Lipid transfers between reconstituted high density lipoprotein complexes and low density lipoproteins: effects of plasma protein factors

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Abstract In this study we examined the transfer of lipids between reconstituted high density lipoprotein discs (r-HDL) and human low density lipoproteins (LDL) in the presence and absence of lecithin:cholesterol acyltransferase (LCAT) or of plasma phospholipid transfer protein (PLTP). We found that spontaneous transfer of phospholipids from r-HDL to LDL occurred by an apparent first order reaction with a half-time of 5.8 to 6.9 hr depending on the phospholipid. During the time of incubation of r-HDL with LDL (from 0 to 25 hr), the phospholipid content of r-HDL decreased more than 30%, the free cholesterol content increased 2.5-fold, and low levels of cholesteryl esters appeared in r-HDL. These compositional changes gave rise to small discoidal particles with a limiting diameter of 77 Å and two molecules of apoA-I per particle. When LCAT was included in the reaction mixture, the r-HDL lost even more phospholipid, lost some free cholesterol, and gained cholesteryl esters relative to the apolipoprotein content, due to the enzymatic reaction. The products of the LCAT reaction had a diameter of 93 Å and three, rather than two, apoA-I molecules per particle. Inclusion of PLTP into the reaction mixture accelerated the transfer of phospholipids (half-time of 1.7 hr) and the formation of the 77 Å product. In addition to these compositional and morphological changes, which may be important in the interconversions of native HDL subspecies, the prolonged incubations revealed some slow reactions, such as the esterification of LDL cholesterol by LCAT, a background formation of cholesteryl esters in r-HDL, and an apparent hydrolysis of cholesteryl esters in LDL in the presence of r-HDL.—**Jonas, A., K. E. Kézdy, M. I. Williams, and K-A. Rye.** Lipid transfers between reconstituted high density lipoprotein complexes and low density lipoproteins: effects of plasma protein factors. *J. Lipid Res.* 1988. **29:** 1349–1357.

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Spontaneous transfers of unesterified cholesterol and phospholipids are known to occur between plasma lipoproteins (1). It is now well established that cholesterol transfers occur through the aqueous medium, at rates that are limited by cholesterol desorption from the donor particle interface (1). The transfers of phospholipids have been mostly investigated in systems consisting of donor and acceptor vesicles or reconstituted high density lipoprotein (r-HDL) particles (2, 3). These studies showed that exchanges of labeled phospholipids also proceed through the aqueous medium, but occur at much slower rates than exchanges of cholesterol, as a result of the lower solubility of phospholipids in water (1). Because the measured half-times for physiological phospholipid exchange in such synthetic systems at equilibrium were in excess of 10 hr, it has been generally accepted that in vivo phospholipid transfers between lipoproteins must be facilitated by plasma lipid transfer proteins (1, 4). However, in the plasma compartment transfers of phospholipids occur between homologous and heterologous particles, under nonequilibrium conditions due to the entry of nascent lipoproteins into the system and the action of lipid-metabolizing enzymes such as lecithin:cholesterol acyltransferase (LCAT). In this study we examined a relatively simple nonequilibrium system consisting of nascent HDL analogs and human low density lipoprotein (LDL) in the presence and absence of pure LCAT or partially purified phospholipid transfer protein (PLTP). The objective was to assess the extent, time course, and structural consequences of spontaneous lipid transfers and the effects of the LCAT reaction or plasma phospholipid transfer protein on this simple, yet physiologically relevant system.

Abbreviations: r-HDL, reconstituted high density lipoprotein complexes; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; apoA-I, apolipoprotein A-I; egg-PC, egg phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; r-HDL-egg PC, recombinant HDL complexes prepared with egg PC; r-HDL-DPPC, recombinant HDL complexes prepared with DPPC; SDS, sodium dodecyl sulfate; PLTP, plasma phospholipid transfer protein; BSA, bovine serum albumin.

EXPERIMENTAL PROCEDURES

Materials and preparations

Human apolipoprotein A-I (apoA-I), LCAT, and LDL were prepared in our laboratory by well-established methods (5, 6). The human phospholipid transfer protein was partially purified by using the infranatant of plasma centrifuged at density 1.210 g/ml, and a series of column steps including Pharmacia Phenyl Sepharose CL-4B, Whatman CM-52, and Pharmacia Heparin Sepharose CL-6B media (7). The fraction corresponding to the LTP-II protein of Tollefson and Albers (7) and Albers and co-workers (8) was used in our experiments. Human plasma was a generous gift from the Champaign County Blood Bank. Apolipoprotein A-I and LCAT preparations were pure as judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis using Coomassie protein stain, which revealed a single protein band (> 95% of the stain) of the appropriate molecular weight. LDL appeared free of HDL and plasma proteins as judged by nondenaturing gradient gel electrophoresis on Pharmacia PAA 4/30 gels. Before use, LDL was heated at 60°C for 1 hr in order to inactivate any endogenous LCAT and lipid transfer activities.

The lipids used in the preparation of discoidal nascent HDL analogs, egg phosphatidylcholine (egg-PC), dipalmitoylphosphatidylcholine (DPPC), and cholesterol, as well as fatty acid-free bovine serum albumin (BSA) were obtained from Sigma Chemical Company; [4-^{14}C]cholesterol was purchased from New England Nuclear.

The discoidal complexes of egg-PC or DPPC with apoA-I and cholesterol, designated r-HDL, were prepared by the sodium cholate dialysis method (9), using initial molar ratios of 80:8:1 egg-PC–cholesterol–apoA-I, and 100:10:1 DPPC–cholesterol–apoA-I. The reaction mixtures contained from 2 to 15 mg of apoA-I and approximately 2000 cpm/nmol of [^{14}C]cholesterol. The r-HDL complexes were prepared and stored in 10 mM Tris HCl, 150 mM NaCl, 1 mM NaN_3, 0.01% EDTA, pH 8.0 buffer. All of the incubations and analyses were performed in this standard buffer, except the PLTP assay, which was carried out at pH 7.4.

Incubations and separation of products

Incubations of the r-HDL complexes with LDL were performed at concentrations near the normal plasma concentrations of HDL and LDL. A typical reaction mixture contained 0.6 mg/ml apoA-I in r-HDL, 0.6 mg/ml LDL protein, 24 mg/ml BSA, 6 mM β-mercaptoethanol, and 8 μg of LCAT in a total volume of 3.3 ml of standard buffer. The incubations with PLTP and the corresponding controls had the same concentrations of particles and included 14 μg of the partially purified PLTP, but contained no BSA or β-mercaptoethanol, in a total reaction volume of 0.6 ml of the standard buffer. The samples were incubated under N_2 at 37°C from 0 to 25 hr.

The products of the incubations were isolated by sequential ultracentrifugation in a Ti 50 rotor (Beckman Instruments) at 50,000 rpm and 10°C. The initial spin for 22 hr at d 1.070 g/ml floated LDL which was free of contaminating r-HDL as judged by gradient gel electrophoresis and radioimmunoassay for apoA-I (kindly performed by Dr. E. Krul, Washington University, St. Louis). A second spin at the same density removed most residual LDL from the subnatant fractions. These were then adjusted to d 1.210 g/ml and were centrifuged for 40 hr in order to float HDL density particles. The top 1.5–2.0 ml was removed in the LDL and HDL spins, dialyzed against standard buffer, and stored at 4°C for subsequent analysis. The recovered r-HDL complexes prepared with DPPC were free of BSA but did contain 3–5% of LDL protein. The estimates of LDL contamination were performed by gradient gel electrophoresis and radioimmunoassay for LDL (the latter was performed by Dr. E. Krul, Washington University). Most of the r-HDL samples prepared with egg-PC were essentially free of LDL; those containing DPPC were further purified by precipitating LDL with dextran sulfate (ICN Biochemicals) and $MgCl_2 \cdot 6H_2O$ (10). In the experiments including PLTP, the separations of r-HDL and LDL were performed by selectively precipitating LDL with heparin (Sigma Chemical Company) and $MnCl_2$ at concentrations of 0.1% heparin and 0.05 M Mn^{2+} in a total volume of 0.69 ml. The mixtures were allowed to stand 10 min at room temperature, and were then centrifuged at 5°C for 1 hr at 10,000 rpm (10, 11). The supernatant contained over 90% of the r-HDL and less than 5% of the LDL mass. Only the supernatant was used for the subsequent chemical and gradient gel electrophoresis analyses.

Analysis of r-HDL and LDL

The size distribution and purity of the lipoproteins was determined by gradient gel electrophoresis under nondenaturing conditions using Pharmacia PAA 4/30 gels. A Tris–boric acid (pH 8.4) buffer was used during electrophoresis which was conducted at 150 V for 19 hr. The gels were stained with Coomassie blue stain and were scanned with an LKB Ultro Scan XL laser densitometer. Each gel included standard proteins for size calibration: BSA, lactate dehydrogenase, catalase, horse ferritin, and thyroglobulin, as supplied in the Pharmacia calibration kit.

The chemical composition of LDL and r-HDL complexes was determined by the procedures of Lowry et al. (12) for protein determination, Chen, Toribara, and Warner (13) for phospholipid determination, and an enzymatic fluorometric assay for free and total cholesterol determination (14).

The fatty acid composition of the acyl esters present in the r-HDL-DPPC complexes before and after incubation with LDL and the fatty acid composition of the corresponding LDL lipids were determined by gas–liquid chromatog-

raphy using a Perkin Elmer Sigma 3B gas chromatograph and a Supelco SP-2310 (3%), SP-2300 (2%) Chromosorb WAW column to separate the fatty acid methyl esters. The fatty acid methyl esters were prepared by a modification of the procedure described by Ast (15).

The content of apoA-I molecules per HDL complex particle was determined by cross-linking the protein with bis(sulfosuccinimidyl)suberate and analyzing the products of the reaction by sodium dodecyl sulfate (SDS) polyacrylamide gradient gel electrophoresis, using a modification of the method described by Staros (16).

LCAT activity assay

The initial r-HDL, incubated r-HDL, and LDL isolated from incubation mixtures were examined as substrates for LCAT by using the same assay procedure used in previous studies (17). The resulting [^{14}C]cholesterol and [^{14}C]cholesteryl esters were separated by thin-layer chromatography and the spots were scraped and counted in a Beckman LS-3801 scintillation counter.

The products of the incubations, which included LCAT, were similarly extracted with chloroform–methanol 2:1 (v/v), and the radiolabeled [^{14}C]cholesterol and [^{14}C]cholesteryl esters determined in order to assess [^{14}C]cholesterol transfers and [^{14}C]cholesteryl ester synthesis during the incubations. The PLTP activity was assessed by methods described previously (7, 18).

RESULTS

Recovery and purity of the incubation products

Three independent incubation experiments were performed with different preparations of r-HDL complexes, LDL, and LCAT. All the results were similar; therefore a single representative experiment was chosen for presentation in this paper.

The recoveries of r-HDL, after the three ultracentrifugal spins, were 50–60% in terms of apoA-I and lipid; LDL recoveries after one spin were 80%. Since our objective was to obtain pure lipoproteins rather than to optimize recoveries, the 20–40% losses are mostly due to incomplete removal of fractions. Controls of r-HDL or LDL subjected to the same series of ultracentrifugal steps, with or without prior incubation at 37°C for 25 hr, gave the same recoveries as the actual samples. Therefore, specific losses of lipoprotein particles or of their components during ultracentrifugation do not appear to be significant. Control r-HDL complexes retained their original composition and particle distribution, as determined by gradient gel electrophoresis, when incubated at 37°C for 25 hr in the presence and in the absence of BSA and β-mercaptoethanol. Similarly, the composition of control LDL was not affected. Thus, the conditions of the incubation alone do not change the lipoprotein particle distribution or their composition. The heparin–Mn^{2+} separation method gave high yields of r-HDL and did not significantly change the composition of control r-HDL complexes.

The methods for the determination of lipoprotein purity after ultracentrifugation, and the results we obtained are given in the Experimental Procedures section. In short, LDL and recombinant HDL complexes containing egg-PC (r-HDL-egg PC) were essentially pure, whereas the DPPC-containing HDL complexes (r-HDL-DPPC) were contaminated with up to 5% LDL in terms of protein. Further purification was attained by dextran sulfate–$MgCl_2$ precipitation of the residual LDL.

Time course experiments

Incubations carried out from 0 to 25 hr in the presence and absence of LCAT resulted in changes in the chemical compositions of both the r-HDL complexes and of LDL, as shown in Figs. 1 and 2, respectively. Changes in composition between the end of the incubation and the isolation of the products do occur but they are minor, corresponding to changes seen after about one-half hour at 37°C in the reaction mixtures. Evidently, at the lower temperature of ultracentrifugation and in the presence of high salt concentrations, the phospholipid transfers and LCAT action are markedly decreased.

Fig. 1 shows the change in composition of r-HDL-egg PC complexes exposed to LDL in the presence and in the absence of LCAT. In the absence of LCAT, the r-HDL lost over 30% of the phospholipid in an apparent first order transfer reaction, with a half-time of 6.9 hr. The free cholesterol increased about 2.5-fold in less than 2 hr and the protein content remained essentially constant during the entire experiment. After prolonged incubation at 37°C, control r-HDL-egg PC retained the original composition, but had measurable levels of cholesteryl esters, up to 3 moles per mol of apoA-I; some cholesteryl esters were also detected in the complexes exposed to LDL.

When LCAT was included in the incubation mixture, even more phospholipid was lost from the HDL complexes, due to the enzymatic reaction. Concomitantly, free cholesterol, after an initial increase, was lost and cholesteryl esters were formed. Incorporation of PLTP into the incubation mixture of r-HDL-egg PC with LDL increased the rate of the phospholipid transfer markedly, giving a half-time of 1.7 hr.

The experiments with the r-HDL-DPPC complexes gave results analogous to those shown in Fig. 1, but the half-time for the phospholipid transfer was 5.8 hr and the starting and final compositions of the complexes were slightly different, as indicated in **Table 1**.

Fig. 2 shows the changes with time in the composition of the LDL incubated with r-HDL-egg PC complexes. As expected, the phospholipid content of LDL increased be-

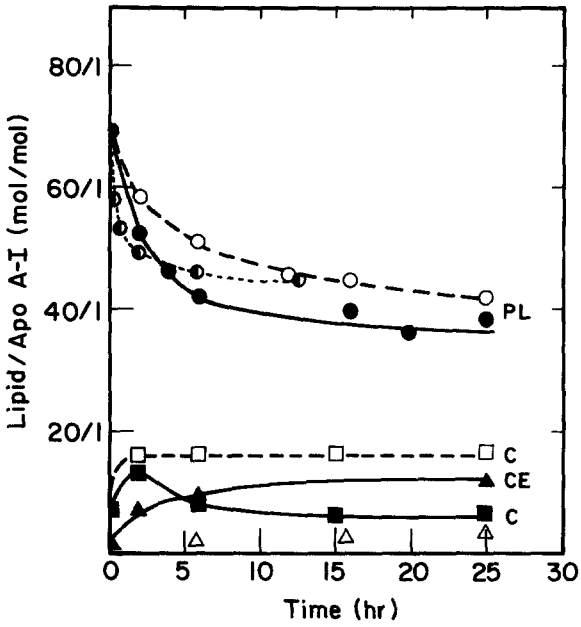

Fig. 1. Composition changes, as a function of time, of r-HDL-egg PC incubated with LDL in the presence of LCAT (filled symbols), in the absence of LCAT (open symbols), or in the presence of the PLTP (half-filled symbols). The lipid contents are expressed as mol of lipid per mol of apoA-I: (○, ●, ◐) phospholipid, (□, ■) free cholesterol, and (△, ▲) cholesteryl esters. The experimental errors are on the order of 10% for the molar ratios, and the limits of detection are approximately ± 2 mol of lipid/mol of apoA-I. The incubations were performed at 37°C using 0.6 mg/ml of apoA-I and LDL protein, 8 μg of LCAT, plus BSA and β-mercaptoethanol in 3.3 ml of standard buffer. Separation of the lipoproteins was carried out by sequential ultracentrifugal flotation.

cause of phospholipid transfer from the r-HDL complexes. The calculated half-time for the phospholipid incorporation was 6.8 hr. However, the changes in free cholesterol and cholesteryl ester content were unexpected. Instead of a loss of free cholesterol and constant cholesteryl ester levels, we observed an increase in the former and a decrease in the latter. These changes, reproduced in five independent experiments, do not occur in control LDL samples incubated at 37°C for 25 hr; they are tentatively attributed to a slow hydrolysis of the LDL cholesteryl esters, catalyzed by an unknown factor. Our laboratory is actively investigating this intriguing observation.

In the presence of LCAT, only minor changes occur in the composition of LDL over time. The only significant change is an initial loss of free cholesterol followed by a gradual increase, such that after 25 hr the final composition of the LDL is very similar to the original particles (see Table 1). The results of the experiments that were performed with the r-HDL-DPPC complexes were similar in most respects to those shown in Fig. 2.

The compositions of the original r-HDL and LDL and of the incubated lipoproteins are summarized in Table 1. As previously described by us, the r-HDL-DPPC complexes contain higher levels of phospholipid than do the r-HDL-egg PC complexes (18). The two kinds of r-HDL complexes, when incubated with LDL in the presence or absence of LCAT, retain a similar difference in phospholipid levels.

In order to estimate the exchange of phospholipids between the r-HDL complexes and LDL, the fatty acids present in r-HDL-DPPC and in the LDL that had been incubated with them for 25 hr were converted to methyl esters and analyzed by gas–liquid chromatography. The r-HDL-DPPC complexes contained 83% palmitic acid, 4% stearic acid, and 13% unsaturated fatty acids derived from LDL. The LDL fatty acids, in turn, were markedly en-

TABLE 1. Compositions of r-HDL complexes and of LDL incubated at 37°C for 25 hr and separated by sequential ultracentrifugation

Lipoprotein	Incubated with	Protein	Phospholipid	Cholesterol	Cholesteryl Esters
		mol		*mol/mol apoA-I*	
r-HDL-egg PC	Control[a]	1	69	6	2
r-HDL-egg PC	LDL	1	44	16	3
r-HDL-egg PC	LDL, LCAT	1	39	6	12
r-HDL-DPPC	Control[a]	1	87	7	2
r-HDL-DPPC	LDL	1	60	18	3
r-HDL-DPPC	LDL, LCAT	1	53	11	16
				mg/ml	
LDL	Control[a]	0.218	0.244	0.112	0.414
LDL	r-HDL-egg PC	0.218	0.344	0.140	0.345
LDL	r-HDL-egg PC, LCAT	0.218	0.253	0.085	0.407
LDL	r-HDL-DPPC	0.218	0.352	0.142	0.330
LDL	r-HDL-DPPC, LCAT	0.218	0.246	0.098	0.390

The protein content in the r-HDL and LDL samples remained essentially constant; therefore it was used as the normalizing factor in expressing the lipid composition of the lipoproteins. The experimental errors in the determinations were on the order of 10%. The detection limit for the lipids was approximately ± 2 mol/mol apoA-I in the r-HDL complexes.

[a]No additions.

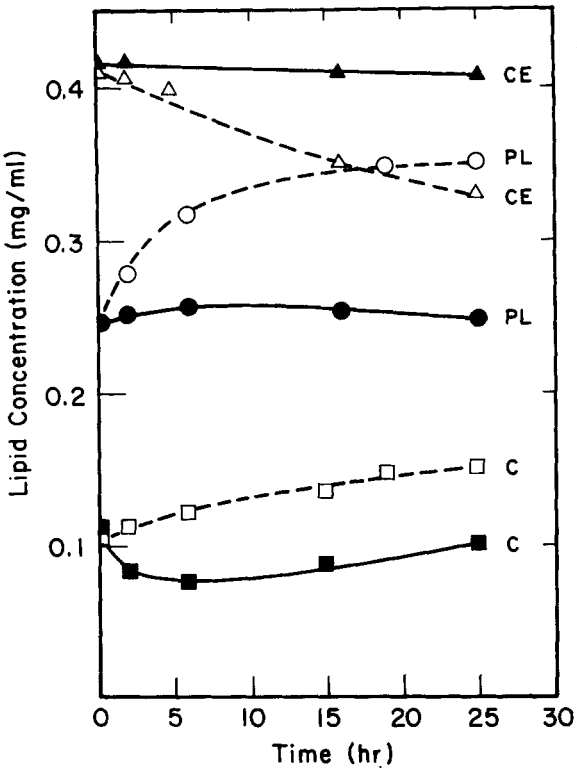

Fig. 2. Composition changes, as a function of time, of LDL incubated with r-HDL-egg PC in the presence (filled symbols) and in the absence (open symbols) of LCAT. The lipid contents are expressed in mg/ml concentrations that have been normalized to an LDL protein concentration of 0.218 mg/ml: (○, ●) phospholipid, (□, ■) free cholesterol, (△, ▲) cholesteryl esters. The experimental errors and the incubation conditions are the same as those given in the legend to Fig. 1.

riched in palmitic acid (doubled) as a result of the transfer of DPPC from the r-HDL-DPPC complexes. While there is clearly some exchange of phospholipids, net transfer from the r-HDL complexes to LDL predominates under the conditions of our experiment.

LCAT reaction

Since a significant, neutral lipid transfer activity should not be present in our incubation mixtures, the appearance of ^{14}C-labeled cholesteryl esters in LDL can be attributed to the reaction of LCAT, following a redistribution of free [^{14}C]cholesterol among all the lipoprotein particles. **Fig. 3** shows the formation of ^{14}C-labeled cholesteryl esters in r-HDL-egg PC complexes and in LDL incubated in the presence of LCAT. Radiolabeled free cholesterol equilibrated very rapidly among the lipoprotein particles in the system, so that the kinetics of cholesteryl ester formation reflect the rate of the LCAT reaction. Esterification of cholesterol in the r-HDL complexes occurs relatively rapidly and is essentially complete after 6 hr of reaction. Short term experiments (30 min to 1 hr) using appropriate dilutions of LCAT, so that a linear rate of product formation was obtained, indicated that LDL reacts with LCAT at rates only 2 to 5% of those of the r-HDL-egg PC complexes. Nevertheless, after prolonged incubations the formation of cholesteryl esters by LCAT on LDL becomes quite significant, as shown in Fig. 3. LDL alone incubated with LCAT for 16 hr gives about 65% conversion of [^{14}C]cholesterol into cholesteryl esters, compared to 50% conversion in the presence of r-HDL-egg PC complexes. Evidently the HDL complexes saturated with cholesteryl esters still compete with LDL for LCAT binding.

In the absence of LCAT, there is no measurable esterification of cholesterol on LDL, but there is some endogenous esterification of cholesterol on r-HDL complexes after 25 hr of incubation. Whether this is due to endogenous LCAT activity that persists in the synthetic r-HDL complexes or to a spontaneous background reaction has not been determined. In any event, this experiment shows that extended incubations (25 hr) at 37°C can lead to appreciable reactions that are not significant over short time periods.

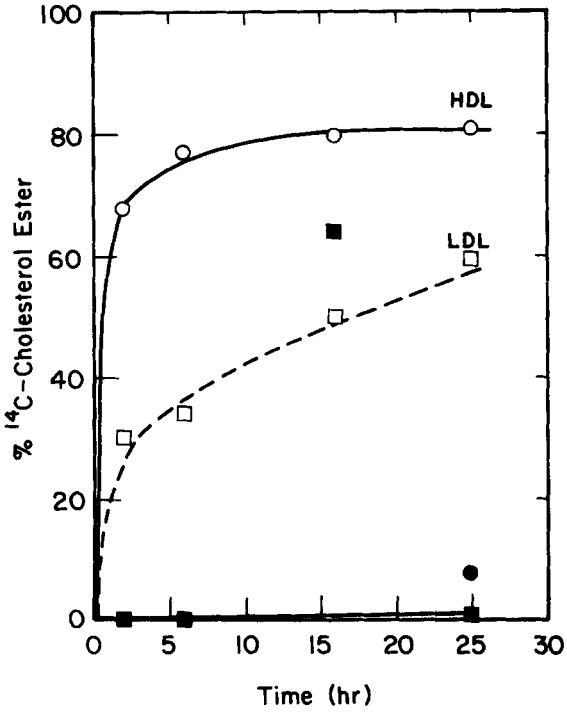

Fig. 3. Percent of [^{14}C]free cholesterol converted into [^{14}C]cholesteryl esters by LCAT during the incubation of r-HDL-egg PC (initially labeled with [^{14}C]free cholesterol) with LDL. Percent of conversion in r-HDL-egg PC (○) and in LDL (□) under the same incubation conditions as those given in the legend to Fig. 1. The data point (■) shown at 16 hr represents the formation of cholesteryl esters in isolated LDL that had been labeled with [^{14}C]free cholesterol by preincubation with r-HDL complexes. The LCAT reaction was then performed under the same conditions as above. Background reaction of radiolabeled r-HDL-egg PC (●) and LDL (■) in the absence of LCAT.

Size and apoA-I content of r-HDL complexes

The r-HDL complexes exposed to LDL in the presence or absence of LCAT or of PLTP were analyzed further to determine their size and apoA-I content. **Fig. 4** shows the densitometer traces of nondenaturing gradient gels run on r-HDL-egg PC complexes after incubation with LDL in the presence or absence of LCAT. The Stokes radii shown on the figure were determined by reference to standard proteins run on the same gel. The insert shows a representative nondenaturing gel pattern of the r-HDL complexes. Exposure of the somewhat heterogeneous r-HDL-egg PC complexes, having a main band at 96 Å, to LDL produced a single major population of particles with diameters of 77 Å. Incorporation of LCAT into the reaction mixture resulted in the formation of particles with diameters of 93 Å. Cross-linking of the apoA-I in these three classes of particles with bis(sulfosuccinimidyl)suberate and analysis by SDS gradient gel electrophoresis showed that the original r-HDL-egg PC particles and those exposed to LDL contained predominantly two apoA-I molecules per particle.

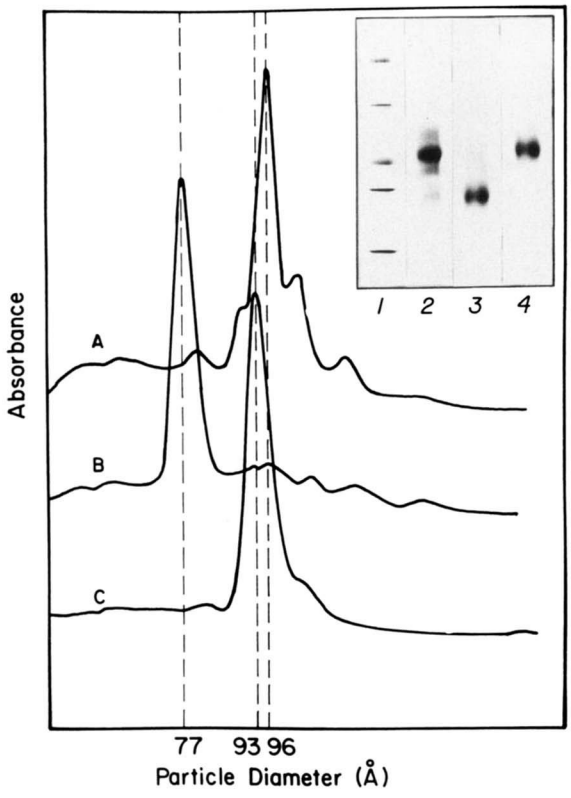

Fig. 4. Laser densitometer traces corresponding to nondenaturing gradient gel patterns of isolated r-HDL-egg PC complexes: A, incubated r-HDL-egg PC control; B, r-HDL-egg PC incubated with LDL; C, r-HDL-egg PC incubated with LDL and LCAT. The insert shows the gradient gel pattern for: lane 1, protein standards; lane 2, incubated control; lane 3, sample incubated with LDL; lane 4, sample incubated with LDL and LCAT. The experimental conditions were identical to those given in the legend to Fig. 1; the incubations were performed for 25 hr.

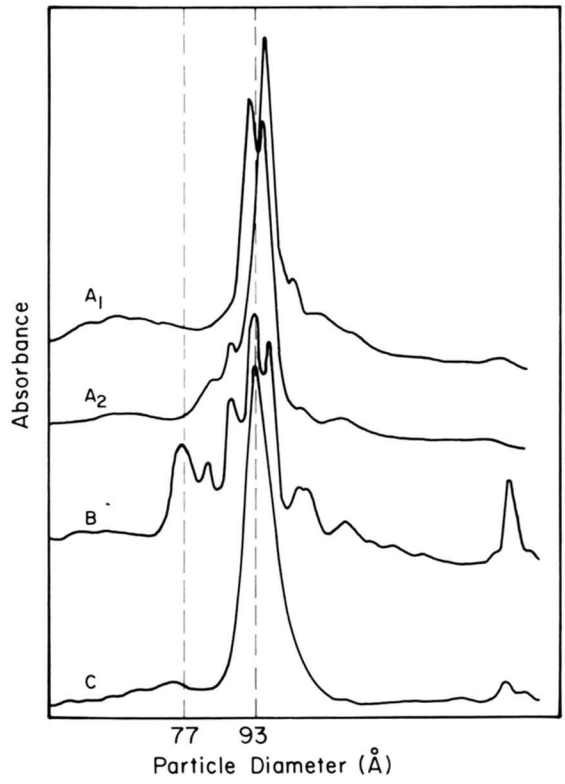

Fig. 5. Laser densitometer traces corresponding to nondenaturing gradient gel patterns of isolated r-HDL-DPPC complexes: A_1, original r-HDL-DPPC complexes; A_2, incubated control r-HDL-DPPC complexes; B, r-HDL-DPPC incubated with LDL; C, r-HDL-DPPC incubated with LDL and LCAT. The experimental conditions are the same as those given in the legends to Figs. 1 and 4.

The sample reacted with LCAT, in contrast, mostly had three apoA-I molecules per r-HDL complex.

The isolated particles with diameters of 77 Å, when exposed to LCAT for 16 hr, gave rise to 93-Å particles and some residual 77-Å species, suggesting that the 93-Å product particles produced in the reaction mixtures containing r-HDL-egg PC, LDL, and LCAT could be formed via a 77-Å intermediate particle. When phospholipid transfer protein was included with r-HDL-egg PC and LDL, the major HDL particle that was produced was again the 77-Å species, but the rate of its formation was accelerated. The results of these experiments are not shown.

Fig. 5 shows the same gradient gel electrophoresis analysis for the r-HDL-DPPC complexes exposed to LDL in the presence or absence of LCAT. Qualitatively, the changes in the particles are similar to those depicted in Fig. 4. Incubation with LDL produces, on the average, smaller but heterogeneous particles. Reaction with LCAT forms a single product particle, 93 Å in diameter. It is interesting to note that the original complexes that give a characteristic doublet of particles with diameters of 92 and 96 Å, rearrange upon incubation at 37°C for 16 hr to give one major species of 95 Å. **Table 2** summarizes the sizes and apoA-I contents of the particles represented in Figs. 4 and 5.

TABLE 2. Stokes radii and apoA-I contents of r-HDL-complexes exposed to LDL in the presence or absence of LCAT or PLTP

r-HDL Complex	Incubated with	Stokes Radius	ApoA-I/Particle
		Å	
r-HDL-egg PC	Original[a]	97, (112)	2
r-HDL-egg PC	Control	96, (110)	2
r-HDL-egg PC	LDL	77	2
r-HDL-egg PC	LDL, LCAT	93	3
r-HDL-egg PC	LDL, PLTP	77, (96), (110)	2
r-HDL-DPPC	Original[a]	92, 96	2
r-HDL-DPPC	Control	95	2
r-HDL-DPPC	LDL	(77), (87), 93, 97, 110	2
r-HDL-DPPC	LDL, LCAT	93	3

The Stokes radii were determined by gradient gel electrophoresis with reference to standard proteins. The results given in parentheses correspond to minor gel bands or densitometric peaks. The reproducibility of the densitometric peak positions was ± 2 Å. The content of apoA-I per particle was determined by cross-linking the protein with bis(sulfosuccinimidyl)suberate and SDS polyacrylamide gradient gel electrophoresis (16).

[a]Unincubated complex.

DISCUSSION

In this study we show that a relatively rapid spontaneous transfer of phospholipids occurs from r-HDL complexes into LDL, with half-times from 5.8 to 6.9 hr in an apparent first order process. Phospholipid transfer protein increases the rate of the transfer reaction fourfold, giving a half-time of 1.7 hr. These results clearly indicate that the spontaneous, net phospholipid transfers and exchanges among lipoproteins have considerably higher rates than those observed for the exchange or transfer of similar phospholipids between vesicles (half-times of 63 to 83 hr) (1). Spontaneous transfers of phospholipids may become important in the equilibration of phospholipids among lipoproteins in species with low or impaired PLTP activity. The difference between the rates of phospholipid transfer among vesicles or lipoproteins could be explained by the smaller size of the donor r-HDL complexes and the presence of an apolipoprotein that may accelerate the phospholipid desorption from the particle surface. Most of the reports on the activity of the human PLTP, to date, examined the transfer of radiolabeled phospholipids from synthetic vesicles into HDL (19,20) or between VLDL and HDL (8, 21), and noted the net transfer of phospholipid from VLDL into HDL (21). Here we show that the transfer of phospholipid mass from HDL-like particles into LDL is also catalyzed by this plasma factor. Clearly, the direction of the transfer must be a function of the thermodynamic gradient of the phospholipid activity in the system. Since the bulk concentration of the phospholipids in r-HDL complexes and LDL were very similar in our incubation experiments, it follows that the activity of the phospholipid in the HDL complexes is higher than in LDL by virtue of the different surface composition, size, and curvature of these two particle classes.

The consequences of the lipid transfers, phospholipid loss, and cholesterol enrichment on the size and shape of the r-HDL complexes can be predicted from our previous observations on these synthetic r-HDL complexes (22). We showed previously that lowering the phospholipid content or increasing the free cholesterol content of r-HDL-egg PC particles produces smaller subclasses of discoidal particles. The limiting size is 78 ± 3 Å in diameter. Therefore, the exposure of r-HDL-egg PC to LDL, with the concomitant loss of phospholipid and increase in free cholesterol, also leads to the formation of the small discoidal products. Phospholipid transfer protein only accelerates the process, without changing the morphology or the composition of the 77-Å product particles. It is of interest that these 77-Å particles resemble in size and in composition the "small spherical HDL" observed in LCAT-deficient plasma (23) or secreted by Hep G2 cells (24). In fact, Nichols and colleagues (25) reported that discoidal r-HDL of small diameters (72 to 78 Å) frequently appear as spherical particles upon negative-stain electron microscopy. By analogy with our observations in this study, it is possible that the "small spherical HDL" arise in vivo, in the absence of LCAT, from larger nascent HDL discs containing apoA-I by the transfer of phospholipids to LDL. Simultaneously, LDL could become enriched in phospholipids and free cholesterol and depleted in cholesteryl esters. The increased triglyceride content of LDL could be due to transfers from VLDL to compensate for the net decrease of cholesteryl esters (23).

Our experiments show that the incorporation of LCAT into the incubation mixture of r-HDL complexes and LDL affects the composition, size, and shape of the r-HDL. A single population of r-HDL particles is produced which is depleted in phospholipids and in free cholesterol, enriched in cholesteryl esters, and contains three instead of two

molecules of apoA-I per particle. In composition and size they resemble native HDL_{2a} (26) and are probably of spheroidal shape because of the presence of a substantial cholesteryl ester core. The mechanism by which discoidal particles containing two apoA-I molecules are converted to spherical particles with three apoA-I molecules is a challenging area for future investigation relating to the question of the genesis of HDL subclasses. A similar conversion of particles from species containing two into species containing three apoA-I molecules per particle was reported by Nichols and his co-workers (25, 27) in a system consisting of r-HDL made with egg-PC, LDL, and a partially purified LCAT preparation. They also observed a predominant 78-Å particle in the absence of LCAT activity, and followed its conversion into a larger spherical species in the presence of LCAT activity.

Our observation, that in the presence of LCAT the composition of LDL apparently becomes normalized relative to that of LDL incubated with the r-HDL complexes alone, is in agreement with the reports that LCAT replacement in LCAT-deficient plasma returns all the lipoprotein classes to near normal compositions (28). In this study we show that the direct reaction of LCAT on LDL apparently replenishes the cholesteryl ester content and decreases the excess phospholipid and free cholesterol in these lipoproteins due to the exposure to r-HDL complexes.

In addition to the possible physiological relevance of the lipoprotein transformations discussed above, this study identified some of the slow reactions that may become significant during prolonged incubations of plasma or isolated lipoproteins at 37°C. We noted that although LDL are very poor substrates for LCAT under ordinary reaction conditions (30 min to 1 hr reaction times) they can give rise to significant amounts of cholesteryl esters by the LCAT reaction after several hours. HDL complexes, prepared with pure apoA-I and lipids, had measurable levels of cholesteryl esters formed after extended incubations in the absence of LDL or LCAT. Whether this reaction is due to a residual LCAT activity in the HDL complexes or to a spontaneous transesterification has not yet been determined. Most intriguing is the apparent deesterification of the cholesteryl esters of LDL when exposed to r-HDL complexes for several hours. Since the composition of control LDL does not change, the degradation of cholesteryl esters and increase in free cholesterol may be a consequence of the increased phospholipid content of LDL and/or the presence of r-HDL. This observation is currently under active investigation in our laboratory. In any event, the results summarized above indicate that long term incubations can lead to a significant manifestation of slow reactions that may or may not have physiological importance.

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