Elevated CETP Activity Improves Plasma Cholesterol Efflux Capacity From Human Macrophages in Women

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Objective—We aim to identify the impact of endogenous cholesteryl ester transfer protein (CETP) activity on plasma capacity to mediate free cholesterol efflux from human macrophages.

Methods and Results—Endogenous plasma CETP activity was measured in a population of 348 women. We defined a low CETP group corresponding to subjects displaying an endogenous plasma CETP activity within the first tertile and a high CETP group corresponding to subjects with an endogenous plasma CETP activity within the third tertile. Subjects from the high CETP activity group displayed a significant increase in the capacity of their plasma (+8.2%; \( P=0.001 \)) to mediate cholesterol efflux from human acute monocytic leukemia cell line human macrophages and from ATP-binding cassette transporter A1-dependent pathway (+23.4%; \( P=0.0001 \)) as compared with those from the low CETP activity group. Multivariate analyses revealed that the impact of CETP activity was independent of plasma lipids levels. Pre-\( \beta \)-high-density lipoprotein concentrations were significantly elevated (+29.6%; \( P=0.01 \)) in the high CETP activity group as compared with the low CETP activity group. A positive correlation between pre-\( \beta \)-high-density lipoprotein levels and plasma efflux efficiency from human acute monocytic leukemia cell line human macrophages was observed (\( r=0.29, \ P=0.02 \)).

Conclusion—CETP leading to the improvement of plasma efflux capacity, as a result of efficient pre-\( \beta \)-high-density lipoprotein formation and ATP-binding cassette transporter A1 efflux, should be preserved to prevent lipid accumulation in human macrophages. (Arterioscler Thromb Vasc Biol. 2012;32:2341-2349.)

Key Words: ATP binding cassette transporter ■ lipoproteins ■ macrophages ■ cholesteryl ester transfer protein ■ cellular cholesterol efflux

Elevated high-density lipoprotein cholesterol (HDLC) level represents a protective factor against development of atherosclerosis. Indeed, it is well established that HDL particles exhibit numerous potentially antiatherogenic activities, including reverse cholesterol transport (RCT), antioxidative, and anti-inflammatory activities. Such specific metabolic route allows the removal of excess cholesterol accumulated within the peripheral tissues, including arterial wall, and its transport back to the liver for excretion. The initial step of RCT represents cellular free cholesterol (FC) efflux to various extra-cellular acceptors, such as lipid-poor/lipid-free apolipoprotein (apo) AI or mature HDL particles. By this way, cellular FC efflux can prevent lipid accumulation in macrophages and foam cell formation. In the arterial wall, foam cell formation represents a key step in atherosclerosis. In this context, it is relevant to note that an inverse relationship between the capacity of apoB-depleted sera or of isolated HDL particles to mediate cholesterol efflux from macrophages and intima-media thickness or cardiovascular risk has been recently demonstrated.

To date, several mechanisms for cellular FC efflux from macrophages have been described, corresponding to both transporter/receptor dependent pathways via ATP-binding cassette transporter A1 (ABCA1), ABCG1-dependent pathway, and scavenger receptor class B member 1-scraper receptor class B member 1 (SR-BI) and a transporter/receptor independent process by passive aqueous diffusion. The relative contribution of each efflux pathway involved in the release of FC from cholesterol-loaded macrophages remains controversial and appears to be species dependent. Indeed, in mouse macrophages, ABCA1- and ABCG1-dependent efflux represent major pathways for the release of cholesterol. By contrast, in human macrophages cholesterol efflux primarily requires ABCA1 and in second importance human homologue CD36- and LIMPII-analogous 1 whereas ABCG1 appears to have no determinant role in the export of cholesterol. Within the plasma, both quantitative and qualitative features of HDL particles are continuously modulated by the action of a number of lipid transfer proteins and enzymes, which play a
determinant role in the formation, maturation, and intravascular remodeling of HDL particles. After FC efflux process, lipiddated apoAI is rapidly converted into spherical HDL particles through the action of Lecithin-Cholesterol Acyl Transferase. Then, the cholesteryl ester transfer protein (CETP) promotes the redistribution of neutral lipids, cholesteryl esters, and triglycerides (TG), between plasma lipoprotein particles. In humans, CETP is responsible for the transfer of cholesteryl ethers (CE) from HDL to very-low-density lipoprotein and low-density lipoprotein (LDL), which occurs concomitantly with the transfer of TG in the opposite direction. Under fasting conditions, it is well established that endogenous plasma CETP activity is primarily modulated by apoB containing lipoprotein concentrations. On a quantitative basis, LDL particles represent the major cholesteryl ester acceptor, whereas on a qualitative basis TG-rich lipoprotein particles displayed the highest capacity to accept cholesteryl esters from HDL.1,11 In good agreement with these latter observations, in vivo evaluation of cholesterol fluxes between circulating lipoproteins revealed that in normolipidemic subjects very-low-density lipoprotein represent the earliest CE acceptor from HDL as compared with LDL.13 CETP allows the formation of TG-rich HDL particles which become a substrate for hepatic lipase. Thus, hydrolysis of TG by hepatic lipase induces the reduction in HDL particle size and the formation of unstable HDL particles.14,15 The determinant role of CETP in HDL metabolism has been clearly demonstrated by the identification of CETP-deficient patients. These patients are characterized by elevated levels of HDL-C as a result of a specific increase in large HDL particles and a reduced turnover of apoAI.16,17 In this context, although the impact of endogenous CETP activity on plasma levels of HDL-C and intravascular HDL remodeling is well established,15,19 the consequence of physiological CETP activity variability on HDL particle structure and function remains largely unknown.

During the past few years, pharmacological CETP inhibitors have been developed to raise plasma HDL-C levels.20 CETP inhibitors, such as torcetrapib or anacetrapib, increase the capacity of HDL2 particles to mediate efflux from human acute monocytic leukemia cell line (THP-1) macrophages.21,22 Thus, the impact of pharmacological CETP inhibition on HDL functionality highlights the need to further analyze the relationship between physiological endogenous plasma CETP activity and HDL function.

The aim of the present study was to evaluate the relationship between endogenous plasma CETP activity and the capacity of the whole plasma or isolated HDL particles to mediate FC efflux from human cholesterol–loaded macrophages.

Patients and Methods

Subjects and Plasma Sample

The study was conducted in a total of 348 women aged >18 years displaying a metabolic disorder characterized by a dyslipidemia (total cholesterol ≥250 mg/dL or LDL-C≥160 mg/dL or TG≥150 mg/dL) or by a low HDL-C phenotype (HDL-C<50 mg/dL). Major clinical and biochemical characteristics of the population are presented in Table 1. Thirty-five percent of the population displayed a hypercholesterolemia (25% without hypertriglyceridemia and 10% with hypertriglyceridemia), and 13% displayed a hypertri glyceridemia. Thirty-nine percent of the population displayed a low HDL-C phenotype. The frequency of patients with a fasting blood glucose >6.1 mmol/L was 15%. Twenty-four percent of the subjects were receiving a lipid-lowering therapy with 90% of treated patients receiving a statin, and 7% were treated for diabetes mellitus. Fifty-eight percent of the population was considered as obese (body mass index ≥30). Among obese subjects, 16% were receiving a lipid-lowering therapy. Patients were separated into 3 groups with respect to their alcohol consumption: abstention or low alcohol intake (<10 g/day), moderate consumption (10–30 g/day), and elevated consumption (>30 g/day). Heavy drinkers represented only 8% of the population. Approximately 16% of women were smokers, and 34% declared to have a regular physical practice defined as at least 30 minutes exercise training per day.

Blood samples were obtained from subjects after a 12-hour overnight fast and were collected into sterile EDTA-containing tubes (final concentration 1 mg/mL). Plasma was separated from blood cells by low-speed centrifugation at 2500 rpm for 20 minutes at 4°C and frozen at −80°C until used. The study was performed in accordance with the ethical principles set forth in the Declaration of Helsinki. Written informed consent was obtained from all subjects.

FC Efflux Assays

Efflux assays were performed using human THP-1 macrophages and several cellular models Fu5AH, Chinese hamster ovary (CHO) -K1, CHO-hABC1, and CHO-hABCA1 as previously described.8,24–26 3H-cholesterol-labeled cells were incubated 4 hours at 37°C in the presence of 40-fold diluted total plasma and, when indicated, in the presence of purified CETP. Cholesterol efflux to isolated HDL particles was measured by incubating cells in the presence of 10% of the total HDL2 and HDL3 particles isolated from 1 mL of plasma or in the presence of fixed HDL-phospholipids concentrations: 15 µgPL/mL for cholesterol loaded–THP-1 macrophages; 10 µgPL/mL for Fu5AH (SR-BI–dependent efflux); 5 µgPL/mL for CHO-K1, and CHO-K1 overexpressing human ABCG1 gene. ABCG1-dependent efflux was calculated as the difference between efflux to CHO-hABC1 and CHO-K1 cells. In CHO-hABCA1, the expression of ABCA1 was induced by tetracycline (1 µg/mL). The ABCA1-dependent efflux was calculated as the amount of the label recovered in the medium divided by the total label in each well. The background cholesterol efflux obtained in the absence of any acceptor was subtracted from the efflux obtained with samples. A standard pool of human plasma was tested in all efflux experiments. Plasma efflux capacity was expressed as a relative efflux obtained by dividing the fractional efflux of the sample value by those obtained with the standard plasma. The capacity of individual HDL subfractions to mediate FC efflux is expressed as a percentage of cholesterol efflux per mole of acceptor particle as previously described.24–26 All efflux experiments were performed in triplicate for each sample. Others methods are described in the online-only Data Supplement.

Results

CETP Activity Represents an Independent Predictor of Plasma Cholesterol Efflux Capacity From Human Macrophages

We observed that the capacity of total plasma to mediate FC efflux was significantly correlated with plasma HDL-C (β=0.12, P=0.02) and apoA1 (β=0.19; P<0.001) levels (Table 1 in the online-only Data Supplement). Interestingly, plasma LDL-C, apoB, and TG levels were positively associated with plasma cholesterol efflux capacity (β=0.15, P=0.006; β=0.17, P=0.003; and β=0.11, P=0.05, respectively).
As shown in Table 2, univariate regression analyses revealed that endogenous CETP activity was associated with an increase in plasma cholesterol efflux capacity from human macrophages ($\beta=0.16$, $P=0.003$; Figure I in the online-only Data Supplement). In multiple regression analyses, adjusted for age, body mass index, lipid-lowering therapy, smoking, alcohol consumption, and physical activity, the regression coefficient between CETP activity and plasma efflux capacity remained significant ($\beta=0.15$, $P=0.006$). After additional adjustment for HDL-C, LDL-C, and TG, CETP activity remained a significant predictor of THP-1 plasma cholesterol efflux efficiency ($\beta=0.015$, $P=0.001$). It is important to note that interaction test revealed no significant impact of either obesity ($P=0.17$) or lipid-lowering therapy ($P=0.46$) on multiple regression analyses between endogenous plasma CETP activity and cholesterol efflux from cholesterol-loaded macrophages.

To further analyze the relationship between endogenous plasma CETP activity and the ability of plasma to mediate THP-1 FC efflux, we constituted 2 subgroups of subjects according to their endogenous plasma CETP activity as follows: the low CETP group corresponding to subjects displaying an endogenous plasma CETP activity within the first tertile (<27.4%) and the high CETP group corresponding to subjects displaying an endogenous plasma CETP activity within the third tertile (>34%). As shown in Table 1, the 2 groups were matched for age, body mass index, lipid-lowering therapy, smoking, alcohol consumption, and physical activity. Both plasma HDL-C and apoA1 levels were significantly reduced in women from the high CETP activity group as compared with those from the low CETP activity group. By contrast, plasma total cholesterol, LDL-C, apoB, and TG levels were significantly elevated in the high CETP group as compared with those from the low CETP group. In addition, plasma from women with high CETP activity displayed a significant increased capacity (+8.2%, $P=0.001$) to mediate cholesterol efflux from human THP-1 macrophages as compared with those from the low CETP activity group. Such a relationship between endogenous plasma CETP activity and the capacity of plasma to mediate cholesterol efflux from human macrophages remained significant after adjustment for lipid parameters, including LDL-C, HDL-C, and TG (Table 2). In this context, it is relevant to consider that in addition to HDL particles, apoB-containing lipoproteins have been shown to represent potential cellular cholesterol acceptors for both the SR-BI– and the ABCG1–mediated cholesterol efflux.27–29 We, thus, evaluated the potential contribution of apoB-containing lipoproteins by measuring cholesterol efflux from human
cholesterol efflux in subjects from the high CETP group as in the capacity of plasma to mediate ABCA1-dependent efflux. Indeed, we observed a marked increase (+23.4%, \(P<0.0001\)) in the capacity of plasma to mediate ABCA1-dependent efflux, SR-BI, ABCG1, and ABCA1. As shown in Table 3, CETP activity was associated with an increased capacity of plasma to mediate ABCA1-dependent cellular FC efflux (\(\beta=0.14, P=0.01\)). Indeed, we observed a marked increase (+23.4%, \(P=0.0001\)) in the capacity of plasma to mediate ABCA1-dependent cholesterol efflux in subjects from the high CETP group as compared with those from the low CETP group. In multivariate analyses adjusted for clinical parameters (age, body mass index, and lipid-lowering therapy), lifestyle factors (smoking, alcohol consumption, and physical activity), or for additional biological parameters (HDL-C, LDL-C, and TG) as covariates, difference in plasma efflux capacity between the 2 subject groups remained significant (\(P=0.02\)). By contrast, neither the ABCG1 nor human homologue CD36- and LIMPII-analogous 1–/– nor human homologue CD36- and LIMPII-analogous 1–/– SR-BI–dependent efflux was significantly associated with endogenous plasma CETP activity. These latter observations suggest that increased capacity of plasma from subjects with elevated CETP activity to mediate cholesterol efflux from macrophages might result, at least in part, from an increased ABCA1-dependent plasma efflux capacity. Because lipid-free/low apoAI particles, such as pre–β-HDL, have been shown to represent the preferential cholesterol acceptor for the ABCA1-dependent efflux, \(^{30}\) we have quantified plasma pre–β1-HDL levels in subjects from both low and high CETP groups. As shown in Figure 1A, pre–β1-HDL concentrations appeared to be significantly increased (+29.6%, \(P=0.01\)) in the plasma from women displaying a high CETP activity as compared with those displaying a low CETP activity (130.5±9.1 µg/L and 100.7±6.8 µg/mL in women from the high and the low CETP group, respectively). In addition, we observed a significant positive relationship between pre–β1-HDL levels and the capacity of total plasma to mediate FC efflux from human THP-1 macrophages (\(r=0.29, P=0.02\)). In conclusion, a high endogenous plasma CETP activity is associated with elevated pre–β1-HDL levels, which likely account for the observed increase of plasma efflux capacity through the ABCA1-dependent efflux pathway.

### Table 2. Univariate and Multivariate Analyses of the Impact of Plasma Endogenous CETP Activity on the Ability of Plasma to Mediate Free Cholesterol Efflux From Human Macrophages

<table>
<thead>
<tr>
<th>CETP Activity Group</th>
<th>Total Population (n=348) Per 1 SD Increase in CETP Activity</th>
<th>L-CETP Group (n=116) CETP Activity &lt;27.4%</th>
<th>H-CETP Group (n=116) CETP Activity &gt;34%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-Regression Coefficient</td>
<td>0.16</td>
<td>7.81 (2.60)</td>
<td>0.01</td>
</tr>
<tr>
<td>(P) value</td>
<td>0.003</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Adjusted for clinical/lifestyle factors</td>
<td>0.15</td>
<td>7.26 (2.63)</td>
<td>0.006</td>
</tr>
<tr>
<td>(P) value</td>
<td>0.006</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>+Adjustment for HDL-C, LDL-C, and TG</td>
<td>0.15</td>
<td>7.87 (3.18)</td>
<td>0.01</td>
</tr>
<tr>
<td>(P) value</td>
<td>0.01</td>
<td></td>
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</tbody>
</table>

CETP indicates cholesteryl ester transfer protein; L-CETP, low CETP; H-CETP, high CETP; TG, triglycerides. Clinical parameters used for multivariate analyses are age, body mass index, and lifestyle factors used for multivariate analyses are alcohol consumption, smoking, and physical activity. The L-CETP activity group corresponds to subjects displaying an endogenous plasma CETP activity within the first tertile (<27.4%), and the H-CETP activity group corresponds to subjects displaying endogenous plasma CETP activity within the third tertile (>34%).

To demonstrate the influence of CETP on cellular cholesterol efflux process, we performed efflux experiments in the presence of purified CETP (Figure III in the online-only Data Supplement). The capacity of whole plasma to mediate FC efflux from cholesterol-loaded THP-1 macrophages was significantly increased (+29.6%, \(P<0.0001\)) in subjects displaying a high CETP activity (12.8±1.1% and 7.3±0.7% in women from the high CETP group and from the low CETP group, respectively; Figure II in the online-only Data Supplement), indicating that elevation in endogenous plasma CETP activity represents a positive predictor of the capacity of plasma to mediate FC efflux from human macrophages independent of plasma lipid variations.

To further analyze the mechanism underlying the relationship between the capacity of plasma to mediate cellular FC efflux and endogenous plasma CETP activity, we used various cellular models, each representative of one efflux pathway: CLA1, SR-BI, ABCG1, and ABCA1. As shown in Table 3, CETP activity was associated with an increased capacity of plasma to mediate ABCA1-dependent cellular FC efflux (\(\beta=0.14, P=0.01\)). Indeed, we observed a marked increase (+23.4%, \(P=0.0001\)) in the capacity of plasma to mediate ABCA1-dependent cholesterol efflux in subjects from the high CETP group as compared with those from the low CETP group. In multivariate analyses adjusted for clinical parameters (age, body mass index, and lipid-lowering therapy), lifestyle factors (smoking, alcohol consumption, and physical activity), or for additional biological parameters (HDL-C, LDL-C, and TG) as covariates, difference in plasma efflux capacity between the 2 subject groups remained significant (\(P=0.02\)). By contrast, neither the ABCG1 nor human homologue CD36- and LIMPII-analogous 1–/– SR-BI–dependent efflux was significantly associated with endogenous plasma CETP activity. These latter observations suggest that increased capacity of plasma from subjects with elevated CETP activity to mediate cholesterol efflux from macrophages might result, at least in part, from an increased ABCA1-dependent plasma efflux capacity. Because lipid-free/low apoAI particles, such as pre–β-HDL, have been shown to represent the preferential cholesterol acceptor for the ABCA1-dependent efflux, \(^{30}\) we have quantified plasma pre–β1-HDL levels in subjects from both low and high CETP groups. As shown in Figure 1A, pre–β1-HDL concentrations appeared to be significantly increased (+29.6%, \(P=0.01\)) in the plasma from women displaying a high CETP activity as compared with those displaying a low CETP activity (130.5±9.1 µg/L and 100.7±6.8 µg/mL in women from the high and the low CETP group, respectively). In conclusion, a high endogenous plasma CETP activity is associated with elevated pre–β1-HDL levels, which likely account for the observed increase of plasma efflux capacity through the ABCA1-dependent efflux pathway.

### Impact of Endogenous Plasma CETP Activity on Quantitative and Qualitative Features of Mature HDL Particles

As shown in Figure 1B, plasma concentrations of large HDL2 subfraction were significantly reduced (−17.8%, \(P=0.009\)) in women from the high CETP activity subgroup as compared with those from the low CETP subgroup (135.4±7.8 mg/dL and 164.8±7.8 mg/dL, respectively; Figure 1B). By contrast,
HDL3 levels were significantly increased by 14.2% \((P=0.007)\) in subjects with elevated plasma CETP activity in comparison with those with lower CETP activity \((122.8±4.7 \text{ mg/dL and } 107.5±2.9 \text{ mg/dL}, \text{respectively}; \text{Figure } 1C)\). The chemical composition of each HDL subfraction, expressed as percentage of FC, esterified cholesterol, TG, phospholipids, and protein contents determined in women subjects from the low and high CETP groups is presented in Table II in the online-only Data Supplement. A significant mean reduction in the CE/TG ratio in both HDL2 \((-36.3%; \text{P}<0.0001)\) and HDL3 \((-16.5%; \text{P}=0.0108)\) was observed in women displaying a high CETP activity as compared with those displaying a low CETP activity.

**Elevated Plasma CETP Activity Is Associated With an Increased Capacity of HDL2 to Accept FC From Human Macrophages**

Considering latter evidences that CETP activity modulates HDL particle composition, we further explored the capacity of individual HDL particles to accept FC efflux from human THP-1 macrophages. Fractional cholesterol efflux expressed as a function of HDL mole number revealed that HDL2 particles isolated from the plasma of women from the high CETP activity group displayed a significant elevated capacity \((+24.3%; P=0.003)\) to mediate FC efflux from macrophages than those from the low CETP activity group (Figure 2A). By contrast, we observed that the capacity of HDL3 subfraction for FC efflux from macrophages was similar in both groups.

We equally measured the efflux capacity of the overall HDL2 and HDL3 subfractions, reflecting both quantitative and qualitative features of HDL particles, by performing efflux experiments in the presence of 10% of the total HDL2 or HDL3 mass of individual subject’s plasma (Figure 2B). Under these conditions, total HDL2- or HDL3-mediated efflux from macrophages was similar in the 2 subject groups. We thus conclude that in subjects with elevated endogenous plasma CETP activity, the observed increased capacity of HDL2 particles to mediate FC efflux from macrophages counterbalanced the reduced number of circulating HDL2 particles in those individuals, leading to a similar total HDL2 efflux capacity than subjects with lower CETP activity.

**Impact of Plasma CETP Activity on HDL-Mediated SR-BI and ABCG1-Dependent Efflux**

Mature HDL subfractions have been described to represent efficient FC acceptors through ABCG1 and SR-BI efflux pathways, their relative efflux capacity being associated with HDL particle size and composition.

27,31,32 To better understand the mechanisms underlying the relationship between plasma CETP activity and HDL efflux capacity, we evaluated the capacity of individual HDL subfraction from both high CETP and low CETP groups, to mediate SR-BI– and ABCG1-dependent FC efflux using our specific cell models (Figure 3). We observed that HDL2 particles isolated from subjects with a high CETP activity displayed a decreased capacity \((-11.7%,\text{P}=0.0108)\) to mediate FC efflux from macrophages compared to those from the low CETP group.

Figure 1. Impact of endogenous cholesteryl ester transfer protein (CETP) activity on plasma levels of pre-\(\beta\)-1-high-density lipoprotein (pre-\(\beta\)-1-HDL; A), HDL2 (B), and HDL3 (C) determined in subjects \((n=49)\) displaying a CETP activity within the first tertile (low CETP \([\text{L-CETP}]\); CETP activity \(<27.4\%); \text{open bar}) and in subjects \((n=49)\) displaying a CETP activity within the third tertile (high CETP \([\text{H-CETP}]\); CETP activity \(>34\%); \text{closed bar}). Values are means±SEM. **\(P<0.01\) and *\(P<0.05\) vs the L-CETP group.
of HDL2 particles (Figure IV and Table II in the online-only Data Supplement). The capacity of HDL2 to mediate cholesterol efflux via the ABCG1 pathway was unaffected by CETP activity (Figure 3B). By contrast, elevated endogenous plasma CETP activity appeared to be associated with a significant reduction (~13.2%, \( P=0.007 \)) in the capacity of HDL3 particles to mediate cholesterol efflux via the ABCG1 pathway (Figure 3B). However, concomitant increase in plasma levels of HDL3 led to counterbalance the reduced HDL3 efflux capacity observed in high CETP activity group (Figure IVB in the online-only Data Supplement).

Discussion

The present study identified endogenous plasma CETP activity as a key modulator of the ability of whole plasma to mediate FC efflux from human macrophages. Elevated endogenous CETP activity is associated with increased plasma levels of pre-\(\beta\)-HDL, which allow an increased plasma efflux capacity from cholesterol-loaded THP-1 macrophages likely via the ABCA1-dependent pathway. Thus, despite the CETP-induced reduction in plasma HDL-C, we report that cholesterol efflux from macrophages is favored in women subjects with high CETP. These observations highlight that HDL-C rising strategy using CETP inhibitor could impair the capacity of whole plasma to manage macrophage RCT.

First, we explored the impact of endogenous CETP activity on whole plasma efflux capacity. The observed impact of endogenous plasma CETP activity on the distribution of HDL particles is entirely consistent with the known mechanism of action of CETP. In particular, CETP favors the formation of small HDL and pre-\(\beta\) HDL particles at the expense of large HDL2.\(^6\) Earlier findings have reported a significant relationship between plasma pre-\(\beta\)-HDL levels and ABCA1-dependent cellular cholesterol efflux.\(^{33}\) In addition, it has been recently demonstrated that for equivalent HDL-cholesterol levels, the capacity of plasma to mediate macrophage cholesterol efflux is related to pre-\(\beta\)-HDL concentrations and occurs via an ABCA1-dependent process.\(^{34}\) In line with those latter observations, here we report a positive relationship between elevated endogenous CETP activity and the capacity of whole plasma to mediate ABCA1-dependent efflux, which appeared to be related to plasma pre-\(\beta\)-HDL levels. Our findings provide strong evidence of the beneficial action of endogenous plasma CETP activity on intravascular remodeling of HDL particles and pre-\(\beta\)-HDL formation and its major role in maintaining efficient removal of cholesterol from human macrophages despite reduced levels of circulating HDL-C. We demonstrated that the relationship between endogenous plasma CETP activity and plasma efflux capacity of human macrophages is independent of plasma levels of LDL-C, HDL-C, and TG. However, it has been demonstrated that very-low-density lipoprotein and LDL particles may contribute significantly to cholesterol efflux from cells to plasma. Indeed, in addition to HDL, apoB-containing lipoprotein particles have been demonstrated to equally mediate cellular cholesterol from human macrophages.\(^{35,36}\) Moreover, several studies have demonstrated that a continuous transfer of cholesterol from HDL to apoB-containing lipoprotein is required to maintain efficient HDL-mediated cholesterol efflux from cells.\(^{35,36}\)
Second, we focused on intrinsic HDL efflux functionality. Interestingly, we observed that elevated CETP activity allows the formation of HDL2 particles with reduction of CE/TG ratio, which display a diminished capacity to mediate cellular cholesterol efflux via SR-BI pathway. Indeed, it has been demonstrated that a reduced CE/TG ratio in HDL particles is associated with an impaired interaction between HDL and SR-BI,\(^4,5\) the latter being improved after CETP inhibition therapy.\(^5\) Because HDL2-mediated ABCG1 efflux did not differ between the 2 CETP groups, the reduced ability of HDL2 particles for SR-BI-mediated efflux may appear controversial with the observed increased macrophage cholesterol efflux to HDL2 particles isolated from patients with elevated CETP activity. In addition to receptor- or transporter-mediated efflux pathways, removal of FC from cells can occur by a passive aqueous transfer mechanism, which is influenced by acceptor size and FC content.\(^6,7\) However, changes in chemical composition of HDL2 particles observed as a function of CETP activity do not support modification in the passive diffusion process. Indeed, in women subjects presently studied, elevated CETP activity was associated with the formation of HDL2 particles slightly enriched in FC, displaying no significant variation in the surface to core lipid ratio as compared with women with lower CETP activity.

Here, we report that an elevation in plasma CETP activity is associated with an enhanced capacity of whole plasma to promote cholesterol efflux from human macrophages. This observation is entirely consistent with a recent study showing that reduction of circulating CETP concentration following after caloric restriction induced a significant reduction of plasma efflux capacity from cholesterol-loaded human THP-1 macrophages.\(^8\) Equally, bariatric surgery–induced weight loss has been shown to significantly reduce both endogenous plasma CETP activity and the capacity of whole plasma to mediate cellular cholesterol efflux via an ABCA1-dependent pathway.\(^9\) By contrast, it has been demonstrated that pharmacological inhibition of CETP activity by torcetrapib or anacetrapib enhanced HDL-mediated efflux from macrophages resulting from increased HDL2 functionality and quantity.\(^10\) However, using total plasma as acceptor, torcetrapib had no significant impact on the capacity of whole plasma to mediate cholesterol efflux from human macrophages.\(^11\) It is likely that these apparent conflicting observations might result from the mechanistic action of CETP inhibitors that may impair pre-β-HDL formation\(^12\) Indeed, torcetrapib and anacetrapib have been shown to block major lipid transfer mediated by CETP including not only hetero exchange of neutral lipid between HDL and apoB-containing lipoproteins but also lipid redistribution among HDL subspecies, thus impairing the intravascular HDL remodeling process.\(^13\) In contrast with torcetrapib or anacetrapib, dalcetrapib modulates plasma CETP activity by limiting CE transfer from HDL to very-low-density lipoprotein and LDL but maintains pre-β-HDL formation in human plasma.\(^14\) However, although in vivo animal studies have demonstrated that dalcetrapib can significantly improve fecal sterol elimination as compared with other CETP inhibitors, clinical evaluation of dalcetrapib in humans has recently been stopped due to the lack of cardiovascular benefits.\(^15\)

It is important to note that conclusions reached from the present study conducted in women might not be transposed to men. Analysis of a mixed population was not appropriate because expression of most of the key genes involved in the RCT pathway, including CETP, hepatic lipase, or CLA1/ SR-BI, is influenced by sex hormones.\(^16,17\) In addition, we have previously demonstrated that gender-specific mechanisms involved in the intravascular remodeling of HDL particles contribute significantly to modulate the capacity of circulating HDL particles to mediate the initial step of the RCT pathway.\(^18\) Interestingly, similar analyses performed in men \(n=409\) did not reveal significant \(P=0.80\) association between endogenous plasma CETP activity and plasma efflux capacity from either cholesterol-loaded human macrophages or specific efflux pathways, (ie, ABCA1, ABCG1, or SR-BI [data not shown]).

Epidemiological studies currently available do not converge to a clear conclusion that reduction of CETP activity and concomitant elevation in circulating HDL-C levels result in a significant reduction of cardiovascular risk.\(^19\) This reflects that CETP exerts both proatherogenic and antiatherogenic actions.\(^20\) The present study, focused on the first step of RCT, affords new insight on the antiatherogenic function of physiological CETP. We suggest that the action of endogenous CETP activity leading to the improvement of plasma efflux capacity from human macrophages, as a result of efficient pre-β-HDL formation and ABCA1 efflux stimulation, should be preserved to prevent lipid accumulation in macrophages within atherosclerotic plaques. Indeed, ABCA1 is crucial in the removal of cholesterol from human macrophages.\(^21\) Numerous studies conducted in mice have well demonstrated the determinant role of ABCA1 in prevention of foam cell formation and atherosclerosis development via the promotion of macrophage RCT.\(^22\) In humans, infusion of recombinant apoA-I Milano is associated with a significant regression in atheroma volume in treated patients.\(^23\) Sera from apoA-I Milano patient, characterized by a relative abundance of pre-β like HDL particles as compared with large HDL species, have been described to display an increased capacity to remove cholesterol from macrophage through the ABCA1 pathway.\(^24\) CETP inhibition constitutes a major target for therapy against cardiovascular disease; however, the present study provides evidence for the importance to maintain sufficient CETP activity to preserve the first step of macrophage RCT. Regulation of CETP activity to an optimal level, leading to both reduction of atherogenic CETP actions and also preservation of its beneficial properties, will constitute a future therapeutic challenge.

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Disclosures
None.

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Elevated CETP Activity Improves Plasma Cholesterol Efflux Capacity From Human Macrophages in Women
Elise F. Villard, Petra El Khoury, Emilie Duchene, Dominique Bonnefont-Rousselot, Karine Clement, Eric Bruckert, Randa Bittar, Wilfried Le Goff and Maryse Guerin

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Statistical analyses

Statistical analyses were performed with GraphPad Prism and the R statistical software 2.12.2. Numerical variables were presented as means ± SD. Non normally distributed variables (triglycerides) were presented as median and interquartiles range. Mean difference between two groups were compared using unpaired t-test. For categorical variables, differences between the two CETP subgroups were evaluated with Chi2 test. Univariate linear regression analyses were used to determine relationships between two numerical variables or one numerical and one categorical variable. Multivariate linear regression analyses for the impact of endogenous CETP activity on plasma efflux capacity were performed in a first model with age, BMI, lipid-lowering drugs, smoking, alcohol consumption and physical activity practice as covariates. In a second model, HDL-C, LDL-C and logTG were included. Endogenous plasma CETP activity in univariate and multivariate regression analyses was considered as continuous variable or as categorical variable (H-CETP versus L-CETP). In the case of continuous CETP values, regression results were expressed as beta-regression coefficient corresponding to the impact of 1SD increase of the predictive variable (CETP) on the variability of the explained variable (plasma efflux); in the case of CETP groups (H-CETP/L-CETP) results correspond to the slope of explained variable according to H-CETP group with L-CETP group as referent. P-value <0.05 were considered statistically significant.
References


3. de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJ, Rothblat GH. The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein cholesterol to remove cholesterol from macrophages. *Arterioscler Thromb Vasc Biol.* 2010;30:796-801.


Supplemental Table I:

Factors impacting the capacity of plasma to mediate free cholesterol efflux from human macrophages

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<tr>
<td>Age</td>
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<td>Body Mass Index</td>
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<td>Smoking</td>
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<tr>
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</table>

Beta regression coefficient are shown. BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein.
The table below shows the chemical composition of major HDL subfractions according to endogenous plasma CETP activity, with data expressed as mean± SD.

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<tr>
<td><strong>HDL2</strong></td>
<td></td>
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</tr>
<tr>
<td>Phospholipids (%)</td>
<td>32.4 ± 3.3</td>
<td>31.6 ± 3.4</td>
<td>+2.5%</td>
<td>ns</td>
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<tr>
<td>Triglycerides (%)</td>
<td>4.9 ± 1.4</td>
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<tr>
<td>Cholesteryl Esters (%)</td>
<td>18.8 ± 3.1</td>
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<td>Free Cholesterol (%)</td>
<td>5.2 ± 1.3</td>
<td>5.5 ± 2.2</td>
<td>+5.8%</td>
<td>ns</td>
</tr>
<tr>
<td>Total Protein (%)</td>
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</tr>
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<td>CE/TG (PL+FC)/(TG+CE)</td>
<td>3.83</td>
<td>2.44</td>
<td>-36.3%</td>
<td>&lt;0.0001</td>
</tr>
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<td><strong>HDL3</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospholipids (%)</td>
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<td>Triglycerides (%)</td>
<td>4.8 ± 1.7</td>
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<td>Cholesteryl Esters (%)</td>
<td>14.8 ± 2.1</td>
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<td>2.5 ± 0.7</td>
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<td>1.63</td>
<td>+8.7%</td>
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The chemical composition of HDL subfraction was determined in subjects displaying endogenous plasma CETP activity within the first tertile (L-CETP; CETP activity <27.4%) and in subjects displaying endogenous plasma CETP activity within the third tertile (H-CETP; CETP activity > 34%). Data are expressed as mean± SD.
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Supplemental Figure IV

Bar graph showing the capacity HDL2 and HDL3 subfractions isolated from subjects displaying a CETP activity in the first tertile (L-CETP, open bar) and from subjects displaying a CETP activity in the third tertile (H-CETP, closed bar) to mediate cellular free cholesterol efflux via SR-BI (panel A) or via ABCG1 (panel B). Fractional cholesterol efflux was determined after 4 hours incubation in the presence of 10% of total isolated HDL particles. Values are mean ± SEM. *p<0.05 versus L-CETP group.