Plasma cholesteryl ester transfer activity is modulated by the phase transition of the lipoprotein core

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Abstract Previous studies have shown that lipid transfer protein (LTP) activity is strongly temperature dependent, demonstrating a dramatic rise in activity near 37°C. We have investigated the origin of this rapid rise in LTP activity. LTP-mediated transfers of radiolabeled cholesteryl ester (CE) from LDL to HDL, HDL to LDL, LDL to biotin-LDL, HDL to biotin-HDL, and between liposomes were determined as a function of assay temperature. Only assays containing LDL demonstrated this rapid rise in CE transfer activity. In contrast, TG transfer was almost linear with assay temperature. As human LDL CE undergoes a thermal phase transition near 37°C, we investigated whether the rapid rise in CE transfer was dependent on this transition. Monkey LDL were isolated from animals consuming diets containing cholesterol and enriched in saturated, monounsaturated, or polyunsaturated fatty acids. With these LDL as substrate, the CE transfer between 21° and 49°C could be described by two straight lines, the intersection of which defined the inflection temperature. Among eight LDL samples, the inflection temperature was highly correlated with the CE phase transition determined by differential scanning calorimetry (r^2 = 0.86). Both calorimetry and CE transfer activity inflection values were correlated with the saturated + monoene/polyene ratio of the LDL cholesteryl esters (r^2 = 0.733 and 0.612, respectively). For LDL with inflection temperatures below 37°C, CE transfer activity at 37°C increased 10–14% for each 1°C decrease in the inflection temperature. We conclude that LTP activity is markedly affected by the physical state of the core CE. Diets rich in saturated fatty acids may result in LDL that are poor LTP substrates, which may hinder LTP's ability to promote normal lipoprotein remodeling.—**Morton, R. E., and J. S. Parks.** Plasma cholesteryl ester transfer activity is modulated by the phase transition of the lipoprotein core. *J. Lipid Res.* 1996. **37:** 1915–1923.

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Plasma lipoproteins are dynamic particles that undergo constant remodeling during their circulatory lifetime. This remodeling is driven by several factors including lipases, lecithin:cholesterol acyltransferase (LCAT) and lipid transfer proteins (LTP). Lipid transfer protein (cholesteryl ester transfer protein) mediates the equilibration of cholesteryl ester (CE) and triglyceride (TG) molecular species and also facilitates the mass exchange of CE and TG (1). CE and TG transfers occur largely through an equimolar heteroexchange mechanism where each lipid is transferred down its concentration gradient resulting in alterations in the ratio of these two lipids but no change in their sum. LTP activity requires the binding of LTP to the surface of substrate lipoproteins, where it interacts with CE and TG dissolved in the phospholipid monolayer of the lipoprotein particle (2, 3); the precise mechanism of transfer remains unclear.

LTP activity in normal and dyslipidemic plasma is controlled at multiple points including variations in LTP protein mass (4), inhibition by an endogenous suppressor protein (5, 6), and variations in the quantity and quality of the lipoprotein substrates (7–9). Changes in the composition of lipoproteins may affect the solubility of CE and/or TG in the surface phospholipid monolayer, and thus, directly alter LTP activity (8).

Atherogenic diets lead to the appearance in plasma of abnormal lipoproteins, such as β-very low density lipoproteins and HDL_c (10). Diet-induced modifications of lipoproteins also include changes in fatty acid composition of phospholipid, CE, and TG, and in alterations of the relative amounts of lipid components (11–14). One consequence of these diet-induced changes is an

Abbreviations: LDL, low density lipoprotein (1.019 < d < 1.063 g/ml); HDL, high density lipoprotein (1.063 < d < 1.21 g/ml); LTP, lipid transfer protein; LCAT, lecithin:cholesterol acyltransferase; PC, phosphatidylcholine; CE, cholesteryl ester; TG, triglyceride; T_m, transition temperature; BSA, bovine serum albumin; EDTA, ethylene diaminetetraacetic acid.

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alteration in the physical state of lipoprotein lipids. The CE of LDL isolated from normolipemic monkeys and humans shows a peak phase transition temperature (a smectic liquid crystal to liquid phase transition) below body temperature (15, 16), indicating that physiologically a large portion of core CE is in the more fluid (less ordered) state. In contrast, in LDL isolated from monkeys fed an atherogenic diet, the CE phase transition is shifted to temperatures considerably higher than body temperature (13, 15). Similarly, in subjects fed a high saturated fat diet, all lipoprotein classes show a decrease in microscopic fluidity as measured by fluorescence polarization and paramagnetic resonance (12, 17). These altered physical properties appear to be due, in large part, to an increase in the ratio of CE to TG and an enrichment in saturated and monounsaturated fatty acids esterified to cholesterol (13, 15, 16). By inference, the altered ratio of CE to TG in hyperlipemic LDL may considerably alter the physical state of the lipids and subsequently impair normal metabolism.

The regulation of LTP activity by the physical state of the core lipids has been suggested, but not demonstrated directly, by several studies. Long-term feeding of diets enriched in saturated fatty acids, which leads to a decrease in lipoprotein fluidity (13), results in a loss of the normal equilibration of LDL and HDL core lipids (18), suggesting that LTP is depressed by increased lipoprotein lipid microviscosity. Furthermore, Ihm et al. (19) demonstrated that CE transfer from LDL to HDL is minimal below 30°C but undergoes a marked activation around body temperature. However, it is unclear whether this enhanced activity reflects properties of LTP or its lipoprotein substrates. In this study we have characterized in detail the relationship between lipid transfer activity and assay temperature to elucidate the mechanism of this temperature-dependent response and to further understand the mechanisms whereby LTP activity is regulated.

EXPERIMENTAL PROCEDURES

Materials

Glycerol tri[9,10-^{3}H]oleate (26.8 Ci/mmol) was obtained from New England Nuclear (Boston, MA), and [1α,2α(n)-^{3}H]cholesterol (45.6–48.4 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL). Radiolabeled TG and CE with purities of <98% were repurified by thin-layer chromatography.

BSA (fraction V), diethyl *p*-nitrophenyl phosphate, egg PC, butylated hydroxytoluene, dithiothreitol, biotin, avidin, CNBr-Sepharose and all reagents for salt and buffer solutions were obtained from Sigma Chemical Co. (St. Louis, MO). Cholesterol, cholesteryl oleate, and triolein were purchased from Nu-Chek (Elysian, MN), and cardiolipin (beef heart) was from Serdary Research Labs, Inc. (Port Huron, MI). Lipid solutions were prepared in chloroform containing 10 μg/ml butylated hydroxytoluene and stored at -20°C. Phenyl Sepharose CL-4B, Con A-Sepharose, and dextran sulfate (M_r = 500,000) were from Pharmacia Fine Chemicals (Piscataway, NJ), and CM52-cellulose was from Whatman Chemical Separations, Inc. (Clifton, NJ).

Isolation of LTP

Partially purified LTP was isolated from lipoprotein-deficient human plasma by hydrophobic and ion exchange chromatography as previously described (20). During the purification of LTP, lipid transfer activity was routinely assayed by determining the extent of radiolabel transferred from [^{3}H]CE-labeled LDL to unlabeled HDL (10 μg cholesterol each) in the presence of 0.5% BSA in a total volume of 0.7 ml (21, 22).

Lipoprotein isolation, biotinylation, and radiolabeling

Fresh human plasma from the Blood Bank of the Cleveland Clinic Foundation was the source of LDL and HDL. Lipoproteins were isolated at 4°C by sequential ultracentrifugation (23), extensively dialyzed against 0.9% NaCl, 0.02% EDTA, pH 8.5, and stored at 4°C. LDL (1.019 < d < 1.063 g/ml) and HDL (1.063 < d < 1.21 g/ml) were quantitated based on their total cholesterol content. In some instances, before isolation from plasma, lipoproteins were radiolabeled by a lipid dispersion technique (24). Under these labeling conditions, lipoproteins typically contained ≥1.6 × 10^3 dpm ^{3}H/μg cholesterol. HDL and LDL were biotinylated with NHS-biotin (Pierce, Rockford, IL) as previously described (25).

Monkey diet and LDL isolation

Blood samples for LDL isolation were obtained from a subset (n = 8) of cynomolgus monkeys (*Macaca fascicularis*) that were part of an ongoing atherosclerosis progression study. The animals consumed diets consisting of 35% of calories as fat and 0.4 mg cholesterol per kcal. Four dietary fat groups were established: saturated (lard), monounsaturated (oleic acid-enriched safflower oil [oleinate]), polyunsaturated (safflower oil), and fish oil. The fish oil diet consisted of 8.2 g fish oil and 8.2 g lard/100 g diet, whereas the other three diets contained 16.4 g of the respective fat/100 g diet. The animals had consumed their respective diets for 3 years when the present study was initiated. All animals were individually housed in the animal facility (Bowman Gray School of Medicine), which is approved by the American Association for the Accreditation of Laboratory Animal Care

and supervised by a veterinary staff. All procedures were approved by the institutional animal care and use committee.

Blood was obtained from animals after an overnight fast. Ketamine HCl was administered (10 mg/kg) to anesthetize the animals and blood was taken by venipuncture from the femoral vein into sterile evacuator tubes containing 0.1% EDTA and 0.1% NaN_3. Tubes were immediately chilled to 4°C and plasma was promptly separated by centrifugation at 1000 *g* for 30 min at 4°C. Aprotinin (1 mg/l; final concentration) and phenylmethyl sulfonyl fluoride (80 mg/l) were then added to the fresh plasma. LDL were isolated from plasma by combined ultracentrifugation (d < 1.225 g/ml) and high pressure liquid chromatography as described previously (26) and LDL cholesterol was quantified by an enzymatic procedure (27). Aliquots of the LDL samples were blanketed with argon and shipped on ice via overnight mail to The Cleveland Clinic. An aliquot of the LDL was extracted with chloroform–methanol 2:1; the cholesteryl esters were separated by thin layer chromatography; and the fatty acids were transmethylated for analysis as described previously (26).

Liposome preparation

PC-cholesterol-CE liposomes with or without radiolabeled CE, and PC-cholesterol-CE liposomes containing radiolabeled CE plus 10 mole% cardiolipin were prepared by cholate dialysis using a modification (3) of the method of Brunner, Skrabal, and Hauser (28). Egg PC was used in all liposomes. The cholesteryl ester content and the specific activity of liposome preparations varied depending on the experimental design; specific details are provided in the figure legends. Liposomes were routinely characterized with respect to radiolabel and phospholipid phosphorus content.

LTP assays

Lipid transfer assays between [^{3}H]CE-LDL or [^{3}H]TG-LDL (donor) and HDL (acceptor) were carried out as previously described (21, 22). Radiolabeled LDL and unlabeled HDL (10 μg cholesterol of each) were incubated with or without LTP at 37°C for 1 h. The assay was concluded by selectively precipitating the donor lipoprotein (22) and counting the radioactivity in the supernatant (acceptor). Assays in the reverse direction, i.e., from [^{3}H]CE-HDL to LDL were terminated in the same manner but the pellet containing the acceptor was washed once with "equilibrated top phase" (the supernatant derived from precipitated assay blanks not containing labeled lipoprotein) and its radiolabel content was determined. Homologous transfer assays between [^{3}H]CE-LDL and unlabeled, biotinylated LDL were essentially the same as that described above except that the assays were terminated by affinity adsorption of the biotinylated lipoprotein with avidin–agarose (25). Lipid transfer assays from [^{3}H]CE-PC liposomes (150–190 nmol PC) to LDL (10 μg cholesterol) were performed as previously described (25). For [^{3}H]CE-PC liposome to HDL transfer reactions, biotin-HDL was used as the acceptor and it was precipitated by avidin–agarose (25). To measure CE transfer from [^{3}H]CE-cardiolipin-PC to PC liposomes, donor and acceptor liposomes (~155 nmol phospholipid) were added to 3.5% BSA (20 μl), ± LTP and buffer to a final volume of 90 μl. Samples were preincubated at 4°C for 30 min prior to the start of the assay. Donor and acceptor liposomes were separated by electrophoresis on pre-made agarose gels (Ciba-Corning Diagnostics Inc., Palo Alto, CA). Two 2-μl aliquots of the assay mixture were applied and the gels were electrophoresed at 70 volts for 30 min. Each lane was cut into two 4-cm strips and their radioactivity content was determined by scintillation counting in the presence of 0.1% SDS.

All assays were carried out in a shaking water bath (Bellco Glass Inc., Vineland, NJ) at the indicated temperature. For assessing the temperature response of LTP in the various assays, samples were held at 4°C prior to the incubation and were placed on ice for at least 15 min after the incubation before the donor/acceptor separation protocol was started. In all assays the radiolabel content of the acceptor fraction was determined after separation of the donor and acceptor as referenced above. The fraction of radiolabeled, donor lipid that was transferred (kt) to the acceptor particle was calculated as described previously (21), and reported as percent lipid transfer (%kt). Radiolabel "transfer" in the absence of LTP was subtracted from the values reported unless otherwise indicated. Duplicate values generally differed by <10%.

Differential scanning calorimetry

LDL transition temperatures (T_m) were measured using a MicroCal ultrasensitive differential scanning calorimeter (MicroCal Inc., Northhampton, MA) interfaced with a personal computer. LDL preparations were concentrated to ≥5 mg cholesterol/ml using Centriflow ultrafiltration membranes (Amicon, Danvers, MA). Solutions were then degassed under vacuum. The sample cell was then filled with 1.3 ml of the LDL sample and the reference cell was filled with an equal volume of 0.9% NaCl, 0.01% EDTA, 0.01% NaN_3, pH 7.4, buffer. The samples were pre-equilibrated at 5°C for 45 min before initiation of the heating scan from 5° to 60°C at a rate of 90°C/h. During the pre-equilibration run, the sample was maintained under a positive pressure N_2 atmosphere. Peak transition temperatures were deter-

mined digitally from the computer output of each run. Transition temperature accuracy of the instrument was monitored with dimyristoyl PC vesicles.

Analytical procedures

Protein was quantitated by the method of Lowry et al. (29) as modified by Peterson (30), with bovine serum albumin as standard. Total cholesterol of lipoproteins was assayed by a colorimetric, enzymatic method using Reagent-Set (Boehringer-Mannheim). The cholesterol content of organic solutions of lipids was assayed by the method of Zak et al. (31). TG was measured by a colorimetric, enzymatic kit (Sigma) and lipid phosphorus was quantitated by the method of Bartlett (32).

RESULTS

Role of assay temperature in lipid transfer between lipoproteins and liposomes

The transfer of CE from LDL to HDL by LTP was strongly influenced by the assay temperature (**Fig. 1**). CE transfer remained very low from 4–25°C, increased in a nearly linear fashion until about 35–37°C, and then increased rapidly thereafter. Essentially identical kinetics were observed with CE transfer in the reverse direction, i.e., from HDL to LDL. Thus, the effect of assay temperature was independent of the source of CE in this two-lipoprotein assay system.

To assess whether these kinetics were a property of LTP or the lipoprotein substrates, transfer assays were performed with LTP and synthetic acceptors. CE transfer from PC liposomes to PC-CL liposomes was linear with assay temperature (**Fig. 2**). These results suggest that the strong dependence of CE transfer on assay temperature depicted in Fig. 1 was not a property of LTP itself, but rather due to the lipoprotein substrates. To determine whether LDL or HDL alone could account for this temperature dependence, CE transfer assays were performed with liposomes as the donor of CE to either LDL or HDL. As shown in **Fig. 3,** CE transfer from liposomes to HDL increased in a slightly curvilinear fashion over the temperatures tested. In

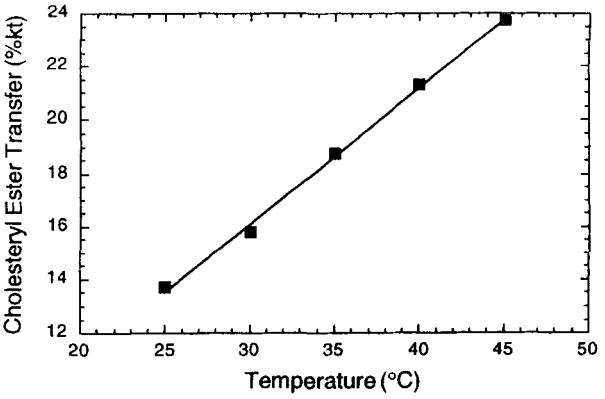

Fig. 2. Temperature dependence of CE transfer between liposomes. CE transfer between liposomes was measured as described in Methods. In short, [^{3}H]CE-labeled cardiolipin liposomes (100 nmol lipid phosphorus of PC liposomes containing 10 mol% cardiolipin, 0.04 mol% CE, and 1.2×10^6 cpm/nmol CE) and PC liposomes (100 nmol lipid phosphorus, 490 pmol CE) were incubated ± LTP (485 ng) for 1 h. Label transfer to the acceptor in the absence of LTP has been subtracted.

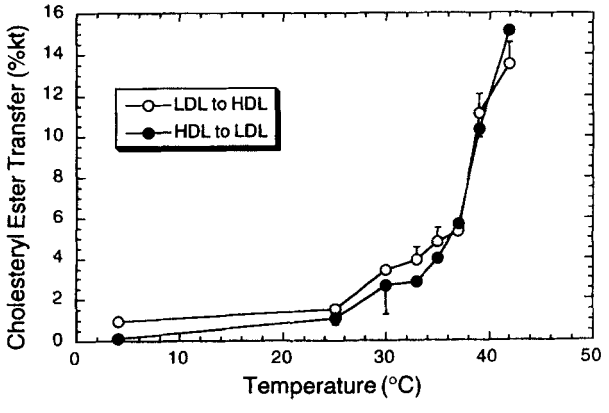

Fig. 1. Temperature dependence of CE transfer between LDL and HDL. Human LDL and HDL (10 μg cholesterol each) were incubated in the presence of LTP (14 μg protein) and the transfer of [^{3}H]CE was measured. CE transfer from [^{3}H]CE labeled LDL to HDL (○) and from [^{3}H]CE labeled HDL to LDL (●) was determined as described in Methods. Label transfer to the acceptor in the absence of LTP has been subtracted. Values plotted are mean ± SD; error bars not visible are smaller than the symbols.

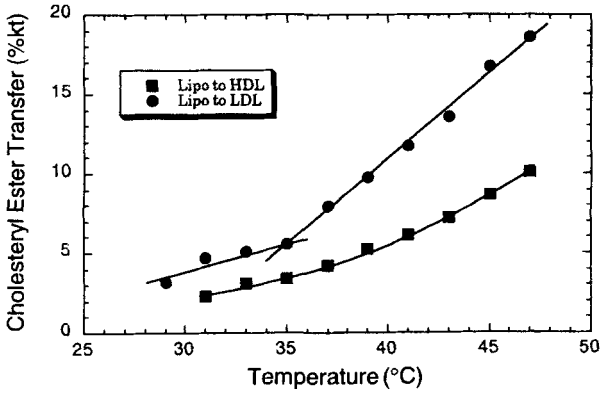

Fig. 3. Temperature dependence of CE transfer from liposomes to LDL or HDL. [^{3}H]CE transfers from PC liposomes to LDL (●) and HDL (■) were measured as described in Methods. PC liposomes (100 nmol lipid phosphorus, 270 pmol CE, and ~4.4×10^3 cpm [^{3}H]CE) were incubated with LDL (10 μg cholesterol) ± 229 ng LTP, or with HDL (10 μg cholesterol) ± 458 ng LTP. Label transfer to the acceptor in the absence of LTP has been subtracted.

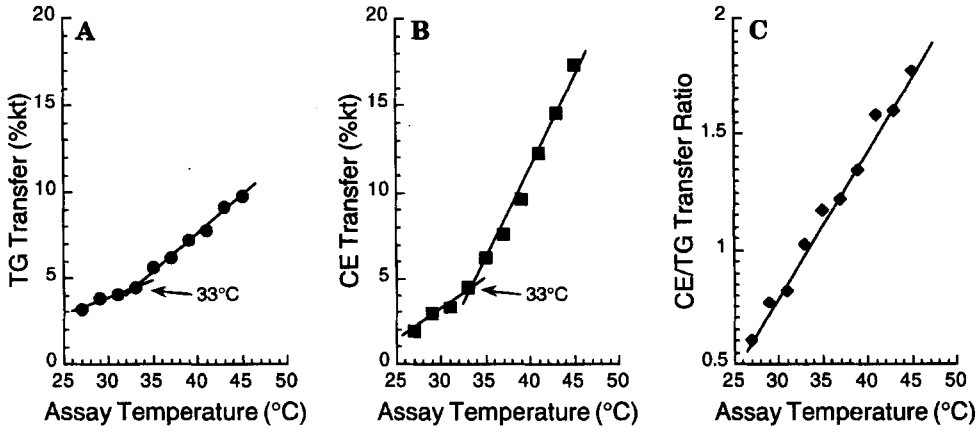

Fig. 4. Temperature dependence of TG and CE transfer from LDL to HDL. Radiolabeled LDL (10 μg cholesterol) containing [^{3}H]TG (3.53 × 10^4 cpm) or [^{3}H]CE (1.62 × 10^4 cpm) was incubated with HDL (10 μg cholesterol) ± LTP (5.6 μg protein) as described in Methods. Both radiolabeled LDL preparations were derived from the same donor plasma. Panel A: TG transfer; panel B: CE transfer; panel C: ratio of CE to TG transfer. Label transfer to the acceptor in the absence of LTP has been subtracted.

contrast, CE transfer from liposomes to LDL demonstrated a strong temperature dependence, which was best described by two lines with slopes of 0.35 and 1.03% kt/°C. The intercept of these two lines, termed the inflection point, was similar to the point where CE transfer activity began to increase rapidly in Fig. 1. Similar results to those shown in Fig. 3 were obtained when transfers were measured from LDL to biotinylated LDL (data not shown). Thus, LDL, rather than LTP or HDL accounted for the marked change in transfer rates near physiological temperature. The temperature of this inflection point was reproducible for a given LDL preparation but varied from 32.7 to 38.2°C among LDL from six different donors (mean = 35.3°C).

The transfer of both CE and TG by LTP demonstrated similar inflection points (**Fig. 4**). However, compared to CE, TG transfer from human LDL to HDL was subtly affected by assay temperature with a transfer slope of 0.27% kt/°C below the inflection point and 0.43% kt/°C above (Fig. 4A). The slope for TG transfer below the inflection point was very similar to that also noted for CE transfer; however, above the inflection point the slope for CE transfer was 1.04% kt/°C (Fig. 4B). These slopes, determined for CE transfer from LDL to HDL, were very similar to those noted above with CE transfer from liposomes to LDL. Because of the differential temperature response of TG and CE, the ratio of CE to TG transferred by LTP changed 3-fold over the 25–45°C interval (Fig. 4C).

Influence of core lipid transition temperature on CE transfer kinetics

The preceding data demonstrate that LTP activity is often characterized by a marked increase in CE transfer activity near physiological temperature and that this enhanced transfer is a property of the LDL used in the assay. As CE, but not TG, in the core of LDL undergoes a thermotropic phase transition (liquid-crystalline to disordered transition) in the region where the inflection in CE transfer activity is frequently noted, we investigated whether these events were correlated. LDL was isolated from cynomolgus monkeys consuming diets enriched with saturated or polyunsaturated fats. The CE fatty acid composition of these LDL is shown in **Table 1.** The saturation ratio (0 + 1 double bond species/2+ double bond species) of these LDL varied from 4.6 for saturated fat-fed monkeys to 0.34 for polyunsaturated fat-fed monkeys. This provided LDL preparations that underwent CE phase transitions (T_m) ranging from 32.4 to 46.9°C, as determined by differential scanning calorimetry. T_m correlated well (r^2 = 0.733) with the CE fatty acid saturation ratio as previously reported (15).

CE transfer studies were carried out using these LDL as acceptors of CE from radiolabeled PC liposomes. This assay approach permitted two desired conditions: *1)* the mass of radiolabeled CE (cholesteryl oleate) transferred was small compared to the acceptor CE pool, and *2)* the donor liposome contained only CE (i.e., no TG). Thus, during the transfer assay the CE/TG ratio of the acceptor monkey LDL did not change and the CE fatty acid composition was altered by less than 1% even when label transfer was high (30%kt). As with human LDL, the temperature response of CE transfer to monkey LDL was defined by two straight lines. CE transfer to LDL isolated from a polyunsaturated fat-fed monkey demonstrated an inflection point (the intercept of the two lines) of 32.6°C (**Fig. 5**). Transfer in the absence of LTP was low and relatively unaffected by assay tempera-

TABLE 1. Fatty acid content of LDL cholesteryl esters

	Monkey # and Diet Consumed							
Fatty Acid	3372 Sat	3392 Sat	3406 Sat	3407 Mono	3379 Mono	3385 FO	3368 Poly	3409 Poly
16:0	10.9	13.2	12.4	9.0	5.4	15.4	5.0	6.1
16:1	3.6	4.8	3.7	1.5	1.2	7.2	0.4	0.3
18:0	7.5	7.4	5.4	2.6	1.6	5.9	1.2	1.6
18:1	54.5	52.4	50.0	64.4	58.2	31.9	21.2	16.1
18:2	17.3	16.0	22.9	18.4	30.8	14.8	69.1	70.9
20:4	0.8	0.8	1.3	0.6	0.3	2.0	0.3	0.7
20:5	-	-	-	-	-	13.3	-	-
22:6	-	-	-	-	-	1.8	-	-
other	5.4	5.4	4.3	3.5	2.5	7.7	2.8	4.3
S + M/P[a]	4.23	4.63	2.95	4.08	2.14	1.89	0.40	0.34

Cynomolgus monkeys consumed diets containing 35% of calories as fat and 0.4 mg cholesterol per kcal for 3 years. Diets were enriched in either saturated fat (Sat), monounsaturated fat (Mono), polyunsaturated oil (Poly), or fish oil (FO). LDL were isolated from plasma by a combination of ultracentrifugation and high pressure liquid chromatography. LDL CE fatty acids were quantitated by gas–liquid chromatography and reported as % of total weight.

[a]S + M/P = 0 + 1 double bond species/2+ double bond species.

ture. By comparison, the inflection point observed with LDL from a saturated fat-fed animal was more than 8°C higher (**Fig. 6**). Again, CE transfer in the absence of added LTP was low and independent of temperature. Arrhenius plots of the data in Figs. 5 and 6 (see insets) were non-linear and demonstrated breaks at the inflection temperatures previously noted. For the eight monkey LDLs studied, the CE transfer activity inflection point was highly correlated with the peak phase transition temperature of the core CE (r^2 = 0.86, **Fig. 7**). Inflection temperatures correlated less well with the saturation ratio of CE fatty acids (r^2 = 0.612).

Among six human LDL samples studied, three of which are illustrated in the figures referenced above, the inflection temperatures varied over a 6 degree range; two samples demonstrated inflections at or above physiologic temperature. In the single human LDL sample analyzed by calorimetry, a T_m value of 31°C was correlated with a 34°C inflection point. The strong temperature dependence of CE transfer activity above the inflection temperature means that for most samples, small

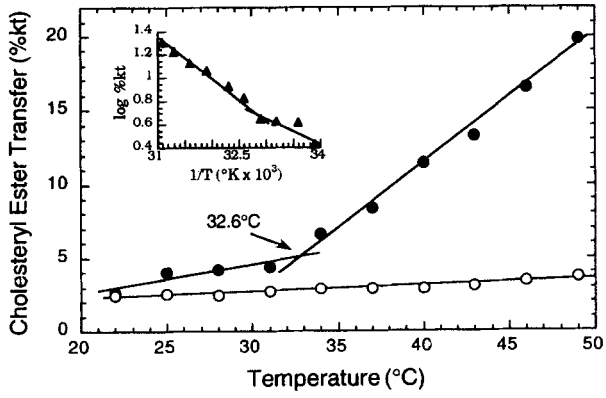

Fig. 5. Temperature dependence of CE transfer from liposomes to monkey LDL (low T_m). [^{3}H]CE transfer from PC liposomes (100 nmol lipid phosphorus, 270 pmol CE, and ~4.4 × 10^3 cpm [^{3}H]CE) to monkey LDL (10 μg cholesterol) was measured in the presence and absence of LTP (411 ng protein) as described in Methods. Transfer due to LTP (●, total label transfer minus transfer in the absence of LTP) and background transfer (○, transfer in the absence of LTP) are shown. LDL was isolated from an animal that had consumed a polyunsaturated fat diet for more than 3 years. The arrow denotes the temperature (inflection point) where LTP activity abruptly changes. Inset, Arrhenius plot of the data presented in the main figure.

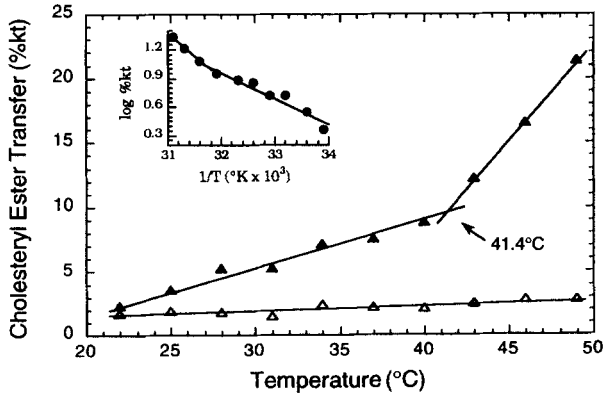

Fig. 6. Temperature dependence of CE transfer from liposomes to monkey LDL (high T_m). [^{3}H]CE transfer from PC liposomes (100 nmol lipid phosphorus, 270 pmol CE, and ~4.4 × 10^3 cpm [^{3}H]CE) to monkey LDL (10 μg cholesterol) was measured in the presence and absence of LTP (411 ng protein) as described in Methods. Transfer due to LTP (▲, total label transfer minus transfer in the absence of LTP) and background transfer (△, transfer in the absence of LTP) are shown. LDL was isolated from an animal that had consumed a saturated fat diet for more than 3 years. The arrow denotes the temperature (inflection point) where LTP activity abruptly changes. Inset, Arrhenius plot of the data presented in the main figure.

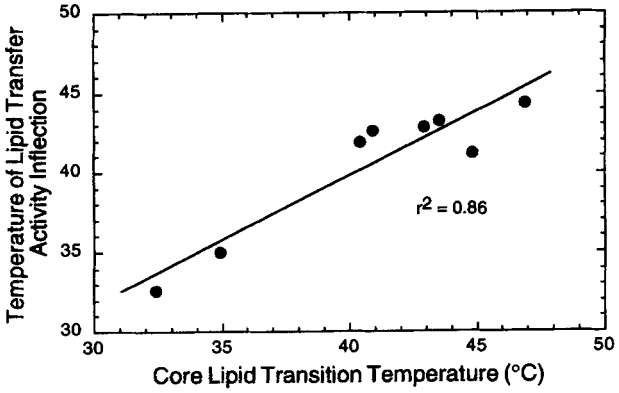

Fig. 7. Correlation of LDL core lipid transition temperature and CE transfer activity inflection point. Inflection temperatures, determined as shown in Figs. 5 and 6, were defined for LDL isolated from eight monkeys consuming diets containing various fats. Core lipid transition temperatures were determined by differential scanning calorimetry as described in Methods. Although the data points shown reflect single determinations, replicate T_m and inflection point determinations typically had SD values of ± 1.2 and ± 0.7°C, respectively.

variations in the inflection temperature would result in significant changes in LTP activity at body temperature. Overall, when normalized for transfer activity at 25°C, CE transfer activity at 37°C, from either human or monkey LDL, increased 10–14% for each 1°C decrease in the inflection temperature.

DISCUSSION

In these studies we have demonstrated that CE transfer by LTP is strongly influenced by assay temperature when LDL is either the donor or acceptor of the transferred lipid. Our data show that the previously reported temperature effect on LTP activity is not due to thermodynamic effects on LTP itself (19), but rather due to LDL. Neither HDL nor liposome substrates demonstrated this temperature dependence. With monkey LDL there was a strong correlation between the inflection point for CE transfer and the peak temperature of the ordered-disordered CE transition. Likewise, the inflection points observed with various human LDL preparations overlapped the phase transition temperatures reported for normolipidemic human LDL (16). However, the fact that we observed inflection points above body temperature whereas reported T_m values for normolipidemic human LDL are below 37°C suggests that in human LDL the inflection temperatures may not correlate precisely with the peak transition temperature but may require greater disordering of CE for optimum CE transfer activity. This may be due to the presence of significant amounts of TG in human, but not monkey, LDL (15, 33), which significantly broadens the CE transition peak (16). Alternatively, there may be more variability in the T_m values for individual human LDL than has previously been shown. It is notable that with human LDL, CE transfer activity (Fig. 1, for example) began to increase at a temperature near the onset of the CE phase transition (16), suggesting that the physical state of the core CE can affect the rate of CE transfer over a temperature range encompassing the entire phase transition. Overall, the data show that the physical state of the LDL CE core is the defining influence on the temperature response of CE transfer.

The phase transition temperature of LDL is largely defined by the ratio of CE to TG and the fatty acid content of CE (15, 16). These properties are readily influenced by dietary lipids and thus the capacity of LDL to participate in CE transfer events is subject to dietary modification, as exemplified by the monkey studies herein.

Lipid transfer activity requires the physical binding of LTP to the lipoprotein surface, where it interacts with CE and TG dissolved in the phospholipid surface monolayer (2, 3). Perturbations in either binding of LTP or surface availability of lipid substrates can alter transfer activity. It seems unlikely that the inflection point reflects a change in the interaction of LTP with the LDL surface. TG transfer, which is also mediated by LTP, was affected to only a minor extent over the temperature range studied, indicating that the binding of LTP with the LDL surface does not change abruptly at the inflection point. Likewise, the nearly identical temperature curves of CE transfer from LDL to HDL and from HDL to LDL (Fig. 1) suggest that the distribution of LTP among LDL and HDL particles does not change over these temperatures. It also seems unlikely that the capacity of the phospholipid surface to specifically solubilize CE, but not TG, would be altered as the phospholipid has no phase transition over this temperature range (16). On the other hand, the surface availability of CE for transfer could be limited by the physical ordering of CE within the core. Although TG does not undergo phase transition near body temperature (16), the identical inflection point noted for CE and TG transfers suggests that the ordering of the CE core may also alter the availability of TG for transfer to a minor extent. Overall, these data are consistent with a temperature-dependent solvation of CE in the phospholipid monolayer where the amount of CE available for solvation increases markedly at the T_m. We hypothesize that in LDL with an ordered or partially order CE core, the rate at which CE partitions between the core and the surface may be rate-limiting to the transfer process.

An important function of LTP is to equilibrate the molecular species of CE among circulating lipoproteins.

This relatively rapid process homogenizes diet-derived CE originating in nascent VLDL and the LCAT-derived CE species, which are principally cholesteryl linoleate (34). When LDL core CE is physically restricted due to a high transition temperature, this equilibration process is hindered because the CE carried by LDL is poorly available for this process. Notably, once the core CE of LDL, and presumably intermediate density lipoproteins as well since they also undergo CE phase transitions (35), becomes ordered due to a high transition temperature, subsequent LTP activity may actually enhance the problem. This is because these particles will be better TG donors than LDL with less ordered CE cores as the ratio of TG to CE transferred is higher below the T_m (Fig. 4C). As transfer progresses in these CE-restricted LDL, the TG/CE mass ratio will be decreased which will further raise the phase transition temperature and additionally compromise LDL remodeling. It is not known how modifications in the surface TG/CE ratio might affect the activity of other enzymes that interact with the lipoprotein surface such as LCAT and lipases.

In conclusion, these results demonstrate that the physical state of CE in the LDL core is a strong determinant of CE transfer activity. The non-linear response of CE transfer to assay temperature is due to the order-disorder phase transition of CE that alters its mobility within the LDL particle. We propose that the high structural organization of core CE that exists below the phase transition temperature causes the partitioning of CE from the core to the surface to become rate-limiting to the transfer process. Factors that increase either the saturation of CE fatty acids or the CE/TG ratio of LDL will directly increase the transition temperature and decrease CE transfer activity with these particles. In addition to LDL, intermediate density lipoproteins (35) also undergo CE phase transitions near body temperature and therefore the capacity of LTP to participate in their catabolism to LDL (1) may be significantly altered by small compositional changes.

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