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Reversible flow of cholesteryl ester between high-density lipoproteins and triacylglycerol-rich particles is modulated by the fatty acid composition and concentration of triacylglycerols

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Reversible flow of cholesteryl ester between high-density lipoproteins and triacylglycerol-rich particles is modulated by the fatty acid composition and concentration of triacylglycerols

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Abstract

We determined the influence of fasting (FAST) and feeding (FED) on cholesteryl ester (CE) flow between high-density lipoproteins (HDL) and plasma apoB-lipoprotein and triacylglycerol (TG)-rich emulsions (EM) prepared with TG-fatty acids (FAs). TG-FAs of varying chain lengths and degrees of unsaturation were tested in the presence of a plasma fraction at $d > 1.21$ g/mL as the source of CE transfer protein. The transfer of CE from HDL to FED was greater than to FAST TG-rich acceptor lipoproteins, 18% and 14%, respectively. However, percent CE transfer from HDL to apoB-containing lipoproteins was similar for FED and FAST HDL. The CE transfer from HDL to EM depended on the EM TG-FA chain length. Furthermore, the chain length of the monounsaturated TG-containing EM showed a significant positive correlation of the CE transfer from HDL to EM ($r = 0.81$, $P < 0.0001$) and a negative correlation from EM to HDL ($r = -0.41$, $P = 0.0088$). Regarding the degree of EM TG-FAs unsaturation, among EMs containing C18, the CE transfer was lower from HDL to C18:2 compared to C18:1 and C18:3, 17.7%, 20.7%, and 20%, respectively. However, the CE transfer from EMs to HDL was higher to C18:2 than to C18:1 and C18:3, 83.7%, 51.2%, and 46.3%, respectively. Thus, the EM FA composition was found to be the rate-limiting factor regulating the transfer of CE from HDL. Consequently, the net transfer of CE between HDL and TG-rich particles depends on the specific arrangement of the TG acyl chains in the lipoprotein particle core.

Key words: Cholesteryl ester transfer protein; Fatty acid; Emulsion; Triacylglycerol; HDL; Lipoproteins

Introduction

Lipid transfer proteins mediate the exchange and net transfer of cholesteryl ester (CE), triacylglycerol (TG) and phospholipid molecules among plasma lipoproteins. The regulation of CE transfer protein (CETP) and its role in cardiovascular disease have been dealt with previously (1). The mechanisms of action of CETP are of interest because of its effect on the transfer between lipoproteins of other hydrophobic molecules (such as vitamin E) that harbor potentially anti-atherogenic effects ascribed to their antioxidant activity (2,3).

Although considerable *in vivo* and *in vitro* information

about the regulation of CETP activity by drugs (4) and diet (5-11) has been gathered from studies on humans (12-14), animal models (7-10,15) and *in vitro* models (16-24), little is known about the influence of dietary modifications on the chemical and physical processes whereby plasma CETP exchanges lipids between lipoproteins. *In vitro* studies have shown that the activity of CETP is modified by fatty acid species (12,16-18) and quantity (19), the surface concentration of CE (20) and unesterified cholesterol (21), as well as by the TG content (22) of the acceptor lipoprotein. Other lipoprotein modifications that influence CETP activity include

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alterations of the CETP sulfhydryl group (23), increased lipoprotein negative charge (24), glycosylation (25), and desialylation (26). However, because TG quantity, chain length (C11-C18) and degree of unsaturation vary, it is not known how fasting condition compared to being well-fed influences the rate of CE transfer between the TG-rich lipoproteins and the high-density lipoproteins (HDL) particles. This knowledge is critical to understand the regulation of the reverse cholesterol transport system whereby cholesterol from tissues is delivered to the liver as CE directly via HDL or indirectly through TG-rich lipoproteins.

Material and Methods

Material

[4-¹⁴C]-cholesteryl oleate ([¹⁴C]-CO, specific activity 45-60 mCi/mmol) was purchased from New England Nuclear (USA). The purity of the isotope and lipids was checked by silica gel thin layer chromatography and was shown to be higher than 98%. Cholesterol, cholesteryl oleate, triundecenoic (C11:1), triundecanoic (C11:0), trilaurin (C12:0), tridodecenoic (C12:1), tripalmitin (C16:0), tripalmitolein (C16:1), and triolein (C18:1) were obtained from Nu-Check Prep (USA), and trimyristin (C14:0), trimyristolein (C14:1), trilinolein (C18:2), and trilinolenin (C18:3) were from Sigma (USA). Phosphatidylcholine (egg yolk lecithin) was purchased from Lipid Products (UK). All other chemicals were of analytical grade or equivalent.

Enzyme kits for the determination of TG were purchased from E. Merck (Germany) and kits for the determination of free cholesterol and total cholesterol were from Boehringer-Mannheim (Germany).

Preparation of triacylglycerol-rich emulsions

Emulsions were prepared with cholesterol (2%), cholesteryl oleate (6%), phosphatidylcholine (23%), and TG (69%) of various fatty acid chain lengths and extents of unsaturation (C11:0, C12:0, C14:0, C16:0, C11:1, C12:1, C14:1, C16:1, C18:1, C18:2, and C18:3) dissolved in chloroform/methanol (2:1). The solvent of the lipid mixture with and without [¹⁴C]-CO was evaporated under a nitrogen stream. Using a Branson Cell Disruptor 450 (Branson Sonifier, USA), the lipid mixture was then emulsified in a NaCl solution (d = 1.101 g/mL, pH 7.4) with a 1-cm probe at 70-80 W for 30 min under a nitrogen flow. Ultracentrifugation was used to isolate the triacylglycerol-rich particles after a discontinuous gradient, as previously described (27).

All emulsions presented nearly identical levels of TG (80%), cholesteryl oleate (4%), unesterified cholesterol (1%), and lecithin (15%). The incorporation of TG into each emulsion was checked by gas-liquid chromatography (CG Analítica, Brazil). We chose an emulsion composition with good physical stability and with the absence of visual evidence of crystallization or phase separation after standing overnight at room temperature; namely, only one phase

was observed. Stabilization was achieved with emulsions containing triolein (34.5 mg%) together with another TG (34.5 mg%) belonging to the series C11:0 to C16:0 or C11:1 to C16:1. Emulsions without triolein but containing only one unsaturated TG species (69 mg%) were prepared with C11:1 to C18:1 or C18:2 and C18:3.

Isolation and labeling of lipoproteins

The study was approved by the Ethics Committee of Faculdade de Medicina, Universidade de São Paulo. Blood from 5 normolipidemic healthy male donors (mean age (±SD) 29 ± 8 years), nonsmokers, and taking no medications, was drawn either after fasting (FAST) or 4 h after an *ad libitum* meal (postprandial period, FED) on EDTA (1 mg/mL) and immediately centrifuged at 1000 g, 4°C for 15 min for plasma separation. HDL (d = 1.063-1.21 g/mL) and lipoprotein-deficient serum (d > 1.21 g/mL) were isolated by preparative sequential ultracentrifugation at 100,000 g, 4°C, in the 50 Ti rotor of an L-8 Beckman ultracentrifuge (Beckman Instruments, USA) (28) and dialyzed at 4°C against phosphate-buffered saline with 0.01% EDTA, pH 7.4. HDL was labeled with [¹⁴C]-CO according to Gavish et al. (29), with slight modifications, using 10 μCi (0.37 Mbq). The labeling efficiency was about 30-35% and more than 95% of the radioactivity was present as [¹⁴C]-CO.

Total cholesterol, free cholesterol, and TG were determined enzymatically with commercial kits. Esterified cholesterol was estimated as the difference between the total and free cholesterol values. Phospholipids were determined by Bartlett's method (30) and protein was measured by the method of Lowry et al. (31) using bovine serum albumin (BSA) as the standard.

Assay of CE transfer activity

Lipoprotein transfer assays (32) were carried out in triplicate on samples obtained from each of the 5 donors because the observed interquartile coefficient of variation was below 10%, representing low dispersion. Transfer assays were carried out with FAST or FED HDL [¹⁴C]-CO from each donor with autologous FAST or FED total plasma (1 mL) in the presence of 5,5'-dithio-bis-(2)-nitrobenzoic acid (DTNB) at 37°C for 4 h. After incubation, the mixtures were adjusted to d = 1.21 g/mL with saline (1.8 mL of d = 1.33 g/mL) plus saline (0.6 mL of d = 1.21 g/mL) for a final volume of 3.5 mL. Lipoprotein separation was carried out by discontinuous density gradient ultracentrifugation (33). Radioactivity was measured in aliquots of each lipoprotein isolated, namely, chylomicron (CM), very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and HDL.

Transfer assays of a pool of total HDL to the emulsion or from the emulsion to HDL were carried out using emulsions (500 μg TAG) prepared with fatty acids of variable chain length (C11-C18) and degree of unsaturation in the presence of HDL (120 μg total cholesterol) labeled with

[¹⁴C]-CO obtained from a pool of normolipidemic plasma donors together with plasma ($d > 1.21$ g/mL, 1 mL) as the CETP source in the presence of an inhibitor of the lecithin-cholesterol acyltransferase enzyme (0.5 μ g/mL DTNB) at 37°C for 4 h. The same incubation mixture was used as a control reaction at 4°C. After incubation, the mixtures were adjusted to $d = 1.21$ g/mL. Samples were then immediately transferred to ultracentrifuge tubes, and the volume was completed to 7 mL with NaCl ($d = 1.101$ g/mL) and overlaid with 4 mL saline, $d = 1.006$ g/mL. This gradient was ultracentrifuged at 190,000 g in an SW41 Ti rotor at 4°C for 30 min (28). The top phase (3 mL) containing the emulsion and the infranate HDL fraction (8 mL) was vacuum aspirated and radioactivity was measured. The percentage of radioactivity transferred from HDL to the emulsion or from the emulsion to HDL was calculated as the ratio between radioactivity present in the emulsion fraction and total radioactivity, and was corrected using the control reaction assay. The melting points of the TGs were kindly provided by Professor Donald M. Small (Department of Physiology and Biophysics, Boston University School of Medicine, Boston, MA, USA).

Analysis of emulsion droplet size

Electron microscopy was performed after cryofracture with a small droplet of fresh emulsion (of different composition) frozen by rapid immersion in the liquid phase of nitrogen slush (-210°C). Freeze-fracture was carried out at -100°C at 2×10^{-6} torr in a Blazers 301 freeze-etch apparatus. The replicas were placed on a gold/iridium disc and examined with a Joel 1010 model transmission electron microscope operating at 80 kV. The images were obtained with 8.1 x 10 cm Kodak negatives from a high-resolution cathode ray tube. The droplet size of each emulsion sample was calculated as the average of three measurements with at least 100 dispersed particles (34).

Statistical analysis

Data are reported as medians and percentiles (25-75). The Wilcoxon matched pair test was used for statistical comparison of two groups. The Kruskal-Wallis test with the

Dunn contrast post test was used for statistical comparison of the various groups. Correlations between [¹⁴C]-CO percent transfer and TG-fatty acid chain length were determined by the Spearman correlation test. A 0.05 confidence level was considered to be significant.

Results

The percentage of CETP-mediated transfer of [¹⁴C]-CO HDL was greater with TG-rich acceptor lipoproteins obtained during the postprandial period (FED) than with those obtained in the fasting period (FAST; Table 1). However, when the percent transfer values were related to the plasma TG concentration during both periods, this difference was no longer observed (data not shown). Thus, the rate-limiting step of CE transfer depends on the amount of TG, which is known to increase in VLDL and in CM during the feeding phase (33). In contrast, the percent transfer from HDL to apoB-containing lipoproteins (CM+VLDL and IDL+LDL) was similar for HDL obtained during the FED and the FAST periods (Table 1).

We also examined the role of the acceptor lipoprotein TG composition when the fatty acid chain length and degree of unsaturation were modified. For this purpose, artificial emulsions mimicking TG-rich particles were used. These emulsions were prepared with TGs consisting of a series of pure unsaturated fatty acids with variable chain lengths and also a series of variable species of saturated (SAT) fatty acids admixed with one species of monounsaturated (MONO) fatty acid (C18:1). The latter addition was necessary in order to maintain the stability of the emulsion containing SAT fatty acids. In all these experiments, the percent transfer was reported with respect to the mass of the emulsion TG because the molar contents of the TG emulsions used could have influenced the transfer values. Nonetheless, data based on the percent values (not shown) were similar to those when the latter was corrected for the molarity of TG.

We found that the percent transfer of [¹⁴C]-CO from HDL to TG-rich emulsions depended to some extent on the chain length of the emulsion TGs. The percent [¹⁴C]-

Table 1. Transfer of cholesteryl oleate ([¹⁴C]-CO) from HDL to chylomicrons (CM) plus VLDL and to IDL plus LDL using lipoproteins obtained during the fasting (FAST) or the postprandial period (FED) of the donors.

	FAST			FED		
	CM+VLDL	IDL+LDL	HDL	CM+VLDL	IDL+LDL	HDL
[¹⁴ C]-CO HDL-FAST	14.0 (11.2-19.7)	36.0 (31.0-40.0)	47.5 (40.7-59.2)	18.0 (18.0-32.0)*	37.0 (32.0-40.0)	40.0 (30.0-49.0)
[¹⁴ C]-CO HDL-FED	16.0 (11.2-20.5)	34.5 (27.7-41.2)	44.5 (40.0-60.5)	22.0 (20.0-32.2)*	36.0 (30.0-39.2)	39.6 (29.0-50.0)

Data are reported as median (percent) and percentiles (25-75) for N = 14-15. HDL = high-density lipoprotein; VLDL = very low-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein. *.*P < 0.05 for CM+VLDL (FED) compared to CM+VLDL (FAST) (Wilcoxon matched pairs test).

CO transfer to 11- and 12-carbon chain emulsion TG was lower than the transfer to the C14 and C16 emulsions prepared with 50% SAT fatty acids and also lower than the transfer to the 16 carbon chain of the MONO fatty acid species (Table 2). The data obtained here did not permit a comparison between pairs of SAT x MONO fatty acids because the experiments with this series were not carried out simultaneously.

In addition, the [^{14}C]-CO-HDL transfer rate to the emulsion did not correlate with the TG saturated fatty acid chain length (Figure 1A), but was positively correlated with the chain length of the 50% MONO emulsions (Figure 1B). This was confirmed in experiments that used emulsions consisting of pure MONO fatty acids (Table 3 and Figure 2A). However, the reverse flow of [^{14}C]-CO from emulsion to HDL was inversely correlated with the length of the MONO fatty acids (Table 3 and Figure 2B). These results agree with a previous study showing that the transfer rate depended on the esterified cholesterol acyl chain length (35).

Regarding the degree of unsaturation of the TG-fatty acids, we observed that when the emulsion contained C18:2 the transfer rate of [^{14}C]-CO from HDL to the emulsion was lower and the percent transfer from the emulsion to HDL was

higher than with C18:1 or C18:3 (Table 4).

Because it has been shown that the CE transfer activity is related to LDL particle diameter (9), we investigated whether modifications in the emulsion TG-fatty acid chain length might influence the physical state of the emulsion by examining the particle size and the TG-fatty acid melting point. In the emulsion series consisting of TG with 100% MONO fatty acids, the particle diameter did not alter the CE transfer, and did not correlate with the CE transfer rate from HDL (data not shown). However, there was a positive cor-

Table 2. Transfer of cholesteryl oleate ([^{14}C]-CO) from HDL to lipid emulsions (EMs) containing 50% of various saturated (SAT) or monounsaturated (MONO) TG-fatty acids.

[^{14}C]-CO transfer from HDL to EM (%/ μmol TG)			
SAT-EM [34.5 mg% SAT (C11:0-C16:0) + 34.5 mg% (C18:1) TG-fatty acid]		MONO-EM [34.5 mg% MONO (C11:1-C16:1) + 34.5 mg% (C18:1) TG-fatty acid]	
C11:0	13.1 (11.4-13.7)	C11:1	10.7 (9.1-13.1)
C12:0	12.2 (10.8-15.4)	C12:1	10.6 (9.9-12.6)
C14:0	17.2 (15.6-18.6)*	C14:1	10.9 (8.9-13.3)
C16:0	16.4 (14.5-18.3) [†]	C16:1	20.4 (17.3-22.2) [‡]

Data are reported as median (percent) and percentiles (25-75) for N = 12-23. HDL = high-density lipoprotein; TG = triacylglycerol. SAT-EM (P < 0.05): *C14:0 compared to C11:0 or C12:0; [†]C16:0 compared to C11:0 or C12:0. MONO-EM (P < 0.05): [‡]C16:1 compared to C11:1, C12:1 or C14:1 (Kruskal-Wallis test with the Dunn contrast post test).

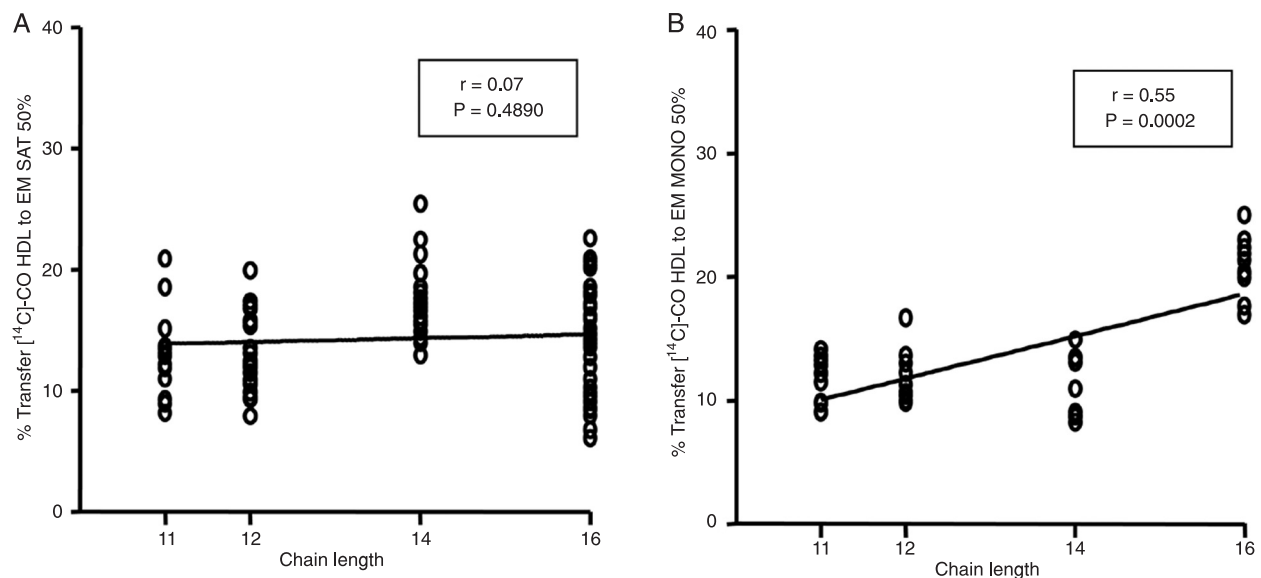


Figure 1. Transfer of cholesteryl oleate ([^{14}C]-CO) from HDL to lipid emulsions (EM) does not correlate with the saturated TG-fatty acid chain length. A, $r = 0.07$; $P = 0.4890$; $N = 9$, but is positively correlated with the chain length of emulsions containing monounsaturated TG-fatty acid. B, $r = 0.55$; $P = 0.0002$; $N = 40$, by Spearman correlation test. TG = triacylglycerol; HDL = high-density lipoprotein; MONO = monounsaturated fatty acids; SAT = saturated fatty acids.

relation between the particle diameter of the emulsion and the TG-fatty acid melting point (Figures 3 and 4). Therefore, the fatty acid composition of the emulsion rather than the TG melting point was the rate-limiting step that regulated the transfer rate of CE between HDL and the TG-rich particles mediated by plasma lipid transfer protein.

Table 3. Transfer of cholesteryl oleate ($[^{14}\text{C}]$ -CO) between HDL and lipid emulsions (EMs) containing 100% of various mono-unsaturated TG-fatty acids.

$[^{14}\text{C}]$ -CO transfer (%/ μmol TG)			
HDL to EM [69 mg% MONO (C11:1-C18:1) TG-fatty acid]		EM to HDL [69 mg% MONO (C11:1-C18:1) TG-fatty acid]	
C11:1	8.9 (8.1-9.1)	C11:1	86.2 (84.3-86.9)
C12:1	9.4 (8.8-10.0)	C12:1	85.2 (83.8-89.6)
C14:1	10.7 (8.2-14.0)	C14:1	86.3 (82.8-90.7)
C16:1	17.5 (16.1-20.2)*	C16:1	87.9 (84.7-91.4)
C18:1	20.7 (17.3-25.0)†	C18:1	51.2 (45.4-57.7)‡

Data are reported as median (percent) and percentiles (25-75) for N = 8-21. TG = triacylglycerol; HDL = high-density lipoprotein; MONO = monounsaturated fatty acids. HDL to EM (P < 0.05): *C16:1 compared to C11:1, C12:1 or C14:1; †C18:1 compared to C11:1, C12:1 or C14:1. EM to HDL (P < 0.05): ‡C18:1 compared to C11:1, C12:1, C14:1 or C16:1 (Kruskal-Wallis test with the Dunn contrast post test).

Discussion

Several factors influence the rate of CE transfer between HDL and TG-rich particles, including the medium fatty acid content (16-18,27), lipoprotein charge (24), dietary fatty acid composition (9-12), the lipoprotein CE content (20), and *in*

Table 4. Transfer of cholesteryl oleate ($[^{14}\text{C}]$ -CO) between HDL and lipid emulsions (EMs) containing 100% of various TG-unsaturated fatty acids of 18 chains.

$[^{14}\text{C}]$ -CO transfer (%/ μmol TG)			
HDL to EM [69 mg% (C18:1-C18:3) G-fatty acid]		EM to HDL [69 mg% (C18:1-C18:3) TG-fatty acid]	
C18:1	20.7 (17.3-25.0)	C18:1	51.2 (45.4-57.7)
C18:2	17.7 (15.8-19.3)*	C18:2	83.7 (79.0-88.7)†
C18:3	20.0 (16.5-25.3)	C18:3	46.3 (43.0-50.8)

Data are reported as median (percent) and percentiles (25-75) for N = 8-21. TG = triacylglycerol; HDL = high-density lipoprotein. HDL to EM (P < 0.05): *C18:2 compared to C18:1 or C18:3; EM to HDL (P < 0.05): †C18:2 compared to C18:1 or C18:3 (Kruskal-Wallis test with the Dunn contrast post test).

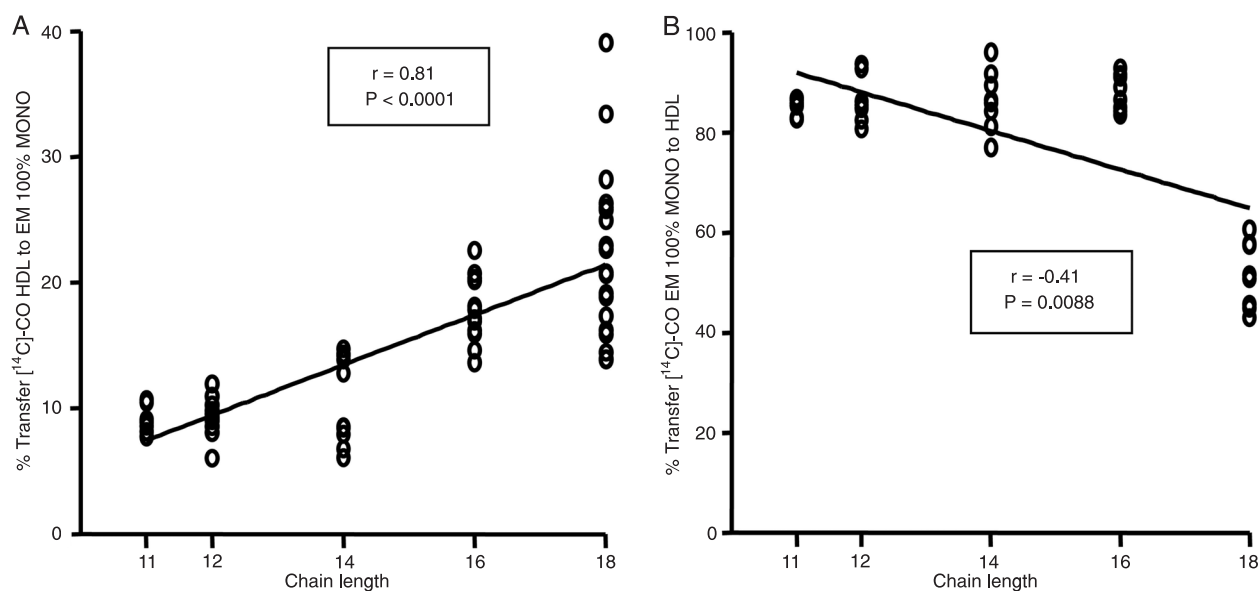


Figure 2. Transfer of cholesteryl oleate ($[^{14}\text{C}]$ -CO) from HDL to the emulsion (A) and from the emulsion to HDL (B). The percent transfer from HDL to the emulsion showed a highly significant positive correlation of the triacylglycerol fatty acid chain length. A, $r = 0.81$, $P < 0.0001$; $N = 69$, and inversely from emulsion to HDL. B, $r = -0.41$; $P = 0.0088$; $N = 40$ (Spearman correlation test). HDL = high-density lipoprotein; EM = lipid emulsions; MONO = monounsaturated fatty acids.

vivo lipoprotein chemical modifications (25,26). Furthermore, the expression of human CETP in mice increases postprandial plasma TG levels by delaying its clearance (15). These results imply that the transfer rate is regulated by a combination of the amount of CETP available and the quantity and quality of plasma TG-rich lipoproteins. In this regard, the dependence of HDL-CE transfer on the acceptor MONO TG chain length shown here may represent a mechanism for reducing plasma HDL cholesterol that, to some extent, might counterbalance the dietary MONO fatty acid-elicited rise of plasma HDL cholesterol that occurs when the plasma CETP concentration is reduced (36).

Previous studies had shown that fatty acids influence the CE transfer rate between HDL and the TG-rich lipoproteins (16-18,27). However, in these investigations free fatty acids were added to the *in vitro* incubations. Nonetheless, because *in vivo* fatty acids are inherent to the lipoprotein particles as TG moieties, we investigated the effect of the TG-rich particles that varied according to their fatty acid composition.

The data show that the fatty acid composition of the emulsion was the rate-limiting factor regulating the transfer of CE from HDL because the CETP-mediated net transfer of CE from HDL particles correlates positively with the TG-rich particle content as well as with its fatty acid chain length and, to a lesser extent, with the degree of fatty acid unsaturation. Consequently, the net transfer of CE between HDL and

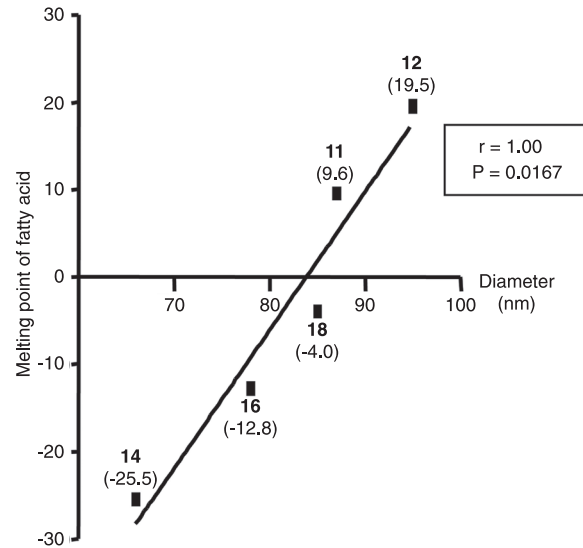


Figure 3. Diameter of triacylglycerol (TG)-rich emulsion particles containing one monounsaturated fatty acid as a function of chain lengths (bold numbers inside the figure) and respective TG melting points ($^{\circ}\text{C}$ in parentheses). Results are from one representative experiment (three similar experiments were performed). A significant positive correlation between TG melting point and emulsion particle size is observed ($r = 1.00$; $P = 0.0167$; $N = 5$; Spearman correlation test). Melting point data provided by Donald M. Small (Boston, MA, USA).

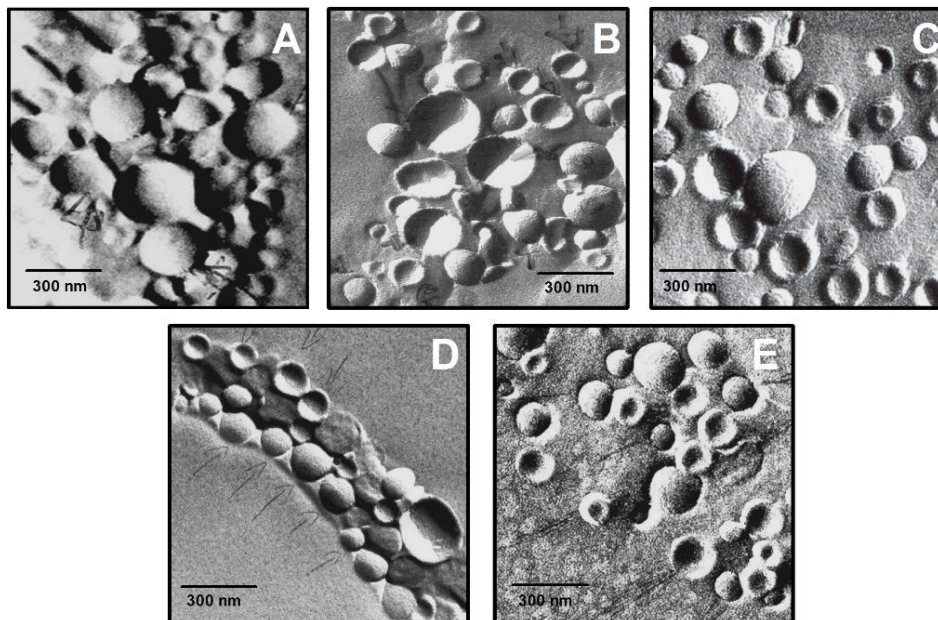


Figure 4. Electron micrograph of emulsions each containing 100% monounsaturated triacylglycerol-fatty acids. The replicas were examined with a Joel 1010 model transmission electron microscope operating at 80 kV. The images were obtained with 8.1 x 10 cm of Kodak negatives from a high-resolution cathode ray tube. The droplet size of each emulsion was calculated as the average of the three measurements. Data are reported as means \pm SD in nm. A, C11:1 = 87 ± 26 ; B, C12:1 = 95 ± 22 ; C, C14:1 = 66 ± 18 ; D, C16:1 = 78 ± 34 , and E, C18:1 = 85 ± 29 .

TG-rich particles depends on the specific arrangement of the TG acyl chains in the lipoprotein particle core.

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References

- Schaefer EJ, Asztalos BF. Increasing high-density lipoprotein cholesterol, inhibition of cholesteryl ester transfer protein, and heart disease risk reduction. *Am J Cardiol* 2007; 100: n25-n31.
- Hacquebard M, Vandenbranden M, Malaisse WJ, Ruyschaert JM, Deckelbaum RJ, Carpentier YA. Vitamin E transfer from lipid emulsions to plasma lipoproteins: mediation by multiple mechanisms. *Lipids* 2008; 43: 663-671.
- Kwong M, Wasan KM. Cholesteryl ester transfer protein facilitates the movement of water-insoluble drugs between lipoproteins: a novel biological function for a well-characterized lipid transfer protein. *Biochem Pharmacol* 2002; 64: 1669-1675.
- Kontush A, Guerin M, Chapman MJ. Spotlight on HDL-raising therapies: insights from the torcetrapib trials. *Nat Clin Pract Cardiovasc Med* 2008; 5: 329-336.
- Li TY, Zhang C, Asselbergs FW, Qi L, Rimm E, Hunter DJ, et al. Interaction between dietary fat intake and the cholesterol ester transfer protein TaqIB polymorphism in relation to HDL-cholesterol concentrations among US diabetic men. *Am J Clin Nutr* 2007; 86: 1524-1529.
- Cheema SK, Agarwal-Mawal A, Murray CM, Tucker S. Lack of stimulation of cholesteryl ester transfer protein by cholesterol in the presence of a high-fat diet. *J Lipid Res* 2005; 46: 2356-2366.
- Dorfman SE, Wang S, Vega-Lopez S, Jauhiainen M, Lichtenstein AH. Dietary fatty acids and cholesterol differentially modulate HDL cholesterol metabolism in Golden-Syrian hamsters. *J Nutr* 2005; 135: 492-498.
- de Silva PP, Agarwal-Mawal A, Davis PJ, Cheema SK. The levels of plasma low density lipoprotein are independent of cholesterol ester transfer protein in fish-oil fed F1B hamsters. *Nutr Metab* 2005; 2: 8.
- Fusegawa Y, Kelley KL, Sawyer JK, Shah RN, Rudel LL. Influence of dietary fatty acid composition on the relationship between CETP activity and plasma lipoproteins in monkeys. *J Lipid Res* 2001; 42: 1849-1857.
- Khosla P, Hajri T, Pronczuk A, Hayes KC. Replacing dietary palmitic acid with elaidic acid (t-C18:1 delta9) depresses HDL and increases CETP activity in cebus monkeys. *J Nutr* 1997; 127: 531S-536S.
- Hirano R, Igarashi O, Kondo K, Itakura H, Matsumoto A. Regulation by long-chain fatty acids of the expression of cholesteryl ester transfer protein in HepG2 cells. *Lipids* 2001; 36: 401-406.
- Richelle M, Carpentier YA, Deckelbaum RJ. Long- and medium-chain triacylglycerols in neutral lipid-exchange processes with human plasma low-density lipoproteins. *Biochemistry* 1994; 33: 4872-4878.
- Guerin M, Egger P, Soudant C, Le Goff W, van Tol A, Dupuis R, et al. Cholesteryl ester flux from HDL to VLDL-1 is preferentially enhanced in type IIB hyperlipidemia in the postprandial state. *J Lipid Res* 2002; 43: 1652-1660.
- Paniagua JA, de la Sacristana AG, Sanchez E, Romero I, Vidal-Puig A, Berral FJ, et al. A MUFA-rich diet improves postprandial glucose, lipid and GLP-1 responses in insulin-resistant subjects. *J Am Coll Nutr* 2007; 26: 434-444.
- Salerno AG, Patricio PR, Berti JA, Oliveira HC. Cholesteryl ester transfer protein (CETP) increases postprandial triglyceridaemia and delays triacylglycerol plasma clearance in transgenic mice. *Biochem J* 2009; 419: 629-634.
- Morton RE, Greene DJ. The capacity of various non-esterified fatty acids to suppress lipid transfer inhibitor protein activity is related to their perturbation of the lipoprotein surface. *Biochim Biophys Acta* 2000; 1486: 275-284.
- Lagrost L. Differential effects of cis and trans fatty acid isomers, oleic and elaidic acids, on the cholesteryl ester transfer protein activity. *Biochim Biophys Acta* 1992; 1124: 159-162.
- Lagrost L, Florentin E, Guyard-Dangremont V, Athias A, Gandjini H, Lallemand C, et al. Evidence for nonesterified fatty acids as modulators of neutral lipid transfers in normolipidemic human plasma. *Arterioscler Thromb Vasc Biol* 1995; 15: 1388-1396.
- Sammatt D, Tall AR. Mechanisms of enhancement of cholesteryl ester transfer protein activity by lipolysis. *J Biol Chem* 1985; 260: 6687-6697.
- Morton RE, Greene DJ. The surface cholesteryl ester content of donor and acceptor particles regulates CETP: a liposome-based approach to assess the substrate properties of lipoproteins. *J Lipid Res* 2003; 44: 1364-1372.
- Rajaram OV, Chan RY, Sawyer WH. Effect of unesterified cholesterol on the activity of cholesteryl ester transfer protein. *Biochem J* 1994; 304 (Part 2): 423-430.
- Lee DM, Alaupovic P, Knight-Gibson C, Bagdade JD. Apolipoprotein-B subclasses as acceptors of cholesteryl esters transferred by CETP. *Eur J Clin Invest* 2008; 38: 734-742.
- Epps DE, Vosters AF. The essential role of a free sulfhydryl group in blocking the cholesteryl site of cholesteryl ester transfer protein (CETP). *Chem Phys Lipids* 2002; 114: 113-122.
- Morton RE, Greene DJ. CETP and lipid transfer inhibitor protein are uniquely affected by the negative charge density of the lipid and protein domains of LDL. *J Lipid Res* 2003; 44: 2287-2296.
- Lemkadem B, Loiseau D, Larcher G, Malthiery Y, Foussard F. Effect of the nonenzymatic glycosylation of high density lipoprotein-3 on the cholesterol ester transfer protein activity.

- Lipids* 1999; 34: 1281-1286.
26. Harada LM, Carvalho MD, Passarelli M, Quintao EC. Lipo-protein desialylation simultaneously enhances the cell cholesterol uptake and impairs the reverse cholesterol transport system: *in vitro* evidences utilizing neuraminidase-treated lipoproteins and mouse peritoneal macrophages. *Atherosclerosis* 1998; 139: 65-75.
 27. Oliveira HC, Quintao EC. 'In vitro' cholesteryl ester bi-directional flow between high-density lipoproteins and triglyceride-rich emulsions: effects of particle concentration and composition, cholesteryl ester transfer activity and oleic acid. *J Biochem Biophys Methods* 1996; 32: 45-57.
 28. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955; 34: 1345-1353.
 29. Gavish D, Oschry Y, Eisenberg S. *In vivo* conversion of human HDL3 to HDL2 and apoE-rich HDL1 in the rat: effects of lipid transfer protein. *J Lipid Res* 1987; 28: 257-267.
 30. Bartlett GR. Colorimetric assay methods for free and phosphorylated glyceric acids. *J Biol Chem* 1959; 234: 469-471.
 31. Lowry OH, Rosebrough NJ, Farr AL, Ramdall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265-275.
 32. Guerin M, Dolphin PJ, Chapman MJ. A new *in vitro* method for the simultaneous evaluation of cholesteryl ester exchange and mass transfer between HDL and apoB-containing lipoprotein subspecies. Identification of preferential cholesteryl ester acceptors in human plasma. *Arterioscler Thromb* 1994; 14: 199-206.
 33. Redgrave TG, Roberts DC, West CE. Separation of plasma lipoproteins by density-gradient ultracentrifugation. *Anal Biochem* 1975; 65: 42-49.
 34. Tannuri U, Sesso A, Coelho MC, Maksoud JG. Long-term stability of lipid emulsions with parenteral nutrition solutions. *Nutrition* 1992; 8: 98-100.
 35. Green SR, Pittman RC. Comparative acyl specificities for transfer and selective uptake of high density lipoprotein cholesteryl esters. *J Lipid Res* 1991; 32: 457-467.
 36. Jansen S, Lopez-Miranda J, Castro P, Lopez-Segura F, Marin C, Ordovas JM, et al. Low-fat and high-monounsaturated fatty acid diets decrease plasma cholesterol ester transfer protein concentrations in young, healthy, normolipemic men. *Am J Clin Nutr* 2000; 72: 36-41.