Active and low-active forms of serum phospholipid transfer protein in a normal Finnish population sample

Minna T. Jänis,* Sarah Siggins,* Esa Tahvanainen,* Riikka Vikstedt,* Kaisa Silander,* Jari Metso,* Arpo Aromaa,† Marja-Riitta Taskinen,§ Vesa M. Olkkonen,* Matti Jauhiainen,* and Christian Ehnholm[1,*]

Department of Molecular Medicine* and Department of Health and Functional Capacity,† National Public Health Institute, Biomedicum, Helsinki, Finland; and Division of Cardiology,§ Department of Medicine, University of Helsinki, Helsinki, Finland

Abstract Human serum phospholipid transfer protein (PLTP) exists as a catalytically active (HA-PLTP) and a low-active (LA-PLTP) form. In this study, the association of PLTP activity and the concentrations of both forms with lipid and carbohydrate parameters were investigated. In a random Finnish population sample, serum PLTP concentration (n = 250) was 6.56 ± 1.45 mg/l, the mean lipoprotein-independent ($PLTP_{exo}$) phospholipid transfer activity was 6.59 ± 1.66 μmol/ml/h, and the mean lipoprotein-dependent ($PLTP_{endo}$) activity was 1.37 ± 0.29 μmol/ml/h. Of the serum PLTP concentration, ~46% was in a catalytically active form. HA-PLTP concentration correlated positively with serum $PLTP_{exo}$ activity ($r = 0.380$, $P < 0.001$), HDL cholesterol ($r = 0.291$, $P < 0.001$), and apolipoprotein A-I ($r = 0.187$, $P < 0.01$). Of the potential regulatory factors for PLTP, apolipoprotein E showed a weak positive correlation with serum $PLTP_{exo}$ ($r = 0.154$, $P < 0.05$) and $PLTP_{endo}$ ($r = 0.192$, $P < 0.01$) activity but not with PLTP concentration. Weak associations were also observed between PLTP parameters and determinants of glucose homeostasis (glucose, insulin, and homeostasis model assessment for insulin resistance). The present data on PLTP activity and concentration reveal novel connections of the two PLTP forms to lipid and carbohydrate metabolism.—Jänis, M. T., S. Siggins, E. Tahvanainen, R. Vikstedt, K. Silander, J. Metso, A. Aromaa, M-R. Taskinen, V. M. Olkkonen, M. Jauhiainen, and C. Ehnholm. **Active and low-active forms of serum phospholipid transfer protein in a normal Finnish population sample. *J. Lipid Res.* 2004.** 45: **2303–2309.**

Supplementary key words apolipoproteins • lipid transfer proteins • enzyme-linked immunosorbent assay

Increased HDL concentration protects against coronary heart disease (1, 2), primarily by removing cholesterol from peripheral tissues (3). One important regulator of HDL metabolism is phospholipid transfer protein (PLTP) (4). It transfers phospholipids between different lipoproteins (5) and mediates HDL conversion (6, 7). Recent observations of the presence of PLTP in macrophage foam cells of atherosclerotic lesions suggest that PLTP could function either as an antiatherogenic molecule by facilitating cholesterol efflux or as a proatherogenic molecule by mediating lipid retention (8, 9). The involvement of PLTP in the reverse cholesterol transport process was recently suggested by Oram and colleagues (10), who demonstrated that PLTP interacts with ABCA1 on macrophages and facilitates cholesterol and phospholipid efflux. In addition to lipid metabolism, PLTP may also have a role in carbohydrate metabolism (11–13). In HepG2 cells, high glucose concentration increases both PLTP mRNA and activity levels (14).

Human plasma contains two forms of PLTP, a high-active form (HA-PLTP) and a low-active form (LA-PLTP). These two forms are associated with macromolecular complexes of different size (15, 16). At present, the processes that regulate the distribution of the HA- and LA-PLTP forms are unknown.

Although gene-targeted animal models (17–22) have been studied extensively, the physiological role of PLTP in human lipid metabolism is far from resolved. The present study was specifically carried out to clarify the relationships of PLTP concentration and activity with lipoprotein and carbohydrate metabolism in normolipidemic Finnish individuals participating in the Health 2000 Health Examination Survey. By measuring the concentration of both HA- and LA-PLTP forms (23) and studying the relation-

Abbreviations: apoB, apolipoprotein B; BMI, body mass index; CRP, C-reactive protein; CV, coefficient of variation; HA, high-active form; HDL-C, HDL-cholesterol; HOMA IR, homeostasis model assessment for insulin resistance; LA, low-active form; LDL-C, LDL-cholesterol; PLTP, phospholipid transfer protein; TC, total cholesterol; TG, triglyceride; WHR, waist-to-hip ratio.

[1] To whom correspondence should be addressed.
e-mail: christian.ehnholm@ktl.fi

Manuscript received 1 July 2004 and in revised form 19 August 2004.

Published, JLR Papers in Press, September 1, 2004.
DOI 10.1194/jlr.M400250-JLR200

ships between PLTP concentration and activity and selected serum parameters, we will increase our understanding of the regulation of PLTP activity and the distribution of the two PLTP forms.

The aims of this study were to investigate the distribution of the two PLTP forms in a population sample, to test how the two PLTP forms relate to lipid and carbohydrate metabolism, and to find possible regulatory factors for PLTP.

MATERIALS AND METHODS

Subjects

For this study, 250 subjects (125 men and 125 women), age range 30–94 years, were randomly chosen as a subsample of the Health 2000 Health Examination Survey study (24) carried out in Finland. Serum samples were stored at −70°C before analysis.

Determination of PLTP concentration

Measurement of human serum PLTP concentration was performed essentially as described earlier (23). Briefly, the serum samples were incubated with 0.5% SDS for 30 min at 22°C in the sample buffer [10 mM Na-phosphate, 150 mM NaCl (PBS), pH 7.4, containing 0.1% Tween 20]. After the SDS pretreatment, the mixtures were diluted in the sample buffer and added in duplicate to microtiter plate wells coated with anti-human PLTP monoclonal antibody JH66. All subsequent steps were performed according to the PLTP ELISA method as described (25). The intra-assay and interassay coefficients of variation (CVs) were 8.3% (n = 8) and 9.7% (n = 12), respectively.

Separation of the two PLTP forms from each other was carried out by dextran sulfate-$CaCl_2$ precipitation using the method of Kato et al. (26), with minor modifications. Briefly, 200 μl of serum was mixed with 300 μl of water and 200 μl of 1% dextran sulfate (Dextran Sulfate Sodium Salt; dextran molecular weight ~500,000; Amersham Pharmacia Biotech; dialyzed against water before use). The mixture was incubated on ice for 20 min with intermittent mixing. After this, $CaCl_2$ was added to a final concentration of 0.1 M. The mixture was centrifuged (16,000 *g*) for 5 min at room temperature, and the clear supernatant was collected and assayed for PLTP activity and concentration. NaCl was added to the supernatant to a final concentration of 1 M before the PLTP activity assay. PLTP activity in the supernatant after precipitation was ~90% of that in the original serum sample. The LA-PLTP concentration was obtained by subtracting the HA-PLTP concentration measured in the dextran sulfate-$CaCl_2$ supernatant from total serum PLTP concentration.

Assay of PLTP activity

PLTP-facilitated phospholipid transfer activity was measured in serum using two different radiometric methods.

1) In the exogenous, lipoprotein-independent assay ($PLTP_{exo}$), [^{14}C]phosphatidylcholine (PC)-liposomes (150 nmol of dipalmitoylphosphatidylcholine in 10 mM Tris-HCl, 150 mM NaCl, and 1 mM EDTA, pH 7.4) were incubated with isolated HDL_3 (250 μg of protein) for 45 min at 37°C in a final volume of 400 μl, containing either 10 μl of serum or dextran sulfate-$CaCl_2$ supernatant (both diluted 1:7.2). Excess liposomes and apolipoprotein B (apoB)-containing lipoproteins originating from serum samples were subsequently precipitated with 300 μl of 230 mM $MnCl_2{\cdot}4H_2O$ and 530 mM NaCl containing 172 U/ml heparin, as described (6, 27). The intra-assay CV was 9.4% (n = 11), and the interassay CV was 12% (n = 15).

2) In the endogenous, lipoprotein-dependent assay ($PLTP_{endo}$), [^{14}C]PC-liposomes were mixed with 30 μl of undiluted serum and incubated for 30 min at 37°C in a final volume of 80 μl, containing 1.5 mM iodoacetate (28). After incubation, 320 μl of 10 mM Tris-HCl, 150 mM NaCl, and 1 mM EDTA, pH 7.4, were added, and then excess liposomes and apoB-containing lipoproteins were precipitated with 300 μl of 215 mM $MnCl_2{\cdot}4H_2O$ and 500 mM NaCl containing 445 U/ml heparin. The intra-assay and interassay CVs were 3.2% (n = 20) and 6.3% (n = 14), respectively.

In both assays, radioactivity was measured from the supernatants with a liquid scintillation counter (Wallac, Turku, Finland).

ApoE genotyping

ApoE-3937 and apoE-4075 variations were genotyped using the MassARRAY System (Sequenom, San Diego, CA), with the following protocol modifications: 30 ng of genomic DNA was amplified in a 5 μl reaction, using 0.25× BD Titanium Taq DNA Polymerase (BD Biosciences Canada, Mississauga, Ontario). The PCR program involved eight cycles at 95°C for 30 s and 72°C (−0.5°C/cycle) for 1 min, 37 cycles at 95°C for 30 s and 68°C for 1 min, followed by a 3 min extension at 68°C. PCR products were diluted 1:3 before the variation-specific extension reaction. Biplex extension reactions were done using the protocol specified by the manufacturer, with 100 cycles. Genotypes were automatically called with SpectroCALLER software (Sequenom) and checked manually. The PCR primers used were 5′-ACGTTGGATGAGACGCGGGCACGGCTGTCCAAG-3′ and 5′-ACGTTGGATGGCCCCGGCCTGGTACACTGC-3′. The extension primer used for apoE-3937 was 5′-GCGGACATGGAGGACGTG-3′, and that used for apoE-4075 was 5′-TGCCGATGACCTGCAGAAG-3′. The terminator mix used was ACG (Sequenom).

General procedures

Protein concentration was determined by the method of Lowry et al. (29). ApoA-I, apoA-II, and apoB concentrations were measured by immunoturbidometric methods using commercial kits (Orion Diagnostica, Espoo, Finland; Boehringer-Mannheim, Mannheim, Germany) and a clinical chemistry analyzer (Olympus Diagnostica GmbH). ApoE concentration was measured using an ELISA (30).

Serum total cholesterol (TC) and triglyceride (TG) were analyzed using fully enzymatic methods (Olympus Diagnostica). HDL- and LDL-cholesterol (HDL-C and LDL-C) were measured using direct enzymatic methods (Roche Diagnostics GmbH).

Plasma glucose concentration was analyzed by a hexokinase method (Olympus Diagnostica), and insulin was analyzed by a microparticle enzyme immunoassay (Abbott Diagnostics Division, Axis-Shield, Oslo, Norway). Concentration of C-reactive protein (CRP) was determined by an immunoturbidometric method (Orion Diagnostica). The homeostasis model assessment for insulin resistance (HOMA IR) was calculated from the fasting plasma glucose and serum insulin concentrations as follows: fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5 (31).

Statistical analysis

Comparisons of continuous variables between genders and apoE genotypes were tested for statistical significance with one-way ANOVA. Correlations of PLTP mass and activity with age, body mass index (BMI), and serum lipids were tested with the Pearson correlation test. All statistical analyses were performed using SPSS version 11.5.

RESULTS

Characteristics of the study population

The mean age of the study population, consisting of 125 male and 125 female subjects, was 55 years; the BMI

was 26.1 ± 4.0 (mean ± SD). The age, BMI, and CRP values did not differ significantly between genders (**Table 1**). The same was true for serum TG, LDL-C, apoB, apoA-II, apoE, and insulin, but there was a significant gender difference in TC, HDL-C, apoA-I, and glucose. The waist-to-hip ratio (WHR) was significantly higher among men.

Serum PLTP activities

PLTP activity assays were performed by two methods: the exogenous assay ($PLTP_{exo}$) measures phospholipid transfer to exogenously added HDL (27), and the endogenous assay ($PLTP_{endo}$) measures phospholipid transfer to endogenous serum HDL (28). Serum PLTP activity values, as measured by the two assays, are shown in **Table 2**.

TABLE 1. Basic characteristics of the study subjects (n = 250)

Variable	Mean ± SD
Age (years)	
Men	55.29 ± 15.29
Women	54.94 ± 15.14
BMI (kg/m^2)	
Men	25.62 ± 3.12
Women	26.58 ± 4.73
WHR	
Men	0.96 ± 0.05
Women	0.86 ± 0.06[a]
CRP (mg/l)	
Men	2.51 ± 7.31
Women	1.92 ± 5.23
TC (mmol/l)	
Men	5.45 ± 1.08
Women	5.81 ± 0.94[b]
TG (mmol/l)	
Men	1.54 ± 1.32
Women	1.40 ± 0.66
LDL-C (mmol/l)	
Men	3.53 ± 0.91
Women	3.70 ± 0.82
apoB (g/l)	
Men	1.13 ± 0.26
Women	1.15 ± 0.26
HDL-C (mmol/l)	
Men	1.26 ± 0.35
Women	1.48 ± 0.38[a]
apoA-I (g/l)	
Men	1.47 ± 0.27
Women	1.68 ± 0.29[a]
apoA-II (g/l)	
Men	0.35 ± 0.08
Women	0.36 ± 0.09
apoE (mg/l)	
Men	25.76 ± 16.57
Women	27.02 ± 14.92
Glucose (mmol/l)	
Men	5.62 ± 0.82
Women	5.33 ± 0.62[b]
Insulin (mU/l)	
Men	7.82 ± 8.37
Women	7.26 ± 6.94
HOMA IR	
Men	2.01 ± 2.30
Women	1.78 ± 1.55

apoB, apolipoprotein B; BMI, body mass index; CRP, C-reactive protein; HDL-C, HDL-cholesterol; HOMA IR, homeostasis model assessment for insulin resistance; LDL-C, LDL-cholesterol; TC, total cholesterol; TG, triglyceride; WHR, waist-to-hip ratio.

[a] $P < 0.001$.

[b] $P < 0.01$.

TABLE 2. PLTP activity and mass in men (n = 125) and women (n = 125)

Variable	Mean ± SD
Total PLTP mass (mg/l)	
Men	6.65 ± 1.44
Women	6.47 ± 1.46
HA-PLTP mass (mg/l)	
Men	3.15 ± 1.24
Women	2.85 ± 1.16
LA-PLTP mass (mg/l)	
Men	3.50 ± 1.07
Women	3.61 ± 1.21
$PLTP_{exo}$ activity (μmol/ml/h)	
Men	6.79 ± 1.58
Women	6.40 ± 1.71
$PLTP_{endo}$ activity (μmol/ml/h)	
Men	1.34 ± 0.37
Women	1.39 ± 0.18

HA, high-active form; LA, low-active form; PLTP, phospholipid transfer protein.

There were no significant gender differences in these activities. The serum $PLTP_{exo}$ activity was 6.59 ± 1.66 μmol/ml/h (mean ± SD), and the serum $PLTP_{endo}$ activity 1.37 ± 0.29 μmol/ml/h.

Quantitation of serum HA- and LA-PLTP concentrations

Using a method that enables us to quantitate separately the concentrations of the high- and low-active forms of serum PLTP (23), we evaluated the distribution of the two PLTP forms in sera from 250 randomly selected Finnish subjects. The mean PLTP concentration in serum was 6.56 ± 1.45 mg/l (range, 2.78–10.89 mg/l), and the mean LA-PLTP and HA-PLTP concentrations were 3.56 ± 1.14 mg/l (range, 1.20–8.43 mg/l) and 3.00 ± 1.21 mg/l (range, 0.92–7.63 mg/l), respectively (Table 2). The correlations between serum PLTP activity and concentration values are presented in **Fig. 1**. Serum $PLTP_{exo}$ activity showed a significant positive correlation with serum PLTP concentration ($r = 0.45$, $P < 0.001$; Fig. 1A) and HA-PLTP concentration ($r = 0.38$, $P < 0.001$; Fig. 1B), whereas the correlation with LA-PLTP concentration was weaker ($r = 0.17$, $P < 0.01$; Fig. 1C). $PLTP_{endo}$ activity demonstrated a weak positive correlation with HA-PLTP concentration, whereas no correlation to serum total PLTP or LA-PLTP concentration was evident (**Table 3**). A significant negative correlation was detected between serum LA- and HA-PLTP concentrations (**Fig. 2**).

Correlation of PLTP with clinical and biochemical parameters

None of the PLTP parameters correlated with gender or the inflammation marker, CRP (**Table 4**). Serum PLTP concentration correlated positively with age, and $PLTP_{exo}$ activity correlated with BMI and WHR, in accordance with earlier findings (12, 25).

$PLTP_{endo}$ activity showed a positive correlation with serum TC, TG, and LDL-C, whereas $PLTP_{exo}$ activity associated only weakly with TC and TG and not at all with LDL-C. ApoE concentration showed a positive but weak correlation with both $PLTP_{exo}$ and $PLTP_{endo}$ activity (Table 4). As

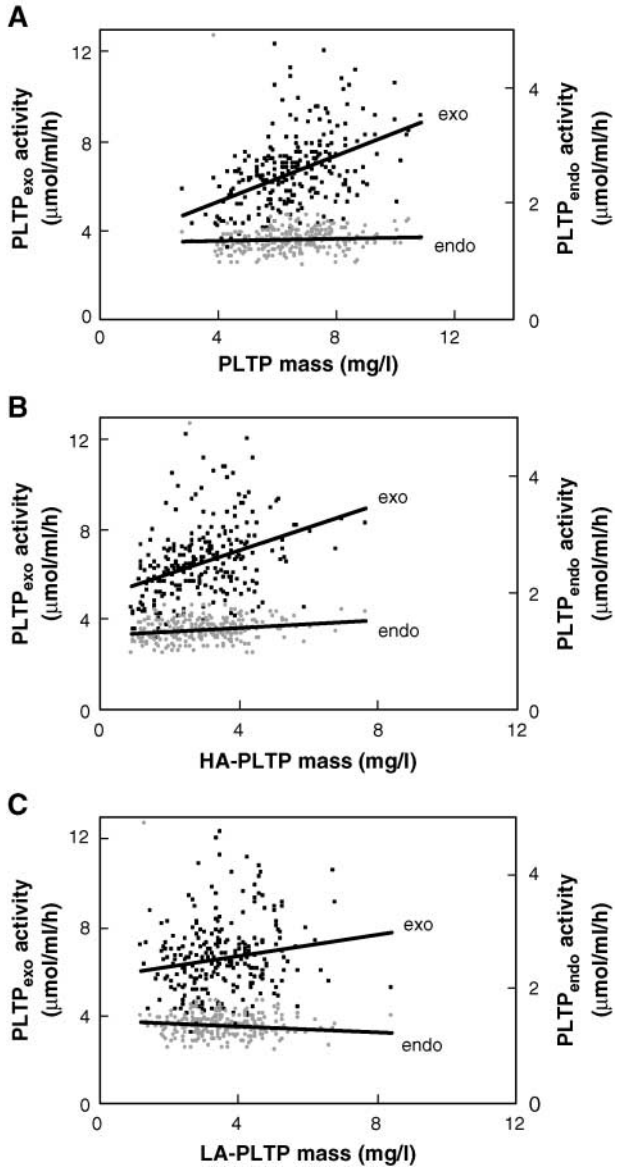

Fig. 1. Correlations between serum phospholipid transfer protein (PLTP) activities and concentrations. A: Correlation between serum PLTP concentration and PLTP activities as measured by the two assays. Black dots, exogenous assay ($PLTP_{exo}$); gray dots, endogenous assay ($PLTP_{endo}$). B: Correlation between PLTP activities and high-active form (HA)-PLTP concentration. Symbols as in A. C: Correlation between PLTP activities and low-active form (LA)-PLTP concentration. Symbols as in A.

previous studies (32) have demonstrated that serum apoE concentration is dependent on the apoE polymorphism, we analyzed apoE allele distribution in the study population. ApoE allele frequencies were similar to those reported previously for Finns (33). Correlations between apoE genotypes and PLTP parameters were performed by subgrouping apoE isoforms as follows: group 1, E3/E3 (n = 137); group 2, E3/E4, E4/E4 (n = 78); group 3, E2/E2, E2/E3, E2/E4 (n = 17). Serum apoE concentration was highest in group 3, carriers of the ε2 allele, followed by groups 1 and 2 [group 1, 28.1 ± 14.5 mg/l; group 2, 20.7 ± 15.8 mg/l; group 3, 39.9 ± 18.6 mg/l (mean ± SD, $P < 0.001$)]. $PLTP_{exo}$ activity and PLTP concentrations did not differ between the groups. The ε2 allele carriers (group 3), however, had increased serum TG levels [group 1, 1.45 ± 0.84 mmol/l; group 2, 1.42 ± 0.87 mmol/l; group 3, 2.12 ± 2.55 mmol/l ($P < 0.05$)] and increased $PLTP_{endo}$ activity [group 1, 1.35 ± 0.18 μmol/ml/h; group 2, 1.35 ± 0.19 μmol/ml/h; group 3, 1.64 ± 0.88 μmol/ml/h ($P < 0.001$)].

A statistically significant positive correlation was observed between HDL-C and serum PLTP concentration, HA-PLTP concentration, and $PLTP_{endo}$ activity. $PLTP_{exo}$ activity, however, did not correlate with HDL-C. The main apolipoprotein component of HDL, apoA-I, correlated positively with HA-PLTP concentration and $PLTP_{endo}$ activity. No correlation between LA-PLTP concentration and any measured lipid parameter was evident (Table 4).

PLTP activity has in previous studies been associated with carbohydrate metabolism (11, 34, 35). Therefore, we studied the correlations between PLTP parameters, glucose, and insulin. $PLTP_{exo}$ activity correlated positively with serum glucose, and serum total PLTP concentration and HA-PLTP concentration showed a similar trend. However, the LA-PLTP concentration tended to associate with serum insulin. Interestingly, the insulin resistance calculated as the HOMA index displayed a trend of positive correlation with $PLTP_{exo}$ activity in this normolipidemic, nondiabetic population sample.

DISCUSSION

PLTP is found in human serum in two distinct forms, one active in phospholipid transfer (HA-PLTP) and the

TABLE 3. Pearson correlation coefficients between different PLTP parameters

	Total PLTP Mass	HA-PLTP Mass	LA-PLTP Mass	Serum $PLTP_{exo}$ Activity	Serum $PLTP_{endo}$ Activity
		mg/l		*μmol/ml/h*	
Total PLTP mass (mg/l)	1	0.64[a]	0.59[a]	0.45[a]	0.05
HA-PLTP mass (mg/l)	0.64[a]	1	−0.24[a]	0.38[a]	0.15[b]
LA-PLTP mass (mg/l)	0.59[a]	−0.24[a]	1	0.17[c]	−0.10
Serum $PLTP_{exo}$ activity (μmol/ml/h)	0.45[a]	0.38[a]	0.17[c]	1	0.14[b]
Serum $PLTP_{endo}$ activity (μmol/ml/h)	0.05	0.15[b]	−0.10	0.14[b]	1

[a] $P < 0.001$.
[b] $P < 0.05$.
[c] $P < 0.01$.

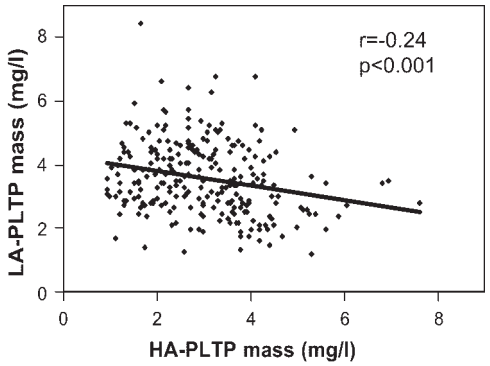

Fig. 2. Correlation between LA-PLTP and HA-PLTP concentrations.

other low-active (LA-PLTP). Both forms associate with macromolecular complexes of different size, which, in turn, affects the reactivity of PLTP antibodies (23, 36). To quantitate HA- and LA-PLTP accurately, a pretreatment of samples with SDS, to denature the protein and reveal epitope, was used for the efficient detection of both forms of PLTP. The new ELISA (23) is an essential tool for addressing the mechanisms by which the two forms of PLTP influence lipoprotein metabolism and atherogenesis.

The mean serum PLTP concentration of 250 normolipidemic subjects was 6.6 ± 1.5 mg/l, which is similar to the values reported by Desrumaux et al. (34). Of this, ~50% represents the LA-PLTP form. A large individual variation, however, exists between the relative amounts of HA- and LA-PLTP. The prevalence of HA- and LA-PLTP in subjects with different forms of dyslipidemia cannot be concluded from this study, as the sample was composed of normolipidemic individuals.

We used two different radiometric assays to determine PLTP activity. The two activity assays resulted in quite different values for serum PLTP activity: 6.6 μmol/ml/h ($PLTP_{exo}$) and 1.4 μmol/ml/h ($PLTP_{endo}$). The two methods also resulted in different correlations to lipoprotein parameters and serum glucose. The $PLTP_{endo}$ assay is dependent on endogenous HDL levels (28) and will therefore, by definition, correlate with the HDL parameters apoA-I and HDL-C. The correlations with TC, TG, and LDL-C suggest that other lipoproteins also significantly affect the outcome of the assay. The $PLTP_{endo}$ activity does not correlate with serum PLTP concentration or LA-PLTP concentration, and only a marginal correlation was seen with HA-PLTP concentration. These data suggest that the $PLTP_{endo}$ assay reflects more the activity of PLTP as modulated by the composition of endogenous lipoproteins (28), whereas the $PLTP_{exo}$ assay reflects more directly the amount of active PLTP in serum.

Among normolipidemic Finnish subjects, relatively weak correlations were detected between $PLTP_{exo}$ activity and serum TC, TG, apoB, apoE, and glucose. Murdoch and colleagues (37) reported a similar correlation between PLTP activity and apoB. They also observed a positive correlation between PLTP activity and buoyant LDL (38). In the present study, no correlation between $PLTP_{exo}$ activity and LDL-C or HDL-C was evident. The correlations with TC, apoB, and TG suggest that PLTP activity correlates with VLDL, which is in agreement with the hypothesis of Murdoch et al. (38) that as VLDL apoB increases, PLTP activity also increases to facilitate the transfer of postlipolytic surface remnants to HDL. Subjects with hypertriglyceridemia display an increase in PLTP activity (12). This increase has been suggested to be attributable to insulin resistance. The correlation of $PLTP_{exo}$ activity to the surrogate marker for insulin resistance (HOMA IR) suggested by the present study is in agreement with these findings.

Because HA- and LA-PLTP in the circulation exist as different macromolecular complexes, their interaction with other proteins and lipids seems to modulate their function and distribution. The observations that apoE concen-

TABLE 4. Pearson correlation coefficients between PLTP parameters and selected study parameters

	Total PLTP Mass	HA-PLTP Mass	LA-PLTP Mass	Serum $PLTP_{exo}$ Activity	Serum $PLTP_{endo}$ Activity,
		mg/l		*μmol/ml/h*	
Gender	−0.062	−0.123	0.050	−0.120	0.096
Age (years)	0.175[a]	0.097	0.119	0.012	−0.148[b]
BMI (kg/m^2)	0.051	−0.002	0.067	0.165[a]	0.081
WHR	0.009	0.078	−0.072	0.197[a]	−0.008
CRP (mg/l)	0.005	0.022	−0.017	0.008	−0.010
TC (mmol/l)	0.028	0.120	−0.091	0.136[b]	0.423[c]
TG (mmol/l)	−0.132[b]	−0.076	−0.087	0.154[b]	0.458[c]
LDL-C (mmol/l)	0.009	0.061	−0.054	0.092	0.255[c]
apoB (g/l)	−0.078	−0.029	−0.068	0.193[a]	0.044
HDL-C (mmol/l)	0.215[c]	0.291[c]	−0.034	−0.010	0.310[c]
apoA-I (g/l)	0.107	0.187[a]	−0.062	0.063	0.404[c]
apoA-II (g/l)	0.015	0.028	−0.008	0.073	0.515[c]
apoE (mg/l)	0.003	0.024	−0.021	0.154[b]	0.192[a]
Glucose (mmol/l)	0.130[b]	0.162[b]	−0.006	0.182[a]	0.055
Insulin (mU/l)	0.049	−0.065	0.132[b]	0.095	−0.078
HOMA IR	0.054	−0.031	0.102	0.133[b]	−0.061

[a] $P < 0.01$.
[b] $P < 0.05$.
[c] $P < 0.001$.

tration correlates positively with PLTP activity but not with PLTP concentration, and that apoA-I does not correlate with PLTP activity but correlates with PLTP concentration, suggest that these apolipoproteins may be actively involved in the distribution of PLTP between the LA-PLTP and HA-PLTP forms. In accordance with this is the observation that LA-PLTP isolated from serum is associated with apoA-I and the HA-PLTP form copurifies with apoE (16). Also, the observations that active PLTP secreted by HepG2 cells is associated with apoE (30) and that PLTP colocalizes exclusively with apoE-containing particles during inflammation (39) are in agreement with the suggestion that apoE may be involved in maintaining PLTP in a catalytically active form.

The presence of three apoE isoforms (E2, E3, and E4) has physiological implications regarding lipoprotein metabolism (40–42). Because HA-PLTP preferentially associates with apoE during purification from human plasma (16) and there is a correlation between serum apoE concentration and PLTP activity, we analyzed the apoE isoform distribution in the study population and its correlation with PLTP activity and concentration. The ε2-carrying genotypes displayed the highest serum levels of apoE and had the highest serum $PLTP_{endo}$ activity. Because we could not demonstrate any differences in the $PLTP_{exo}$ activity versus the apoE isoforms, we consider that the increased $PLTP_{endo}$ activity is attributable to a difference in the serum lipid/lipoprotein profile that is reflected in the outcome of the endogenous PLTP activity assay.

Studies have set out to assess the impact of PLTP on atherosclerosis, type II diabetes, and the interaction of PLTP with cholesterol-lowering drugs (11, 43–45). The number of patients in these studies has been low, and either the PLTP concentration has not been determined or the methods of measuring PLTP serum concentration have not taken into account the existence of the HA- and LA-PLTP forms in the circulation. However, recent reports suggest that high PLTP activity is associated with high-risk patients who display lipid profiles characteristic of atherosclerosis and type II diabetes. In the present study, serum glucose correlated positively with $PLTP_{exo}$ activity, total PLTP concentration, and HA-PLTP concentration but not with LA-PLTP concentration. These data suggest that PLTP activity and the distribution of the two PLTP forms in serum are influenced not only by lipid but also by glucose metabolism. Tu and Albers (14) have shown that expression of the PLTP gene is subject to regulation by glucose via a peroxisome proliferator-activated receptor-mediated pathway. However, the detailed mechanisms by which glucose and insulin could modulate the PLTP subclass distribution remain an interesting target for future studies.

In conclusion, we still await large, preferably prospective studies on PLTP to assess whether PLTP concentration, activity, or both combined can be used as independent markers for cardiovascular risk evaluation and whether it is possible to reduce the risk by targeted drug treatment.

This work was supported by the International HDL Research Awards Program (C.E., M.J., and V.M.O.), the Finnish Foundation for Cardiovascular Research (M.T.J., M.J., and V.M.O.), the Sigrid Juselius Foundation (M.J. and V.M.O.), the Finska Vetenskaps-Societeten, the Magnus Ehrnrooths Stiftelse, the Finska Läkaresällskapet (S.S.), and the Wihuri Research Foundation (M.T.J.).

REFERENCES

1. Castelli, W. P., R. J. Garrison, P. W. Wilson, R. D. Abbott, S. Kalousdian, and W. B. Kannel. 1986. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *J. Am. Med. Assoc.* **256:** 2835–2838.
2. Kannel, W. B. 1983. High-density lipoproteins: epidemiologic profile and risks of coronary artery disease. *Am. J. Cardiol.* **52:** 9B–12B.
3. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
4. Huuskonen, J., V. M. Olkkonen, M. Jauhiainen, and C. Ehnholm. 2001. The impact of phospholipid transfer protein (PLTP) on HDL metabolism. *Atherosclerosis.* **155:** 269–281.
5. Tollefson, J., S. Ravnik, and J. Albers. 1988. Isolation and characterisation of a phospholipid transfer protein (LTP-II) from human plasma. *J. Lipid Res.* **29:** 1593–1602.
6. Jauhiainen, M., J. Metso, R. Pahlman, S. Blomqvist, A. van Tol, and C. Ehnholm. 1993. Human plasma phospholipid transfer protein causes high density lipoprotein conversion. *J. Biol. Chem.* **268:** 4032–4036.
7. Tu, A. Y., H. I. Nishida, and T. Nishida. 1993. High density lipoprotein conversion mediated by human plasma phospholipid transfer protein. *J. Biol. Chem.* **268:** 23098–23105.
8. Desrumaux, C. M., P. A. Mak, W. A. Boisvert, D. Masson, D. Stupack, M. Jauhiainen, C. Ehnholm, and L. K. Curtiss. 2003. Phospholipid transfer protein is present in human atherosclerotic lesions and is expressed by macrophages and foam cells. *J. Lipid Res.* **44:** 1453–1461.
9. Laffitte, B. A., S. B. Joseph, M. Chen, A. Castrillo, J. Repa, D. Wilpitz, D. Mangelsdorf, and P. Tontonoz. 2003. The phospholipid transfer protein gene is a liver X receptor target expressed by macrophages in atherosclerotic lesions. *Mol. Cell. Biol.* **23:** 2182–2191.
10. Oram, J. F., G. Wolfbauer, A. M. Vaughan, C. Tang, and J. J. Albers. 2003. Phospholipid transfer protein interacts with and stabilizes ATP-binding cassette transporter A1 and enhances cholesterol efflux from cells. *J. Biol. Chem.* **278:** 52379–52385.
11. Riemens, S. C., A. van Tol, W. J. Sluiter, and R. P. Dullaart. 1998. Plasma phospholipid transfer protein activity is related to insulin resistance: impaired acute lowering by insulin in obese type II diabetic patients. *Diabetologia.* **41:** 929–934.
12. Jonkers, I. J., A. H. Smelt, H. Hattori, L. M. Scheek, T. van Gent, F. H. de Man, A. van der Laarse, and A. van Tol. 2003. Decreased PLTP mass but elevated PLTP activity linked to insulin resistance in HTG: effects of bezafibrate therapy. *J. Lipid Res.* **44:** 1462–1469.
13. Colhoun, H. M., M. R. Taskinen, J. D. Otvos, P. Van Den Berg, J. O'Connor, and A. Van Tol. 2002. Relationship of phospholipid transfer protein activity to HDL and apolipoprotein B-containing lipoproteins in subjects with and without type 1 diabetes. *Diabetes.* **51:** 3300–3305.
14. Tu, A. Y., and J. J. Albers. 2001. Glucose regulates the transcription of human genes relevant to HDL metabolism: responsive elements for peroxisome proliferator-activated receptor are involved in the regulation of phospholipid transfer protein. *Diabetes.* **50:** 1851–1856.
15. Oka, T., T. Kujiraoka, M. Ito, T. Egashira, S. Takahashi, M. N. Nanjee, N. E. Miller, J. Metso, V. M. Olkkonen, C. Ehnholm, M. Jauhiainen, and H. Hattori. 2000. Distribution of phospholipid transfer protein in human plasma: presence of two forms of phospholipid transfer protein, one catalytically active and the other inactive. *J. Lipid Res.* **41:** 1651–1657.
16. Kärkkäinen, M., T. Oka, V. M. Olkkonen, J. Metso, H. Hattori, M. Jauhiainen, and C. Ehnholm. 2002. Isolation and partial characterization of the inactive and active forms of human plasma phospholipid transfer protein (PLTP). *J. Biol. Chem.* **277:** 15413–15418.
17. Jiang, X. C., C. Bruce, J. Mar, M. Lin, Y. Ji, O. L. Francone, and

A. R. Tall. 1999. Targeted mutation of plasma phospholipid transfer protein gene markedly reduces high-density lipoprotein levels. *J. Clin. Invest.* **103:** 907–914.
18. Foger, B., S. Santamarina-Fojo, R. D. Shamburek, C. L. Parrot, G. D. Talley, and H. B. Brewer, Jr. 1997. Plasma phospholipid transfer protein. Adenovirus-mediated overexpression in mice leads to decreased plasma high density lipoprotein (HDL) and enhanced hepatic uptake of phospholipids and cholesteryl esters from HDL. *J. Biol. Chem.* **272:** 27393–27400.
19. Jiang, X., O. L. Francone, C. Bruce, R. Milne, J. Mar, A. Walsh, J. L. Breslow, and A. R. Tall. 1996. Increased prebeta-high density lipoprotein, apolipoprotein AI, and phospholipid in mice expressing the human phospholipid transfer protein and human apolipoprotein AI transgenes. *J. Clin. Invest.* **98:** 2373–2380.
20. Ehnholm, S., K. W. van Dijk, B. van't Hof, A. van der Zee, V. M. Olkkonen, M. Jauhiainen, M. Hofker, L. Havekes, and C. Ehnholm. 1998. Adenovirus mediated overexpression of human phospholipid transfer protein alters plasma HDL levels in mice. *J. Lipid Res.* **39:** 1248–1253.
21. van Haperen, R., A. van Tol, P. Vermeulen, M. Jauhiainen, T. van Gent, P. van den Berg, S. Ehnholm, F. Grosveld, A. van der Kamp, and R. de Crom. 2000. Human plasma phospholipid transfer protein increases the antiatherogenic potential of high density lipoproteins in transgenic mice. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1082–1088.
22. Qin, S., K. Kawano, C. Bruce, M. Lin, C. Bisgaier, A. R. Tall, and X. Jiang. 2000. Phospholipid transfer protein gene knock-out mice have low high density lipoprotein levels, due to hypercatabolism, and accumulate apoA-IV-rich lamellar lipoproteins. *J. Lipid Res.* **41:** 269–276.
23. Siggins, S., M. Kärkkäinen, J. Tenhunen, J. Metso, E. Tahvanainen, V. M. Olkkonen, M. Jauhiainen, and C. Ehnholm. 2004. Quantitation of the active and low-active forms of human plasma phospholipid transfer protein by ELISA. *J. Lipid Res.* **45:** 387–395.
24. Aromaa, A., and S. Koskinen. 2002. Health and Functional Capacity in Finland: Baseline Results of the Health 2000 Health Examination Survey. National Public Health Institute, Helsinki, Finland.
25. Huuskonen, J., M. Ekstrom, E. Tahvanainen, A. Vainio, J. Metso, P. Pussinen, C. Ehnholm, V. M. Olkkonen, and M. Jauhiainen. 2000. Quantification of human plasma phospholipid transfer protein (PLTP): relationship between PLTP mass and phospholipid transfer activity. *Atherosclerosis.* **151:** 451–461.
26. Kato, H., T. Nakanishi, T. Arai, H. I. Nishida, and T. Nishida. 1988. Purification, microheterogeneity, and stability of human lipid transfer protein. *J. Biol. Chem.* **264:** 4082–4087.
27. Damen, J., J. Regts, and G. Scherphof. 1982. Transfer of [^{14}C] phosphatidylcholine between liposomes and human plasma high density lipoprotein. Partial purification of a transfer-stimulating plasma factor using a rapid transfer assay. *Biochim. Biophys. Acta.* **712:** 444–452.
28. Lagrost, L., A. Athias, N. Lemort, J. L. Richard, C. Desrumaux, L. Chatenet-Duchene, M. Courtois, M. Farnier, B. Jacotot, S. Braschi, and P. Gambert. 1999. Plasma lipoprotein distribution and lipid transfer activities in patients with type IIb hyperlipidemia treated with simvastatin. *Atherosclerosis.* **143:** 415–425.
29. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
30. Siggins, S., M. Jauhiainen, V. M. Olkkonen, J. Tenhunen, and C. Ehnholm. 2003. PLTP secreted by HepG2 cells resembles the high-activity PLTP form in human plasma. *J. Lipid Res.* **44:** 1698–1704.
31. Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* **28:** 412–419.
32. Smit, M., P. de Knijff, M. Rosseneu, J. Bury, E. Klasen, R. Frants, and L. Havekes. 1988. Apolipoprotein E polymorphism in the Netherlands and its effect on plasma lipid and apolipoprotein levels. *Hum. Genet.* **80:** 287–292.
33. Ehnholm, C., M. Lukka, T. Kuusi, E. Nikkilä, and G. Utermann. 1986. Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. *J. Lipid Res.* **27:** 227–235.
34. Desrumaux, C., A. Athias, G. Bessede, B. Verges, M. Farnier, L. Persegol, P. Gambert, and L. Lagrost. 1999. Mass concentration of plasma phospholipid transfer protein in normolipidemic, type IIa hyperlipidemic, type IIb hyperlipidemic, and non-insulin-dependent diabetic subjects as measured by a specific ELISA. *Arterioscler. Thromb. Vasc. Biol.* **19:** 266–275.
35. Albers, J. J., and M. C. Cheung. 2004. Emerging roles for phospholipid transfer protein in lipid and lipoprotein metabolism. *Curr. Opin. Lipidol.* **15:** 255–260.
36. Murdoch, S. J., G. Wolfbauer, H. Kennedy, S. M. Marcovina, M. C. Carr, and J. J. Albers. 2002. Differences in reactivity of antibodies to active versus inactive PLTP significantly impacts PLTP measurement. *J. Lipid Res.* **43:** 281–289.
37. Murdoch, S. J., M. C. Carr, J. E. Hokanson, J. D. Brunzell, and J. J. Albers. 2000. PLTP activity in premenopausal women. Relationship with lipoprotein lipase, HDL, LDL, body fat, and insulin resistance. *J. Lipid Res.* **41:** 237–244.
38. Murdoch, S. J., M. C. Carr, H. Kennedy, J. D. Brunzell, and J. J. Albers. 2002. Selective and independent associations of phospholipid transfer protein and hepatic lipase with the LDL subfraction distribution. *J. Lipid Res.* **43:** 1256–1263.
39. Barlage, S., D. Frohlich, A. Bottcher, M. Jauhiainen, H. P. Muller, F. Noetzel, G. Rothe, C. Schutt, R. P. Linke, K. J. Lackner, C. Ehnholm, and G. Schmitz. 2001. ApoE-containing high density lipoproteins and phospholipid transfer protein activity increase in patients with a systemic inflammatory response. *J. Lipid Res.* **42:** 281–290.
40. Dallongeville, J., S. Lussier-Cacan, and J. Davignon. 1992. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. *J. Lipid Res.* **33:** 447–454.
41. Ehnholm, C., R. W. Mahley, D. A. Chappell, K. H. Weisgraber, E. Ludwig, and J. L. Witztum. 1984. Role of apolipoprotein E in the lipolytic conversion of beta-very low density lipoproteins to low density lipoproteins in type III hyperlipoproteinemia. *Proc. Natl. Acad. Sci. USA.* **81:** 5566–5570.
42. Mahley, R. W., Y. Huang, and S. C. Rall, Jr. 1999. Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia): questions, quandaries, and paradoxes. *J. Lipid Res.* **40:** 1933–1949.
43. Riemens, S. C., A. Van Tol, W. J. Sluiter, and R. P. Dullaart. 1999. Acute and chronic effects of a 24-hour intravenous triglyceride emulsion challenge on plasma lecithin:cholesterol acyltransferase, phospholipid transfer protein, and cholesteryl ester transfer protein activities. *J. Lipid Res.* **40:** 1459–1466.
44. Kaser, S., A. Sandhofer, B. Foger, C. F. Ebenbichler, B. Igelseder, L. Malaimare, B. Paulweber, and J. R. Patsch. 2001. Influence of obesity and insulin sensitivity on phospholipid transfer protein activity. *Diabetologia.* **44:** 1111–1117.
45. Cheung, M. C., G. Wolfbauer, H. Kennedy, B. G. Brown, and J. J. Albers. 2001. Plasma phospholipid transfer protein activity in patients with low HDL and cardiovascular disease treated with simvastatin and niacin. *Biochim. Biophys. Acta.* **1537:** 117–124.