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Plasma Cholesteryl Ester Transfer, But Not Cholesterol Esterification, Is Related to Lipoprotein-Associated Phospholipase A₂: Possible Contribution to an Atherogenic Lipoprotein Profile

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Context: Plasma lipoprotein-associated phospholipase A₂ (Lp-PLA₂) predicts incident cardiovascular disease and is associated preferentially with negatively charged apolipoprotein B-containing lipoproteins. The plasma cholesteryl ester transfer (CET) process, which contributes to low high-density lipoprotein cholesterol and small, dense low-density lipoproteins, is affected by the composition and concentration of apolipoprotein B-containing cholesteryl ester acceptor lipoproteins.

Objective: We tested relationships of CET with Lp-PLA₂ in subjects with and without metabolic syndrome (MetS).

Design and Setting: In 68 subjects with MetS and 74 subjects without MetS, plasma Lp-PLA₂ mass, cholesterol esterification (EST), lecithin:cholesterol acyltransferase (LCAT) activity level, CET, CET protein (CETP) mass, and lipoproteins were measured.

Results: EST, LCAT activity, CET (∼0.001 for all), and CETP (∼0.030) were increased, and Lp-PLA₂ (∼0.043) was decreased in MetS. CET was correlated positively with Lp-PLA₂ in subjects with and without MetS (∼0.005 for both). EST and LCAT activity were unrelated to Lp-PLA₂, despite a positive correlation between EST and CET (∼0.001). After controlling for age, sex, and diabetes status, CET was determined by Lp-PLA₂ in the whole group (∼0.245; ∼0.001), and in subjects with (∼0.304; ∼0.001) and without MetS (∼0.244; ∼0.006) separately, independently of triglycerides and CETP.

Conclusions: Plasma CET is related to Lp-PLA₂ in subjects with and without MetS. The process of CET, but not EST, may be influenced by Lp-PLA₂. These findings provide a rationale to evaluate whether maneuvers that inhibit Lp-PLA₂ will reduce CET, and vice versa to document effects of CETP inhibition on Lp-PLA₂. (J Clin Endocrinol Metab 96: 1077–1084, 2011)
droperoxides, which results in the generation of proinflammatory oxidized nonesterified fatty acids, fatty acid hydroperoxides, and lysophospholipids (1–3, 5, 6). The potential pathogenic role of Lp-PLA2 in cardiovascular disease development has received much attention in the past few years (1–3). A recent meta-analysis, comprising previously published and unpublished data from more than 79,000 individuals, has unequivocally demonstrated independent positive relationships between plasma Lp-PLA2 mass and activity levels and incident cardiovascular disease (7).

Despite growing interest in the role of Lp-PLA2 in atherosclerosis, its possible effects on lipoprotein metabolism are largely unknown. The process of cholesteryl ester transfer (CET), mediated by CET protein (CETP), represents an important pathway whereby cholesteryl esters are transported from HDL toward apo B-containing lipoproteins (8–12). Plasma CET will decrease cholesterol in HDL particles and increase the cholesterol content in apo B-containing lipoproteins, as well as contribute to the formation of small, dense LDL. This provides a rationale for the development of novel drugs that inhibit CETP (11–14). Importantly, the rate of CET toward apo B-containing acceptor lipoproteins is affected by their size, protein content, and lipid composition (8, 9, 15–17). In this regard, it is relevant that the CET process is also governed by the charge characteristics of the acceptor lipoproteins, in such a way that negatively charged particles preferentially accept cholesteryl esters (16, 18). This may explain why CET is enhanced upon association of nonesterified fatty acids with cholesteryl ester acceptor lipoproteins (19). In turn, several reports indicate that Lp-PLA2 associates preferentially with negatively charged apo B-lipoproteins, including small, dense LDL (3, 20, 21). Therefore, it is plausible to hypothesize that the plasma Lp-PLA2 level is a determinant of CET.

The present study was carried out to determine whether plasma CET is related to Lp-PLA2, independently of plasma lipoproteins. Enhanced CET has been shown in subjects with the metabolic syndrome (MetS) (22) and accordingly in insulin-resistant individuals (23). We therefore decided to study relationships of plasma CET with Lp-PLA2 in subjects with and without MetS. Second, because plasma CET and cholesteryl esterification (EST) are thought to be coordinately regulated processes (24–26), we also evaluated relationships of plasma EST with Lp-PLA2.

### Subjects and Methods

The protocol was approved by the medical ethics committee of the University Medical Center Groningen, The Netherlands. The participants (aged >18 yr) were recruited by advertisement in local newspapers and provided written informed consent. Physical examination did not reveal pulmonary or cardiac abnormalities. Clinically manifest cardiovascular disease, renal insufficiency, thyroid disorders, liver diseases, current pregnancy, smoking, consumption of more than three alcoholic drinks per day, and statin therapy were exclusion criteria. The use of antihypertensive drugs was allowed. Subjects with type 2 diabetes mellitus (previously diagnosed by primary care physicians using glucose cutoff values as defined by the World Health Organization) were not excluded, except when using insulin or thiazolidinediones.

Body mass index (BMI) was calculated as weight divided by height squared (in kilograms/meter²). Waist circumference was measured between the 10th rib and the iliac crest. Systolic and diastolic blood pressure was measured in a sitting position with a sphygmomanometer after a 15-min rest. All subjects were studied after an overnight fast.

MetS was defined according to the revised National Cholesterol Education Program Adult Treatment Panel III criteria (27). Three or more of the following criteria were required for categorization of subjects with MetS: waist circumference larger than 102 cm for men and larger than 88 cm for women; hypertension (blood pressure ≥130/85 mm Hg or use of antihypertensive drugs); fasting plasma triglycerides of at least 1.7 mmol/liter; HDL cholesterol below 1.0 mmol/liter for men and below 1.3 mmol/liter for women; and fasting glucose of at least 5.6 mmol/liter or known diabetes.

### Laboratory analyses

Venous blood samples were collected into EDTA-containing tubes (1.5 mg/ml). Samples were prepared by centrifugation at 1400 × g for 15 min at 4 °C. Glucose was measured shortly after blood collection. Samples for other assays were kept frozen at −80 °C until assay.

Cholesterol and triglycerides were assayed by routine enzymatic methods (Roche/Hitachi catalog nos. 11875540 and 11876023, respectively; Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol was measured with a homogeneous enzymatic colorimetric test (Roche/Hitachi catalog no. 04713214; Roche Diagnostics GmbH). Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. Apo B was assayed by immunoturbidimetry (Roche/Cobas Integra Tinaquant catalog no. 03032574; Roche Diagnostics GmbH).

Plasma CET was assayed by a radioisotope method as described previously (26, 28). In short, [3H]cholesterol was equilibrated for 24 h with plasma cholesterol at 4 °C, followed by incubation at 37 °C for 3 h. Apo B-containing lipoproteins were then precipitated by the addition of phosphotungstate/MgCl2. Lipids were extracted from the precipitate, and the labeled cholesteryl esters were separated from labeled unesterified cholesterol on silica columns. This isotope method is an accurate measure of net mass transfer of cholesteryl esters from HDL to apo B-containing lipoproteins (29). Plasma EST in total plasma was measured as the generation of cholesteryl esters after addition of [3H]cholesterol to plasma according to a previously described procedure (26, 28). EST was assayed using the same incubation system as for the CET assay. The EST rate is linear with time for 5 h, indicating an excess of unesterified cholesterol in the assay system. All assays were performed in duplicate. The CET and EST measurements are expressed in nanomoles per milliliter per hour.

Subjects and Methods

The protocol was approved by the medical ethics committee of the University Medical Center Groningen, The Netherlands. The
hour. The within-assay coefficients of variation of plasma CET and EST are less than 7.5%.

Plasma CETP mass was measured with a double-antibody sandwich ELISA (courtesy of Dr. G. M. Dallinga-Thie, Amsterdam, The Netherlands) (30). A combination of monoclonal antibodies TP1 and TP2 was employed as coating antibodies, and monoclonal antibody TP20, labeled with digoxigenine, was the secondary antibody. The CETP control samples were validated using a RIA (carried out by Dr. R. M. McPherson, Montreal, Canada). The plasma CETP concentration is closely correlated using a comparable exogenous substrate method has been shown to be strongly correlated with its concentration in plasma (35, 36). CETP activity was related to the activity measured in human pool plasma; 100 AU is equivalent to 87 nmol cholesterol esterified per ml of plasma per hour. CETP mass and CETP activity were measured in duplicate, and their intraassay coefficients of variation are less than 5.0%.

Lp-PLA2 mass was assayed using a commercially turbidimetric immunoassay (PLAC Test, diaDexus catalog no. 10-0112; diaDexus Inc., San Francisco, CA) on the Modular P. The manufacturer’s instructions for thawing samples were strictly followed. In samples with a measured concentration exceeding 360 μg/liter, the presence of interfering heterophilic antibodies was excluded. The intraassay coefficient of variation was 1.7%, using the EP5 protocol (Evaluation of Precision Performance of Quantitative Measurement Methods) and buffered quality control at a concentration of 150 and 360 μg/liter.

Glucose was measured with an APEC glucose analyzer (APEC Inc., Danvers, MA).

**Statistical analysis**

Data are given as mean ± SD or as median (interquartile range) and were compared by unpaired t tests. Because of skewed distribution, logarithmically transformed values for triglycerides were used. Differences in proportions of variables were determined by χ² analysis. Univariate correlations were calculated using linear regression analysis using Pearson’s correlation coefficients. Multiple linear regression analysis was performed to disclose independent contributions of variables. Interactions were assessed in additional analyses. To this end, the group mean value of the continuous variable of interest was subtracted from the measured value to obtain a distribution centered on the mean. Product terms between the variables of interest were then calculated. Two-sided P values <0.05 were considered significant. For interaction terms, the level of significance was taken at P < 0.10.

**Results**

The study population consisted of 142 Caucasian subjects, of whom 68 fulfilled the criteria for MetS (Table 1). Subjects with MetS tended to be older, but sex distribution was not different between subjects with and without MetS. Twenty-five of the MetS subjects (37%) and five of the subjects without MetS (7%) were on antihypertensive medication (P < 0.001) (mostly angiotensin-converting enzyme inhibitors, angiotensin II antagonists, diuretics, and beta-blockers). Type 2 diabetes mellitus was more

**TABLE 1. Clinical characteristics, glucose, plasma lipids and lipoproteins, EST, CET, LCAT activity level, CETP concentration, and Lp-PLA2 in 68 subjects with and 74 subjects without MetS**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MetS present (n = 68)</th>
<th>MetS absent (n = 74)</th>
<th>P value</th>
<th>P value adjusted for age and sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>58 ± 9</td>
<td>55 ± 10</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>37/31</td>
<td>42/32</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes mellitus (n)</td>
<td>52</td>
<td>23</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>145 ± 19</td>
<td>132 ± 21</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>89 ± 9</td>
<td>81 ± 10</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.8 ± 4.4</td>
<td>25.1 ± 3.3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>104 ± 13</td>
<td>86 ± 11</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/liter)</td>
<td>8.6 ± 2.6</td>
<td>6.2 ± 1.5</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/liter)</td>
<td>5.59 ± 1.02</td>
<td>5.49 ± 0.92</td>
<td>0.54</td>
<td>0.58</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/liter)</td>
<td>4.40 ± 1.02</td>
<td>3.95 ± 0.96</td>
<td>0.008</td>
<td>0.007</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/liter)</td>
<td>1.19 ± 0.34</td>
<td>1.54 ± 0.38</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/liter)</td>
<td>1.94 (1.66–2.49)</td>
<td>1.12 (0.79–1.46)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apo B (g/liter)</td>
<td>1.00 ± 0.23</td>
<td>0.89 ± 0.21</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>EST (mmol/ml h)</td>
<td>66.8 ± 17.2</td>
<td>52.6 ± 13.9</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CET (mmol/ml h)</td>
<td>25.7 ± 8.6</td>
<td>19.3 ± 6.3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CETP mass (mg/liter)</td>
<td>2.52 ± 0.94</td>
<td>2.21 ± 0.70</td>
<td>0.030</td>
<td>0.034</td>
</tr>
<tr>
<td>LCAT activity (AU)</td>
<td>117 ± 15</td>
<td>105 ± 15</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lp-PLA2 (μg/liter)</td>
<td>290 ± 65</td>
<td>313 ± 73</td>
<td>0.048</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD or median (interquartile range). M, Male; F, female.
In all subjects combined, the plasma Lp-PLA₂ concentration was correlated positively with total cholesterol and non-HDL cholesterol, but not significantly with HDL cholesterol, triglycerides, LCAT activity levels, and CETP mass (Table 2). Comparable correlations were observed in subjects with and without MetS separately (Table 2), as well as in subjects without diabetes (data not shown).

The univariate correlations of EST with LCAT activity levels, lipids, lipoproteins, and plasma Lp-PLA₂ mass are shown in Table 3. Plasma EST was correlated positively with CET in all subjects combined, as well as in the separate groups of subjects with and without MetS. In all subjects together, in subjects with and without MetS separately (Table 3), and in non-diabetic subjects only (n = 67, data not shown), plasma EST was correlated positively with LCAT activity, triglycerides, and non-HDL cholesterol, but no relationship with Lp-PLA₂ was observed. In multiple linear regression analysis, plasma EST was found to be predicted independently and positively by plasma triglycerides (β = 0.444; P < 0.001) and LCAT activity levels (β = 0.341; P < 0.001) after controlling for age (β = 0.001; P = 0.99), sex (β = −0.084; P = 0.143), and diabetes status (β = 0.137; P = 0.035). This analysis again did not show a significant contribution of the Lp-PLA₂ concentration to plasma EST (β = 0.037; P = 0.55). Further analysis demonstrated that the absence of a relationship of Lp-PLA₂ with EST (β = 0.013; P = 0.83) was not confined by the use of antihypertensive and glucose-lowering drugs (data not shown). Additional multiple linear regression analyses in subjects with and without MetS separately also revealed no independent positive relationship of plasma EST with Lp-PLA₂ (β = 0.114, P = 0.276; and β = −0.091, P = 0.310, respectively, data not shown).

Plasma CET was found to be correlated positively with triglycerides and non-HDL cholesterol in all subjects together and in subjects with and without MetS separately.

### Table 2. Univariate correlations of the plasma Lp-PLA₂ concentration with plasma lipids and lipoproteins, LCAT activity level, and CETP mass in all subjects combined (n = 142) and in subjects with (n = 68) and without MetS (n = 74)

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n = 142)</th>
<th>MetS present (n = 68)</th>
<th>MetS absent (n = 74)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lp-PLA₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total cholesterol</td>
<td>0.294&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.204&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Non-HDL cholesterol</td>
<td>0.292&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.226&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol</td>
<td>−0.030</td>
<td>−0.070</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>−0.046</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>LCAT activity</td>
<td>−0.043</td>
<td>−0.109</td>
</tr>
<tr>
<td></td>
<td>CETP mass</td>
<td>0.064</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Pearson’s correlation coefficients are shown.

<sup>a</sup> P < 0.10; <sup>b</sup> P < 0.001.

### Table 3. Univariate correlations of plasma EST and CET with LCAT activity level, CETP mass, triglycerides, non-HDL cholesterol and plasma Lp-PLA₂ concentration in all subjects combined (n = 142) and in subjects with (n = 68) and without MetS (n = 74)

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n = 142)</th>
<th>MetS present (n = 68)</th>
<th>MetS absent (n = 74)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EST</td>
<td>CET</td>
<td>EST</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CET</td>
<td>0.664&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.521&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>LCAT activity</td>
<td>0.673&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.529&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>CETP mass</td>
<td>0.266&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.321&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>0.695&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.765&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Non-HDL cholesterol</td>
<td>0.432&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.649&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Lp-PLA₂</td>
<td>−0.057</td>
<td>0.169&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.246&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Pearson’s correlation coefficients are shown.

<sup>a</sup> P < 0.05; <sup>b</sup> P < 0.01; <sup>c</sup> P < 0.001.
In all subjects together and in subjects with MetS, plasma CET was also positively correlated with CETP mass. In the combined subjects and subjects with and without MetS separately, plasma CET was correlated positively with Lp-PLA2 (Table 3). A comparable trend was found in nondiabetic subjects only (n = 67; r = 0.187; P = 0.129). Multiple linear regression analysis demonstrated that in the combined subjects plasma CET was independently and positively related to plasma triglycerides, CETP, and Lp-PLA2 after controlling for age, sex, and diabetes status (Table 4). A graphical presentation of the relationships of plasma CET with Lp-PLA2 and triglycerides, CETP, and Lp-PLA2 after adjusting for age, sex, and diabetes status is provided in Figs. 1 and 2, respectively. Additional multiple linear regression analyses in subjects with and without MetS (Table 4), as well as in subjects without diabetes (β = 0.213; P = 0.025), showed similar independent relationships of plasma CET with Lp-PLA2. The relationship of CET with Lp-PLA2 remained significant (β = 0.167; P = 0.003) after further adjustment for non-HDL cholesterol and apo B, as well as after controlling for the use of medication (antihypertensives, sulfonylurea, and metformin) (β = 0.236; P = 0.001; data not shown). No interactions of plasma Lp-PLA2 with either triglycerides (β = 0.019; P = 0.70) or CETP mass (β = 0.001; P = 0.99) on plasma CET were observed.

**Discussion**

This study shows for the first time that plasma CET is correlated positively with the Lp-PLA2 concentration in subjects with and without MetS. This effect could not be ascribed to a relationship between plasma triglycerides, CETP, and Lp-PLA2 after adjusting for age, sex, and diabetes status (Table 4). A graphical presentation of the relationships of plasma CET with Lp-PLA2 and triglycerides, CETP, and Lp-PLA2 after adjusting for age, sex, and diabetes status is provided in Figs. 1 and 2, respectively. Additional multiple linear regression analyses in subjects with and without MetS (Table 4), as well as in subjects without diabetes (β = 0.213; P = 0.025), showed similar independent relationships of plasma CET with Lp-PLA2. The relationship of CET with Lp-PLA2 remained significant (β = 0.167; P = 0.003) after further adjustment for non-HDL cholesterol and apo B, as well as after controlling for the use of medication (antihypertensives, sulfonylurea, and metformin) (β = 0.236; P = 0.001; data not shown). No interactions of plasma Lp-PLA2 with either triglycerides (β = 0.019; P = 0.70) or CETP mass (β = 0.001; P = 0.99) on plasma CET were observed.

**TABLE 4.** Multiple linear regression analyses showing relationships of plasma CET with CETP mass, triglycerides, and Lp-PLA2 in all subjects combined (n = 142), subjects with MetS (n = 68), and subjects without MetS (n = 74)

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>Subjects with MetS</th>
<th>Subjects without MetS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>P value</td>
<td>β</td>
</tr>
<tr>
<td>Diabetes (yes/no)</td>
<td>0.178</td>
<td>0.003</td>
<td>0.143</td>
</tr>
<tr>
<td>Ln triglycerides</td>
<td>0.723</td>
<td>&lt;0.001</td>
<td>0.666</td>
</tr>
<tr>
<td>CETP mass</td>
<td>0.148</td>
<td>0.005</td>
<td>0.265</td>
</tr>
<tr>
<td>Lp-PLA2</td>
<td>0.245</td>
<td>&lt;0.001</td>
<td>0.304</td>
</tr>
</tbody>
</table>

All models are adjusted for age and sex. β, Standardized regression coefficient.

**FIG. 1.** Graphical presentation of the relationships of plasma CET with Lp-PLA2 and triglycerides. Standardized regression coefficients (β values) from the multiple linear regression analysis model given in Table 4 are used.

**FIG. 2.** Graphical presentation of the relationships of plasma CET with Lp-PLA2 and CETP mass. Standardized regression coefficients (β values) from the multiple linear regression analysis model given in Table 4 are used.
Lp-PLA₂. In contrast, plasma EST was unrelated to Lp-PLA₂, despite strong interrelations between EST and CET.

We decided to compare subjects with and without MetS in the expectation that plasma CET is increased in MetS (22, 23), as confirmed in this report. Plasma CET was not only affected by the triglyceride concentration, as a reflection of the concentration of triglyceride-rich lipoproteins, but to some extent also by CETP mass. Notably, the effect of CETP mass on CET was not significant among subjects without MetS. This finding is consistent with the proposition that CETP may become rate limiting in the CET process in hypertriglyceridemia (37). In agreement, it was found earlier that plasma CET is only weakly related to CETP activity, measured using an exogenous substrate assay that is closely correlated with CETP mass (25). Likewise, in subjects without diabetes, no independent contribution of plasma CETP mass on CET was observed in another report (17).

Of further relevance, our study showed a moderately decreased plasma Lp-PLA₂ concentration in MetS subjects. Increased Lp-PLA₂ concentration and activity levels were found previously in nondiabetic subjects with MetS (38), and an increased Lp-PLA₂ concentration was found in diabetic patients with MetS compared with those without MetS (39). It is well established that there is sufficient agreement between the concentration and the activity level of Lp-PLA₂ in plasma (7). Nonetheless, it has been shown that MetS exerts greater effects on the Lp-PLA₂ activity level compared with its plasma concentration (38). Of further interest, the recent meta-analysis revealed lower plasma Lp-PLA₂ mass but unaltered activity in diabetes mellitus (7). In line with a decreasing effect of the diabetic state in Lp-PLA₂, the difference in its concentration between subjects with and without MetS was not significant after controlling for diabetes status. Thus, the apparent discrepancy with respect to Lp-PLA₂ concentration with other reports (38, 39) could be explained at least in part by the considerable number of diabetic subjects in our study population. Although measurement of Lp-PLA₂ activity could have provided additional insight in its relation with CET, multiple linear regression analysis did reveal that the plasma Lp-PLA₂ concentration contributed to CET independently of diabetes status. Furthermore, comparable relationships of CET with the Lp-PLA₂ concentration were seen in the separate groups of subjects with and without MetS as well as in nondiabetic subjects only. Therefore, the conclusion is allowed that the positive and independent relation of CET with the Lp-PLA₂ concentration is not confined to subjects with MetS and is not confounded by the presence of type 2 diabetes mellitus.

An increasing body of evidence supports the notion that nonesterified fatty acids and lipid peroxides are accumulated in electronegative LDL particles to which Lp-PLA₂ associates preferentially (3, 20, 21). In turn, the CET process is stimulated by negatively charged cholesteryl ester acceptor lipoproteins, although a delicate balance in the concentration and structure of various long chain nonesterified fatty acids seems to be required for maximal stimulation of CET (16, 18, 40). Additionally, a higher affinity of CETP for oxidized LDL has been demonstrated in vitro, which may be attributable to the generation of negatively charged oxidized lipids during LDL oxidation (16, 41). Association of CETP with negatively charged LDL may affect the CET process by targeting transfer of HDL-derived cholesteryl esters more preferentially toward LDL (9, 16). Thus, it is likely that the composition of apo B-containing lipoproteins, including their charge characteristics, affect both plasma CET and their association with Lp-PLA₂ in the same direction. It seems plausible that the independent relationship of plasma CET with Lp-PLA₂ is not merely associative, but could imply a causal role of Lp-PLA₂ in CET regulation or vice versa. It can be anticipated that generation of negatively charged oxidized nonesterified fatty acids elicited by Lp-PLA₂ may increase directly the electronegativity of apo B-containing lipoproteins (3) and hence stimulate the CET process. In this respect, it is noteworthy that Lp-PLA₂ is able to hydrolyze long acyl chain oxidized phospholipids, thereby generating long acyl chain oxidized nonesterified fatty acids and fatty acid hydroperoxides (3). Conversely, the CET process contributes to the formation of (electronegative) small, dense LDL (8, 9, 10), which may preferentially bind Lp-PLA₂ (3, 21). Furthermore, CETP action could also promote generation of oxidized LDL in vitro (42). Accordingly, resistance of LDL particles to oxidative modification has been observed after immunological inhibition of CETP in vitro (43). We therefore hypothesize that the relationship of plasma CET with Lp-PLA₂, as shown in this report, may represent implication of Lp-PLA₂-mediated generation of oxidized nonesterified fatty acids in the CET process. Alternatively, this relationship could imply that the CET process could contribute to generation of oxidized lipids rendering apo B-containing lipoprotein (subfractions) more electronegative, which may enhance Lp-PLA₂ association with LDL particles.

Both possibilities can be anticipated to promote the atherogenicity of plasma lipoproteins. If Lp-PLA₂ enhances the CET process, then the contribution of circulating Lp-PLA₂ to atherosclerosis susceptibility could in part be explained by a hitherto unappreciated contribution to an unfavorable distribution of cholesterol between HDL and apo B-containing lipoproteins (9, 10, 12, 17). Conversely, if plasma CET stimulates binding of Lp-PLA₂ to apo B-containing lipoproteins, then this process may
aggravate the prooxidative and proinflammatory potential of these lipoproteins, including small, dense LDL (3, 44). Obviously, the precise mechanisms responsible for the relationship of plasma CET with Lp-PLA₂ should be determined in future experiments. Clearly, the present findings provide a rationale to test whether pharmacological inhibition of Lp-PLA₂ (45, 46) will selectively reduce the plasma CET process. Furthermore, it may be relevant to know whether CETP inhibitors, which are anticipated to increase LDL size (12–14), affect Lp-PLA₂ binding to LDL and its prooxidant activity.

Early studies have demonstrated that EST in incubated plasma is stimulated by cholesteryl ester acceptor lipoproteins (24). This mechanism explains why the plasma triglyceride concentration is a determinant of EST, besides an effect of the LCAT activity level per se (25, 26). Our study entirely agrees with this finding (25) and reiterates previously documented increases in plasma EST (22) and CET activity (15, 47) in MetS and dyslipidemic subjects. The observation that CET can become rate limiting for EST (24) most likely also underlies the strong relationship of EST with CET (25, 26). In this respect, it is noteworthy that there was no positive association of plasma EST with Lp-PLA₂. Although HDL size and lipid compositional characteristics affect their binding affinity for CETP (48), there are to our knowledge no reports that demonstrate preferential binding of CETP to electronegative HDL particles. Moreover, only a minor proportion of Lp-PLA₂ is associated with HDL (3), even in dyslipidemic and diabetic plasma where Lp-PLA₂ may be redistributed from apo B-containing lipoproteins toward HDL (4). Thus, the present report suggests that effects of Lp-PLA2 on components of the reverse cholesterol transport pathway are confined to CET.

In conclusion, this study has revealed robust independent relationships of plasma CET, but not EST, with the Lp-PLA₂ level in subjects with and without the MetS. This finding raises the possibility that Lp-PLA₂ may specifically affect the CET process, or alternatively that CETP inhibition could affect Lp-PLA₂ activity.

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