Long- and Medium-Chain Triacylglycerols in Neutral Lipid-Exchange Processes with Human Plasma Low-Density Lipoproteins†

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ABSTRACT: To explore the effects of various molecular species of triacylglycerols on neutral lipid exchange processes, we compared the *in vitro* transfer of triacylglycerols and cholesteryl esters between phospholipid-stabilized emulsions of medium-chain triacylglycerols (MCT) or long-chain triacylglycerols (LCT) and human plasma low-density lipoproteins (LDL). Incubations were performed with MCT, LCT, or mixed MCT/LCT (1:1, w/w ratio) emulsions with LDL over varying ratios and/or time periods, in the presence or absence of human cholesteryl ester transfer protein (CETP). Relative triacylglycerol mass content increased up to 5–6-fold in LDL with all emulsions, but LDL cholesteryl ester loss was about 33–50% lower with MCT as compared to LCT emulsion. In the absence of CETP, there was a significant transfer of MCT but not of LCT to LDL, and cholesteryl ester exchange never occurred. Spontaneous transfer of MCT in the absence of CETP accounted for half of the total MCT transfer. In fact, CETP-mediated transfer of MCT was less than that of LCT when normalized to the molar concentrations of MCT and LCT in the incubations. Net triacylglycerol accumulation in LDL was accompanied by LDL cholesteryl ester depletion (i.e., exchange), while the greater MCT accumulation could be related to increased solubility at the LDL phospholipid surface (Deckelbaum et al., 1990). This property may also have contributed to some back transfer of MCT from LDL and decreased LDL cholesteryl ester removal, in the presence of MCT emulsions. The results suggest that the different properties of MCT compared to LCT contribute to the differences in neutral lipid exchange processes in the presence of these two triacylglycerols, as well as to the ability of LDL to accommodate greater molar concentrations of MCT.

Mass transfers of cholesteryl esters and triacylglycerols between cholesteryl ester-rich lipoproteins and triacylglycerol-rich particles largely affect lipid transport and lipoprotein remodeling in human plasma (Deckelbaum et al.,1984). These exchanges are mediated by the M_r 74 000 cholesteryl ester transfer protein (CETP)[1] (Hesler et al., 1987), which transfers cholesteryl esters from cholesteryl ester-rich lipoproteins (LDL, HDL) to triacylglycerol-rich lipoproteins (VLDL, chylomicrons). Concomitantly, CETP transfers triacylglycerols from VLDL and chylomicrons into LDL and HDL (Deckelbaum et al., 1979, 1986; Tall, 1986; Barter, 1990). We and others have demonstrated that phospholipid-stabilized artificial emulsions made of long-chain triacylglycerols (LCT) can fully substitute for human endogenous triacylglycerol-rich lipoproteins in these exchange processes *in vitro* (Granot et al., 1985). Moreover, during *in vivo* infusion into normal subjects, we observed substantially different effects on LDL and HDL lipid composition between a LCT emulsion and a medium-chain triacylglycerol (MCT) containing emulsion, suggesting differences in neutral lipid exchange processes (Richelle, 1992).

The concentration of neutral lipid substrates at phospholipid interfaces is an important determinant of transfer protein activity (Morton et al., 1990). Moreover, the molecular fatty acid composition of cholesteryl esters also affects their transfer rate between particles (Morton et al., 1986). No data is available, however, as to how the molecular species of triacylglycerol molecules might affect neutral lipid transfer processes.

We recently reported that MCTs are hydrolyzed by lipoprotein and hepatic lipases at substantially higher rates than LCTs stabilized with the same phospholipid emulsifier (Deckelbaum et al., 1990). This is possibly due to the a 4–5-fold greater solubility of MCT compared to LCT in phospholipid surfaces and presumably at emulsion–water interfaces.

In the present study, we questioned the relative importance of the triacylglycerol concentration at the particle surface and of the molecular species of triacylglycerols in neutral lipid exchanges between donor and acceptor particles. In particular, we explored whether LCT, MCT, and mixed emulsions would show different behaviors as triacylglycerol donors to human LDL and cholesteryl ester acceptors in *in vitro* transfer reactions, in the presence and absence of CETP. Considerable differences found between MCT and LCT in transfer processes seem to result from differences in both the physical properties of MCT and LCT molecules and their CETP-mediated transfer.

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[1] Abbreviations: MCT, medium-chain triacylglycerols; LCT, long-chain triacylglycerols; MCT/LCT, medium- and long-chain triacylglycerols in 1:1 (w/w) ratio; CETP, cholesteryl ester transfer protein; FA, fatty acid.

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EXPERIMENTAL PROCEDURES

Preparation of Lipoproteins. Blood was drawn in glass tubes containing disodium EDTA [1 mg/(mL of blood)] from fasting normal subjects. Plasma was immediately separated at 4 °C by low-speed centrifugation (1000*g*) for 10 min, using a Beckman refrigerated centrifuge (J2-21, Beckman Instruments Inc., Fullerton, CA). Sodium azide [1 mg/(mL of plasma)] was added prior to lipoprotein separation. LDL ($1.019 < d < 1.063$ g/mL) and lipoprotein-deficient plasma ($d > 1.21$ g/mL) were isolated by salt-density ultracentrifugation in a L8-55 ultracentrifuge (Beckman Instruments Inc.) using a fixed-angle Ti 50 rotor (Beckman Instruments Inc.) at 40 000 rpm for 18 and 44 h, respectively (Havel et al., 1955). Plasma LDL and lipoprotein-deficient plasma containing CETP were washed by one additional ultracentrifugation run and then dialyzed at 4 °C in the dark for 24 h against five changes of 0.15 M NaCl/1 mM EDTA solution, pH 8.5 (1:100, v/v).

Emulsions. Three emulsions were studied. Each contained 200 g/L triacylglycerols, 12 g/L fractionated egg phospholipids, and 25 g/L glycerol. They differed only in the type of triacylglycerols emulsified. The LCT emulsion contained soybean triacylglycerols with the following fatty acid composition (wt %): $C_{16:0}$, 10%; $C_{18:0}$, 4%; $C_{18:1}$, 23%; $C_{18:2}$, 55%; $C_{18:3}$, 6%; $C_{20:4}$ and others, 2%. The MCT emulsion contained MCT oil extracted from coconut oil (triacylglycerol fatty acid composition: C_6, 1%; C_8, 56%; C_{10}, 42%; C_{12}, 1%). The mixed MCT/LCT emulsion was prepared from a blended homogeneous 1:1 weight mixture of MCT and LCT oils. Mean particle sizes determined by laser spectroscopy were 0.29, 0.27, and 0.29 μm for LCT, MCT, and blended MCT/LCT emulsions. All emulsions were prepared by B. Braun AG (Melsungen, Germany) following the same homogenization methods as detailed previously (Deckelbaum et al., 1990).

Incubations. Incubations of LDL with triacylglycerol emulsions were performed as previously described for VLDL (Deckelbaum et al., 1979) and Intralipid (Granot et al., 1985). Usually, LDL (4–11 μmol of LDL cholesteryl esters) was incubated with 15–50 mg of each triacylglycerol emulsion, in the presence or absence of CETP from lipoprotein-deficient plasma (240 mg of protein), in a total volume of 4 mL containing 0.15 M NaCl and 1 mM EDTA (pH 8.5). After mixing, incubations were performed under N_2 in a slowly shaking water bath at 37 °C over 0.5–20 h. Then salt-density ultracentrifugation was used to separate the emulsion fraction at $d < 1.019$ and LDL at $d = 1.019$–1.063 g/mL (Havel et al., 1955).

Analyses. Protein content of lipoprotein fractions was measured with bovine serum albumin as a standard (Lowry et al., 1951). Triacylglycerols were enzymatically assayed using the Triacylglycerol GPO-PAP kit (Boehringer Mannhein GmbH, Mannheim, Germany). This assay measures the glycerol moiety of triacylglycerols, so that all samples were extensively dialyzed to remove free glycerol before determination. The mean M_r of MCT is 500, while that of LCT is 875, and the mean molecular weight of triacylglycerols in MCT/LCT is 635. The following calculations were made for determination of triacylglycerols where MCT and MCT/LCT solutions were used. We assumed that increments in LDL triacylglycerols represented new molecules transferred from the emulsion particles. Thus triacylglycerols in the LDL fraction after incubation can be calculated as

$$TG_o + TG_p = TG_{corrected}$$

and

$$TG_p = \frac{(M_r \text{ of TG in emulsion})}{(M_r \text{ of LCT})} \times (TG_{measured} - TG_o) \quad \text{(mg, by enzyme assay)}$$

where TG_o is the triacylglycerol concentration of LDL before incubation and TG_p is the calculated net triacylglycerol increase in LDL after incubation.

Phospholipid concentration was measured by the Test Combination Phospholipid kit (Boehringer Mannheim GmbH). Concentrations of total and free cholesterol were measured using Monotest Cholesterol and Test combination free cholesterol kits (Boehringer Mannheim GmBh), respectively. Because the free cholesterol assay did not give consistent results in the presence of emulsions, free and esterified cholesterol were determined in the $d < 1.019$ g/mL fraction after extraction with chloroform/methanol (2:1, v/v) (Folch et al., 1957) and thin-layer chromatography (TLC) to separate free and esterified cholesterol with a *n*-hexane/diethyl ether/acetic acid (140:60:2) solvent system. Esterified cholesterol was calculated by subtracting the amount of free cholesterol from total cholesterol and multiplying by 1.67.

Triacylglycerol present in LDL after incubation was separated from other lipids by TLC, and the concentration of individual fatty acids was analyzed by gas–liquid chromatography (Lepage & Roy, 1986). In brief, 400 μL of the LDL fraction was transferred into a 15-mL borosilicate test tube containing 100 μg of tripentadecanoin dissolved in chloroform/methanol (2:1, v/v); this was followed by the addition of 7.8 mL of chloroform/methanol (2:1, v/v). After 10 min of vortexing, 1.7 mL of 17 mM saline solution containing 1 mM sulfuric acid was added. After 10 min of centrifugation at 3000*g*, the clear chloroform lower phase was separated and concentrated to a volume of 20–40 μL under a gentle stream of nitrogen. The whole lipid extract was then applied to a TLC plate (Merck 5721; E. Merck, Darmstadt, Germany) presprayed with rhodamine 6 G (0.01% in ethanol). The plate was immediately developed in a TLC tank as described above. The fraction corresponding to triacylglycerols was located under UV light, scraped, and poured into a Teflon-lined screw-capped test tube containing 2 mL of methanol/hexane (4:1, v/v). The saponification of triacylglycerols and methylation of liberated fatty acids was performed by using acetyl chloride. Two microliters of the hexane upper phase, obtained at the end of the reaction after addition of 6 mL of 6% K_2CO_3 solution, was injected into an HP 5890 gas chromatograph (Hewlett-Packard, Avondale, PA). Blanks of triacylglycerols were processed using the same procedure but with distilled water instead of the LDL fraction. Each fatty acid methyl ester was identified and quantitated by comparison to the corresponding fatty acid standard. The blank value corresponding to each fatty acid was subtracted when determining the concentration of individual fatty acids.

To calculate MCT transfer from emulsions to LDL, we measured fatty acids $C_{8:0}$ and $C_{10:0}$ in MCT and $C_{18:1n-9}$ and $C_{18:2n-6}$ in LCT. $C_{8:0}$ plus $C_{10:0}$ accounted for 98% of fatty acids present in MCT, and $C_{18:1n-9}$ plus $C_{18:2n-6}$ accounted for 78% of fatty acids in LCT. Net transfer was measured after subtraction of the initial amounts of these fatty acids present in LDL triacylglycerols before incubation. Thus MCT transfer to LDL was calculated from transfer of $C_{8:0}$ and $C_{10:0}$, $([C_{8:0}] + [C_{10:0}])/3 \times 100/98$, and LCT transfer, from net transfer of $C_{18:1n-9}$ and $C_{18:2n-6}$, $([C_{18:1n-9}] + [C_{18:2n-6}])/3 \times 100/78$.

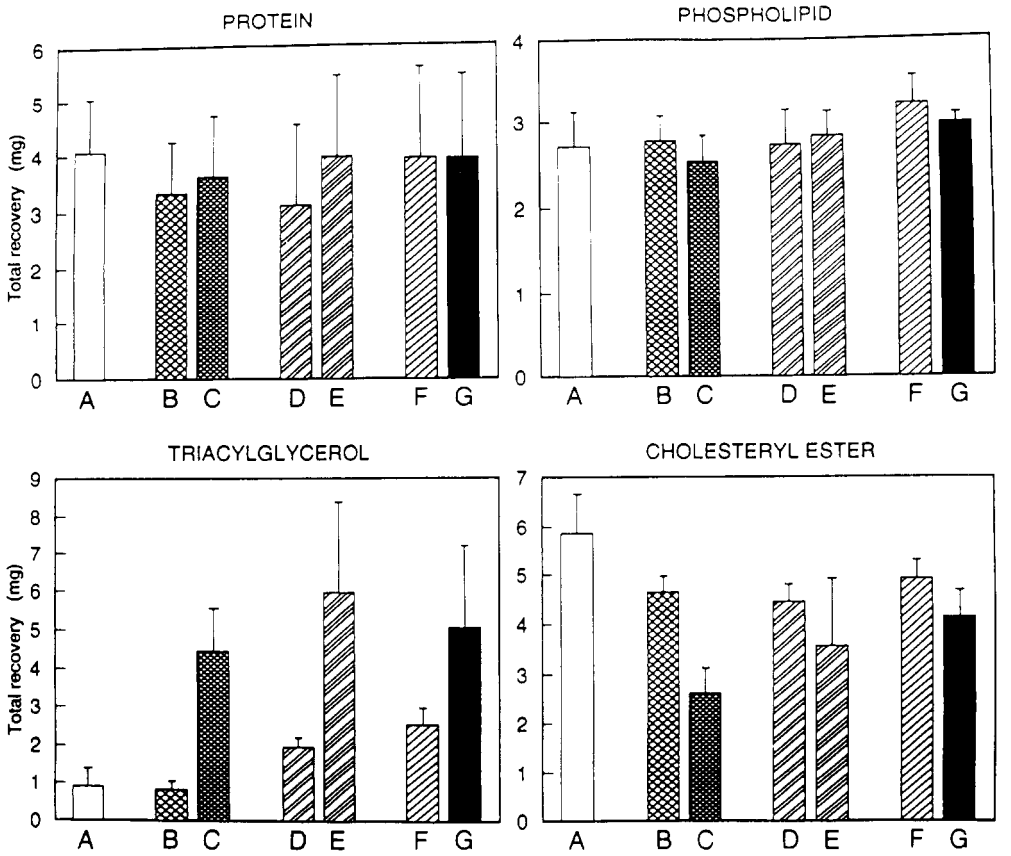

FIGURE 1: Recovery of LDL constituents in the LDL density fraction ($1.019 < d < 1.063$ g/mL) after incubation with the different lipid emulsions in the absence or presence of CETP activity. LDL (10.8 μmol of cholesteryl ester) was incubated with LCT, MCT, or MCT/LCT emulsions (50 mg of triacylglycerol) in the absence or presence of CETP activity contained in $d > 1.21$ g/mL lipoprotein-deficient plasma (240 mg of protein) for 20 h. The total recovery of each lipoprotein constituent in LDL was assayed as indicated for a control LDL incubation (A, $n = 4$), LDL + LCT without CETP (B, $n = 5$) and with CETP (C, $n = 5$); MCT/LCT without CETP (D, $n = 4$) and with CETP (E, $n = 4$), and MCT without CETP (F, $n = 3$) and with CETP (G, $n = 3$). Results are expressed as mean + SD. Statistical differences were analyzed using unpaired the Student's t test. For triacylglycerol the parameters were C vs A, $p < 0.005$; D vs A, $p < 0.005$; E vs A, $p < 0.001$; F vs A, $p < 0.01$; and G vs A, $p < 0.025$. For cholesteryl ester the parameters were B vs A, $p < 0.01$; C vs A, $p < 0.001$; E vs A, $p < 0.01$; F vs A, $p < 0.05$; and G vs A, $p < 0.01$.

Statistical Analysis. The results are expressed as mean values + SD. Statistical analysis was performed using Student's unpaired *t* test.

RESULTS

Incubations of LDL together with the different emulsions and CETP substantially modified LDL core neutral lipid composition with little change in surface constituents, i.e., protein, phospholipids, and free cholesterol (Figure 1). Although the relative LDL triacylglycerol content increased to a similar extent (30–36%) during incubation with all emulsions, the cholesteryl ester content decreased more markedly with LCT than with MCT, in the presence of CETP in lipoprotein-deficient plasma. These results suggest that while CETP does mediate transfers and exchanges of cholesteryl esters in the presence of both MCT and LCT, the type of triacylglycerols present also modifies the transfer process.

In the absence of CETP, LDL composition remained unchanged after incubation with LCT emulsion. In contrast, incubation with MCT more than doubled the relative and absolute triacylglycerol mass content of LDL, even in the absence of CETP, and some enrichment was also observed with the mixed MCT/LCT emulsion. However, LDL cholesteryl ester content was affected little, if at all, and this held true for all emulsions. This indicates that, while neither LCT nor cholesteryl ester transfers can occur in the absence of CETP, transfer of MCT can.

Qualitative and quantitative analyses of the emulsions incubated in the presence of LDL and CETP confirmed that cholesteryl esters derived from LDL were transferred into the emulsion particles (Table 1). The modest amount of cholesteryl esters found in emulsions after incubations in the absence of CETP likely resulted from contamination with LDL.[2] CETP-mediated cholesteryl ester transfer to emulsions was at least 2–3-fold greater with LCT compared to MCT emulsions.

To determine whether LCAT activity in $d > 1.21$ g/mL plasma influenced the above results, experiments were performed with lipoprotein-deficient plasma preheated at 56 °C for 30 min in order to inactivate LCAT activity. As previously reported (Yen et al., 1989), LCAT activity did not alter qualitative and quantitative changes associated with the neutral lipid transfer reaction in lipoprotein or emulsion

[2] When triacylglycerol-rich and LDL fractions are washed, neither cholesteryl esters or apoprotein B is transferred to either VLDL or triacylglycerol emulsions in the absence of CETP (Granot et al., 1982; Deckelbaum et al., 1986). As shown in Table 1, however, about 7–10% of the original cholesteryl esters in the incubation mixture was recovered in the $d < 1.019$ g/mL top (or emulsion) fraction after incubation with LDL in the absence of CETP. SDS–PAGE of the emulsion fraction after incubation in the absence of CETP revealed the presence of small amounts of apoprotein B (data not shown), suggesting that, after ultracentrifugation at a density of 1.019 g/mL, a modest LDL contamination of the emulsion fraction was responsible for the presence of small amounts of cholesteryl esters.

Table 1: Compositional Changes in Emulsions after Incubation with LDL[a]

	quantitative cholesteryl ester recovery (mg)	protein	triacylglycerol	free cholesterol	cholesteryl ester	phospholipid
LCT (2)	0	0	95.1	<0.2	0	4.9
LCT + LDL (5)	0.64 ± 0.16[b]	1.5 ± 0.6	90.0 ± 0.8***	1.9 ± 0.1	1.3 ± 0.2***	5.3 ± 0.2
LCT + LDL + CETP (5)[c]	2.86 ± 0.57	2.1 ± 0.5	83.1 ± 3.7*	2.0 ± 0.3	7.5 ± 3.2*	5.4 ± 0.7
MCT (2)	0	0	95.5	<0.2	0	4.5
MCT + LDL (3)	0.64 ± 0.25	1.4 ± 0.6	90.7 ± 1.5*	1.8 ± 0.2	1.6 ± 0.5***	4.6 ± 0.5
MCT + LDL + CETP (3)	0.98 ± 0.09	1.4 ± 0.7	88.3 ± 1.3**	1.9 ± 0.2	4.1 ± 2.1*	4.3 ± 0.3
MCT/LCT (2)	0	0	95.5	<0.2	0	4.5
MCT/LCT + LDL (4)	0.57 ± 0.18	1.2 ± 0.6	91.5 ± 1.3*	1.7 ± 0.2	1.2 ± 0.3**	4.4 ± 0.5
MCT/LCT + LDL + CETP (4)	2.17 ± 0.85	1.9 ± 0.5	81.0 ± 5.7*	2.7 ± 0.4	7.9 ± 5.1	6.5 ± 0.6

[a] In each experiment, LDL (11 μmol of cholesteryl ester), emulsion (50 mg of triacylglycerol), and CETP present in lipoprotein-poor, $d > 1.21$ g/mL plasma (240 mg of protein) were incubated for 19 h at 37 °C under N_2 in a total volume of 4 mL containing 0.15 M NaCl and 1 mM EDTA (pH 8), in the combinations indicated. The numbers in parentheses indicate the number of separate incubations for each condition. Student's unpaired t test was used to determine the statistical significance of differences between emulsion and emulsion incubated with LDL in the presence or absence of CETP: (*) $p < 0.050$; (**) $p < 0.010$; (***) $p < 0.001$). [b] Composition is expressed as relative weight composition (percentage of total emulsion mass). Results are stated as mean ± SD. [c] CETP as present in the $d > 1.21$ g/mL plasma.

constituents (data not shown). Thus changes observed were due to neutral lipid transfers, with no significant contribution from cholesteryl esters newly formed during incubation.

To better characterize exchange mechanisms between LDL and MCT or LCT emulsions, we studied neutral lipid transfer over varying incubation times at fixed emulsion to LDL ratios. Marked differences were observed between emulsions in their ability to remove cholesteryl esters from LDL over time (Figure 2A). Net transfer of cholesteryl esters was higher with LCT compared to MCT emulsions. Cholesteryl ester depletion from LDL was intermediate with the mixed MCT/LCT emulsion and did not occur in the absence of CETP (data not shown). Net transfer of triacylglycerols to LDL over time was 2-fold higher from MCT than from LCT emulsion and was again intermediate with the MCT/LCT emulsion (Figure 2B). Again, substantial triacylglycerol transfers occurred from MCT and MCT/LCT emulsions, but not from LCT emulsions, in the absence of CETP activity. Spontaneous MCT transfer was 1/3 to 1/2 that achieved when CETP was present. The higher transfer of MCT vs LCT into LDL therefore represents the sum of spontaneous plus CETP-mediated transfers. As shown in Figure 2C, CETP-mediated transfer of triacylglycerols was not substantially greater for MCT than for LCT, when spontaneous transfer is taken into account.

After incubating emulsions containing equal weight ratios of MCT or LCT to LDL as illustrated in Figure 2, we next evaluated net triacylglycerol transfer when LDL was incubated with equal numbers of MCT and LCT molecules. As shown in Figure 3, total triacylglycerol transfer to LDL was augmented with increases in the molar ratio of MCT or LCT to LDL. However, when spontaneous transfer was subtracted, CETP-mediated net transfer of MCT appeared to be slightly lower than that of LCT (after a 20-h incubation).

We evaluated triacylglycerol transfer to LDL by analyzing individual medium- and long-chain triacylglycerol fatty acids as markers for MCT and LCT (Figure 4). The shorter (0–4 h) time period allowed exploration of both CETP-mediated initial transfer of MCT and LCT to LDL and the potential contribution of triacylglycerol back transfer from LDL to emulsions (Figure 4). The mass ratios of MCT, LCT, and MCT/LCT emulsions to LDL were similar, so that MCT molecules were available for transfer in a 1.75-fold excess over LCT molecules [(M_r of LCT):(M_r of MCT) = 875:500 = 1.75]. Figure 4A compares the rates of CETP-mediated transfer of triacyglycerols from MCT and LCT emulsions to LDL over time. Note that the apparently higher transfer rate from MCT relative to that from LCT decreases after 1 h. Figure 4B also shows higher transfer of MCT over LCT from the MCT/LCT emulsion over the first hour. Figure 4C compares the ratios of MCT and LCT transfer to LDL obtained with pure MCT or LCT emulsion as shown in Figure 4A or the mixed MCT/LCT emulsion as shown in Figure 4B. The dashed line represents the ratio of 1.75 which would be expected between MCT and LCT if molar transfer rates promoted by CETP were equal for both triacylglycerols when offered in the same MCT or LCT over LDL mass ratio. The observed ratio was indeed close to this theoritical value at early time points (1 h or less), but later dropped below this value. Thus, despite a solubility of MCT 4-fold greater than that of LCT at the phospholipid surface (Deckelbaum et al., 1990), the ratio of CETP-mediated MCT to LCT transfer was only 2-fold greater over the first hour of incubation, and then it decreased. This suggests that, after 1 h, back transfer (spontaneous plus CETP-mediated) of triacylglycerols from LDL to emulsions is greater for MCT than LCT.

We examined the hypothesis that more MCT molecules than LCT molecules could accumulate in LDL at equilibrium because of (a) the smaller MCT molecular volume as compared to that of LCT and (b) the greater solubility of MCT at the LDL particle surface. Figure 5 compares the ability of MCT and LCT to replace cholesteryl esters lost from LDL during neutral lipid transfer in all experiments where CETP was present. At each level of cholesteryl ester depletion in LDL, about 2-fold more MCT molecules, as compared to LCT molecules, can be taken up by LDL (Figure 5). Indeed, the ratio of the regression slopes comparing LDL triacylglycerol enrichment from MCT and LCT to LDL cholesteryl ester loss is 2.16 (1.62:0.75 for MCT:LCT), a figure slightly higher than the ratio of their molecular volumes (evaluated from the M_r ratio as being 875:500, or 1.75). This is consistent with the greater solubility of MCT compared to LCT at the phospholipid surface–water interface (Deckelbaum et al., 1990). Thus, differences in triacylglycerol molecular volumes and higher surface solubility are likely to contribute to the larger net transfer of MCT to LDL, compared to that of LCT, at similar levels of cholesteryl ester depletion from the LDL particle core.

DISCUSSION

A number of factors modulate cholesteryl ester and triacylglycerol transfers between plasma lipoproteins. These include mass concentrations of CETP (Yen et al., 1989; Brown et al., 1989), concentration and relative mass ratios of lipoproteins (Deckelbaum et al., 1979, 1986; Morton & Zilversmit, 1983), concentration of neutral lipids at the

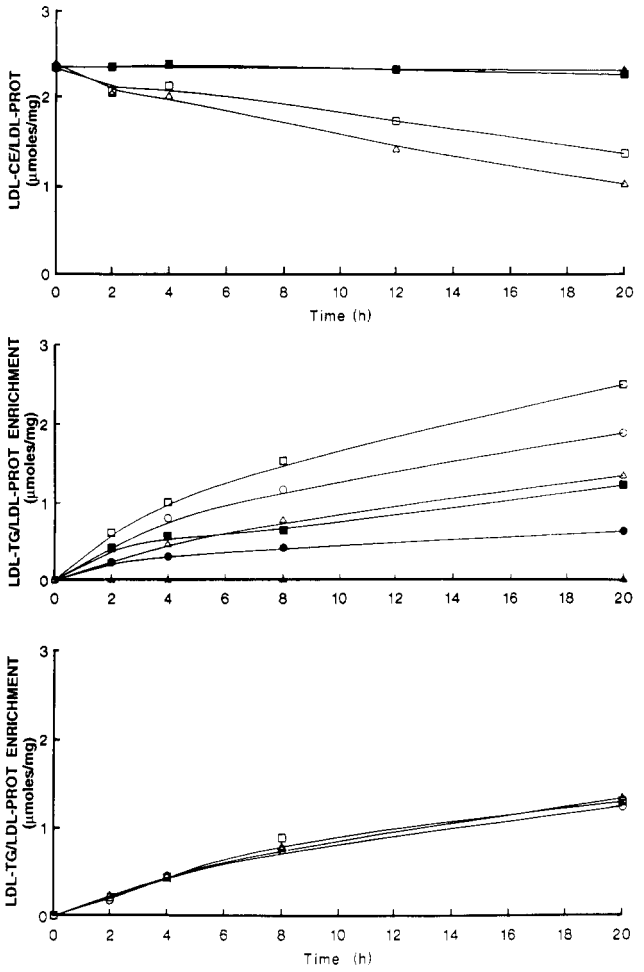

FIGURE 2: Transfer of cholesteryl esters and triacylglycerols between LDL and different lipid emulsions over time. LDL (6.2 μmol of cholesteryl ester) was incubated with LCT, MCT, or MCT/LCT emulsions (32 mg of triacylglycerol) in the presence or absence of CETP (240 mg of protein) over increasing time periods. To correct for variation in LDL recovery, neutral lipid content in LDL is expressed relative to the LDL–protein concentration. (A, top) Changes of LDL cholesteryl ester content with LCT and MCT, in the presence and absence of CETP activity. Symbols for incubation in the presence of CETP are as follows: LCT (△) and MCT (□). Symbols for incubation without CETP are as follows: LCT (▲) and MCT (■). (B, middle) Net transfer of triacylglycerol from emulsions to LDL in the presence and absence of CETP activity. Symbols for incubation in the presence of CETP are as follows: LCT (△), MCT (□), and MCT/LCT (○). Symbols for incubation without CETP are as follows: LCT (▲), MCT (■), and MCT/LCT (●). (C, bottom) CETP-mediated net transfer of triacylglycerol from emulsions to LDL. Results are calculated by subtracting triacylglycerol transfer from emulsions in the absence of CETP from total transfer measured in the presence of CETP: LCT (△), MCT (□), and MCT/LCT (○).

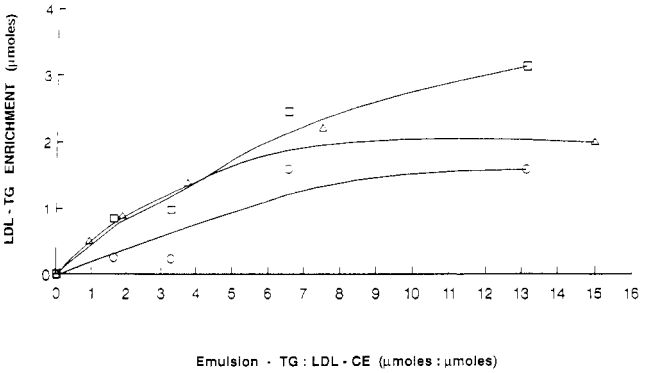

FIGURE 3: Transfer of triacylglycerol to LDL from LCT and MCT at different molar ratios of emulsion triacylglycerol to LDL cholesteryl ester. Emulsions were incubated with LDL (4 μmol of cholesteryl ester) for 20 h in the presence or absence of CETP (240 mg of protein). Total MCT transfer in the presence of CETP is shown by (□). Net CETP-mediated triacylglycerol transfer to LDL is shown for LCT + CETP (△) and MCT + CETP (○) after subtraction of triacylglycerol transfer in the absence of CETP. Since no spontaneous LCT transfer occurs in the absence of CETP, the data shown for LCT transfer represents CETP-mediated transfer.

phospholipid surface of substrate particles (Morton & Steinbrunner, 1990), presence or absence of free fatty acids (Tall et al., 1984; Barter, 1990), and fatty acid composition of cholesteryl esters (Morton, 1986). Relatively little is known about the potential for different triacylglycerols to modify neutral lipid transfer processes.

Our data emphasizes the potential for different species of triacylglycerols to affect triacylglycerol as well as cholesteryl ester transfers. We observed that the transfer of MCT from emulsion particles to human LDL was greater than that of LCT. At the same time, the presence of MCT decreased transfer of cholesteryl esters from LDL to acceptor emulsion particles. The higher level of MCT transfer to the LDL acceptor particles was not due to a higher rate of CETP-mediated mass transfer, but rather to a spontaneous MCT transfer to LDL. The higher surface solubility of MCT in the emulsion surface (Deckelbaum et al., 1990) along with its ability to partition significantly into water likely contributes to the spontaneous transfer of MCT to LDL. In addition, due to their smaller molecular volume (plus their greater solubility), more MCT molecules can be accepted by LDL as compared to LCT.

Differences in long-chain fatty acid composition affect transfer rates of different cholesteryl esters (Morton, 1986). It was therefore surprising to observe only small differences in CETP-mediated net transfer between MCT and LCT, triacylglycerol molecules with relatively large differences in both chain length and saturation. The cholesteryl esters tested by Morton were all long-chain and apolar with probably similar degrees of hydrophobicity and solubility in phospholipid surfaces. In our experiments, MCT had a predicted 4-fold greater availability at the phospholipid surface as compared to LCT (Deckelbaum et al., 1990) and in most incubations exceeded LCT in terms of molecular number by a factor of 1.75. Despite this higher concentration of "available" MCT molecules, the initial MCT transfer rate from emulsions was only 1.5–2.0-fold greater than that for LCT. Thus, although not directly measured, net CETP-mediated transfer rates are likely to be lower for MCT than for LCT. In several experiments, CETP was in contact with equal surface areas. To directly compare actual CETP-dependent transfer rates of MCT and LCT, experiments similar to those of Morton (Morton, 1986; Morton & Steinbrunner, 1990) would be required in which radiolabeled MCT and LCT are incorporated at equal concentrations into phospholipid liposomes.

If MCT affinity to CETP was similar to that of LCT, one would predict that the 4-fold higher surface solubility of MCT could promote a 4-fold higher rate of MCT transfer (Morton & Steinbrunner, 1990). This, however, was not observed. Possibly, the attachment of MCT to CETP was short-lived, with substantially higher losses of MCT from CETP in the water phase. Another possibility is that CETP may act by temporarily joining particles and allowing for direct "contact" transfer of neutral lipids and that the presence of MCT decreases the ability of CETP to approximate the particles. Since our experiments were not necessarily performed under first-order-kinetic conditions, we cannot clearly define the exact mechanism for differences in the CETP-mediated transfer rates recorded for MCT and LCT.

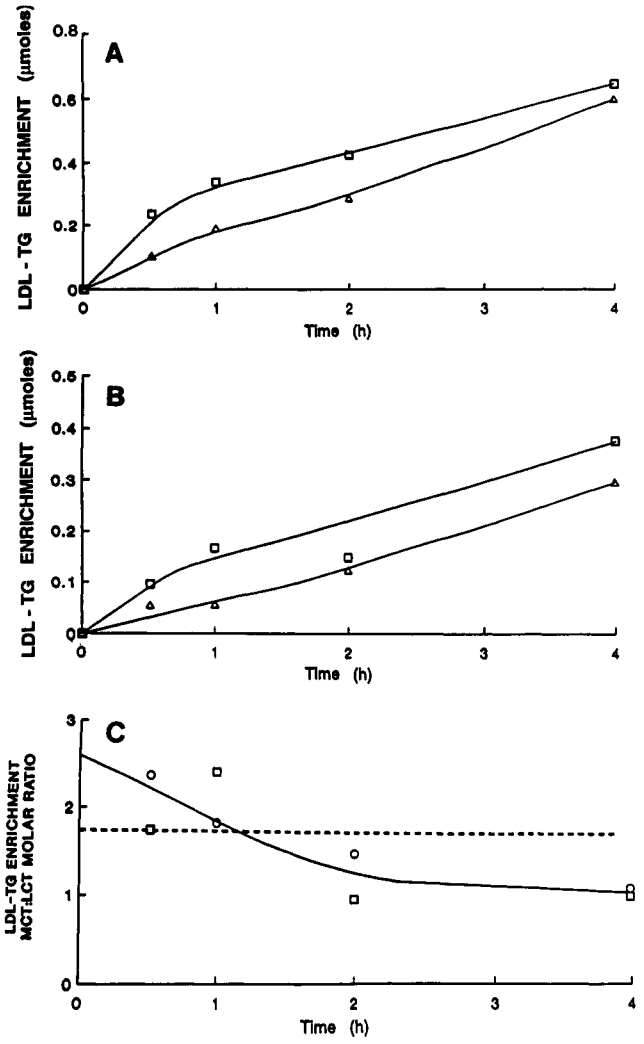

FIGURE 4: CETP-mediated transfer of MCT and LCT to LDL measured by net transfer of triacylglycerol fatty acids over time. LDL (5 μmol of cholesteryl ester) was incubated with three different emulsions, LCT, MCT, or MCT/LCT (25 mg of triacylglycerol), in the presence or absence of CETP (240 mg of protein) over increasing time periods. Transfer of ($C_{8:0}$ + $C_{10:0}$) and ($C_{18:1n-9}$ + $C_{18:2n-6}$) to LDL was measured for MCT and LCT emulsions. In panels A and B, total triacylglycerol transfer was calculated after subtraction of (i) the basal amounts of each of these triacylglycerol fatty acids present in LDL before incubation and (ii) the transfer in the absence of CETP, as described under Experimental Procedures. (A) CETP-mediated transfer to LDL of LCT (△) and MCT (□) from LCT and MCT emulsions, respectively. (B) MCT (□) and LCT (△) transfer to LDL from a blended MCT/LCT emulsion. (C) Ratios of CETP-mediated MCT to LCT enrichment of LDL from pure MCT and LCT emulsion (○) and a blended MCT/LCT emulsion (□). The dashed line represents the expected 1.75 molar ratio of MCT to LCT if transfer occurred solely on the basis of the relative number of MCT and LCT molecules present in the incubation mixture.

The decreased ability of MCT-containing particles (relative to LCT-containing particles) to act as acceptors for LDL cholesteryl esters is a matter of interest. The presence of MCT at the LDL particle surface could conceivably exclude cholesteryl esters. In fact, while we have recently shown that MCT does compete to decrease LCT surface solubility in phospholipid liposomes, such an effect of MCT on cholesteryl ester surface solubility did not occur (Hamilton et al., 1992). Therefore, the predicted 4-fold greater solubility of MCT as compared to LCT or cholesteryl esters (Deckelbaum et al., 1990) is likely to result in CETP having access to more MCT than cholesteryl esters at the LDL surface, inducing to-and-from shuttling of MCT between emulsions and LDL. This

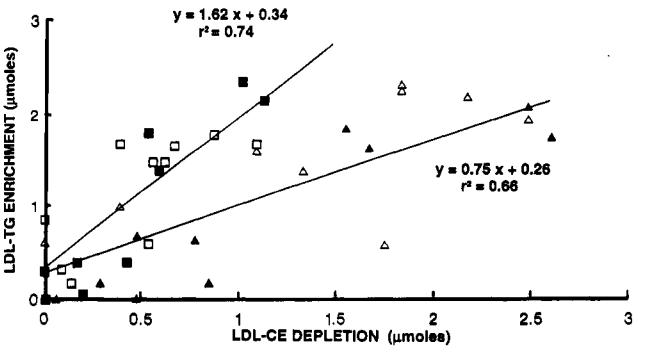

FIGURE 5: Relationship between CETP-mediated triacylglycerol enrichment in LDL and cholesteryl ester loss from LDL in the presence of MCT and LCT emulsions. Results are shown for a number of experiments where MCT emulsion or LCT emulsions were incubated with LDL over varying time periods (▲ and △ for LCT and ■, and □ for MCT) from 2 to 20 h at a fixed emulsion triacylglycerol to LDL cholesteryl ester weight ratio (7:1) with varying time (close symbols) or over a fixed time (20 h) with varying emulsion triacylglycerol to LDL cholesteryl ester weight ratios (open symbols). The equations for each regression line are as follows: for LCT, $y = 0.75x + 0.26$, $r^2 = 0.66$; for MCT, $y = 1.62x + 0.34$, $r^2 = 0.74$.

is supported by our observation of progressively lower net transfer of MCT (as compared to LCT) to LDL with increasing incubation time and suggests back transfer of MCT, from LDL to emulsion, to be substantially greater than that of LCT.

Our *in vitro* observations contribute to an understanding of previous *in vivo* data obtained during intravenous infusion of LCT and MCT/LCT emulsions into both normal human volunteers (Richelle, 1992) and patients requiring long-term nutritional support (Richelle et al., 1993). In subjects infused with different emulsions, more triacylglycerols transfer to both LDL and HDL and fewer cholesteryl esters accumulate in the triacylglycerol-rich particle fraction during MCT/LCT infusions than during LCT infusions (Carpentier et al., 1990). Thus, both *in vitro* and *in vivo* data raise the possibility that the presence of certain lipid molecules, e.g., MCT, may diminish the transfer of cholesteryl ester molecules from cholesteryl ester-rich particles, such as LDL and HDL, to triacylglycerol-rich particles, such as chylomicrons and VLDL, and provide one mechanism for decreasing the cholesteryl ester content in both chylomicron and VLDL remnants.

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