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Original Article

Plasma Phospholipid Transfer Protein Activity Is Decreased in Type 2 Diabetes During Treatment With Atorvastatin

A Role for Apolipoprotein E?

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Plasma phospholipid transfer protein (PLTP) plays an important role in lipoprotein metabolism. PLTP activity is elevated in patients with diabetes, a condition with strongly elevated risk for coronary heart disease. The aim of this study was to test the hypothesis that statins reduce PLTP activity and to examine the potential role of apolipoprotein E (apoE). PLTP activity and apoE were measured in patients with type 2 diabetes from the DALI (Diabetes Atorvastatin Lipid Intervention) Study, a 30-week randomized double-blind placebo-controlled trial with atorvastatin (10 and 80 mg daily). At baseline, PLTP activity was positively correlated with waist circumference, HbA_{1c}, glucose, and apoE (all $P < 0.05$). Atorvastatin treatment resulted in decreased PLTP activity (10 mg atorvastatin: −8.3%, $P < 0.05$; 80 mg atorvastatin: −12.1%, $P < 0.002$). Plasma apoE decreased by 28 and 36%, respectively ($P < 0.001$). The decrease in apoE was strongly related to the decrease in PLTP activity ($r = 0.565$, $P < 0.001$). The change in apoE remained the sole determinant of the change in PLTP activity in a multivariate model. The activity of PLTP in type 2 diabetes is decreased by atorvastatin. The association between the decrease in PLTP activity and apoE during statin treatment supports the hypothesis that apoE may prevent PLTP inactivation.
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apo, apolipoprotein; DALI, Diabetes Atorvastatin Lipid Intervention; PLTP, phospholipid transfer protein.

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Patients with type 2 diabetes have a severely increased risk for cardiovascular morbidity and mortality (1,2). An atherogenic lipoprotein profile, characterized by increased plasma levels of triglycerides, decreased levels of HDL cholesterol, and presence of small dense LDL particles, is a major determinant, although the exact underlying molecular mechanisms remain to be established (3). HDL plays a central role in lipoprotein metabolism. The process of reverse cholesterol transport, i.e., the transport of cholesterol from peripheral tissues to the liver for degradation and excretion, is mediated through HDL (4). Furthermore, HDL functions as an antioxidant and anti-inflammatory agent. All functions of HDL have a similar tenacity: they protect against the development of atherosclerosis. Thus, decreased levels of plasma HDL cholesterol as seen in type 2 diabetes are pro-atherogenic.

Plasma lipid transfer proteins have an important role in remodeling of HDL. Two major lipid transfer proteins are described: cholesterylester transfer protein and phospholipid transfer protein (PLTP) (5). Plasma PLTP resides on HDL particles and is involved in phospholipid transfer activity (6). In vitro, its activity results in HDL conversion, generating large HDL_2 and small pre-β HDL particles that participate in cellular cholesterol efflux (7,8). This action implies a potential antiatherogenic function of PLTP (9). In vivo studies in PLTP-deficient mice have illustrated the physiological importance of PLTP in transfer of surface fragments to HDL that originate from triglyceride-rich lipoproteins during lipolysis by the enzyme lipoprotein lipase (10–12). Furthermore, PLTP was shown to be involved in the hepatic secretion of apolipoprotein B (apoB)-containing lipoproteins (13,14). This may explain the decreased susceptibility for diet-induced atherosclerosis in PLTP-deficient mice (14). Studies with transgenic mice revealed increased atherogenesis (15–17). Thus, PLTP appears to be pro-atherogenic in vivo in mice. In line with these animal studies, a study in humans provided evidence for a positive association between PLTP activity and coronary artery disease (18).

Plasma PLTP activity levels are elevated in type 2 diabetes (19), type 1 diabetes (20), hypertriglyceridemia (21), obesity (22,23), and patients with systemic inflamma-

TABLE 1
Characteristics of the study cohort

Characteristics	Placebo	10 mg atorvastatin	80 mg atorvastatin
n	71	72	72
Age (years)	59 ± 8	60 ± 8	60 ± 8
BMI (kg/m^2)	32.2 ± 6.1	30.0 ± 3.8	30.4 ± 4.5
Waist (cm)	108 ± 16	106 ± 12	106 ± 14
A1C (%)	8.76 ± 1.25	8.67 ± 1.27	8.86 ± 1.22
Glucose (mmol/l)	10.6 ± 3.6	10.5 ± 3.1	10.6 ± 2.9
Cholesterol (mmol/l)	6.03 ± 0.88	5.94 ± 0.95	6.04 ± 0.90
Triglycerides (mmol/l)	2.76 ± 0.95	2.68 ± 0.91	2.99 ± 1.14
HDL cholesterol (mmol/l)	1.05 ± 0.21	1.05 ± 0.26	1.03 ± 0.24
ApoAI (g/l)	1.41 ± 0.19	1.39 ± 0.20	1.39 ± 0.21
ApoB (g/l)	1.24 ± 0.20	1.19 ± 0.20	1.23 ± 0.22

Data are the means ± SD.

tion (24). Recently, PLTP has also been implicated to play a role in lipid metabolism in the brain (25–28). Not all PLTP molecules in plasma are active because PLTP mass is present in two distinct forms, one active in phospholipid transfer and one inactive (29,30). These forms react differently to PLTP antibodies used in PLTP enzyme-linked immunosorbent assay, and they may explain the poor correlation between PLTP mass and PLTP activity (31). Data in the literature suggest a potential relationship between apoE and PLTP activity. The active form of PLTP copurifies with apoE (32). In addition, secretion of active PLTP and apoE from HepG2 cells are associated (33). Interestingly, in a recent in vitro study, it was shown that apoE is able to activate the low-activity form of PLTP, suggesting a role for apoE in the regulation of PLTP activity (34).

The effect of statins on PLTP has not been studied. We hypothesized that statins influence PLTP activity by a mechanism involving changes in the level of circulating mass of apoE. Therefore, we investigated the influence of atorvastatin on PLTP activity and mass and the relation with plasma apoE in patients with type 2 diabetes of the Diabetes Atorvastatin Lipid Intervention (DALI) study. The DALI study was a double-blind randomized placebo-controlled trial comparing effects of atorvastatin 10 and 80 mg daily on plasma lipids and apos (35).

RESEARCH DESIGN AND METHODS

This study comprised 215 patients enrolled in the DALI study. DALI was a double-blind randomized placebo-controlled multicenter study evaluating the effect of 10 vs. 80 mg atorvastatin daily on lipid metabolism, endothelial function, coagulation, and inflammatory factors in unrelated men and women with type 2 diabetes. The protocol and eligibility criteria have been described in detail elsewhere (35). Briefly, men and women aged 45–75 years with duration of diabetes for at least 1 year and an HbA_{1c} (A1C) ≤10% were eligible. The diagnosis of type 2 diabetes was defined according to the American Diabetes Association classification (36). Lipid inclusion criteria were total cholesterol between 4.0 and 8.0 mmol/l and fasting triglycerides between 1.5 and 6.0 mmol/l. Patients were recruited in Leiden, Rotterdam, and Utrecht, the Netherlands. The ethical committees of the participating centers approved the study protocol, and written informed consent was obtained from all subjects.

Laboratory measurements. After an overnight fast for a minimum of 12 h, blood was drawn into EDTA-containing tubes for analysis of lipid profiles at baseline and after treatment for 30 weeks. Plasma was prepared by immediate centrifugation (3,000*g*, 15 min, 4°C), and samples were stored at −80°C for further analyses. Cholesterol and triglycerides were determined by enzymatic colorimetric methods on a Hitachi 911 automatic analyzer (Boehringer Mannheim, Mannheim, Germany). ApoB and apoAI were assayed, using automated immunoturbidimetric assays (Tina-Quant; Roche Diagnostics, Mannheim, Germany). Plasma apoE was analyzed automatically with a nephelometric assay (Wako, Osaka, Japan). Plasma HDL cholesterol was

TABLE 2
Plasma PLTP mass and activity and apoE and apoAI levels before and after treatment

Variable	Baseline	Week 30	% change
PLTP mass (mg/l)			
Placebo	8.88 ± 2.57	8.83 ± 2.70	−0.6
10 mg atorvastatin	9.00 ± 3.28	9.26 ± 3.15	2.8*
80 mg atorvastatin	8.63 ± 3.11	9.63 ± 3.09	11.6†
PLTP activity (%)			
Placebo	106.7 ± 18.0	107.3 ± 18.8	0.9
10 mg atorvastatin	107.8 ± 18.3	99.7 ± 16.1	−8.3‡
80 mg atorvastatin	108.8 ± 21.8	96.8 ± 18.6	−12.1†
ApoE (mg/100 ml)			
Placebo	4.46 ± 0.80	4.52 ± 1.12	0.9
10 mg atorvastatin	4.34 ± 1.02	3.15 ± 0.83	−28†
80 mg atorvastatin	4.68 ± 1.17	2.99 ± 1.06	−36†
ApoAI (mg/100 ml)			
Placebo	141 ± 19	137 ± 18	−2.8
10 mg atorvastatin	139 ± 20	138 ± 20	−0.71
80 mg atorvastatin	139 ± 21	134 ± 20	−3.6

Data are the means ± SD, and PLTP activity is % of reference plasma. Placebo: $n = 71$; A10: $n = 72$; A80: $n = 72$. Test was for difference among the three groups, adjusted for baseline value. *$P < 0.003$ A10 vs. A80; †$P < 0.001$ vs. placebo; ‡$P < 0.01$ vs. placebo.

TABLE 3
Plasma apoE and plasma PLTP activity levels per apoE genotype group

ApoE genotype	*n*	Plasma apoE (mg/100 ml)	Plasma PLTP activity
ApoE 2/3	34	5.24 ± 1.14	108.8 ± 17.8
ApoE 3/3	133	4.31 ± 0.85	108.0 ± 21.2
ApoE 2/4	6	5.18 ± 0.80	104.4 ± 11.1
ApoE 3/4	37	4.17 ± 0.92	106.2 ± 16.5
ApoE 4/4	4	4.53 ± 0.92	99.3 ± 12.2

Data are the means ± SD. Plasma PLTP activity is percent of reference plasma.

measured by a direct enzymatic method based on polyethylene glycol–modified enzymes, using a Hitachi 911 autoanalyzer (Roche Diagnostics). LDL cholesterol was estimated with the Friedewald formula (37). Fasting plasma glucose was determined on a Hitachi 917 analyzer using an UV-hexokinase method (catalog no. 18766899; Boehringer Mannheim). A1C was determined by high-pressure liquid chromatography, using the Variant method (catalog no. 270-0003; BioRad).

PLTP mass and activity. Plasma PLTP mass was analyzed as previously described (29). The between-assay coefficient of variation (CV) and within-assay CV were <3.5 and <2.8%, respectively. Plasma PLTP activity was measured in a liposome vesicles–HDL system as described previously (38,39). In short, plasma samples were incubated for 45 min, 37°C, with [^{14}C]dipalmitoyl-phosphatidylcholine–labeled liposomes and an excess of HDL, followed by precipitation of the liposomes with a mixture of NaCl, $MgCl_2$, and heparin (final concentrations 230 mmol/l, 92 mmol/l, and 200 units/ml, respectively). The measured PLTP activities are linearly related to the amount of plasma used in the incubations (0.5–1.0 μl). The method is not influenced by the phospholipid transfer–promoting capacities of cholesterylester transfer protein (38). Plasma PLTP activity was related to the activity measured in a reference plasma pool, based on plasma collected from 200 randomly selected healthy individuals, and is expressed as the percentage of the activity measured in this reference plasma, which was included in every assay. The PLTP activity in this reference plasma was 21.2 $\mu mol \cdot ml^{-1} \cdot h^{-1}$. The between- and within-assay CVs were 4.8 and 3.5%, respectively.

Statistical analysis. All data are expressed as the means ± SD. All statistical analyses were carried out with SPSS software (version 12.0). Mean differences between the groups were analyzed, using ANOVA. To analyze determinants of PLTP activity and PLTP mass, we used the following strategy. First, univariate linear regression analyses was performed in the total cohort with PLTP activity and PLTP mass as dependent variables and the determinant of interest as an independent variable. Second, multivariate regression analysis was performed with PLTP activity and PLTP mass as dependent outcome variables, sex and waist as confounders, and statistically significant variables as independent variables. Third, the effect of treatment on PLTP activity, PLTP mass, and apoE was analyzed with two-way ANCOVA, using a placebo-controlled setting. Throughout, a two-tailed *P* value of <0.05 was interpreted as indicating a statistically significant difference.

TABLE 4
Correlation between plasma PLTP activity and baseline parameters

Parameter	Effect (β)	*P* value
Age	−0.116	NS
Sex	0.033	NS
Waist*	0.243	<0.001
Glucose*	0.169	0.015
A1C*	0.169	0.014
ApoE*	0.208	0.004

*All analyses were performed using multiple linear regression analyses, adjusted for age and sex and each determinant separately. HDL cholesterol, apoAI, and triglycerides do not contribute significantly to the variation in PLTP activity.

RESULTS

The characteristics of the 215 patients have been extensively described (35) and are summarized in Table 1. The atherogenic lipoprotein profile of the patients was characterized by elevated plasma triglycerides (2.81 ± 1.01 mmol/l) and decreased plasma HDL cholesterol (1.04 ± 0.24 mmol/l) levels. The mean plasma cholesterol levels were 6.0 ± 0.9 mmol/l. Average A1C was 8.3 ± 1.1%, and plasma glucose was 10.6 ± 3.2 mmol/l. The average duration of diabetes was 10.5 years.

Baseline findings. Baseline plasma PLTP mass concentrations varied from 2.50 to 24.50 mg/l, with an average of 8.84 ± 3.01 mg/l, and were similar in the three treatment groups (Table 2). Plasma PLTP activity at baseline varied from 64.4 to 187.1%, with an average of 107.6 ± 19.4%, and was identical in the three treatment groups. The correlation between baseline plasma PLTP mass and activity was low ($r = 0.120$, NS). Baseline plasma apoE concentrations were 4.49 ± 1.02 mg per 100 ml. One important determinant for plasma apoE levels is the apoE genotype. In the present study cohort, the apoE genotypes were in Hardy-Weinberg equilibrium ($\chi^2 = 2.916$, degrees of freedom = 3, $P = 0.4$): 62% of the patients had the apoE3/E3 genotype, 17% apoE2/E3, 3% apoE2/E4, 17% apoE3/apoE4, and 1% apoE4/E4. The average apoE and PLTP activity levels per apoE genotype group are shown in Table 3. No significant effect of apoE genotype on PLTP activity was found.

Waist circumference, plasma glucose, A1C, and plasma apoE were associated with baseline PLTP activity (Table 4, Fig. 1). In multivariate analyses, adjusted for age and

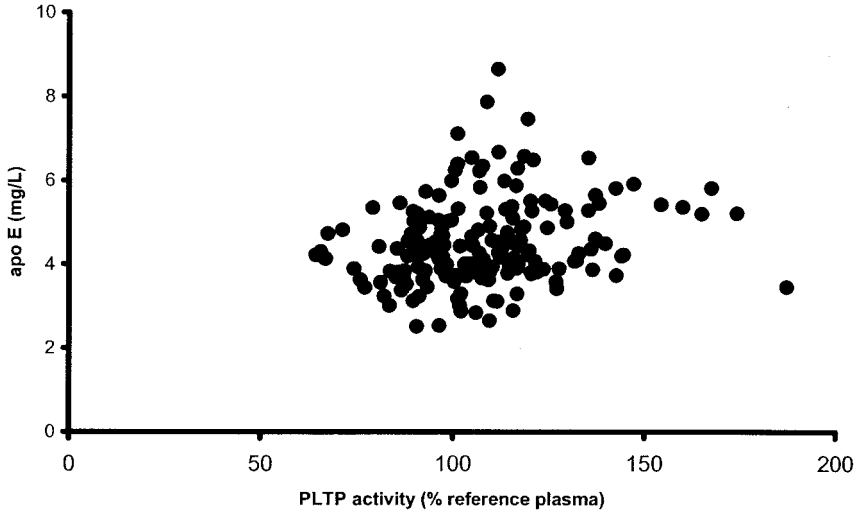

FIG. 1. Relationship between plasma PLTP activity and plasma apoE levels at baseline. Spearman correlation coefficient $r_s = 0.21$, $P < 0.05$.

TABLE 5
Correlation between plasma PLTP mass and baseline parameters

Parameter	Effect (β)	*P* value
Age	0.269	0.001
Sex	0.144	0.044
Waist*	−0.017	NS
HDL cholesterol*	0.719	0.001
ApoAI*	0.594	0.001
ApoE*	−0.164	0.019
Triglycerides*	−0.395	0.001

*All analyses were performed using linear regression analyses, adjusted for age and sex and each determinant separately. A1C and glucose do not contribute significantly to the variation of PLTP mass.

sex, only waist circumference and plasma apoE remained major determinants of plasma PLTP activity. None of the other lipid (including cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides) and apo (apoAI and apoB) parameters showed a significant correlation. Plasma PLTP mass is known to be predominantly determined by the levels of plasma HDL cholesterol and apoAI (21). In our analyses both HDL cholesterol and apoAI contributed to the variation of plasma PLTP mass, independent of age, sex, and waist circumference. Plasma apoE and triglyceride concentrations were less important determinants (Table 5).

Treatment with atorvastatin. As expected, adjusted for the placebo effect, treatment with atorvastatin resulted in a dose-dependent decrease in plasma total cholesterol, LDL cholesterol, and apoB (all $P < 0.001$) (35). Plasma HDL cholesterol increased by 6.0 and 5.2% with 10 and 80 mg atorvastatin, respectively ($P < 0.05$), whereas plasma apoAI levels remained unchanged (Table 2). Plasma PLTP activity decreased by 8.3% ($P < 0.01$) and 12.1% ($P < 0.001$), respectively. The difference between the two groups did not reach statistical significance, but the trend was significant ($P < 0.01$). In contrast, plasma PLTP mass increased by 2.8% (NS) and 11.6% ($P < 0.001$), respectively. The difference between 10 and 80 mg atorvastatin was significant ($P < 0.005$). Plasma apoE levels were strongly decreased after atorvastatin treatment by 28 and 36%, respectively (both $P < 0.001$ vs. control). In a multivariate placebo-controlled analyses, including all lipid and apo parameters, the decrease in PLTP activity was only predicted by the decrease in apoE (standardized coefficient $\beta = 0.529$, $P < 0.0001$) (Fig. 2). Similar results were obtained when only individuals with the apoE3/E3 genotype were analyzed ($n = 133$, $r = 0.513$, $P < 0.001$), illustrating that the effect is independent of the apoE genotype.

DISCUSSION

In patients with type 2 diabetes, we found that atorvastatin treatment resulted in a decrease in PLTP activity and an increase in PLTP mass, leading to a substantial change in mass-adjusted activity. Statistical analysis revealed that the change in apoE and the change in PLTP activity are associated, thereby supporting the concept that apoE may be essential for the stability of the PLTP protein and its activity.

It has been shown that patients with type 2 diabetes have increased levels of plasma PLTP activity (19,40). Positive associations have been reported for PLTP activity and parameters of body composition, such as body fat, BMI, and waist circumference (22,23,41). Also in the present study, both waist circumference and BMI were positively correlated with PLTP activity. In multivariate analysis, however, waist circumference was the better predictor. Increase in abdominal fat accumulation, as represented by increase in waist circumference, strongly relates to insulin resistance (42). Therefore, it is not surprising that patients with hypertriglyceridemia without diabetes have increased levels of PLTP activity as well (21). However, neither plasma glucose levels nor A1C were strong predictors for PLTP activity in the present study, even though glycemic control was suboptimal. Also, no sex-specific difference in PLTP activity or mass was found.

Mice with a genetic deficiency of PLTP have impaired hepatic secretion of apoB-containing particles, which is in favor of a role for PLTP in the biosynthetic pathway of VLDL by enabling donation of lipids to the nascent particles (14). In accordance with this mechanism, overexpression of human PLTP in transgenic mice results in elevated rates of hepatic VLDL secretion (43). Indeed, a number of clinical studies have reported the existence of a link between plasma apoB or LDL cholesterol and PLTP activity (20,41), but in the present study, we were unable to find evidence for this association. It has to be noted that we selected our patients on the presence of raised plasma triglycerides. As a result, we observed relatively small ranges in plasma apoB and cholesterol levels (Table 1), which could have obscured a relationship between PLTP activity and LDL cholesterol levels. Positive correlations with plasma triglycerides and cholesterol have been reported in a Finnish population study (44), but this could not be replicated in the present study, where correlations with triglycerides (r = 0.10, NS) and cholesterol (r = 0.00, NS) were only weak or absent.

Plasma apoE levels at baseline were associated with PLTP activity in both univariate and multivariate analyses. None of the other lipid variables or determinants of

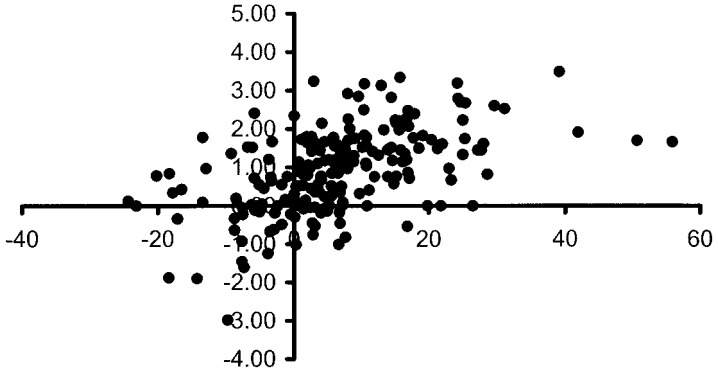

FIG. 2. Relationship between the change in apoE (*y*-axis) and the change in PLTP activity (*x*-axis) during atorvastatin treatment. Spearman correlation coefficient $r_s = 0.595$, $P < 0.001$.

glucose homeostasis, like glucose levels and A1C, showed any significant contribution. PLTP mass, on the other hand, strongly positively correlated with plasma HDL cholesterol and apoAI and negatively with plasma triglycerides in univariate analysis. Multivariate analysis revealed that PLTP mass was predominantly explained by HDL cholesterol levels. Plasma PLTP is present in two forms: one form with little, if any, phospholipid transfer activity and another form with high activity. Both are associated with macromolecular complexes of different size (30,32). The inactive form is present on larger HDL particles, compared with the active form. The currently used PLTP mass assay has been extensively validated and is capable of analyzing both the catalytically active and inactive forms (45). Because the greater part of plasma PLTP is inactive and because inactive PLTP copurifies with apoAI (32), it can be appreciated that PLTP mass and apoAI, as well as HDL cholesterol, are related (21).

Atorvastatin treatment significantly reduced plasma apoE levels and plasma PLTP activity, whereas total PLTP mass was increased. Evidence for a possible role for apoE in PLTP homeostasis has been obtained in a number of different studies. The active form of PLTP copurifies with apoE, whereas the inactive form is associated with apoAI (32). In addition, active PLTP secreted from HepG2 cells coelutes with apoE but not with apoAI (46). Furthermore, apoE but not apoAI is able to convert inactive PLTP into the active form (34). In light of these and our present observations, we hypothesize that atorvastatin, via its lowering effect on apoE concentration mediated through elevation of hepatic LDL receptors, destabilizes the active form of PLTP. As a consequence, PLTP could be partly inactivated, resulting in lowering of PLTP activity. Finally, more inactive PLTP may have contributed to the observed elevation of plasma PLTP mass. In conclusion, the present study shows, for the first time, that a statin is able to decrease PLTP activity and supports our hypothesis that apoE may stabilize PLTP in vivo.

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APPENDIX

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