Preferential enrichment of large-sized very low density lipoprotein populations with transferred cholesteryl esters

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Abstract The effect of lipid transfer proteins on the exchange and transfer of cholesteryl esters from rat plasma HDL_2 to human very low (VLDL) and low density (LDL) lipoprotein populations was studied. The use of a combination of radiochemical and chemical methods allowed separate assessment of [^{3}H]cholesteryl ester exchange and of cholesteryl ester transfer. VLDL-I was the preferred acceptor for transferred cholesteryl esters, followed by VLDL-II and VLDL-III. LDL did not acquire cholesteryl esters. The contribution of exchange of [^{3}H]cholesteryl esters to total transfer was highest for LDL and decreased in reverse order along the VLDL density range. Inactivation of lecithin:cholesterol acyltransferase (LCAT) and heating the HDL_2 for 60 min at 56°C accelerated transfer and exchange of [^{3}H]cholesteryl esters. Addition of lipid transfer proteins increased cholesterol esterification in all systems. The data demonstrate that large-sized, triglyceride-rich VLDL particles are preferred acceptors for transferred cholesteryl esters. It is suggested that enrichment of very low density lipoproteins with cholesteryl esters reflects the triglyceride content of the particles.—**Eisenberg, S.** Preferential enrichment of large-sized very low density lipoprotein populations with transferred cholesteryl esters. *J. Lipid Res.* 1985. **26:** 487–494.

Supplementary key words lipid transfer proteins • very low density lipoprotein • low density lipoprotein • high density lipoprotein • cholesterol • triglyceride • lecithin:cholesterol acyltransferase

Plasma lipid transfer is a reaction mediated by a protein, or a group of proteins, that shuttles cholesteryl ester, triglyceride, and possibly phospholipid molecules among lipoproteins (1–9). The protein-catalyzed reaction may cause two distinctly different effects: the first is bidirectional transfer of cholesteryl ester or triglyceride molecules between lipoproteins (an exchange phenomenon) (1) and the second is unidirectional ("net") transfer of molecules from one lipoprotein to another (10–16). This second reaction is responsible for net movement of cholesteryl ester molecules from HDL to lower density lipoproteins and of triglycerides from chylomicrons and VLDL to LDL and HDL. The reaction, moreover, allows transfer of LCAT-produced cholesteryl esters from HDL and thereby relieves the enzyme of product inhibition effects (11, 17).

A major consequence of the lipid transfer reaction is supply of cholesteryl esters to the VLDL → IDL → LDL interconversion process. Since VLDL is a precursor of LDL (18–20), it follows that the number of cholesteryl ester molecules transferred to VLDL also determines their number in LDL. Hence, if VLDL, and especially large-sized VLDL, subpopulations are preferred acceptors for cholesteryl ester molecules, the activity of the lipid tranfer reaction is of major metabolic significance. These considerations prompted us to initiate the present investigation on the comparative properties of different VLDLs and of LDL to serve as acceptors for both transferred and exchanged cholesteryl ester molecules.

METHODS

Preparation of human VLDL density subfractions and of LDL

Human plasma was obtained from an antecubital vein after an overnight (12–14 hr) fast. The blood was collected in citrate-phosphate-dextrose (CPD) solution, and blood cells were sedimented within 60 min by centrifugation at 2000 *g* for 20 min. VLDL was separated in a Beckman ultracentrifuge at plasma density after 16 hr centrifugation in a 60Ti rotor at 45,000 rpm. VLDL density subfractions were prepared in a zonal ultracentrifugation system following published procedures (21). In short, the VLDL was applied to the bottom of a Ti14 zonal rotor and eluted after 45 min centrifugation at 42,000 rpm in a 1.0–1.15 g/ml NaBr gradient. With this procedure, the VLDL spans over the first 350 ml of the rotor effluent volume. The initial 25 ml containing large aggregates (and chylomicrons, if present) was discarded. The remaining 325 ml was divided by volume into VLDL den-

Abbreviations: VLDL, very low density lipoproteins (d < 1.006 g/ml); LDL, low density lipoproteins (d 1.019–1.063 g/ml); HDL_2, high density $lipoprotein_2$ (d 1.085–1.21 g/ml); LCAT, lecithin:cholesterol acyltransferase; [^{3}H]CE, [^{3}H]cholesteryl esters; [^{3}H]FC, [^{3}H]free cholesterol.

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sity subfractions. VLDL-I contained particles isolated at the zonal rotor volume interval of 26-125 ml, VLDL-II at volumes of 126 to 225 ml, and VLDL-III, the remaining 125 ml. The pooled VLDL fractions were dialyzed against 0.9% NaCl, 0.01% EDTA solution, pH 7.4, and concentrated by vacuum ultrafiltration (22) to volumes of 3-6 ml. LDL was prepared from plasma of normolipidemic subjects by preparative ultracentrifugation at the density interval of 1.019-1.063 g/ml, and was extensively dialyzed as above. The chemical composition and mean diameter of the VLDL subfractions and of LDL are shown in **Table 1**. The data for VLDL are similar to those reported for density subfractions prepared on NaCl gradients (23).

Preparation of [^{3}H]cholesterol-labeled rat HDL

To prepare [^{3}H]cholesterol-labeled rat plasma HDL, biosynthetic labeling procedures were employed (24). Male rats of the Hebrew University strain were injected intravenously with 200-400 μCi of [7(n)-^{3}H]cholesterol (Amersham, England) and were exsanguinated through the abdominal aorta 6 hr later. [^{3}H]Cholesterol-labeled HDL was separated by ultracentrifugation at the density interval of 1.085-1.21 g/ml. Previous studies from our laboratory have recently demonstrated that the major lipoprotein present in that plasma density fraction is HDL_2 (22). Unlabeled HDL_2 was isolated likewise from the plasma of male rats not injected with any radioactive precursor. The HDL was dialyzed against 0.9% NaCl, 0.01% EDTA solution, pH 7.4, and was used within 1 week of preparation. The chemical composition of the HDL was similar to that previously reported (22). In [^{3}H]cholesterol-labeled HDL_2, 70-75% of the radioactivity was associated with [^{3}H]cholesteryl esters ([^{3}H]CE) and 25-30% with [^{3}H]free cholesterol ([^{3}H]FC).

Preparation of partially purified lipid transfer protein(s)

Lipid transfer proteins were partially purified from human plasma fractions of density 1.21-1.25 g/ml following published procedures (3, 9). The plasma fraction, in KBr solution of d 1.21 g/ml was applied to a phenyl-Sepharose column, washed extensively with 4 M NaCl solution, and the core lipid transfer proteins were then eluted with water. Partially purified lipid transfer proteins were then isolated by ion exchange chromatography on DEAE (3, 9). The preparation thus obtained was free of LCAT activity and, as compared to plasma, the purification (activity/mg protein) was 300-500-fold. Lipid transfer activity was assayed by the percent transfer of [^{3}H]-cholesteryl esters from human plasma HDL_3 (labeled by the LCAT reaction) to a combined fraction of VLDL and IDL (d < 1.02 g/ml) prepared from human plasma.

Analytic procedures

Lipoprotein protein was determined by the procedure of Lowry et al. (25), phospholipids by the Bartlett method (26), and triglycerides by the AutoAnalyzer procedure (27). Cholesteryl esters and free cholesterol were measured in plasma or lipoproteins by the cholesterol oxidase-cholesterol esterase method (28), using a commercial kit (Boehringer-Mannheim, Germany). Radioactive cholesteryl esters and free cholesterol were extracted in organic solvents and separated by thin-layer chromatography on silica gel-coated cellulose plates, using a solvent system of petroleum ether-diethyl ether-acetic acid 160:40:2 (v/v/v) (24). Radioactivity was determined in a Packard model 2660 liquid scintillation spectrometer. Electron micrographs of VLDL density subfractions and of LDL were obtained as previously described (22), in a Phillips 300 electron microscope at 60 kV and instrument magnification of 90,000 diameters.

Experimental procedures

Transfer of labeled and unlabeled cholesteryl esters between HDL and VLDL density subfractions or LDL was studied in in vitro incubation systems. The systems contained rat plasma HDL_2 and one of the acceptor lipoproteins, each with a final cholesteryl ester content of 0.5 mg. Three ml of rat lipoprotein-deficient plasma (d > 1.21 g/ml) served as an LCAT source. LCAT activity in these samples was 3-5-fold more than that in the human lipoprotein-deficient plasma (d > 1.21 g/ml). The systems also contained [^{3}H]cholesterol-labeled rat plasma HDL_2

TABLE 1. Chemical composition and diameters of very low density lipoprotein subfractions and low density lipoprotein

Lipoprotein	Protein	Triglyceride	Cholesteryl Ester[a]	Free Cholesterol	Phospholipid	Diameter
	mg/100 mg lipoprotein					Å
VLDL-I	7.8 ± 0.4	67.0 ± 2.5	7.6 ± 1.2	4.1 ± 0.5	13.5 ± 1.1	494 ± 26.0
VLDL-II	11.3 ± 0.5	57.3 ± 2.5	9.1 ± 1.3	5.2 ± 0.3	17.1 ± 0.9	386 ± 9.0
VLDL-III	13.6 ± 0.8	47.2 ± 1.7	14.9 ± 1.2	6.1 ± 0.3	18.2 ± 1.2	310 ± 6.7
LDL	20.9 ± 0.5	8.0 ± 1.6	39.9 ± 1.6	9.4 ± 0.3	21.8 ± 0.6	213 ± 4.2

Data are mean ± SEM of four preparations.
[a]Calculated for molecular weight of 650.

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(1-2 × 10^5 dpm), [^{14}C]free cholesterol (added in ethanolic solution to unlabeled HDL_2) (1-2 × 10^4 dpm), and 0.05 M Tris buffer, pH 7.4. One ml of human lipoprotein-deficient plasma (d > 1.21 g/ml) or an amount of lipid transfer protein of similar transfer activity was added to one-half of the samples. The other half of the samples served as controls (without added transfer activity) and they were supplemented with either rat lipoprotein-deficient plasma (control for samples containing human d > 1.21 g/ml fractions), or with 0.9% NaCl solution (control for samples containing purified lipid transfer protein preparation). Heat inactivation (56°C for 60 min) served to abolish LCAT activity. Thus, four different conditions were tested: with and without LCAT, and each with or without added lipid transfer proteins.

Incubations were carried out at 37°C, in a slowly shaking water bath, for 8 hr (see, however, below). The final volume was 5 ml and the reaction was carried out in cellulose nitrate ultracentrifugation tubes. At the end of the incubation period, 0.5 ml of the medium was taken for lipid extraction and determination of [^{14}C]cholesterol esterification, while the other 4.5 ml served for separation of lipoproteins. VLDL was separated at density of 1.019 g/ml, and LDL at density of 1.07 g/ml by ultracentrifugation in a 50.3 rotor at 45,000 rpm for 18 hr. Lipoproteins were isolated by the tube slicing technique, VLDL or LDL being present in the lighter top fraction and HDL_2 in the heavier bottom fraction. Aliquots were then taken for determination of total fraction radioactivity, thin-layer chromatography of VLDL, LDL, or HDL_2 labeled lipids and determination of cholesteryl ester content in the VLDL or LDL. Radioactivity was determined on undialyzed lipoproteins; cholesteryl ester content was determined after dialysis against 0.9% NaCl solution.

Initial experiments were performed with incubation periods of 2, 4, 8, and 24 hr. [^{3}H]CE transfer was already substantial (10-20%) after 2 hr and increased during the time of incubation (**Fig. 1**). After 2 hr incubation, however, increments of cholesteryl ester content in the acceptor lipoproteins were very small (2-10%) and were regarded as unreliable. Therefore, the 8-hr incubation period was regarded as an optimal period for determining the properties of different lipoproteins to serve as acceptors for transferred labeled and unlabeled cholesteryl esters. During that period, 15-25% of the free cholesterol became esterified, and some newly formed [^{3}H]CE of lower specific activity was generated. However, because [^{3}H]CE contributed 70-75% of total HDL radioactivity, the generation of small amounts of lower specific activity esters (4-6% of total [^{3}H]CE) had only negligible effect on distributions of the labeled esters.

RESULTS

VLDL was obtained from the plasma of three subjects with mild to moderate hypertriglyceridemia (plasma triglyceride concentrations between 300 and 500 mg/dl) and two normolipidemic humans. Cholesteryl ester transfer was initiated with lipoprotein-deficient plasma in three experiments and with partially purified lipid transfer proteins in two experiments. Very similar results were obtained in all five experiments. The data from all five experiments were, therefore, analyzed together.

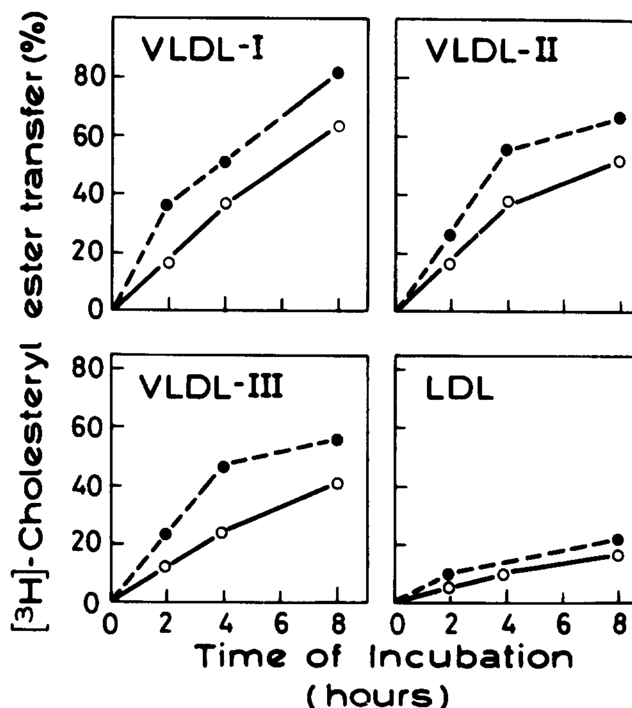

Fig. 1. Transfer of [^{3}H]CE from labeled rat plasma HDL_2 to human VLDL-I, VLDL-II, VLDL-III, and LDL as a function of time of incubation. Incubations were carried out as detailed in Methods. Closed circles, data for heat-inactivated samples; open circles, native samples.

Distribution of [^{3}H]cholesteryl esters from labeled rat plasma HDL_2 to VLDL density subfractions and to LDL is shown in **Table 2**. In the absence of lipid transfer activity, minimal amounts of radioactivity separated with the VLDL or LDL consistent with absence, or near absence, of lipid transfer activity in rat plasma. Yet, it is interesting to note that with each lipoprotein tested, slightly lower amounts separated with the VLDL or LDL when the HDL_2 had been heated (56°C for 60 min). Pronounced transfer of [^{3}H]cholesteryl esters to either VLDL or LDL was observed when lipid transfer activity was added, and that transfer was higher with heat-inactivated as compared to untreated samples. VLDL-I and VLDL-II served as preferred acceptors to transferred [^{3}H]CE, followed by VLDL-III; transfer of [^{3}H]CE to LDL was the lowest.

The observation that more [^{3}H]CE molecules were transferred (by lipid transfer proteins) from heated as compared to non-heated HDL_2 raised the question whether that phenomenon was due to changing specific activity of the [^{3}H]CE in non-heated samples. To that end, the experiments were repeated with another LCAT

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TABLE 2. Effect of lipid transfer proteins on transfer of [^{3}H]cholesteryl esters from biosynthetically [^{3}H]cholesterol-labeled HDL to VLDL subfractions and LDL

Lipoprotein	Untreated Lipoproteins		Heat-Inactivated Lipoproteins	
	−LTP	+LTP	−LTP	+LTP
	[^{3}H]CE in VLDL subfractions or LDL, % of total [^{3}H]CE			
VLDL-I	4.9 ± 2.3	46.7 ± 6.5	3.1 ± 1.7	59.6 ± 7.9
VLDL-II	5.0 ± 2.2	45.5 ± 10.0	3.5 ± 2.7	57.4 ± 10.6
VLDL-III	4.2 ± 1.6	35.1 ± 7.2	3.0 ± 2.3	47.1 ± 10.1
LDL	3.1 ± 0.7	16.4 ± 8.2	3.4 ± 1.7	21.1 ± 5.2

Data are mean ± SEM of five experiments. Incubations were carried out for 8 hr in the absence (−) and presence (+) of lipid transfer proteins (LTP) with either untreated or heat-inactivated (56°C, 60 min) HDL and lipoprotein-deficient plasma. Transfer of [^{3}H]CE to VLDL subfractions and LDL was determined as described in Methods.

inhibitor, 2 mM DTNB. **Fig. 2** presents the data from an experiment comparing [^{3}H]CE transfer to the various acceptors using three systems: native lipoproteins, heat-inactivated HDL_2, and native lipoproteins plus DTNB. As shown in the figure, identical results were obtained in systems with and without DTNB (ratios of [^{3}H]CE transfer 0.98 and 1.03 after 2 and 8 hr of incubation, respectively), while transfer from heated HDL_2 was appreciably higher than from the native lipoprotein (ratios of [^{3}H]CE transfer of 1.37 and 1.33 after 2 and 8 hr of incubation, respectively). Thus, the enhanced transfer observed with heated lipoproteins must have been due to the heating procedure and not to the inhibition of the LCAT reaction.

The cholesteryl ester content of VLDL-I, VLDL-II, VLDL-III, and LDL isolated after incubation, with and without added lipid transfer activity, is shown in **Table 3**. Without added lipid transfer activity, (−LTP, Table 3), the recovery of cholesteryl esters in VLDL was between 0.36 and 0.39 mg of cholesterol, i.e., 80–90%. In the presence of lipid transfer proteins, pronounced enrichment of VLDL-I with cholesteryl esters was evident, followed by VLDL-II and VLDL-III. Cholesteryl ester content of LDL, in contrast, remained almost unchanged. Differences between heat-inactivated and untreated samples were small. The increments of lipoprotein cholesteryl ester content were significant for VLDL-I ($P < 0.025$) and VLDL-II ($P < 0.025$), but nonsignificant for VLDL-III ($0.05 < P < 0.1$) and LDL.

Transfer of labeled cholesteryl esters to acceptor lipoproteins can be associated with back-transfer of unlabeled ester molecules (an exchange phenomenon) or of other molecules, e.g., triglycerides or phospholipids. With the former process, no enrichment of the acceptor lipoprotein with cholesteryl esters would have occurred, while enrichment is observed with the latter. Comparison of increments of mass and of radioactive cholesteryl esters in the acceptor lipoproteins enables assessment of the contribution of net transfer and of exchange processes to total [^{3}H]CE transfer. **Table 4** presents the mean percentage increments (based on individual experiments) of [^{3}H]-cholesteryl esters and of mass cholesteryl esters in the acceptor lipoproteins. With each acceptor lipoprotein, percentage transfer of [^{3}H]CE exceeded the percentage increment of cholesteryl ester mass. Comparison of the two allows calculation of ratios between transferred cholesteryl ester molecules and transfer of [^{3}H]cholesteryl ester to each lipoprotein. This calculation reveals that 88.5% of the labeled cholesteryl esters transferred to VLDL-I in untreated plasma and 77.9% transferred in heat-inactivated plasma represent enrichment of this lipoprotein with cholesteryl ester molecules. In VLDL-II, the values

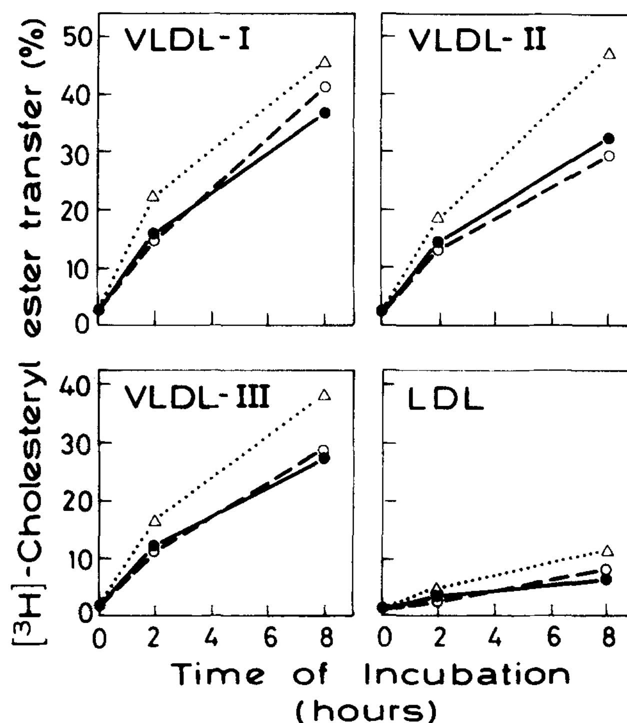

Fig. 2. Comparison of transfer of [^{3}H]CE to acceptor lipoproteins in native incubation mixtures (●), DTNB (2 mM)-containing mixtures (○), and systems with heat-inactivated (56°C for 60 min) rat plasma HDL_2 (△). All systems contained human acceptor lipoproteins (0.5 mg cholesteryl ester), rat plasma [^{3}H]cholesterol-labeled HDL_2 (0.5 mg cholesteryl ester), and 3 ml of native heated rat lipoprotein-deficient plasma fraction of d > 1.21 g/ml. Partially purified human plasma lipid transfer proteins were added at time zero, and transfer of [^{3}H]CE was determined after 2 and 8 hr of incubation at 37°C. No transfer was observed in samples incubated without added lipid transfer proteins.

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TABLE 3. Effects of lipid transfer proteins on the cholesteryl ester content of very low density lipoprotein subfractions and low density lipoproteins

Lipoprotein	Untreated Lipoproteins			Heat-Inactivated Lipoproteins		
	– LTP	+ LTP	+ LTP/ – LTP Ratio	– LTP	+ LTP	+ LTP/ – LTP Ratio
	mg/sample			*mg/sample*		
VLDL-I	0.38 ± 0.028	0.53 ± 0.048	1.37 ± 0.04	0.36 ± 0.023	0.52 ± 0.037	1.44 ± 0.03
VLDL-II	0.39 ± 0.028	0.48 ± 0.019	1.24 ± 0.08	0.39 ± 0.025	0.46 ± 0.034	1.19 ± 0.03
VLDL-III	0.36 ± 0.029	0.39 ± 0.013	1.10 ± 0.10	0.37 ± 0.023	0.41 ± 0.024	1.11 ± 0.08
LDL	0.31 ± 0.049	0.32 ± 0.033	1.02 ± 0.12	0.36 ± 0.032	0.36 ± 0.027	1.01 ± 0.05

Data are mean ± SEM of five experiments. Incubations were carried out for 8 hr in the absence (–) and presence (+) of lipid transfer proteins (LTP) with either untreated or heat-inactivated (56°C, 60 min) HDL and lipoprotein-deficient plasma. Cholesteryl ester content of acceptor lipoprotein (VLDL subfractions and LDL) was determined as described in Methods.

were 59.3% and 35.3% and in VLDL-III, 42.1% and 24.9%. For LDL, most of the [^{3}H]CE molecules represented an exchange process, as almost no transfer of unlabeled molecules was observed.

[^{14}C]Cholesteryl ester formation during the 8-hr incubation is shown in **Table 5**. Because no attempt was made to reach equal amounts of free cholesterol with the different acceptors, cholesterol esterification is presented as percent of total [^{14}C]cholesterol introduced to the incubation mixture. A slight increase of [^{14}C]cholesterol esterification was found in all systems, when lipid transfer activity was added.

DISCUSSION

The lipid exchange and transfer reaction has been studied mainly with reference to its effects on the cholesteryl ester-rich lipoproteins, LDL and HDL. At first, the lipid transfer proteins were believed to initiate only an exchange process (1, 29). Subsequent studies, however, have established that the proteins are also responsible for net transfer of cholesteryl ester molecules from LDL and HDL (3, 4, 7, 8, 10–15) to other lipoproteins. VLDL and chylomicrons were shown to be important acceptors for transferred cholesteryl esters (12–15, 30, 31) and the reaction serves as a major source of these molecules in triglyceride-rich lipoproteins (10). The data reported here lend further support to the concept that VLDL becomes enriched with cholesteryl ester via the activity of the lipid transfer proteins.

The main aim of the present investigation was to compare the ability of different lipoproteins to serve as acceptors for transferred cholesteryl esters. The experimental procedures used allowed separate assessment of exchange of labeled cholesteryl esters and of unidirectional transfer of these same molecules between HDL and the different acceptors. Total [^{3}H]CE transfer (exchanged *and* unidirectionally transferred molecules) decreased progressively from VLDL-I to LDL. Enrichment of acceptor lipoproteins with cholesteryl esters also followed the same order: substantial enrichment was found in VLDL-I and VLDL-II, but none in LDL. The contribution of exchange to total [^{3}H]CE transfer, in contrast, followed an opposite order, i.e., was highest in LDL (85–95% of total [^{3}H]CE) and lowest in VLDL-I (10–20%, Table 4). These observations are in very good agreement with current concepts on the mode of action of the lipid transfer proteins (3). Morton and Zilversmit (3) have recently demonstrated that cholesteryl esters and triglycerides compete for transfer in the donor lipoproteins and that the extent of transfer for each lipid is determined by its relative concentration. The study, moreover, indicated that the transfer protein does not distinguish between cholesteryl ester and triglyceride molecules and that transfer proceeds by a reciprocal movement of triglycerides and cholesteryl esters

TABLE 4. Comparison of transfer of cholesteryl esters and [^{3}H]cholesteryl esters to different acceptor lipoproteins

Lipoprotein	Untreated Lipoproteins			Heat-Inactivated Lipoproteins		
	Transferred CE	Transferred [^{3}H]CE	CE/[^{3}H]CE Ratio	Transferred CE	Transferred [^{3}H]CE	CE/[^{3}H]CE Ratio
	%			%		
VLDL-I	37	41.8	0.885	44	56.5	0.779
VLDL-II	24	40.5	0.593	19	53.9	0.353
VLDL-III	13	30.9	0.421	11	44.1	0.244
LDL	1.5	13.3	0.111	0.5	16.7	0.030

Data calculated from results shown in Tables 2 and 3. The values are percent of HDL cholesteryl esters or [^{3}H]CE transferred to different acceptors. Ratios are between the increment of cholesteryl ester content and the increment of [^{3}H]CE content (both expressed as percents above values observed in the absence of lipid transfer proteins) in acceptor lipoproteins.

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TABLE 5. Effect of lipid transfer proteins on [^{14}C]cholesterol esterification

Lipoprotein	[^{14}C]Cholesterol Esterification	
	−LTP	+LTP
	%	
VLDL-I	18.8 ± 2.9	25.0 ± 3.5
VLDL-II	13.4 ± 0.7	19.4 ± 1.2
VLDL-III	12.3 ± 1.7	17.4 ± 1.0
LDL	14.0 ± 1.7	16.7 ± 3.1

Data are mean ± SEM of four experiments. [^{14}C]Cholesterol esterification was determined by thin-layer chromatography of lipids extracted at the end of the incubation period.

between donor and acceptor lipoproteins (3). The data reported here offer an opportunity to test some of these concepts, although transfer of triglycerides was not determined. Using four acceptors along the VLDL-LDL density range, it was found that total ^{3}H transfer could be best explained by the different surface areas of the lipoproteins, while unidirectional transfer could be explained by the triglyceride to cholesteryl ester ratios (**Fig. 3**). If lipid transfer is a surface reaction, then indeed the larger surface area must influence the amount of transferred molecules, at least in part. Enrichment of a lipoprotein with cholesteryl esters, however, depends on the nature of the molecule that is back-transferred to the donor lipoprotein (3). Because LDL contains a predominance of cholesteryl ester molecules, these molecules are likely to be transferred back to HDL, and the reaction results mainly in an exchange phenomenon, i.e., replacement of an unlabeled cholesteryl ester molecule in LDL with a labeled molecule of HDL origin. With VLDL, however, the likelihood of attachment of a triglyceride molecule is great and increases proportionally to the increase of triglyceride to cholesteryl ester molar ratio along the VLDL density range. Thus, surface characteristics seem to affect total lipid transfer, whereas core-composition determines unidirectional transfer of cholesteryl esters.

Heat-inactivation of plasma or plasma fractions has been shown to inactivate the LCAT, but not the cholesteryl ester transfer reaction (12). As well, initiation of cholesteryl ester transfer increases rates of cholesterol esterification (11, 17). The present findings confirm the former observations. With each lipoprotein acceptor tested, however, it was found that the amount of labeled cholesteryl ester molecules transferred was 20–30% larger when the plasma fractions used were inactivated by heating, while cholesteryl ester enrichment was not decreased. These findings differ from those reported by other investigators (11, 17) and by us (15), when more cholesteryl esters were shown

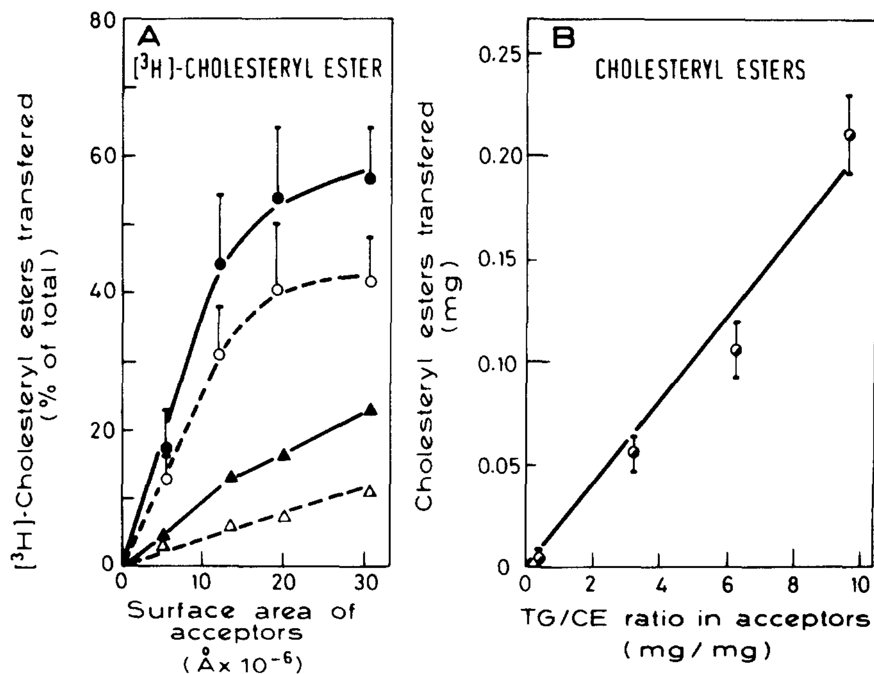

Fig. 3. A. Relationships between transfer of [^{3}H]cholesteryl esters (exchange *and* unidirectional transfer) and surface area of acceptor lipoproteins. Data for untreated samples are shown by open symbols, 2 hr (△) and 8 hr (○) incubation. The data for heated samples are shown by closed symbols. B. Relationships between unidirectional transfer of cholesteryl esters (based on mass measurements) and the triglyceride to cholesteryl ester mass ratio of acceptor lipoproteins. Because similar degrees of enrichment with cholesteryl esters were observed in untreated and heat-inactivated samples, the data were combined. Linear regression analysis of the curve yielded an r value of 0.968.

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to be transferred to acceptor lipoproteins in systems containing active LCAT. It is therefore suggested that heating the HDL at 56°C for 60 min alters the physicochemical properties of the lipoprotein such that cholesteryl ester transfer is facilitated. The nature of the physicochemical alterations of HDL that may facilitate cholesteryl ester transfer and their possible physiological roles in lipoprotein metabolism are currently being investigated.

Regardless of mechanism, the present results demonstrate preferential enrichment of large VLDL with cholesteryl ester molecules via the lipid transfer reaction. With the system used here, an increment of about 40% of the cholesteryl ester content of the VLDL-I was observed. In in vivo situations, however, it is conceivable that much larger amounts of cholesteryl esters will be transferred to the VLDL, in particular large VLDL subpopulations. The sources of this cholesteryl ester are LDL, the major plasma cholesteryl ester-carrying lipoprotein, and the LCAT reaction. Indeed, we have recently shown that cholesteryl ester-rich VLDL is regularly found in patients with hypertriglyceridemia (32), a situation where both LDL and HDL contain less than normal amounts of these molecules (32-34). In another study (35), we moreover provided evidence that this phenomenon reflects mainly cholesteryl ester accumulation in VLDL-I and VLDL-II and suggest that the abnormally rich VLDL subpopulations cannot complete the VLDL to LDL conversion process. It is proposed that the mechanism discussed in the present report, i.e., preferential delivery of cholesteryl esters to large VLDL particles, is responsible for this phenomenon and indicates its potential role in lipoprotein metabolism.

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