Metabolism of lysolecithin in vivo: effects of hyperlipemia and atherosclerosis in squirrel monkeys

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ABSTRACT We have studied the effect of long-term hyperlipemia and atherosclerosis in squirrel monkeys on the metabolism of lysolecithin-^{14}C (1-palmitoyl-1′-^{14}C *sn*-glycerol 3-phosphorylcholine) in order to explain elevated plasma and arterial concentrations of lysolecithin. The die-away curves of lysolecithin-^{14}C from plasma and the timing of appearances of other ^{14}C-labeled moieties in plasma and other tissues demonstrated a complex pattern of metabolic reactions. There was a rapid equilibration of specific activities of lysolecithin of plasma, liver, and aortic intima plus inner media. The specific activities of lecithin peaked first in liver, then in plasma, and rose slowly in aortic intima plus inner media. The appearance of lecithin-^{14}C in heart and skeletal muscle was also slower than in the liver and some other tissues. Triglycerides, and to a lesser extent, cholesteryl esters contained radioactivity. The concentrations of aortic lysolecithin in the atherosclerotic aortas were several times greater than comparable values for control aortas, and the time of equilibration of plasma and aorta lysolecithin-^{14}C was much greater for the atherosclerotic group. The quantities of lysolecithin in plasma and in the pool of which the plasma was a part, were increased with hyperlipemia and atherosclerosis, as was the rate of lysolecithin production in the fast pool. Hyperlipemia was also associated with an early increase in plasma lecithin:cholesterol acyltransferase (LCAT) activity in vitro. Furthermore, nutritional hyperlipemia influenced the distribution of lysolecithin-^{14}C and lecithin-^{14}C between different plasma lipoproteins. The increase in concentrations of lysolecithin in the aorta occurred more slowly than that in plasma after we had induced hyperlipemia in the monkeys.

SUPPLEMENTARY KEY WORDS aortic intima plus inner media · lecithin:cholesterol acyltransferase (LCAT) · lecithin · thin-layer chromatography · turnover

Concentrations of lysolecithin in the aortic intima plus inner media are greatly increased with nutritionally induced atherosclerosis in squirrel monkeys (1, 2) and rabbits (3). This elevation may be related to increased concentrations of lecithin when atherosclerosis develops in several species of experimental animals (e.g., 4–7) and in man (e.g., 8–10). The rate of incorporation of different precursors of lecithin, including fatty acids, by arterial tissue in vitro and in vivo is also elevated (e.g., 11–14). The fatty acyl CoA:lysolecithin fatty acyltransferase pathway (15) is active in arterial tissue (16), and we (2, 17) have shown in vitro that endogenous levels of lysolecithin partly determine the level of fatty acyl incorporation into the lecithin of the arterial wall.

There are at least two possible sources of the lysolecithin found in arterial and other tissues. One is the tissue itself where lysolecithin may be found by the action of phospholipase on lecithin. Phospholipase A is widely distributed and exists in several forms, as evidenced by different subcellular locations, pH optima, and fatty acyl specificities (18–20). To what degree nutritionally induced atherosclerosis affects the level of phospholipase A activity in the arterial wall is debatable (3, 21). Like Eisenberg, Stein, and Stein (3), we found that atherosclerosis did not greatly influence the levels of lysolecithin formation nor its hydrolysis by arterial homogenates (22, 23). Moreover, the phospholipase activity present in disrupted tissue with Ca^{++} added may be largely latent in intact healthy tissue.

Abbreviations: LCAT, lecithin:cholesterol acyltransferase.

Another source of arterial lysolecithin is the plasma where the lecithin:cholesterol acyltransferase (LCAT) enzyme (24, 25) catalyzes lysolecithin formation. Lysolecithin seems to be reduced in the plasma to one-third or one-fourth of normal when LCAT is not active (26). We have shown that the concentrations of lysolecithin and LCAT activity (22) are higher in the plasma of squirrel monkeys with nutritionally induced atherosclerosis than in plasma of normal monkeys. Stein and Stein (27) have shown in vivo that lysolecithin is rapidly removed from the plasma of rats and taken up by various tissues where it is esterified or hydrolyzed. Eisenberg, Stein, and Stein (28) have demonstrated in vitro the uptake, esterification, and hydrolysis of lysolecithin by arteries.

In the experiments described here, we measured in vivo the rates of lysolecithin metabolism in the plasma of squirrel monkeys with and without atherosclerosis as well as the levels of LCAT activity in vitro. We also compared lysolecithin metabolism in vivo in other tissues of the same animals with particular emphasis on the lysolecithin metabolism in the aortic intima plus inner media which is profoundly changed by atherosclerosis.

METHODS

Preparation of Lysolecithin-^{14}C

We prepared 1-palmitoyl-1′-^{14}C glycerophosphorylcholine (lysolecithin-^{14}C) as follows. Five flasks, each containing a 1000 *g* supernatant fraction from 0.8 g of rat liver, were incubated with 100 μCi of palmitic acid-1-^{14}C, 5 μmoles CTP, 10 μmoles ATP, 50 μmoles choline, 24 μmoles $MgCl_2$, 40 μmoles potassium hydrogen phosphate (pH 7.4), 2 μmoles CoA, and 20 μmoles glycerol-3-PO_4 in a total volume of 2.2 ml. The lecithin was isolated and treated with phospholipase A_2 (*Crotalus adamanteus* venom from Ross Allen's Reptile Institute, Inc., Silver Springs, Fla.); the lecithin was then isolated by thin-layer chromatography (2, 29). The specific activities of the lysolecithin preparations (each beginning with a total of 500 μCi at about 50 μCi/μmole) were 1.92, 1.56, and 0.96 μCi/μmole. At least 99% of the radioactivity chromatographed with lysolecithin.

Dietary Treatment of Squirrel Monkeys

In several previous publications (2, 7, 30, 31) we have described the semipurified diets used to induce atherosclerosis in squirrel monkeys (*Saimiri sciureus*). 62 female squirrel monkeys (525–727 g) were used in these studies. The control group of monkeys, whose diet supplied 15% of calories as corn oil, had a very low incidence of atherosclerotic lesions. The second group was fed a diet in which 45% of the calories was supplied as butter plus crystalline cholesterol (0.1 g/100 kcal). We maintained most animals on these diets for 8–12 months, except for one experiment which lasted only 2 months. In this latter experiment, the animals had hypercholesterolemia but a low incidence of gross atherosclerotic lesions.

Injection of Lysolecithin-^{14}C and Preparation of Tissue Samples

To prepare the test material for each experiment, we dried samples of lysolecithin-^{14}C (which had been stored in CH_3OH at −20°C) under nitrogen, added 2.1 ml of Krebs–Ringer phosphate buffer (pH 7.4), and subjected the micellar solution, which formed spontaneously, to ultrasound to insure complete solution. 1 ml of the lysolecithin preparation was then mixed with 1 ml of control plasma for injection into each control monkey. A similar preparation of lysolecithin-^{14}C and hypercholesterolemic plasma was used with each atherosclerotic monkey. Each monkey received 1.6 μCi of ^{14}C.

Femoral catheters (PV 3 tubing), two venous and one arterial, were implanted under local procaine anesthesia and maintained full with 0.9% saline in restrained unanesthetized monkeys. Lysolecithin samples, together with a wash-in aliquot of saline, were injected simultaneously into two monkeys between time zero minus 30 sec and time zero. Blood samples were withdrawn from the contralateral femoral vein and sometimes from the femoral artery catheter between time *t* and time *t* plus 10 sec. The withdrawal times were usually 2, 10, 20, and 30 min, and 1, 2, 3, and 5 hr. Continuous fluid and electrolyte replacement infusions were maintained through the catheters used for withdrawals; these infusions were also useful in keeping the catheters patent without anticoagulants. The catheters were removed within 5 hr after lysolecithin injection from animals that were not killed, and the animals were caged. The blood samples were added to heparin, and the tubes were placed in ice for 2 min. The plasma was separated in a refrigerated centrifuge (2000 *g* for 10 min), and aliquots of the plasma were added to 8 volumes of methanol and 16 volumes of chloroform. No new lysolecithin was formed in the plasma by the lecithin:cholesterol acyltransferase (LCAT) activity.

After the injection of 0.3 mg of phencyclidine per kg of body weight, the peritoneal and thoracic cavities were opened, and the animals were exsanguinated from the iliac bifurcation of the aorta. Small aliquots of selected organs were rapidly weighed for analysis and stored in liquid nitrogen (for 2 hr or less) until they could be homogenized in chloroform–methanol 2:1. The aortas were opened *in situ* with a ventral midline incision, flushed with iced 0.9% saline, and transferred

to iced saline. With a dissecting microscope, a layer of intima plus inner media (25–30 μ thick) was stripped from the inner surface of the saline-moistened artery, which was in a Petri dish resting on crushed ice. We have already described our methods for preparing lipid extracts of tissues for radioassay and chemical determination of different components (2, 29, 32).

Radioactivity and Chemical Determinations

We have also described our methods for assaying radioactivity (2, 29) and for measuring of small quantities of different lipids including lysolecithin. These methods are based on thin-layer chromatography; the determinations are based mainly on a charring technique (2, 29). One modification of the assays (2, 29) during the latter part of this study was the use of the Farrand VIS-UV thin-layer spectrophotometer, which is much more sensitive than the instrument previously used. The thin-layer chromatographic systems used were those of Belfrage, Elovson, and Olivecrona (33), and Skipski, Peterson, and Barclay (34). For lysolecithin determinations, the plates were overloaded with total lipid and developed in chloroform–methanol–water 62:38:7.

LCAT activity in plasma (rapidly harvested at 2°C) of monkeys not injected with lysolecithin-^{14}C was determined by the method of Portman and Sugano (35). Cholesterol-^{14}C solubilized in heat-inactivated (55°C for 30 min) plasma was used as a substrate. LCAT activity measured in this way is similar to that measured by the formation of lysolecithin in plasma. For determination of LCAT activity from lysolecithin formation, one aliquot of plasma was added directly to chloroform–methanol 2:1, and a second was heated at 55°C for 30 min and then added to chloroform–methanol. A third aliquot, which had been heated at 55°C for 30 min, and a fourth, which had not been heated, were incubated at 37° C for 3 hr before chloroform–methanol was added. The lysolecithin concentrations of the first three aliquots of plasma, which were determined by previously described procedures (2, 29), were equal, that of the fourth aliquot was increased by an amount about equal on a molar basis to the amount of cholesterol esterified as determined by the method of Portman and Sugano (35).

RESULTS

Sequence of Appearance of Lysolecithin-^{14}C and Products in Plasma and Tissues

Lysolecithin-^{14}C, which had been complexed with plasma proteins and injected intravenously into squirrel monkeys, rapidly disappeared from plasma and appeared in other tissues. Fig. 1 shows the mean specific

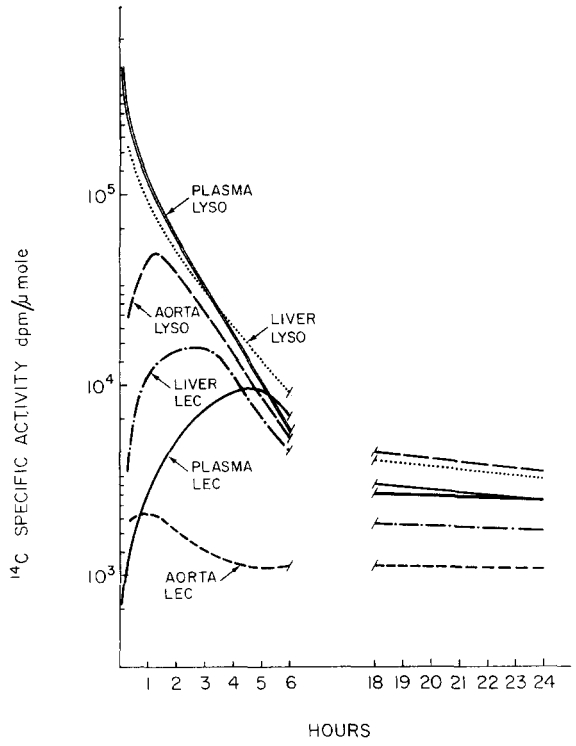


FIG. 1. The specific activities of lecithin and lysolecithin in plasma, liver, and aorta as a function of time after the intravenous administration of lysolecithin-^{14}C to squirrel monkeys which had been maintained for 8–12 months on a semipurified diet selected for the maintenance of normal plasma lipid values. *Lyso*, lysolecithin; *lec*, lecithin.

activities of lysolecithin and lecithin in the plasma, liver, and aorta of 12 monkeys on control, semipurified diets (containing corn oil) for 8–12 months before the injection of lysolecithin-^{14}C. Plasma values are based on determinations of 70 samples; tissue values are based on 12 terminal samples. There was a very rapid drop in plasma lysolecithin-^{14}C specific activity after injection of lysolecithin-^{14}C, and the specific activities of plasma, liver, aorta, and of other tissue lysolecithin were similar by 2 hr after the injection of lysolecithin. However, there may not have been free equilibration between the three compartments, since the specific activities for liver and aorta were higher than those for plasma by 18 hr after the injection of lysolecithin-^{14}C. This pattern of labeling could indicate that lysolecithin enters a compartment that does not freely equilibrate with the total plasma compartment.

The sequence of specific activity peaks shows that liver lysolecithin is a precursor of liver lecithin, which in turn is a precursor of plasma lecithin. The failure of the specific activity curves of the precursor to intersect the precise peaks of the curves of the product (36) is probably due to a low turnover rate of part of the liver lecithin. Zilversmit and Van Handel (37) and Stein

TABLE 1 THE DISTRIBUTION OF ^{14}C IN LIVER LIPIDS AFTER THE INTRAVENOUS ADMINISTRATION OF LYSOLECITHIN-^{14}C INTO SQUIRREL MONKEYS

Diet Group	No. of Monkeys	Time	LL*	Lec	PE	MG	1,2DG	1,3DG	FFA	TG	CE
Control†	1	20 min	27.7	42.4	1.0	0.1	0.6	0.3	1.5	26.0	0.3
	3	3 hr	4.0	57.9	1.4	0.1	0.8	0.4	0.5	35.1	0.9
	3	18–24 hr	0.6	42.5	2.0	0.5	0.9	1.7	1.7	48.5	1.4
Atherosclerotic‡	1	20 min	13.6	51.5	1.2	0.1	0.8	0.1	0.8	31.4	0.7
	3	3 hr	3.8	60.0	1.2	0.2	0.8	0.2	2.4	32.0	0.3
	3	18–24 hr	0.7	18.2	2.4	0.2	0.5	0.5	0.8	72.5	4.0

* LL, lysolecithin; Lec, lecithin; PE, phosphatidylethanolamine; MG, monoglyceride; 1,2DG, 1,2-diglyceride; 1,3DG, 1,3-diglyceride; FFA, free fatty acid; TG, triglyceride; CE, cholesteryl esters.
† Fed a diet containing 15% of the calories as corn oil. See text for details.
‡ Fed a diet containing 45% of the calories as butter and cholesterol at a level of 0.1 g/100 kcal. See text for details.

and Stein (38) have shown that biliary lecithin has a higher specific activity than total liver lecithin. We found in the present study that from 1 to 24 hr after lysolecithin-^{14}C administration lecithin in gall bladder bile has a higher specific activity than lecithin in the liver, although we did not collect common duct bile and have no values for freshly secreted lecithin. Thus a more active pool of liver lecithin, including bile lecithin, probably shows a classical product–precursor relationship (36) with liver lysolecithin and is a precursor of plasma lecithin.

The small peak contributed by two points in the aorta lecithin specific activity curve, although low, occurred before the lysolecithin peak and did not support a lysolecithin:lecithin precursor:product relationship for the aortic intima plus inner media.

During the first 24 hr after lysolecithin-^{14}C injection, the specific activity curve of plasma lysolecithin has at least three components. The first fast part of the die-away curve from zero to 1 hr presumably reflects distribution to a freely miscible pool in other tissues. The second part, from 1 to 5 hr after lysolecithin-^{14}C administration, represents turnover before the period when significant quantities of newly formed lecithin-^{14}C are present in plasma to form lysolecithin-^{14}C by the LCAT reaction. The third part from 5 to 24 hr suggests that there was recycling of lysolecithin-^{14}C formed in part from lecithin-^{14}C (formed in the liver and released to plasma) via the LCAT reaction.

Distribution of Radioactivity in Different Lipid Subfractions

Table 1 shows the percentage distribution of total radioactivity in different lipid classes of liver at three periods after injection of lysolecithin-^{14}C. There was not only a rapid appearance of radioactivity in lecithin but also a considerable incorporation of ^{14}C into triglycerides, which increased during the 24 hr period, and to a lesser extent into cholesteryl esters. The radioactivity in lecithin was shown by phospholipase A-catalyzed hydrolysis to be largely in position 1 (88–97%). Triglyceride-^{14}C is presumably formed by hydrolysis of lysolecithin and incorporation of the fatty acid-^{14}C into triglyceride, since there is no evidence for phospholipase C activity which could result in the formation of diglyceride-^{14}C from lecithin-^{14}C and subsequent esterification.

In studies on rats, Stein and Stein have already shown (27) that after lysolecithin-^{14}C administration, ^{14}C is variously distributed between lysolecithin and lecithin in different tissues. This, perhaps, is an indication of the different rates at which the lysolecithin-^{14}C is converted to lecithin-^{14}C. Table 2 shows the ratios of radioactivities of lecithin to those of lysolecithin in various tissues at different times after lysolecithin-^{14}C administration to control and atherosclerotic animals. Liver, lung, and kidney rapidly acquired a high ratio, whereas heart and skeletal muscle and plasma had

TABLE 2 THE RATIOS OF LECITHIN-^{14}C TO LYSOLECITHIN-^{14}C IN DIFFERENT TISSUES AFTER ADMINISTRATION OF LYSOLECITHIN-^{14}C TO SQUIRREL MONKEYS

Diet Group	20 min	80 min	3 hr	5 hr	18–24 hr
Control					
Plasma	0.02	0.29	1.8	6.8	11.0
Liver	1.5	11.3	14.7	35.5	49.7
Heart	0.57	0.83	2.8	3.8	6.0
Skeletal muscle	0.41	0.46	0.85	3.3	2.9
Kidney	0.86	4.3	14.8	16.6	42.0
Lung	0.98	1.3	6.8	14.2	67.6
Atherosclerotic					
Plasma	0.09	0.18	0.86	6.8	13.5
Liver	3.7	4.1	18.8	21.6	18.5
Heart	0.54	0.61	1.1	3.3	4.2
Skeletal muscle	0.33	0.21	0.69	1.7	4.4
Kidney	4.4	2.5	13.0	17.3	18.2
Lung	1.1	2.8	8.8	13.0	24.3

Samples of plasma and liver lecithin from animals on each diet and at different times after administration of lysolecithin-^{14}C were treated with phospholipase A_2. From 3.3 to 11.8% of the ^{14}C activity was in free fatty acids (from position 2 of lecithin), and the remainder was in lysolecithin.

low ratios during the early period after lysolecithin-^{14}C administration.

Effect of Nutritionally Induced Hypercholesterolemia on Lysolecithin-^{14}C and Lecithin-^{14}C in Aortic Intima Plus Inner Media

The control and the atherosclerotic monkeys differed significantly with regard to the incorporation of lysolecithin-^{14}C into aortic intima plus inner media (Fig. 2). The peak of lysolecithin radioactivity occurred much later in the atherosclerotic arteries than in the control arteries, reflecting a slower rate of equilibration of plasma and arterial lysolecithin. The specific activities of lysolecithin in the atherosclerotic intima plus inner media were lower than those for the control tissues since the concentrations of unlabeled lysolecithin were much higher in the atherosclerotic tissue (mean values 0.80 vs. 0.14 mg/g). Thus, the atherosclerotic arteries have a larger pool of lysolecithin, but the fractional turnover of the pool is less than that of the control arteries.

Similar curves for lecithin are shown in Fig. 3, and there were differences here also. In both groups the radioactivity of lecithin was steady or even rising at 24 hr after the injection of lysolecithin-^{14}C, and the specific activities of lecithin still had not reached the values for lysolecithin.

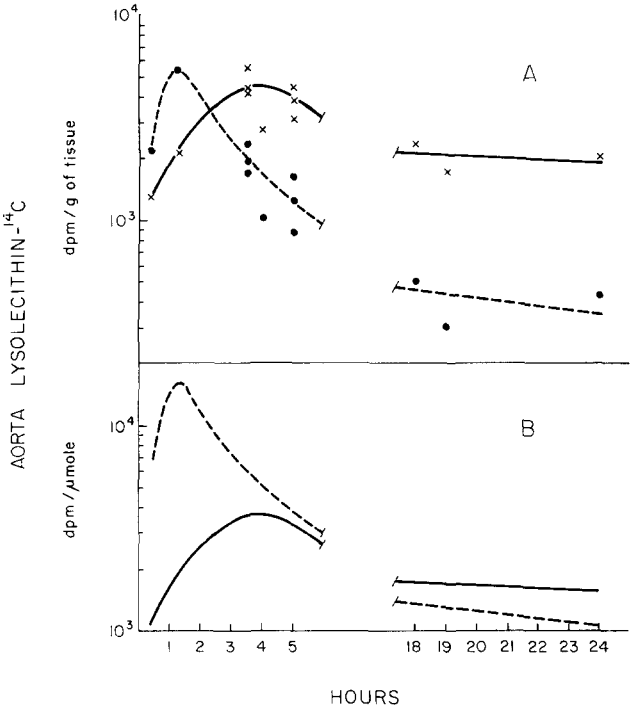


FIG. 2. Radioactivity in lysolecithin in the aortic intima plus inner media as a function of time after administration of lysolecithin-^{14}C to squirrel monkeys. Two groups are shown: ● ----●, control; x — x, atherosclerotic (hyperlipidemia and atherosclerosis). The upper portion of the figure (*A*) shows dpm in lysolecithin per g of tissue while the lower portion (*B*) shows lysolecithin specific activities.

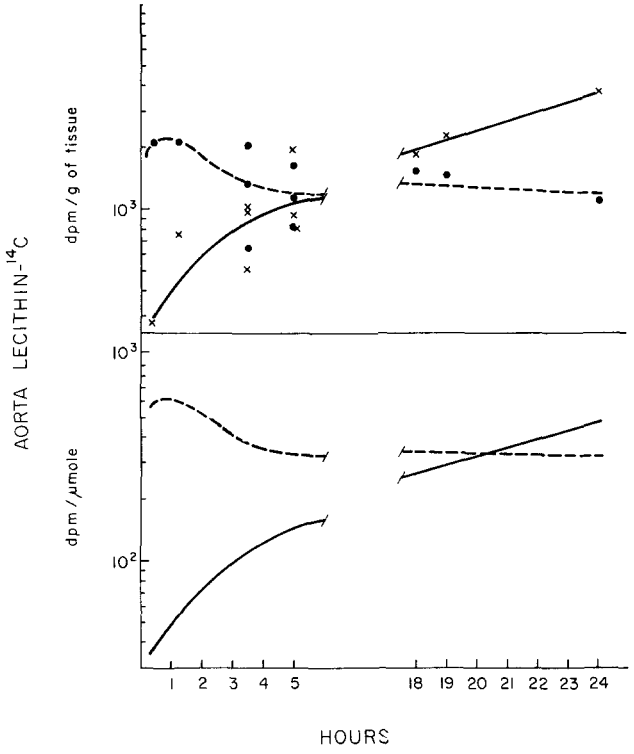


FIG. 3. Radioactivity in lecithin in the aortic intima plus inner media as a function of time after administration of lysolecithin-^{14}C to squirrel monkeys (see Fig. 2). ● ---- ●, values for the monkeys fed the control diet; x — x, values for the atherosclerotic monkeys.

The Effect of Nutritional Pretreatment on Concentrations, Compartment Sizes, and Turnover Rates of Plasma Lysolecithin

The markedly different patterns of uptake of lysolecithin-^{14}C by control and atherosclerotic aortas may result from differences in the properties of the arterial wall or in the rate of clearance of lysolecithin-^{14}C from the plasma. Fig. 4 shows the relative specific activities of plasma lysolecithin-^{14}C for the control group and for the group with hypercholesterolemia and atherosclerosis. Each curve is the composite of 12 individual curves.

We used the method of Gurpide, Mann, and Sandberg (39) and of Goodman and Noble (40) to analyze the semilogarithmic plots of plasma lysolecithin-^{14}C specific activities vs. time for each monkey. The flat portions of the turnover curves (1–5 hr) were extended back to time zero, and the points obtained by subtracting the extrapolated from the observed values formed straight lines. Thus until at least 5 hr after injection of lysolecithin-^{14}C, the die-away curves conformed to a two-pool model (39). We have calculated compartment sizes and turnover rates from these two exponentials using

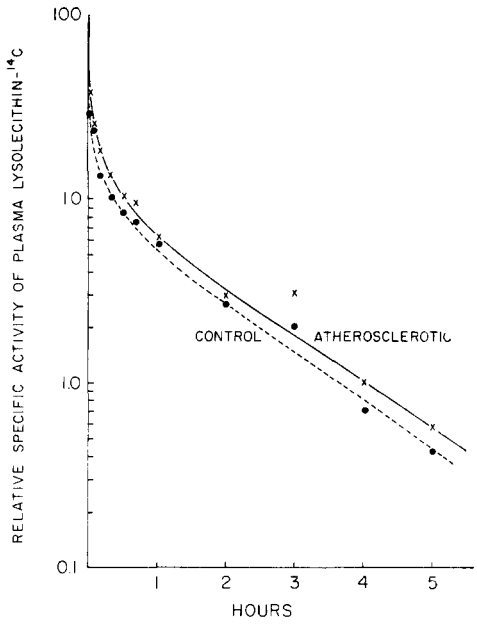
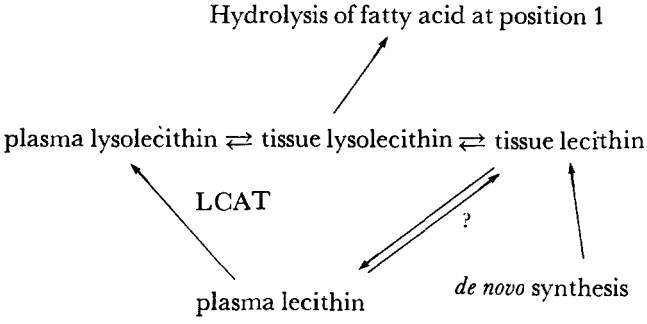


FIG. 4. Relative specific activities of plasma lysolecithin. The specific activities of zero time were set at 100. The zero-time absolute specific activity for the atherosclerotic group was lower than that for the control group because the absolute concentrations of lysolecithin were higher in the plasma from the atherosclerotic group, and the plot of absolute lysolecithin specific activities for the atherosclerotic group was below that for the control group. Each line is a composite of the values for 12 monkeys and consists of 70 different measurements.

equations 2–5, as well as the symbols, listed by Goodman and Noble (40).

These compartments and the associated turnover rates do not conform to precise anatomical entities and metabolic reactions. We may, however, be able to approximate the biological meaning of these calculated values from the sequence of appearance of ^{14}C in lipids of different tissues. The following is a simplified representation of the reactions involving lysolecithin-^{14}C:



Presumably the fast pool of the two-pool system includes the lysolecithin of plasma and that of liver and other organs which is exchangeable with plasma lysolecithin. The second pool may be equivalent to the more slowly exchangeable lysolecithin of the arterial wall and skeletal muscle and to lecithin.

We made too few measurements after 5 hr to determine accurately the slope of the line after that time, but a flat third component of the die-away curve is evident. This third component may result partly from a significant recycling of plasma lysolecithin-^{14}C via lecithin-^{14}C formed in the liver and delivered to the plasma.

Some of the values obtained from the kinetic studies of lysolecithin turnover in the plasma of monkeys from the two dietary groups are shown in Table 3. The mean half-lives and turnover rates of both exponentials and k_{AA} and k_{BB}, the rate constants for total rates of removal from the fast and slow pools, respectively, were not markedly influenced by diet. k_A, the rate constant for net removal of lysolecithin from the fast pool, calculated on the assumption of a negligible net rate of removal of lysolecithin from the slow pool (k_B), was also unaffected by diet. However, the mean lysolecithin content of the fast pool, M_A, as well as the lysolecithin content of the plasma, was higher for the group with hypercholesterolemia and atherosclerosis. The production rate of lysolecithin, PR_A, in the fast pool was also significantly higher for the atherosclerotic group. As might be expected, the values for production rates in the fast pool could be obtained by equation 5 of Goodman and Noble (40) or by multiplying the size of the fast pool, M_A by the net rate of removal from the fast pool, k_A. Plasma lysolecithin was only a part of the lysolecithin in the fast pool.

TABLE 3 SOME CONSTANTS DESCRIBING THE KINETICS OF LYSOLECITHIN METABOLISM

	Control	Atherosclerotic
Half-life of fast exponential (*hr*)	0.201 ± 0.050	0.167 ± 0.010
Half-life of slow exponential (*hr*)	1.32 ± 0.07	1.33 ± 0.10
α, turnover, fast exponential, (hr^{-1})	3.47 ± 0.17	4.15 ± 0.24
β, turnover, slow exponential (hr^{-1})	0.548 ± 0.027	0.538 ± 0.039
k_{AA}, rate constant, total removal from fast pool (hr^{-1})	2.23 ± 0.18	2.50 ± 0.18
k_{BB}, rate constant, total removal from slow pool (hr^{-1})	1.79 ± 0.15	2.19 ± 0.19
k_A, rate constant, net removal from fast pool (hr^{-1})	1.06	1.02
M_A, lysolecithin in fast pool (*μmoles*)	25.5 ± 1.6	41.5 ± 1.8
PR_A, production rate in fast pool (*μmoles/hr*)	27.1 ± 1.9	42.2 ± 2.9
Plasma lysolecithin (*μmoles/ml*)	0.209 ± 0.025	0.355 ± 0.036
(*μmoles/total plasma*)	5.33	9.05

The figures shown are mean values based on eight individual die-away curves per group. Symbols are those used by Goodman and Noble (40).

Level of Lecithin: Cholesterol Acyltransferase (LCAT) Activity in Plasma

The levels of LCAT activity, measured in vitro (Table 4), were also elevated in the plasmas of the atherosclerotic and hypercholesterolemic monkeys. However, the LCAT activities in the total blood volume (estimated), 2.47 μmoles/hr for the control group and 4.37 μmoles/hr for the hypercholesterolemic group, account for only a small part of the production rate of lysolecithin in the fast pool. Thus lecithinase activities in tissues, as well as tissue LCAT activity, must contribute to the lysolecithin of the fast pool. The values of LCAT activity, determined by adding cholesterol-^{14}C in heated plasma to the active plasma to be assayed (35), were similar to those based on the direct measurements of lysolecithin formation. The measurements of LCAT activity in experiments 1, 2, and 3 were carried out at different times, and different levels of substrate (heated plasma) were used. Nevertheless the measurements were performed using cholesterol-4-^{14}C with very small quantities of heated plasma, and the values are endogenous levels of activity which are lower than maximum values with saturating concentrations of substrate. The elevations of plasma LCAT activity were as pronounced in animals on atherogenic diets for 5 days as in those on atherogenic diets for 8–12 months.

Distribution of Lysolecithin-^{14}C and Lecithin-^{14}C in Different Plasma Lipoprotein Subfractions

There was also a difference between control and atherosclerotic animals in the distribution of lysolecithin-^{14}C and lecithin-^{14}C in the plasma lipoproteins (Table 5). Although lysolecithin-^{14}C was found predominantly in the d > 1.21 subnatant fraction of the plasma from control animals (in agreement with Phillips [41]), significant amounts were found in the HDL and LDL plus VLDL fractions of hypercholesterolemic plasma. This latter effect could not be reproduced by simply adding lysolecithin-^{14}C to hypercholesterolemic plasma. There was also a greater proportion of the lecithin-^{14}C in the LDL plus VLDL of hypercholesterolemic plasma than in the corresponding fraction of control plasma. Because of technical difficulties in desalting the lipoprotein fractions for thin-layer chromatography without loss of lysolecithin, we could not determine lysolecithin concentrations or lysolecithin specific activities in the fractions. It is possible that specific activities of lysolecithin and lecithin in LDL plus VLDL from the two groups of animals were similar.

TABLE 4 LYSOLECITHIN CONCENTRATIONS AND LCAT ACTIVITIES IN PLASMA OF SQUIRREL MONKEYS FED DIFFERENT DIETS

	No. of Experiment	Control	Short-Term Hypercholesterolemia*	Atherosclerosis†
Lysolecithin concentration (nmoles/ml)		209 ± 12	466 ± 28	355 ± 18
LCAT‡ (nmoles lysolecithin formed/hr/ml plasma)	1	72 ± 5		118 ± 7
	2	137 ± 8		199 ± 9
	3	76 ± 7	182 ± 10	183 ± 8
	4§	104 ± 11	238 ± 22	186 ± 13

* Animals were fed the atherosclerosis-inducing diet for 2–8 wks.
† Animals were fed the atherosclerosis-inducing diet for 8–12 months.
‡ Measurement of LCAT activity in experiments 1, 2, and 3 was based on the esterification of a trace quantity of cholesterol-4-^{14}C (35); in experiment 4 the increase in lysolecithin was determined. Each value is the mean of nine or more plasma values.
§ Based on lysolecithin formed.

TABLE 5 DISTRIBUTION OF LYSOLECITHIN-^{14}C AND OF LECITHIN-^{14}C IN PLASMA LIPOPROTEINS AFTER THE INTRAVENOUS INJECTION OF LYSOLECITHIN-^{14}C INTO SQUIRREL MONKEYS

	Control*		Atherosclerotic*	
	Lysolecithin	Lecithin	Lysolecithin	Lecithin
	%			
LDL plus VLDL (d < 1.065)	7.7	15.2	33.4	33.2
HDL (d 1.065–1.21)	5.8	62.9	26.9	50.2
Subnatant (d > 1.21)	86.6	21.9	39.6	16.6

* Values are the means for two 3-hr plus two 5-hr samples from each diet group. Each vertical column totals 100%.

Effect of Duration of Hypercholesterolemia and Severity of Atherosclerosis on the Concentrations of Lysolecithin in the Aorta

Since the concentrations of lysolecithin and LCAT activity increase within a few days after starting the hypercholesterolemic diet, an early increase in arterial lysolecithin concentrations could result from changes in plasma composition. On the other hand, a delay in the rise of lysolecithin concentrations in aorta until atherosclerosis is clearly established would show that the rise is perhaps secondary to altered permeability of the arterial wall to lysolecithin. Table 6 shows lysolecithin and total cholesterol concentrations in the aortic intima plus inner media from control monkeys,

TABLE 6 CHOLESTEROL AND LYSOLECITHIN CONCENTRATIONS IN AORTIC INTIMA PLUS INNER MEDIA IN THREE DIET GROUPS

Diet Group	Total Cholesterol	Lysolecithin
	mg/g	
Control	3.51 ± 0.38	0.142 ± 0.034
Short-term hypercholesterolemia	5.25 ± 0.50	0.449 ± 0.10
Atherosclerosis	17.17 ± 2.01	0.800 ± 0.16

from monkeys with hypercholesterolemia but without atherosclerosis (on atherogenic diets from 2 to 9 wk), and from monkeys with both hypercholesterolemia and atherosclerosis (on atherogenic diets from 8 to 12 months). The aorta lysolecithin and cholesterol values for the short-term hypercholesterolemia group were intermediate between the values for the other two groups. Short-term hyperlipemia is, therefore, associated with an early increase in lysolecithin in the arterial wall. This lysolecithin may be involved in the development of the first lesions.

DISCUSSION

The results of this study and of others (27, 28, 42) show that lysolecithin in plasma is rapidly taken up by many tissues and converted to lecithin, almost certainly by direct acylation. In all samples of tissue lecithin-^{14}C examined, most of the ^{14}C was in position 1, an indication that the pathway of Erbland and Marinetti (43) (condensation of two lysolecithin molecules to form one molecule of lecithin and one molecule of glycerol-3 phosphorylcholine) was not the major pathway of lecithin formation. The fatty acid in position 1 of lysolecithin appears also in other lipid classes, particularly in the triglycerides. Some of the difficulties encountered with transfer of the labeled moiety to other classes of lipids could have been avoided if we had used lysolecithin labeled in the glycerol or choline residues. Another complexity in studying the metabolism of lysolecithin-^{14}C is due to recycling whereby lysolecithin in plasma is removed and converted to lecithin by the liver; the lecithin-^{14}C is then released into the plasma in high density lipoproteins where it is again converted to lysolecithin by the LCAT reaction.

Lysolecithin, a normal constituent of plasma (44, 45), is partly a product of the LCAT reaction. When the LCAT enzyme is absent from the plasma, lysolecithin is greatly reduced (26). Moreover, the conversion of lecithin to lysolecithin by the LCAT reaction has been demonstrated in vivo (27) and in vitro (25). Nevertheless, the plasma lysolecithin seemed to be part of a much larger pool, and the total production rate of lysolecithin in this pool was much larger than the LCAT activity in the plasma alone. This discrepancy between the calculated lysolecithin production rate in vivo and the level of LCAT activity suggests that much of the lysolecithin in the fast pool, which is exchangeable with the plasma lysolecithin, results from tissue phospholipase activity and tissue LCAT activity.

Conclusive evidence for a net unidirectional flow into tissues of lysolecithin is difficult to obtain from plasma lysolecithin-^{14}C concentrations alone. Careful determination of arteriovenous differences in lysolecithin-^{14}C concentrations during the second phase of the die-away curve might reveal whether there is a net flow of lysolecithin to tissues. Since the lysolecithin fractional turnover in this period is about 0.5 per hr compared with an estimated 130 blood volumes perfusing the capillary bed per hr, one could not expect an arteriovenous difference of more than 0.4%. Of 14 arteriovenous differences measured, nine were positive, and five were negative.

Two observations by Stein and Stein (42) on rat hearts perfused with lysolecithin-^{14}C are pertinent. The lecithin-^{14}C formed in the heart during pulse labeling with lysolecithin-^{14}C could not be removed by a subsequent perfusion. Moreover, the specific activity of lysolecithin-^{14}C in the initial perfusate did not drop even though a large amount of lysolecithin-^{14}C was taken up by the heart. Thus lysolecithin-^{14}C uptake was not a simple exchange with tissue lysolecithin.

Monkeys with hypercholesterolemia and diet-induced atherosclerosis have increased concentrations of lysolecithin and lecithin in the aorta and plasma as well as increased levels of lysolecithin formed by the LCAT reaction (1, 2, 22, 23). Since aortic lysolecithin was a part of the fast pool which included the plasma, the increased concentrations of lysolecithin in atherosclerotic intima plus inner media and plasma compared with normal tissues could result from increased LCAT activity in the plasma. On the other hand, lysolecithin was formed and hydrolyzed by a particulate fraction of homogenate of aortic intima plus inner media from squirrel monkeys at alkaline pH and with Ca^{++} (22, 23), as described by Scherphof and Van Deenen (18) for liver, although the effect of atherosclerosis on maximum rates of activity was not significant. One other report confirms (3) and one denies (21) that atherosclerosis does not affect arterial phospholipase activity. The possible importance of lysosomal (optimal activity at acidic pH) enzymes in lysolecithin metabolism (20) by aorta has not been investigated. Although it is impossible to evaluate the in vivo importance of hydrolytic enzymes from in vitro studies, aortic lysolecithin hydrolysis was possible, based on the appearance of some free palmitate-^{14}C in the intima plus inner media after lysolecithin-^{14}C administration.

Lysolecithin concentrations in normal-appearing aortas from animals with short-term hypercholesterolemia were somewhat greater than concentrations in aortas from normal animals but much less than those in atherosclerotic aortas.

Lysolecithin is acylated to form lecithin most actively in the endoplasmic reticulum of aortic smooth muscle cells,[1] as well as in hepatic cells (38). Lecithin is the

[1] Portman, O. W., and M. Alexander. Manuscript in preparation.

primary phospholipid of the endoplasmic reticulum as distinct from the plasmalemma of the smooth muscle cell where sphingomyelin predominates (23, 46, 47). Changes in lysolecithin and lecithin concentrations and metabolism in the aortic wall may be related to the extraordinary proliferation of endoplasmic reticulum and mitochondria of aortic smooth muscle cells that Parker, Ormsby, Peterson, Odland, and Williams. (48) have shown in atherosclerosis. Additional factors that might account for changes in the concentrations and metabolism of these membrane-bound phospholipids in arteries are changes in the types of cells, for example, increases in foam cells (49) and in the rate of cell division (50, 51).

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