Cholesterol Transfer from Low Density Lipoproteins to Reconstituted High Density Lipoproteins Is Determined by the Properties and Concentrations of Both Particles†

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ABSTRACT: Cholesterol spontaneously transfers from low density lipoproteins (LDL) to high density lipoproteins (HDL). This transfer is important physiologically as it supplies the major portion of cholesterol for the lecithin:cholesterol acyltransferase reaction and is one mechanism for the reduction of atherogenic LDL cholesterol. The objective of this work was to examine the properties of both HDL and LDL which modulate cholesterol transfer, as well as to obtain the relevant kinetic constants for the transfer at concentrations of lipoproteins approaching those existing *in vivo*. To examine the effects of HDL structural parameters on cholesterol transfer, we prepared reconstituted HDL particles with saturated or unsaturated phospholipid, with apolipoprotein AI or apolipoprotein AII, with increasing size and phospholipid content, and with increasing initial contents of cholesterol. We also prepared five LDL subfractions of variable density and size. The kinetics of cholesterol mass transfer were measured by incubating LDL with rHDL at 37 °C, separating the lipoproteins by dextran sulfate/Mg^{2+} precipitation of LDL at timed intervals, and analyzing rHDL cholesterol content. The cholesterol content of rHDL at equilibrium, C_{eq}, and the half-time for transfer, $t_{1/2}$, as well as the ratio of the lipid surface areas of LDL to rHDL were used in the analysis of the kinetic data by the aqueous diffusion model for lipid transfer developed by Nichols and Pagano [(1982) *Biochemistry 21*, 1720−1726]. The only variables that significantly affect the C_{eq} and/or $t_{1/2}$ are the phospholipid content and composition of the rHDL and the size or density of the LDL particles. These effects are mostly related to the differences in the ratio of donor to acceptor surface areas. Experiments as a function of LDL and rHDL concentrations gave the off-rate constants, as well as the ratio of the affinity constants and the ratio of the on-rate constants for cholesterol in equilibrium with LDL and rHDL. We found that rHDL has 0.7-fold the affinity constant of LDL, but a 3.7-fold higher off-rate constant, and 2.7-fold higher on-rate constant for cholesterol adsorption than does LDL. In addition, we determined that over 40% of the cholesterol in LDL is not available for transfer and confirmed that these rHDL have a limited capacity for cholesterol compared to a phospholipid bilayer.

High density lipoproteins (HDL)[1] remove cholesterol both from peripheral tissues and from other lipoproteins, including low density lipoproteins (LDL), in the process of reverse cholesterol transport from the peripheral tissues to the liver. Recent work suggests that certain HDL subclasses may preferentially accept cholesterol from cells; for example, pre-β HDL are thought to be the initial acceptors of cellular cholesterol (Castro & Fielding, 1988). Also, according to some reports, the apolipoprotein composition of HDL may affect its ability to accept cholesterol from cells. These studies show that HDL containing only apolipoprotein AI (apoAI) are more effective acceptors of cholesterol from cells than HDL containing both apoAI and apolipoprotein AII (apoAII) (Barkia et al., 1991; Barbaras et al., 1987), while other studies dispute this finding (Oikawa et al., 1993; Johnson et al., 1991). Since much of the cholesterol esterified on HDL by the enzyme lecithin:cholesterol acyltransferase (LCAT) comes from LDL (Huang et al., 1993; Miida et al., 1990), the objective of this study was to identify the physical properties of both HDL and LDL which modulate the ability of HDL to accept cholesterol from LDL, and to investigate the mechanism and kinetic parameters for the transfer of cholesterol between LDL and HDL under conditions approaching those *in vivo*.

Since native HDL is a heterogeneous mixture of particles, it is difficult to isolate reasonable quantities of HDL homogeneous in lipid content, protein composition, shape, and size. Reconstituted HDL (rHDL) (Matz & Jonas, 1982; Jonas, 1986) provide a convenient model system to examine the role of each of these parameters separately. For this study, rHDL were made with saturated or unsaturated phospholipids (PL), with apoAI or apoAII, in three discrete sizes, and with varying amounts of cholesterol. Most of these particles have been well characterized previously (Jonas et al., 1989, 1990).

LDL is also a heterogeneous mixture of particles spanning a spectrum of densities, lipid compositions, and sizes (Shen et al., 1981). LDL subfractions, which differ measurably in all of these parameters (Chapman et al., 1988), can be isolated by density gradient ultracentrifugation. These subfractions are defined by the fractionation method used, rather than by any naturally occurring separations between

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[1] Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; apoAI, apolipoprotein AI; apoAII, apolipoprotein AII; LCAT, lecithin:cholesterol acyltransferase; rHDL, reconstituted HDL; PL, phospholipid; POPC, palmitoyloleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; eggPC, phosphatidylcholine from egg yolk; C, unesterified cholesterol; CE, cholesterol ester; TG, triglyceride.

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distinct particles. Previous work has shown that these subfractions differ metabolically in their rate of degradation (Swinkels et al., 1988), in their ability to bind to the LDL receptor (Swinkels et al., 1990), and in their ease of oxidation (Tribble et al., 1992; de Graaf et al., 1991). In addition, several studies have demonstrated that the distribution of LDL subfractions differs between individuals (Austin et al., 1988; Austin & Krauss, 1986): patients with heart disease are more likely to show a predominance of small, dense LDL particles than are healthy controls (Austin et al., 1988). The ability of these subfractions to donate cholesterol to HDL is examined for the first time in this study.

The mechanism of cholesterol transfer between vesicles or lipoproteins has been a subject of debate for many years (Brown, 1992; Backer & Dawidowicz, 1981). Recently, the aqueous diffusion model has gained acceptance (Phillips et al., 1987). According to this mechanism, cholesterol first leaves the donor surface, diffuses through the aqueous medium, and finally adsorbs to the acceptor surface. When cholesterol or any other lipid is able to desorb or adsorb to either donor or acceptor with similar rate constants, the physical properties and concentrations of both influence transfer (Nichols & Pagano, 1981, 1982; Phillips et al., 1987). Steck et al. (1988) have challenged the aqueous diffusion mechanism for cholesterol transfer from red blood cells. They proposed an activation−collision model, in which cholesterol first becomes activated within the donor particle (a first order reaction) and then is transferred during collision with the acceptor particle (a second order process). Since the transfer rate depends on both donor and acceptor concentrations in either model, it is difficult to unambiguously distinguish the activation collision mechanism from the aqueous diffusion mechanism by simply varying the donor and acceptor concentrations. However, our results are highly consistent with the aqueous diffusion mechanism which allows the determination of rate constants and affinity constants for the equilibration of cholesterol between LDL and rHDL.

MATERIALS AND METHODS

Palmitoyloleoylphosphatidylcholine (POPC), dipalmitoylphosphatidylcholine (DPPC), phosphatidylcholine from egg yolk (eggPC), cholesterol, and sodium cholate were purchased from Sigma Chemical Co. ApoAI and apoAII were isolated from human plasma, obtained from the Champaign County Blood Bank, by routine methods used in our laboratory (Matz & Jonas, 1982; Schumaker & Puppione, 1986; Edelstein et al., 1972; Nichols et al., 1976). All experiments were performed in a 0.01 M Tris buffer (pH 8.0) containing 0.15 M NaCl, 1 mM NaN_3, and 0.01% EDTA.

Preparation of rHDL. The sodium cholate dialysis method (Matz & Jonas, 1982; Jonas, 1986) was used to prepare rHDL particles. Typically, particles were made without initial cholesterol using a 100:1 molar ratio of eggPC to apoAI. These particles were used with the LDL subfractions and in the concentration dependence studies. To study the effect of lipid packing, particles were prepared from either POPC or DPPC in a 100:1 PL to apoAI ratio. To examine the effect of initial cholesterol (C) content, particles were made with molar ratios of eggPC:C:apoAI of 80:0:1 (0%), 80:2.4:1 (3%), 80:4.8:1 (6%), 80:7.2:1 (8%), and 80:9.6:1 (11%). Particles with apoAI or apoAII were prepared with POPC. The apoAII particles contained the same mass of POPC per milligram of protein as apoAI particles made in a 100:1 molar ratio of POPC:apoAI.

Preparation of LDL. Unfractionated LDL was isolated from human plasma by sequential ultracentrifugation between the densities of 1.019 and 1.063 g/mL, while LDL for subfractionation was prepared between the densities of 1.024 and 1.050 g/mL to reduce contamination with IDL and Lp(a). Subsequent density gradient ultracentrifugation was performed according to the procedure of Chapman et al. (1988) using a SW 41 Ti swinging bucket rotor at 10 °C for 44 h. After centrifugation, 15 fractions of 0.8 mL each were manually removed from the top of the tube. All LDL preparations were heat inactivated at 60 °C for 1 h and sterilized by filtration before use.

Measurement of Cholesterol Transfer. Appropriate amounts of LDL and rHDL were mixed and incubated at 37 °C; aliquots of the reaction mixture were removed at timed intervals. Except for the concentration dependence studies, LDL and rHDL were present in equal protein concentrations of either 1 or 0.5 mg/mL. Following the procedure of Bachorik and Albers (1986), dextran sulfate/Mg^{2+} was added to the reaction mixture aliquots to precipitate LDL. A lower concentration of reagents is needed in the absence of plasma proteins (Burstein et al., 1970); thus a 1:20 dilution of the working reagent was used. Typical recoveries of POPC-rHDL and eggPC-rHDL in the supernatant were 70−80%, while recovery of DPPC-rHDL was 40−50%. In contrast, more than 95% of the LDL was precipitated. Cholesterol was measured in the rHDL supernatant by an enzymatic method (Heider & Boyett, 1978); protein was determined with a Lowry assay modified for lipoproteins by the addition of SDS (Markwell et al., 1978). All experiments were repeated at least twice. The reported composition values are the means ± SD.

Kinetic Data. In most experiments, the cholesterol contents of rHDL at different time points were normalized to a protein concentration of 1 mg/mL by dividing the cholesterol content of each rHDL sample by its protein content after precipitation of LDL. This corrected for varying rHDL recoveries after precipitation. In the concentration dependence studies, cholesterol contents were normalized to the initial rHDL protein concentration.

As described by Gains (1992), the rHDL cholesterol values were fitted to the first order exponential expression:

$$C_t = C_0 + C_{max}(1 - e^{-"k"t}) \quad (1)$$

where C_t is the normalized cholesterol content of the rHDL particles at time t, expressed as the bulk concentration of cholesterol in mg/mL; C_0 is the initial cholesterol content of the particles in mg/mL; C_{max} is the maximum amount of cholesterol transferred from the LDL to the rHDL particles, in mg/mL; "k" is an apparent rate constant for transfer which is actually a combination of several rate constants and concentrations as indicated below (Nichols & Pagano, 1981, 1982); and t is time. The computer program Kaleidagraph was used to fit the data to eq 1 and yielded C_{max} and "k" directly. The half-time of transfer, $t_{1/2}$, was calculated from "k"; $t_{1/2} = (\ln 2)/"k"$. The cholesterol content of rHDL at equilibrium, C_{eq}, is the sum of C_0 and C_{max}. For many of the experiments C_0 was 0 mg/mL; thus C_{eq} and C_{max} were equivalent. The C_{eq} values are a measure of the capacity of

the rHDL to accept cholesterol under the conditions of the experiment, while $t_{1/2}$ values (the time required to come halfway to equilibrium) are a measure of the efficiency of transfer. In the measurement of the kinetic parameters, summarized in Tables 2 and 4, the concentration of apoAI was fixed; therefore, all the comparisons of kinetic parameters in the text refer to equal apoAI concentrations.

Kinetic Analysis. As reviewed by Phillips et al. (1987), the aqueous diffusion mechanism is the most widely accepted model for cholesterol transfer between lipid surfaces. The following equation applies for the transfer of cholesterol from LDL to rHDL via aqueous diffusion:

$$\text{LDL-C} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{C} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \text{rHDL-C}$$

where k_1 and k_4 are the off-rate constants from LDL and rHDL, and k_2 and k_3 are the on-rate constants to LDL and rHDL, respectively. $K_{LDL} = k_2/k_1$ and $K_{HDL} = k_3/k_4$ are the association or affinity constants of cholesterol for LDL and rHDL, respectively. Here, the terms "association or affinity constant" do not imply specific binding, but rather adsorption of cholesterol to the surface of the lipoproteins.

Nichols and Pagano (1981, 1982) derived a rate equation for lipid transfer by aqueous diffusion between phospholipid vesicles where the rate of transfer is expressed in terms of rate constants and surface areas available for lipid adsorption. Their integrated rate equation (Nichols & Pagano, 1982) has the same form as eq 1 and contains, in the exponent, the expression for "k", which converted to the $t_{1/2}$ for the LDL and HDL system becomes:

$$t_{1/2} = \frac{\ln 2}{\text{"}k\text{"}} = (\ln 2)\left[\frac{[(\text{LDL})_{surf}/(\text{HDL})_{surf}](1/k_4) + K_{HDL}/K_{LDL}(1/k_1)}{K_{HDL}/K_{LDL} + (\text{LDL})_{surf}/(\text{HDL})_{surf}}\right] \quad (2)$$

In eq 2 $(\text{LDL})_{surf}$ and $(\text{HDL})_{surf}$ are the lipid surface areas of these particles. The integrated rate equation also gives C_{eq}:

$$C_{eq} = \frac{\alpha[\text{LDL}]_C}{1 + [k_2k_4(\text{LDL})_{surf}]/[k_1k_3(\text{HDL})_{surf}]} \quad (3)$$

which rearranges to

$$\frac{[\text{LDL}]_C}{C_{eq}} = \frac{K_{LDL}(\text{LDL})_{surf}}{\alpha K_{HDL}(\text{HDL})_{surf}} + \frac{1}{\alpha} \quad (4)$$

In eqs 3 and 4, α is the fraction of LDL cholesterol available for transfer, and $[\text{LDL}]_C$ is the initial concentration of cholesterol in LDL. Using eq 4, a linear plot of $[\text{LDL}]_C/C_{eq}$ *versus* $(\text{LDL})_{surf}/(\text{HDL})_{surf}$ can give α and the ratio of the association constants of cholesterol for LDL and HDL. Inspection of eq 2 shows that when $(\text{HDL})_{surf}$ is much greater than $(\text{LDL})_{surf}$, the equation reduces to $t_{1/2} = (\ln 2)/k_1$, and when $(\text{LDL})_{surf}$ is much greater than $(\text{HDL})_{surf}$, the equation approaches $t_{1/2} = (\ln 2)/k_4$. Thus two rate constants, k_1 and k_4, and the ratio of the affinity constants as well as k_2/k_3 can be obtained from cholesterol transfer experiments as a function of donor and acceptor concentrations expressed as surface area ratios.

Table 1: Variables and Comparisons of rHDL Acceptors of Cholesterol from LDL

variable	r-HDL	composition,[a] PL/C/protein (mol/mol)
phospholipid composition	POPC-rHDL	63/0/1
	DPPC-rHDL	64/0/1
apolipoprotein	apoAI-POPC-rHDL	63/0/1[b]
	apoAII-POPC-rHDL	40/0/1
size, phospholipid content	apoAI-POPC-rHDL[c]	
	78 Å diameter	32/3/1
	96 Å diameter	73/4/1
	108 Å diameter	82/9/1
initial cholesterol content	apoAI-eggPC-rHDL	50/0.0/1
		56/1.2/1
		55/2.7/1
		58/4.4/1
		57/6.0/1

[a] PL, phospholipid; C, free cholesterol. The chemical compositions were determined as described in the Materials and Methods section. The means are given, and standard deviations of measurements performed in triplicate are ±5−10%. At least 2 particle preparations were made for each variable; representative data are shown. [b] ApoAI-POPC-rHDL have a PL/protein mass ratio of 1.76; apoAII-POPC-rHDL have a PL/protein ratio of 1.84. [c] Jonas et al., 1994. Except for this series, all other particles had a major particle component with a diameter of 96 ± 3 Å, representing >80% of all the protein in each preparation.

RESULTS AND DISCUSSION

Effects of rHDL Variables on Cholesterol Transfer from LDL to rHDL. Reconstituted HDL particles differing in phospholipid composition, apolipoprotein type, size, and initial cholesterol content were prepared. Table 1 lists the chemical composition of the various rHDL preparations used in this study. The POPC-rHDL were identical to the DPPC-rHDL, except for their phospholipid composition. The apoAI-POPC-rHDL and apoAII-POPC-rHDL particles were made with similar weight ratios of PL/protein rather than similar molar ratios of PL/protein since apolipoproteins interact with phospholipid through amphipathic helical lipid-binding units (Jonas, 1992). All of these preparations had a major rHDL component (≥80% of protein mass) around 96 ± 3 Å in diameter.

Differences in size of rHDL particles cannot be separated from differences in the amount of phospholipid, since larger particles always contain more phospholipid than smaller ones. Particles prepared with POPC and apoAI but differing in size have been described in previous publications (Jonas et al., 1989, 1994). Both the 78 and 108 Å particles are exposed to LDL during their preparation. A series of particles with different initial cholesterol contents were prepared with eggPC and apoAI. All particles in this series were predominantly 96 ± 3 Å in diameter and were similar except for their initial cholesterol contents.

Cholesterol spontaneously transfers from LDL to all of the rHDL particles described above. Figure 1 shows characteristic time course results for cholesterol transfer from LDL to rHDL, and eq 1 given in the Materials and Methods section describes this transfer. Table 2 summarizes the C_{max}, C_{eq}, and $t_{1/2}$ kinetic parameters for all the rHDL particles described above.

DPPC-rHDL accepts somewhat less cholesterol than POPC-rHDL at equilibrium, as indicated by the C_{eq} of 0.082 mg/mL for DPPC-rHDL *versus* 0.12 mg/mL for POPC-

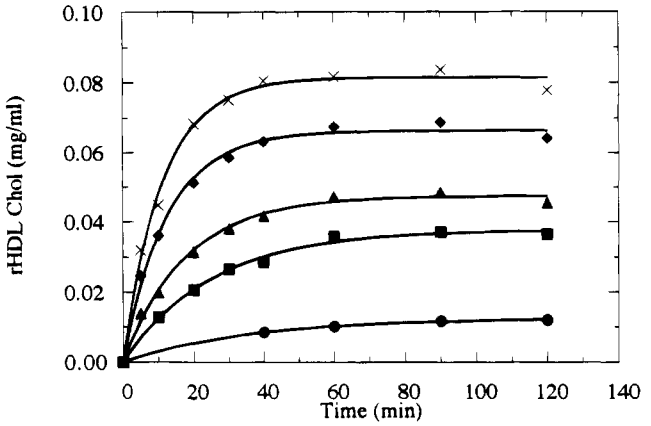

FIGURE 1: Cholesterol transfer kinetics from LDL to rHDL at variable concentrations of LDL. Cholesterol transfer from LDL to rHDL was measured as described in Materials and Methods and Table 2. The [rHDL] protein concentration was fixed at 0.5 mg/mL, while [LDL] varied from 0.05 to 2.5 mg/mL: (●) 0.05 mg/mL; (■) 0.25 mg/mL; (▲) 0.5 mg/mL; (♦) 1.25 mg/mL; and (×) 2.5 mg/mL. The curves represent the computer fit of the data to eq 1.

Table 2: Kinetic Parameters for Cholesterol Transfer from LDL to Different rHDL[a]

r-HDL[b]	C_{max} (mg/mL)	C_{eq} (mg/mL)	$t_{1/2}$ (min)
POPC-rHDL	0.12 ± 0.02	0.12 ± 0.02	14 ± 2
DPPC-rHDL	0.082	0.082	15
apoAI-rHDL	0.12	0.12	14
apoAII-rHDL	0.10	0.10	14
78 Å[c]	0.038	0.078	12
96 Å	0.069	0.12	15
108 Å	0.036	0.16	19
0% Chol[d]	0.094	0.094	12
2% Chol	0.074	0.095	12
5% Chol	0.068	0.10	14
7% Chol	0.061	0.11	12
10% Chol	0.052	0.10	12

[a] LDL isolated between d = 1.019−1.063 g/mL. LDL (1 mg/mL) and rHDL (1 mg/mL) were incubated at 37 °C, aliquots were removed at timed intervals, LDL was precipitated, and the cholesterol was measured in the rHDL supernatant. Kinetic parameters were obtained using eq 1 given in the Materials and Methods section. [b] Compositions of the rHDL particles are given in Table 1. [c] The standard error in C_{max} from the fit of the data to the exponential curve is ±10%; standard error for $t_{1/2}$ is ±15%. [d] % Chol is [C/(C + PL)] × 100, in terms of moles of lipids.

rHDL, but the $t_{1/2}$ values for the two particles are identical. Thus, according to eq 4 the association constant of cholesterol for the DPPC-rHDL particles appear to be slightly lower than for the POPC-rHDL particles. These results suggest that in rHDL particles an unsaturated POPC matrix is a slightly better acceptor for cholesterol than a gel phase, saturated DPPC matrix.

When apoAII replaces apoAI in POPC-rHDL, the differences in C_{eq} are small (0.10 mg/mL for AII-POPC-rHDL *versus* 0.12 mg/mL for AI-POPC-rHDL), and the $t_{1/2}$ values are identical for these two rHDL particles. Thus, changing the apolipoproteins in the rHDL particles does not significantly affect the capacity of rHDL to accept cholesterol (relative to the 0.02 mg/mL error of measurement), the cholesterol affinity constant, nor the rate constants for cholesterol transfer. This result is in contrast with cellular studies which report that HDL particles containing only apoAI are better acceptors of cholesterol from cells than HDL containing both apoAI and apoAII (Barkia et al., 1991; Barbaras et al., 1987). However, there remains the possibility that cholesterol transfers from cells to HDL acceptors involve a mechanism more complex than aqueous diffusion.

The effect of rHDL size and phospholipid content on cholesterol transfer was investigated using the series of rHDL particles with 78, 96, and 108 Å diameters. Since these particles contained cholesterol initially, the C_{max} values given in Table 2 are lower than the C_{eq} values. The C_{eq} values and $t_{1/2}$ values increase with increasing size and phospholipid content of the particles. In fact, by reference to eqs 2 and 3, these changes in the kinetic parameters can be accounted for by the increase in $(HDL)_{surf}$ with increasing particle diameter and lipid content. Thus, the smaller rHDL, with the smaller $t_{1/2}$ values, accept cholesterol more rapidly from LDL. These results may be relevant physiologically since the smallest pre-β HDL are reported to be the initial acceptors of cholesterol from cells (Castro & Fielding, 1988; Kunitake et al., 1985). According to the dependence of $t_{1/2}$ on the ratio of donor to acceptor particle surface areas observed in this experiment, it can be predicted that individual small HDL particles would accept cholesterol more rapidly than larger particles; however, their capacity for cholesterol at equilibrium would be lower than that of the larger HDL particles containing more phospholipid. This hypothesis agrees with the results of Agnani and Marcel (1993), who showed that larger rHDL particles have, indeed, a greater capacity to accept cellular cholesterol.

A series of rHDL particles with 0−10 mol % cholesterol was prepared to determine how varying the amount of initial cholesterol influences cholesterol transfer. Predictably, particles with the lowest initial content of cholesterol acquire the largest amount of cholesterol from LDL, i.e., have the highest C_{max}. The C_{eq} values are all very similar, demonstrating that the amount of cholesterol in the rHDL at equilibrium does not depend on its initial cholesterol content. The $t_{1/2}$ values are also similar; therefore, for these initial contents of cholesterol the rate constants for cholesterol transfer to the rHDL have not been affected. The cholesterol transfer properties of these particles confirm that the differences in C_{eq} and $t_{1/2}$ observed with the 78, 96, and 108 Å particles are not due to differences in their initial cholesterol contents but rather to differences in their phospholipid contents and/or sizes.

Cholesterol Transfer from LDL Subfractions Having Different Densities. Density gradient ultracentrifugation of LDL in the density range from 1.024 to 1.050 g/mL produced five LDL subfractions in sufficient quantity for cholesterol transfer analysis. Table 3 lists their densities, chemical compositions, and C/PL ratios. The subfractions differ measurably in their chemical compositions; as expected, the more dense subfractions contain less lipid. The C/PL molar ratio for all subfractions is very similar. Electrophoresis of the subfractions on a Pharmacia 4−15% Phast system gradient gel verified that the subfractions become progressively smaller as they increase in density (data not shown).

The five LDL subfractions were incubated with predominantly 96 Å apoAI-eggPC-rHDL containing no initial cholesterol, and the kinetic parameters were obtained for cholesterol transfer (Table 4). The maximum amount of cholesterol transferred from LDL to rHDL (C_{eq}) decreases with increasing LDL subfraction density. This trend may be explained by the changes in $(LDL)_{surf}$ and $[LDL]_C$ of the

Table 3: Characterization of LDL Subfractions[a]

LDL subfraction	density (g/mL)[b]	PL/C/CE/TG/protein (molar ratios)[c]	C/PL (molar ratios)
LDL 7	1.035	1340/600/1480/131/1	0.45
LDL 8	1.037	1260/554/1350/108/1	0.44
LDL 9	1.040	1110/541/1200/82/1	0.49
LDL 10	1.043	1050/463/1120/72/1	0.44
LDL 11	1.048	1030/464/1090/74/1	0.45

[a] Isolated from LDL, d = 1.024−1.050 g/mL. [b] Densities were measured from a gradient prepared without LDL. The mass of twelve 500 μL aliquots of each fraction was averaged. [c] PL, phospholipid; C, free cholesterol; CE, cholesterol ester; TG, triglyceride. Compositions were measured as described in Materials and Methods. Mean values are given in the table; measurments performed in triplicate have standard deviations of ±5−10%.

Table 4: Kinetic Parameters for Cholesterol Transfer from Different LDL Subfractions to rHDL[a]

LDL subfraction	C_{eq} (mg/mL)	$t_{1/2}$ (min)	LDL subfraction	C_{eq} (mg/mL)	$t_{1/2}$ (min)
LDL 7	0.13	14	LDL 10	0.11	16
LDL 8	0.12	16	LDL 11	0.10	15
LDL 9	0.12	15			

[a] ApoAI-eggPC-rHDL was incubated at 37 °C with LDL subfractions as described in Table 2 except that both LDL and rHDL were at a protein concentration of 0.5 mg/mL. Kinetic parameters were obtained as described in Table 2.

different LDL subfractions if the effect of $[LDL]_C$ predominates. Since $t_{1/2}$ values remain constant, the rate constants for cholesterol on the LDL particles are not significantly affected by their core composition and size differences.

Concentration Dependence Studies of Cholesterol Transfer. Three experiments were performed to determine the kinetic constants for cholesterol transfer from LDL to rHDL and to determine if the aqueous diffusion model adequately describes this process. In these experiments either [rHDL], [LDL], or the ratio of [LDL]/[rHDL] was held constant, and [LDL], [rHDL], or total concentration was varied, respectively. Figure 1 shows the kinetic curves for the first type of experiment ([rHDL] constant), and Table 5 summarizes the measured kinetic parameters for all three experiments. The rHDL used in these experiments contained no initial cholesterol; thus $C_{max} = C_{eq}$.

The C_{eq} at an infinite [LDL] can be obtained from the y-intercept of a plot of $1/C_{eq}$ *versus* 1/[LDL] (not shown). This is the maximum amount of cholesterol that can be transferred to 96 Å eggPC-apoAI-rHDL from LDL and is 0.085 mg/mL cholesterol for an [rHDL] of 0.5 mg/mL. For the 96 Å rHDL used in this study, this is equivalent to a molar ratio of 80/12/1 (PL/C/apoAI) or 13 mol % cholesterol. In agreement with this result, we have previously observed that 96 Å rHDL particles cannot be made with cholesterol contents higher than about 16 mol % (Jonas & McHugh, 1983). This limit for the cholesterol content of the rHDL is well below the 50 mol % cholesterol contents observed for eggPC liposomes. The low capacity of the rHDL phospholipid bilayer to solubilize cholesterol may be due to the exclusion of cholesterol from the phospholipid adjacent to the apolipoprotein (Tall et al., 1977; Jonas & McHugh, 1983). Alternatively, the ordering and restriction of the phospholipid by the apolipoprotein ring may decrease the volume available for the incorporation of cholesterol throughout the bilayer. A prediction of this hypothesis is that larger rHDL particles, with larger phospholipid to apolipoprotein ratios, will be able to accommodate more cholesterol. Also, mixtures of phospholipids found in native HDL may provide a more favorable matrix for cholesterol incorporation.

Figures 2 and 3 show the plots of $[LDL]_C/C_{eq}$ and $t_{1/2}$ as a function of $(LDL)_{surf}/(HDL)_{surf}$, respectively. The $(LDL)_{surf}/(HDL)_{surf}$ values were calculated from the concentration of the LDL and HDL expressed in terms of the bulk surface phospholipids, assuming that these lipids have equivalent surface areas on LDL and rHDL and that they represent the surfaces available for cholesterol adsorption. The plots of $[LDL]_C/C_{eq}$ against $(LDL)_{surf}/(HDL)_{surf}$ are linear (see Figure 2) and quite similar for the two experiments performed at constant [HDL] or constant [LDL]. Interestingly, the y-intercept gives an average α value of 0.55, which indicates that over 40% of LDL cholesterol is not available for transfer under the conditions of our experiments. This could be cholesterol that is tightly bound to apoB or some specific phospholipid, such as sphingomyelin, or cholesterol that does not exchange between the core and surface of the LDL particle. The ratio of the association (affinity) constants, K_{LDL}/K_{HDL}, obtained from Figure 2 and eq 4, is on average 1.4. Thus the affinity of cholesterol in the aqueous medium is 1.4-fold greater for LDL than for rHDL. Figure 3 shows the dependence of $t_{1/2}$ on the ratio of $(LDL)_{surf}/(HDL)_{surf}$ and gives $k_1 = 0.026$ min^{-1} and $k_4 = 0.097$ min^{-1}. In other words, the off-rate constant for cholesterol from rHDL is 3.7-fold greater than the off-rate constant for cholesterol from LDL. The ratio of on-rate constants, k_2/k_3, can be calculated from the association constant ratio and the off-rate constants. The calculated relationship is that the on-rate constant of cholesterol for rHDL is 2.7-fold greater than the on-rate constant for LDL. These constants are summarized below:

$$\underset{(\alpha = 0.55)}{\text{LDL-C}} \underset{k_2}{\overset{t_{1/2} = 27\text{ min};\ k_1 = 0.026\text{ min}^{-1}}{\rightleftharpoons}} \text{C} \underset{k_4 = 0.097\text{ min}^{-1};\ t_{1/2} = 7\text{ min}}{\overset{k_3 = 2.7\,k_2}{\rightleftharpoons}} \text{HDL-C}$$

Our experimental results agree very well with the behavior predicted for the aqueous diffusion model and kinetic equations of Nichols and Pagano (1982). Clearly, the extent (C_{eq}) and efficiency ($t_{1/2}$) of cholesterol transfer between LDL and rHDL depend on the ratio of the surfaces of donor and acceptor particles available for cholesterol transfer. Another prediction of the diffusion model is that $t_{1/2}$ values would remain constant as the donor and acceptor particle concentrations increase proportionately (eq 2) (Nichols & Pagano, 1981, 1982). In fact, the third experiment summarized in Table 5 shows that $t_{1/2}$ remains constant at 9.4 (±0.8 SD) min as the total lipoprotein concentration changes at a fixed ratio of LDL to HDL. Although the activation−collision model of Steck et al. (1988) cannot be ruled out completely by our data, the evidence for the diffusion-mediated mechanism is very strong.

Both the on- and off-rate constants for cholesterol adsorption to rHDL are considerably higher than to LDL, probably due to the more fluid nature of the rHDL surface lipids than those of LDL (Jonas, 1975). In fact, several studies of cholesterol exchange between unilamellar phospholipid vesicles have shown that the off-rate constant for cholesterol depends on the nature of the vesicle phospholipids and decreases in the order: unsaturated PC > saturated PC >

Table 5: Kinetic Parameters from Concentration Dependence Studies[a]

study	[LDL donor] (mg/mL)	[rHDL acceptor] (mg/mL)	[LDL]:[rHDL]	C_{eq} (mg/mL)	$t_{1/2}$ (min)
[rHDL][b] constant	0.05	0.5	0.1:1	0.013 ± 0.02	24 ± 2
	0.25	0.5	0.5:1	0.038	17
	0.5	0.5	1.0:1	0.047	12
	1.25	0.5	2.5:1	0.066	8.8
	2.5	0.5	5.0:1	0.082	7.8
[LDL] constant	0.5	0.1	5.0:1	0.013	10
	0.5	0.2	2.5:1	0.022	11
	0.5	0.5	1.0:1	0.042	12
	0.5	1	0.5:1	0.058	17
	0.5	2.5	0.2:1	0.068	19
[LDL]:[rHDL][c] constant	0.25	0.1	2.5:1	0.011	8.9
	0.375	0.15	2.5:1	0.018	9.8
	0.5	0.2	2.5:1	0.022	8.6
	0.625	0.25	2.5:1	0.029	10
	1.25	0.5	2.5:1	0.054	9.2
	1.875	0.75	2.5:1	0.092	9.2
	2.5	1	2.5:1	0.13	11
	3.75	1.5	2.5:1	0.18	8.8
	5	2	2.5:1	0.23	9.2

[a] Kinetic parameters were obtained as described in Table 2. The errors are as indicated in Table 2. [b] [rHDL] and [LDL] refer to protein concentrations. [c] The mean and SD for these $t_{1/2}$ measurements is 9.4 ± 0.8 min.

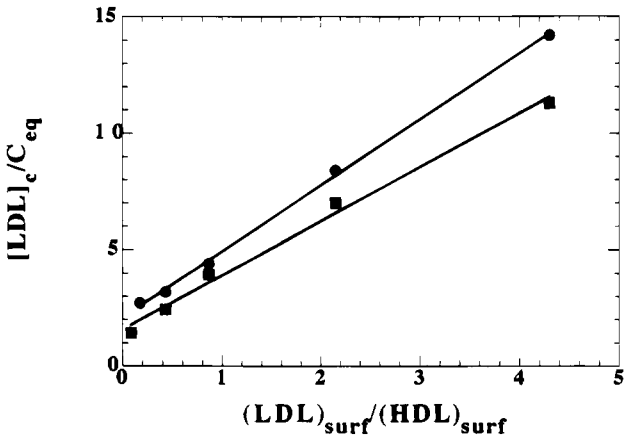

FIGURE 2: Plot, according to eq 4, of the ratio of initial cholesterol in LDL, $[LDL]_C$, over the cholesterol concentration in rHDL at equilibrium, C_{eq} (Table 5), as a function of the ratio of donor $(LDL)_{surf}$ to acceptor $(HDL)_{surf}$ phospholipid surface areas. To calculate $[LDL]_C$ and the $(LDL)_{surf}/(HDL)_{surf}$ ratio, we used the average cholesterol and phospholipid contents of the particles from Tables 1 and 3. We assumed that the surface areas available for cholesterol adsorption are proportional to the phospholipid concentrations. Data obtained for constant [rHDL] (■); data obtained for constant [LDL] (●). The inverse of the y-intercept from the linear regression analysis gives α, the fraction of cholesterol on LDL available for transfer, and the slope × α gives the K_{LDL}/K_{HDL} ratio (see eq 4).

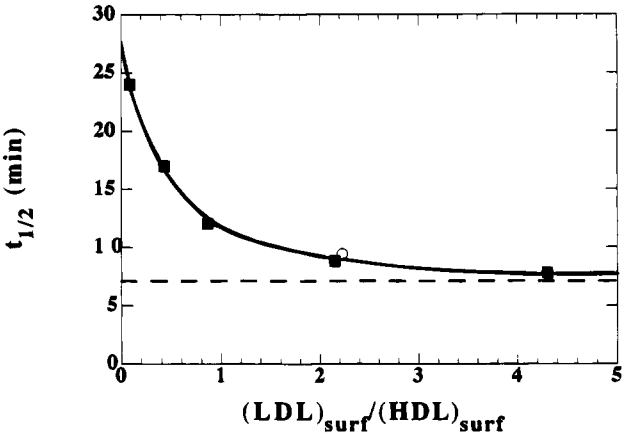

FIGURE 3: Plot, according to eq 3, of half-time, $t_{1/2}$, from Table 5, *versus* the ratio of donor to acceptor surface areas $(LDL)_{surf}/(HDL)_{surf}$, calculated as explained in the legend to Figure 2. Data for constant [HDL] (■); data for a constant [LDL]/[rHDL] ratio (○). The $t_{1/2}$ value at the y-intercept gives k_1, and the asymptotic value of $t_{1/2}$, as $(LDL)_{surf}/(HDL)_{surf}$ becomes very large, gives k_4.

sphingomyelin (Fugler et al., 1985; Lund-Katz et al., 1988; Bar et al., 1987). Previous reports of $t_{1/2}$ values for cholesterol desorption from native HDL in the presence of excess acceptor were 2.9 and 4 min for HDL_3 and HDL_2 at 37 °C, respectively (Lund-Katz et al., 1982). For a series of PC complexes with apoAI, the $t_{1/2}$'s reported for cholesterol desorption range from 14 to 130 min, increasing with the size and phospholipid content of the complexes (Letizia & Phillips, 1991). The published $t_{1/2}$ for cholesterol desorption from LDL is 45 min at 37 °C (Lund-Katz et al., 1982). These off-rate constants are in reasonable agreement with the values determined in this work; however, they were all obtained under experimental conditions where the acceptor particle is present in large excess. Under the more physiological conditions of similar LDL and rHDL concentrations, we show that the measured $t_{1/2}$ is a function of the ratio of both particles; we also demonstrate that the affinity of rHDL for cholesterol is similar to that of LDL. Finally, our results suggest that only 55% of the cholesterol in LDL is available for transfer to the rHDL. Also, we confirm that the rHDL have a more limited capacity for cholesterol than their phospholipid content would predict. This property of the discoidal rHDL probably depends on the phospholipid composition of the particles, because nascent discoidal HDL of various sizes, isolated from LCAT deficient patients, have considerably higher cholesterol contents (Chen et al., 1984; Soutar et al., 1982) relative to their phospholipid contents compared with the rHDL described in this report.

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