High Plasma Phospholipid Transfer Protein Levels as a Risk Factor for Coronary Artery Disease

Axel Schlitt, Christoph Bickel, Prathima Thumma, Stefan Blankenberg, Hans J. Rupprecht, Juergen Meyer, Xian-Cheng Jiang

Objective—Plasma phospholipid transfer protein (PLTP) mediates both net transfer and exchange of phospholipids between different lipoproteins. Animal studies have shown that it is closely related to the development of atherosclerosis. PLTP-deficient mice have demonstrated increased antioxidation potential as well as a decrease in apolipoprotein B secretion and atherosclerotic lesions. In humans, high PLTP is associated with type II diabetes and obesity.

Methods and Results—To assess the relationship between PLTP activity and coronary artery disease (CAD), a novel, high-throughput method to measure plasma PLTP activity was used, relating it to CAD in 1102 cases and 444 controls. This demonstrated that PLTP activity in patients with CAD was significantly higher than in controls (25.5 versus 22.4 pmol/μL per h; P < 0.0001). Using multivariate logistic regression analysis, PLTP activity was found to have independent predictive value for CAD. Patients within the highest quintile of PLTP activity revealed a 1.9-fold increase in risk for CAD compared with patients within the lowest quintile.

Conclusions—These findings indicate that PLTP activity is positively and independently related to CAD and suggest that (1) prospective studies to evaluate this relationship are warranted and (2) PLTP should be considered a therapeutic target. (Arterioscler Thromb Vasc Biol. 2003;23:1857-1862.)

Key Words: phospholipid transfer protein ■ risk factors ■ coronary artery disease

There is accumulating in vitro evidence indicating that plasma phospholipid transfer protein (PLTP) plays an important role in the remodeling of lipoproteins. During lipolysis of triglyceride-rich lipoprotein (TRL), partially purified PLTP has been shown to mediate both the transfer and the exchange of phospholipids between these particles and HDL.1,2 PLTP can also cause conversion of HDL3 into both larger and smaller particles in a time- and concentration-dependent fashion.3,4

Genetic mouse models have played a crucial role in elucidating the role of PLTP in lipoprotein metabolism. We and others5-7 have shown that within PLTP transgenic mice, PLTP overexpression increases the influx of phospholipid and secondarily of cholesterol into HDL, leading to an increase in pre-β-HDL particles. PLTP gene knockout mice have recently provided the first in vivo evidence of a crucial role for PLTP-mediated lipid transfer in the maintenance of lipoprotein levels and in atherosclerosis development. PLTP deficiency resulted in markedly decreased atherosclerosis8 through at least 2 mechanisms, (1) decreased production and levels of TRL,8 and (2) increased antioxidation potential.9,10

Human studies on PLTP activity have presented a complicated and sometimes conflicting picture. Plasma PLTP activity was significantly higher in patients with type II diabetes.11,12 It was associated with insulin resistance in conjunction with altered nonesterified fatty acid and triglyceride metabolism.13 In regard to plasma HDL cholesterol (HDL-C) levels, which have powerful antiatherogenic properties,14 existing reports are contradictory. In one set of studies, PLTP activity was positively related to HDL-C,15 and in patients with low HDL and cardiovascular disease, this activity was positively correlated with the concentration of HDL particles containing apolipoprotein (apo) A-I but not apoA-II.15 However, in another study, opposite results were reported.16 These conflicting results might be attributable to (1) too small a sample size, (2) subjects that were not controlled very well, and (3) the method for PLTP measurement not being precise enough.

Both animal and human studies suggest that plasma PLTP levels are an important factor in lipoprotein metabolism and atherosclerosis development. However, these levels have never been systematically assessed as a risk factor for atherosclerosis in humans. Because there are two forms of PLTP in human plasma,16-18 one being catalytically active and the other not, activity measurement of PLTP is more...
relevant than its mass measurement. In this report, we have used a novel, high-throughput method to test the hypothesis that PLTP activity levels are associated with coronary artery disease (CAD).

Methods

Study Population
Between November, 1996, and July, 2000, we recruited male and female patients experiencing stable (n=830) or unstable (n=222) angina pectoris according to Braunwald classifications II and III or recent (<48 hours) acute myocardial infarction (MI) (n=50) admitted to the second medical department of the Johannes Gutenberg-University Mainz and the Bundeswehrzentralkrankenhaus Koblenz for diagnostic coronary angiography. The sole inclusion criterion was the presence of a stenosis greater than 30% in at least 1 major coronary artery. The study is described in detail elsewhere.\(^\text{19}\) Exclusion criteria were lack of CAD as defined above and evidence of significant concomitant disease, in particular severe valvular heart disease, known cardiomyopathy, malignancy, or febrile condition. Patients completed a questionnaire about smoking habits, history of diabetes mellitus, hypertension, hyperlipoproteinemia, current drug use, and family history of premature CAD (documented in 1 first-degree relative before age 65 years). Diabetes mellitus was diagnosed in patients who had previously undergone dietary treatment or received additional oral antidiabetic or insulin medication or who had a present fasting blood sugar level >125 mg/dL; hypertension was diagnosed in patients who had received antihypertensive treatment or had been diagnosed as hypertensive (blood pressure >160/90 mm Hg); hyperlipoproteinemia was diagnosed in patients who had been given lipid-lowering medication or had a history of total cholesterol (TC) levels >240 mg/dL.

Healthy control subjects (n=444) were recruited either from general practitioners’ offices in the course of routine check-up visits or by newspaper announcement. This announcement briefly described the study design and invited healthy individuals aged ≥40 years to participate in the AtheroGene study as control subjects. Of the individuals who presented, we selected those without any classical and treatable risk factors and the laboratory personnel was unaware of the study individual assignation.

Serum lipid levels (TC, Roche Diagnostics; HDL-C, Rolf Greiner Biochemical; LDL cholesterol (LDL-C), calculated according to the Friedewald formula; triglyceride, Roche Diagnostics) were determined immediately. ApoA-I and apoB concentrations were determined by an immunoturbidimetric assay (Tina-quant, Roche Diagnostics). The Lipoprotein(a) [Lp(a)] concentration was determined using an enzyme-linked immunosorbent assay-based method supplied by Immuno Ltd (Dunton Green). C-reactive protein (CRP) was determined by a highly sensitive, latex particle–enhanced immunoassay (detection range of 0 to 20 mg/L); the between-day imprecision coefficient of variation of this assay (n=21) was 2.14% and 1.44% at mean levels of 1.90 and 4.33 mg/L (Roche Diagnostics). Homocysteine was determined by established high-pressure liquid chromatography method.

Statistical Analysis
Plasma levels of PLTP activity were normally distributed, and results are presented as mean values. Demographic and clinical variables of cases and controls were compared by χ² test for categorical and ANOVA test for continuous variables. Because of skewed distribution for triglyceride, median values were presented and Mann-Whitney test was applied for this variable. For correlation of PLTP activity with additional lipid variables, Pearson correlation coefficients were calculated using log-transformed triglyceride values. Association between PLTP activity—considered as continuous variable—and CAD was additionally tested in the overall population as well as stratified by gender using ANOVA test, additionally adjusting for age, sex, classical risk factors, and lipid variables by using logistic regression analysis (for details, see Table 3). We aimed to assess any evidence of association between PLTP and CAD in models assuming both linear and nonlinear effects. We thus divided cases and controls into quintiles on the basis of the overall study population. We applied 4 logistic regression models, first controlling for age and sex and, in additional models, for conventional risk factors (body mass index [BMI], smoking [current smoking as dichotomous variable], history of diabetes, and hypertension as well as the lipid parameters HDL-C, LDL-C, Lp(a), and apoA-I levels as continuous variables) as well as hs-CRP and homocysteine as log-transformed continuous variables. With respect to multicolinearity, we only included LDL-C as the cluster representative of the variables TC, LDL-C, and apoB into the logistic regression model. The Wald χ² test (4 degrees of freedom) was used to test for differences across quintiles, and the association was quantified by means of the odds ratio. P<0.05 was considered to be significant. All analyses were carried out using SPSS 10.01 software.

Results
Baseline data regarding case and control subjects are outlined in Table 1. As expected, prevalence of classical risk factors such as current smoking (25.8% versus 12.6%), history of diabetes mellitus (32.8% versus 9.9%), hypertension (72.3% versus 28.6%), and family history of premature CAD (38.7% versus 20.8%) were more frequent in cases compared with controls. Furthermore, a significantly higher prescription rate of statins was noted in cases than in controls, and this might well have caused the significantly lower levels of TC and LDL-C levels that were noted in the cases. LDL-C and apoA-I levels were lower in cases than in controls, as expected. Lp(a) and triglyceride but not apoB levels were higher in cases.

To investigate the possibility that PLTP activity could act as a marker for HDL and apoB-containing particles, we performed correlation analyses as outlined in Table 2. In control subjects, there was a moderate but significant nega-
TABLE 1. Baseline Characteristics in Cases and Controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n=1102)</th>
<th>Controls (n=444)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61.5±10.2</td>
<td>59.9±7.4</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.1±3.8</td>
<td>26.7±4.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Male, %</td>
<td>74.3</td>
<td>72.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>25.8</td>
<td>12.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>32.8</td>
<td>9.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>72.3</td>
<td>28.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family history of CAD, %</td>
<td>38.7</td>
<td>20.8</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Categorical variables are presented as percentage of patients. P values were obtained by using the χ² test (two-sided) for association. Continuous variables are presented as mean±SD or median values (25%/75% interquartiles) because of skewed distribution. P values were obtained by using ANOVA or Mann-Whitney test.

The Figure demonstrates the distribution of PLTP activity in cases and controls. CAD cases were found more commonly at higher levels than controls were found. However, there was no statistically significant difference of PLTP activity between patients with stable angina and acute coronary syndrome (25.9±9.6 versus 24.7±10.2 pmol/µL per h).

Patients with CAD had significantly higher plasma PLTP activity levels (Table 3). Analyzed by sex, female patients with CAD had levels of 25.1±10.0 pmol/µL per h and female controls had levels of 19.4±6.7 pmol/µL (P<0.0001), whereas male patients with CAD had levels of 25.6±9.8 pmol/µL per h and male controls had levels of 23.5±7.4 pmol/µL per h (P<0.0001).

To evaluate the risk associated with increasing plasma PLTP activity, we calculated odds ratios for each quintile (based on the whole study sample) relative to the first. The odds ratio for CAD for the fourth and fifth quintile was significantly higher than for the first (Table 4).

To evaluate whether the association of PLTP activity with CAD was independent of other known risk factors, we carried out various logistic regression models. As outlined in Table 5, model 1, each increase of PLTP activity quintile was associated with an 1.2-fold increase in CAD risk (95% CI, 1.1 to 1.3; P for trend <0.0001). Individuals within the highest quintile of PLTP activity were at significantly higher risk of CAD than individuals in the lowest quintile (odds ratio, 1.21; 95% CI, 1.11 to 1.31; P<0.0001).

The Figure demonstrates the distribution of PLTP activity in cases and controls. CAD cases were found more commonly at higher levels than controls were found.

TABLE 2. Coefficients of Correlation Between PLTP and Further Lipid Variables

<table>
<thead>
<tr>
<th>Lipid Variable</th>
<th>PLTP Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
</tr>
<tr>
<td>Age, y</td>
<td>−0.02</td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>0.03</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>0.04</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>0.02</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>0.11†</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.01</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>−0.01</td>
</tr>
</tbody>
</table>

Pearson correlation coefficients: *P<0.05, †P<0.01. ‡Log-transformed triglyceride levels were assessed.
PLTP quintile had a 1.9-fold (95% CI, 1.3 to 2.8; \( P < 0.0001 \)) increase in risk of CAD compared with those in the lowest. This association remained nearly unaffected after controlling for potential risk factors like BMI, history of hypertension, diabetes, current smoking, and family history of CAD, as well as the lipid factors LDL-C, HDL-C, and triglyceride (Table 5, model 2). After apoA-I and Lp(a), which were, in contrast to apoB, significantly associated with prevalence of CAD, were further forced into the model, the association between PLTP activity and CAD risk was not materially altered; also, additional adjustment for hs-CRP and homocysteine did not weaken this association (Table 5, models 3 and 4). However, because of missing variables for homocysteine and hs-CRP, the latter model consists only of 805 individuals.

We additionally evaluated the association between quintiles of PLTP activity and CAD in a sex-specific manner based on sex-specific quintiles in controls. In these analyses, each PLTP quintile increase was associated with a 1.4-fold (95% CI, 1.2 to 1.6; \( P = 0.0001 \)) increase in CAD risk for women and a 1.2-fold (95% CI, 1.02 to 1.2; \( P = 0.014 \)) increase in CAD for men.

**Discussion**

Traditional measurements have focused on TC and apoB-containing particles as indicators of atherogenesis and HDL-C as an indicator of antiatherogenesis. However, a body of in vitro and in vivo evidence has suggested that the measurement of plasma enzyme activities, including PLTP and cholesteryl ester transfer protein,\(^{20}\) which control the lipoprotein metabolism, could also be relevant to atherosclerosis and even more relevant to antiatherogenic therapy.\(^{21}\) However, the relationship between plasma PLTP activity and atherosclerosis has never been systematically assessed, partly because of the difficulties of measuring PLTP activity on a large scale. To overcome this problem, we used a novel high-throughput kit to measure plasma PLTP activity in a relatively large scale. In the present study, we demonstrated for the first time that plasma PLTP activity levels were higher in cases with CAD than in controls, and this difference was found to be independent of other risk factors. Although these findings are biologically plausible, it will be important to confirm them in other samples and in a prospective study.

Why was PLTP activity higher in CAD cases than in controls? A significantly higher prescription rate of statins was noted in cases compared with controls, and this might possibly have caused the increase of PLTP activity. However, this seems unlikely, because no significant change in such activity (as measured by an endogenous lipoprotein–independent method) was observed in simvastatin-treated patients compared with placebo.\(^{15,22}\) It was also noted that the percentage of diabetes and unfavorable BMI were significantly higher in cases than in controls (Table 1), and this might have caused the increase in PLTP activity, because such an association has been observed.\(^{11-13,23}\) This also seems to be unlikely, though, because when we considered both factors in the multivariate analysis, PLTP activity was independent of them (Table 4).

Several different mechanisms could explain the relationship between plasma PLTP activity and CAD case versus control status. First, high plasma PLTP activity may decrease HDL concentrations, which are a well-known antiatherogenic marker. Overexpression of PLTP in mice, either by adenovirus or transgene, has been seen to decrease HDL levels.\(^{6,7,24}\) Huuskonen et al\(^{16}\) reported that

<table>
<thead>
<tr>
<th>Quintiles of PLTP, pmole/( \mu )L per h</th>
<th>No. of Subjects</th>
<th>No. (%)</th>
<th>Odds Ratio</th>
<th>Upper 95% CI</th>
<th>Lower 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;16.5</td>
<td>317</td>
<td>215 (67.8)</td>
<td>1</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>16.5 to 21.5</td>
<td>321</td>
<td>198 (61.7)</td>
<td>0.8</td>
<td>0.6</td>
<td>1.1</td>
</tr>
<tr>
<td>&gt;21.5 to 26.2</td>
<td>312</td>
<td>222 (71.2)</td>
<td>1.2</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>&gt;26.2 to 32.6</td>
<td>297</td>
<td>226 (76.1)</td>
<td>1.5</td>
<td>1.1</td>
<td>2.2</td>
</tr>
<tr>
<td>&gt;32.6</td>
<td>299</td>
<td>241 (80.6)</td>
<td>2.0</td>
<td>1.4</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Data presented are odds ratios of PLTP quintiles in a logistic regression model including only PLTP as a covariate.
the activity of PLTP in human plasma showed a negative correlation with HDL-C and apoA-I, a major apolipoprotein in HDL. However, conflicting reports also exist. In this study, we found that there was a mild negative correlation between PLTP activity and HDL-C in controls ($r = -0.15$, $P < 0.001$) and between PLTP activity and apoA-I ($r = -0.14$, $P < 0.05$), but not in cases, indicating that HDL-C lowering may play only a minor role here.

Second, high plasma PLTP activity may increase TRL concentrations, which is well-known atherogenic risk factor. We have previously reported that PLTP deficiency in mice resulted in reduced production and levels of TRL and markedly decreased atherosclerosis. Murdoch et al. reported that PLTP activity in premenopausal women was significantly and positively correlated with LDL-C and apoB ($r = 0.44$). However, our study did not arrive at such easy and clear-cut conclusions. What we found was a mild positive correlation between PLTP activity and triglyceride levels ($r = 0.16$, $P < 0.001$) in controls but not in cases, indicating that in this study increased TRL levels may play only a minor role here, if any.

Third, high PLTP activity may provide a prolipoprotein oxidation status. We recently measured $\alpha$-tocopherol content and oxidation parameters of lipoproteins from PLTP-deficient mice in apoE-deficient, LDL receptor–deficient, or apoB/CETP transgenic backgrounds. In all 3 backgrounds, the vitamin E content of VLDL or LDL was significantly increased in PLTP-deficient mice compared with controls having normal PLTP activity. Moreover, PLTP deficiency produced a dramatic delay in generation of conjugated dienes in copper-oxidized TRL as well as markedly lower titers of plasma IgG autoantibodies to conjugated dienes.25 It is likely that higher PLTP activity in cases might promote TRL oxidation.

Fourth, high plasma PLTP activity may transfer more oxidized phospholipid from TRL to HDL, which then becomes less protective against atherosclerosis than normal HDL. We found that the ability of HDL from PLTP-deficient mice to inhibit LDL oxidation and LDL-induced monocyte chemotactic activity in human artery wall cell cocultures significantly decreased with time after inoculation compared with their controls (X.C. Jiang and M. Navab, unpublished data, 2003).

Although presently known risk factors have some predictive value for CAD, a major part of the variability in this process remains unexplained.26 Also, therapy aimed at lowering LDL cholesterol reduces only a fraction ($\approx 30\%$) of the burden of atherosclerosis.27 Our data are hypothesis-generating rather than proving and have to be evaluated additionally in prospective studies. Although our discovery that high plasma PLTP activity is a risk factor for CAD needs to be confirmed by prospective studies, it holds the promise of a simple test that may have independent predictive value for CAD and provide a novel therapeutic target.

Acknowledgments

This study was supported by National Institute of Health grants HL-64735 and HL-69817.

References

active forms of human plasma phospholipid transfer protein (PLTP).


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Arterioscler Thromb Vasc Biol. 2003;23:1857-1862; originally published online August 28, 2003; doi: 10.1161/01.ATV.0000094433.98445.7F

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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