) blood was drawn into plastic syringes containing 100 μL EDTA (100 mmol/L) and 5,5′-dithiobis nitrobenzoic acid (DTNB, ICN Biomedicals Inc, USA) as LCAT inhibitor, the final concentration of DTNB was 1.4 mmol/L. The plasma was separated immediately by microfuge and was extracted for lipids as soon as they were obtained. The plasma was extracted in 20 volumes of chloroform:methanol (1:1, v/v) containing 0.005% BHT at 4°C for 1 hr. LPC was separated from other phospholipids by high performance liquid chromatography (HPLC).[13] A Shimadzu SPD 6 A HPLC equipped with a Nucleosil 50-5 column and a UV-detector set at 205 nm was used. The mobile phase was acetonitrile:methanol:water containing 1.5 μM lithium acetate (50:45:6.5 v/v), and the flow rate was 1 mL/min. The collected LPC fractions were methylated by sodium methoxide-catalyzed transesterification[14] and the composition of the fatty acid methyl esters determined by gas chromatography and integration of peak areas (Perkin Elmer Model 30 apparatus). L-α-heptadecanoyl lysophosphatidylcholine (17:0-LPC) was added as internal standard. The mass distribution of different fatty acids into LPC in rat plasma was then calculated and related to the known amount of internal standard.

## Results

### *Clearance of 2-[$^{14}C$]AA-LPC in plasma*

The kinetics for the disappearance of 2-[$^{14}C$]AA-LPC from plasma was determined. At 30 sec after injection, the total radioactivity in plasma LPC was 57 to 75% of the injected dose, at 60 sec 46 to 49%, at 180 sec 27 to 35%, and at 10 min only 11 to 15%. The radioactivity in LPC was 74.4% of the total plasma radioactivity at 10 min. Most of the remaining part was in plasma PC (9%) and nonpolar lipids (7.1% in FFA, 1.4% in CE, 1.2% in TG, 2.9% in DG, and 0.8% in MG). The rate of clearance was estimated by a linear regression analysis of the amounts of 2-[$^{14}C$]AA-LPC radioactivity remaining in serum at different time intervals. The initial half-life calculated using the logarithm values of percent radioactivity of injected dose in plasma LPC plotted against time was 1.07 min (*Figure 1*). The labeled LPC was mostly cleared within 10 min. The average concentration of LPC in rat plasma was 512.5 ± 23.0 nmol/mL (*Table 1*) estimated by gas chromatographic analysis of the plasma LPC fraction. The concentration of 2-AA-LPC was 9.9% of the total LPC (50.5 ± 2.5 nmol/mL) in the plasma (*Table 1*). With the assumption that the plasma volume is 4% of the body weight the average plasma pool of 2-AA-LPC was

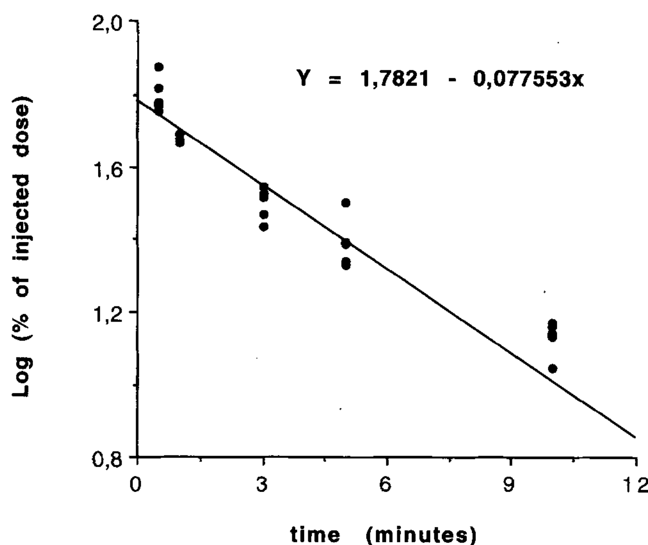


**Figure 1** Linear regression of clearance of 2-[$^{14}$C]AA-LPC in plasma. Rats were injected intravenously with 0.5 mL labeled serum containing 2.2 × $10^6$ dpm 2-[$^{14}$C]AA-LPC. 0.3-0.4 mL of blood was collected by cutting the tails of the rats at the time intervals indicated. The correlation coefficient is 0.97, $P < 0.0001$. Data are from five individual observations.

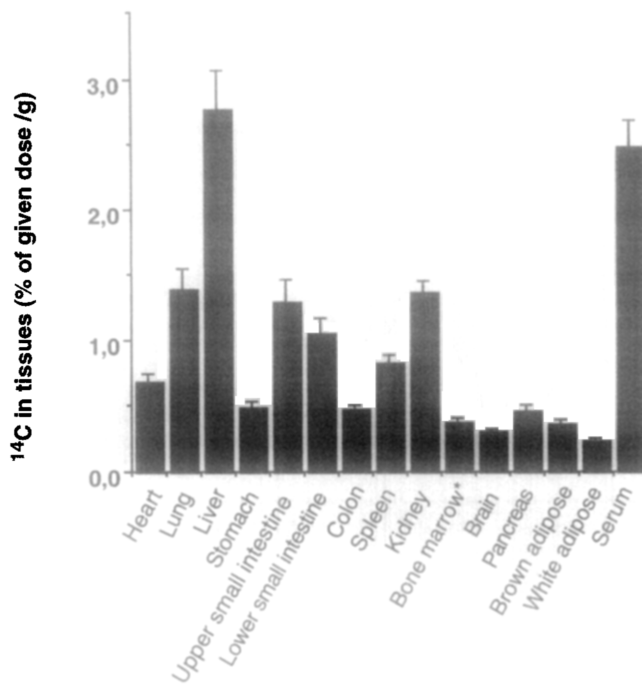


**Figure 2** Lipid radioactivity per gram tissue in different organs. Rats were injected intravenously with 0.5 μCi 2-[$^{14}$C]AA-LPC as described in "Methods and materials". Values are means ± SEM of five observations. * Bone marrow radioactivity per gram was calculated as radioactivity per total weight of both femurs, tibias, and humerus.

calculated to be 505.3 ± 24.7 nmol. From the half-life of 1.07 min it could be calculated that 236.1 nmol/min 2-AA-LPC is cleared from plasma.

### *Tissue retention of 2-[$^{14}$C]AA-LPC*

The rats were killed 10 min after intravenous injection of 2-[$^{14}$C]AA-LPC. The total $^{14}$C radioactivity in serum was 21% of injected dose, and the $^{14}$C-LPC amounted to 15.6% of injected dose. The retention of $^{14}$C in lipids of different tissues is shown in *Figure 2*. The retention of $^{14}$C per gram tissue in lung, upper small intestine, lower small intestine, and kidney was higher than that in the heart, brain, and adipose tissues but lower than that in the liver (*Figure 2*). The retention of $^{14}$C was 2.0 to 3.3%/g tissue in the liver, 0.4 to 0.6%/g in stomach, 1.1 to 1.3%/g in small intestine, 0.4 to 0.6%/g in colon, 0.3 to 0.4%/g in bone marrow, and 0.7 to 1.0%/g in spleen. The total retention in whole liver was 25.8% of injected dose. The total uptake in the gastrointestinal tract including stomach, upper part small intestine, lower part small intestine, and colon was 10.6% of injected dose. We assume that the degree of retention of 2-[$^{14}$C]AA-LPC is the same as that of unlabeled 2-AA-LPC continuously taken up from the plasma. Based on this assumption, the rate of retention of plasma 2-AA-LPC in different tissues was calculated from the mass of 2-AA-LPC that disappeared from blood per time unit and the percentage of this amount retained in the tissues over the time span studied. The rate of uptake of AA from plasma LPC were 6.5 ± 0.7 nmol/(g.min) in liver, 1.2 ± 0.1 nmol/(g.min) in the stomach, 3.1 ± 0.4 nmol/(g.min) in the upper small intestine, 2.5 ± 0.3 nmol/(g.min) in lower small intestine, 1.1 ± 0.1 nmol/(g.min) in the colon, and 0.9 ± 0.1 nmol/(g.min) in bone marrow (*Table 2*).

From the data described above, the rates of tissue retention of AA originating from the plasma 2-AA-LPC pool in different organs were calculated (*Table 2*). The retention of AA in the liver was 61.0 ± 7.3 nmol/min and in the GI tract including stomach, small intestine, and colon 25.0 nmol/min. Based on the assumption that bone marrow accounts for 3% of the body weight, it can be calculated that the total AA obtained in bone marrow was 5.7 ± 0.5 nmol/min.

**Table 1** Fatty acids composition of the LPC in the plasma of rats

| | nmol/mL Plasma | % of total FA |
|---|---|---|
| Total FA | 512.5 ± 26.5 | |
| 16:0 | 141.4 ± 16.0 | 27.4 ± 1.8 |
| 18:0 | 109.5 ± 4.6 | 21.5 ± 1.4 |
| 18:1 | 54.5 ± 3.9 | 10.6 ± 0.3 |
| 18:2 | 149.6 ± 5.0 | 29.3 ± 0.9 |
| 20:4 | 50.5 ± 2.8 | 9.9 ± 0.8 |
| 20:5 | 7.1 ± 3.0 | 1.3 ± 0.5 |

Means ± SEM, $n$ = 3 to 6

### *Distribution of $^{14}$C between lipid classes*

The distribution of $^{14}$C between nonpolar lipids (NL) and individual phospholipids (LPC, PC, PI: phosphatidylinositol, PE: phosphatidylethanolamine) is given in *Table 3*. The proportion of $^{14}$C radioactivity in PC was highest in the liver, and in tissues at the GI tract. In the heart, lung, and spleen more of the radioactivity remained in LPC. Percent

**Table 2** Retention of plasma 2-AA-LPC in different tissues ($n = 5$)*

| | nmol/min per gram tissue (Means ± SEM) | nmol/min in whole organ (Means ± SEM) |
|---|---|---|
| Heart | 1.6 ± 0.1 | 1.4 ± 0.1 |
| Lung | 3.3 ± 0.4 | 4.3 ± 0.7 |
| Liver | 6.5 ± 0.7 | 61.0 ± 7.3 |
| Spleen | 2.0 ± 0.1 | 1.6 ± 0.2 |
| Stomach | 1.2 ± 0.1 | 1.7 ± 0.1 |
| Upper small intestine | 3.1 ± 0.4 | 13.0 ± 1.5 |
| Lower small intestine | 2.5 ± 0.3 | 8.6 ± 1.0 |
| Colon | 1.1 ± 0.1 | 1.7 ± 0.1 |
| Pancreas | 1.1 ± 0.1 | 0.7 ± 0.1 |
| Brown adipose | 0.9 ± 0.1 | N.D.✣ |
| Bone marrow | 0.9 ± 0.1† | 5.7 ± 0.5‡ |
| Kidney | 3.2 ± 0.2 | 6.2 ± 0.5 |
| Brain | 0.7 ± 0.1 | 1.1 ± 0.1 |

*Calculations are based on the finding that the mass of plasma 2-AA-LPC that disappeared from blood was 236.1 nmol/min and on the assumption that the degree of retention of 2-[$^{14}$C]AA-LPC is the same as that of 2-AA-LPC delivered to the tissues. The retention of plasma 2-AA-LPC in different tissues was thus calculated by multiplying percent retention of 2-[$^{14}$C]AA-LPC in different tissues with 236.1 nmol/min.
✣N.D., not determined.
†The weight of bone marrow based on the total weight of the both femurs, tibias, and humerus, including both bone and bone marrow.
‡The weight of bone marrow based on the assumptions that bone marrow accounts for 3% of body weight and for 25% of the weight of femurs, tibias, and humerus.

radioactivity in phosphatidylserine was less than 2% and in cardiolipin less than 2.5% (data not shown).

## Discussion

Eicosanoids are produced from arachidonic acid (AA) released from tissue phospholipids by phospholipase A2, when cells are stimulated by agonists.[15–18] How eicosanoid formation is influenced by the supply of dietary fatty acids requires a knowledge about the transport and synthetic pathways by which different tissues acquire their AA pools. This question is particularly important in case of the tissues that have a rapid cell turnover and/or a high rate of eicosanoid formation, such as bone marrow and the mucosa of the gastrointestinal tract. There are a number of different pathways by which the extrahepatic tissues might acquire their AA: (a) Absorption and transport by the chylomicrons of dietary AA,[19] (b) formation of AA by desaturation-elongation of linoleic acid (LA) in the liver, succeeded by transport to the tissues by secreted lipoproteins or by other pathway,[20,21] and (c) uptake of linoleate as free fatty acids (FFA)[6,10] or as glycerolipids via lipoprotein receptors succeeded by local desaturation-elongation in the extrahepatic tissues.[22] When evaluating the different possibilities, one has to expect considerable species differences, because on one extreme carnivores like the cat and the lion do not produce AA by desaturation-elongation and are thus obligately dependent on supply of AA in the diet, and on the other extreme herbivores do not ingest AA and thus must form the whole AA pools by interconversion of LA.

Recently it was suggested by Brindley[7] that 2-AA-LPC secreted from the liver may be an important pathway for the transport of AA to extrahepatic tissues. Teleologically an advantage with this pathway would be that the biologically potent AA is transported in esterified form by a mechanism that may be regulated differently than the lipoprotein receptors. In this study we took advantage of the high hydrolytic activity of recombinant guinea pig PLRP2[8,9] against the 1-ester bond of glycerophospholipids to conveniently prepare 2-[$^{14}$C]-AA-LPC and inject it intravenously. The clearance rate of the 2-[$^{14}$C]AA-LPC was fast, the initial half-life was 1.07 minutes; this data is in line with the results of 2-[$^{14}$C]AA-LPC($^{3}$H-choline) perfusing into rat study. They found the disappearance increased with the unsaturation degree of the fatty acid moiety.[11] The rapid kinetics for the disappearance in combination with our data on tissue uptake 2-[$^{14}$C]AA-LPC demonstrate that the pathway may supply substantial amounts of AA to extrahepatic tissues. Although the highest uptake was observed for the liver, as much as 15% of the recovered radioactivity was found in the small intestine and the retention of AA-LPC was calculated as 25 nmol/min in GI tract. Also the kidney and lung contained radioactivity amounting to 30 to 40% of the hepatic radioactivity (per gram tissue). The conclusion is that although this pathway supplies AA to several extrahepatic tissues its quantitative importance is largest in case of the small intestine compared with other pathways. In bone

**Table 3** Percentage distribution of $^{14}$C in phospholipid subclasses and neutral lipid in different tissues at 10 minutes after injection (Mean ± SEM, $n = 5$)

| | LPC + SM | PC | PI | PE | NL |
|---|---|---|---|---|---|
| Heart | 65.1 (60) | 18.3 (1.3) | 0.9 (0.7) | 1.7 (1.2) | 4.5 (1.2) |
| Lung | 67.8 (5.8) | 22.8 (3.9) | 0.4 (0.2) | 1.5 (0.7) | 4.5 (0.5) |
| Liver | 12.9 (0.8) | 72.0 (0.7) | 0.4 (0.1) | 3.1 (0.4) | 10.2 (0.2) |
| Stomach | 10.3 (0.8) | 64.3 (1.2) | 3.8 (0.4) | 6.1 (1.9) | 13.9 (1.9) |
| Upper small intestine | 10.8 (1.2) | 60.1 (0.6) | 4.6 (0.2) | 3.8 (0.8) | 17.0 (1.0) |
| Lower small intestine | 9.7 (0.5) | 67.5 (1.2) | 3.3 (0.2) | 8.2 (2.5) | 9.7 (1.4) |
| Colon | 34.7 (3.1) | 53.9 (2.2) | 0 | 2.8 (0.7) | 6.1 (1.1) |
| Bone marrow | 29.1 (0.5) | 51.4 (1.2) | 0.6 (0.3) | 2.4 (0.8) | 11.5 (1.6) |
| Spleen | 55.7 (2.7) | 37.6 (2.5) | 0.5 (0.1) | 1.1 (0.1) | 3.7 (0.4) |
| Kidney | 23.9 (1.0) | 57.6 (0.8) | 1.9 (0.3) | 7.6 (1.5) | 6.5 (1.9) |
| Plasma | 74.4 (1.9) | 9.0 (0.3) | N.D. | N.D. | 14.3 (1.6) |

N.D., not determined.

marrow tissue, uptake and local interconversion of unesterified plasma LA pathway[6] may be more important than uptake of 2-AA-LPC compared with the early study.[6]

In the liver and GI tract a high reacylation of LPC into PC was observed, whereas in other organs it proceeded at a slower rate (*Table 3*), similar results were found when $^{32}P$ labeled total plasma LPC was injected.[23] Partial hydrolysis of 2-[$^{14}C$]AA-LPC might occur in plasma and tissues, because 7% of total plasma radioactivity was found in FFA at 10 min and some radioactivity in other phospholipids in tissues. An early study[11] found that doubly labelled 2-acyl-LPC could be taken up by brain tissues without previous hydrolysis and reacylated into PC. Partial hydrolysis of 2-AA-LPC occurred in the brain tissue, and the isotopic ratio of the fatty acid to the choline moiety in PC fraction quickly increased by reesterification the labeled fatty acid derived from the hydrolysis of the injected LPC. Morash et.[24] examined the fate of exogenous 1-acyl and 2-acyl-LPC specifically radiolabelled with choline and/or fatty acid incubated ether singly or as equimolar with NIE-115 neuroblastoma and C6 rat glioma cells in culture. They found the 2-acyl species was taken up and acylated to PC faster than the 1-acyl species in both cell lines. Both species turn over actively, but only the 1-Acyl species accumulates, whereas 2-acyl-LPC is likely to be reacylated to form PC. Thiès et al.[11] found the brain uptake of the unesterified fatty acids from blood reached a plateau at 5 to 15 min and was maximal for AA (0.45% of the perfused dose). But the brain uptake 2-acyl-LPC increased with the degree of unsaturation and was 6- to 10-fold higher than that of the corresponding unesterified fatty acid. The ability to take up and acylate 2-acyl-LPC thus is a general feature of many cell types. The most likely interpretation of our findings is thus, that 2-acyl-LPC is an efficient delivery form of unsaturated fatty acids to some extrahepatic tissues, although we did not exclude that some of the tissue $^{14}C$ originated from FFA released intravascularly from the 2-acyl-LPC.

Secretion of AA-LPC has been demonstrated most clearly in the rat.[1,2,7] Brindley could not demonstrate secretion of AA-LPC from sheep hepatocytes.[2] In the guinea pig we demonstrated that the formation of AA from unesterified plasma LA in several extrahepatic tissues was much larger than in the rat in comparison to the hepatic AA formation (L. Zhou and Å Nilsson, unpublished data). Other studies in this laboratory have shown that the AA pool of chylomicrons, chylomicron remnants, and rat plasma lipoproteins equilibrate very slowly with that of the gastrointestinal mucosa except in the upper part of the small intestine that reabsorbs AA from bile phospholipids.[19] Furthermore chylomicron AA esters are relatively resistant to the action of lipoprotein lipase and AA is preferentially incorporated into chyle phospholipids during absorption.[25] Hereby AA is preferentially transported to the liver without being released as FFA or stored in adipose tissue.

On the basis of these findings and the present data we suggest the following hypothesis: Uptake and local interconversion of unesterified plasma LA and secretion of 2-AA-LPC by the liver are two important alternative pathways for the supply of AA to extrahepatic tissues. Their relative importance is expected to vary with genetic factors (carnivore/herbivore status of the species), with the dietary supply of AA, and LA and with other dietary factors affecting the fatty acid composition of the plasma FFA, adipose tissue TG, and liver phospholipids.

## Acknowledgments

The recombinant guinea pig $PLRP_2$ was kindly supplied by dr. Frédéric Carrière, Laboratoire de Lipolyse Enzymatique UPR 9025, Centre National de la Recherche Scientifique, 13402 Marseille cedex 20, France.

This work was supported by grants from the Swedish Medical Research Council (3969), The Albert Påhlsson Foundation, The Anna-Lisa and Sven-Erik Lundgrens Foundation, The Nutrition Foundation of the Swedish Margarin Industry, and The Medical Faculty University of Lund Foundation.

## References

1 Sekas, G., Patton, G.M., Lincoln, E.C., and Robin, S.J. (1985). Origin of plasma lysophosphatidylcholine: evidence for direct hepatic secretion in the rat. *J. Lab. Clin. Med.* **105,** 190–194
2 Graham, A., Zammit, V.V., Christie, W.W., and Brindley, D.N. (1991). Sexual dimorphism in the preferential secretion of unsaturated lysophosphatidylcholine by rat hepatocytes but no secretion by sheep hepatocytes. *Biochim. Biophys. Acta* **1081,** 151–158
3 Nelson, G.J. (1969). The phosphalipid composition of plasma in various mammalian species. *Lipids* **2,** 323–328
4 Norum, K.R. and Gjore, E. (1967). Familial plasma lecithin: Cholesterol acyltransferase deficiency. *Scand. J. Clin. Lab. Invest.* **20,** 231–243
5 Esko, J.D., Matsuoka, K.Y. (1983). Biosynthesis of phosphatidylcholine from serum phospholipids in Chinese hamster ovary cells deprived of choline. *J. Biol. Chem.* **258,** 3051–3057
6 Xu, N. and Nilsson, Å. (1996). Uptake and interconversion of plasma unesterified $^{14}C$ linoleic acid by gastro intestinal tract and blood forming tissues: an experimental study in the rat. *J. Nutr. Biochem.* **7,** 16–22
7 Brindley, D.N. (1993). Hepatic secretion of lysophosphatidylcholine: a novel transport system for polyunsaturated fatty acids and choline. *J. Nutr. Biochem.* **4,** 442–449
8 Jennens, M.L. and Lowe, M.E. (1995). Rat GP-3 is a pancreatic lipase with kinetic properties that differ from colipase- dependent pancreatic lipase. *J. Lipid Res.* **36,** 2374–2382
9 Hjorth, A., Carriere, F., Cudrey, C., Woldike, H., Boel, E., Lawson, D.M., Ferrato, F., Cambillau, C., Dodson, G.G., and Thim, L. (1993). A structural domain (the lid) found in pancreatic lipases is absent in the guinea pig (phospho)lipase. *Biochemistry* **32,** 4702–4707
10 Andersson, L., Carrière, F., Lowe, M.E., Nilsson, Å, and Verger, R. (1996). Pancreatic lipase-related protein 2 but not classical pancreatic lipase hydrolyzes galactolipids. *Biochim. Biophys. Acta.* **1302,** 236–240
11 Thiès, F., Delachambre, M.C., Bentejac, M., Lagarde, M., and Lecerf, J. (1992). Unsaturated fatty acids esterified in 2-acyl-1-Lysophosphatidylcholine bound to albumin are more efficiently taken up by the young rat brain than the uneasterified form. *J. Neurochem.* **59,** 1110–1116
12 Nilsson, Å. and Becker, W. (1988). Uptake and interconversion of plasma unesterified (n-3) polyunsaturated fatty acids by the gastrointestinal tract of the rat. *Am. J. Physiol.* **268,** (Gastrointest. Liver Physiol.), G732–G738
13 Christie, W.W. and Hunter, M. (1984). High-performance liquid chromatography in the analysis of the products of phospholipase A hydrolysis of phosphatidylcholine. *J. Chromatog.* **294,** 489–493
14 Christie, W.W. (1982). A simple procedure for rapid tranmethylation of glycerolipids and cholesteryl esters. *J. Lipid Res.* **23,** 1072–1075
15 Withnall, M.T., Pennington, A., and Wiseman, D. (1995). Characterisation of cytosolic phospholipase $A_2$ as mediator of the enhanced

arachidonic acid release from dimethyl sulphoxide differentiated U937 cells. *Biochem. Pharmacol.* **50**(11), 1893–1902

16 Fonteh, A.N., Bass, D.A., Marshall, L.A., Seeds, M., Samet, J.M., and Chilton, F.H. (1994). Evidence that secretory phospholipase $A_2$ plays a role in arachidonic acid release and eicosanoid biosynthesis by mast cells. *J. Immunol.* **152,** 5438–5446

17 Gustafson-Svard, C., Lilja, I., Sjodahl, R., and Tagesson, C. (1995). Cytosolic phospholipase $A_2$ and cyclooxygenase-2 mediate release and metabolism of arachidonic acid in tumor necrosis factor-alpha-primed cultured intestinal epithelial cells (INT 407). *Scand. J. Gastroenterol.* **30**(10), 1000–1007

18 Ambs, P., Baccarini, M., Fitzke, E., and Dieter, P. (1995). Role of cytosolic phospholipase $A_2$ in arachidonic acid release of rat-liver macrophages: regulation by Ca2+ and phosphorylation. *Biochem. J.* **311,** 189–195

19 Melin, T., Chen, Q., and Nilsson, Å. (1996). Bile but not chyle lipoprotein is an important source of arachidonic acid for the rat small intestine. *Prostaglandins, Leukotrienes Essential Fatty Acids.* **55,** 337–343

20 Salbach, P.B., Specht, E. Hodenberg, E.V., Kossmann, J., Janssen-Timmen, U., Schneider, W.J., Hugger, P., King, W.C., Glomset, J.A., and Habenicht, A.J. (1992). Differential low density lipoprotein receptor-dependent formation of eicosanoids in human blood-derived monocytes. *Proc. Natl. Acad. Sci. USA* **89,** 2439–2443

21 Pomerantz, K.B., Tall, A.R., Feinmark, S.J., and Cannon, P.J. (1984). Stimulation of Vascular smooth muscle cell prostacyclin and prostaglandin $E_2$ synthesis by plasma high and low density lipoprotein. *Circ. Res.* **54,** 554–565

22 Salbach, P.B., Janssen-Timmen, U., Blattner, C., Ziegler, R., and Habeniche, A.J. (1991). A new role for the low density lipoprotein receptor. *Z. Gastroenterol. Verh.* **26,** 107–109

23 Stein, Y. and Stein, O. (1966). Metabolism of labeled lysolecithin, lysophosphatidyl ethanolamine and lecithin in the rat. *Biochim. Biophys. Acta* **116,** 95–107

24 Morash, S.C., Cook, H.W., and Spence, M.W. (1989). Lysophosphatidylcholine as an intermediate in phosphatidylcholine metabolism and glycerophosphocholine synthesis in cultured cells: An evaluation of the 1-acyl and 2-acyl lysophosphatidylcholine. *Biochim. Biophys. Acta* **1004,** 221–229

25 Nilsson, Å., Landin, B., and Schotz, M.C. (1987). Hydrolysis of chylomicron arachidonate and linoleate ester bonds by lipoprotein lipase and hepatic lipase. *J. Lipid Res.* **28,** 510–517