Lysophosphatidylcholine concentrations and metabolism in aortic intima plus inner media: effect of nutritionally induced atherosclerosis

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ABSTRACT The concentration of lysophosphatidylcholine (monoacyl *sn*-glycerol 3-phosphorylcholine) in intima plus inner media of atherosclerotic aorta from squirrel monkeys was nearly eight times that in comparable control tissue. Plasma levels of the same compound were somewhat elevated in the atherosclerotic group.

The metabolism of fatty acyl CoA's and lysophosphatides was studied in cell-free preparations of intima plus inner media from squirrel monkey aorta. Linoleic acid was incorporated predominantly into phosphatidylcholine (as opposed to other phospholipids) when linoleoyl-1-^{14}C CoA was the substrate. The extent of this reaction was dependent on the concentration of lysophosphatidylcholine. Lysophosphatidylethanolamine (monoacyl *sn*-glycerol 3-phosphorylethanolamine) stimulated the incorporation of linoleate into phosphatidylethanolamine. 1-Palmitoyl-1′-^{14}C *sn*-glycerol 3-phosphorylcholine (^{14}C-lysophosphatidylcholine) was incorporated into phosphatidylcholine only in the presence of acyl CoA's or ATP plus CoA. Incorporation of ^{14}C with ^{14}C-lysophosphatidylcholine plus linoleoyl CoA equaled that with linoleoyl-1-^{14}C CoA and lysophosphatidylcholine.

Various other lines of evidence are presented to support the importance of the fatty acyl CoA:lysophosphatide fatty acyl transferase mechanism in aortic phospholipid metabolism. Cell-free preparations of aortic intima plus inner media from squirrel monkeys with early, nutritionally-induced atherosclerosis utilized linoleoyl-1-^{14}C CoA more than preparations from control monkeys when incubations were carried out without added lysophosphatidylcholine and for long periods (30 min). With optimum levels of labeled linoleoyl CoA and unlabeled lysophosphatidylcholine, or unlabeled linoleoyl CoA and labeled lysophosphatidylcholine, there were no differences in substrate utilization between control and atherosclerotic tissues.

We conclude that the concentrations of lysophosphatidylcholine, which are higher in atherosclerotic than in control aortic tissues, could be a factor controlling rates of fatty acid incorporation into phosphatidylcholine.

SUPPLEMENTARY KEY WORDS squirrel monkeys · linoleoyl CoA · palmitoyl CoA · fatty acyl CoA: lysophosphatide fatty acyl transferase

SINCE LANDS (1) demonstrated a transferase mechanism for phosphoglyceride formation from fatty acyl CoA and "lysophosphatides" (monoacyl glycerophosphoryl compounds), evidence of the importance and distribution of this activity has continued to accumulate (e.g., 2–6). Furthermore, the prevalence of phospholipase activity in tissues (e.g., 7) has focused attention on the possible role of phospholipase and transferase mechanisms in the renewal of tissue phospholipid fatty acids. The demonstration by Eisenberg, Stein, and Stein (8) that lysophosphatidylcholine passes rapidly from plasma to tissues suggests that the large quantity of this compound formed in plasma by the phospholipid:cholesterol fatty acyl transferase mechanism (9, 10) could also act as substrate for net synthesis of tissue phospholipids.

The synthesis of phospholipids in aorta has been the subject of several studies (e.g., 11, 12). Stein, Stein, and Shapiro (13) first demonstrated in cell-free preparations of aorta that lysophosphatidylcholine stimulates the incorporation of fatty acids into phospholipids. We showed that endogenous levels of lysophosphatidylcholine could account for the different rates of incorporation of fatty acyl moieties into phosphatidylcholine by normal and atherosclerotic aorta preparations when lysophosphatidylcholine is not added to the incubation medium (14–16). The incorporation of fatty acids into phosphatidylcholine by cell-free preparations of atherosclerotic aorta was higher than in control aortas when assays were carried out without exogenous lysophos-

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phatidylcholine. At the time of these previous studies, however, we were unable to accurately measure low levels of endogenous lysophosphatidylcholine.

The concentrations of phosphatidylcholine in aortic intima plus inner media are increased in nutritionally-induced atherosclerosis of squirrel monkeys (17). It is possible that these increases are also related to the increased rates of incorporation of fatty acids into phosphatidylcholine. The rates of fatty acyl incorporation could, in turn, be controlled by concentrations of lysophosphatidylcholine. In the present study we have showed that lysophosphatidylcholine concentrations in the aorta are increased several-fold and the plasma concentrations are increased somewhat with experimental atherosclerosis. Studies were also carried out with fatty acyl CoA's and lysophosphatides to determine if these increased concentrations of endogenous lysophosphatidylcholine explain increased rates of fatty acid incorporation into aortic phospholipid in atherosclerosis.

MATERIALS AND METHODS

Unlabeled fatty acids were obtained from The Hormel Institute, Austin, Minn. Linoleic acid-1-^{14}C and palmitic acid-1-^{14}C were obtained from the Nuclear-Chicago Corporation. Radiopurity of the fatty acids was investigated by gas–liquid cochromatography of their methyl esters with aliquots of N.I.H. fatty acid standard mixtures and collection of the peaks. More than 98% of the recovered radioactivity was in the correct peak. Labeled and unlabeled palmitoyl CoA and linoleoyl CoA were synthesized by the methods of Seubert (18), modified to minimize the time of synthesis and exposure to conditions likely to alter the products. After the products had been washed three times by centrifugation from cold (0°C) 0.8% perchloric acid, three times with cold acetone, and once with ether, a water-soluble white powder was obtained. We characterized the products by measuring the absorption at 232 mμ and 260 mμ. The ratios of optical densities at these wavelengths ranged from 0.545 to 0.590 [theoretical (18), 0.573]. The specific activities of all preparations on a molar basis were within 10% of those of the precursor fatty acids. Samples of the labeled fatty acyl CoA compounds were hydrolyzed with KOH, extracted with petroleum ether, methylated with diazomethane, and cochromatographed (gas–liquid) with standard mixtures of methyl esters of fatty acids on ethylene glycol succinate. More than 93% of the recovered radioactivities for linoleate and 95% for palmitate were in the correct fractions. No specific labeled contaminants were identified. Fatty acyl CoA preparations in weakly acidified (0.001 M KH_2PO_4) water were stored under nitrogen at −20°C.

Lysophosphatidylcholine and lysophosphatidylethanolamine were obtained from Sigma Chemical Co., St. Louis, Mo. and Pierce Chemical Co., Rockford, Ill., respectively.

^{14}C-Lysophosphatidylcholine

1-Palmitoyl-1′-^{14}C glycerophosphoryl choline (^{14}C-lysophosphatidylcholine) was prepared biosynthetically as follows. A 1000 *g* supernatant fraction of rat liver (equivalent to 0.8 g) was incubated with 50 μc of palmitic acid-1-^{14}C (45 μc/μmole), which had been dispersed in water by ultrasonication. Incubations were for 4 hr at 37°C and contained the following additions for a total volume of 2.4 ml: $MgCl_2$ 24 μmoles; potassium hydrogen phosphate (pH 7.4), 40 μmoles; choline 50 μmoles; ATP 10 μmoles; cytidine triphosphate (CTP) 5 μmoles; CoA 2 μmoles; and glycerol 3-PO_4 20 μmoles. At the end of the incubation period, approximately 25 volumes of chloroform–methanol 2:1 was added to the incubation vessels. The preparations were kept at 37°C for 1 hr, filtered through Whatman No. 41 paper, and washed with water by the procedure of Sperry and Brand (19).

The subnatant chloroform layer was concentrated and applied as a continuous band to two 20 cm × 20 cm thin-layer plates bearing a 400 μ thick coating of specially prepared (20) Silica Gel H. Areas along both sides of each plate were used for reference standards. The plates were then chromatographed in the system described by Skipski, Peterson, and Barclay (21). The locations of individual phospholipids were determined from the positions of the reference compounds, after exposure of only the reference area of the plates to Rhodamine 6 G spray. The zone corresponding to phosphatidylcholine was scraped into a 10 ml graduated pipette that had been closed with a tightly packed plug of chloroform-extracted glass wool. The phospholipid was eluted with 10 ml of methanol followed by 10 ml of methanol–water 9:1. Solvent was removed from the phosphatidylcholine under a stream of nitrogen.

The residue was treated with phospholipase A (*Crotalus adamanteus* venom from the Ross Allen's Reptile Institute, Inc., Silver Springs, Fla.) for 18 hr under conditions similar to those described by Long and Penny (22). The reaction was stopped by the addition of ethanol. The total volume was reduced under a stream of nitrogen and the residue was subjected to thin-layer chromatography (chloroform–methanol–water 62:37:7). The lysophosphatidylcholine was located by comparison with the position of reference standard lysophosphatidylcholine, and eluted from the silica gel with methanol–water 9:1. The specific activities of the three lysophosphatidylcholine preparations used in the experiments were 0.4, 1.0, and 1.2 μc/μmole.

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Preparation of Animals and Tissues

Sexually mature female Brazilian squirrel monkeys (*Saimiri sciureus*) weighing 575–650 g were purchased from the Tarpon Zoo, Tarpon Springs, Fla. The conditions for maintaining these animals, particularly the dietary composition, have been described (17). Semipurified diets that differed in the level and type of fat and in the presence or absence of cholesterol were used; one diet not associated with atheroscolerosis supplied 15% of calories as corn oil. The diet consistently associated with atherosclerosis supplied 45% of calories as butter and 0.1 g of additional cholesterol per 100 kcal.[1] The animals were killed and aortic intima plus inner media was obtained as previously described (17).

Fig. 1 is a low-power photomicrograph of squirrel monkey aortas in which the intima plus inner media has been partially dissected from the outer media and adventitia.[2] One aorta is from the control group and one from the atherosclerotic group. The atherosclerotic aorta had a diffusely roughened intimal surface and the intima plus inner media was stiffer and could be dissected away more easily than that of the corresponding control tissue. In both atherosclerotic and control aortas the media constituted more than 90% of the samples chosen for study. Nevertheless the absolute weight of the intima from the atherosclerotic aorta was probably greater than that from the control aorta. Thus differences in metabolic activity of aortic preparations from the two groups of animals could reflect differences in the quantity of intima if the intima were unusually active for the reaction studied.

Atherosclerosis in these squirrel monkeys is characterized by diffuse intimal and inner medial lipid (intra- and extracellular) and increased numbers of smooth muscle cells in the intima. The total cholesterol concentration of aortic intima plus inner media, which has been shown to be a good index of aortic sudanophilia (17), was used to estimate the severity of atherosclerosis.

50–80 mg of tissue (wet weight) was obtained. This tissue was homogenized in 0.25 M sucrose with a glass–glass Potter–Elvehjem homogenizer and brought to exactly 3 ml. 25 μl was taken for protein determination (23); 1.5 ml was added to 50 ml (final volume) of chloroform–methanol 2:1. The rest of the tissue homogenate was centrifuged at 1000 *g* for 10 min and the supernatant fraction (about 1 ml) was used for the metabolic studies described here. Protein determinations (23) were also carried out on these cell-free preparations.

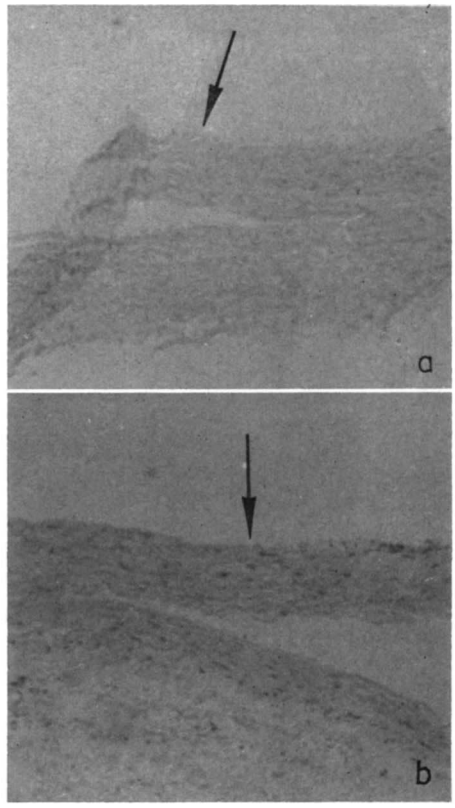

FIG. 1. Normal (*a*) and atherosclerotic (*b*) squirrel monkey aortas. The intima plus inner media (arrows) has been partially dissected free from the underlying outer media and adventitia. The quantity of intima plus inner media obtained from the atherosclerotic aortas was usually 25–30% greater than that obtained from "normal" aortas. This reflects primarily a difference in the depth of planes of dissection in the media. Magnification ×300.

Study of In Vitro Metabolism of Fatty Acyl CoA's and Lysophosphatides

Aorta cell-free preparations in total volumes of 0.5 ml were incubated at 37°C in an atmosphere of air on a Dubnoff metabolic incubator. Each tube contained about 0.2 ml (depending on protein concentration) of the cell-free preparation, 0.1 ml of Ringer's phosphate buffer including $MgCl_2$, 0.03 ml of additional 0.2 M K–PO_4 buffer (pH 7.4), and various amounts of other substrates and supplements. Reactions were terminated by the addition of 12 ml of chloroform–methanol 2:1 plus, in some instances, carrier lipid. The methods for processing these samples and for subsequent assays of

[1] The composition of the control diet was "vitamin-free" casein 25%; sucrose 61%; corn oil 8%; salt mixture (33) 4%; and vitamin mixture in dextrose 2%. Composition of the atherogenic diet was vitamin-free casein 25%; sucrose 43%; butter 25.5%; cholesterol 0.5%; salt mixture 4%; and vitamin mixture in dextrose 2%. The vitamin supplement per kg of diet included vitamin A acetate 12,500 units, α-tocopherol 0.1 g, crystalline vitamin D_3 100 μg (4000 units), menadione 0.04 g, ascorbic acid 0.5 g, inositol 1.0 g, choline chloride 5.0 g, niacin 0.049 g, riboflavin 0.01 g, thiamine 0.01 g, pyridoxine 0.01 g, calcium pantothenate 0.03 g, biotin 0.2 mg, folic acid 1.0 mg, and vitamin B_{12} 0.02 mg.

[2] We are most grateful to Dr. Alexsander Knezevich for various histological preparations to verify our tissue sampling methods.

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radioactivity in substrates and reaction products have been previously described (14).

Concentration of Aortic Lipids

The concentrations of individual phospholipids and of cholesterol and cholesteryl esters in aorta were determined by thin-layer chromatography on specially washed Silica Gel H (20), followed by controlled charring of the plates. The solvent system used for separation of lysophosphatidylcholine from the other phospholipids was chloroform–methanol–water 62:37:7. This combination of solvents was the only one that allowed us to obtain a good separation of lysophosphatidylcholine from sphingomyelin in a one-dimensional chromatograph. Because of the low concentrations of lysophosphatidylcholine, it was necessary to overload the plates to obtain good densitometric (see below) measurements. 120 μg of total phospholipid was applied across a 0.6 mm path on plates in which the silica gel was about 350 μ thick. For determination of the other phospholipids we used 50 μg of total phospholipid and the solvent system of Skipski et al. (21). The solvent system of Belfrage, Elovson, and Olivecrona (24) was used to separate the major lipid classes, including cholesterol and cholesteryl esters. After being freed of solvent, the plates were placed in a thin-layer tank containing 100 ml of a solution of NH_4HSO_4 (25), which was allowed to travel to near the top of the plates. The plates were then heated at exactly 225°C for 30 min. Densitometric measurements were made from a continuous scan of thin-layer plates essentially according to Privett, Blank, Codding, and Nickell (26) and compared with densitometric scans of graded levels of standards. Measurements on the standard mixtures gave closely similar results to direct determination by previously described methods (20) based on a combination of thin-layer and gas–liquid chromatography. The minimum quantity of lysophosphatidylcholine that could be detected, with a difference between duplicates of <10%, was 1.0 μg. Plates with 15 lanes, 1.2 cm wide, marked in the silica gel were used for all quantitative thin-layer chromatography.

RESULTS

Aortic Concentrations of Endogenous Lysophosphatidylcholine

Mean concentrations of lysophosphatidylcholine (and of cholesterol) in the total homogenates of aortic intima plus inner media and in plasma are shown in Table 1. The atherosclerotic aortas had much higher concentrations of lysophosphatidylcholine than the normal aortas. Lysophosphatidylcholine as a percentage of total phospholipid in aorta was also markedly elevated. Protein concentrations of aorta were the same for the two groups (results not shown). The absolute concentrations of lysophosphatidylcholine in plasma were somewhat increased in the atherosclerosis group, but the percentage of it in total phospholipid was decreased. In a few instances the concentrations of lysophosphatidylcholine were also measured in cell-free aorta preparations. The mean lysophosphatidylcholine concentration of two cell-free preparations of atherosclerotic aortas was 11.2 μg/mg protein compared to 2.2 μg/mg protein for controls.

Incubation of Cell-Free Aortic Preparations with Linoleoyl and Palmitoyl CoA

We studied the metabolism of fatty acyl CoA's and lysophosphatidylcholine in cell-free preparations of normal and atherosclerotic aortas in order to evaluate the significance of the different levels of lysophosphatidylcholine. We first compared linoleoyl-1-^{14}C CoA and palmitoyl-1-^{14}C CoA as substrates for enzymes of normal aorta. The percentage distributions of the added radioactivities in the presence and absence of unlabeled lysophosphatidylcholine are shown in Table 2. Two thin-layer chromatographic separations were used. Phosphatidylcholine was the major product from linoleoyl-1-^{14}C CoA. The addition of lysophosphatidylcholine caused a marked increase of radioactivity in the phosphatidylcholine peak regardless of the fatty acyl CoA substrate used. A component with high levels of radioactivity, particularly after addition of palmitoyl-1-^{14}C CoA, was tentatively identified as ceramide by com-

TABLE 1 Levels of Lysophosphatidylcholine and Total Cholesterol in the Complete Homogenate of Aortic Intima Plus Inner Media and in Plasma from Squirrel Monkeys With and Without Nutritionally Induced Atherosclerosis

Diet Group	Aorta			Plasma		
	Lysophosphatidylcholine		Cholesterol	Lysophosphatidylcholine		Cholesterol
	*mg/g**	*% total phospholipid*	*mg/g*	*mg/ml*	*% total phospholipid*	*mg/g*
Basal	0.066 ± 0.01	0.81	3.1 ± 0.3	0.098 ± 0.008	6.3	1.6 ± 0.1
Atherosclerotic	0.51 ± 0.10	4.2	9.2 ± 1.2	0.132 ± 0.010	4.5	3.3 ± 0.2

Values are means ± SEM (n = 9–11).
* Wet weight.

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TABLE 2 INCORPORATION OF RADIOACTIVITY (% OF RADIOACTIVITY ADDED) IN DIFFERENT LIPID FRACTIONS AFTER 30-MIN INCUBATION OF AORTIC CELL-FREE PREPARATIONS WITH RADIO-LABELED LINOLEOYL CoA OR PALMITOYL CoA (150 μM)

Product	Substrate: Linoleoyl-1-^{14}C CoA, With LPC	Linoleoyl-1-^{14}C CoA, Without LPC	Palmitoyl-1-^{14}C CoA, With LPC	Palmitoyl-1-^{14}C CoA, Without LPC
	% of radioactivity added			
Phosphatidylcholine	19.5	1.5	1.36	0.39
Phosphatidylethanolamine	0.52	0.60	0.49	0.35
Sphingomyelin	0.31	0.30	0.37	0.44
Ceramide	2.80	2.67	5.63	6.13
Triglyceride + cholesterol ester	0.03	0.04	0.13	0.15
Free fatty acid	36.5	43.2	32.9	33.1

Most of the radioactivity not recovered in these lipid fractions was found in a water-soluble fraction (19) and was thus unreacted acyl CoA. Incubations were carried out with and without added lysophosphatidylcholine (LPC) (20 μM). Values are the means of six incubations for each combination of substrates. Six aortas were used and each aorta provided tissue for four incubations.

parison with the R_f values of authentic ceramides in thin-layer chromatographs.

Total fatty acids from lipid extracts of similar cell-free homogenates of aorta that had been incubated with linoleoyl-1-^{14}C CoA or palmitoyl-1-^{14}C CoA were methylated and separated by gas–liquid chromatography. There was no significant conversion of the labeled fatty acyl moieties to other fatty acids.

Substrate–Product Curves for Fatty Acyl CoA's in the Formation of Phosphatidylcholine

The graph (Fig. 2) relating the concentrations of palmitoyl-1-^{14}C CoA used to the amount of labeled phosphatidylcholine formed agrees with studies on other tissues in showing that high concentrations of palmitoyl CoA were inhibitory (27, 28). On the other hand, the relationship of linoleoyl CoA concentrations to phosphatidylcholine formed was nearly linear throughout the range tested. Fig. 2 also shows the inhibitory effects of different concentrations of unlabeled palmitoyl CoA on the incorporation of radioactivity into phosphatidylcholine from a fixed concentration (120 μM) of linoleoyl-1-^{14}C CoA. Fig. 3 shows the relationships of the reciprocals of linoleoyl-1-^{14}C concentrations and enzyme activities when unlabeled palmitoyl CoA was absent or present at one of two concentrations. On the basis of the very different ordinates for the three lines when $1/S = 0$, palmitoyl CoA appeared to noncompetitively inhibit the formation of labeled phosphatidylcholine from linoleoyl-1-^{14}C CoA.

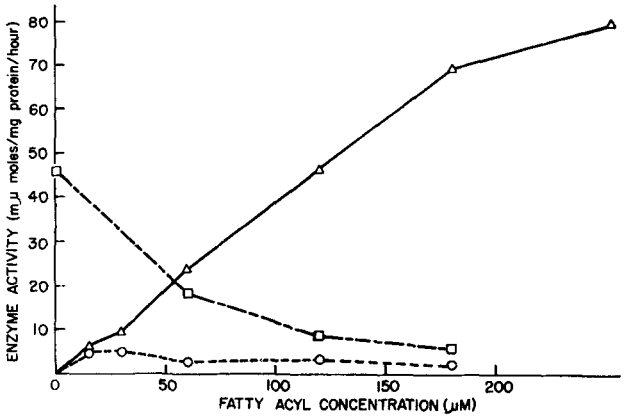

FIG. 2. Relationship of concentration of fatty acyl-1-^{14}C CoA to activity of cell-free preparations of aortic intima plus inner media in forming ^{14}C-phosphatidylcholine. Lysophosphatidylcholine was present at 40 μM. Incubation time, 30 min; protein, 0.2–0.25 mg in 0.5 ml. △—△, linoleoyl-1-^{14}C CoA; ○- - -○, palmitoyl-1-^{14}C CoA; □- - -□, 120 μM linoleoyl-1-^{14}C CoA plus concentrations of unlabeled palmitoyl CoA indicated on abscissa.

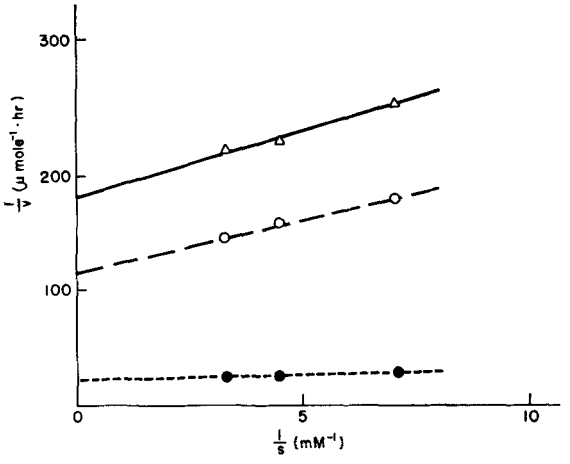

FIG. 3. Relationship of the reciprocals of linoleoyl-1-^{14}C CoA concentration and activity of cell-free preparations of aortic intima plus inner media in forming ^{14}C-phosphatidylcholine. ●, No added palmitoyl CoA; ○, 140 μM palmitoyl CoA (unlabeled); △, 300 μM palmitoyl CoA.

The preferential incorporation of unsaturated fatty acyl moieties into phosphatidylcholine is consistent with observations on other tissues (4, 5) and aorta (14).

Relative Reaction Rates with Different Substrates

A comparison of rates of incorporation of linoleic acid-1-^{14}C, linoleoyl-1-^{14}C CoA, and ^{14}C-lysophosphatidylcholine, in the presence of other substrates and supplements, into the glycerophosphatides should help to establish the major pathways of incorporation of fatty acids by aortic tissues. The results in Table 3 show that the amount of labeled phosphatidylcholine synthesized by cell-free preparations of aorta from linoleoyl-1-^{14}C CoA and unlabeled lysophosphatidylcholine (line 2) was

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TABLE 3 ACTIVITIES OF AORTIC CELL-FREE PREPARATIONS IN FORMING PHOSPHATIDYLCHOLINE (PC) AND PHOSPHATIDYLETHANOLAMINE (PE) FROM VARIOUS LABELED SUBSTRATES

Number	Substrates						Activities	
	LPC (40 μM)	LPE (40 μM)	Linoleic Acid (110 μM)	Linoleoyl CoA (150 μM)	CoA (3 mM)	ATP (20 mM)	PC	PE
							mμmoles/mg protein/hr	
1	—	—	—	*	—	—	5.16 ± 0.6	2.50 ± 0.2
2	+	—	—	*	—	—	54.8 ± 4.5	1.89 ± 0.1
3	—	+	—	*	—	—	3.63 ± 0.2	17.5 ± 1.7
4	+	—	—	*	+	+	52.9 ± 6.4	2.12 ± 0.2
5	—	—	*	—	—	—	0.03 ± 0.01	0.08 ± 0.02
6	—	—	*	—	+	+	1.57 ± 0.2	0.40 ± 0.05
7	+	—	*	—	+	+	5.27 ± 0.4	0.37 ± 0.02
8	*	—	—	—	—	—	1.27 ± 0.1	<0.1
9	*	—	—	+	—	—	53.5 ± 6.7	<0.1
10	*	—	+	—	+	+	27.6 ± 2.1	<0.1
11	*	—	—	—	+	+	24.1 ± 2.3	<0.1
12	*	—	—	—	+	—	2.72 ± 0.4	<0.1
13	*	—	—	—	—	+	3.10 ± 0.4	<0.1
14	*	—	—	+	+	+	54.7 ± 6.8	<0.1

The substrates for a given compound which contain a ^{14}C label are indicated by an asterisk (*); the presence of unlabeled substrates are indicated by a plus (+). Incubation times = 30 min. Approximately 0.25 mg protein in a total volume of 0.5 ml was used. Each value is the mean of six or more determinations.

equal to the amount formed from ^{14}C-lysophosphatidylcholine and unlabeled linoleoyl CoA (line 9). The rates of incorporation of either substrate into phosphatidylcholine when the second substrate was not present were very low (lines 1 and 8). ATP and CoA together could partially replace the requirement for linoleoyl CoA for incorporation of labeled lysophosphatidylcholine into phosphatidylcholine (lines 8, 10, and 11). Lysophosphatidylcholine had little effect on the rate of formation of phosphatidylethanolamine from linoleoyl CoA (lines 1 and 2). On the other hand, the addition of lysophosphatidylethanolamine markedly stimulated the formation of phosphatidylethanolamine but had little effect on the rate of incorporation of linoleoyl groups into phosphatidylcholine (lines 1 and 3). CTP (0.01 M) and glycerol 3-phosphate (0.0066 M) had no stimulatory effect on the formation of glycerophosphatides from linoleoyl CoA, although this system was not studied with a range of substrate concentrations (results not shown). Furthermore, both linoleic and palmitic acids were incorporated almost exclusively into the 2-position of phosphatidylcholine. These observations suggest that the cell-free preparations of aortic intima plus inner media incorporated substrate into phospholipids predominantly by the fatty acyl CoA:lysophospholipid fatty acyl transferase mechanism described by Lands (1). The extremely low activity for incorporation of lysophosphatidylcholine into phosphatidylcholine when fatty acyl CoA and ATP + CoA were omitted (line 8) tends to minimize the possible importance (in this preparation and under these conditions) of the mechanism of Erbland and Marinetti (29), whereby 2 moles of lysophosphatidylcholine yield 1 mole of phosphatidylcholine and 1 mole of glycerol 3-phosphorylcholine.

Free linoleic acid with ATP and CoA was incorporated into phospholipids with considerably less efficiency than was linoleic acid in thiol linkage with CoA (compare lines 7 and 2). On the other hand, lysophosphatidylcholine was efficiently incorporated into phosphatidylcholine by cell-free preparations of aortic intima plus inner media when ATP and CoA were added, regardless of whether exogenous linoleic acid was present (lines 10 and 11). This suggests that the inefficient incorporation of linoleic acid-1-^{14}C into phospholipid can be attributed to an unfavorable physical state of the substrate (micelles), or to its chemical state (free rather than an appropriate ester), or to failure of linoleic acid-1-^{14}C to mix with the pool of fatty acids available to the fatty acyl activation and transferase enzymes.

Effects of Atherosclerosis on Aortic Metabolism of Linoleoyl CoA and Phosphatidylcholine

The activities for incorporating linoleate (from linoleoyl CoA) and lysophosphatidylcholine into phosphatidylcholine were measured in cell-free preparations of aortic intima plus inner media from squirrel monkeys that had been fed different diets. The values shown in Table 4 were determined from animals killed after 3–6 months on the indicated diets. Fifteen animals were used from each group and duplicate determinations carried out on each. One animal from each diet group was used on a given day. In order to make intergroup comparisons and compensate for day-to-day variations in the enzyme methods, we expressed the values as a proportion of the

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TABLE 4 EFFECT OF NUTRITIONALLY INDUCED ATHEROSCLEROSIS ON ACTIVITY OF AORTIC CELL-FREE PREPARATIONS IN CONVERTING LINOLEOYL-1-^{14}C CoA (150 μM) OR LYSOPHOSPHATIDYLCHOLINE (40 μM) TO PHOSPHATIDYLCHOLINE

Group	Linoleoyl-1-^{14}C CoA		Linoleoyl-1-^{14}C CoA Plus LPC		LPC-^{14}C Plus Linoleoyl CoA
	6 min	30 min	6 min	30 min	6 min
			mμmoles/mg protein/hr		
Basal	14.3 ± 1.58*	3.6 ± 0.84	101.8 ± 18.3	36.4 ± 4.7	98.0 ± 13.9
Atherosclerotic	17.1 ± 1.83	10.7 ± 2.2	106.8 ± 15.7	46.0 ± 6.8	85.6 ± 15.1

Incubations were for 6 or 30 min. LPC = lysophosphatidylcholine from Sigma Chemical Co.; LPC-^{14}C = synthetic 1-palmitoyl-1′-^{14}C glycerophosphoryl choline.
* Means ± SEM (n = 16–30).

absolute activity observed for the animals fed the basal diet. The normalized absolute values listed were obtained by multiplying these proportions by the mean value for all of the measurements on the basal group preparations. The group with grossly detectable atherosclerosis had a higher mean activity in formation of phosphatidylcholine from linoleoyl CoA than the other group. This was only true of the measurements made without exogenous lysophosphatidylcholine. The group differences were also more apparent after 30 min measurements than after 6 min. These times were selected for measurement of the radioactive products because reaction rates were always linear until 6 min even without added lysophosphatidylcholine, and the 30 min measurement accentuated differences in activity due to substrate limitations. These results can thus be best explained by intergroup differences in endogenous lysophosphatidylcholine concentrations. The level of fatty acyl CoA:lysophosphatide fatty acyl transferase did not seem to be elevated by atherosclerosis.

DISCUSSION

The limiting factors in synthesis of aortic glycerophosphatides seem to be the concentration of monoacylated (lyso-) forms and the level of ATP and CoA for formation of activated fatty acids. The ATP and CoA present in the cell-free preparations of aorta seemed to be low compared to the endogenous fatty acids available to the fatty acyl CoA synthetase. This study does not cast any light on the rate of supply of ATP and CoA to the fatty acyl activating system. With added ATP and CoA (with or without exogenous fatty acid) or fatty acyl CoA, the synthesis of phosphatidylcholine or phosphatidylethanolamine depends on the concentration of the corresponding lyso forms (endogenous + added).

The cell-free system of aorta incorporated fatty acids predominantly into the 2-position even when lysophosphatidylcholine was not added to the system. Under identical incubation conditions, cell-free preparations of liver incorporated palmitate into the 1- and 2-positions to about the same extent. The positional specificity of the aortic enzyme, together with the dependence of its activity on the concentration of lysophosphatidylcholine, shows that the rate of de novo synthesis (30) of phospholipids is probably low compared to that for incorporation of fatty acids by the transferase mechanism (1). Nevertheless, the in vitro incorporation of phosphate-^{32}P into aorta phospholipids (31) and the stimulation of phospholipid synthesis in cell-free preparations of aorta by glycerol 3-phosphate (8) suggest that de novo synthesis via Kornberg–Pricer (32) and Kennedy (30) pathways occurs.

We have confirmed (results not shown) the observations of Eisenberg et al. (8) that lysophosphatidylcholine is rapidly removed from plasma by arteries in vitro. Thus, it is probable that aorta phospholipid is synthesized partly from plasma-derived lysophosphatidylcholine, which may be largely a product of plasma phospholipid:cholesterol fatty acyl transferase activity (9).

Ultimately, the stimulus for increased phosphatidylcholine synthesis and concentrations in atherosclerotic squirrel monkey aortas (17) may be higher lysophosphatidylcholine concentrations. There are several possible explanations of the increases in aortic lysophosphatidylcholine with atherosclerosis: (*a*) increased synthesis by tissue lecithinase (7); (*b*) decreased utilization by lysolecithinase (7) or fatty acyl CoA: lysophosphatide fatty acyl transferase (1) enzymes; and (*c*) increased plasma concentrations and (or) aortic uptake from plasma lysophosphatidylcholine. We have investigated some of these possibilities. We observed no differences in the hydrolysis of phosphatidylcholine (16) or lysophosphatidylcholine by cell-free preparation of atherosclerotic and of control tissues. On the other hand, we have shown in the present study that plasma lysophosphatidylcholine concentrations were higher in the atherosclerotic than in the control group. Plasma phospholipid:cholesterol fatty acyl transferase activity was also higher in the atherosclerotic group (16). We have also shown that the uptake of lysophosphatidylcholine by everted aortas is proportional to the concentration in the incubation medium.

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We conclude that the high concentration of aortic lysophosphatidylcholine in atherosclerotic squirrel monkeys may be a controlling factor of the rate of incorporation of fatty acids into aortic phosphatidylcholine by the Lands mechanism (1). The high concentrations of lysophosphatidylcholine in atherosclerotic aorta may result from increased concentrations of lysophosphatidylcholine in plasma. If there is a larger influx of lysophosphatidylcholine from plasma to aorta in the atherosclerotic than in the control group, a greater net synthesis of aortic phosphatidylcholine could result.

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