Specificity of lipid transfer protein for molecular species of cholesteryl ester

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Abstract The capacity of the plasma-derived lipid transfer protein to facilitate the transfer of various cholesteryl ester species has been investigated. Four different molecular species of cholesteryl ester were incorporated into either reconstituted high density lipoproteins or phosphatidylcholine liposomes, and the resulting particles were used as donors in standardized lipid transfer assays. With reconstituted high density lipoproteins as substrate, the rate of transfer of cholesteryl esters was cholesteryl oleate > cholesteryl linoleate > cholesteryl arachidonate > cholesteryl palmitate. The transfer rate for cholesteryl oleate was 154% of that for cholesteryl palmitate. Liposome substrates gave similar results. It is concluded that lipid transfer protein transfers all major species of cholesteryl ester found in plasma; however, the relative rates of transfer were significantly affected by acyl chain composition. The transfer rates appeared to reflect substrate specificity rather than substrate availability within the donor particle. —**Morton, R. E.** Specificity of lipid transfer protein for molecular species of cholesteryl ester. *J. Lipid Res.* 1986. **27:** 523–529.

Supplementary key words substrate specificity • human plasma • reconstituted lipoproteins • liposomes

The protein-facilitated transfer/exchange of apolar lipids between lipoproteins has been studied extensively in the recent past. Several laboratories (1–3) have purified a protein from human plasma that is responsible for the majority of core lipid transfer activity. This protein, designated lipid transfer protein, is characterized as a hydrophobic glycoprotein of 58,000–63,000 molecular weight with an isoelectric point of 5.2 (1–3). Purified lipid transfer protein facilitates the transfer of cholesteryl ester, triglyceride, and phospholipids (2). Cholesteryl ester and triglyceride appear to compete for transfer, with the extent of transfer for each lipid being determined by its relative concentration in the donor lipoprotein (4). Through this competition mechanism, lipid transfer protein facilitates two distinct processes—a homoexchange reaction in which cholesteryl ester is exchanged for cholesteryl ester or triglyceride for triglyceride, and a heteroexchange reaction in which cholesteryl ester is exchanged for triglyceride.

Although the factors affecting the transfer of cholesteryl ester and triglyceride are partially understood, little is known about the specificity of the lipid transfer protein for different molecular species of these lipids. In the only study to date to address this issue, Akanuma and Glomset (5) observed that during short-term incubation of cholesteryl ester-labeled high density lipoprotein (d > 1.063 g/ml fraction of human plasma) with very low density lipoprotein, a slightly greater proportion of monounsaturated cholesteryl esters was transferred than saturated or polyunsaturated species. However, these measurements were made in the presence of *p*-chloromercuriphenyl sulfonate, which has been shown to alter lipid transfer protein function (2). In studies reported here, the specificity of the lipid transfer protein for various molecular species of cholesteryl ester has been investigated in detail with several well-defined assay systems.

EXPERIMENTAL PROCEDURES

Materials

[4-^{14}C]Cholesterol (57.8 mCi/nmol) and [1α,2α(n)-^{3}H]-cholesterol (44 Ci/nmol) were purchased from Amersham Corp. (Arlington Heights, IL). Radiolabeled cholesteryl esters were synthesized from [^{3}H]- or [^{14}C]cholesterol and the appropriate acid chloride as described by Pinter, Hamilton, and Muldrey (6). Palmitoyl-, stearoyl-, oleoyl-, linoleoyl-, and arachidonoyl-chloride as well as cholesteryl palmitate (C16:0), cholesteryl stearate (C18:0), cholesteryl oleate (C18:1), cholesteryl linoleate (C18:2), and cholesteryl arachidonate (C20:4) were obtained from Nu-Chek-Prep, Inc. (Elysian, MN). The radiochemical purity of labeled cholesteryl esters was monitored by thin-layer chromatography on silica gel G plates (E. Merck, Darmstadt, Germany) containing 2.5% $AgNO_3$ in a developing system of hexanes–diethyl ether 90:10 (v/v). Cholesteryl esters were visualized under ultraviolet light after spraying the plate with 0.2% 2,7-dichlorofluoroscein in 95% ethanol. By this technique, the initial radiochemical purity of all labeled esters was ≥95%, except for [^{3}H]-cholesteryl arachidonate which averaged 92%. When necessary, radiolabeled cholesteryl esters were purified by thin-layer chromatography on SG-60 plates (E. Merck) in

a developing system of hexanes–diethyl ether 80:20 (v/v).

Bovine serum albumin (fraction V), egg phosphatidylcholine, triolein, and butylated hydroxytoluene were purchased from Sigma Chemical Co. (St. Louis, MO). Phenyl-Sepharose and dextran sulfate ($M_r \cong 500{,}000$) were from Pharmacia Fine Chemicals (Piscataway, NJ). Cholesterol was purified as the dibromide derivative and crystallized from methanol. All lipids were stored at −20°C in chloroform containing butylated hydroxytoluene (10 μg/ml).

Isolation of lipid transfer protein

Partially purified lipid transfer protein was isolated from human plasma as previously described (2). Briefly, citrated human plasma was made lipoprotein-deficient by the dextran sulfate–$MnCl_2$ precipitation procedure of Burstein, Scholnick, and Morfin (7). Lipid transfer protein was then purified by sequential chromatography on phenyl-Sepharose and CM-cellulose (CM-52, Whatman Inc., Clifton, CA). This preparation was routinely enriched 700- to 900-fold in transfer specific activity. Recent studies have shown that the lipid transfer protein in these preparations is indistinguishable from highly purified transfer protein with respect to its specificity for different lipid classes and its interaction with plasma lipoproteins (2, 8, 9). Three preparations were used in the present studies.

Liposome and reconstituted high density lipoprotein preparations

Phosphatidylcholine-cholesterol liposomes were prepared by a modification (2) of the cholate procedure described by Brunner, Skrabal, and Hausen (10). A typical preparation consisted of egg phosphatidylcholine (10 μmol), cholesterol (2.5 μmol), and cholesteryl ester (20 nmol). In some preparations a single cholesteryl ester was added, while in others an equimolar mixture of cholesteryl palmitate, cholesteryl oleate, cholesteryl linoleate, and cholesteryl arachidonate was added. When a single cholesteryl ester species was used, trace amounts of the same ester labeled either with ^3H (1.7×10^5 cpm) *or* ^{14}C (4.6×10^4 cpm) were added during the liposome preparation. Liposomes containing all four esters were labeled with *both* [^{14}C]cholesteryl oleate (9.1×10^4 cpm) *and* one of the four cholesteryl esters tagged with ^3H (3.4×10^5 cpm). Thus, [^{14}C]cholesteryl oleate served as an internal control to preparations containing different ^3H-labeled cholesteryl esters. Typically, 70–80% of the initial radioactivity was recovered in liposomes; when present together, ^3H and ^{14}C were recovered to an almost identical extent. Upon gel filtration on a Bio-Gel A-15m column, the ^3H and ^{14}C of each liposome preparation co-eluted as a single peak just after the column void volume ($k_{av} = 0.11$), with a peak width slightly larger than that obtained for plasma low density lipoprotein ($k_{av} = 0.46$). Across the elution peak of each liposome preparation, the ratio of $^3H/^{14}C$ varied less than 10% and was the same as the starting material; isotope recovery averaged 95%. These results indicate that there is no apparent heterogeneity in cholesteryl ester distribution.

Reconstituted high density lipoprotein (r-HDL) was prepared essentially as described by Ritter and Scanu (11). In a typical preparation, solutions containing 825 nmol of egg phosphatidylcholine, 210 nmol of cholesterol, 102 nmol of triolein, and 142 nmol *each* of cholesteryl palmitate, cholesteryl oleate, cholesteryl linoleate, and cholesteryl arachidonate were mixed in a 15-ml Corex tube (Corning Glass Works, Corning, NY). Each r-HDL preparation also contained trace amounts of [^{14}C]cholesteryl oleate (1.7×10^5 cpm) and one of the four cholesteryl ester species labeled with ^3H (8.0×10^5 cpm). Organic solvents were removed under a stream of N_2. The lipids were dispersed in 20 mM EDTA, pH 8.6, using a Branson sonifier model 185 (Heat Systems Co., Plainview, NY) with a standard microtip for 15 s at 75 W and 25°C. The dispersion was then incubated at 41°C for 30 min. Apolipoprotein A-I (600 μg of protein), isolated and purified by ion exchange chromatography (12), was added and the mixture was sonicated for 5 min at 65 W. The temperature of the sonication mixture was maintained near 41°C by constant cooling in a 24°C water bath.

r-HDL were isolated as the $1.063 < d < 1.21$ g/ml fraction by ultracentrifugation (4) and dialyzed against 50 mM Tris-HCl, 150 mM NaCl, 0.01% EDTA, 0.02% NaN_3, pH 7.4. Bovine serum albumin was added to dialyzed preparations to yield a 0.35% albumin solution. The recovery of ^{14}C in the r-HDL fraction was 20–30% of the initial radioactivity. For a given preparation, the percent recovery of the ^3H-labeled cholesteryl ester was essentially the same as [^{14}C]cholesteryl oleate, except for [^3H]cholesteryl palmitate which was incorporated at an average extent of 48% of that for [^{14}C]cholesteryl oleate. The lipid and protein composition of these r-HDL was very similar to that previously reported by Morton and Zilversmit (4) for r-HDL of the same cholesteryl ester and triglyceride content. As shown below in Fig. 1, these r-HDL were similar in size to plasma high density lipoprotein (HDL) and their hydrated density resembled that of HDL_2 (13).

The radiochemical purity of cholesteryl esters after incorporation into liposomes and r-HDL was quantitated by argentation thin-layer chromatography (see above). With liposome and r-HDL preparations, >93% of [^3H]-cholesteryl palmitate, [^3H]cholesteryl oleate, [^{14}C]cholesteryl oleate, and [^3H]cholesteryl linoleate each co-migrated with their respective unlabeled authentic standard. For each cholesteryl ester, this reflected a decrease of ≤3% in purity compared to the original labeled compound. The radiochemical purity of [^3H]cholesteryl

arachidonate was decreased from its original purity of 92% to 76 and 84% when it was incorporated into r-HDL and liposomes, respectively. As shown later, these contaminants appeared to have no detectable effect on transfer rates over a wide range of percent lipid transfer.

Lipid transfer assays

For the routine monitoring of lipid transfer protein purification, lipid transfer activity was measured from labeled low density lipoprotein to unlabeled high density lipoprotein according to a previously described procedure (14). Labeled lipoproteins, prepared according to the procedure of Morton and Zilversmit (15), and unlabeled human lipoproteins were isolated at 4°C by sequential ultracentrifugation (16). Lipid transfer assays were terminated by selectively precipitating the radiolabeled low density lipoprotein by the addition of PO_4^{3-} and Mn^{2+} (14). The extent of lipid transfer (kt) was calculated as previously described (17) from the radioactivity presented in the acceptor lipoprotein (supernatant fraction) and is expressed as percent lipid transfer (kt × 100).

Lipid transfer from radiolabeled phosphatidylcholine liposomes or r-HDL to unlabeled low density lipoprotein was assayed in triplicate according to the procedure described for native lipoproteins (14, 17). Transfer from liposomes to low density lipoprotein was terminated (2) by precipitation of the lipoprotein with ConA-Sepharose (Pharmacia), whereas transfer from r-HDL to low density lipoprotein was stopped by the standard PO_4^{3-}–Mn^{2+} precipitation procedure. In both cases, the donor particle remained in the supernatant, and the extent of lipid transfer was calculated from the decrease in supernatant radioactivity as previously described (4).

Analytical procedures

Protein was quantitated by the method of Lowry et al. (18) as modified by Peterson (19) with bovine serum albumin as standard. Lipids were extracted from lipoproteins and liposomes by the method of Thompson et al. (20), or by the method of Bligh and Dyer (21) when quantitative phospholipid extraction was required. Cholesterol was determined by the ferric chloride procedure (22) after saponification (23). Triglyceride was measured by the method of Sardesai and Manning (24) after isolation by thin-layer chromatography on silica gel H plates in a developing system of hexanes–diethyl ether 70:30 (v/v). Lipid phosphorus was measured by the method of Bartlett (25).

RESULTS

When r-HDL, containing a mixture of cholesteryl ester species, was incubated with lipid transfer protein and low density lipoprotein (acceptor), lipid transfer protein showed a marked preference among the four major cholesteryl ester species normally present in plasma lipoproteins (**Table 1**). Cholesteryl oleate was transferred preferentially, with cholesteryl linoleate being transferred at a slightly slower rate. Cholesteryl palmitate was transferred at a rate that was only 65% of that for cholesteryl oleate. In separate experiments, it was shown that the markedly lower transfer rate for this steryl ester was not due to a shorter acyl chain length but reflected acyl chain unsaturation since cholesteryl stearate, a minor plasma cholesteryl ester, was also transferred at a significantly lower rate relative to that for cholesteryl oleate (0.71 ± 0.01, mean ± SE, n = 3). Similar to that observed for saturated cholesteryl esters, polyunsaturated cholesteryl arachidonate was also a less preferred substrate for lipid transfer protein. The relative transfer rates shown in Table 1 were essentially the same over a wide range of [^{14}C]cholesteryl oleate transfer (12–45%), indicating that the radiolabel for each cholesteryl ester appeared to be homogeneous. Additionally, since the cholesteryl ester composition of the r-HDL is modified during the incubation by the reciprocal transfer of uncharacterized cholesteryl esters from the acceptor lipoprotein, these constant relative transfer rates suggest that the *percent* transfer of the various cholesteryl ester species is unaffected by their relative concentrations

TABLE 1. Specificity of lipid transfer protein for cholesteryl ester species: r-HDL substrates containing a *mixture* of cholesteryl esters

	Lipid Transfer Activity (% kt, t = 1.5 hr)			
	[^{3}H]-CP/[^{14}C]-CO	[^{3}H]-CO/[^{14}C]-CO	[^{3}H]-CL/[^{14}C]-CO	[^{3}H]-CA/[^{14}C]-CO
Representative data	17.5/26.4	28.2/27.1	20.9/23.2	18.0/25.4
Average ratio (mean ± SE, n = 5)	0.65 ± 0.03	1.06 ± 0.02	0.89 ± 0.03	0.73 ± 0.01

r-HDL were prepared as described in Experimental Procedures to contain cholesteryl palmitate, cholesteryl oleate, cholesteryl linoleate, *and* cholesteryl arachidonate, plus trace amounts of [^{14}C]cholesteryl oleate and one of the indicated esters labeled with ^{3}H. r-HDL (2 μg of cholesteryl ester) as donor, low density lipoprotein (10 μg of cholesterol) as acceptor, and lipid transfer protein (6–12 μg of protein) were incubated for 1.5 hr as described in Experimental Procedures. The representative data shown are the values from a single r-HDL preparation for each cholesteryl ester species assayed in triplicate. The average transfer ratios were calculated from values obtained from assays on three separate r-HDL preparations for each ^{3}H-labeled cholesteryl ester. Abbreviations: CP, cholesteryl palmitate; CO, cholesteryl oleate; CL, cholesteryl linoleate; and CA, cholesteryl arachidonate.

in the donor lipoprotein. This conclusion is consistent with our recent observation that the transfer of cholesteryl palmitate relative to that of cholesteryl oleate was still 0.65 even when the donor r-HDL contained only these two cholesteryl ester species, and this value was unchanged by varying the ratio of these cholesteryl esters in the r-HDL threefold (R. E. Morton and A. K. Osborne, unpublished results).

In order to determine whether the apparent cholesteryl ester species specificity shown in Table 1 could have been due to a heterogeneous distribution of cholesteryl ester species in the r-HDL donor substrates, these reconstituted lipoproteins were characterized by gel filtration chromatography (**Fig. 1A**) and density gradient ultracentrifugation (Fig. 1B). As shown in Fig. 1, by either technique, the radioisotopes in r-HDL labeled with [^{3}H]cholesteryl palmitate and [^{14}C]cholesteryl oleate co-eluted; across both elution profiles the ratio of these lipids remained constant and the recovery of both isotopes was the same. Analysis of other r-HDL preparations labeled with different cholesteryl ester species gave profiles very similar to those shown in Fig. 1A and B, consistent with the fact that these r-HDL were prepared from the same lipid components and differed only in the cholesteryl ester species that were radiolabeled (see Experimental Procedures). Thus, by these fractionation techniques, there was no detectable heterogeneity in cholesteryl ester species distribution in the r-HDL preparations.

To investigate the possibility that the relative transfer rates noted for the various cholesteryl esters reflect differential availability of these esters within the lipoprotein rather than a true substrate specificity, liposomes were utilized as substrates. Previous studies (26) have shown that cholesteryl esters incorporated into phosphatidylcholine liposomes at concentrations up to 10-fold higher than that used here are homogeneously dispersed within the bilayer, and thus, all cholesteryl esters should be equally available for transfer. As seen in **Table 2**, when liposomes containing a mixture of cholesteryl ester species were used as donor, lipid transfer protein still demonstrated a similar preference for cholesteryl ester species. As with r-HDL, cholesteryl oleate was transferred most rapidly followed by cholesteryl linoleate. Cholesteryl palmitate was transferred less effectively, although its relative transfer rate was higher than that for cholesteryl arachidonate. Again, the relative transfer rates for cholesteryl palmitate (Table 2) and cholesteryl stearate (0.74 ± 0.03, mean ± SE, n = 4) were the same. Relative transfer rates were essentially the same over a wide range of absolute transfer rates of [^{14}C]cholesteryl oleate (16–45%).

When phosphatidylcholine liposomes containing a *single* cholesteryl ester species were used as donors in lipid transfer assays, all four cholesteryl esters studied were transferred at nearly the same rate (**Table 3**). These results show that, in the absence of other cholesteryl esters, each cholesteryl ester species is bound and transferred at the same rate.

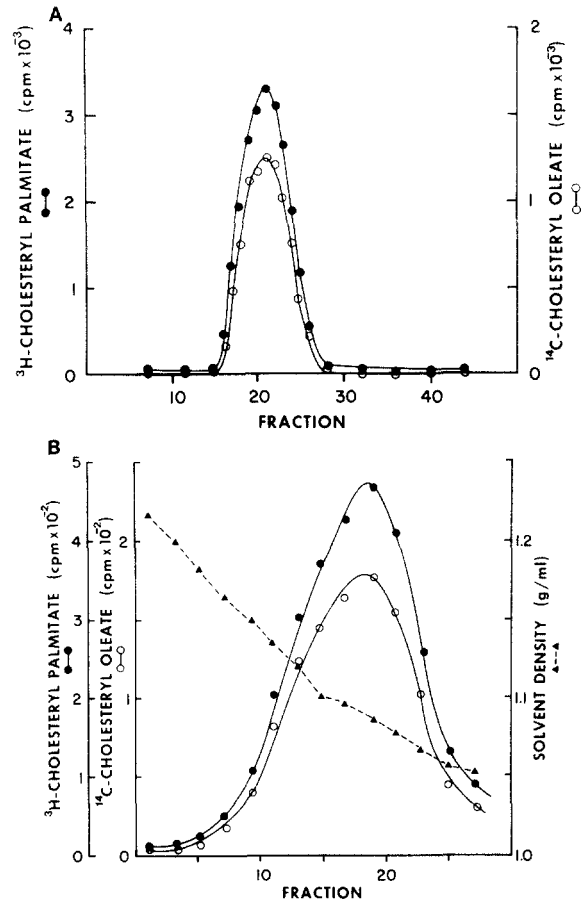

Fig. 1. Characterization of r-HDL substrates. r-HDL were prepared as described in Experimental Procedures and then subjected to gel filtration chromatography (panel A) or density gradient ultracentrifugation (panel B). In panel A, an aliquot (750 μl, 50 nmol of cholesteryl ester) of r-HDL was applied to a 1 × 39 cm column of Bio-Gel A-0.5m equilibrated in 50 mM Tris-HCl, 150 mM NaCl, 0.1% bovine serum albumin, 0.02% NaN_3, pH 7.4. The column was eluted at 7 ml/hr and 750-μl fractions were collected. The distribution of [^{3}H]cholesteryl palmitate (●) and [^{14}C]cholesteryl oleate (○) was determined by scintillation counting on 500-μl aliquots. The recovery of ^{3}H and ^{14}C radioactivity was 87.9 and 87.6% of that applied, respectively. The elution profile for normolipemic HDL (1.063 < 1.21 g/ml) peaked at fraction 23. For density gradient ultracentrifugation (panel B), a 300-μl aliquot of r-HDL was layered on top of a gradient consisting of 7.6 ml each of density 1.006, 1.063, 1.137, 1.21 and 1.264 g/ml NaBr solutions. Samples were centrifuged at 17°C in a 60 Ti rotor at 60,000 rpm for 18 hr. Samples (1.3 ml) were removed from the bottom of the tube by a peristaltic pump at a flow rate of 40 ml/hr. The distribution of [^{3}H]cholesteryl palmitate (●) and [^{14}C]cholesteryl oleate (○) was determined on 500-μl aliquots by scintillation counting. Fraction density (▲) was determined by refractometry. The recovery of ^{3}H and ^{14}C radioactivity was 80.0 and 80.2% of that applied, respectively.

DISCUSSION

These studies demonstrate that the plasma-derived lipid transfer protein facilitates the exchange of cholesteryl esters that differ in acyl chain length and extent of un-

TABLE 2. Specificity of lipid transfer protein for cholesteryl ester species: liposome substrates containing a *mixture* of cholesteryl esters

	Lipid Transfer Activity (% kt, t = 1.5 hr)			
	[^{3}H]-CP/[^{14}C]-CO	[^{3}H]-CO/[^{14}C]-CO	[^{3}H]-CL/[^{14}C]-CO	[^{3}H]-CA/[^{14}C]-CO
Representative data	17.4/24.5	19.5/18.3	20.8/23.3	12.3/21.6
Average ratio (mean ± SE, n = 6)	0.76 ± 0.03	1.03 ± 0.02	0.87 ± 0.03	0.65 ± 0.03

Phosphatidylcholine liposomes containing 25 mol % free cholesterol and 0.05 mol % of cholesteryl-palmitate, -oleate, -linoleate, *and* -arachidonate were prepared by cholate dialysis (see Experimental Procedures). Each preparation also contained [^{14}C]cholesteryl oleate and one of the indicated cholesteryl esters labeled with ^{3}H. Liposomes (0.25 μmol of phospholipid) were incubated for 1.5 hr with low density lipoprotein (10 μg of cholesterol) as acceptor plus lipid transfer protein (1.5–3.0 μg of protein) as described in Experimental Procedures. The representative data shown are typical values from a single liposome preparation for each cholesteryl ester species assayed in triplicate. The average transfer ratios were calculated from values obtained from assays on three liposome preparations for each ^{3}H-labeled cholesteryl ester. Abbreviations: CP, cholesteryl palmitate; CO, cholesteryl oleate; CL, cholesteryl linoleate; and CA, cholesteryl arachidonate.

saturation; however, the acyl group is a major determinant of the rate of transfer. With lipoprotein substrates, the relative rates of transfer for different cholesteryl ester species were: cholesteryl oleate > cholesteryl linoleate > cholesteryl arachidonate > cholesteryl palmitate = cholesteryl stearate. A similar preference for monoene species has been previously reported (5). This specificity does not appear to reflect substrate availability, i.e., differential partitioning of cholesteryl ester species between the coat and core regions of the lipoprotein, since a similar order of substrate preference was noted with phosphatidylcholine substrates—an assay system in which the cholesteryl esters are dispersed in the phospholipid bilayer (26). These studies cannot exclude the possibility that subtle differences in the localization of cholesteryl esters within the liposome phospholipid bilayer may affect substrate availability. However, this seems unlikely since the transfer rates for cholesteryl ester species incorporated individually into liposomes were the same, indicating that the binding (or availability) of the various cholesteryl ester species to the lipid transfer protein is not rate limiting to the transfer reaction. Collectively, these data suggest that the preferential transfer of cholesteryl ester species from a mixture of esters is the consequence of substrate competition for interaction with the lipid transfer protein.

The capacity of lipid transfer protein to facilitate the exchange of various molecular species of cholesteryl ester could have at least two physiological consequences. First, during the homoexchange of cholesteryl esters, i.e., the exchange of one cholesteryl ester for another, lipid transfer protein may facilitate the equilibration of cholesteryl ester species between lipoproteins that have long plasma residence times. Such a role for the lipid transfer protein is supported by the in vivo observation of others (27) that the cholesteryl ester species of low and high density lipoproteins from animals with lipid transfer protein in their plasma are in chemical equilibrium, even though these sterol species have different metabolic origins, whereas the cholesteryl ester composition of these lipoproteins is markedly different in animals lacking appreciable lipid transfer activity in their plasma (28). The equilibration of cholesteryl esters by lipid transfer protein permits greater control of lipoprotein core composition by mixing cholesteryl ester species of both hepatic and extrahepatic origin.

Second, the preferential transfer of certain cholesteryl ester species may have important consequences during the heteroexchange reaction of lipid transfer protein, i.e., the exchange of cholesteryl ester for triglyceride. It has been proposed that lipid transfer protein facilitates the conversion of very low density lipoprotein to low density lipoprotein by promoting the exchange of excess cholesteryl ester present in the core of the very low density lipoprotein remnant with triglyceride from other lipoproteins; the triglyceride is then hydrolyzed by lipoprotein lipase (29). The result of this heteroexchange is the reduction of the remnant core size by the net removal of cholesteryl ester. Due to the specificity of lipid transfer protein, this unidirectional flux of cholesteryl ester mass would preferentially involve mono- and di-unsaturated cholesteryl ester species, resulting in an enrichment of the remnant with saturated cholesteryl esters. One consequence of enriching lipoproteins with saturated cholesteryl esters is a decrease in the fluidity of lipoprotein core lipids (30, 31). This increased microviscosity could alter remnant metabolism by inhibiting the activity of enzymes that remodel the remnant. In this regard, it is notable that saturated fat feeding, which leads to a decrease in lipoprotein core fluidity (30), also results in a loss of the normal equilibration of low- and high-density lipoprotein core lipids (32), suggesting that lipid transfer activity is inhibited by increased core lipid microviscosity. Whether the enrichment of a very low density lipoprotein remnant with saturated cholesteryl esters adversely affects its structure and subsequent metabolism is likely to depend on the cholesteryl ester composition of the parent very low density lipoprotein. For example, very low density lipoprotein from animals fed saturated fatty acid-rich diets is enriched in saturated cholesteryl esters (33). Thus, further enrichment of very low density lipoprotein remnants from these animals with saturated cholesteryl esters during the lipid

TABLE 3. Specificity of lipid transfer protein for cholesteryl ester species: liposome substrates containing a *single* cholesteryl ester species

Relative Lipid Transfer Activity			
[^{3}H]Cholesteryl Palmitate	[^{3}H]Cholesteryl Oleate	[^{3}H]Cholesteryl Linoleate	[^{3}H]Cholesteryl Arachidonate
1.04 ± 0.06	1.07 ± 0.09	1.01 ± 0.05	0.97 ± 0.07

Phosphatidylcholine liposomes containing 25 mol % free cholesterol and 0.2 mol % of a single radiolabeled cholesteryl ester were prepared by cholate dialysis (see Experimental Procedures). Liposomes (0.25 μmol of phospholipid) were incubated for 1.5 hr with low density lipoprotein (10 μg cholesterol) and lipid transfer protein (1.5–3.0 μg of protein) as described in Experimental Procedures. Data are the mean ± SE (n = 6) of values from three liposome preparations, each assayed at two levels of lipid transfer protein. The data presented are the ratios of the transfer rates observed for the indicated [^{3}H]cholesteryl ester-labeled liposomes divided by the rate observed for [^{14}C]cholesteryl oleate-labeled liposomes.

transfer protein heteroexchange reaction may be sufficient to lead to a shift in the physical state of the lipoprotein core at physiological temperatures. On the other hand, the cholesteryl ester specificity of lipid transfer protein probably has no adverse effect on very low density lipoproteins initially poor in saturated cholesteryl esters, such as those from animals fed polyunsaturated fatty acid-rich diets (33).

It is speculated that, under certain conditions, lipid transfer protein may facilitate the enrichment of very low density lipoprotein remnants in saturated cholesteryl esters, leading to the formation of lipoproteins that are chemically and physically altered, and are poor lipid transfer protein substrates. Thus, further depletion of the lipoprotein core of cholesteryl ester by the lipid transfer protein heteroexchange reaction would be prevented. Such a mechanism may be important in the formation of the large low density lipoprotein-like lipoproteins that accumulate in the plasma of animals fed diets enriched in saturated fat and cholesterol (31, 34). Consistent with the above discussion, these abnormal lipoproteins are characterized by an increased number of cholesteryl ester molecules/particle, an enrichment in saturated cholesteryl esters, and a shift in the cholesteryl ester phase transition temperature to above body temperature (31). These large molecular weight low density lipoproteins have been shown to be highly correlated with the severity of atherosclerosis (34).

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