Elevation of plasma phospholipid transfer protein increases the risk of atherosclerosis despite lower apolipoprotein B-containing lipoproteins

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Abstract Plasma phospholipid transfer protein (PLTP) transfers phospholipids between lipoproteins and mediates HDL conversion. PLTP-overexpressing mice have increased atherosclerosis. However, mice do not express cholesteryl ester transfer protein (CETP), which is involved in the same metabolic pathways as PLTP. Therefore, we studied atherosclerosis in heterozygous LDL receptor-deficient ($LDLR^{+/-}$) mice expressing both human CETP and human PLTP. We used two transgenic lines with moderately and highly elevated plasma PLTP activity. In $LDLR^{+/-}$/huCETPtg mice, cholesterol is present in both LDL and HDL. Both are decreased in $LDLR^{+/-}$/huCETPtg/huPLTPtg mice (>50%). An atherogenic diet resulted in high levels of VLDL+LDL cholesterol. PLTP expression caused a strong PLTP dose-dependent decrease in VLDL and LDL cholesterol (−26% and −69%) and a decrease in HDL cholesterol (−70%). Surprisingly, atherosclerosis was increased in the two transgenic lines with moderately and highly elevated plasma PLTP activity (1.9-fold and 4.4-fold, respectively), indicating that the adverse effect of the reduction in plasma HDL outweighs the beneficial effect of the reduction in apolipoprotein B (apoB)-containing lipoproteins. The activities of the antiatherogenic enzymes paraoxonase and platelet-activating factor acetyl hydrolase were both PLTP dose-dependently reduced (∼ −33% and −65%, respectively). We conclude that expression of PLTP in this animal model results in increased atherosclerosis in spite of reduced apoB-containing lipoproteins, by reduction of HDL and of HDL-associated antioxidant enzyme activities.—Lie, J., R. de Crom, T. van Gent, R. van Haperen, L. Scheek, F. Sadeghi-Niaraki, and A. van Tol. **Elevation of plasma phospholipid transfer protein increases the risk of atherosclerosis despite lower apolipoprotein B-containing lipoproteins. *J. Lipid Res.* 2004.** 45: **805–811.**

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Plasma levels of HDL are inversely correlated with the risk for development of atherosclerosis (1). HDLs are considered to have various atheroprotective effects, including anti-inflammatory and antioxidant properties and the potential to transport excess cholesterol from peripheral cells to the liver for degradation and excretion via the bile (1–4).

Phospholipid transfer protein (PLTP) plays several key roles in HDL metabolism (5–8). PLTP facilitates the transfer of phospholipids, α-tocopherol, and possibly unesterified cholesterol from triglyceride-rich lipoproteins to HDL particles during lipolysis (9, 10). PLTP is able to modulate HDL size and composition (11–13) and may also be involved in HDL cellular-mediated efflux of phospholipids and cholesterol (14).

Elevation of PLTP in transgenic mice results in a PLTP dose-dependent decrease in plasma HDL levels, coinciding with an increased susceptibility to diet-induced atherosclerosis (15). These results are in agreement with the previous study by Jiang et al. (16), who demonstrated that PLTP-deficient mice are less prone to atherosclerosis. However, the explanations for the results were different in these cases; the changes in atherosusceptibility in PLTP-deficient mice were explained by a decrease of hepatic VLDL secretion, not by an HDL effect. Elevation of PLTP results in a stimulation of VLDL secretion (17), but this effect was found to be PLTP dose independent. Therefore, it was considered to be a contributor to but not the main cause for the atherogenic effect of elevated PLTP (15).

Mice do not have cholesteryl ester transfer protein (CETP). Thus, the previously described genetically modified mice might be considered poor models in which to study the relation between PLTP and atherosclerosis, inas-

Abbreviations: apoB, apolipoprotein B; CETP, cholesteryl ester transfer protein; HFHC, high fat, high cholesterol; LDLR, LDL receptor; PAF-AH, platelet-activating factor acetyl hydrolase; PLTP, phospholipid transfer protein; PON, paraoxonase.

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much as CETP is thought to be critically involved in atherogenesis (18). Moreover, although PLTP and CETP function independently (19, 20), CETP affects the metabolism of both HDL and apolipoprotein B (apoB)-containing lipoproteins (18). Therefore, we decided to study the effect of elevated PLTP activity on atherosclerosis using mice genetically modified to express CETP. We found that also in this context, PLTP increases the susceptibility to diet-induced atherosclerosis. Surprisingly, this effect was found in spite of lowered levels of apoB-containing lipoproteins.

MATERIALS AND METHODS

Animals

Human PLTP transgenic mice were obtained as described previously (15). In this study, we used mice with moderately and highly elevated PLTP activity (lines huPLTPtgP4 and huPLTPtgA2, respectively). LDL receptor (LDLR) knockout mice were purchased from Jackson Laboratory. The huCETPtg mice were kindly provided by Dr. A. R. Tall (Columbia University, New York). All mice were in the C57BL/6J background. We created $LDLR^{+/-}$/huCETPtg mice, which were used as control mice. By crossbreeding these mice with the PLTP transgenic mouse lines expressing various levels of PLTP, we obtained $LDLR^{+/-}$/huCETPtg/huPLTPtgP4 and $LDLR^{+/-}$/huCETPtg/huPLTPtgA2 mice. Male mice were used in all experiments.

After weaning, animals were kept on a standard chow diet (Hope Farms, The Netherlands). At 4 months of age, all groups of mice were put on a high-fat, high-cholesterol (HFHC) diet for 14 weeks, which contained 40% (w/w) sucrose, 15% (w/w) fat, and 1% (w/w) cholesterol, and 0.5% (w/w) cholate to increase uptake of fat and cholesterol, leading to high plasma cholesterol levels. Animals had free access to water and food. After animals were fasted overnight, blood samples were collected from the orbital plexus by using Vitrex™ sodium-heparinized micropipettes (80 IU) (Modulohm A/S, Copenhagen, Denmark) and immediately stored on ice. Blood was centrifuged at 2,700 rpm for 15 min at 4°C. Plasma was either used immediately or stored in small aliquots at −80°C until analysis. All experiments conformed to national and institutional guidelines.

Separation of plasma lipoproteins by gel filtration

Plasma from transgenic mice was analyzed by gel filtration on two HR10/30 fast-protein liquid chromatography columns in tandem (Superdex 200 prepgrade, Superose 6 prepgrade, Pharmacia Biotech, Uppsala, Sweden). The columns were equilibrated with 2 mM NaH_2PO_4/Na_2HPO_4, pH 7.4 [containing 0.9% NaCl (w/v), 0.02% NaN_3 (w/v), and 5 mM EDTA]. Combined plasma samples were passed through 0.45 μm filters from Millipore S.A. (Molsheim, France), and 0.5 ml was subjected to gel filtration. The columns were run at 4°C with a flow rate of 0.1 ml/min. Fractions of 0.8 ml were collected. Recoveries were >90% for all analyses.

Separation of plasma lipoproteins by density gradient centrifugation

Lipoprotein fractions were obtained by density gradient ultracentrifugation of plasma samples in a Beckman SW60 Ti rotor (36,500 rpm, 21 h 12 min, 12°C) (21). Alternatively, HDL and VLDL+LDL lipoprotein fractions were obtained by density gradient ultracentrifugation of plasma samples in a Beckman 42.2 Ti rotor (42,000 rpm, 3 h 12 min, 12°C) at d = 1.063 g/ml. The lipoprotein fractions were collected by tube slicing.

Quantification of cholesterol

Cholesterol was determined enzymatically with the Free Cholesterol C kit no. 274-47109 (WAKO, Neuss, Germany) after hydrolysis of cholesteryl esters with cholesterol esterase from *Candida cylindracea* (Boehringer, Mannheim, Germany).

Plasma activity assays

CETP and PLTP activity assays were performed as described previously (22). The activities are expressed as arbitrary units (AUs); 1 AU is the activity found in human reference pool plasma. The activities are: CETP, 216 nmol/ml/h; PLTP, 14 μmol/ml/h.

Paraoxonase (PON) activity toward paraoxon was quantified spectrophotometrically essentially as described (23). In short, 10–20 μl of serum or heparin-plasma was added to 1 ml incubation medium with 50 mM Tris-HCL buffer (pH 8.0) containing 1 mM $CaCl_2$ and 6.0 mM paraoxon. The reaction was monitored for up to 30 min at 25°C by measuring the appearance of *p*-nitrophenol at 412 nm in a CARY 1E UV visible spectrophotometer. PON activity is expressed as U/ml.

Platelet-activating factor acetyl hydrolase (PAF-AH) was determined as described by Tselepis et al. (24), using 2-[^{3}H-acetyl]PAF as substrate. In short, 80 nmol of substrate (final concentration 0.33 mM) was incubated with 10 μl diluted (10-fold) plasma samples in incubation medium (total volume 0.24 ml) with 0.1 M Tris-HCl buffer, pH 7.4, containing 0.9% NaCl and 1 mM EDTA. After incubation for 20 min at 37°C, the reaction was stopped on ice and 300 μl $CHCl_3$ and 600 μl methanol were added. After mixing, another 300 μl of $CHCl_3$ was added with 300 μl 0.9% NaCl, pH 2.0. After mixing again, phase separation was obtained by centrifugation for 30 min. Seven hundred microliters of the upper layer was used for counting radioactive acetic acid. PAF-AH activity is expressed as nmol/min/ml plasma.

Histological assessment of atherosclerosis

After 14 weeks of feeding the HFHC diet, mice were sacrificed following collection of blood as described above. The hearts were dissected, stored in phosphate-buffered 4% formaldehyde until processing, and embedded in paraffin. Serial cross sections of 5 μm throughout the aortic valve area were used for histological analysis. Sections were stained with hematoxylin and eosin. Five sections per mouse, with intervals of 60 μm, were used for quantification of atherosclerotic lesion areas as described previously (15). To evaluate whether PLTP has an effect on accumulation of free cholesterol in atherosclerotic lesions, the number of sections containing free cholesterol clefts in each group was counted as described by Delsing et al. (25).

Statistical analysis

Data are expressed as means ± SE. Differences were analyzed by two-sample Wilcoxon rank sum tests by using Intercooled Stata 6.0 software (Stata Corporation, College Station, TX).

RESULTS

Elevation of PLTP activity lowers plasma cholesterol

Plasma levels of total cholesterol, VLDL+LDL cholesterol, and HDL cholesterol were measured in $LDLR^{+/-}$/huCETPtg, $LDLR^{+/-}$/huCETPtg/huPLTPtgP4, and $LDLR^{+/-}$/huCETPtg/huPLTPtgA2 mice, respectively (**Table 1**). The PLTP-overexpressing mouse lines were compared with $LDLR^{+/-}$/huCETPtg mice, which are referred to as control mice throughout the present study. Overexpression of PLTP resulted in a dramatic reduction of plasma levels of total cholesterol (−50% and −70%

TABLE 1. Plasma cholesterol levels in mice fed normal chow diet

Transgenic Mouse Line	LDLR$^{+/-}$/huCETPtg (n = 19)	LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 (n = 16–20)	LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 (n = 16–18)
		mM	
TC	2.7 ± 0.1	1.4 ± 0.1[a]	0.8 ± 0.1[a]
HDL-TC	1.7 ± 0.1	0.7 ± 0.1[a]	0.04 ± 0.01[a]
VLDL+LDL-TC	0.8 ± 0.1	0.4 ± 0.1[a]	0.44 ± 0.06[a]

CETP, cholesteryl ester transfer protein; PLTP, phospholipid transfer protein; TC, total cholesterol.
[a] Significantly different from LDLR$^{+/-}$/huCETPtg, $P < 0.001$.

in LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 and LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice, respectively, compared with control). This reduction in plasma cholesterol was found in both VLDL+LDL and in HDL lipoprotein classes. In a chow diet, the majority of the plasma cholesterol is in HDL, in which class the strongest reduction in cholesterol was found.

Overexpression of PLTP increases susceptibilty to atherosclerosis

After the animals had been fed an HFHC diet for 14 weeks, the areas of the atherosclerotic lesions were quantified in cross sections of the aortic valves from individual mice. As shown in **Fig. 1A**, atherosclerosis was more severe in LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 mice as compared

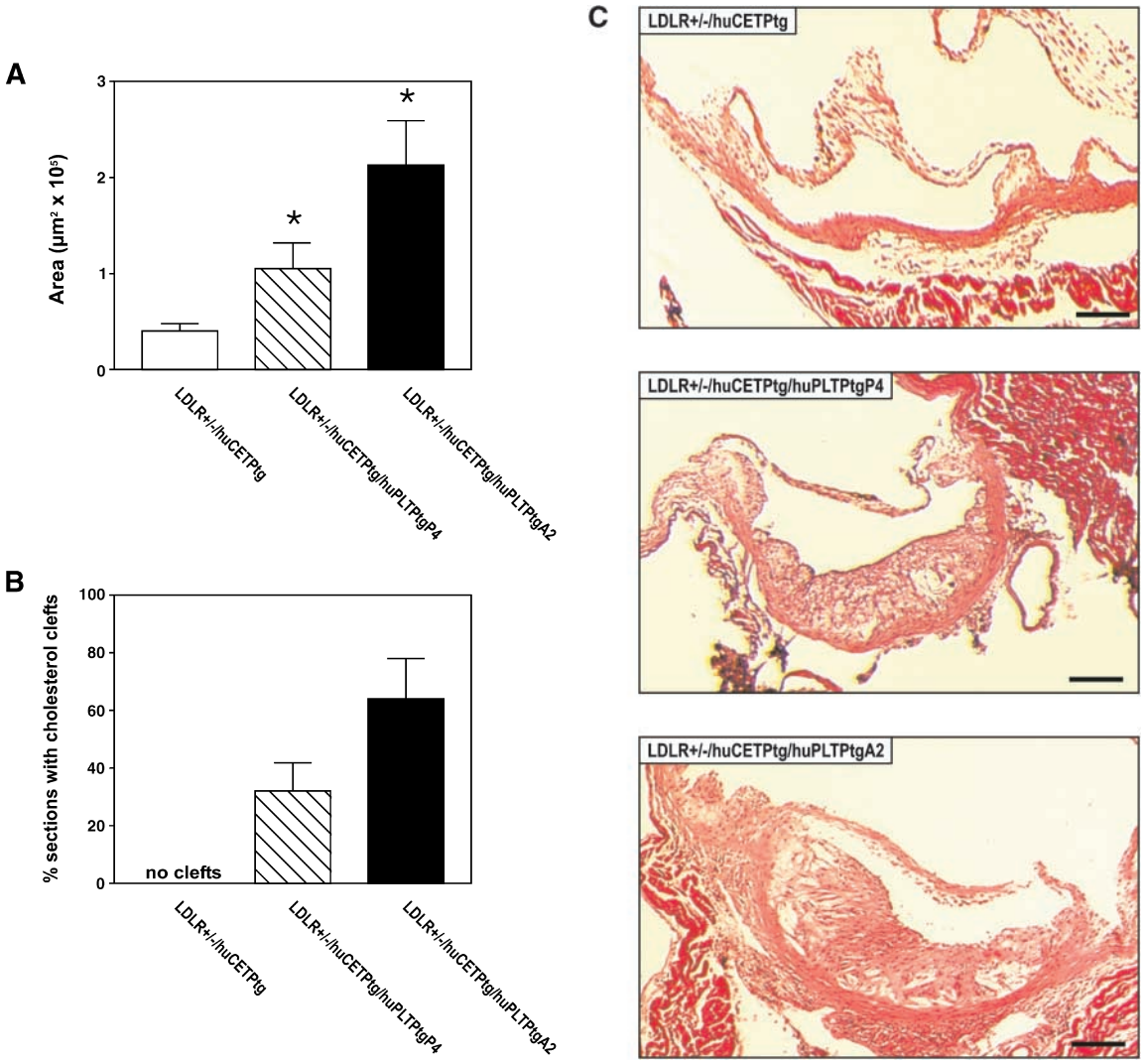

Fig. 1. Atherosclerosis in transgenic mice after 14 weeks of a high-fat, high-cholesterol (HFHC) diet. Mice (n = 9–13 per group) were sacrificed, and aortas were removed and atherosclerotic lesion areas in the aortic roots were determined as described in Materials and Methods. A: Aortic valve lesion areas; B: percentage of sections containing free cholesterol clefts in the aortic lesions; C: representative lesion areas of LDLR$^{+/-}$/huCETPtg, LDLR$^{+/-}$/huCETPtg/huPLTPtgP4, and LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice. Cholesterol clefts are particularly conspicuous in the lowest panel. Values represent means ± SE. * $P < 0.05$ compared with LDLR$^{+/-}$/huCETPtg mice.

TABLE 2. Plasma cholesterol levels in mice fed an HFHC diet

Transgenic Mouse Line	LDLR$^{+/-}$/huCETPtg (n = 17)	LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 (n = 18)	LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 (n = 13–17)
		mM	
TC	11.3 ± 0.8	8.3 ± 0.6[a]	5.5 ± 1.1[b]
HDL-TC	1.0 ± 0.1	0.3 ± 0.02[b]	0.3 ± 0.1[b]
VLDL + LDL-TC	10.8 ± 0.7	8.0 ± 0.7[a]	3.4 ± 0.3[b]

HFHC, high fat, high cholesterol.
[a] Significantly different from LDLR$^{+/-}$/huCETPtg, $P < 0.05$.
[b] Significantly different from LDLR$^{+/-}$/huCETPtg, $P < 0.001$.

with control mice, while a further increase was found in LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice.

We also evaluated whether PLTP overexpression increased the number of sections containing cholesterol clefts (Fig. 1B). In control mice, cholesterol clefts were not observed. However, in mice overexpressing human PLTP cholesterol, clefts were present. Moreover, atherosclerotic lesions from LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice showed more cholesterol clefts than those from LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 mice ($P = 0.05$). Fig. 1C shows representative photographs of the atherosclerotic lesions from mice of the various genotypes.

PLTP expression reduces diet-induced hypercholesterolemia

To determine why LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 and LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice have more atherosclerosis than do control mice, we analyzed the levels of plasma cholesterol after feeding the animals an HFHC cholesterol diet for 14 weeks (**Table 2**). As expected, the diet resulted in a strong increase of the plasma levels of total cholesterol in all three genotypes (compare Tables 1 and 2). However, overexpression of PLTP reduced total cholesterol in plasma compared with control mice. As a consequence of the diet, the bulk of the plasma cholesterol was in VLDL+LDL in all three genotypes analyzed. A PLTP dose-dependent decrease in VLDL+LDL cholesterol was found in PLTP-overexpressing mice compared with control mice (−26% and −69% in LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 and LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice, respectively). HDL cholesterol was also strongly reduced in the PLTP-overexpressing animals (−70% in both genotypes compared with control mice).

To examine the effects on lipoprotein classes in more detail, plasma samples from the various transgenic mouse lines were analyzed by gel filtration chromatography (**Fig. 2**). The HFHC diet resulted in an increase of total plasma cholesterol in all lipoprotein fractions of all transgenic mouse lines compared with the cholesterol profiles found on chow diet (results not shown). The HFHC diet also induced a strong shift of the cholesterol profiles to the more atherogenic lipoproteins, intermediate density lipoproteins and VLDLs. The effect of PLTP expression was a decrease in cholesterol in these lipoprotein fractions. The biggest effect was found in the mice with the highest plasma PLTP activity level, i.e., the LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice. HDL cholesterol was almost absent after feeding the diet for 14 weeks in both PLTP-expressing lines (Fig. 2).

Therefore, overexpression of PLTP in the presence of CETP results in an elevation of diet-induced atherosclerosis in spite of markedly reduced levels of apoB-containing lipoproteins. This suggests that the effect on atherosclerosis is caused by the strong reduction in HDL. However, LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 and LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice hardly differ in HDL cholesterol levels after the animals have been fed the HFHC diet for 14 weeks, while the atherosclerotic lesion size is considerably higher in the LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice. Therefore, we proceeded to examine HDL-associated lipid transfer and enzymatic activities.

CETP and PLTP activities

After the animals had been fed an HFHC diet for 14 weeks, CETP activities were slightly decreased in PLTP-overexpressing mice compared with control mice (**Fig. 3A**). However, no difference was found in plasma CETP activity between mice with moderate and those with high PLTP expression. Differences in plasma PLTP activity levels were maintained after the diet period (Fig. 3B).

Effect of PLTP overexpression on PON and PAF-AH activities

PON and PAF-AH are HDL-associated enzymes that may have antiatherogenic properties (26, 27). Because PLTP overexpression lowers the plasma HDL concentrations in our mice, we examined whether this might affect

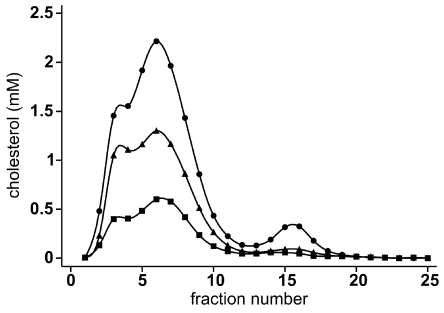

Fig. 2. Distribution of lipoproteins in plasma from transgenic mice. Equal amounts of plasma from transgenic mice (n = 16–20) fed an HFHC diet for 14 weeks were pooled and subjected to gel filtration on Superose 6 and Superdex 200 columns connected in tandem as described in Materials and Methods. Fractions 1–5 contain VLDL, fractions 6–11 contain LDL, fractions 12–20 contain HDL, and fractions 21–25 contain albumin. Circles, LDLR$^{+/-}$/huCETPtg mice; triangles, LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 mice; squares, LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice.

these enzymes as well (**Fig. 4**). Indeed, we found that PLTP overexpression was associated with decreased activities of both PON and PAF-AH. In LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 and LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice, respectively, PON activity was 33% and 60% lower compared with control mice (Fig. 4A). The reduction in PAF-AH activity was 33% and 70%, respectively (Fig. 4B). The difference in activity between the two lines of PLTP-expressing mice was statistically significant for both enzymes, suggesting PLTP dose-dependent lowering effects.

DISCUSSION

In the present study, we corroborated our previous finding that elevated PLTP activity in plasma results in increased atherosclerosis in mice (15). However, the crucial difference between this study and the previous study is that we now used transgenic mouse models that express CETP in addition to PLTP. Increased CETP activity has been found to result in increased atherosclerosis in mice (28, 29), rats (30), and rabbits (31). We demonstrated that variations in PLTP activity modulate the susceptibility to diet-induced atherosclerosis in addition to the CETP effect. Surprisingly, the atherogenic effect of PLTP was found despite a decrease of plasma cholesterol in apoB-containing lipoproteins. Plasma levels of HDL were also lower in PLTP-overexpressing mice. This is in agreement with results reported previously in other mouse models with elevated PLTP activity from our laboratory as well as other laboratories (15, 32, 33). In the present study, we found that the decrease in plasma cholesterol in both lipoprotein classes studied, i.e., apoB-containing lipoproteins and HDL, persist after the animals have been fed the HFHC diet for 14 weeks. These findings suggest that the reduction in plasma HDL affects the development of atherosclerosis to a greater extent than does the reduction in apoB-containing lipoproteins.

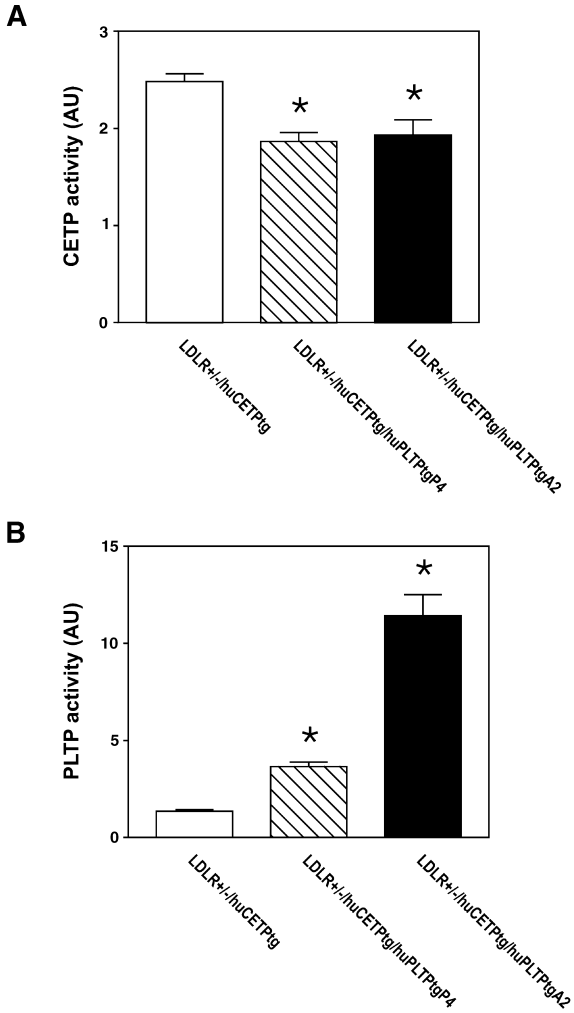

Fig. 3. Cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) activities in plasma from transgenic mice. Plasma samples from the various transgenic mouse lines were analyzed for CETP (A) and PLTP (B) activities as described in Materials and Methods after the mice had been fed an HFHC diet for 14 weeks. Values shown are means ± SE obtained from >13 mice per group. * $P < 0.05$ compared with LDLR$^{+/-}$/huCETPtg mice.

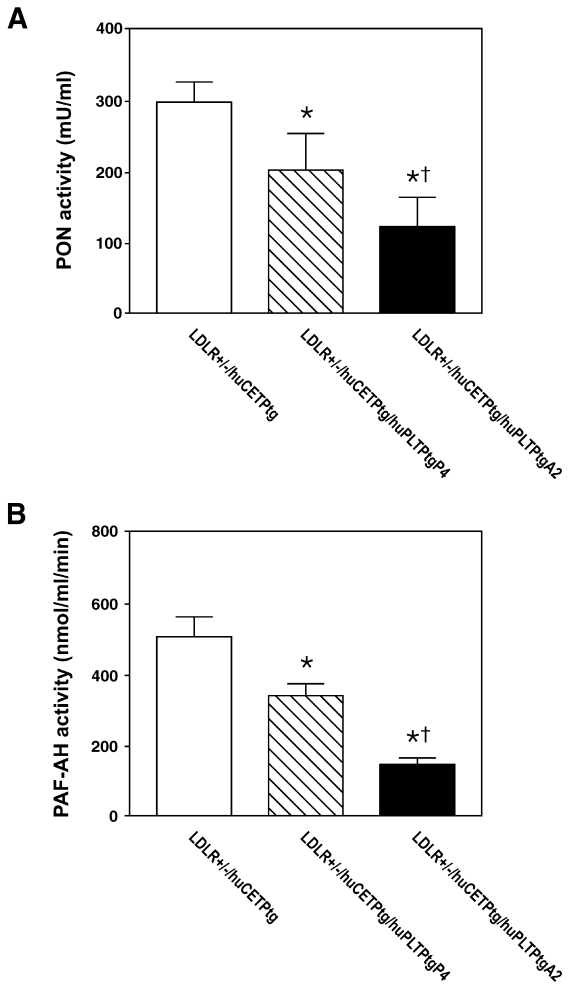

Fig. 4. Antioxidant enzyme activities in plasma from transgenic mice. Plasma samples from the various transgenic mouse lines were analyzed for paraoxonase (A) and platelet-activating factor acetyl hydrolase (B) activities as described in Materials and Methods. Values shown are means ± SE obtained from 4–7 mice per group. * $P < 0.05$ compared with LDLR$^{+/-}$/huCETPtg mice; † $P < 0.05$ compared with LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 mice.

We used two lines of transgenic mice overexpressing human PLTP to different levels. The LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice have the highest level of PLTP activity and also have the highest level of atherosclerosis. However, the levels of plasma HDL cholesterol are similar between the two lines at the end of the 14 week period of HFHC diet. On the other hand, on a chow diet, the LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice have lower levels of plasma HDL cholesterol than the LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 mice. Therefore, the variation in atherosclerosis could be caused by the difference in HDL cholesterol, even though this equals out during the diet period. The presence of a larger number of cholesterol clefts in LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice compared with LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 mice is indicative of a less-efficient removal of cholesterol from the atherosclerotic lesions by reverse cholesterol transport, allowing the formation of extracellular cholesterol crystalline deposits, which are inert and cannot be mobilized (34). However, there could also be a qualitative difference between the HDL from the LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 and that from the LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice. Therefore, we analyzed the activity of transfer proteins and enzymes that have been found to be associated with HDL in various mouse models (17, 35, 36).

A small but statistically significant decrease in CETP activity was found in the mice overexpressing PLTP. This is in agreement with previous findings from our laboratory (20) and may be caused by the drop in HDL levels caused by PLTP expression, inasmuch as HDL is the carrier of CETP. As outlined above, CETP is atherogenic in various animal models. Therefore, the small decrease in CETP activity cannot explain the increase in atherosclerosis found in PLTP-overexpressing animals. Moreover, CETP activity is not different between LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 and LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice. As expected, the mice have different levels of plasma PLTP activity, which persist at the end of the diet period.

In addition, the activities of PON and PAF-AH were found to be decreased in PLTP-overexpressing mice. Both enzymatic activities are lower in LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice than in LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 mice. Elevation of the activity of either PON (37) or PAF-AH (38) results in decreased atherosclerosis in mice. Moreover, PON-deficient mice have increased atherosclerosis (39), while decreased activities of both enzymes have been found in mouse models susceptible to atherosclerosis (36). It is likely that the decrease in PON and PAF-AH activities is related to the decrease in HDL, because in mice, these enzymes are mainly associated with HDL (35, 36), even in animals with extremely elevated plasma cholesterol in apoB-containing lipoproteins.

At present, it is not clear why overexpression of PLTP results in a decrease in VLDL+LDL cholesterol in plasma in LDLR$^{+/-}$/huCETPtg/huPLTPtgP4 and LDLR$^{+/-}$/huCETPtg/huPLTPtgA2 mice. In the absence of CETP, PLTP overexpression does not affect VLDL+LDL cholesterol levels (15). On the other hand, in mice that have no mutations in the LDLR (LDLR$^{+/+}$), VLDL+LDL cholesterol levels in huCETPtg mice and in huCETPtg/huPLTPtg mice are similar (20). Therefore, the LDLR seems to play a critical role in the effect of PLTP in CETP-transgenic mice. An alternative explanation is that the effect of PLTP on apoB-containing lipoproteins exists only above a certain threshold level of these lipoproteins. Both LDLR$^{+/-}$ mice (15, 40) and CETP transgenic mice (20, 29) show an increase in plasma levels of apoB-containing lipoproteins. Consequently, the levels of these lipoproteins are higher in the mice analyzed in the present study than in those analyzed in the earlier studies.

PLTP deficiency has been shown to increase the vitamin E content of VLDL and LDL, thereby protecting these lipoproteins from oxidation (41). Conversely, elevated PLTP expression might result in decreased vitamin E in VLDL+LDL and thus in a higher susceptibility to oxidative modification of these lipoproteins, resulting in a more rapid removal from the plasma compartment. This mechanism could contribute to the atherogenicity of PLTP overexpression, despite a decrease in plasma VLDL+LDL cholesterol concentration.

In conclusion, elevated expression of PLTP results in decreased plasma cholesterol in both atherogenic apoB-containing lipoproteins and antiatherogenic HDL. Inasmuch as the susceptibility to atherosclerosis is increased in a PLTP dose-dependent fashion, the effect on HDL apparently outweighs the effect on apoB-containing lipoproteins. The mechanism may include various atheroprotective properties of HDL, including the involvement in reverse cholesterol transport and the association with antioxidant enzymes.

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