Measurement of Human Plasma Phospholipid Transfer Protein by Sandwich ELISA

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Methods: A sandwich ELISA for PLTP has been developed, using two monoclonal antibodies against recombinant human PLTP (rhPLTP) expressed in Chinese hamster ovary cells. The ELISA allows for the quantification of PLTP in the range 0.625–15.0 ng/assay (1.2–30.0 mg/L). Intra- and interassay CVs were <3.0% and <4.2% respectively. The assay was used to quantify plasma PLTP concentrations in 132 Japanese subjects (75 males and 57 females).

Results: PLTP concentrations were 12.0 ± 3.0 mg/L (mean ± SD; range, 4.9–20.5 mg/L). No sex difference was observed. Plasma PLTP concentration was positively correlated with HDL-cholesterol (r = 0.72; P <0.001), apolipoprotein (apo) A-I (r = 0.62; P <0.001) and HDL2-cholesterol (r = 0.72; P <0.001), and was negatively correlated with triacylglycerol (r = −0.45; P <0.001). There was no correlation with plasma apo A-II. These results agree with other evidence that plasma PLTP is associated with large apo A-I-containing lipoproteins. There was no correlation (r = −0.01) between plasma PLTP and plasma phosphatidylcholine transfer activity (range, 3.5–10.5 μmol · mL⁻¹ · h⁻¹), suggesting that PLTP may exist in active and inactive forms.

Conclusion: This new ELISA will be of value for further studies of PLTP in health and disease.

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Received May 2, 2000; accepted June 27, 2000.

Plasma phospholipid transfer protein (PLTP)3 plays an important role in lipid transport. It mediates a net movement of phospholipids between vesicles and plasma HDLs (1) and between acceptor-donor pairs of HDLs, LDLs, and VLDLs (2, 3). It also generates from spheroidal HDLs small lipid-poor apolipoprotein (apo) A-I-containing particles (4–6), which are thought to act as the primary acceptors of cell-derived cholesterol from peripheral tissues (7). PLTP-mediated transfer of phospholipids is most efficient between HDL particles, presumably reflecting its physiologic function in the remodeling of HDLs. Pussinen et al. (8) have shown that the apo A-II/apo A-I molar ratio in HDLs influences PLTP-mediated particle interconversions, and have located the PLTP-binding domain in the NH₂-terminal region of apo A-I (9).

Cholesteryl ester transfer protein (CETP) also catalyzes the transfer of phospholipids between plasma lipoproteins (10), and PLTP has been found to increase CETP-mediated transfer of cholesteryl esters between VLDLs and HDLs (11). Nevertheless, PLTP and CETP show neither cooperativity nor competition in the transfer of phospholipids between HDLs and LDLs (11), suggesting that the two proteins transfer phospholipids by different mechanisms.

The apparent molecular mass of PLTP purified from human or pig plasma is between 69 and 81 kDa (4, 11–14). Human PLTP cDNA has been cloned and is 1750 bp in length, coding a signal sequence of 17 amino acids and a mature protein of 476 residues (13).

Plasma PLTP activity has been reported to be increased in patients with non-insulin-dependent diabetes mellitus (15, 16) and obesity (17), and to be increased by cigarette

3 Nonstandard abbreviations: PLTP, phospholipid transfer protein; apo, apolipoprotein; CETP, cholesteryl ester transfer protein; mAb, monoclonal antibody; rhPLTP, recombinant human phospholipid transfer protein; CHO, Chinese hamster ovary; PC, phosphatidylcholine; PBS, phosphate-buffered saline; and SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
Materials and Methods

Egg phosphatidylcholine (PC) and bovine phosphatidylserine were purchased from Sigma Chemical Company. 1-Palmityl-2-[1-14C]-palmityl phosphatidylcholine (80–120 mCi/mmol) was from NEN™ Life Science Products. Heparin (5000 KIU/L) was from Mochida Pharmaceutical Co. Block Ace was from Snow Brand Milk Products. Heparin (5000 KIU/L) was from Mochida Pharmaceutical Co. Block Ace was from Snow Brand Milk Products.

Subjects

Blood samples were collected into EDTA-containing glass tubes (final concentration, 1 g/L; Terumo), and samples were immediately centrifuged at 2500 × g for 10 min. Plasma was obtained from 132 apparently healthy subjects (75 males and 57 females). Their lipid profiles are summarized in Table 1. Plasma samples were stored at −80 °C. No subject was taking any medication known to affect plasma lipoproteins.

Isolation of Lipoproteins

The HDL₃ fraction (d = 1.125–1.21 kg/L) was isolated from fresh human plasma by sequential preparative ultracentrifugation in a Beckman Ti 50.2 rotor, using solid KBr to adjust the density (20). The washed HDL₃ was refloated at d = 1.21 kg/L (14); dialyzed against phosphate-buffered saline (PBS); pH 7.4, containing 10 mmol/L Tris-HCl, 150 mmol/L NaCl, and 1 mmol/L EDTA; and stored at 4 °C. Plasma HDL₂-cholesterol (d = 1.063–1.125 kg/L) and HDL₃-cholesterol were quantified after preparative ultracentrifugation as described previously (20).

Preparation of rhPLTP

rhPLTP was prepared from CHO cell culture medium and purified using phenyl-Sepharose, Ni-NTA agarose, and heparin-Sepharose chromatography, as described previously (21, 22). As a primary calibrator, heparin-purified rhPLTP was used. The purity of purified rhPLTP, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by silver staining, was determined by gel scanning using the Intelligent Quantifier system (BioImage). This showed one 80-kDa protein band, representing >90% of total protein (Fig. 1A). Purified rhPLTP has been shown to be similar to plasma PLTP in PC transfer activity, remodeling of HDLs, and apo A-I cleavage activity (22).

Preparation of mAbs Against rhPLTP

Balb/c mice were immunized with 25 μg of purified rhPLTP (22) and spleen cells from the mice were fused with Sp2/0 cells (23). The supernatants of hybridoma cells were screened by ELISA using plates coated with purified rhPLTP (100 ng/well) and by immunoblotting. Positive hybridoma cells were cloned three times by limiting dilution and injected intraperitoneally into pristane-primed Balb/c mice. The IgG fraction was isolated from ascitic fluid using protein A-Sepharose CL-4B according to the manufacturer’s instructions, dialyzed at 4 °C against PBS, and stored at −80 °C. The specificities of mAb113 and mAb114 were confirmed by immunoblotting against purified human plasma PLTP and human plasma. mAb isotype was characterized using the Mouse mAb Isotyping kit (Boehringer Mannheim) and was IgG2b and IgG1 for mAb113 and mAb114, respectively.

Measurement of Phospholipid Transfer Activity

Phospholipid transfer activity was measured as described by Damen et al. (24), using [14C]PC liposomes prepared as described by Cheung et al. (25). A diluted plasma sample

| Table 1. Clinical characteristics of the subjects (mean ± SD). |
|-----------------|-----------------|-----------------|
| **Males**       | **Females**     | **Total**       |
| **Age, years**  | 45 ± 11         | 49 ± 13         | 47 ± 12         |
| **Total cholesterol, mmol/L** | 5.29 ± 0.64 | 5.22 ± 0.64 | 5.26 ± 0.64 |
| **Triglycerides, mmol/L** | 1.21 ± 0.43 | 1.03 ± 0.44 | 1.13 ± 0.44 |
| **LDL-cholesterol, mmol/L** | 3.44 ± 0.63 | 3.11 ± 0.62 | 3.30 ± 0.64 |
| **HDL-cholesterol, mmol/L** | 1.41 ± 0.31 | 1.61 ± 0.48 | 1.50 ± 0.40 |
| **LDL₃-cholesterol, mmol/L** | 0.84 ± 0.22 | 1.07 ± 0.38 | 0.94 ± 0.32 |
| **HDL₂-cholesterol, mmol/L** | 0.46 ± 0.07 | 0.42 ± 0.06 | 0.44 ± 0.07 |
| **apo B, g/L**  | 1.01 ± 0.19     | 0.93 ± 0.19     | 0.97 ± 0.19     |
| **apo A/I, g/L** | 1.43 ± 0.25     | 1.51 ± 0.30     | 1.47 ± 0.27     |
| **apo A/II, g/L** | 0.35 ± 0.06     | 0.31 ± 0.06     | 0.33 ± 0.06     |

*Males, n = 75; females, n = 57.*
(100 μL of a 1:50 dilution) was added to 400 μL of reaction mixture containing washed HDL₃ (250 μg of protein) and liposomes (75 nmol of PC) and incubated at 37 °C for 30 min. Liposomes were then precipitated by the addition of 300 μL of a solution containing 230 mmol/L NaCl, 92 mmol/L MnCl₂, 150 IU/L heparin (24), and the radioactivity in the supernatant was measured. Phospholipid transfer activity was expressed as μmol of PC transferred to HDL₃ per milliliter of plasma per hour. All assays were performed using the same batches of liposomes and HDL₃. The intra- and interassay CVs (n = 8) were 5.9% and 7.7%, respectively. The PC transfer activity of rhPLTP was a linear function of concentration up to 13.0 μmol/L. Polyclonal antibody raised against the rhPLTP in rabbits almost completely inhibited the PC transfer activities of both rhPLTP and normal human plasma (data not shown).

The PC transfer assay was tested by adding to each of three plasma samples of differing PC transfer activities (6.3, 9.5, and 11.0 μmol·mL⁻¹·h⁻¹) increasing amounts of rhPLTP of known transfer activities (0.7, 1.8, and 3.2 μmol·mL⁻¹·h⁻¹), and then measuring the PC transfer activities of the mixtures. The final measured PC transfer activities increased in a dose-dependent manner and were within 4–20% of the predicted values (mean, 8.4%).

### MEASUREMENT OF PLTP CONCENTRATION

mAb114 (100 μL of a 5 mg/L solution in PBS) was coated on a microtiter plate (Nunc Immunoplate II) by incubation at 4 °C overnight. The wells were then blocked with 200 μL of PBS containing 40 g/L Block Ace (Snow Brand Milk Products) for 2 h at room temperature. After the plate was washed with 200 μL of PBS containing 1 mL/L Tween 20, 100 μL of the calibrator solution and diluted plasma samples (1:200) was added and incubated for 2 h at room temperature. After the plate was washed five times, 100 μL of 1 mg/L biotinylated mAb113 was added to each well, and the mixture was incubated for 2 h at room temperature. After the plate was washed five times, 100 μL of 1 mg/L horseradish peroxidase-conjugated streptavidin (Vector Laboratories) was added, and the mixture was incubated for 1 h. After the plate was washed, 100 μL of substrate solution containing 0.25 g/L o-phenylenediamine and 0.15 mL/L H₂O₂ was added to each well. After 30 min, the reaction was stopped by addition of 50 μL of 4 N H₂SO₄. The absorbance at 492 nm was measured with a microplate reader.
addition of 100 μL of 4 mol/L H₂SO₄. The absorbance was measured immediately at 492 nm by a microplate reader. Pooled culture medium from CHO cells expressing rhPLTP served as a secondary calibrator (1.2–40 mg/L), which was calibrated against the heparin-purified rhPLTP primary calibrator.

OTHER ANALYTICAL METHODS
Measurements of plasma total cholesterol, triacylglycerol, and HDL-cholesterol concentrations were performed in a Hitachi 7450 automated analyzer using commercial kits. Total HDL-cholesterol was measured after precipitation of apo B-containing lipoproteins with dextran sulfate and magnesium chloride. The LDL-cholesterol concentration was measured after precipitation of apo B-containing lipoproteins with dextran sulfate and magnesium chloride. The LDL-cholesterol concentration was calculated according to Friedewald et al. (26). The protein content of purified rhPLTP was determined with the BCA protein assay kit (Pierce), using bovine serum albumin as the calibrator. SDS-PAGE was performed by the Laemmli method (27) and immunoblotting as described by Towbin et al. (28).

Table 2. Recovery of PLTP in the ELISA.

<table>
<thead>
<tr>
<th>Amount of rhPLTP added, mg/L</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured, mg/L</td>
<td>Recovery, %</td>
<td>Measured, mg/L</td>
<td>Recovery, %</td>
</tr>
<tr>
<td>None</td>
<td>5.8</td>
<td></td>
<td>8.8</td>
</tr>
<tr>
<td>2.5</td>
<td>8.3</td>
<td>100</td>
<td>11.4</td>
</tr>
<tr>
<td>5.6</td>
<td>11.3</td>
<td>98</td>
<td>14.5</td>
</tr>
<tr>
<td>9.2</td>
<td>14.1</td>
<td>90</td>
<td>19.1</td>
</tr>
<tr>
<td>13.2</td>
<td>19.5</td>
<td>103</td>
<td>21.1</td>
</tr>
</tbody>
</table>

a Purified rhPLTP was added to three samples of plasma in sufficient amounts to raise the concentrations of total PLTP by the values shown in the first column. The measured final concentration was then expressed as a percentage of the predicted final concentration. Each result is the mean of triplicates.

STATISTICAL ANALYSIS
Results are expressed as means ± SD. Analysis of variance was used for group comparisons. Correlations were assessed by least-squares regression analysis. P < 0.05 was considered statistically significant.

RESULTS
CHARACTERIZATION OF ANTI-PLTP mAbs
Two mAbs specific for PLTP were established: mAb113 and mAb114. Their specificities were examined by SDS-PAGE and immunoblotting under reducing and non-reducing conditions. When human plasma, purified plasma PLTP, and purified rhPLTP were subjected to SDS-PAGE, the mAbs reacted with a single protein (Fig. 1B), the molecular mass of which (~80 kDa) was similar to that previously reported for human PLTP (12). Neither mAb inhibited PC transfer activity (data not shown). A sandwich ELISA for PLTP was established using mAb114 for capture and biotinylated mAb113 for detection. This system showed a dose-dependent response to heparin-purified rhPLTP (1:64 000 to 1:200), rhPLTP culture medium (1:2560 to 1:20), and plasma (1:2560 to 1:40; Fig. 2).

STANDARDIZATION OF ELISA FOR PLTP CONCENTRATION
For calibration of the ELISA, rhPLTP was purified from the culture medium of transfected CHO cells (22). When subjected to SDS-PAGE and visualized by silver staining, the heparin-purified rhPLTP showed a single major 80-kDa band (Fig. 1A), which represented >90% of the total protein in the preparation (as determined by gel scanning using the Intelligent Quantifier system). The protein concentration of this primary rhPLTP calibrator, assayed using a BCA protein kit with bovine serum albumin as calibrator, was typically 80–90 mg/L. To obtain a calibration curve for the ELISA, dilutions of the primary calibrator were made in PBS containing 1 mL/L Tween 20 to provide 0.156–20.0 ng of rhPLTP protein per well (0.31–40.0 mg/L). As shown in Fig. 3, the ELISA was linear up to 30 mg/L and suitable for quantifying PLTP concentrations as low as 1.2 mg/L. When the
rhPLTP culture medium, as a secondary calibrator (1:2560 to 1:20), was diluted in PBS containing 1 mL/L Tween 20 to cover the PLTP concentration range 1.2–40.0 mg/L, the curve was identical to that attained with the primary calibrator (Fig. 3). To avoid potential nonlinearity caused by very low or high absorbance, the PLTP concentrations in plasma samples were measured using several dilutions (1:2560 to 1:40), and the least diluted aliquot that gave an absorbance value between 0.8 and 1.2 was chosen.

The detergent Tween 20 was included in the diluent to avoid any effects of differences between samples in lipid/absorbance value between 0.8 and 1.2 was chosen. (1:2560 to 1:40), and the least diluted aliquot that gave an absorbance value between 0.8 and 1.2 was chosen.

When purified rhPLTP was added to samples of plasma in sufficient amounts to raise the total PLTP concentration to 2.5–13.2 mg/L, the final concentrations given by the ELISA averaged 102% of those predicted (Table 2). The intra- and interassay CVs of the ELISA were <3.0% and 4.2%, respectively (n = 8). No interference with the ELISA was observed with hemoglobin (0.2 g/L), bilirubin (0.2 g/L), or triacylglycerol (4.25 g/L).

### Discussion

Plasma PLTP activity was first described as mediating the transfer of phospholipids from triglyceride-rich lipoproteins to HDLs during lipolysis (2). Phospholipid exchange activity is also possessed by CETP (29), lipopolysaccharide-binding protein, and soluble CD14 (30). An important difference between PLTP and CETP is that only the former transfers PC from liposomes to isolated HDLs (10, 11, 31, 32). This forms the basis of plasma PLTP activity assays (24) and can be completely blocked by anti-PLTP antibodies (12, 32). Recent studies in vitro have shown that PLTP catalyzes the remodeling of spheroidal HDLs into larger HDLs and small lipid-poor apo A-I-containing particles (pre-β HDLs) (4, 5, 12), the primary acceptors of cell-derived cholesterol in tissue culture (7). Other findings in vitro and in transgenic animals have supported a role of PLTP in HDL remodeling and reverse cholesterol transport (6, 33–37). PC transfer activity has been found in the plasma of all vertebrate species studied to date (38). PLTP has also been shown to facilitate the transfer of cholesterol (36), lipopolysaccharides (39), and α-tocopherol (40) between lipoproteins.

### Table 3. PLTP concentration, PC transfer activity, and PLTP specific activity in plasma from healthy subjects (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Males*</th>
<th>Females*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLTP concentration, mg/L</td>
<td>11.6 ± 2.8</td>
<td>12.7 ± 3.1</td>
<td>12.0 ± 3.0</td>
</tr>
<tr>
<td>PC transfer activity, μmol · mL⁻¹ · h⁻¹</td>
<td>6.3 ± 1.3</td>
<td>6.2 ± 1.3</td>
<td>6.3 ± 1.3</td>
</tr>
<tr>
<td>PLTP specific activity, μmol · h⁻¹ · μg⁻¹</td>
<td>0.59 ± 0.22</td>
<td>0.52 ± 0.16</td>
<td>0.56 ± 0.20</td>
</tr>
</tbody>
</table>

* Males, n = 75; females, n = 57.

a PLTP specific activity was calculated as PC transfer activity divided by PLTP concentration.

### Table 4. Correlation coefficients between PLTP concentration and other measured variables in plasma.

<table>
<thead>
<tr>
<th></th>
<th>Males*</th>
<th>Females*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC transfer activity</td>
<td>0.06</td>
<td>−0.14</td>
<td>−0.10</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.12</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.37</td>
<td>−0.49</td>
<td>−0.45</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>−0.09</td>
<td>−0.32</td>
<td>−0.23</td>
</tr>
<tr>
<td>Total HDL-cholesterol</td>
<td>0.60</td>
<td>0.78</td>
<td>0.72</td>
</tr>
<tr>
<td>HDL₂-cholesterol</td>
<td>0.64</td>
<td>0.79</td>
<td>0.72</td>
</tr>
<tr>
<td>HDL₃-cholesterol</td>
<td>0.21</td>
<td>0.07</td>
<td>0.10</td>
</tr>
<tr>
<td>apo B</td>
<td>0.25</td>
<td>−0.51</td>
<td>−0.40</td>
</tr>
<tr>
<td>apo A-I</td>
<td>0.56</td>
<td>0.67</td>
<td>0.62</td>
</tr>
<tr>
<td>apo A-II</td>
<td>0.23</td>
<td>0.17</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Males, n = 75; females, n = 57.

b P < 0.005.

c P < 0.001.
To date there have been few studies of the function of PLTP in the regulation of lipid transport in humans in vivo. In the absence of a reliable immunoassay of plasma PLTP concentration, most clinical studies have been limited to measurements of phospholipid transfer activity (15–19). For a better understanding of PLTP function, there is a need for reliable and sensitive methods for measuring PLTP concentration in plasma and other biological fluids. To this end, we prepared a series of mAbs against rhPLTP and used two of them to develop the sandwich ELISA. By SDS-PAGE and immunoblotting, both mAbs recognized a single protein of ~80 kDa under both reducing and nonreducing conditions, indicating that they react with a linear epitope of PLTP protein. We found that mAb113 cross-reacted with pig plasma PLTP, the amino acid sequence of which has 93% homology with human PLTP (41). However, mAb114 did not cross-react with pig PLTP, demonstrating that the two mAbs recognize different epitopes on the PLTP molecule. The ELISA was found to be suitable for assaying plasma PLTP concentrations in the range 1.2–30.0 mg/L. No differences in dilution curves were observed between purified rhPLTP (primary calibrator), rhPLTP culture medium (secondary calibrator), and human plasma. Intra- and interassay CVs were <5%. These results indicate that our ELISA is specific, accurate, reproducible, and sufficiently sensitive for a wide range of applications.

We used our ELISA to measure plasma PLTP concentrations in 132 healthy Japanese subjects. No sex difference was observed. No correlation existed with the PC transfer activity of plasma, suggesting that factors other than total PLTP concentration are more important as determinants of the rate of transfer PC into HDLs. Plasma PLTP concentration was positively correlated with the plasma HDL-cholesterol, HDL2-cholesterol, and apo A-I concentrations and negatively correlated with plasma triacylglycerol concentration. The correlation with HDL2-cholesterol is consistent with a report that on gel filtration chromatography PLTP coeluted with particles of similar size to HDL2 (42). Although PLTP has been shown to bind to both apo A-I and apo A-II in vitro (9), our finding that the PLTP concentration correlated positively with the concentration of apo A-I but not with that of apo A-II suggests that binding to apo A-I-containing particles may predominate in vivo. Such particles might capture newly secreted PLTP, thereby retaining it in the plasma compartment. The apo A-I/apo A-II ratio of HDLs has been shown to be a determinant of PLTP-mediated HDL remodeling (8), and PLTP cannot mediate the conversion of particles that lack apo A-I (9, 21).

Fig. 4. Correlations between plasma PLTP concentration and plasma PC transfer activity (A) or PLTP specific activity (B) in 132 healthy Japanese subjects.

Fig. 5. Correlations between the plasma concentrations of PLTP and HDL-cholesterol (A) or apo A-I (B) in 132 healthy Japanese subjects.
Two immunoassays for plasma PLTP concentration have been developed by other groups. Desrumaux et al. (43) described a competitive ELISA that used a polyclonal rabbit antibody to purified human plasma PLTP. As determined by this assay, the plasma PLTP concentration in 30 healthy subjects was 3.95 ± 1.04 mg/L (range, 1.98–5.71 mg/L), which is much lower than the values obtained in the present study. No significant correlation was found between plasma PLTP concentration and either the HDL-cholesterol or triacylglycerol concentration. No measurements were made of apolipoproteins. Also contrasting with our results is the fact that Desrumaux et al. (43) found a strong correlation \( r = 0.79 \) \( P < 0.001 \) between plasma PLTP concentration and plasma PC transfer activity. More recently, Huuskonen et al. (44) developed an assay that uses a mAb for capture and a polyclonal rabbit antibody for detection, both raised against rhPLTP expressed in *Escherichia coli*. This assay gave plasma PLTP concentrations of 15.6 ± 5.1 mg/L (range, 2.3–33.4 mg/L) in 159 Finnish subjects, which is similar to those obtained by us in Japanese subjects with our assay. As in the present study, Huuskonen et al. (44) found no correlation between plasma PLTP concentration and plasma PC transfer activity \( r = -0.06 \).

Thus, our results are in good agreement with those obtained by Huuskonen et al. (44) in relation to both the absolute values of plasma PLTP concentration in healthy subjects and the absence of any correlation with plasma PC transfer activity. The apparent inconsistency between these results and those reported by Desrumaux et al. (43) probably cannot be explained by differences in sample sizes or in the genetic background, dietary habits, or life-styles of the subjects. A theoretical possibility is that there are catalytically active and inactive forms of PLTP in plasma and that both our immunoassay and that of Huuskonen et al. (44) quantify both forms, but that of Desrumaux et al. (43) quantifies only the active form. We recently studied the distribution of PLTP mass and PC transfer activity in human plasma fractions separated by size exclusion chromatography. Evidence was obtained that there are indeed active and inactive species of PLTP, that the active form constitutes only ~20% of the total, and that the two species are associated with particles of different sizes [Oka et al. The distribution of phospholipid transfer protein (PLTP) in human plasma: presence of two forms of PLTP, one catalytically active and the other inactive, submitted for publication].

We thank Drs. Matti Jauhiainen and Christian Ehnholm (Department of Biochemistry, National Public Health Institute, Helsinki, Finland) for kindly supplying purified human plasma and pig plasma PLTP.

References

36. Nishida HI, Nishida T. Phospholipid transfer protein mediates transfer of not only phosphatidylcholine but also cholesterol from phosphatidylcholine-cholesterol vesicles to high density lipoproteins. J Biol Chem 1997;272:6959–64.