Exchange of phospholipids between low and high density lipoproteins of squirrel monkeys

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Abstract Ultracentrifugal analysis of the plasma of squirrel monkeys at various times after the injection of [*Me*-^{14}C]choline revealed the specific activities of lecithin in both high (HDL) and low (LDL) density lipoproteins to be similar. This was also true for sphingomyelin.

The exchange of phospholipids in vitro was studied by incubating unlabeled plasma with labeled LDL and HDL isolated 40 hr after the injection of [*Me*-^{14}C]choline. Recentrifugation of plasma immediately after the addition of either ^{14}C-labeled LDL or HDL demonstrated that significant exchanges of both lecithin and sphingomyelin had occurred. In further studies, ^{14}C-labeled LDL or HDL were incubated with plasma and the low density lipoproteins were rapidly isolated by precipitation with heparin–Mn^{2+}. Complete equilibration of lecithin and sphingomyelin between LDL and HDL was attained after 4 and 5 hr, respectively. The fractional exchange rates for lecithin and sphingomyelin of LDL to HDL were 0.60 hr^{-1} and 0.45 hr^{-1}. Corresponding values for HDL to LDL were 0.51 hr^{-1} and 0.53 hr^{-1}. Inhibition of plasma lecithin:cholesterol acyltransferase reduced the exchange of sphingomyelin but had no effect on lecithin exchange. The rates of exchange of four lecithin subfractions of different unsaturation between LDL and HDL were the same.

Supplementary key words lecithin · sphingomyelin · lysolecithin · lecithin:cholesterol acyltransferase · lecithin subfractions

Although earlier studies have demonstrated an in vitro exchange between the total ^{32}P-labeled phospholipids of human (1–3) and rabbit (4) α- and β-lipoproteins, little quantitative information about the rates of exchange of individual phospholipids between the major lipoproteins is available. Minari and Zilversmit (5) observed an exchange of ^{32}P-labeled phospholipids between dog chylomicrons and serum; the rates of exchange of sphingomyelin and lysolecithin, expressed as a percentage of their initial specific activities, were somewhat faster than those for lecithin. In quantitative terms, however, the exchange of lecithin was considerably greater.

Similar studies have also revealed an analogous exchange reaction between the phospholipids of plasma lipoproteins and those of isolated erythrocytes (6–9), mitochondria (10, 11), and microsomes (12), although in these cases both the relative and absolute exchange rates of lecithin exceeded those of sphingomyelin. The exchange of lecithin between plasma HDL and brain tissue has also been proposed (13) to explain the low linoleate content of this lipid in cerebrospinal fluid.

In the present study, the in vivo turnover and in vitro rates of exchange of the lecithin and sphingomyelin constituents of plasma lipoproteins from squirrel monkeys have been examined. The influence of the plasma lecithin:cholesterol acyltransferase (LCAT) enzyme on the exchange of lipoprotein phospholipids in vitro was also studied. In view of the known heterogeneity of plasma lecithins (14, 15), the exchange rates of four principal lecithin species, differing in degree of unsaturation, between low and high density lipoproteins were also determined.

MATERIALS AND METHODS

Isolation of labeled plasma lipoproteins

Sexually mature female squirrel monkeys (*Saimiri sciureus*) maintained on a semipurified diet (16) were

Abbreviations: VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); LCAT, lecithin:cholesterol acyltransferase; PCMPS, sodium *p*-chloromercuriphenyl sulfonate; TLC, thin-layer chromatography.

injected intraperitoneally with either 100 or 150 μCi of [*Me*-^{14}C]choline chloride (specific activity 54 or 61 mCi/mmole, Amersham/Searle Corp.) in 0.5 ml of 0.9% NaCl. For studies on the in vivo turnover of plasma phospholipids, serial samples of 2.5 ml of blood were withdrawn from the femoral vein into heparinized syringes, and the plasma and cells were separated by centrifugation at 2,000 *g* for 15 min. LCAT was inhibited by adding sodium *p*-chloromercuriphenyl sulfonate (PCMPS) to a final concentration of 0.002 M, which was maintained throughout the ultracentrifugal separation of plasma.

Control experiments, in which the esterification of [26-^{14}C]cholesterol was determined (17, 18) in both the presence and absence of 0.002 M PCMPS, demonstrated that addition of the latter inhibited plasma LCAT activity by a mean of 97.8%.

When plasma lipoproteins were to be isolated for subsequent in vitro studies, blood was withdrawn 40 hr after the injection of [*Me*-^{14}C]choline and the ^{14}C-labeled plasma was mixed with unlabeled plasma from similar animals; the final volume was 11.0 ml. Lipoproteins were isolated in the 50 rotor of a Spinco model L ultracentrifuge operated at 15°C. After the plasma had been overlaid with d 1.006 NaCl, chylomicrons were isolated by ultracentrifugation for 1 hr at 30,000 *g* (avg). Very low density lipoproteins (VLDL) were isolated from the infranatant fraction by further centrifugation for 16 hr at 122,000 *g* (avg). After the removal of VLDL, the density of the infranatant fraction was adjusted to 1.063 by the addition of solid KBr, and low density lipoproteins (LDL) were separated by centrifugation for 20 hr at 122,000 *g* (avg). The infranatant fraction was finally adjusted to a density of 1.21 with solid KBr, and high density lipoproteins (HDL) were isolated by centrifugation at 122,000 *g* (avg) for 40 hr. In all cases, resolved lipoprotein fractions were removed from the top of the centrifuge tubes after the latter had been cut with a tube slicer.

Before the exchange studies, LDL and HDL were dialyzed overnight against 200 vol of Tris buffer (0.005 M, pH 7.4)–0.001 M EDTA. Whereas LDL was stored at 4°C for 2 days before incubation, samples of HDL were utilized immediately.

Incubation

Aliquots of the [^{14}C]phospholipid-labeled LDL or HDL (usually 2.0 ml) were added to 5.0 ml of fresh, unlabeled plasma from similar animals that had been fasted overnight. After mixing, the samples were incubated at 37°C in a metabolic shaker. Serial samples were then withdrawn at different times and diluted with 2.0 ml of water, and the lipoproteins were separated by heparin precipitation. Further details of the individual experiments are given in the legends to the figures.

Separation of lipoproteins from the incubation media

Preliminary work (see Results) showed that a considerable exchange of phospholipids occurred during the isolation of plasma lipoproteins by ultracentrifugation. Previous work (19) has demonstrated that the addition of heparin and Mn^{2+} to human serum results in the selective precipitation of chylomicrons, VLDL, and LDL and that this method offers a means of rapidly separating these lipoproteins from those of higher density. The validity of this method for the plasma of nonhuman primates was checked by adding 50 μl of 5% heparin (170 USP units/mg; Sigma Chemical Co., St. Louis, Mo.) and 50 μl of 1 M $MnCl_2$ solution per ml of plasma. The precipitate, which sedimented at 4,000 *g* for 20 min, was washed with 1 ml of heparin (0.25%)–Mn^{2+} (0.05 M) and redissolved in 1 ml of 1% NaCl–$NaHCO_3$ solution. The redissolved precipitate and supernatant fractions were then electrophoresed on both Whatman 3MM paper and cellulose acetate strips (Sepraphore III, Gelman Instrument Co., Ann Arbor, Mich.) with a veronal buffer (I 0.05, pH 8.6). The equivalent of 0.3 ml and 10–15 μl of plasma was normally applied to the paper and to the cellulose acetate strips, respectively. The presence of lipids in HDL and LDL could be detected in the equivalent of 8 μl and 5 μl of plasma, respectively, by paper electrophoresis, and in 1–2 μl by electrophoresis on cellulose acetate. Protein was detectable in 0.5 μl of plasma by the latter technique. Subsequent staining of the strips for protein with Ponceau S (20) and for lipid with oil red O (21) revealed that the heparin precipitate contained only LDL with β mobility. The supernatant fraction contained α-lipoprotein (HDL), but the lack of any low density β-lipoproteins demonstrated that precipitation of the latter was complete. These findings were also verified with lipoproteins purified by ultracentrifugation.

For the routine separation of lipoproteins from the incubation medium, 50 μl of 5% heparin and 50 μl of 1 M $MnCl_2$ per ml of diluted plasma were added, and the precipitated LDL were sedimented at 4,000 *g* for 20 min. The precipitate was washed with 1 ml of heparin (0.25%)–Mn^{2+} (0.05 M) prior to extraction. Dilution of plasma with 4 vol of water did not influence the precipitation of low density lipoproteins by the heparin–Mn^{2+} method.

Although the addition of heparin–Mn^{2+} also precipitates chylomicrons and VLDL from plasma (19), ultracentrifugal analysis of plasma from fasting squirrel and rhesus monkeys revealed that in both cases these two fractions together constituted less than 1.5% of the

total plasma phospholipids. Hence, although chylomicrons and VLDL are included in the heparin–Mn^{2+}-precipitated lipoproteins, their influence on total phospholipid exchange, relative to that of LDL, is sufficiently slight to be disregarded. Similarly, the effects of any possible differences in exchange between the small quantities of lecithin and sphingomyelin present in the d > 1.21 fraction on ultracentrifugation (less than 1%) and the much greater amounts present in HDL were considered insignificant. The exchange of lecithin and sphingomyelin between the heparin–Mn^{2+}-precipitable and nonprecipitable lipoproteins was, therefore, taken as an index of exchange occurring between LDL and HDL. This rationale was not, however, valid for lysolecithin, most of which sedimented with the d > 1.21 fraction on ultracentrifugation.

Analysis of phospholipids and assay of radioactivity

Lipids were extracted from lipoproteins with chloroform–methanol 2:1 (v/v) (22) in a ground glass homogenizer, and the total lipid extract was stored in chloroform. For the analysis of individual phospholipids, the total lipids were separated on 0.25-mm layers of washed (23) silica gel H with the solvent system chloroform–methanol–glacial acetic acid–water 75:55:12:6 (by volume). Radioactivity was determined by previously described methods (24, 25), except that for assaying [^{14}C]lysolecithin, 10% BBS3 (Beckman Instruments, Inc., Palo Alto, Calif.) was added to the scintillation fluid as a solubilizer. Another plate was then subjected to controlled charring (24, 25) and was scanned on a Farrand VIS-UV thin-layer spectrophotometer as previously described (26).

After elution with methanol, lecithin samples were further fractionated according to their degree of unsaturation by silver nitrate TLC (27). Individual bands were visualized under UV light after the plate had been sprayed with scintillation fluid (17). Four principal components, corresponding to mono-, di-, tetra-, and penta- + hexaenoic lecithins, were resolved, each band being eluted (27) and assayed for radioactivity.

RESULTS

In vivo turnover of lecithin and sphingomyelin in plasma

As Fig. 1 illustrates, [*Me*-^{14}C]choline injected intraperitoneally was incorporated into the lecithin of ultracentrifugally-isolated lipoproteins considerably more rapidly than into sphingomyelin, maximum specific activities being evident after 8 and 24 hr, respectively. The similar specific activity curves observed for the lecithin and sphingomyelin constituents of LDL and

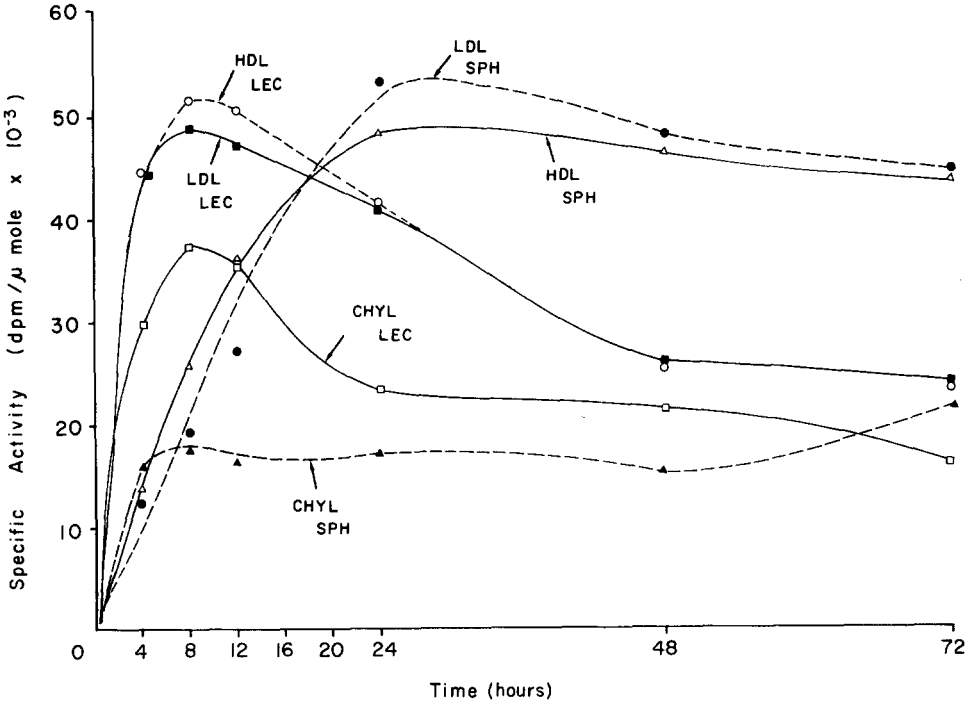

FIG. 1. Specific activities of lecithin (LEC) and sphingomyelin (SPH) in the plasma lipoproteins of squirrel monkeys after injection of 100 μCi of [*Me*-^{14}C]choline. Plasma lipoproteins were separated by ultracentrifugation. Each value represents the mean of three separate experiments. SEM for the lecithin and sphingomyelin of LDL and HDL were 2–11% and for chylomicrons (CHYL) 5–16% of the mean values.

HDL, which are higher than those of chylomicrons, indicate that the turnover of these lipids in LDL and HDL is similar. Another explanation for these results, however, is that a rapid exchange of phospholipids occurs between LDL and HDL and thereby equalizes any differences in specific activity that originally existed between them. Further experiments were, therefore, conducted to evaluate the extent of phospholipid exchange between lipoproteins during both the in vitro incubation and the ultracentrifugation of plasma.

Exchange of phospholipids during ultracentrifugation of plasma

The results of experiments in which squirrel monkey plasma was centrifuged immediately after the addition of [^{14}C]phospholipid-labeled LDL or HDL are shown in Table 1. In either case, significant radioactivity was recovered in both the lecithin and sphingomyelin constituents of chylomicrons and LDL or HDL. Although no net transfer of phospholipids occurred between LDL and HDL, an increase of 9–19% was noted in the phospholipids of the chylomicron fraction. However, because of its small size, the chylomicron fraction was not rewashed after isolation, and the observed increase in phospholipid probably reflects a nonspecific binding of other lipoproteins. At most, this contamination may account for up to 35% of the radioactivity present in chylomicrons; the rest presumably was derived from exchange reactions.

In similar studies, plasma, containing 0.002 M PCMPS to inhibit LCAT, was incubated for 24 hr at 37°C with [^{14}C]phospholipid-labeled LDL or HDL before ultracentrifugation. In both cases, complete equilibration of lecithin between chylomicrons, LDL, and HDL was normally obtained, the exchange of sphingomyelin varying from 85 to 100%.

TABLE 1. Normalized phospholipid specific activities of lipoprotein fractions isolated by ultracentrifugation of plasma immediately after the addition of [^{14}C]phospholipid-labeled LDL or HDL

	Relative Specific Activity					
	Chylomicrons		LDL		HDL	
Sample	Lec	Sph	Lec	Sph	Lec	Sph
Plasma + ^{14}C-labeled LDL	73.0 ±3.5	81.0 ±4.0	100	100	42.7 ±5.3	26.0 ±1.7
Plasma + ^{14}C-labeled HDL	74.2 ±3.7	53.9 ±1.4	49.4 ±1.2	30.6 ±1.4	100	100

In each experiment, ^{14}C-labeled LDL (sphingomyelin 110–145 nmoles, lecithin 330–430 nmoles) or HDL (sphingomyelin 125–160 nmoles, lecithin 780–1000 nmoles) was added to plasma from normal squirrel monkeys (phospholipid content: chylomicrons, sphingomyelin 40 nmoles, lecithin 250 nmoles; LDL, sphingomyelin 150 nmoles, lecithin 440 nmoles; and HDL, sphingomyelin 200 nmoles, lecithin 1250 nmoles). Values are the means ± SEM of four experiments.

In vitro exchange of phospholipids between plasma lipoproteins

So that the exchange of phospholipids between LDL and HDL could be further evaluated, [^{14}C]choline–phospholipid-labeled lipoproteins were incubated with plasma from fasting squirrel monkeys. Serial samples were withdrawn and the low and high density lipoproteins were rapidly separated by precipitation of the former with heparin–Mn^{2+}. Analysis of zero-time controls, in which LDL were precipitated immediately after adding either ^{14}C-labeled LDL or HDL to plasma, demonstrated that, though ^{14}C-labeled LDL was pure, 2–3% of the radioactivity present in ^{14}C-labeled HDL was precipitated by heparin–Mn^{2+}. Appropriate corrections were made.

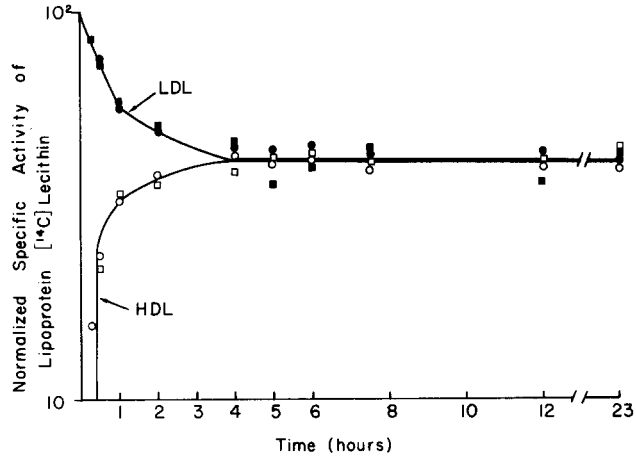

FIG. 2. Exchange of [^{14}C]lecithin between LDL and HDL. 2 ml of [^{14}C]lecithin-labeled LDL (lecithin content 145 nmoles/ml) was incubated with 5.0 ml of plasma (lecithin content of LDL 240 nmoles and of HDL 710 nmoles/ml of plasma), and serial samples of 0.6 ml were withdrawn as indicated. In parallel incubations, 0.002 M PCMPS was added to inhibit LCAT. Normalized specific activity refers to the specific activity of [^{14}C]lecithin in the lipoproteins expressed relative to the specific activity of [^{14}C]lecithin in LDL at time zero being 100. The specific activity of HDL lecithin at time zero is 0. ■——■, LDL [^{14}C]lecithin of normal plasma; ●——●, LDL [^{14}C]lecithin with 0.002 M PCMPS added; ○——○, HDL [^{14}C]lecithin of normal plasma; □——□, HDL [^{14}C]lecithin with 0.002 M PCMPS added.

As Figs. 2 and 3 illustrate, both the lecithin and sphingomyelin constituents of originally labeled LDL were exchanged, and complete equilibration was evident after 4 and 5 hr, respectively. Although not affecting the initial exchange rates, the inhibition of plasma LCAT by 0.002 M PCMPS reduced the total exchangeable sphingomyelin pool by 12–18%. Under these conditions complete equilibration of lipoprotein sphingomyelin was not reached, even after 23 hr of incubation. In contrast, lecithin exchange appeared unaffected by LCAT inhibition. Fractional exchange rates were calculated from the initial slopes of the die-away curves using the equation (28):

$$\frac{1}{K_2} = 1.44t_{1/2}$$

where: K_2 = fractional turnover rate, and
$t_{1/2}$ = half-life of [^{14}C]lecithin or [^{14}C]sphingomyelin in the originally labeled lipoprotein.

Respective values for the lecithin and sphingomyelin of LDL were 0.60 hr^{-1} and 0.45 hr^{-1}.

Results from analogous studies on the exchange of [^{14}C]lecithin and [^{14}C]sphingomyelin between labeled HDL and plasma LDL are shown in Figs. 4 and 5. Although isotopic equilibrium of both phospholipids between HDL and LDL was again attained after about 4 hr, the inhibition of LCAT caused only a 5% reduction

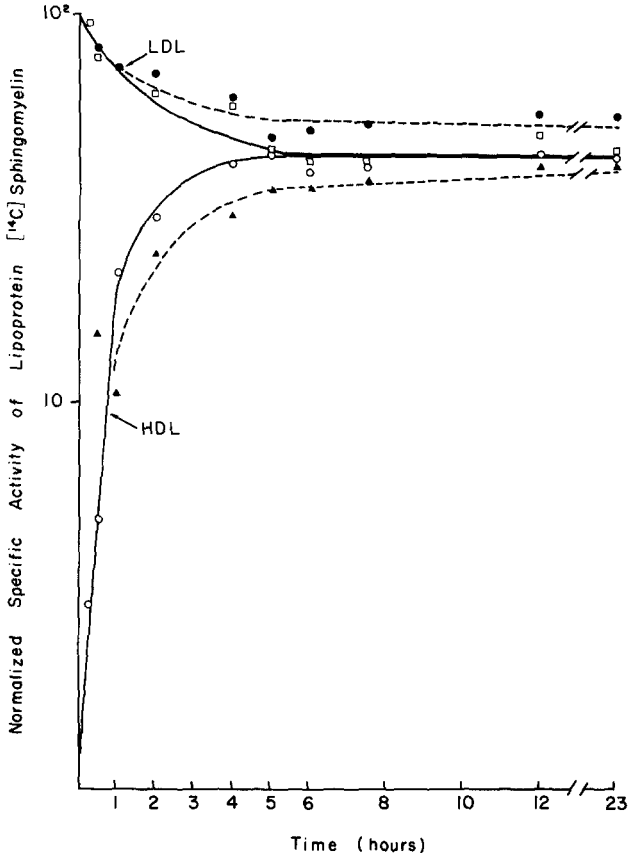

FIG. 3. Exchange of [^{14}C]sphingomyelin between LDL and HDL. 2 ml of [^{14}C]sphingomyelin-labeled LDL (sphingomyelin content 34 nmoles/ml) was incubated with 5.0 ml of plasma (sphingomyelin content of LDL 75 nmoles and of HDL 120 nmoles/ml of plasma), and serial samples of 0.6 ml were withdrawn as indicated. In parallel incubations, 0.002 M PCMPS was added to inhibit LCAT. Normalized specific activity refers to the specific activity of [^{14}C]sphingomyelin in the lipoproteins expressed relative to the specific activity of [^{14}C]sphingomyelin in LDL at time zero being 100. The specific activity of HDL sphingomyelin at time zero is 0. □——□, LDL [^{14}C]sphingomyelin of normal plasma; ●- - -●, LDL [^{14}C]sphingomyelin with 0.002 M PCMPS added; ○——○, HDL [^{14}C]sphingomyelin of normal plasma; ▲- - -▲, HDL [^{14}C]sphingomyelin with 0.002 M PCMPS added.

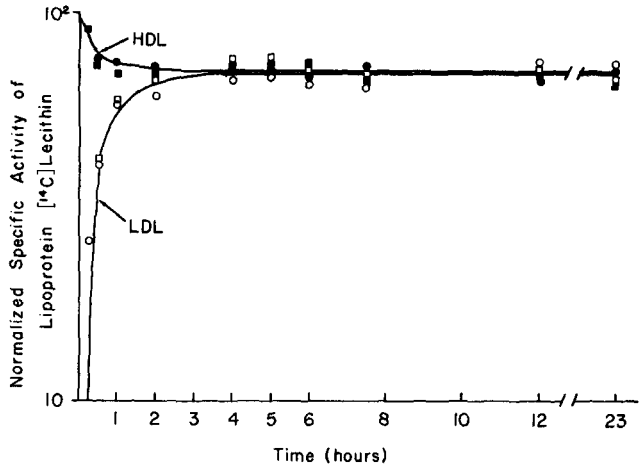

FIG. 4. Exchange of [^{14}C]lecithin between HDL and LDL. 2 ml of [^{14}C]lecithin-labeled HDL (lecithin content 410 nmoles) was incubated with 5.0 ml of plasma (lecithin content of LDL 240 nmoles and of HDL 710 nmoles/ml of plasma), and serial samples of 0.6 ml were withdrawn as indicated. In parallel incubations, 0.002 M PCMPS was added to inhibit LCAT. Normalized specific activity refers to the specific activity of [^{14}C]lecithin in the lipoproteins expressed relative to the specific activity of [^{14}C]lecithin in HDL at time zero being 100. The specific activity of LDL lecithin at time zero is 0. ■——■, HDL [^{14}C]-lecithin of normal plasma; ●——●, HDL [^{14}C]lecithin with 0.002 M PCMPS added; ○——○, LDL [^{14}C]lecithin of normal plasma; □——□, LDL [^{14}C]lecithin with 0.002 M PCMPS added.

in the exchangeable sphingomyelin pool. Fractional exchange rates of lecithin and sphingomyelin from HDL to LDL were 0.51 hr^{-1} and 0.53 hr^{-1}, respectively.

In these experiments, it was also noted that the specific activities of [^{14}C]lysolecithin in the heparin-precipitable and nonprecipitable lipoproteins attained equilibrium 30–60 min after the addition of either labeled LDL or HDL. Although this equilibration clearly demonstrates the lysolecithin constituents of both LDL and HDL to be capable of exchange, the role of albumin, to which the majority of plasma lysolecithin is normally bound (29, 30), in this exchange process is unknown.

Exchange of lecithin subclasses between lipoproteins

In view of the known heterogeneity of plasma lecithins (14, 15), we investigated the rates of exchange of four major subfractions, separated by silver nitrate TLC, between LDL and HDL. 40 hr after the injection of [*Me*-^{14}C]choline into squirrel monkeys, radioactivity in the lecithins of both LDL and HDL was distributed as follows: monoenes 22.2%, dienes 38.2%, tetraenes 32.2%, and pentaenes + hexaenes 7.4% (mean values). Further analysis of serial samples of LDL and HDL, separated after incubation times of up to 4 hr from tubes containing either [^{14}C]lecithin-labeled LDL or HDL and unlabeled plasma, failed to reveal any changes in the

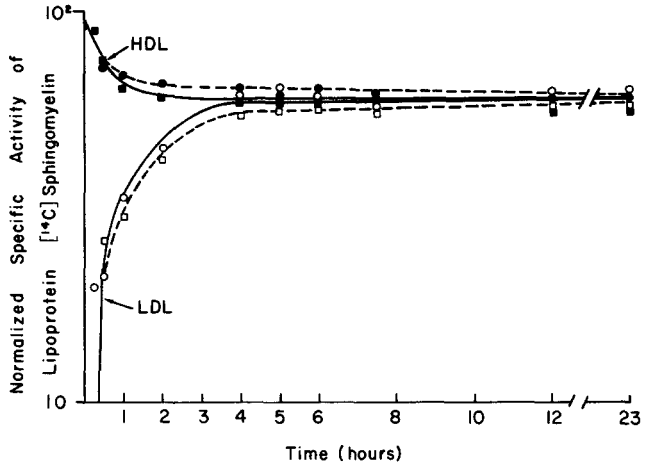

FIG. 5. Exchange of [^{14}C]sphingomyelin between HDL and LDL. 2 ml of [^{14}C]sphingomyelin-labeled HDL (sphingomyelin content 54 nmoles/ml) was incubated with 5.0 ml of plasma (sphingomyelin content of LDL 75 nmoles and of HDL 120 nmoles/ml of plasma), and serial samples of 0.6 ml were withdrawn as indicated. In parallel incubations, 0.002 M PCMPS was added to inhibit LCAT. Normalized specific activity refers to the specific activity of [^{14}C]sphingomyelin in the lipoproteins expressed relative to the specific activity of [^{14}C]sphingomyelin in HDL at time zero being 100. The specific activity of LDL sphingomyelin at time zero is 0. ■——■, HDL [^{14}C]sphingomyelin of normal plasma; ●- - -●, HDL [^{14}C]sphingomyelin with 0.002 M PCMPS added; ○—○, LDL [^{14}C]sphingomyelin of normal plasma; □- - -□, LDL [^{14}C]sphingomyelin with 0.002 M PCMPS added.

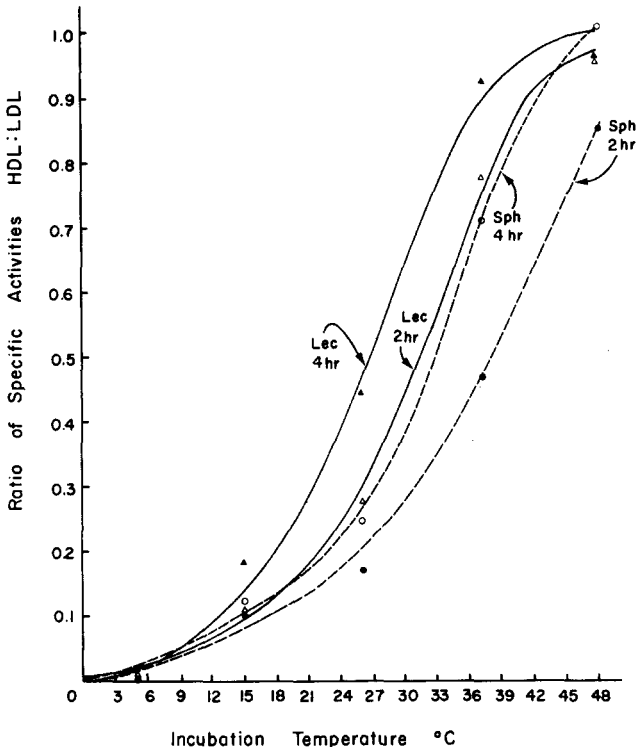

FIG. 6. The effects of temperature on the exchange of [^{14}C]-lecithin (Lec) and [^{14}C]sphingomyelin (Sph) between LDL and HDL. 0.4 ml of LDL, containing 15 nmoles of [^{14}C]sphingomyelin and 58 nmoles of [^{14}C]lecithin, was incubated with 1.1 ml of plasma (sphingomyelin content of LDL and HDL 84 and 132 nmoles and lecithin content 265 and 780 nmoles, respectively) at the temperatures indicated. 0.5-ml samples were withdrawn after 2 and 4 hr.

relative distribution of ^{14}C within lecithin subfractions, despite marked changes in total lecithin specific activities (Figs. 2 and 4). Although small sample sizes prevented us from determining the specific activities of individual lecithin subfractions, the fatty acid composition of lecithin in LDL and HDL was similar and remained essentially unchanged after the incubation of plasma at 37°C for 4 hr. Under these conditions, therefore, values for the relative distribution of radioactivity in lecithin subfractions would be equivalent to those of specific activity. Since the former remained constant throughout, the rates of exchange of lecithins of different unsaturation between LDL and HDL are the same.

Effects of temperature on exchange

The exchange of [^{14}C]lecithin and of [^{14}C]sphingomyelin between plasma lipoproteins was studied over the temperature range 4–48°C. As Fig. 6 illustrates, the exchange of both phospholipids between labeled LDL and plasma HDL, although being virtually abolished at 4°C, increased rapidly as the incubation temperature rose above 25–30°C. However, an appreciable exchange of both [^{14}C]lecithin and [^{14}C]sphingomyelin took place at 15°C, a temperature at which ultracentrifugal separation of plasma lipoproteins is frequently performed. The results of parallel incubations, in which [^{14}C]phospholipid-labeled HDL was added to plasma (results not shown), were similar to those shown in Fig. 6.

Influence of lipoprotein concentrations on phospholipid exchange

We also studied how variations in the quantity of [^{14}C]phospholipid-labeled lipoproteins, added to a constant volume of plasma, influenced the exchange of phospholipids between LDL and HDL. After various amounts of LDL, containing 2–24 nmoles of [^{14}C]-sphingomyelin and 7–100 nmoles of [^{14}C]lecithin, had been incubated for 2 hr with 0.4 ml of plasma (lipid content of LDL, 31 nmoles of sphingomyelin and 98 nmoles of lecithin, and of HDL, 47 nmoles of sphingomyelin and 280 nmoles of lecithin), the ratios of specific activities of lecithin and sphingomyelin in HDL to those in LDL showed only a 3% decrease within the added substrate range. Similar experiments, in which the phospholipid content of added HDL varied from 2 to 38 nmoles of [^{14}C]sphingomyelin and from 20 to 290 nmoles of [^{14}C]lecithin, revealed a 6% decrease in the ratios of the specific activities of lecithin in LDL to those in HDL, although the corresponding ratios for sphingomyelin were

slightly larger, falling from a value of 0.56 with 2 nmoles of [^{14}C]sphingomyelin to 0.46 with 38 nmoles of [^{14}C]-sphingomyelin. These results reveal that, at the concentrations of lipoproteins used throughout this study, the fractional exchange rates remained fairly constant and that the number of lipoprotein–lipoprotein "collisions" was not a limiting factor.

DISCUSSION

The results presented in this paper clearly demonstrate the existence of a rapid in vitro exchange between both the lecithin and sphingomyelin components of plasma LDL and HDL. Using heparin–Mn^{2+} to rapidly precipitate plasma LDL, we were able to circumvent the inherent problems associated with the exchange of phospholipids during the ultracentrifugal separation of plasma. As this study and that of Eder (1) have shown, such exchange is appreciable, whereas it was undetectable when the lipoproteins were separated by heparin–Mn^{2+} precipitation. Nevertheless, it is worth noting that in employing this method of separation, we did not consider any possible differences in exchange rates between the small quantities of lecithin and sphingomyelin present in the chylomicron + VLDL fraction and those in LDL. The possible differences in exchange between the small amounts of lecithin and sphingomyelin in the d > 1.21 fraction and those of HDL were also neglected.

Several investigators (1–4) have studied the exchange of phospholipids between plasma lipoproteins, but the results are conflicting. In the present study we observed that isotopic equilibration of both lecithin and sphingomyelin between plasma LDL and HDL was attained in 4–5 hr. A similar value was obtained by Kunkel and Bearn (3) for the exchange of total ^{32}P-labeled phospholipids between human α- and β-lipoproteins, although in analogous studies (1, 2) isotopic equilibration was not evident even after 20 hr of incubation. Nevertheless, results from this and other (1–4) turnover studies strongly favor the theory that under in vivo conditions phospholipids are rapidly exchanged between lipoproteins.

The fact that the rates of exchange of lecithins of different unsaturation between LDL and HDL were the same is in accordance with the fact that the fatty acid compositions of lecithins from both lipoproteins are similar. Studies by Wirtz, van Golde, and van Deenen (31) also have failed to reveal any differences in the rates of exchange of lecithin subclasses between rat liver microsomes and mitochondria incubated in the presence of the 105,000 *g* supernatant fraction. In the absence of a supernatant fraction, however, species containing arachidonate appeared to be less readily exchanged. Although this 105,000 *g* supernatant fraction or a purified pH 5.1 supernatant fraction derived from it has been shown to stimulate the exchange of phospholipids between plasma and both mitochondria (11) and microsomes (12), it is not known whether the active protein (11, 32) is normally present in plasma or whether the addition of the pH 5.1 supernatant fraction also enhances the exchange of phospholipids between individual plasma lipoproteins.

Recent work has indicated that the phospholipids of human HDL will bind to both of the major peptides of HDL (33), although the association of these lipids with the carboxy-terminal glutamine (R–Gln) peptide appears to be stronger than that occurring with the carboxy-terminal threonine (R–Thr) peptide (34). Both of these peptides, however, appear to be absent from LDL (35). Thus, although at least some of the peptide components of lipoproteins appear capable of exchange (36, 37), the possibility of exchange of phospholipid–peptide complexes between HDL and LDL is unlikely. The theory of a "collision complex," in which the magnitude of exchange is governed by the diffusion of the phospholipids in the complex, seems more plausible, although the participation of a protein carrier cannot be discarded.

Although the inhibition of plasma LCAT by 0.002 M PCMPS did not affect lecithin exchange, it reduced the total exchangeable sphingomyelin pool from 5 to 18%. These observations are compatible with the theory that utilization of HDL lecithin by LCAT (18, 38, 39) unmasks additional sphingomyelin binding sites not previously exposed to the lipoprotein surface. LCAT activity may also be associated with a weakening of the hydrophobic forces normally binding sphingomyelin in the lipoprotein complex.

We conclude that under our in vitro conditions, which closely resemble conditions in vivo, lecithin and sphingomyelin are both exchanged rapidly between the low and high density lipoproteins of squirrel monkey plasma. Such an exchange of phospholipids may be responsible for the apparently similar turnover times observed in vivo.

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REFERENCES

1. Eder, H. A. 1957. The lipoproteins of human serum. *Amer. J. Med.* **23:** 269–282.
2. Florsheim, W. H., and M. E. Morton. 1957. Stability of phospholipid binding in human serum lipoproteins. *J. Appl. Physiol.* **10:** 301–304.
3. Kunkel, H. G., and A. G. Bearn. 1954. Phospholipid

studies of different serum lipoproteins employing P_{32}. *Proc. Soc. Exp. Biol. Med.* **86:** 887–891.
4. Eder, H. A., J. H. Bragdon, and E. Boyle. 1954. The in vitro exchange of phospholipid phosphorus between lipoproteins. *Circulation.* **10:** 603.
5. Minari, O., and D. B. Zilversmit. 1963. Behavior of dog lymph chylomicron lipid constituents during incubation with serum. *J. Lipid Res.* **4:** 424–436.
6. Lovelock, J. E., A. T. James, and C. E. Rowe. 1960. Lipids of whole blood. 2. The exchange of lipids between the cellular constituents and the lipoproteins of human blood. *Biochem. J.* **74:** 137–140.
7. Sakagami, T., O. Minari, and T. Orii. 1965. Behaviour of plasma lipoproteins during exchange of phospholipids between plasma and erythrocytes. *Biochim. Biophys. Acta.* **98:** 111–116.
8. Reed, C. F. 1968. Phospholipid exchange between plasma and erythrocytes in man and dog. *J. Clin. Invest.* **47:** 749–760.
9. Shohet, S. B. 1970. Release of phospholipid fatty acid from human erythrocytes. *J. Clin. Invest.* **49:** 1668–1678.
10. Tarlov, A. 1968. Turnover of mitochondrial phospholipids by exchange with soluble lipoproteins in vitro. *Federation Proc.* **27:** 458. (Abstr.)
11. Wirtz, K. W. A., and D. B. Zilversmit. 1969. Participation of soluble liver proteins in the exchange of membrane phospholipids. *Biochim. Biophys. Acta.* **193:** 105–116.
12. Zilversmit, D. B. 1971. Exchange of phospholipid classes between liver microsomes and plasma: comparison of rat, rabbit, and guinea pig. *J. Lipid Res.* **12:** 36–42.
13. Illingworth, D. R., and J. Glover. 1971. The composition of lipids in cerebrospinal fluid of children and adults. *J. Neurochem.* **18:** 769–776.
14. Kuksis, A., W. C. Breckenridge, L. Marai, and O. Stachnyk. 1969. Molecular species of lecithins of rat heart, kidney, and plasma. *J. Lipid Res.* **10:** 25–32.
15. Marai, L., and A. Kuksis. 1969. Molecular species of lecithins from erythrocytes and plasma of man. *J. Lipid Res.* **10:** 141–152.
16. Portman, O. W., M. Alexander, and C. A. Maruffo. 1967. Nutritional control of arterial lipid composition in squirrel monkeys: major ester classes and types of phospholipids. *J. Nutr.* **91:** 35–46.
17. Illingworth, D. R., and J. Glover. 1970. Lecithin:cholesterol acyl transferase activity in human cerebrospinal fluid. *Biochim. Biophys. Acta.* **220:** 610–613.
18. Sugano, M., and O. W. Portman. 1964. Fatty acid specificities and rates of cholesterol esterification *in vivo* and *in vitro*. *Arch. Biochem. Biophys.* **107:** 341–351.
19. Burstein, M., H. R. Scholnick, and R. Morfin. 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **11:** 583–595.
20. Rottger, H. 1953. Ein neues lichtelektrisches Auswertegerät des Papier-Electrophorese Streifens. *Klin. Wochenschr.* **31:** 85.
21. Jencks, W. P., and E. L. Durrum. 1955. Paper electrophoresis as a quantitative method: the staining of serum lipoproteins. *J. Clin. Invest.* **34:** 1437–1448.
22. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
23. Parker, F., and N. F. Peterson. 1965. Quantitative analysis of phospholipids and phospholipid fatty acids from silica gel thin-layer chromatograms. *J. Lipid Res.* **6:** 455–460.
24. Portman, O. W., and M. Alexander. 1969. Lysophosphatidylcholine concentrations and metabolism in aortic intima plus inner media: effect of nutritionally induced atherosclerosis. *J. Lipid Res.* **10:** 158–165.
25. Portman, O. W., P. Soltys, M. Alexander, and R. E. Behrman. 1968. Plasma lysolecithinase activity in pregnancy. *Biochim. Biophys. Acta.* **167:** 610–612.
26. Portman, O. W., P. Soltys, M. Alexander, and T. Osuga. 1970. Metabolism of lysolecithin in vivo: effects of hyperlipemia and atherosclerosis in squirrel monkeys. *J. Lipid Res.* **11:** 596–604.
27. Arvidson, G. A. E. 1968. Structural and metabolic heterogeneity of rat liver glycerophosphatides. *Eur. J. Biochem.* **4:** 478–486.
28. Zilversmit, D. B. 1960. The design and analysis of isotope experiments. *Amer. J. Med.* **29:** 832–848.
29. Phillips, G. B. 1959. The phospholipid composition of human serum lipoprotein fractions separated by ultracentrifugation. *J. Clin. Invest.* **38:** 489–493.
30. Switzer, S., and H. A. Eder. 1965. Transport of lysolecithin by albumin in human and rat plasma. *J. Lipid Res.* **6:** 506–511.
31. Wirtz, K. W. A., L. M. G. van Golde, and L. L. M. van Deenen. 1970. The exchange of molecular species of phosphatidyl choline between mitochondria and microsomes of rat liver. *Biochim. Biophys. Acta.* **218:** 176–179.
32. Wirtz, K. W. A., and D. B. Zilversmit. 1970. Partial purification of phospholipid exchange protein from beef heart. *FEBS Lett.* **7:** 44–46.
33. Camejo, G., Z. M. Suárez, and V. Muñoz. 1970. The apolipoproteins of human plasma high density lipoprotein: a study of their lipid binding capacity and interaction with lipid monolayers. *Biochim. Biophys. Acta.* **218:** 155–166.
34. Lux, S., A. Nichols, G. Forte, E. Gong, and R. I. Levy. 1970. Molecular transformations of high density lipoprotein. *Circulation.* **42 (Suppl. 3):** 7. (Abstr.)
35. Shore, B., and Shore, V. 1969. Isolation and characterization of polypeptides of human serum lipoproteins. *Biochemistry.* **8:** 4510–4516.
36. Scanu, A. M. 1970. The effect of reduction and carboxymethylation on the circular dichroic spectra of two polypeptide classes of serum high density lipoproteins. *Biochim. Biophys. Acta.* **200:** 570–572.
37. Sodhi, H. S., and R. G. Gould. 1970. Interaction of apoHDL with HDL and with other lipoproteins. *Atherosclerosis.* **12:** 439–450.
38. Sugano, M., and O. W. Portman. 1965. Essential fatty acid deficiency and cholesterol esterification activity of plasma and liver *in vitro* and *in vivo*. *Arch. Biochem. Biophys.* **109:** 302–315.
39. Akanuma, Y., and J. Glomset. 1968. In vitro incorporation of cholesterol-^{14}C into very low density lipoprotein cholesterol esters. *J. Lipid Res.* **9:** 620–626.