Transfer of cholesterol from Ob1771 cells or LDL to reconstituted, defined high density lipoproteins

Ana Jonas,[1] Kathleen Bottum, Nathalie Theret,* Philippe Duchateau,* and Graciela Castro*

Department of Biochemistry, College of Medicine, University of Illinois at Urbana-Champaign, Urbana, IL 61801, and Département d'Etudes des Lipides et des Lipoprotéines,* Institut Pasteur de Lille, Lille, France

Abstract We used defined, reconstituted high density lipoproteins (rHDL) to study the effects of structure and composition of these particles on their role as cholesterol acceptors from cell membranes or from low density lipoproteins (LDL). Three discoidal rHDL and one spherical rHDL with distinct apolipoprotein A-I conformations, diameters, and compositions were used in conjunction with Ob1771 cells to measure the rate of [^{3}H]cholesterol efflux from the cells, direct binding to the cells, and competition with native HDL_3 for binding. In addition, the same rHDL particles were used to study the kinetics of cholesterol mass transfer from LDL. The results show that the rates of cholesterol transfer depend on the nature of the donor ($t_{1/2}$ 11–19 min from LDL, and $t_{1/2}$ 5 h from the cells), on the phosphatidylcholine/cholesterol ratio in the acceptors (the closer this ratio is to the equilibrium value, the slower is the rate), and on the diameter of the acceptors (the smallest particles have the lowest $t_{1/2}$ for cholesterol uptake from LDL, and are the most effective acceptors of [^{3}H]cholesterol from cells after their phospholipid content is taken into account). The cholesterol uptake by the rHDL, both from the cells and from LDL, is determined mostly by the phospholipid pool available in the acceptors. Binding to the cells was equivalent for all the rHDL (K_d = 38–67 μg/ml) and comparable to HDL_3, suggesting that the differences in apoA-I conformation have no effect on the binding to cells. Finally we observed that exposure of rHDL to cells may lead to remodeling of some of the lipoprotein particles.—**Jonas, A., K. Bottum, N. Theret, P. Duchateau, and G. Castro.** Transfer of cholesterol from Ob1771 cells or LDL to reconstituted, defined high density lipoproteins. *J. Lipid Res.* 1994. **35:** 860–870.

Supplementary key words discoidal HDL • apolipoprotein A-I • HDL_3 • HDL binding to cells • remodeling of HDL

It is generally accepted that high density lipoproteins (HDL) are involved in the removal of cholesterol from peripheral cells. The removal is due to the gradient of unesterified cholesterol created between the cell membranes and HDL, by the action of lecithin:cholesterol acyltransferase (LCAT), which converts unesterified cholesterol to cholesterol esters on HDL particles (1). The mechanism for cholesterol transfer between the cell membranes and HDL is, most likely, aqueous diffusion of cholesterol down the chemical potential gradient and redistribution in lipid domains of cells and lipoproteins. This has been amply demonstrated by the work of Rothblat, Phillips, and colleagues (2, 3), who showed that transfers can also occur from HDL into cells if the gradient is in the opposite direction (4). Such a transfer process does not require direct interaction of HDL with cells yet several laboratories have demonstrated the existence of high affinity binding sites for HDL on a variety of cells (5–8), including the Ob1771 adipose cells used in this study (9, 10). In some studies, but not in others, binding of HDL to these membrane sites has been reported to regulate cellular metabolism and release of intracellular cholesterol (11–13). The ligands for the interaction of HDL with cells, either through protein receptors or directly to membrane lipids, are the apolipoproteins, in particular apolipoprotein A-I (apoA-I), which may have different conformations and distinct metabolic roles in different subclasses of HDL. For example, the small preβ-1-HDL, containing only apoA-I, are the first acceptors of cholesterol released from fibroblasts into plasma (14, 15); and apoA-I in HDL containing apoA-II may behave differently from apoA-I in HDL particles without apoA-II, in terms of removal of cellular cholesterol (12) or catabolism (16). These observations suggest that the structure and composition of different HDL may determine their role and efficiency as cholesterol transporters.

Abbreviations: HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; apoA-I, apolipoprotein A-I; preβ-HDL, HDL with preβ electrophoretic mobility on agarose gels; apoA-II, apolipoprotein A-II; LDL, low density lipoproteins; VLDL, very low density lipoproteins; rHDL, reconstituted HDL; POPC, palmitoyloleoylphosphatidylcholine; PC, phosphatidylcholine; PBS, phosphate-buffered saline; CD, circular dichroism.

[1]To whom correspondence should be addressed at: Department of Biochemistry, College of Medicine, University of Illinois at Urbana-Champaign, 506 South Mathews Avenue, Urbana, IL 61081.

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In addition to HDL or reconstituted HDL (rHDL) (17, 18), free apolipoproteins have been shown to remove cellular lipids under certain conditions (19). Hara and Yokoyama (19) reported that free apolipoproteins, including apoA-I, form preβ-HDL-like particles with cellular lipids and mediate lipid transport to lipid microemulsions. Rothblat et al. (3) have proposed that apolipoproteins interact with regions of cell membranes that are depleted in cholesterol, via amphipathic helical domains. Such nonspecific interactions could facilitate cholesterol diffusion between the membranes and HDL or could lead to abstraction of cellular lipids. In this model the conformation of the apolipoproteins may be important in promoting interactions with the membrane lipids.

Similar to their role as acceptors of cellular cholesterol, HDL act as acceptors for unesterified cholesterol from low density lipoproteins (LDL) and very low density lipoproteins (VLDL) in circulation, an important process in the redistribution of cholesterol and the synthesis of cholesteryl esters by LCAT (20). Recently Miida, Fielding, and Fielding (21) reported that α-migrating HDL are the first acceptors of cholesterol from LDL.

In this study we used reconstituted analogs of HDL, including homogeneous discoidal particles that behave like preβ-HDL in their electrophoretic mobility (unpublished results, A. Leroy and A. Jonas) and have high reactivity with LCAT (22, 23), as well as rHDL particles that approach the properties of spherical HDL (24), to study their properties as acceptors of cholesterol from Ob1771 cells and from human LDL. Our objective was to assess how the lipid content and apoA-I conformational states of these well-defined, homogeneous rHDL particles affect their function in cholesterol transport.

EXPERIMENTAL PROCEDURES

Preparation and characterization of rHDL particles

Human apolipoprotein A-I (apoA-I), lecithin:cholesterol acyltransferase (LCAT), and low density lipoproteins (LDL) were isolated from plasma donated by the Champaign County Blood Bank—Regional Health Resource Center. Palmitoyloleoylphosphatidylcholine (POPC), cholesterol, and sodium cholate were obtained from Sigma. These materials were used in the preparation of homogeneous rHDL particles, starting with the sodium cholate dialysis method (25).

The 96 Å discoidal particles were generated from a POPC–cholesterol–apoA-I–Na cholate 120:12:1:120 (mol/mol) reaction mixture, followed by purification on a Pharmacia Superose 6 column (70 × 1.6 cm) driven by a Pharmacia FPLC system (22).

The 78 Å and 108 Å rHDL particles were prepared from a starting mixture of POPC–cholesterol–apoA-I–Na cholate 80:8:1:80 (mol/mol). The resulting rHDL mixture was then incubated with LDL in a 1:1 ratio by protein weight for 24 h at 37°C, in order to effect particle rearrangements due to phospholipid transfers (22, 26). The LDL was removed by ultracentrifugation at d 1.063 g/ml, and rHDL was recovered after ultracentrifugation at d 1.21 g/ml. The rHDL were then fractionated into the 78 Å and 108 Å subclasses by chromatography on the Superose 6 column (22).

The spherical 93 Å rHDL were prepared from a starting reaction mixture of POPC–cholesterol–apoA-I–Na cholate 80:8:1:80 (mol/mol), followed by incubation with LCAT. The enzymatic reaction mixture contained 5 mg of apoA-I in rHDL form, 10 mg of LDL (in terms of protein), 12 mg/ml BSA, 4 mM β-mercaptoethanol, and 20 μg of LCAT. After 48 h at 37°C the reaction mixture was fractionated by ultracentrifugation at densities of 1.063 g/ml and 1.21 g/ml to recover the rHDL product, which was further purified by chromatography on the Superose 6 column (24). A buffer consisting of 0.01 M Tris-HCl, pH 8.0, 0.15 M NaCl, 1 mM NaN_3, and 0.01% EDTA was used in all the preparations, and in storing all purified rHDL particles at 4°C.

The composition of all the particles was determined by a modified Lowry method for protein content (27), by the procedure of Chen, Toribara, and Warner (28) for phosphate determination, and the method of Heider and Boyett (29) for total and unesterified cholesterol determination. The number of apoA-I molecules per particle was determined by cross-linking the protein in rHDL particles transferred into a phosphate buffer, with 10 mM bis(sulfosuccinimidyl) suberate (30) for 3.5 h at room temperature, followed by quenching of the reaction with 0.25 M ethanolamine. Electrophoresis on SDS-PAGE gels revealed the different apoA-I oligomers in the rHDL particles (31).

Adipocyte culture

Ob1771 preadipocyte cell line, a subclone of mouse Ob17 cells (32), was plated at 2.5 × 10^3 cells per cm^2 in 6 multiwell plates (Nunc). Cells were grown to confluence in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 200 U/ml penicillin, 50 μg/ml streptomycin, 33 μM biotin, and 17 μM pantothenate. The cells were differentiated in the same medium supplemented with 17 nM insulin and 2 nM triiodothyronine. 1-Methyl-3-isobutylxanthine (100 μM) was added to the medium during the first 2 days postconfluence. Media were changed every 2 days. The Ob1771 cell line was previously shown to accumulate only unesterified cholesterol upon exposure to LDL and not to internalize HDL (33).

Cholesterol efflux from cells

Differentiated adipocytes were first preincubated for 48 h in lipoprotein-deficient serum and then exposed to the same medium supplemented with [^{3}H]cholesteryl

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linoleate-labeled LDL (34) (150 μg cholesterol/ml). Before the efflux experiments, the cholesterol-loaded cells were washed successively with phosphate-buffered saline (PBS), pH 7.4, containing 1% albumin, and PBS; the cells were then incubated with 50 μg/ml of the rHDLs for up to 6 h at 37°C. A representative concentration analysis using a heterogeneous preparation of rHDL indicated that in the range from 25 to 200 μg/ml (in terms of apoA-I concentration) the rHDL were not saturating for the efflux of [^{3}H]cholesterol. The incubation media were removed and centrifuged before counting radioactivity. The cell monolayers were washed and scraped into 1 ml of 0.1 N NaOH; the alkaline digests were used for counting radioactivity and protein concentration determinations. The results (see Fig. 2) are expressed as the percentage of cell radioactivity released into the medium. Esterification of the radiolabeled cholesterol did not occur during the course of these experiments.

In order to compare the size of the original rHDL with the rHDL after incubation with or without cells, the incubation medium was concentrated on Centricon (Amicon) before analysis by electrophoresis on 4-30% nondenaturating gradient gels from Pharmacia.

Binding studies and competition experiments

Binding studies were performed on adipose cells with ^{125}I-labeled rHDL labeled by the procedure of Bilheimer, Eisenberg, and Levy (35). The specific radioactivity varied between 400 and 600 cpm/ng. The cells were washed with PBS and incubated at 4°C for 2 h with labeled rHDL in medium without serum. The radioactivity was measured on alkaline digests (0.1 M NaOH) of the washed monolayer and the protein concentration was determined by a modification (27) of the Lowry procedure. The nonspecific binding was determined by measuring the radioactivity when experiments were carried out in the presence of a 20-fold excess of unlabeled HDL_3.

Competition experiments were performed under the same conditions as the binding experiments. The competitive displacement of 20 μg/ml ^{125}I-labeled HDL_3 was achieved by addition of increasing amounts of unlabeled rHDL from 0 to 100 μg/ml.

Transfer of cholesterol from LDL into rHDL

LDL and the rHDL particles were incubated in 1:1 ratios by protein weight at 37°C. At appropriate time points from 0 to 2 h, aliquots were removed. LDL was precipitated by the addition of dextran sulfate-Mg^{2+}. The precipitation procedure of Bachorik and Albers (36) was followed, except that in the absence of plasma proteins (37) a 1/20 dilution of the working reagent was required in order to optimize the recovery of rHDL particles in the supernatant. By this method about 80% of the rHDL, but less than 2% of LDL protein, was recovered in the supernatant.

The protein and free cholesterol (FC) content were determined in the rHDL supernatant by the chemical assays described above. Cholesterol values were normalized by dividing by the protein content. A computer program, as described by Gains (38), was used to fit the data to the exponential expression:

$$FC_t = FC_0 + FC_\infty (1 - e^{-kt})$$

where FC_t is the normalized FC content of the rHDL particle at time t, FC_0 is the initial FC content of the particle, FC_∞ is the maximum amount of FC transferred from the LDL to the rHDL particle, k is the rate constant for transfer, and t is the time. The half-time ($t_{1/2}$) of transfer was determined from k: $t_{1/2} = -\ln(0.5)/k$.

RESULTS

Properties of rHDL particles

The rHDL particles used in this study have been well characterized in our previous work (22, 24). **Table 1** summarizes their properties: size, shape, composition, apoA-I content per particle, apoA-I conformation, and their relative reactivities with LCAT. For the discoidal particles, we established previously that their properties do not change uniformly with size, rather there are subclasses of particles within the major classes with 2,3,4... apoA-I per particle, which are homologous. Thus, the 78 Å and 108 Å particles have very similar apoA-I conformations (from circular dichroism (CD) and fluorescence measurements) and have very similar reactivities with LCAT. In contrast, the 96 Å species has a distinct apoA-I conformation and is 13-fold more reactive with LCAT. The 93 Å spherical analog resembles a native HDL species, 88 Å LpAI, which we isolated and described in a recent publication (39). This spherical rHDL retains many of the structural features of apoA-I in discs, and appears from CD measurements to have a conformation of apoA-I intermediate between the 78 Å and 96 Å discs.

The nondenaturing gradient gel electrophoresis patterns of representative rHDL particles used in these experiments are shown in **Fig. 1**, as they appear before and after incubation with cells. The initial particles are quite homogeneous in size except for a minor contaminant of free apoA-I (~5%) in the 78 Å and 108 Å preparations. As the very small amounts of free apoA-I are not expected to have a significant effect on cholesterol efflux from cells or on HDL binding to cells, further purification was not attempted. After exposure to cells for 6 h at 37°C, the size of three of the rHDL particles remained the same, indicating that their protein framework is stable; however, the 96 Å particles showed significant rearrangements to smaller and larger particles. The rearrangements were identical to those we observed previously when the 96 Å

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TABLE 1. Properties of rHDL particles[a]

Size[b]	Shape[c]	Composition[d] PC/C/CE/apoA-I	ApoA-I per Particle[e]	α-Helix Content[f]	Relative reactivity with LCAT[g]
Å		mol/mol		%	%
78 ± 1	disc	32/3/0/1	2	60	6.3
96 ± 2	disc	73/4/0/1	2	72	100
108 ± 3	disc	82/19/0/1	3	58	8.8
93 ± 2	sphere	38/4/15/1	3	67	3.0

[a]The properties of these rHDL were published previously by our laboratory (22, 24) and have been partially confirmed for the three separate preparations used in this study.
[b]Diameters obtained from nondenaturing gradient gel electrophoresis, with the mean ± SD for at least five preparations.
[c]From volume and composition calculations, and from EM.
[d]PC, phosphatidylcholine; C, cholesterol; CE, cholesteryl ester. Average from two to five preparations, errors are ± 5%.
[e]From cross-linking with bis(sulfosuccinimidyl) suberate and SDS-PAGE.
[f]From CD spectra and estimates of % α-helix content from ellipticity at 222 nm.
[g]From relative apparent V_{max}/apparent K_m values (22, 24).

rHDL particles were shown to lose phospholipids spontaneously to different acceptors (26, 40). Apparently loss of phospholipids from these rHDL particles can occur during the 6 h of incubation in the presence of cells.

Efflux of [3H]cholesterol from cells

Fig. 2 shows the efflux of [3H]cholesterol from cells into the medium containing equivalent amounts of the rHDL particles in terms of apoA-I concentration. There appear to be two phases in the efflux kinetics, a rapid one in the first 45 min and a slow one with a half-life on the order of 5 h. This is in agreement with experiments by Mahlberg and Rothblat (18) demonstrating the existence of two kinetic pools of cholesterol in the membranes of J774 macrophages, and other cells. Although the data shown in Fig. 2 reflect the equilibration of the radiolabeled cholesterol throughout the system and do not provide information on mass transfer of this lipid, the results in Fig. 2 reflect the intrinsic transfer rates and the size of the cholesterol pools in the acceptors. Fig. 2 indicates that the 96 Å and 108 Å discs accept about 60% more [3H]cholesterol than the 78 Å discs and 93 Å spheres. **Table 2** shows that these differences are much decreased when the phospholipid content rather than the apoA-I concentration of the particles is the basis for comparison. Apparently the size of the phospholipid pool in the acceptor rHDL is the main factor that determines the rate of [3H]cholesterol efflux from cells, rather than their shape or apoA-I conformation. Size effects are smaller but could account for the higher efficiency of the 78 Å particles as acceptors after the phospholipid concentrations are normalized.

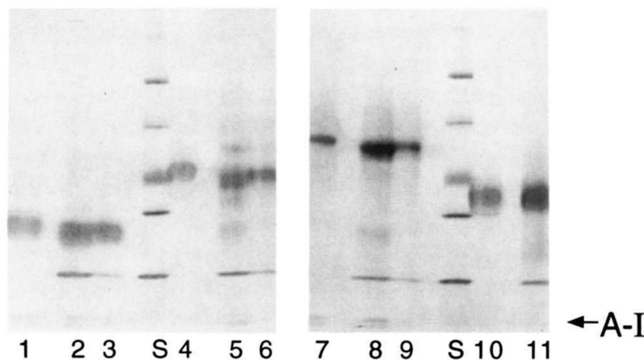

Fig. 1. Non-denaturing gradient gel electrophoresis of rHDL particles before and after incubation with OB1771 cells. Pharmacia 4–30% gradient gels were stained with Coomassie Blue. Lanes 1–3, 78 Å rHDL; lanes 4–6, 96 Å rHDL; lanes 7–9, 108 Å rHDL; lanes 10–11, 93 Å spherical rHDL. The protein standard lanes are designated by an S and free apoA-I with an arrow. The first lane for each rHDL particle (i.e., 1, 4, 7, 10) corresponds to the rHDL sample prior to incubation with cells. The two subsequent lanes for each rHDL particle are from two separate incubations with Ob1771 cells, for 6 h at 37°C. The cell media were concentrated with the Centricon System and 20 μg of protein was applied to each lane.

Binding of rHDL to cells

Fig. 3 shows the total and nonspecific binding curves for radioiodinated rHDL to the Ob1771 cells. The corresponding Scatchard plots for the specific binding are given in **Fig. 4,** and the dissociation constants (K_d) are summarized in **Table 3.** Two separate experiments gave similar results for the binding of the rHDL to the adipocytes and indicated that the K_d values, which range from 21 to 67 μg/ml, are comparable for all the rHDL particles, and for the rHDL particles and the native HDL_3 controls. Evidently, the different morphology of the rHDL particles, different diameters and PC/apoA-I ratios, and the distinct conformations of apoA-I in these particles do not

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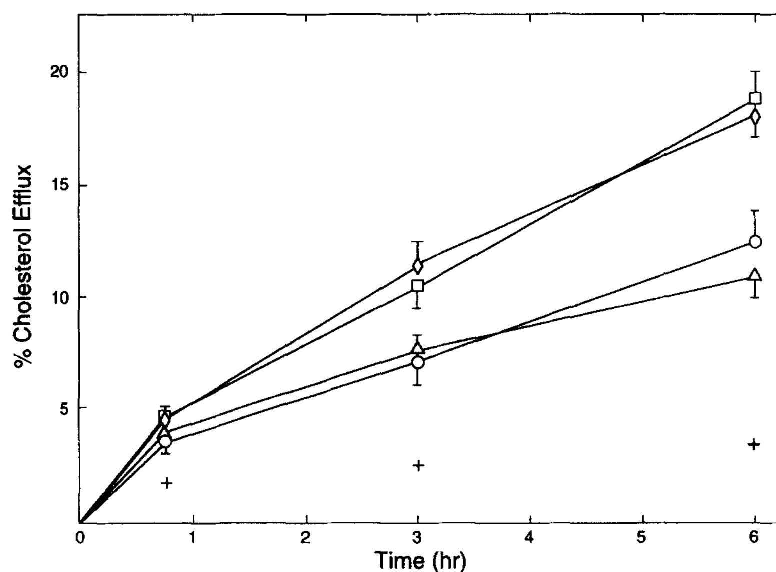

Fig. 2. Percent of [^{3}H]cholesterol efflux from Ob1771 cells into media containing rHDL particles: 78 Å rHDL (○); 96 Å rHDL (◇); 108 Å rHDL (□); 93 Å spherical rHDL (△). Liposomes of DMPC (+) were used as a control. The results from three separate experiments, each performed in duplicate, are given as the mean and standard deviation (error bar).

affect their binding to the Ob1771 cells. Thus, if apoA-I is in fact the major ligand for the binding of HDL to the cell membranes, through a protein receptor or directly to lipid, the recognition structure in the diverse rHDL particles must be the same. As the N-terminal region and the central region of apoA-I have distinct conformations for the different rHDL as indicated by our Trp fluorescence experiments (21, 24) and our studies of the Lys_{107} deletion mutant (41), the most likely apoA-I region for interaction with the cell membranes is in the C-terminal half of the molecule, as suggested by Morrison, Fidge, and Tozuka (42) and Castro et al. (43).

In order to demonstrate that the same binding sites are used by native HDL_3 as by the rHDL particles, competition experiments were performed (**Fig. 5**). Binding of radioiodinated HDL_3 to the Ob1771 cells was measured in the presence of competing cold rHDL particles. All the rHDL particles were able to compete with HDL_3 when present in 50–100 μg/ml concentrations; however, at 6–12 μg/ml concentrations the rHDL apparently increased HDL_3 binding. As the cells, HDL_3, and competing rHDL were coincubated for 2 h, lipid and apolipoprotein exchanges and transfers could occur as the system approached equilibrium (44), resulting in the observed changes in binding. The cold HDL_3 and the 93 Å spheres, which are similar in their properties to HDL_3, show the smallest effects probably because they are closest to equilibrium with the HDL_3 particles.

TABLE 2. Efflux of [^{3}H]cholesterol from adipocytes into rHDL particles

	Percent of Initial Cell [^{3}H]Cholesterol[a]	
HDL particle	Relative to [ApoA-I]	Relative to [PC]
78 Å – disc	12.6 ± 1.4	12.6
96 Å – disc	18.2 ± 1.0	8.0
108 Å – disc	18.8 ± 1.1	7.3
93 Å – sphere	11.0 ± 1.0	9.3
HDL_3	13.5 ± 2.2	

[a]The percent efflux of initial cell [^{3}H]cholesterol values are the 6-h results from Fig. 2, expressed relative to the same apoA-I concentration in the rHDL samples or normalized to the same rHDL phosphatidylcholine (PC) concentration.

Transfer of cholesterol mass from LDL to the rHDL

HDL are acceptors for cholesterol not only from cells but also from LDL and VLDL; therefore, we investigated the transfer of cholesterol mass from LDL to rHDL. **Fig. 6** shows the kinetics of cholesterol transfer up to 2 h. Beyond 2 h (for the 96 Å particles after 1.5 h) phospholipid transfers to LDL become significant (40) and preclude kinetic analysis; however, for the time spans shown in Fig. 6, the transfer of cholesterol mass follows first order kinetics for all the rHDL particles, and gives $t_{1/2}$ values ranging from 11 to 19 min (see Table 4). The initial transfer rates (the initial slopes of the curves in Fig. 6) depend inversely on the initial cholesterol content of the particles: the 108 Å rHDL are most enriched in cholesterol, therefore, their initial rate of cholesterol uptake is slowest. At equilibrium the order of maximum cholesterol incorporation is greatest for the 108 Å and 96 Å particles and lowest for the 78 Å and 93 Å spheres, as in the efflux studies from the Ob1771 cells. When the final cholesterol content is normalized to the concentration of phospholipid, the difference in uptake is essentially eliminated (Table 4), except for the 78 Å particles which appear to have a somewhat higher capacity for cholesterol.

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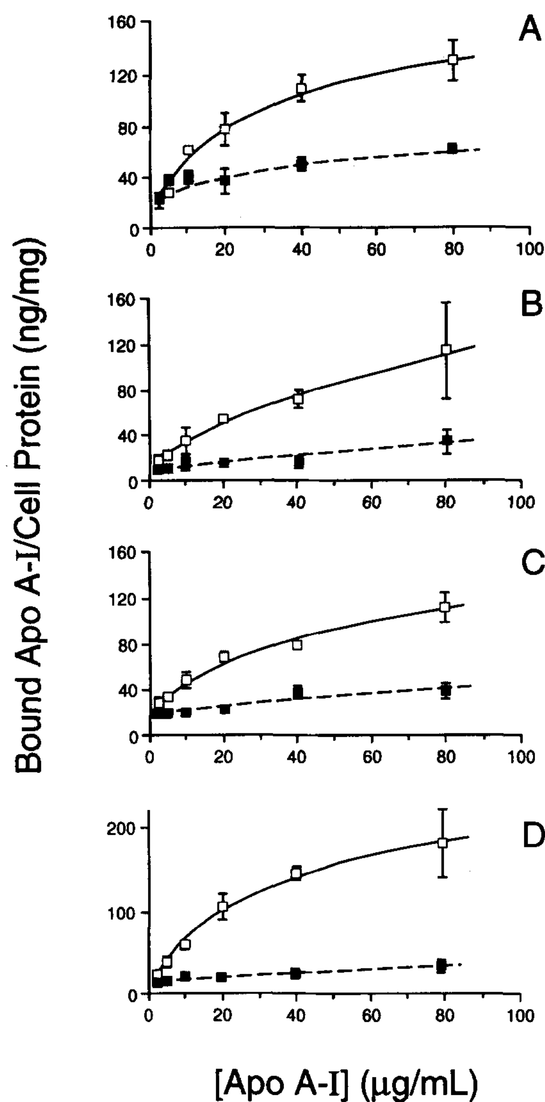

Fig. 3. Total (□) and nonspecific (■) binding of ^{125}I-labeled rHDL to Ob1771 cells. The binding of the rHDL is expressed as μg of bound apoA-I per mg of cell protein, as a function of the apoA-I concentration in the medium. Two separate experiments gave comparable results, a single experiment is shown in this figure, showing the variability in duplicate samples (error bars). A: 78 Å rHDL; B: 96 Å rHDL; C: 108 Å rHDL; D: 93 Å spherical rHDL.

DISCUSSION

For the discoidal rHDL series with 78, 96, and 108 Å diameters, the apoA-I contents were 2, 2, and 3 molecules per particle, and the phospholipid content increased with the size. The conformation of apoA-I was essentially identical for the 78 and 108 Å particles and quite distinct from the 96 Å particle. These conformational differences are well documented by our spectroscopic studies (22, 24) and by our observation that LCAT activation is 13-fold lower for the 78 and 108 Å particles, in contrast to the 96 Å particle (22, 24). Furthermore, Calabresi et al. (45), using monoclonal antibodies against apoA-I, have recently confirmed the structural similarities and differences of apoA-I in the same series of particles. The spherical 93 Å rHDL has three apoA-I molecules per particle, the same surface lipids as the discoidal particles, an apoA-I conformation that is intermediate between the 78, 108 Å, and the 96 Å rHDL (by CD spectroscopy), and a cholesteryl ester core.

From studies of the mutual solubilities of lipoprotein lipids (46), it is well established that unesterified cholesterol partitions predominantly in the surface phospholipids of lipoproteins and only minimally in the core of

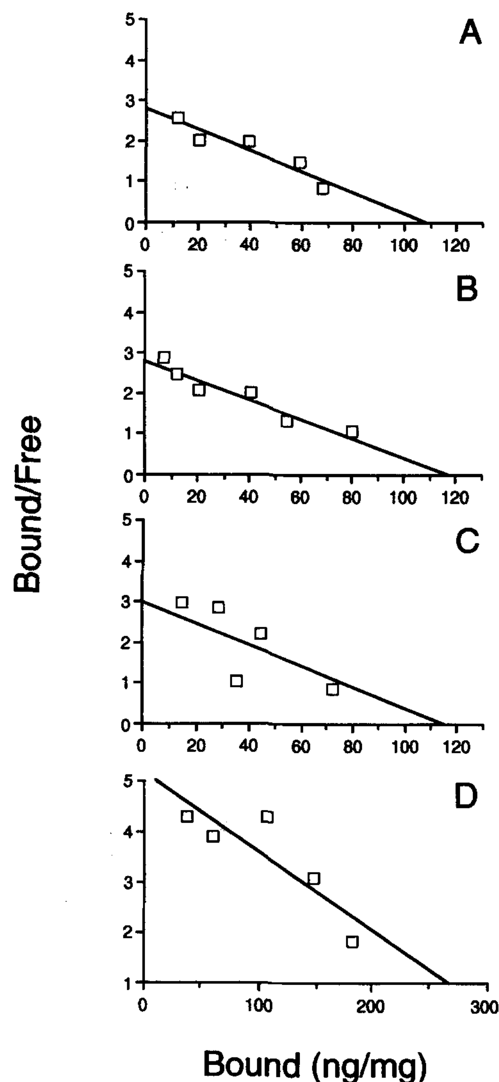

Fig. 4. Scatchard plots for the specific binding results from Fig. 3. A: 78 Å rHDL; B: 96 Å rHDL; C: 108 Å rHDL; D: 93 Å spherical rHDL.

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TABLE 3. Dissociation constants (K_d) for rHDL particles from adipocytes

HDL Particle	K_d µg/ml[a]	K_d M[b]
78 Å – disc	38, 21[c]	6.8×10^{-7}, 3.8×10^{-7}
96 Å – disc	42	7.5×10^{-7}
108 Å – disc	39	4.6×10^{-7}
93 Å – sphere	67, 40[c]	8.0×10^{-7}, 4.8×10^{-7}
HDL_3	30 ± 13[d]	4.0×10^{-7}

[a] K_d determined as a function of apoA-I concentration in the medium.
[b] K_d calculated for the molar concentration of particles, based on the apoA-I content/particle given in Table 1 and on the composition, apoA-I content, and molecular mass of HDL_3 (52).
[c] Values from two completely separate experiments, each performed on duplicate samples.
[d] Mean ± standard deviation from six separate experiments. The range for K_d was 15–51 µg/ml.

HDL. In addition, there is no evidence that cholesterol interacts specifically or directly with apoA-I. In fact Tall and Lange (47), as well as others, showed that cholesterol is excluded from contact with apoA-I in discoidal rHDL. Thus, as all four rHDLs used in this study have the same surface components, they would be expected to attain the same chemical potential for cholesterol in equilibrium with the same donor system. The capacity for cholesterol would be determined by the available phospholipid.

From Figs. 2 and 6, and Tables 2 and 4, it appears that apoA-I contents per particle and distinct apoA-I conformations have little or no effect on cholesterol transfer. The initial PC/cholesterol ratios of the particles only affect the initial mass transfer rates of cholesterol from LDL to rHDL. On the other hand, the contents of PC in the particles determine to a large extent the uptake of cholesterol by the rHDL. This means that the total available pool of

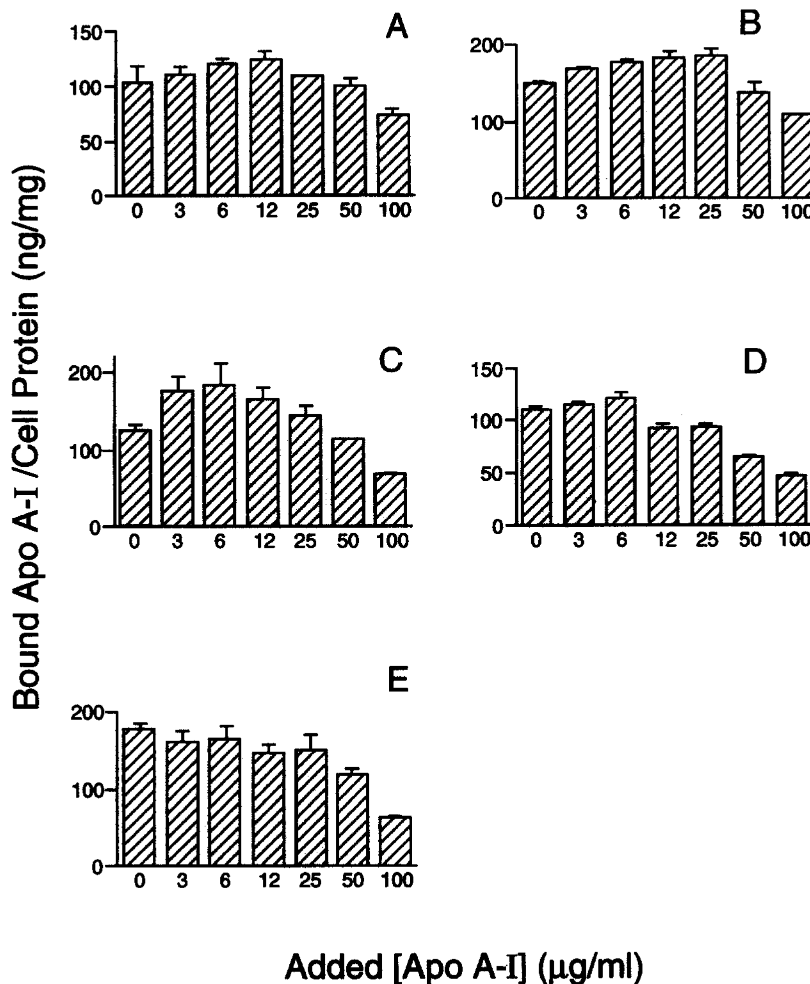

Fig. 5. Competition for ^{125}I-labeled HDL_3 binding to Ob1771 cells by cold rHDL: A: 78 Å rHDL; B: 96 Å rHDL; C: 108 Å rHDL; D: 93 Å spherical rHDL; E: cold HDL_3. The concentration of added cold rHDL is expressed as apoA-I concentration; the concentration of cold HDL_3 is expressed as concentration of protein. The results from a single experiment are the mean and variability (error bars) for duplicate samples.

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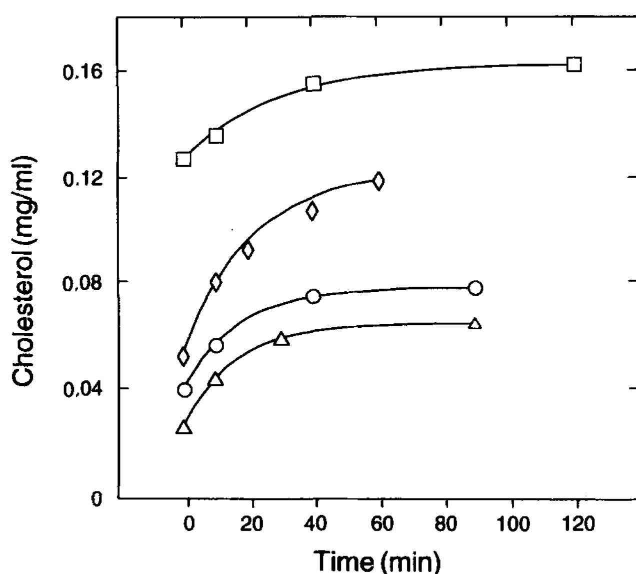

Fig. 6. Cholesterol mass transfer from LDL to rHDL particles. The cholesterol concentration in the rHDL has been normalized to 1.0 mg/ml apoA-I concentration for each data point, which represents duplicate samples. The typical experiment error is 5%. The solid curves represent the fit to the first order kinetic expression given in the Experimental Procedures. The rHDL cholesterol acceptors are: 78 Å rHDL (○); 96 Å rHDL (◇); 108 Å rHDL (□); 93 Å spherical rHDL (△).

PC in the acceptor particles, which can solubilize the cholesterol, is the main factor in the transfer of cholesterol between cells or LDL and rHDL. After accounting for the PC content of the particles (see Table 2), there are some residual size effects that suggest a preferential efflux of cellular cholesterol to the smallest particles. This may explain, in part, the results of Castro and Fielding (14) showing that the smallest preβ-migrating HDL are the initial cholesterol acceptors from fibroblasts. It is possible that there is a limiting size for HDL particles (up to 78 Å) that facilitates diffusion near cells and increases cholesterol uptake. Also, such phospholipid-poor particles may be able to abstract phospholipids from cells (19) and thereby increase cholesterol uptake.

Agnani and Marcel (17) recently reported that the efflux of cholesterol from fibroblasts to defined discoidal rHDL increases in efficiency for particles of increasing diameters, PC, and apoA-I contents. This agrees with our observation that the rHDL presenting the largest extracellular pool of PC are capable of solubilizing the largest proportions of cellular or LDL cholesterol.

In the case of cholesterol transfer from LDL to rHDL, the effects of rHDL sizes are reflected in the $t_{1/2}$ which increases from 11 to 19 min (Table 4). These observations are entirely consistent with the aqueous diffusion model for the transfer of cholesterol as described in the review by Phillips et al. (2): our estimates of $t_{1/2}$ values for transfer from LDL and cells are on the order of 20 min and 5 h, respectively, comparing reasonably well with 45 min and 11-84 h, for different acceptors, cells, and incubation conditions reported by Phillips et al. (2). Huang, von Eckardstein, and Assmann (48) recently examined the transfers of cell-derived cholesterol into pre-β HDL, α-HDL, and LDL, as well as redistribution of cholesterol from LDL into HDL due to the action of LCAT. Their results illustrate that cholesterol will redistribute in a system that is not at equilibrium according to the chemical potential of cholesterol in each of the lipid pools and the intrinsic transfer rates between the pools.

The dependence of the initial mass transfer rates on the PC/cholesterol ratios is due to the system being far or near the equilibrium distribution for cholesterol. This effect cannot be observed in the cell efflux experiments because the [^{3}H]cholesterol transfer rates represent the equilibration of the radiolabeled molecules superimposed on any mass transfers, which under special conditions may even occur in the opposite direction from the [^{3}H]cholesterol movement. For the transfer of cholesterol from LDL there appears to be a single pool of cholesterol, while the transfer from cells may have a rapid phase and a slow phase, suggesting the presence of two pools of cholesterol, as reported by Mahlberg and Rothblat (18) for other cells.

The binding and competition experiments show the presence of relatively high affinity binding sites for all the rHDL on the Ob1771 cells. The K_d values for the rHDL particles are comparable: 38-67 μg/ml based on apoA-I concentration, and 4.6 × 10^{-7} to 8.0 × 10^{-7} M based on particle concentration. These are K_d values similar to those measured for native HDL_3 with the same cells (30 ± 13 μg/ml). Furthermore, the competition of HDL_3 with the rHDL for binding to cells indicates the existence of common binding sites. If the binding to cells is mediated by apoA-I, then it is evident that the N-terminal and central regions of apoA-I, which are folded differently in the 78 Å and 108 Å particles on the one hand and the 96 Å particles on the other, and affect drastically the activation of LCAT, are not involved in the binding to cells.

TABLE 4. Transfer of cholesterol mass from LDL to rHDL particles

rHDL Particle	$t_{1/2}$ [a]	Maximum Cholesterol Uptake [b] C/ApoA-I	C/PC
	min	*mg/mg*	
78 Å – disc	12	0.078	0.090
96 Å – disc	15	0.124	0.063
108 Å – disc	19	0.163	0.073
93 Å – sphere	11	0.064	0.062

[a] From first order kinetic analysis.
[b] From kinetic analysis and the composition of the rHDL particles in Table 1; C, cholesterol; PC, phosphatidylcholine.

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Rather, the C-terminal region of apoA-I, which very likely has the same conformation in all the particles, consisting of antiparallel, amphipathic helices in contact with the acyl chains of the PC (22, 49, 50), may be responsible for the binding. The experiments of Morrison et al. (42) with the C-terminal cyanogen bromide fragment of apoA-I have implicated this region of apoA-I in binding to cell surface receptors. Moreover, Castro et al. (43) using anti apoA-I monoclonal antibodies proposed the region 149–186 of apoA-I as being responsible for the interaction with cells. On the other hand, work by Minnich et al. (51) has shown that certain mutants of apoA-I with deletions in the C-terminus are defective in binding lipid. Therefore, binding to cells could occur via the C-terminal region of apoA-I, directly to lipid, or through a receptor.

The question of whether binding to cells is required for the efflux of cholesterol is not directly addressed by our experiments. However, we find that the binding of rHDLs to cells appears to be independent of apoA-I conformation. In addition, we observe lipid transfers that are entirely consistent with aqueous diffusion of unesterified cholesterol, except perhaps for the smallest 78 Å rHDL. Therefore, it appears that although interactions between the apoA-I-containing rHDL and cells do occur, such interactions are not essential for cholesterol transfer to larger, lipid-rich acceptor particles.

An interesting observation made in this study is that certain rHDL (e.g., the 96 Å disc) may be remodeled when exposed to cells. We have shown in the past that incubations of this rHDL with LDL result in spontaneous transfer of PC from the rHDL to LDL with a $t_{1/2}$ of ~6 h (26, 40). In the process, the 96 Å particles rearrange to 87 Å intermediates, and then to relatively stable 78 Å and 108 Å products. Changes in apoA-I structure are also involved. Fig. 1 shows the same behavior of the 96 Å rHDL in the incubation with cells: they rearrange to the expected intermediates and products. We have shown previously that the 78 Å and 108 Å discs, and the 93 Å spheres may also lose phospholipids (22, 24, 40), but have much more stable apoA-I structures so that they do not rearrange on the same time scale as the 96 Å discs. Thus, the structural changes shown in Fig. 1 suggest that phospholipid transfers probably occur when rHDL are exposed to cells. How would PC transfers affect cholesterol transfer rates and extents? For the LDL incubations with rHDL the cholesterol transfer kinetics are much more rapid than PC transfers and particle rearrangements, so that data obtained for up to 2 h of incubation are unaffected by PC transfers. In the cell incubations with rHDL, similar PC transfer rates can be expected for all the rHDL particles, as the relative [^{3}H]cholesterol uptake is similar for all four particles at 45 min, as well as at 3 or 6 h, of incubation; therefore, the changes in the total PC pool sizes should be comparable. These results suggest that in vitro or in vivo experiments involving the addition of rHDL particles or heterologous lipoproteins to cells or to plasma are likely to result in spontaneous transfers of lipids and particle remodeling until a new equilibrium/steady state is attained.

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