Postprandial chylomicrons: potent vehicles for transporting cholesterol from endogenous LDL+HDL and cell membranes to the liver via LCAT and CETP

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Abstract **We examined whether postprandial (PP) chylomicrons (CMs) can serve as vehicles for transporting cholesterol from endogenous cholesterol-rich lipoprotein (LDL+HDL) fractions and cell membranes to the liver via lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) activities. During incubation of fresh fasting and PP plasma containing [^{3}H]cholesteryl ester (CE)-labeled LDL+HDL, both CMs and VLDL served as acceptors of [^{3}H]CE or cholesterol from LDL+HDL. The presence of CMs in PP plasma suppressed the ability of VLDL to accept [^{3}H]CE from LDL+HDL. In reconstituted plasma containing an equivalent amount of triglycerides from isolated VLDL or CMs, a CM particle was about 40 times more potent than a VLDL particle in accepting [^{3}H]CE or cholesterol from LDL+HDLs. When incubated with red blood cells (RBCs) as a source for cell membrane cholesterol, the cholesterol content of CMs, VLDL, LDL, and HDL in PP plasma increased by 485%, 74%, 13%, and 30%, respectively, via LCAT and CETP activities. The presence of CMs in plasma suppressed the ability of endogenous lipoproteins to accept cholesterol from RBCs. Our data suggest that PP CMs may play an important role in promoting reverse cholesterol transport in vivo by serving as the preferred ultimate vehicle for transporting cholesterol released from cell membranes to the liver via LCAT and CETP.**—Chung, B-H., P. Liang, S. Doran, B. H. S. Cho, and F. Franklin. **Postprandial chylomicrons: potent vehicles for transporting cholesterol from endogenous LDL+HDL and cell membranes to the liver via LCAT and CETP.** ***J. Lipid Res.*** **2004.** 45: **1242–1255.**

Supplementary key words postprandial lipemia • cholesteryl ester transfer protein • lecithin:cholesterol acyltransferase • cholesterol-rich lipoproteins

Reverse cholesterol transport (RCT), a process of transporting cholesterol from cell membranes to the liver for its excretion, occurs in the plasma compartment in vivo. Both lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer proteins (CETPs) may play an important role in regulating the rate of RCT by influencing the rate at which cholesterol released from cell membranes into plasma is trapped in the core of HDL via esterification and the rate at which the trapped HDL-cholesteryl ester (CE) is then transferred to apolipoprotein B (apoB)-containing lipoproteins for delivery to the liver (1–3). CETP promotes the transfer of CE from HDL to various apoB-containing lipoproteins [LDL, VLDL, IDL, and chylomicrons (CMs)] in plasma (3–9), but the catabolic rate and fate of these various apoB-containing lipoproteins differ in vivo (10). Thus, the rate of RCT promoted by LCAT and CETP might then depend on the rate and extent of transfer of HDL-CE to various apoB-containing lipoproteins in plasma in vivo. Although the extent of in vivo transfer of CE from HDL to various apoB-containing lipoproteins is known to be influenced by the levels of individual apoB-containing lipoproteins in plasma (11), the rate and potencies of the various apoB-containing lipoproteins to accept CE from HDL in vivo have not yet been fully elucidated. An earlier study (12) examining the fate of [^{3}H]CE-labeled HDL injected into fasting humans showed that ^{3}H-labeled CE on HDL appeared more rapidly on VLDL than on LDL. In rabbits, ^{3}H-labeled CE on

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Abbreviations: apoB, apolipoprotein B; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CM, chylomicron; CVD, cardiovascular disease; GCRC, General Clinical Research Center; LDL+HDL, cholesterol-rich lipoprotein; PP, postprandial; RBC, red blood cell; RCT, reverse cholesterol transport; TG, triglyceride; TRL, triglyceride-rich lipoprotein; UC, unesterified cholesterol.

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HDL injected intravenously appeared on VLDL and LDL, with peak activity seen between 30 and 60 min and between 60 and 120 min after injection, respectively (11). Goldberg, Beltz, and Pittman (13) reported that in rabbits, 30% of ^{3}H-labeled CE on HDL was cleared from plasma after transfer to VLDL, with 36% cleared after transfer to LDL, although VLDL levels are much lower than those of LDL in rabbit plasma.

In humans, a large amount of dietary fat (>80 g) fluxes daily into circulating blood as CMs during postprandial (PP) lipemic periods (14). Although CMs may serve as acceptors of endogenous CEs from HDL and LDL as well as CEs derived from cell membranes via LCAT, the potential role of CMs in transporting these CEs to the liver has not yet been fully evaluated. In PP plasma obtained from normolipidemic subjects, 72%, 19%, 6%, and 2% of [^{3}H]CE on HDL transferred to LDL, VLDL, CMs, and IDL, respectively (8). CETP in plasma promotes the transfer of CE from LDL to the triglyceride (TG)-rich lipoprotein (TRL) fractions (4). It has also been reported that CEs that were first transferred from HDL to LDL were then transferred secondarily to CMs during PP phases (9). Induction of PP lipemia accompanies an increase in the plasma activities of both LCAT and CETP (15–17) and a net decrease in CE levels on both LDL and HDL, as well as in plasma total CE levels (18), and a shift in the distribution of CEs from endogenous lipoproteins to CMs (19). These observations suggest that CMs appearing in PP plasma may serve as acceptors of CEs from both LDL and HDL. Eisenberg (20) reported that the potency of apoB-containing lipoproteins as acceptors of CEs from HDL increased with the increase in their particle sizes, surface areas, or TG-to-CE ratios. Because CM particles are larger and have a higher TG-to-CE ratio and surface area than any other apoB-containing lipoproteins in plasma, CMs may serve as the preferred acceptors of CEs from HDL via CETP. We observed recently that PP lipemia-mediated increases in plasma CETP activity were primarily due not to the change in the CETP protein mass but to the increased levels of CMs available to serve as acceptors of CEs (21). Castro and Fielding (16) were first to report that human plasma containing PP lipoproteins was better able to promote cholesterol efflux from cultured cells by increasing the extent of LCAT and CETP reactions.

We have reported previously that PP appearance of CMs in plasma increased the potencies of plasma TRL to accept cholesterol released from red blood cells (RBCs) (22). Inasmuch as it is known that the clearance of PP CMs from circulating blood is mediated primarily by hepatic uptake (23), the LCAT- and CETP-mediated acceptance of cholesterol released from cell membranes by PP CMs could possibly lead to an increase in the rate of RCT in vivo. To examine the potential of CMs as vehicles for promoting RCT, we determined the effect of the PP appearance of CMs on *1*) the alteration in the balance of cholesterol between TRL and endogenous cholesterol-rich LDL and HDL fractions in vivo, *2*) the extent of the LCAT- and CETP-mediated transfer of cholesterol from endogenous cholesterol-rich lipoproteins (LDL+HDLs) to TRL in vitro, and *3*) the potencies of endogenous lipoproteins as well as CMs to accept additional cholesterol released from RBC membranes via LCAT and CETP.

EXPERIMENTAL PROCEDURES

Human subjects and collection of fasting and PP blood

Healthy normolipidemic adult men and postmenopausal women were recruited. Interested volunteers underwent a screening examination at the General Clinical Research Center (GCRC), University of Alabama at Birmingham Medical School. Examination included a documentation of a brief medical history, physical examination, and measurement of body weight, height, and fasting plasma lipid and lipoprotein cholesterol levels. Subjects with a fasting TG level above the 75th percentile, plasma cholesterol above the 90th percentile, or HDL-cholesterol below the 10th percentile for their respective age groups were excluded from the study. Eight men aged 33–49 years (35.3 ± 4.5) and eight postmenopausal women aged 45–62 years (51.9 ± 6.6) participated in this study. The mean body mass indexes of men and women were 25.3 ± 4.1 and 29.6 ± 4.5, respectively. Fasting and PP plasma samples used in this study were obtained from subjects after consumption for 16 days of a normal diet rich in polyunsaturated fatty acids, which provided 15%, 50%, and 35% of its calories from proteins, carbohydrates, and fat, respectively and contained 175 mg cholesterol/1,000 kcal. Saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids in the diet provided 7.5%, 12%, and 15.5% of total calories, respectively. All daily meals, prepared by the GCRC Research Kitchen, were provided to the study subjects.

Briefly, on the evening before the fat-loading test, study subjects were admitted to the GCRC and were provided dinner. After fasting overnight at the GCRC, study subjects were given a challenge breakfast. To maximize the PP lipemic response, the challenge meal contained a slightly higher level of fat (40% calories from fat) than the background diet (35% calories from fat) but with the same fatty acid composition. The challenge meal was whole foods, providing 50% of the total daily caloric intake. Fasting and PP blood samples were collected from study subjects immediately prior to the meal (40 ml) and 4 h after the meal (80 ml). The research protocol for using human subjects was approved by the Institutional Review Board at the University of Alabama at Birmingham.

Treatment of blood and plasma samples

Blood samples were collected in tubes containing ethylenediaminetetraacetic acid (0.1%) and immediately placed in an ice bath. After blood samples were spun at 1,000 rpm for 10 min in a precooled (4°C) centrifuge, about two-thirds of the plasma in the tube separated from RBCs, with the remaining one-third becoming trapped within the packed RBCs. After removal of the upper plasma fraction, plasma trapped within the packed RBCs and a small portion of separated plasma were placed in a 37°C water bath for 3–16 h to allow lipoproteins in the plasma to interact with endogenous LCAT, CETP, and/or RBCs. The remaining plasma was kept in an ice bath. After overnight incubation, plasma trapped within the packed RBCs was recovered by centrifuging blood at 3,500 rpm for 25 min. A portion of each plasma sample was stored in several aliquots at −70°C.

Determination of plasma lipoprotein cholesterol and TG profiles and TRL apoB-100 and apoB-48 levels

The plasma lipoprotein cholesterol and TG profiles showing the level and distribution of cholesterol or TG among VLDL,

LDL, and HDL density fractions in fresh and incubated fasting plasma and 4 h PP plasma were determined by the modified lipoprotein cholesterol autoprofiler method developed in this laboratory (24). This method involves: *1*) short (150 min), single-spin, density-gradient ultracentrifugal separation of the major lipoprotein fractions in plasma in a swing-out rotor (Beckman SW 50.1); *2*) continuous-flow online mixing of effluents from density gradient tubes with enzymatic cholesterol and TG reagents; *3*) online incubation of mixtures and online measurement of absorbance produced; and *4*) calculation of lipoprotein cholesterol and TG levels by a computer program. To determine the levels of cholesterol associated with CMs in 4 h PP plasma, CM-free PP plasma was prepared by removing intact CMs in 4 h PP plasma after ultracentrifugation of PP plasma at 30,000 rpm for 30 min in a swing-out rotor (Beckman SW 50.1). After determination of lipoprotein cholesterol profiles of PP plasma and CM-free PP plasma, the levels of cholesterol associated with intact large CMs were then determined by subtracting cholesterol levels of the VLDL density fraction in CM-free PP plasma from those in PP plasma. Following quantitative separation of the VLDL density fraction (0.6 ml) from fasting, 4 h, and CM-free 4 h plasma (4 ml), PP changes in levels of TRL apoB-100 and apoB-48 were determined. TRL apoB-100 and apoB-48 levels were determined using a modified method of the SDS gel electrophoresis method described by Kotite, Bergeron, and Havel (25). Briefly, isolated fasting VLDL solution containing 10 μg of TG and an equivalent volume of the VLDL density fraction separated from 4 h PP plasma and CM-free 4 h plasma were loaded onto a 4–20% SDS gradient gel. Molecular weight standards and apoB-100 standard, prepared from LDL, were also loaded onto the same SDS gel. After electrophoretic separation of apoB-100 and apoB-48 on TRL, gels were stained with gel code blue stain (Pierce Co., Rockford, IL), and then the protein mass of apoB-100 and apoB-48 bands in the gel were quantified based on the apoB-100 standard by using the UVP gel imaging system (Quest Scientific Co., Cumming, GA). PP increases in the number of VLDL, CMs, and CM remnant particles were determined following conversion of the TRL apoB-100 and apoB-48 masses (μg) into molar concentration using molecular weights (MWs) of 555,486 for apoB-100 and 260,416 for apoB-48.

CETP-mediated redistribution among lipoprotein fractions of [^{3}H]CEs on LDL or HDL and cholesterol mass in reconstituted plasma after their incubation

The CETP-mediated redistribution of [^{3}H]CE on HDL and LDL as well as the cholesterol mass among lipoprotein fractions was determined following incubation of reconstituted PP plasma containing trace amounts of [^{3}H]CE-labeled LDL and/or [^{3}H]CE-labeled HDL at 37°C for 16 h. We radiolabeled LDL and HDL in TRL-free fasting or 4 h PP plasma with [^{3}H]CE using the procedure described by Thomas and Rudel (26). The distribution of [^{3}H]CE between LDL and HDL in the TRL-free plasma was observed to be very similar to the distribution of their cholesterol mass. After isolating [^{3}H]CE-labeled LDL and HDL in d>1.006 g/ml plasma fraction by density gradient ultracentrifugation (24), a trace amount of each was added to reconstituted plasma containing isolated VLDL, CMs, TRL, or no TRL. After incubating the [^{3}H]CE-labeled LDL and/or HDL alone or after their addition into reconstituted plasma at 37°C for 3–16 h, the distribution of [^{3}H]CE radioactivity among VLDL, LDL, and HDL density fractions or transfer of [^{3}H]CE from LDL+HDL to the VLDL density fraction was measured after separating VLDL, LDL, and HDL density fractions by density gradient ultracentrifugation (24). Changes in the distribution of cholesterol mass among VLDL, LDL, and HDL density fractions of reconstituted plasma after their incubation at 37°C for 16 h were also measured by obtaining the plasma lipoprotein cholesterol profile using the procedure described above (24).

Potencies of isolated fasting VLDL and PP CMs in reconstituted plasma to accept [^{3}H]CE and cholesterol mass from LDL+HDL via CETP

The potency of VLDL, isolated from fasting plasma, and CMs, isolated from 4 h PP plasma, to act as acceptors of [^{3}H]CE or cholesterol mass from LDL+HDL was examined following incubation of reconstituted plasma containing isolated VLDL or CMs. The reconstituted plasma was prepared by adding equivalent amounts of TG from isolated VLDL or CMs to a common fresh TRL-free plasma containing a trace amount of [^{3}H]CE-labeled LDL+HDL. During the incubation of the above reconstituted plasma at 37°C, the levels of [^{3}H]CE radioactivity transferred from LDL+HDL into the VLDL density fraction and/or the increase in cholesterol content of the VLDL density fraction were measured by the procedures described above. To compare the relative potencies of particles of VLDL and CM for accepting CE from LDL+HDL, the number of TG molecules per particle of VLDL and CM was determined by quantifying apoB-100 or apoB-48 levels on isolated fasting VLDL or 4 h PP CMs by the SDS gradient gel method described above. Briefly, isolated VLDL containing 10 μg of TG and CMs containing 150 μg of TG were loaded onto a 4–20% SDS gradient gel, and apoB-100 and/or apoB-48 on VLDL and CMs were separated from other apolipoproteins. Then, masses of apoB-100 and/or apoB-48 associated with 10 μg of VLDL TG or 150 μg of CM TG were determined based on an apoB-100 standard on the gel. The number of TG molecules per particle of VLDL and CM was then determined by calculating the molar ratio of TG to VLDL apoB-100 or to CM apoB-48 based on the mass ratio. The potency per particle of CM and VLDL to accept CE or cholesterol mass from LDL+HDL was determined based on the potencies of VLDL and CMs containing equivalent TG to accept CE from LDL+HDL and on the number of TG molecules per particle of VLDL and CM. In other experiments, the rate and extent of transfer of ^{3}H-labeled CE from LDL+HDL to VLDL and/or CMs in reconstituted plasma, fasting plasma, PP plasma and/or CM-free plasma were also determined by the procedures described above.

The effect of PP lipemia on the partitioning of CE formed from ^{3}H-labeled unesterified cholesterol by the endogenous LCAT among lipoprotein fractions

To determine the effect of PP lipemia on the extent of esterification of unesterified cholesterol (UC) by endogenous LCAT in plasma and on the extent of partitioning of LCAT-produced CE into TRL, fasting and PP plasma were labeled with [^{3}H]UC by the procedure previously described by Yen and Nishida (27). After incubation of [^{3}H]UC-labeled fasting and PP plasma for 16 h, VLDL, LDL, and HDL density fractions of incubated plasma were fractionated quantitatively by density-gradient ultracentrifugation at 4°C (24). Following the measurement of the total [^{3}H]radioactivity associated with VLDL, LDL, and HDL fractions, lipids were extracted from a portion of plasma and VLDL, LDL, and HDL fractions by using chloroform:methanol (2:1, v/v). UC and CE in the lipid extracts were separated on a silica gel thin-layer plate using chloroform:hexane (3:1, v/v) as a developing solvent. The ratio of [^{3}H]CE to [^{3}H]UC was determined by measuring the ^{3}H radioactivity associated with the CE and UC bands. The distribution of ^{3}H-labeled CE, formed in plasma by LCAT, among VLDL, LDL, and HDL density fractions was then calculated.

Other methods

The levels of total cholesterol, UC, and TG in plasma or in the lipoprotein fraction were measured by using enzymatic assay kits of cholesterol, UC, and TG purchased from Waco Diagnostic Co. (Richmond, VA).

Statistical analysis

Quantitative variables were expressed as mean ± SD. Student *t*-tests were applied to compare the level of lipoproteins or lipids in fasting and PP plasma and control and LCAT- and CETP-reacted plasma.

RESULTS

PP lipemia-mediated alterations in plasma lipoprotein cholesterol and TG profiles, levels of lipids and lipoprotein cholesterol, and the composition and levels of VLDL apoB

Lipoprotein TG profiles and cholesterol profiles of fasting and 4 h plasma from a subject with a brisk PP lipemic response, presented in **Fig. 1A, B**, show the PP lipemia-induced changes in level and distribution of TG and cho-

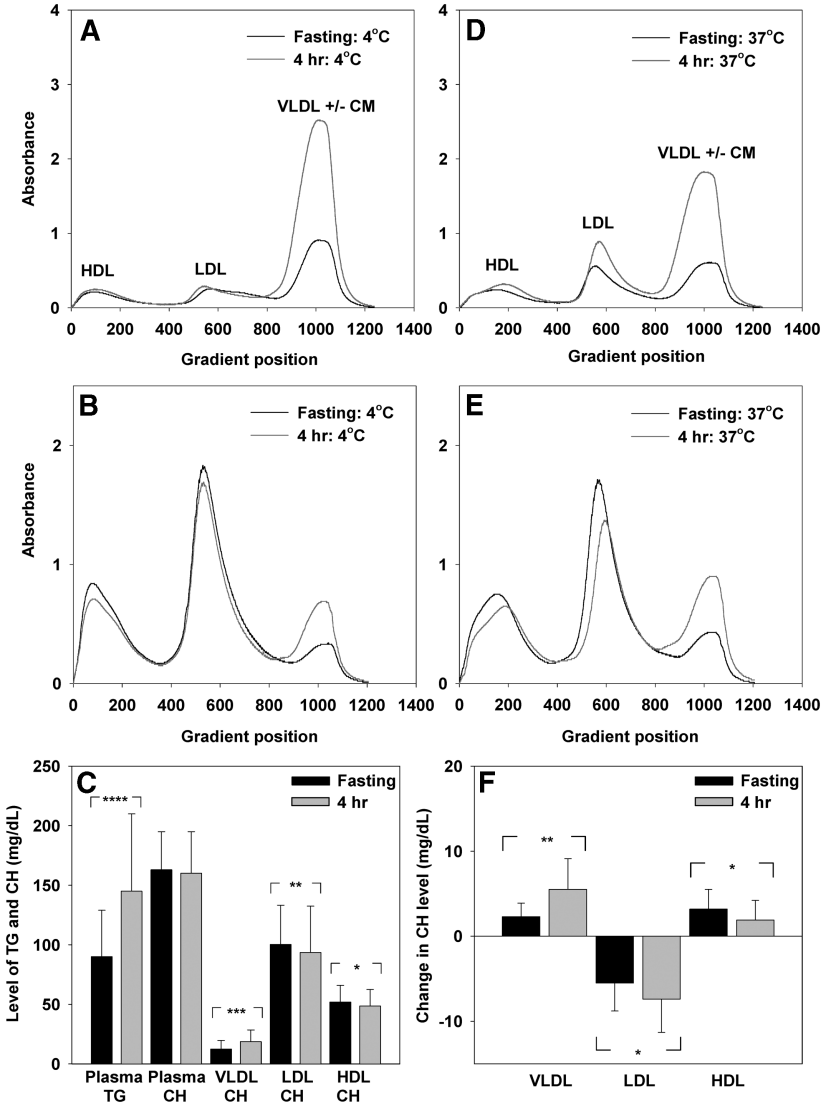

Fig. 1. Alteration in lipoprotein cholesterol and triglyceride (TG) profiles and levels of lipid and lipoprotein cholesterol levels of fasting plasma after the induction of postprandial (PP) lipemia (A–C) and of fasting and PP plasma after in vitro reaction of lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) (D–F). Plasma lipoprotein TG profiles (A) and cholesterol profiles (B) of fresh fasting and 4 h PP plasma obtained from a subject with a brisk PP lipemic response, and mean levels of plasma lipid and lipoprotein cholesterol in fasting and PP plasma obtained from all study subjects (C). Changes in lipoprotein TG profiles (D) and lipoprotein cholesterol profiles (E) of the above fresh fasting and PP plasma after in vitro reaction of endogenous LCAT and CETP (incubation at 37°C for 16 h) and the mean level changes of lipoprotein cholesterol in fasting and PP plasma (F) after in vitro reaction of endogenous LCAT and CETP. Plasma lipoprotein TG and cholesterol profiles showing the relative distribution of TG or cholesterol level among density gradient fractions was measured by the automated (lipoprotein autoprofiler) method as described in Experimental Procedures. Values are mean ± SD (n = 16). Significantly different from fasting plasma value at $*P < 0.05$, $**P < 0.01$, $***P < 0.005$, and $****P < 0.001$.

lesterol among lipoproteins in fasting plasma. The PP lipemia-mediated changes in mean levels of lipids and lipoprotein cholesterol of fasting plasma from all study subjects are also shown (Fig. 1C). The PP lipemia caused a significant increase in plasma TG levels, owing primarily to an increase in the levels of TG associated with the VLDL density fraction (Fig. 1A, C). PP lipemia, although causing no significant change in plasma total cholesterol, did cause a significant increase in the cholesterol level in the VLDL density fraction and a concomitant small but significant decrease in cholesterol levels on both LDL and HDL (Fig. 1A, C). The above data indicate that PP lipemia may shift the distribution of cholesterol from LDL and HDL to PP TRL. Although the data were not included, we observed that decreased cholesterol levels on LDL and HDL in 4 h PP plasma returned to near fasting level at 7 h PP with almost complete clearance of PP TRL from plasma. **Figure 2** shows the SDS gel electrophoregrams of VLDL density fraction isolated from fasting plasma and 4 h PP plasma obtained from a subject with a brisk PP lipemic response (Fig. 2A) and PP change in TRL apoB-100 and apoB-48 of all study subjects (Fig. 2C). To determine the levels of apoB-48 associated with CMs and CM remnants in 4 h plasma, intact CMs were removed centrifugally from 4 h PP plasma, and the levels of apoB-48 and apoB-100 associated with TRL isolated CM-free 4 h PP plasma were also determined. In densitometric scans of gels (Fig. 2A, C), apoB-48 was detectable in fasting plasma VLDL. Determination of particle numbers based on the molar concentration of apoB-48 and apoB-100 in fasting VLDL obtained from all study subjects indicates that the number of apoB-48-containing CM remnant particles in fasting plasma was about 6.1% of apoB-100-containing VLDL particles (Fig. 2C). PP lipemia increased both apoB-100- and apoB-48-containing TRL particles in fasting plasma (Fig. 2). The extent of this increase was much greater for apoB-48-containing particles (170%) than for apoB-100-containing particles (44.8%). However, the total numbers of apoB-100-containing particles resulting from PP increase (Δ 44.8 particles of PP VLDL per 100 fasting VLDL particles) was much greater (4.2-fold) than that of apoB-48-containing particles (Δ 10.4 particles of PP CM per 100 particles of fasting VLDL) (Fig. 2C). Centrifugal removal of CMs from 4 h PP plasma only minimally lowered the TRL apoB-48 level or TRL apoB-48-to-apoB-100 ratio (Fig. 2A–C) despite ~72% decrease of plasma TG level, which had increased in 4 h PP plasma (data not shown). The differences in mean molar concentrations of apoB-48 associated with TRL isolated from 4 h PP plasma and CM-free 4 h PP plasma (Fig. 2C) indicate that the

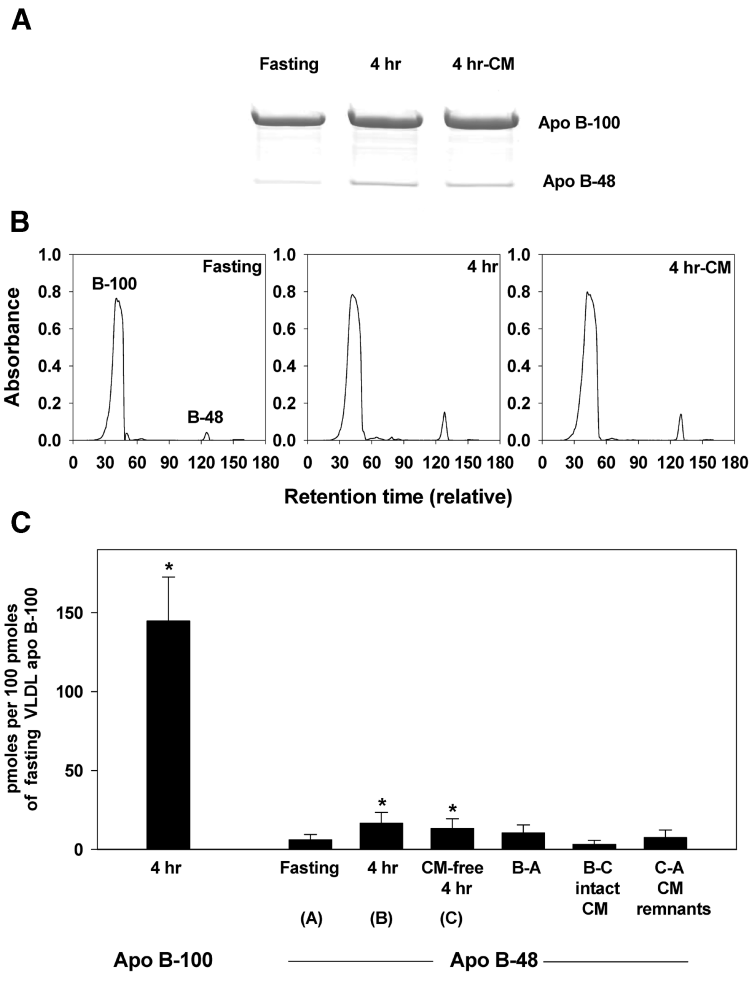

Fig. 2. PP changes in composition and levels of apoB associated with fasting VLDL. Sodium dodecyl sulfate gradient gels (A) and densitometric scans of the gel (B) showing the separation and levels of apoB-100 and apoB-48 in VLDL density fraction isolated quantitatively from fasting plasma, 4 h PP plasma, and chylomicron (CM)-free 4 h PP plasma (4 h −CM). The 4 h −CM plasma was obtained following centrifugal removal of intact CMs from 4 h PP plasma (30,000 rpm for 30 min in a Beckman SW 55 swing-out rotor). C: Mean levels ± SD (pmol) of apoB-100 on 4 h PP triglyceride-rich lipoprotein (TRL) and apoB-48 on the VLDL density fraction of fasting, 4 h PP, and CM-free 4 h PP TRL relative to that of 100 pmol of fasting VLDL apoB-100. Following determination of the masses of apoB-100 and apoB-48 in the same volume of VLDL density fraction separated quantitatively from 4 ml fasting, 4 h PP, and CM-free 4 h PP plasma by SDS gels, the levels of apoB-100 and apoB-48 in PP TRL or apoB-48 in fasting plasma relative to that of fasting VLDL apoB-100 were calculated. Molecular weights of 555,486 for apoB-100 and 260,416 for apoB-48 were used to convert their masses into molar concentration. The net PP increases in levels of apoB-100 and apoB-48 associated with TRL in 4 h PP plasma were calculated by subtracting the apoB-100 and apoB-48 levels in fasting VLDL from those in 4 h PP TRL, and the levels of apoB-48 associated with CMs and CM remnants were calculated by subtracting the levels of apoB-48 in CM-free 4 h TRL from those in 4 h TRL. *Significantly different from fasting value, $P < 0.05$.

numbers of intact CM particles were only ~20% of total apoB-48-containing TRL particles in 4 h PP plasma or 31.7% of apoB-48-containing particles resulting from PP increase. The above data indicate that the major portion (80%) of apoB-48 in 4 h plasma was associated with CM remnants. The data shown in Fig. 2C further show that the number of intact CM particles was ~2% of the total apoB-100- and apoB-48-containing TRL particles in 4 h PP plasma.

LCAT- and CETP-mediated changes in lipoprotein TG and cholesterol profiles and cholesterol content of lipoproteins in fasting and PP plasma in vitro

Because PP lipemia is known to transiently enhance activities of LCAT and CETP (15–17), we examined whether the PP lipemia-mediated shift in distribution of cholesterol from LDL and/or HDL to TRL was due to enhanced LCAT- and CETP-mediated redistribution of cholesterol among lipoproteins. Changes in lipoprotein TG and cholesterol profiles of fasting and PP plasma following in vitro incubation at 37°C revealed that the LCAT and CETP reactions in plasma shifted the distribution of cholesterol from LDL to TRL and the distribution of TG from TRL to LDL and HDL in both fasting and PP plasma (Fig. 1A, B, D, E). The above shifts in the distribution of TG and cholesterol likely reflect the CETP-mediated reciprocal exchange of TG and CE between CE-rich LDL and HDL fractions and TRL. The increase in the cholesterol content of TRL and the decrease in the cholesterol content of LDL were significantly greater in PP plasma than in fasting plasma (Fig. 1F). These data suggest that the PP lipemia-mediated shift in the distribution of cholesterol from LDL and HDL to TRL in vivo may be caused by a PP enhancement of LCAT and CETP activity. The in vitro reaction of LCAT and CETP increased the cholesterol content of HDL, but the extent of that increase was significantly lower in 4 h PP plasma than in fasting plasma (Fig. 1F). We have observed previously that a net decrease in the cholesterol content of HDL in plasma was achieved through the inhibition of LCAT during in vitro incubation (28). Given that a net increase in the cholesterol content of HDL is seen following in vitro reaction of CETP in the presence of active LCAT in fasting and PP plasma, it is likely that the levels of LCAT-derived CE that accumulated in HDL are greater than the levels of HDL-CE transferred to apoB-containing lipoproteins.

We examined further the effect of PP lipemia on the extent of esterification of ^{3}H-labeled UC by endogenous LCAT and the extent to which [^{3}H]CE formed by LCAT was distributed into PP TRL. The appearance of PP TRL in 4 h PP plasma was associated with a small increase in the extent of esterification of [^{3}H]UC by endogenous LCAT, a significant increase in the partitioning of LCAT-produced ^{3}H-labeled CE onto TRL, and a parallel decrease onto LDL (**Table 1**). The above studies suggest further that the presence of CMs in plasma may enhance the transfer of endogenous CE as well as CE formed by LCAT into TRL via CETP in vivo.

TABLE 1. Extent of esterification of ^{3}H-UC added to fasting and postprandial plasma by endogenous LCAT and distribution of the LCAT-produced ^{3}H-CE among lipoproteins

Plasma	Plasma TG Levels	^{3}H-UC Esterified	Distribution of ^{3}H-CE Formed by LCAT among Lipoproteins		
			VLDL	LDL	HDL
	mg/dl		%		
Fasting	91 ± 40	47.1 ± 4.3	12.1 ± 2.9	70.9 ± 6.1	17.0 ± 3.3
4 h PP	171 ± 67	49.4 ± 6.3	20.2 ± 6.9[b]	64.0 ± 7.5[a]	15.8 ± 3.9

PP, postprandial; TG, triglyceride; UC, unesterified cholesterol. Significantly different from fasting plasma. Values are mean ± SD (n = 6).
[a] $P < 0.05$.
[b] $P < 0.01$.

Comparison of the CETP-mediated movement of cholesterol mass of lipoproteins and of ^{3}H-radiolabled CE on LDL or HDL among lipoproteins in reconstituted PP plasma

The CETP-mediated transfer of CE from HDL to apoB-containing lipoproteins has been commonly examined by measuring the transfer of HDL-incorporated [^{3}H]CE to various apoB-containing lipoproteins in plasma. However, it is not yet fully clear whether the transfer of [^{3}H]CE from HDL to apoB-containing lipoproteins truly represents the transfer of its CE mass. When reconstituted 4 h PP plasma containing a trace amount of [^{3}H]CE-labeled HDL or [^{3}H]CE-labeled LDL was incubated at 37°C for 16 h, a significant portion of [^{3}H]CE on HDL (38%) was transferred to LDL, and this transfer was accompanied by the transfer of a substantial portion of [^{3}H]CE on LDL (24%) to HDL (**Fig. 3A, B**). A small portion (6–8%) of [^{3}H]CE on both HDL and LDL was transferred to the VLDL density fraction (Fig. 3A, B). Lipoprotein cholesterol profiles of control and incubated reconstituted 4 h PP plasma showed that the incubation significantly increased the cholesterol mass on VLDL via a net decrease of cholesterol on both LDL and HDL (Fig. 3C). However, when reconstituted plasma containing no TRL was incubated, there was a small net increase of cholesterol level on HDL via the decrease of cholesterol levels on LDL (Fig. 3D), even though the transfer of [^{3}H]CE from HDL to LDL was minimally altered (data not shown). The above data suggest that the extent of CETP-mediated redistribution of [^{3}H]CE differs from that of CE mass on HDL.

The potency of isolated fasting VLDL and 4 h PP CMs to serve as acceptors of CE from LDL+HDL

Because PP TRLs containing both VLDL and CMs may serve as acceptors of [^{3}H]CE as well as cholesterol mass from both LDL and HDL, we determined the potency of VLDL and of CMs to act as acceptors of cholesterol mass or ^{3}H-labeled CE from LDL+HDL. For this study, reconstituted plasma containing equivalent amounts of TG from isolated fasting VLDL or CMs isolated from 4 h PP plasma was incubated with a common source of fresh VLDL-free (d>1.006 g/ml) plasma (± a trace amount of

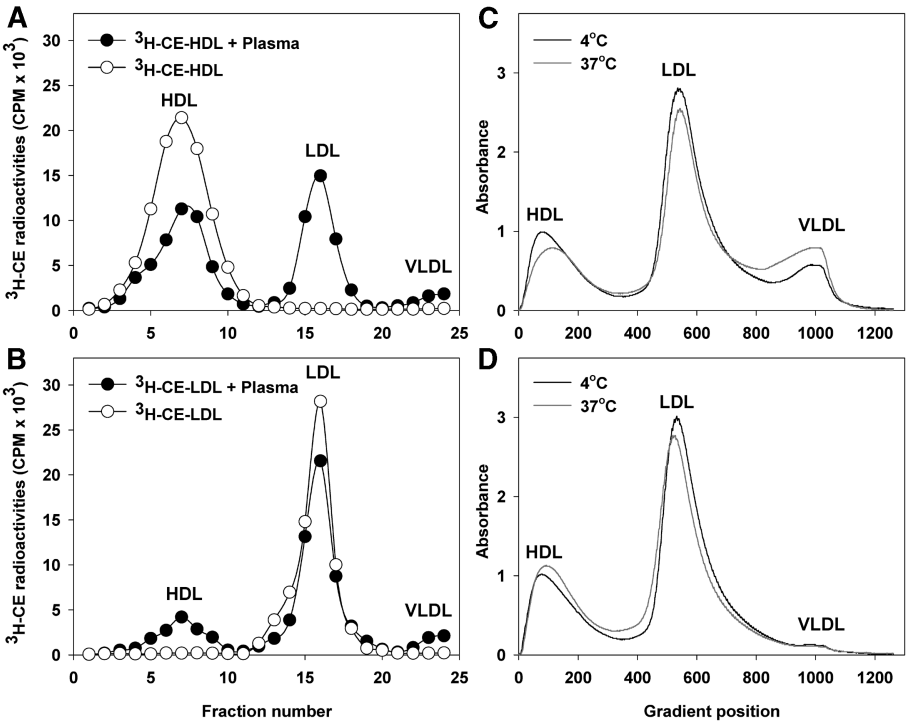

Fig. 3. Change in distribution of [^{3}H]CE radioactivity and cholesterol mass among lipoproteins following in vitro reaction of endogenous LCAT and CETP in reconstituted plasma containing a trace amount of [^{3}H]CE-labeled LDL or [^{3}H]CE-labeled HDL. Change in distribution of [^{3}H]CE radioactivity among density gradient fractions of [^{3}H]CE-labeled HDL (A) or [^{3}H]CE-labeled LDL (B) following incubation with or without reconstituted plasma. Change in lipoprotein cholesterol profiles of reconstituted plasma (4°C) containing PP TRL (C) or lacking PP TRL (D) after their incubation at 37°C for 16 h (37°C). The procedures for this experiment were described in detail in Experimental Procedures.

[^{3}H]CE-labeled LDL+HDL). **Figure 4A** shows that the incubation at 37°C of the above reconstituted plasma containing either VLDL or CMs increased the cholesterol and decreased the TG levels on VLDL or CMs mainly because of a decrease of cholesterol and an increase of TG levels on LDL. VLDL produced a 1.3-fold greater increase of cholesterol level and decrease of TG level than CMs (Fig. 4A). Similarly, after incubation of reconstituted plasma containing [^{3}H]CE-labeled LDL and HDL, the levels of ^{3}H-labeled CE transferred from LDL+HDL to VLDL was about 1.5-fold greater than to CMs (Fig. 4B). Increases to a similar extent of both [^{3}H]CE and cholesterol mass in VLDL and CMs following incubation of reconstituted plasma indicate that transfer of [^{3}H]CE from LDL+HDL to VLDL and CMs does indeed represent the transfer of CE mass.

To further compare the potency of particles of CM and VLDL as acceptors of CE from LDL+HDL, the numbers of TG molecules per particle of fasting VLDL and PP CM were determined by measuring the apoB-100 or apoB-48 content of fasting VLDL or CMs having known TG concentration via SDS gradient gel electrophoresis. Presented in Fig. 4C, the SDS gel electrophoregrams show the levels of the separated apoB-100 band (lanes 1–4) or the apoB-48 band (lanes 7–10) after loading isolated VLDL containing 10 μg (11.4 nmol) TG or isolated CMs containing 150 μg (171 nmol) TG, respectively. Quantification of these levels, based on the apoB-100 standard (lanes 5 and 6), indicates that mean levels of apoB-100 associated with 10 μg VLDL TG and of apoB-48 associated with 150 μg CM TG were 1.07 and 0.191 μg, respectively. Thus, the mass ratios of TG to apoB-100 on VLDL and TG to apoB-48 on CMs were 9.3:1 and 785:1, respectively (**Table 2**). Based on MWs of 555,486 and 260,416 for apoB-100 and apoB-48, respectively, and 866 for TG, the numbers of TG molecules per molecules of apoB-100 on VLDL and per molecules of apoB-48 on CMs were calculated to be 5,906 and 238,493, respectively. Thus, the number of TG molecules per particle of CM was calculated to be about 40 times greater than per particle of VLDL (Table 2). Because CM at the same TG level as that on VLDL was about 77% as potent as VLDL in accepting CE from LDL+HDL (Fig. 4A, B), a CM particle was calculated to be 31-fold more potent than a VLDL particle in accepting CE molecules from LDL+HDL via CETP (Table 2). Although data are not shown, in reconstituted plasma containing physiological levels of VLDL or CM (0.75–3.0 mg VLDL TG or CM TG/ml), the extent of the transfer of ^{3}H-CE from LDL+HDL to VLDL or CMs increased in a dose-dependent manner concurrent with an increase in CM or VLDL levels. Similar differences in potencies of VLDL and CMs to accept ^{3}H-CE from LDL+HDL were also observed at all three different levels of VLDL and CM in the reconstituted plasma.

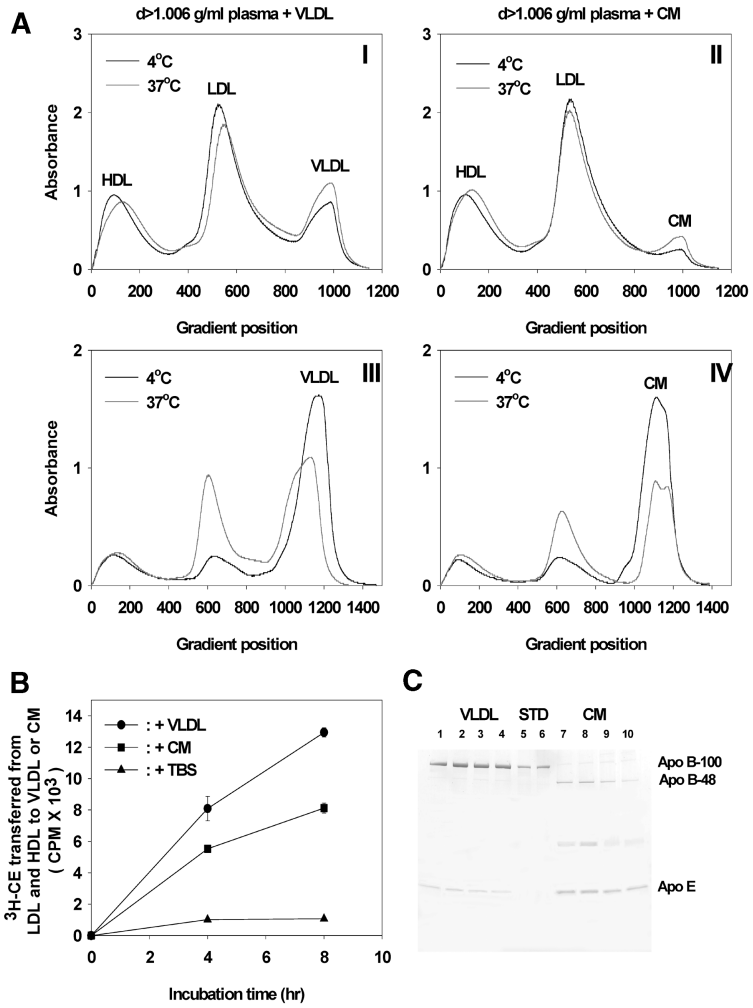

Fig. 4. Determination of the potencies of isolated fasting VLDL and PP CMs to serve as acceptors of CE or donors of TG during in vitro reaction of LCAT and CETP in reconstituted plasma. The potencies of particles of fasting VLDL and PP CMs to serve as acceptors of CE and donors of TG were determined *1*) by measuring the change in TG and cholesterol content of VLDL and CMs (A) and the level of [^{3}H-labeled CE accepted from LDL and HDL by VLDL or CMs (B) following incubation of reconstituted plasma containing equivalent TGs from isolated fasting VLDL or PP CMs and a common VLDL-free (d>1.006 g/ml) plasma (± trace amounts of [^{3}H]CE-labeled LDL and HDL, and *2*) by determining the ratio of TG to apoB-100 on VLDL and TG to apoB-48 on CMs via the SDS gradient gel electrophoretic method (C). A: Change in lipoprotein cholesterol profiles (I and II) and lipoprotein TG profiles (III and IV) of reconstituted plasma containing VLDL (I and III) or CMs (II and IV) after its incubation at 37°C for 16 h. The level of VLDL-TG or CM-TG in reconstituted plasma (A, B) was 1.5 mg/ml. B: Time course transfer of ^{3}H-labeled CE from LDL and HDL to VLDL or CMs during incubation of an equal amount of VLDL TG or CM TG with a common TRL-free (d>1.006 g/ml) plasma containing a trace amount of ^{3}H-CE-labeled LDL and HDL. Values are mean ± SD. C: The SDS gel electrophoregrams showing the levels of apoB-100 on fasting VLDL containing 10 μg TG (lanes 1–4) and levels of apoB-48 on CMs containing150 μg TG (lanes 7–10) isolated from four different individuals. Lanes 5 and 6 show apoB-100 standard (LDL) containing 0.5 μg protein.

The potency of VLDL and CMs in PP plasma to serve as acceptors of [^{3}H]CE from LDL+HDL

Because CMs and VLDL present in 4 h PP plasma would compete to accept CE from LDL+HDL via CETP, we examined the effect of the presence and absence of CMs in PP plasma on the potency of VLDL to accept [^{3}H]CE from LDL+HDL via CETP. For this study, CMs were removed either before or after incubation of PP plasma, and the extent of transfer of [^{3}H]CE from LDL+HDL to CMs and CM-free VLDL density fractions was measured. During the incubation of fasting and PP plasma containing equivalent trace amounts of [^{3}H]CE-labeled LDL+HDL, the transfer of [^{3}H]CE from LDL+HDL to TRL (VLDL density fraction) was much more marked in 4 h PP plasma than in fasting plasma (**Fig. 5**). The levels of [^{3}H]CE accepted by TRL from LDL+HDL in 4 h PP plasma were significantly reduced by the centrifugal removal of CMs from PP plasma prior to incubation (pre-removal). However, these levels of transfer to CM-free TRL remained greater than those accepted by VLDL in fasting plasma (Fig. 5). When CMs were removed from 4 h PP plasma after incubation (post-removal), TRL in the resulting CM-

TABLE 2. Determination of the number of TG molecules per particle of VLDL and CM and of the potencies of a VLDL and CM particle to accept CE from LDL+HDL via LCAT and CETP

Gel Lanes	TRL	ApoB	TG-Loaded		ApoB-100 or apoB-48 Level (Determined by Gel Scan)		TG-to-apoB Ratio		Relative Potency to Accept CE from LDL+HDL	
			μg	*nmol*	*μg*	*nmol (x10^{-3})*[a]	*Mass*	*Molar*	*Per mg TG*[b]	*Per Particle*
1–4	VLDL	B-100	10	11.4	1.07 ± 0.21	1.93 ± 0.31	9.3	5,960 (1)	1.30 ± 0.56	1
7–10	CM	B-48	150	171	0.191 ± 0.033	0.717 ± 0.12	785	238,493 (40.3)	1	31

ApoB, apolipoprotein B; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CM, chylomicron; LCAT, lecithin:cholesterol acyltransferase; LDL+HDL, cholesterol-rich lipoprotein; TRL, triglyceride-rich lipoprotein. Values are mean ± SD (n = 6).

[a] Molecular weights of 866 for TG, 555,486 for apoB-100, and 260,416 for apoB-48 were used to determine nmoles of TG, apoB-100, and apoB-48.

[b] Relative potencies of VLDL and CM to accept CE from LDL+HDL were obtained from the experimental data shown in Fig. 4.

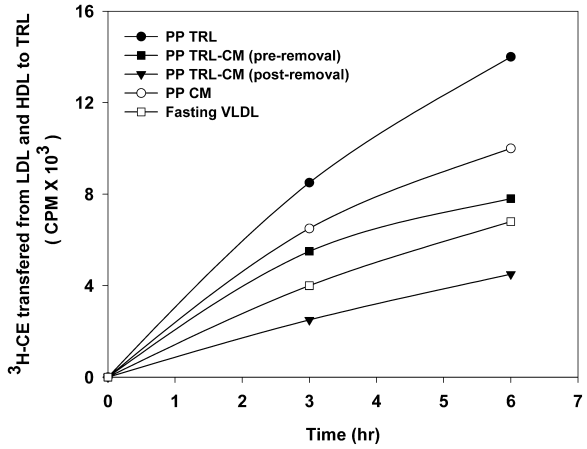

Fig. 5. Effect of the presence and absence of CMs in PP plasma on the transfer of [^{3}H]CE radioactivity from LDL and HDL into the VLDL density fraction. During incubation of fresh fasting plasma (TG: 85 mg/dl), 4 h PP plasma (TG: 147 mg/dl), and CM-free 4 h PP plasma (pre-removal) (TG: 102 mg/dl) containing equal (trace) amounts of [^{3}H]CE-labeled LDL and HDL, aliquots of samples in triplicates were withdrawn 3 h and 6 h after incubation. After further removal of CMs from a portion of the incubated 4 h PP plasma (post-removal), the levels of [^{3}H]CE radioactivity transferred from LDL and HDL into the VLDL density fraction were measured by the method described in Experimental Procedures. The level of [^{3}H]CE radioactivity transferred from LDL and HDL to CMs was determined by subtracting the levels of [^{3}H]CE radioactivity on CM-free PP TRL fractions (post-removal) from those on PP TRL.

free plasma accepted much lower levels of [^{3}H]CE from LDL+HDL than did VLDL in fasting plasma. The above data indicate that the presence of CMs in plasma during incubation suppresses the ability of VLDL and/or CM remnants to accept [^{3}H]CE from LDL+HDL. Although the number of intact CM particles in this 4 h PP plasma was estimated to be much lower (50-fold) than those of VLDL and CM remnant particles (Fig. 2), a greater level of [^{3}H]CE transferred from LDL+HDL to intact CM particles than to VLDL and CM remnant particles (Fig. 5). Taken together, data shown in Table 2 and Fig. 5 demonstrate that CMs in PP plasma may serve as more efficient and potent acceptors of CE from LDL+HDL than from VLDL during in vitro reaction of endogenous LCAT and CETP.

The potency of lipoproteins in fasting and PP plasma to accept cholesterol released from RBC membranes via LCAT and CETP

We examined the potencies of CMs and other lipoproteins in fasting and PP plasma to serve as acceptors of cholesterol released from cell membranes via LCAT and CETP by using RBCs as a source of cell membranes. RBCs are rich in cholesterol, and in vitro and in vivo studies have shown that UC on RBC membranes is in equilibrium with UC on lipoproteins (29) and perhaps also with that on arterial wall cells. RBCs from animals on an atherogenic diet are enriched with UC relative to phospholipids (30), and RBCs from normolipdemic subjects have been shown to act as exceptionally potent acceptors of cholesterol released from cholesterol-loaded, cultured macrophages (31). Although RBCs, unlike nucleated cells, do not synthesize or metabolize cholesterol, studies of cholesterol efflux from RBC membranes into fasting and PP plasma provide pertinent information about the flux of cholesterol from atherosclerotic lesions into plasma in vivo. **Figure 6** shows the effect of incubating fresh fasting and 4 h plasma with an excess amount of autologous RBCs on the change in lipoprotein cholesterol profiles. Incubation of either fasting or 4 h plasma with RBCs increased the cholesterol content of all lipoproteins. Elevation of TRL levels in 4 h PP plasma increased the levels of RBC cholesterol accepted by the VLDL density fraction but lowered that accepted by LDL and HDL (Fig. 6A). To assess the potency of VLDL and CMs in 4 h PP plasma to accept the cholesterol from RBCs via LCAT and CETP, the change in cholesterol content of CMs and VLDL in 4 h PP plasma was examined following incubation with RBCs. To determine the cholesterol content of CMs, CMs in 4 h PP plasma were removed by ultracentrifugation before and after incubation of PP plasma with RBCs. Then, the level of cholesterol associated with the VLDL density fraction in 4 h PP plasma and CM-free 4 h PP plasma was determined from their lipoprotein cholesterol profiles (Fig. 6B). The lipoprotein cholesterol profiles of fresh 4 h PP and CM-free 4 h PP plasma obtained from a hyper-responder of PP lipemia revealed that the removal of CMs from fresh 4 h PP plasma only minimally lowered (−7.5%) the cholesterol level on TRL and had virtually no effect on cholesterol levels of LDL and HDL (Fig. 6B). This small effect indicates that only a small portion of TRL cholesterol in PP plasma is carried by large CM particles. After in vitro incubation of PP plasma with RBCs, CMs in the plasma were also centrifugally removed. Examination of the lipoprotein cholesterol profiles of control and incubated 4 h PP and CM-free 4 h PP plasma (Fig. 6B) showed that the incubation of 4 h PP plasma increased in TRL-cholesterol levels by 107% via an 81% increase in cholesterol levels on VLDL and a 433% increase in cholesterol levels on CMs (Fig. 6B). Although cholesterol levels on CMs in fresh plasma from subjects with a low PP lipemic response were not accurately measurable, the average percent increases in the cholesterol content of CMs, VLDL, LDL, and HDL in PP plasma, obtained from subjects with a normal to brisk PP lipemic response, after incubation with RBCs were 485%, 74%, 13%, and 30%, respectively. The above data indicate that CMs in PP plasma are 6.5-fold, 37-fold, and 16-fold more potent than VLDL, LDL, and HDL, respectively, in accepting additional cholesterol released from cell membranes (Fig. 6C). The percent increases in cholesterol content of VLDL (90%), LDL (21%), and HDL (37%) in fasting plasma were greater than those in 4 h PP plasma (Fig. 6C), suggesting that the presence of CMs in the plasma suppressed endogenous lipoprotein acceptance of cholesterol released from RBC membranes.

Transfer of cholesterol accepted by LDL and HDL from RBCs into CM-rich PP TRL

When fasting plasma containing a very low level of VLDL was incubated with RBCs, most of the RBC-choles-

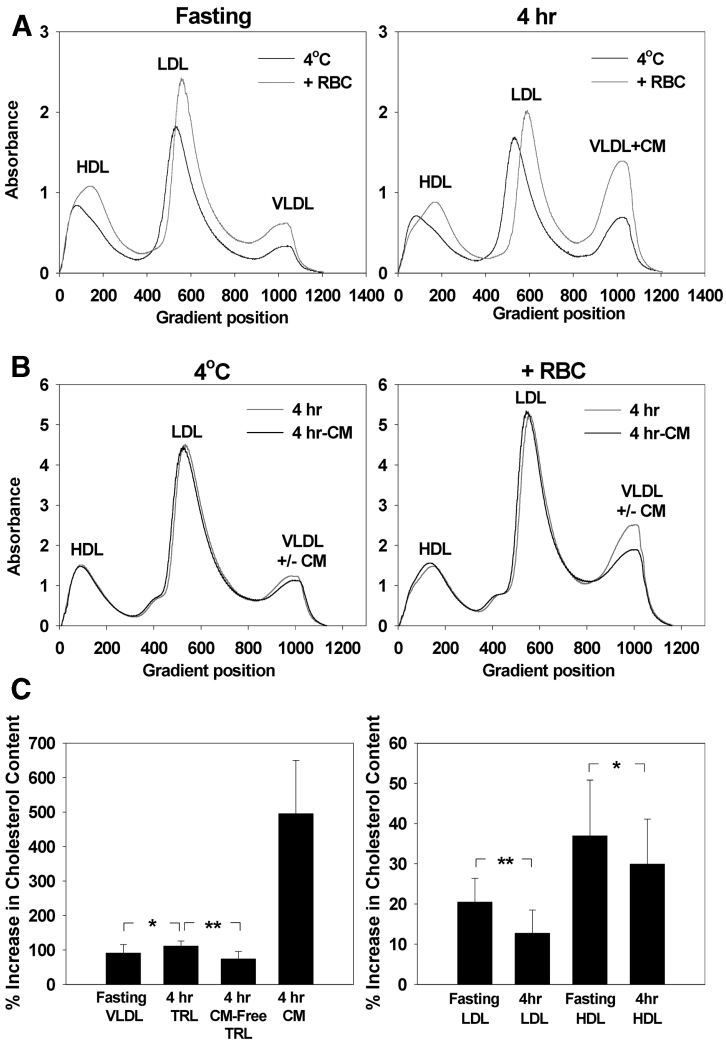

Fig. 6. Potencies of lipoproteins in fasting and PP plasma to serve as acceptors of cholesterol released from red blood cell (RBC) membranes. The potencies of lipoproteins to act as acceptors of cholesterol released from RBCs were determined by measuring the net increase in the cholesterol content of VLDL, LDL, HDL, and/or CMs in fasting and PP plasma through a comparison of the lipoprotein cholesterol profiles of plasma before and after their incubation with RBCs. A: Changes in the lipoprotein cholesterol profile of fresh (4°C) fasting plasma (left) and 4 h PP plasma (right), obtained from a subject with a brisk PP lipemic response, after incubation with RBCs (+RBC). B: Lipoprotein cholesterol profile of fresh 4 h PP plasma (4°C) and 4 h PP plasma incubated with RBCs (+RBC) before removal of CMs (4 h) and after removal of CMs (4 h −CM) The cholesterol levels of the VLDL density fraction in fresh 4 h PP plasma and CM-free 4 h PP plasma prepared before incubation (4°C) were 40 and 37 mg/dl, respectively, and after incubation with RBCs (+RBC) were 83 and 67 mg/dl, respectively. The levels of cholesterol of CMs in fresh 4 h PP plasma and in 4 h PP plasma incubated with RBCs were 3 and 16 mg/dl, respectively. C: The effect of incubating fasting and PP plasma with RBCs on mean percent changes in the cholesterol content of VLDL in fasting plasma and total TRL, CM-free TRL, and CMs in PP plasma (left) and of LDL and HDL in fasting and PP plasma (right). Values are mean ± SD (n = 10). Significantly different at $*P < 0.05$ and $**P < 0.01$.

terol released into the plasma was associated with LDL and HDL (**Fig. 7A**). However, when this RBC-cholesterol-enriched fasting plasma was supplemented with fresh CM-rich 4 h PP TRL and further incubated, a major portion of the RBC-cholesterol partitioned onto LDL and HDL was then transferred into the added CM-rich TRL (Fig. 7B). The above data indicate that PP TRLs are preferred over LDL and HDL as acceptors of cholesterol released from cell membranes

DISCUSSION

It is generally believed that PP CMs may play a role in transporting dietary fat and cholesterol in circulating blood and that the delayed clearance of its remnants may promote atherogenic processes by injuring arterial wall cells, promoting foam cell formation, and altering endothelial function and/or direct arterial retention of CM remnants (32–35). In contrast, our study has provided evidence that the rapid clearance of PP CMs may play a critical role in promoting RCT by transporting cholesterol released from cell membranes and endogenous lipoproteins to the liver via LCAT and CETP. We have shown that the appearance of CMs in PP plasma is associated with a significant increase of cholesterol on TRL and with a concomitant decrease of cholesterol levels in both LDL and HDL, although it has little influence on plasma total cholesterol in vivo (Fig. 1). This shift in the balance of cholesterol from LDL+HDL to TRL is likely a reflection of the CETP-mediated transfer of CE from LDL+HDL to PP CMs appearing in plasma in vivo. It has been suggested that under normal conditions, the rate of the CETP-mediated transfer of CE is more rapid than the catabolic rate of endogenous lipoproteins and that therefore the pools of CE among endogenous lipoproteins in the fasting state approach equilibrium in vivo (36). However, were CETP activity to be enhanced by a PP lipemic state of several hours duration, it is probable that the appearance of PP TRL as an acceptor of CE would alter the equilibrium of CE between PP TRL and endogenous lipoprotein cholesterol levels. Because PP CMs, after their conversion into CM remnants, would be cleared at a rate faster than that of the CETP reaction and the catabolism of endogenous lipoproteins in vivo (10–13), the appearance and clearance of CMs as acceptors of CETP reaction products would lead

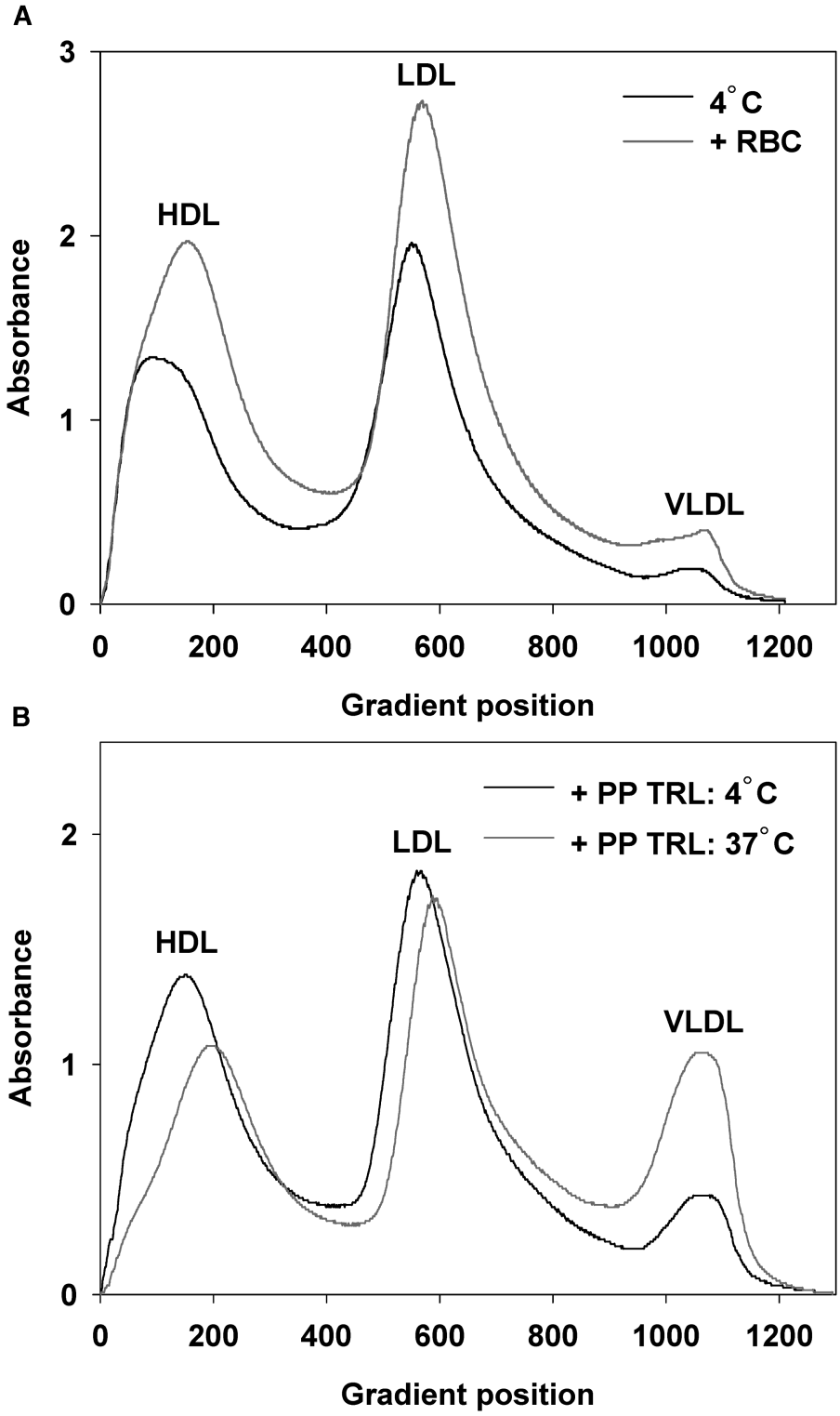

Fig. 7. Change in lipoprotein cholesterol profile of fasting plasma containing a low VLDL level after its incubation with RBCs (top) and subsequent further incubation of the RBC-treated fasting plasma following addition of fresh CM-rich PP TRL (bottom). Top panel: Following incubation of fresh fasting plasma (4°C) containing a low VLDL with RBCs at 37°C for 16 h, the lipoprotein cholesterol profiles of control plasma (4°C) and RBC-cholesterol-enriched plasma (+RBC) were determined. Bottom panel: Following supplementation of RBC-treated fasting plasma with fresh CM-rich PP TRL, lipoprotein cholesterol profiles of RBC-treated fasting plasma containing fresh CM-rich PP TRL were determined after placing it in an ice bath (+PP TRL: 4°C) or in a 37°C incubator (+PP TRL: 37°C). The CM-rich PP TRL was isolated from 4 h PP plasma containing 2-fold excess amount of CM.

to a reduction in the cholesterol levels of LDL and HDL during the PP lipemic state in vivo. A number of recent studies (37, 38) have revealed that dietary cholesterol (deuterated) supplied to healthy humans as a single meal containing fat does not simultaneously appear with TG. In fact, the dietary cholesterol that appeared on PP CMs with dietary TG at the PP peak was only a small portion (1%) of the cholesterol associated with CMs (37), indicating that the bulk of cholesterol on CMs at the PP lipemic peak is derived from endogenous sources. Oliviera et al. (39) reported that in chyluric patients, 58% and 18% of CE on lymph CMs were derived from HDL and LDL, respectively. This finding further supports the hypothesis that the CETP-mediated CE transfer from LDL and HDL to PP CMs occurs in humans in vivo. Radiochemical studies showing that the major portion of radiolabeled CE transferred from HDL to apoB-containing lipoproteins is associated with LDL (6, 7) have led to the generally held belief that LDL is a major acceptor of CE from HDL during the CETP reaction. However, through our in vitro study, we have found that although LDL does indeed accept the major portion of the radiolabeled CE originating from HDL or produced by LCAT, it is TRL that serves as the major acceptor of CE mass, with LDL acting as a net donor of cholesterol to TRL via LCAT and CETP (Fig. 3; Table 1). Our observations suggest that either CE is transferred at greater levels from LDL to TRL than from HDL to LDL or the transfer of ^{3}H-radiolabeled CE from HDL to LDL represents primarily the replacement of unlabeled CE molecules in LDL with labeled molecules of HDL origin. Our study shows that in vitro reactions of LCAT and CETP in fasting and PP plasma involve the transfer of TG from TRL primarily to LDL and only minimally to HDL (Fig. 1). This observation indicates further that the CETP-mediated reciprocal exchange of CE and TG in plasma may occur primarily between TRL and LDL. In the PP state, the CETP-mediated reciprocal exchange of TG and CE in plasma may preferentially occur between CMs and LDL+HDL because the presence of CMs in plasma suppressed the transfer of CE from LDL+HDL to VLDL (Fig. 5). In reconstituted plasma containing equivalent amounts of TG from VLDL and CM, the number of VLDL particles was about 30-fold greater than that of CMs, but the extent of CETP-mediated reciprocal exchange of TG and CE between LDL+HDL and VLDL was only about 1.3–1.5-fold greater than that between LDL+HDL and CMs (Fig. 4; Table 2). This observation suggests that the much greater number of TG molecules on a CM particle may influence the ability of CMs to be more potent acceptors of CE than is VLDL.

It has been proposed that LCAT- and CETP-mediated transfer of cholesterol from cell membranes to apoB-containing lipoproteins for subsequent removal by the liver could inhibit atherosclerosis by enhancing the RCT rate in vivo (1–3). In transgenic mice, diet-induced atherosclerosis was made more severe by an increase in the plasma activity of either CETP or LCAT alone (40, 41), but was prevented by a simultaneous increase in the plasma activity of CETP and LCAT through overexpression of both the LCAT and CETP genes (42). Furthermore, protection against diet-induced atherosclerosis is also afforded to CETP transgenic mice by an increase in PP TRL levels due to overexpression of the human apoC-III gene; however, the overexpression of the human apoC-III gene alone in normal mice is accompanied by a tendency for these mice to develop atherosclerotic lesions (43, 44). These transgenic mouse models suggest that protection against atherosclerosis could be achieved through increasing PP TRL and enhancing both LCAT and CETP simultaneously. During PP lipemia in humans, there are transient increases in the PP CM level and LCAT and CETP activities (15–17). However, the consequence of these PP lipemia-mediated increases of PP CM and LCAT and CETP activities on the human cardiovascular disease (CVD) risk has not yet been fully evaluated. Our current study has demonstrated that *1*) the increase of CMs in PP plasma is primarily responsible for the enhancement of LCAT and CETP activities, *2*) CMs are much more potent than are endogenous lipoproteins in accepting cholesterol released from cell membranes via LCAT and CETP, and *3*) the presence of CMs in plasma suppressed the ability of endogenous lipoproteins to accept cholesterol released from RBC membranes (Fig. 6). Moreover, RBC-cholesterol that was initially accepted by fasting plasma was transferred preferentially to PP CM upon an increase in its level (Fig. 7). These observations suggest that the PP CMs may serve as the preferred ultimate acceptors of cholesterol released from cell membranes. The LCAT- and CETP-mediated transfer of cholesterol from endogenous lipoproteins and cell membranes to CMs via LCAT and CETP would likely lead to the rapid removal of this cholesterol by the liver because PP CMs, after their conversion into remnants, are directly taken up by the liver (23). Numerous case-control studies have shown that PP lipemia is more pronounced and of longer duration in patients with CVD than in normal subjects (45–47). Groot et al. (45) reported that levels of gut-derived lipids in plasma were similar in both CVD patients and control subjects but diverged in the late phases of PP lipemia. The progression of atherosclerosis correlates with the level of apoB-48-containing small remnant particles or TG level in the late PP state but not with the level of large CM particles or TG levels at the peak of PP lipemia (46, 47). These observations suggest a similar production of CMs in both groups, but point to a delayed clearance of PP CMs in CVD patients. Taken together, these findings suggest that it is the delayed clearance, not the increased level of PP CMs, that is atherogenic. Because CMs serve as the ultimate and most avid and potent acceptors of cholesterol transferred from cell membranes into plasma via LCAT and CETP (Fig. 6), the delay in clearance of CMs by the liver may lower the rate of RCT in vivo. As is well established, high levels of HDL in plasma protect against CVD (48). Because the rate of clearance of PP CMs directly correlates with HDL levels in plasma (49), a high HDL level in plasma may enhance the rate of RCT by promoting rapid clearance of PP CMs carrying cholesterol accepted from cell membranes. In circulating blood, a CM particle transports much greater numbers (7- to 300-

fold) of cholesterol molecules than does a particle of endogenous lipoproteins (50) and may have much greater potencies (6- to 37-fold) than do endogenous lipoprotein particles for accepting additional cholesterol released from cell membranes via LCAT and CETP (Fig. 6). It is reasonable to conclude, then, that a CM particle is far more potent than any other endogenous lipoprotein in plasma to deliver cholesterol to the liver via apoE receptors. Although our study examining the role of PP CMs in promoting RCT was limited to studies in vitro, further studies remain to be done to determine the potential role of PP CMs in promoting RCT in vivo.

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