ASBMB
JOURNAL OF LIPID RESEARCH

Influence of lecithin acyl chain composition on the kinetics of exchange between chylomicrons and high density lipoproteins

George M. Patton, Sander J. Robins, Joan M. Fasulo, and Susanne Bennett Clark

Department of Medicine, Boston Veterans Administration Medical Center, Boston, MA 02130, and Biophysics Institute, Boston University School of Medicine, Boston, MA 02118

Abstract The kinetics of lecithin exchange between native lipoproteins was characterized for individual molecular species of lecithins of rat mesenteric lymph chylomicrons and rat plasma HDL. Studies were performed in the absence of lipid transfer proteins. Donor (chylomicrons) and acceptor (HDL) particles were present in ratios of 1:1 and 1:10 with respect to total phospholipid. Biphasic exchange kinetics were observed for all major lecithins common to chylomicrons and HDL at both proportions of donor to acceptor particles. During the early rapid phase of exchange, complete in about 30 min, 40–60% of the total lecithin pool was exchanged. Initial exchange rates were most rapid for the more hydrophilic species of the major lecithins normally present in both lipoproteins. Calculated activation energies correspondingly were least for a diunsaturated lecithin (18:1–20:4), intermediate for lecithins were 16:0 in position-1 (16:0–18:2 and 16:0–20:4), and highest for analogous lecithins with 18:0 in position-1. A 10-fold increase in the ratio of acceptor to donor particles affected neither the biphasic nature of the exchange nor the rates of exchange of individual molecular species (consistent with exchange by diffusion rather than by particle collisions). Total equilibration of individual molecular lecithin species was achieved by 24 hr (37°C, donor to acceptor ratio of 1:1) with only a small change in the relative mass of lecithins in chylomicrons and HDL. Novel lecithins containing 18:3, incorporated into chylomicrons, were found to exchange exceedingly rapidly. The presence of 18:3-containing lecithins did not affect total lecithin exchange between chylomicrons and HDL; however, exchange of these lecithins did result in suppression of exchange of other lecithin species that were normally present in both lipoproteins. These results demonstrate that rapid lecithin exchange can occur between native lipoproteins in the absence of exchange proteins, that individual molecular lecithins exchange at different rates, and that individual rates of exchange are commensurate with the relative hydrophilic strength of individual molecules. By introducing novel lecithins into chylomicrons it was possible to demonstrate that exchange is not restricted to lecithin molecules of identical composition and, more importantly, that the rate of exchange of any *single* lecithin molecule from the surface of a lipoprotein is in large part dependent upon the composition of other lecithin molecules on that same surface.—**Patton, G. M., S. J. Robins, J. M. Fasulo, and S. Bennett Clark.** Influence of lecithin acyl chain composition on the kinetics of exchange between chylomicrons and high density lipoproteins. *J. Lipid Res.* 1985. **26:** 1285–1293.

Supplementary key words lecithin molecular species • non-protein mediated exchange

Lecithin, which is a principal component of all animal cell membranes, has many molecular forms. Although the functional significance of the distinct lecithins is not known, it is clear that lecithins of different composition are synthesized at different rates (see ref. 1 for review) and may interact physically with other membrane components with different efficiencies. Many membranes that do not synthesize phospholipids (PL) acquire preformed PL from other sites by exchange processes[1] and/or net uptake (for reviews, see refs. 2 and 3). It has been demonstrated repeatedly that lecithins are readily exchanged between isolated cellular organelles as well as between plasma lipoproteins, red cell membranes, and liposomes. In addition, in several recent studies (4–6), this exchange process has been characterized for individual lecithin molecules with different acyl chain compositions. However, in no previous study of lecithin exchange has this process been assessed for unmodified, individual molecular species of lecithins present in naturally occurring particles, which contain not only lecithins but also a complex array of other biologically active molecules. It was the purpose of the present study to establish whether lecithins of different composition exchange with quantitatively different rates or qualitatively different patterns between native lipoproteins. To accomplish this aim we employed recently developed HPLC methods to isolate intact molecular species of lecithin (7) and we examined exchange between rat lymph chylomicrons, whose lecithin composition could be markedly changed by diet, and plasma HDL obtained from fasting rats.

The studies we report were performed in the absence of lipid transfer proteins. The results showed that all leci-

Abbreviations: HPLC, high performance liquid chromatography; DTNB, 5,5-dithiobis nitrobenzoic acid; PL, phospholipid(s); HDL, high density lipoprotein(s); ULV, unilamellar vesicles.

[1]Exchange is used in this report to indicate the bidirectional movement of lecithin molecules that occurs without a change in total lecithin mass.

Downloaded from www.jlr.org by guest, on June 18, 2012

thins exchanged between these lipoproteins, but that the rates of exchange were strongly influenced by the acyl chain composition of the lecithins. Furthermore, even in the absence of a PL transfer protein, the two lipoprotein particles ultimately (within 24 hr) equilibrated with respect to lecithin composition. While we have confined our observations to the transfer of lecithins between two lipoproteins in the absence of exchange proteins, general patterns were observed that might have broad applicability for PL transport at other sites in the live animal.

MATERIALS AND METHODS

Preparation of lipoproteins

HDL. Male Sprague-Dawley rats (250-350 g) were fasted overnight, anesthetized, and bled from the abdominal aorta. Plasma was separated in the presence of DTNB (0.5 mg/ml), Na azide (0.5 mg/ml), and Na_2 EDTA (1 mg/ml), and an HDL fraction was isolated in the density range 1.08-1.19 g/ml by sequential ultracentrifugation. HDL were washed once and reisolated at d 1.19 g/ml. They were then dialyzed at 10°C for 48 hr against 10 mM Tris-150 mM NaCl (pH 7.4) buffer containing 1 mM EDTA, and were used immediately.

HDL prepared in this way were shown to be free of PL exchange proteins by a modification of the method of Damen, Regts, and Scherphof (8), as follows. HDL, containing lecithins radiolabeled with choline, were prepared from plasma obtained from a rat 12 hr after a 10-min duodenal pulse of [^{14}C-methyl]choline chloride (New England Nuclear, Boston, MA; 2 μmol; 60 μCi). Unilamellar vesicles (ULV) of egg yolk lecithin (Sigma Chemical Co., St. Louis, MO) were prepared by sonication in 10 mM Tris-150 mM NaCl (pH 7.4) buffer; multilamellar liposomes were removed by ultracentrifugation in a swinging bucket rotor (SW 41, 30 min, 30,000 rpm). Approximately equal amounts of ^{14}C-labeled HDL lecithin and unlabeled ULV lecithin were mixed and incubated at 37°C, with gentle shaking, for 0 or 60 min; control tubes (0 and 60 min) contained an equal volume of buffer in place of ULV. All incubations were performed in duplicate. Immediately after mixing (0 time) or after 60 min, HDL were precipitated from the mixtures with heparin and manganese (8) and the amounts of ^{14}C label in supernatants and precipitates were determined. Less than 3% of the label transferred from HDL to ULV in 60 min, consistent with the absence of a PL transfer protein (8).

Chylomicrons. Mesenteric lymph chylomicrons containing ^{14}C-labeled lecithins were obtained from male Sprague-Dawley lymph fistula rats (270-300 g), prepared with gastric and duodenal infusion cannulas. A continuous gastric saline infusion (NaCl 8.5 g/l and KCl 0.3 g/l) was maintained at 2.7 ml/hr from the time of surgery. The rats were allowed to recover for 6 hr, then a gastric bolus of a lipid mixture (300 mg of corn oil, 300 mg of olive oil, and 10 mg of arachidonic acid) was administered. About 2 hr later, during active chylomicron secretion, the rats received a 10-min duodenal pulse of [^{14}C-methyl]choline chloride (2 μmol, 60-150 μCi). Lymph was then collected for 5-12 hr at 0°C in the presence of DTNB (0.5 mg/ml), Na azide (0.5 mg/ml), and Na_2 EDTA (1 mg/ml, approximate final concentrations). Labeled chylomicrons were isolated from the lymph at 4°C by ultracentrifugation in a swinging bucket rotor (3×10^6 *g*-min). They were harvested by tube slicing, resuspended in an isotonic NaCl solution at 0-4°C and used within 18 hr. Specific activities of individual lecithins ranged from 1 to 10,000 dpm/μg of phosphorus.

In order to enrich chylomicrons with lecithin species not present in fasting plasma HDL for use in specific experiments (see Results), some rats were fed only trilinolenin. These rats were prepared with a bile fistula and were infused via the duodenum with Na taurocholate (30 μmol/hr) and glucose (150 mg/hr) in addition to the maintenance infusion of saline, above. We have shown previously (9) that removal of biliary lipids from the intestinal lumen markedly enhances the incorporation of dietary triglyceride fatty acids into chylomicron lecithins. The bile fistula rats were given a gastric dose of 500 mg of trilinolenin (Applied Science Laboratories, Inc., State College, PA), followed 1.5 hr and 5 hr later by two separate duodenal pulses of 50 μCi (2 μmol) [^{14}C-methyl]choline chloride in order to maximize the lecithin specific activities.

Lipoprotein incubations

Incubations were performed in glass culture tubes (ID 1.2 cm) in temperature-controlled water baths, with continuous shaking at 1 cycle per sec. Individual tubes, prepared in duplicate, were used for each time point. In most experiments the tubes contained, in a total volume of 1.2 ml, equal amounts of chylomicron and HDL lipid phosphorus (approximately 20 μg of each), of which 80-85% was present in the lecithin fraction in both lipoprotein species. In one experiment a ten-fold excess of HDL was present in the same total volume. After incubation, the tubes were immediately cooled to 0°C, which prevented further lecithin exchange (see **Fig. 1**). The contents of each incubation tube were transferred quantitatively to a cellulose nitrate ultracentrifuge tube and the density was adjusted to 1.12 g/ml with KBr. The lipoprotein mixture was overlayered with 2.7 ml of d 1.012 g/ml NaCl and chylomicrons were floated (within 20 min) by ultracentrifugation at 3×10^6 *g*-min in a fixed-angle rotor (50.1) at 0-4°C. The tubes were then frozen in dry ice and the packed chylomicrons were sliced off (approximately 1 ml). The bottom layer contained the HDL (approximately 4 ml). Each lipoprotein fraction was extracted with chloroform-methanol 2:1 (10) and the phosphorus content and radioactivity of individual molecular lecithin species iso-

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

ASBMB
JOURNAL OF LIPID RESEARCH

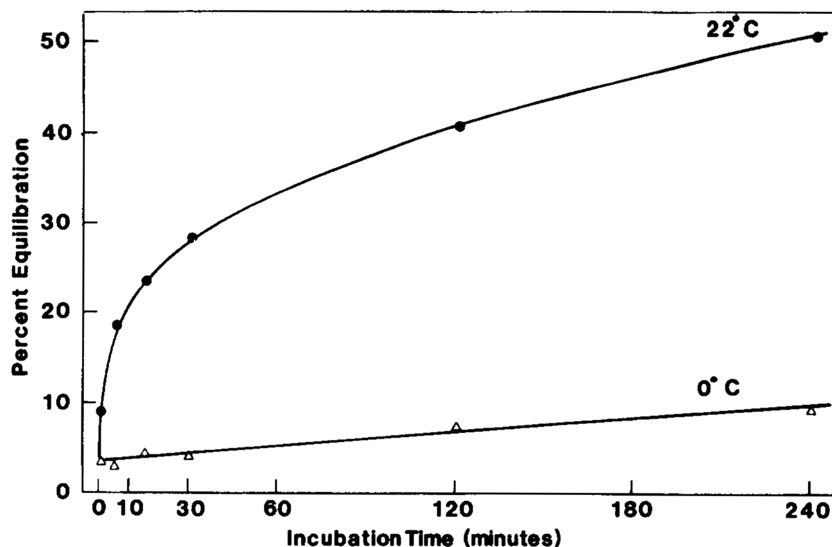

Fig. 1. Comparison of lecithin exchange between chylomicrons and HDL at 22°C and 0°C. Lymph chylomicrons containing [^{14}C-methyl]choline-labeled lecithins and plasma HDL were prepared as described in Methods. Incubation of equal amounts of chylomicrons and HDL total lecithin were performed for up to 4 hr and the lipoproteins were reisolated at 0-1°C.

Downloaded from www.jlr.org by guest, on June 18, 2012

lated from the organic phase were determined as described previously (9).

Chemical methods

Lecithins were first separated from neutral lipids and other PL by HPLC on a silica column (7) and then were further separated into individual molecular species by reverse phase HPLC (7). Individual lecithins were collected for quantitation of both phosphorus (11) and radioactivity (by liquid scintillation spectrometry).

Calculations

Specific activities (dpm per μg P) were calculated for each lecithin species collected from the HPLC column. The specific activity predicted after complete equilibration was calculated for each lecithin species from the total mass of that species in chylomicrons plus HDL and the radioactivity present in that species in the unreacted chylomicrons. Individual data points generally were expressed as a percentage of this theoretical (equilibrium) specific activity found in HDL ("% equilibration").

RESULTS

The lecithin compositions of chylomicrons obtained from rats fed the lipid mixture (see Methods) and of HDL obtained from the plasma of fasted rats differed significantly and individual preparations were each quite reproducible (**Table 1**). All lecithin species in the chylomicrons were labeled in the choline methyl group by a duodenal pulse of [^{14}C-methyl]choline chloride, as outlined in Methods. Transfer of labeled lecithins from chylomicrons to HDL generally was monitored for up to 3 hr at two temperatures, 22°C and 37°C, by following the progressive increase in the specific activity of the total lecithin pool and of each lecithin species in the HDL fraction. Recoveries (measured as total lipid phosphorus) after incubation of chylomicrons and HDL in a 1:1 proportion averaged 97.4 ± 4.1 (SD)% (for 36 samples). In these same incubations, relative changes in the total lipid phosphorus in chylomicrons and HDL were small, averaging 9.8 ± 6.4 (SD)% (with a loss of chylomicron mass and a gain in HDL).

TABLE 1. Major lecithins of chylomicrons and HDL

Lecithin Species	% of Lecithin Species[a]	
	Chylomicrons[b]	HDL[c]
16:0-22:6	1.7 ± 0.6[d]	5.0 ± 0.6[d]
16:0-20:4	13.3 ± 2.2	16.2 ± 0.9
16:0-18:2	30.0 ± 0.8	16.1 ± 1.3
16:0-18:1	7.8 ± 1.1	4.8 ± 0.3
18:0-22:6	4.9 ± 0.5[e]	6.4 ± 0.5
18:0-20:4	9.9 ± 1.2	33.5 ± 2.9
18:0-18:2	16.6 ± 0.7	12.8 ± 1.0
18:2-18:1[f]	12.2 ± 1.3	3.4 ± 0.5
18:2-18:2	3.1 ± 0.6	0.5 ± 0.4

[a]Only lecithins containing >1.5% of the total lecithin phosphorus are shown. The listed lecithins comprise 78-80% of the total lecithins.
[b]Chylomicrons were obtained from mesenteric lymph of rats fed a mixture of corn oil, olive oil, and arachidonic acid, as described in Methods.
[c]HDL were isolated from pooled plasma of rats fasted overnight, as described in Methods.
[d]Mean ± SE of four separate lipoprotein preparations analyzed prior to mixing.
[e]Also contained 18:1-18:1 lecithin.
[f]The specific positions of the acyl groups were not determined for this particular lecithin. In all other lecithins the first fatty acid listed is in position 1 of the lecithin and the second is in position 2.

The fractional equilibration of the total lecithin pool, monitored for 3 hr, was biphasic both in the presence and absence of a large excess of HDL acceptors (**Fig. 2**). Exchange of each individual lecithin also was biphasic and the rates differed among the differing lecithin species. Representative kinetics, expressed as percent equilibration during incubation of equal lipid phosphorus amounts of chylomicron and HDL, are shown for the four major lecithins common to both chylomicrons and HDL in **Fig. 3**. Changes in the specific activities of the chylomicron lecithins also were routinely monitored and were found to be reciprocal to the changes occurring in HDL (data not shown). When equal amounts of chylomicron and HDL total lecithins were present in the incubation, analysis of variance revealed that equilibration of the individual 1-16:0-lecithins was always more complete at each time point and at both 22°C and 37°C than equilibration of the analogous 1-18:0-lecithins ($P < 0.001$). On the other hand, for the four major lecithins shown in Fig. 3, no influence of the 2-acyl fatty acid (18:2 vs 20:4) on the lecithin equilibration rate was observed. Exchange was significantly faster for all lecithins at 37°C than at 22°C.

The data obtained from incubations performed at 37°C for up to 3 hr were fitted to biexponential functions of the form $y = Ae^{-k_1t} + Be^{-k_2t}$, using an iterative curve-fitting procedure (12) to an accuracy of 1%. In this calculation, it was assumed that, at equilibrium, when the lecithin compositions of the two lipoproteins became identical (see Table 4), the specific activities of all HDL lecithin species would be one-half of those of the original chylomicron lecithins when equal amounts of donor and acceptor were present. When, instead, a 10-fold excess of HDL acceptor was present, it was assumed that at equilibrium the specific activities of HDL lecithins would be one-tenth of original chylomicron lecithin specific activities. The computed constants for the most abundant lecithins present in both chylomicrons and HDL are listed in **Table 2**.

To assess the possibility that, despite the demonstrated absence of PL transfer activity in the HDL fraction, such activity may nevertheless have been present in the chylomicron fraction, in one experiment both lipoproteins were heated at 56°C for 1 hr prior to incubation. This treatment inactivates the PL transfer factor of rat plasma (13). Equilibration of total lecithins was similar for heated and unheated lipoproteins (at 15 min, 56.4% and 63.2%, respectively).

Temperature dependence of lecithin exchange

The temperature dependence of lecithin exchange between equal amounts of chylomicrons and HDL was studied at four temperatures, from 22–37°C (**Table 3**). Fractional equilibration of individual lecithin species (% per hr) was calculated from the results obtained after the shortest practical experimental incubation period (5 min). These values were used as approximations of initial exchange rates. During short incubations no change in HDL lecithin composition was detected by phosphorus

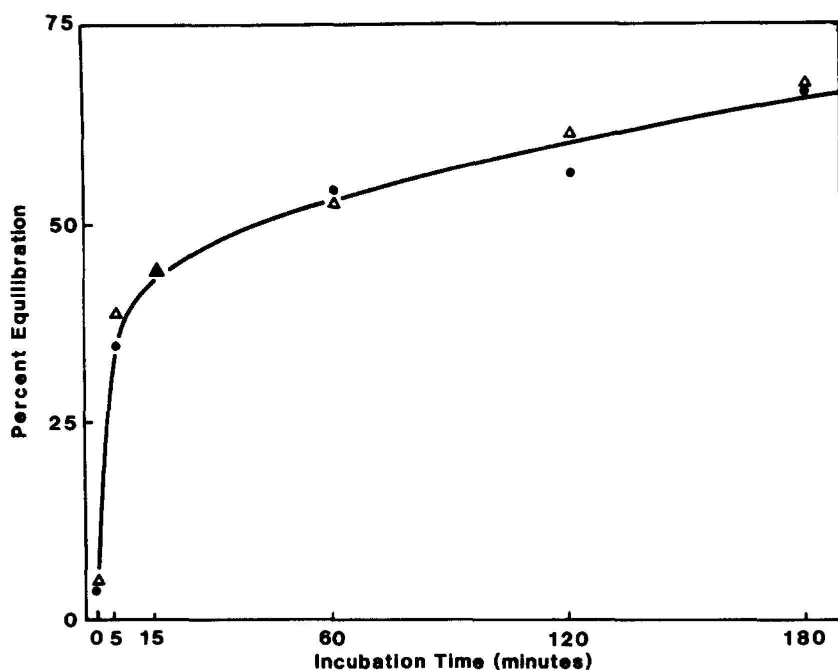

Fig. 2. Lecithin exchange (expressed as percent equilibration) between chylomicrons and HDL with equal amounts of chylomicron and HDL lecithins present (●) and with a 10-fold excess of HDL lecithins present (△). Incubations were performed at 37°C.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH

ASBMB
JOURNAL OF LIPID RESEARCH

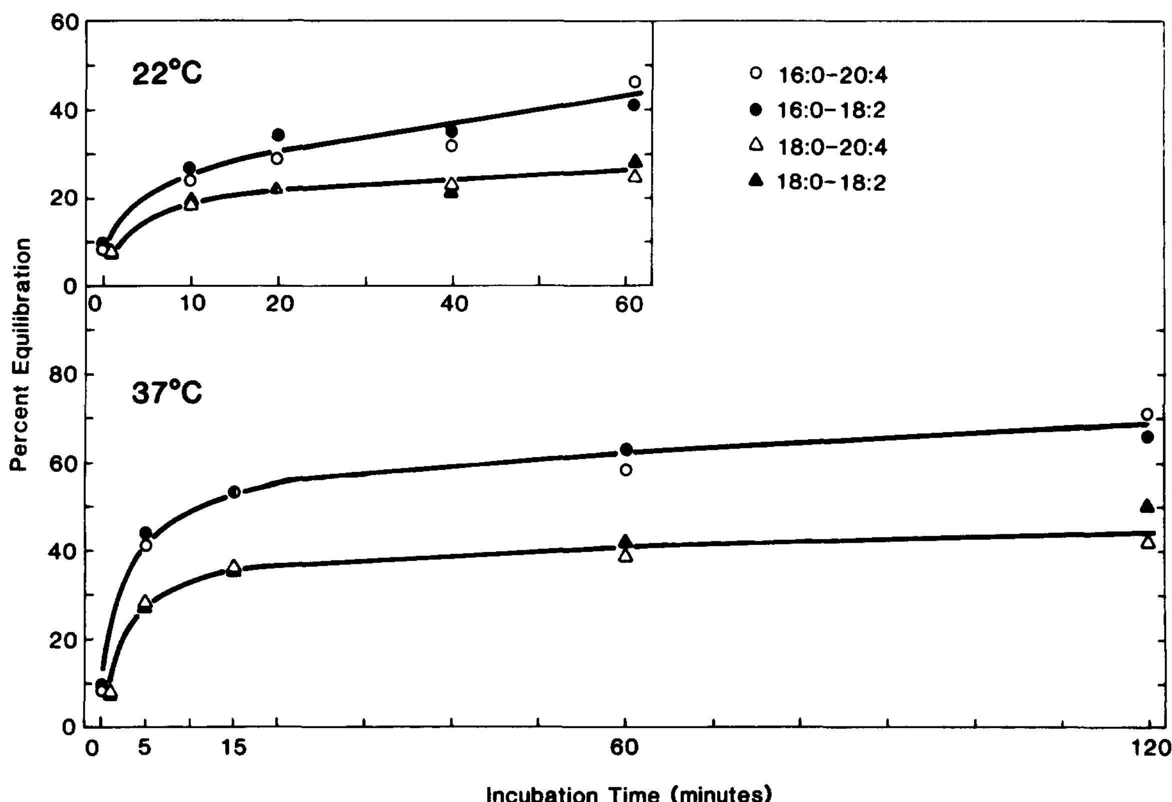

Fig. 3. Exchange of labeled lecithins between chylomicrons and HDL at 22°C (insert) and at 37°C. Lymph chylomicrons and plasma HDL were prepared as described in Methods. The figure shows the appearance of radiolabeled lecithins in HDL expressed as percent equilibration. Only the four major lecithin species common to both lipoproteins are shown.

quantitation of individual lecithin species (see Table 3), so that, within the limits of analytical error, the transfer of radioactivity from chylomicrons to HDL over 5 min represented exchange and not net mass transfer. The apparent acctivation energies calculated from Arrhenius plots of the data are listed in Table 3. As a group, 1-16:0-lecithins demonstrated lower activation energies (44-49 KJ mol^{-1}) than 1-18:0-lecithins (55-75 KJ mol^{-1}). The

TABLE 2. Exchange kinetics for major lecithins of chylomicrons and HDL[a]

Lecithin Species	HDL to Chylomicron Ratio	A	k_1	$t_{1/2}(1)$	B	k_2	$t_{1/2}(2)$	k_e[b]	$t_{1/2}$
				min			*hr*		*hr*
16:0-20:4	1:1	0.43	0.31	2.2	0.50	0.0030	3.9	0.0055	2.1
	10:1	0.39	0.47	1.5	0.45	0.0073	1.6	0.0134	0.9
16:0-18:2	1:1	0.43	0.33	2.1	0.49	0.0036	3.2	0.0067	1.7
	10:1	0.30	0.33	2.1	0.60	0.0024	4.8	0.0036	3.2
18:0-20:4	1:1	0.29	0.26	2.8	0.65	0.0010	11.6	0.0014	8.0
	10:1	0.34	0.21	3.3	0.48	0.0026	4.5	0.0044	2.6
18:0-18:2	1:1	0.28	0.28	2.5	0.66	0.0014	0.8	0.0020	5.8
	10:1	0.31	0.11	6.3	0.55	0.0010	11.6	0.0016	7.4
Total lecithins	1:1	0.37	0.27	2.6	0.56	0.0027	4.3	0.0045	2.6
	10:1	0.31	0.42	1.7	0.58	0.0033	3.5	0.0050	2.3

[a]Individual data points (N = 18-23) from four separate experiments were fitted to the equation $y = Ae^{-k_1t} + Be^{-k_2t}$. For the parameter values shown, $\Sigma(C_i + C_1 - C_1)^2 \leq 0.01$ (where C_i = A, k_1, B, or k_2) and the Residual Error, $\Sigma(Y_{obs} - y_{calc})^2/\Sigma y_{obs}^2 \leq 0.001$.

[b]$K_e = \dfrac{A + B}{A/k_1 + B/k_2}$.

Downloaded from www.jlr.org by guest, on June 18, 2012

TABLE 3. Temperature dependence of lecithin exchange

HDL-Lecithin Species	% of Total HDL Lecithin Phosphorus					% Equilibration of ^{14}C Label[c]				E[d]
	Control[a]	22°C[b]	27°C[b]	32°C[b]	37°C[b]	22°C	27°C	32°C	37°C	KJ mol^{-1}
16:0–22:6	4.6	4.5	5.3	4.4	4.2	16.3		27.5	39.2	43.8
16:0–20:4	17.0	16.7	17.5	17.1	17.0	12.9	17.7	25.1		49.8
16:0–18:2	17.2	16.8	16.7	20.0	18.2	12.0		23.9	31.2	48.9
18:0–22:6	5.9	6.3	5.8	6.2	6.1	8.1	13.4	22.2	25.3	75.4
18:0–20:4	31.7	32.0	30.0	27.9	28.7	7.3	11.3		22.0	55.3
18:0–18:2	10.0	11.7	11.2	10.9	10.9	7.0	11.0	16.5		64.2
18:2–18:1[e]	4.1	4.2	4.1	4.3	6.0	18.0	30.3	35.1	41.3	23.9

[a] ^{14}C-Labeled chylomicrons and unlabeled HDL, each 18–20 μg of phosphorus, were mixed at 0°C and immediately reisolated as described in Methods.
[b] Chylomicrons and HDL were incubated for 5 min at the temperature indicated and were reisolated as described.
[c] Corrected for exchange at zero times, 0°C.
[d] Arrhenius plots (ln percent equilibration vs l/T°K) were fitted to the data points and E values were calculated from the slopes (– E/R) for each lecithin species. All data are means of duplicate incubations.
[e] The specific positions of the acyl groups were not determined.

overall activation energy for the total lecithin mixture was 52.7 KJ mol^{-1} and fell between those of the major 16:0- and 18:0-species.

Transfer of novel lecithins from chylomicrons to HDL

In order to determine whether the exchange of lecithin molecules would ultimately result in the complete equilibration of all lecithin species between chylomicrons and HDL, chylomicrons which contained lecithins that differed radically from those of fasting plasma HDL were prepared for incubation. The lecithin composition of these chylomicrons that were enriched with 18:3 is shown in **Table 4**. Incubations with HDL were performed for up to 24 hr at 37°C. During this time the lecithin compositions of the chylomicrons and the HDL approached one another, demonstrating unequivocally that all individual lecithins are exchangeable between donors and acceptors. There was no evidence that the acquisition of the novel lecithin species by HDL from chylomicrons (18:3–18:3, 16:0–18:3, and 18:0–18:3) preferentially displaced any other HDL lecithin species in particular. Instead, the results suggested that each species ultimately equilibrated without influence from any other lecithin. However, the presence of 18:3-containing lecithins did influence the rapid exchange phase of other lecithins during short incubations. The exchange of 1-16:0- and 1-18:0-lecithins between chylomicrons and HDL was slower when lecithins containing 18:3 were introduced into the chylomicrons. At 5 and 15 minutes, all major 16:0- and 18:0-lecithins appeared to be affected (**Fig. 4**). The fractional equilibration of the total lecithin pool was, however, unaltered.

Transfer at 0°C

All the data tabulated in Tables 1–4 were corrected for a "zero time" transfer of label which occurred with each experiment. When chylomicrons were mixed with HDL

TABLE 4. Equilibration of lecithins among chylomicrons and HDL

Lecithin Species	% of Major Lecithin Species[a]					
	Before Mixing		Zero Time at 0°C		After 24 hr at 37°C	
	Chylomicrons[b]	HDL[c]	Chylomicrons[b]	HDL[c]	Chylomicrons[b]	HDL[c]
16:0–22:6	2.8	6.6	2.9[d]	5.9[d]	4.3	4.9
16:0–20:4	5.0	16.7	6.3	16.3	11.2	11.9
16:0–18:2	12.3	19.3	12.8	16.7	15.1	14.5
16:0–18:1	3.5	4.0	3.2	3.1	4.5	4.3
18:0–22:6	2.8	7.1	3.6	7.0	4.1	5.5
18:0–20:4	7.5	25.8	10.1	24.8	15.7	20.7
18:0–18:2	15.8	13.5	14.8	13.5	14.5	14.5
18:2–18:1[e]	4.8	2.7	5.6	3.0	4.3	3.4
18:3–18:3	13.8	0	12.2	2.1	5.7	6.7
16:0–18:3	12.8	0	11.3	2.1	6.1	5.5
18:0–18:3	17.8	0	16.2	1.6	12.7	7.0

[a] Only lecithins containing >1.5% of the total phosphorus were included. The listed lecithins comprise 78–80% of the total.
[b] Chylomicrons were obtained from mesenteric lymph of rats fed trilinolenin.
[c] HDL were obtained from fasting rat plasma, as described in Methods.
[d] Means of duplicate incubations.
[e] The specific positions of the acyl groups were not determined.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

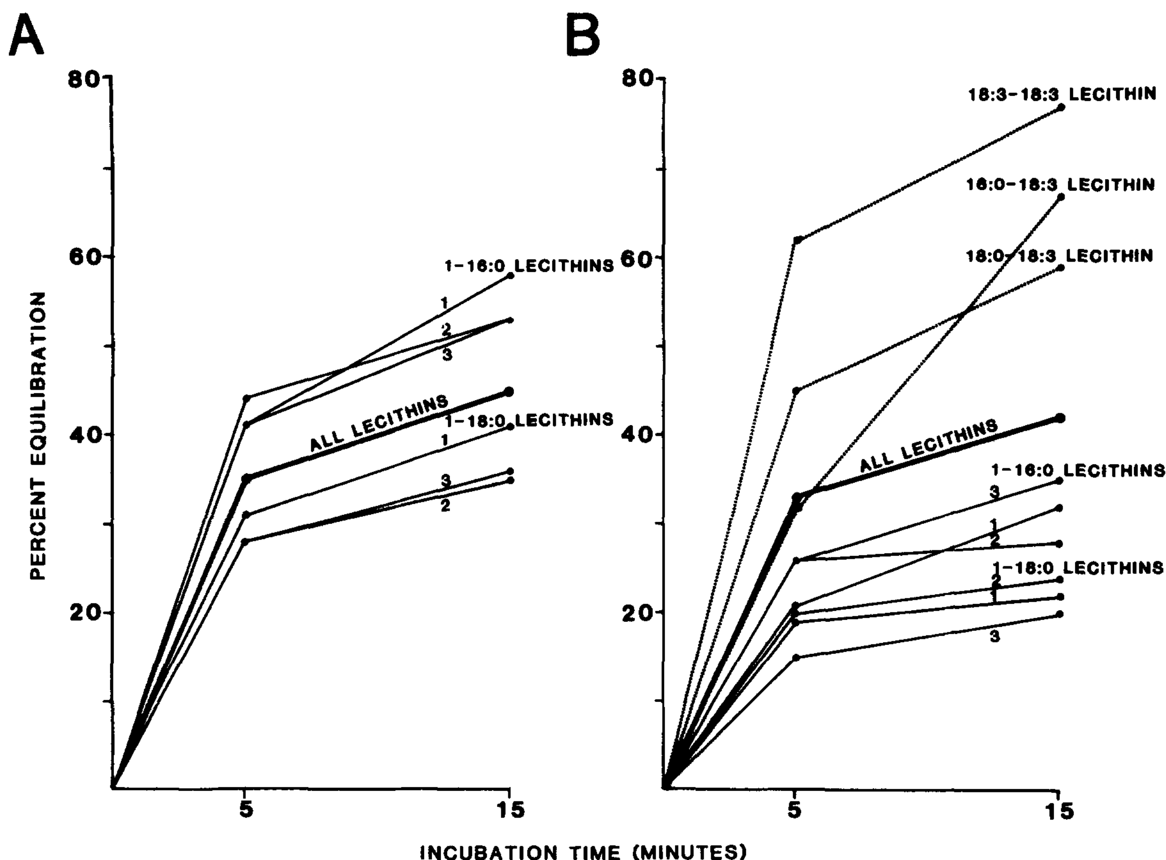

Fig. 4. Effect of lecithins containing 18:3 on lecithin exchange between chylomicrons and HDL. The percent equilibration of the total lecithin pool and individual lecithins was determined using chylomicrons obtained after feeding a mixture of corn oil, olive oil, and arachidonic acid (panel A, mean of four experiments) and after feeding trilinolenin (panel B, single experiment). The composition of chylomicrons used in panel A is shown in Table 1 and the composition used in panel B is shown in Table 4. Incubations were performed at 37°C, with the lecithins in chylomicrons and HDL in a 1:1 proportion. Major 1-16:0 and 1-18:0 lecithin species are numbered as follows: 1, 16:0(18:0)-22:6; 2, 16:0(18:0)-18:2; and 3, 16:0(18:0)-20:4.

at 0°C, there was an immediate exchange of all labeled lecithins to HDL, including species not present in freshly isolated HDL (**Table 5**). The amount exchanged was slightly greater when HDL were present in excess. However, when chylomicrons were incubated with an equal volume of saline at 0°C or 37°C for up to 3 hr before reisolation by ultracentrifugation, there was no loss of label to the infranatant phase. Thus, an acceptor particle (HDL) was required for the small, instantaneous transfer of lecithins from chylomicrons that was observed in all experiments.

DISCUSSION

Many reports (4, 5, 8, 13-24) have established that cholesterol and PL are extensively transferred among lipoproteins and between lipoproteins and tissues. A number of studies has focused on the exchange of the principal PL component of lipoproteins, lecithin (4, 5, 8, 18-23), and more specifically, on individual molecular species of lecithin (4, 5, 19). However, in these studies, lecithins have been chemically modified and/or exchange has been determined in model systems in which vesicles or lipoprotein recombinants have been utilized. We are aware of no previous study of lecithin exchange in which this process has been characterized for unmodified, individual lecithin molecules that are components of native

TABLE 5. Instantaneous transfer of labeled lecithins to HDL at 0°C

Lecithin species	% Equilibration[a] 1:1	% Equilibration[a] 1:10
16:0-22:6	8.9 ± 1.1	13.8 ± 0.6
16:0-20:4	8.4 ± 0.9	16.3 ± 1.6
16:0-18:2	8.9 ± 0.8	9.8 ± 0.3
18:0-22:6	6.5 ± 0.5	9.7 ± 0.8
18:0-20:4	7.1 ± 0.9	17.6 ± 0.4
18:0-18:2	7.0 ± 0.7	9.8 ± 0.7
18:2-18:1[b]	10.5 ± 0.9	12.9 ± 1.3
Total lecithins	6.9 ± 0.7	9.4 ± 1.7

Chylomicrons and HDL, 1:1 and 1:10 with respect to total lecithin content, were mixed at 0°C and immediately reisolated as described in Methods.

[a]Mean ± SE of four to ten experiments.

[b]The specific positions of the acyl groups were not determined.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH

lipoproteins. In the present study, we have examined lecithin exchange between lymph chylomicrons and plasma HDL because these lipoproteins are known to be metabolically related (15) and because, after being mixed in vitro, these lipoproteins can be rapidly separated for analysis.

We have found that the kinetics of exchange of all major lecithins present in both chylomicrons and HDL were qualitatively similar, and occurred in three distinct phases. There was an initial, practically instantaneous, exchange of all lecithin species, which occurred at 0°C (Table 5) and which is as yet unexplained. This phenomenon has been observed previously in studies of lecithin transfer (8, 24) and may be coupled with the transfers of apoproteins E and C and of unesterified cholesterol between lipoproteins, which also occur instantaneously at 4°C (17). The kinetics of the subsequent exchange for all major lecithin species that were common to both classes of lipoproteins was found to be biphasic, suggesting the presence of two functionally distinct populations of lecithins. Addition of excess acceptor particles (in this case, HDL) to the incubations did not alter these kinetics.

Lippiello and Waite (18) recently determined that exchange of the total lecithin pool between chylomicrons obtained from rats fed a chow diet and human plasma HDL is also a biphasic process. Although these authors did not establish that lipid trasnfer proteins were absent in their system, their results are in qualitative agreement with the present results obtained in the unequivocal absence of transfer proteins. They furthermore concluded that lecithin exchange was a "bimolecular", or collisional, event because the rate of the fast phase was dependent on both donor and acceptor concentrations. However, we observed no increase in equilibration rate with a 10-fold increase in acceptor concentration. Thus, and in accord with other studies (4, 20, 23), we conclude that lecithin exchange is not direct from lipoprotein to lipoprotein but, instead, takes place through an intervening aqueous phase. Observed differences in activation energies, calculated for the early rapid phase of exchange, would consequently correspond to differences in the rates at which individual lecithins are initially dissociated from the surface of the lipoproteins into the aqueous medium. In this process the more hydrophilic lecithin species desorbed from lipoproteins most readily (i.e., calculated activation energies were lowest for 18:1-18:2 lecithin, intermediate for all major 16:0-lecithins, and highest for all major 18:0-lecithins (Table 3)).

A pattern of exchange similar to that of the present study, in which more unsaturated lecithins transfer more rapidly than saturated lecithins, has been observed in model systems, e.g., between red cells and liposomes (6, 19) and between lipoprotein recombinants (4). More recently, this same general pattern has also been observed for the exchange of a series of synthetic lecithins incorporated into lipoproteins and model lipoproteins (5). In this last study, although an early rapid phase of exchange was not separately identified, the authors were able to relate overall rates of exchange of individual lecithins to their retention times on a reverse phase (HPLC) column in a sequence that would in general correspond to the relative hydrophilic strength of these particular lecithin molecules. Our findings indicate *1*) that this pattern prevails for naturally occurring lecithins that are present in native, unmodified lipoproteins; and *2*) that the exchange of individual lecithin species can influence the rate of exchange of other individual lecithins that are present in the same population of molecules without affecting the overall rate of exchange of the total population of lecithin molecules. Thus, the introduction (by diet) of rapidly exchanging lecithins containing 18:3 into chylomicrons suppressed exchange of the major 1-16:0- and 1-18:0-lecithins also present in chylomicrons (Fig. 4). These same studies further demonstrated that the exchange of individual lecithins was not restricted to exchange with lecithins of identical composition, since 18:3-containing lecithins were originally present in chylomicrons but not in HDL, yet the two lipoproteins did fully equilibrate in time (Table 4).

Overall, almost total equilibration of both label and mass was observed for all lecithin species within 24 hr at 37°C in spite of the absence of lipid transfer proteins (Table 4). However, PL exchange among lipoproteins in vivo has been thought to be mediated largely by lipid transfer proteins present in plasma. A prominent finding in the present studies was the rapidity with which non-protein-mediated exchange of lecithins occurred among native lipoproteins at a physiological temperature, suggesting that plasma lipid transfer factors may play only a relatively minor role in the exchange of lecithins among lipoproteins in vivo. Thus, in our studies at 37°C, during an initial rapid phase of exchange complete in 20-30 min, fully 40-60% of lecithins had exchanged. Even if non-mediated exchange were restricted only to chylomicrons and HDL in vivo, our findings would have major physiologic significance. Chylomicrons are, of course, freshly generated with every meal, and will contain a large proportion of unsaturated lecithins when a diet rich in polyunsaturated triglycerides is consumed. Through the process of passive exchange of lecithins, chylomicrons may regularly transfer large amounts of these dietary fatty acids to other lipoproteins in the circulation. In view of the differences in the rates of exchange of different lecithins, this process may have a substantial impact on the surface composition and metabolic behavior of all classes of plasma lipoproteins.

This research was supported by the General Medical Research Service of the Veterans Administration and by grants AM28640 and HL26335 from the National Institutes of Health. We are

Downloaded from www.jlr.org by guest, on June 18, 2012

grateful to Ms. Margaret Collins and Mr. Charles Bliss for technical assistance, to Dr. David Atkinson for help with curve fitting, and to Dr. Yvonne Lange for review of this manuscript.

Manuscript received 15 June 1984.

REFERENCES

1. Holub, B. J., and A. Kuksis. 1978. Metabolism of molecular species of diacylglycerophospholipids. *Adv. Lipid Res.* **16:** 1-125.
2. Kader, J-C., D. Douady, and P. Mazliak. 1982. Phospholipid transfer proteins. *In* Phospholipids. J. N. Hawthorne and G. B. Ansell, editors. Elsevier Press, Amsterdam, New York. 279-307.
3. Wirtz, K. W. A. 1982. Phospholipid transfer proteins. *In* Lipid-Protein Interactions. P. C. Jost and O. H. Griffith, editors. Wiley-Interscience, New York. 151-231.
4. Massey, J. B., A. M. Gotto, Jr., and H. J. Pownall. 1982. Kinetics and mechanism of the spontaneous transfer of fluorescent phosphatidylcholines between apolipoprotein-phospholipid recombinants. *Biochemistry.* **21:** 3630-3636.
5. Massey, J. B., D. Hickson, H. S. She, J. T. Sparrow, D. P. Via, A. M. Gotto, Jr., and H. J. Pownall. 1984. Measurement and prediction of the rates of spontaneous transfer of phospholipids between plasma lipoproteins. *Biochim. Biophys. Acta.* **794:** 274-280.
6. Child, P., J. J. Myher, F. A. Kuypers, J. A. F. Op den Kamp, A. Kuksis, and L. L. M. Van Deenen. 1985. Acyl selectivity in the transfer of molecular species of phosphatidylcholines from human erythrocytes. *Biochim. Biophys. Acta.* **812:** 321-332.
7. Patton, G. M., J. M. Fasulo, and S. J. Robins. 1982. Separation of phospholipids and individual molecular species of phospholipids by high-performance liquid chromatography. *J. Lipid Res.* **23:** 190-196.
8. Damen, J., J. Regts, and G. Scherphof. 1982. Transfer of [^{14}C]phosphatidylcholine between liposomes and human plasma high density lipoproteins. Partial purification of a transfer-stimulating plasma factor using a rapid transfer assay. *Biochim. Biophys. Acta.* **712:** 444-452.
9. Patton, G. M., S. Bennett Clark, J. M. Fasulo, and S. J. Robins. 1984. Utilization of individual lecithins in intestinal lipoprotein formation in the rat. *J. Clin. Invest.* **73:** 231-240.
10. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497-509.
11. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **235:** 466-468.
12. Pennington, R. H. 1965. Introductory Computer Methods and Numerical Analysis. MacMillan, New York. 380-386.
13. Albers, J. J., J. H. Tollefson, C-H. Chen, and A. Steinmetz. 1984. Isolation and characterization of human plasma lipid transfer proteins. *Arteriosclerosis.* **4:** 49-58.
14. Minari, O., and D. B. Zilversmit. 1963. Behavior of dog lymph chylomicron lipid constituents during incubation with serum. *J. Lipid Res.* **4:** 424-436.
15. Redgrave, T. G., and D. M. Small. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during catabolism of chylomicrons in the rat. *J. Clin. Invest.* **64:** 162-171.
16. Reed, C. F. 1968. Phospholipid exchange between plasma and erythrocytes in man and the dog. *J. Clin. Invest.* **47:** 749-760.
17. Imaizumi, K., M. Fainaru, and R. J. Havel. 1978. Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. *J. Lipid Res.* **19:** 712-722.
18. Lippiello, P. M., and M. Waite. 1983. Kinetics and mechanism of phosphatidylcholine and cholesterol exchange between chylomicrons and high density lipoproteins. *Biochem. J.* **215:** 279-286.
19. Rindlisbacher, B., and P. Zahler. 1983. Interaction of phosphatidylcholine liposomes and plasma lipoproteins with sheep erythrocyte membranes. Preferential transfer of phosphatidylcholine containing unsaturated fatty acids. *Biochim. Biophys. Acta.* **732:** 485-491.
20. McLean, L. R., and M. C. Phillips. 1981. Mechanism of cholesterol and phosphatidylcholine exchange or transfer between unilamellar vesicles. *Biochemistry.* **20:** 2893-2900.
21. Petrie, G. E., and A. Jonas. 1984. Spontaneous phosphatidylcholine exchange between small unilamellar vesicles and lipid-apolipoprotein complexes. Effects of particle concentrations and compositions. *Biochemistry.* **23:** 720-725.
22. Jonas, A., and G. T. Maine. 1979. Kinetics and mechanism of phosphatidylcholine and cholesterol exchange between single bilayer vesicles and bovine serum high density lipoprotein. *Biochemistry.* **18:** 1722-1728.
23. Roseman, M. A., and T. E. Thompson. 1980. Mechanism of the spontaneous transfer of phospholipids between bilayers. *Biochemistry.* **19:** 439-444.
24. Tall, A. R., L. R. Forester, and G. L. Bongiovanni. 1983. Facilitation of phosphatidylcholine transfer into high density lipoproteins by an apolipoprotein in the density 1.20-1.26 g/ml fraction of plasma. *J. Lipid Res.* **24:** 277-289.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR