Atherosclerosis 227 (2013) 37–42

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Elevated expression of PLTP is atherogenic in apolipoprotein E deficient mice

Rien van Haperen, Teus van Gent, Arie van Tol, Rini de Crom*

Department of Cell Biology & Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 6 July 2012
Received in revised form
1 November 2012
Accepted 25 November 2012
Available online 12 December 2012

Keywords:
Atherosclerosis
PLTP
Lipoproteins

ABSTRACT

Objective: Plasma phospholipid transfer protein (PLTP) plays a key role in lipoprotein metabolism. Its exact function in the development of atherosclerosis is still under debate however. We studied the effect of elevated PLTP expression in one of the most commonly used models of atherosclerosis, the ApoE deficient mouse.

Methods: Experiment 1: Plasma PLTP activity, total cholesterol, HDL cholesterol and atherosclerosis development was measured in ApoE deficient mice with or without elevated expression of PLTP. Experiment 2: The same parameters were measured in ApoE deficient mice after bone marrow transplantation from wild type mice or mice with elevated PLTP expression. Experiment 3: Similar to experiment 2, but using donor mice with an ApoE deficient background.

Results: Experiment 1: ApoE deficient mice have more than two times more atherosclerosis when overexpressing PLTP and a strongly decreased plasma level of HDL. Experiment 2: Bone marrow transplantation with ApoE proficient cells results in a strong reduction of plasma cholesterol in ApoE deficient acceptor mice. Still, elevated PLTP in bone marrow derived cells evoke a reduction of HDL cholesterol and increased atherosclerosis. Experiment 3: Bone marrow transplantation with ApoE deficient cells results in much higher cholesterol levels, but here too HDL cholesterol levels are reduced and atherosclerosis increased.

Conclusion: In all the models with ApoE deficiency, elevated PLTP expression causes higher levels of diet-induced atherosclerosis coinciding with decreased plasma levels of HDL cholesterol.

1. Introduction

The role of plasma phospholipid transfer protein (PLTP) in health and disease has been investigated for many years by several research groups around the world. Still, pivotal questions remain unanswered [1].

PLTP is present in the plasma of all investigated mammalian species, which suggests an essential function. There are no reports on a naturally occurring lack of PLTP activity in humans [2]. Nevertheless, PLTP deficiency that has been induced by gene targeting in mice, does not seem to have any gross abnormalities [3,4].

However, the lipid transfer activity of PLTP, which is not rigidly restricted to phospholipids [1], clearly affects plasma lipoproteins. Therefore, a possible relation with the development of atherosclerosis has been extensively investigated, both in patients and in experimental animals.

In humans, changes in PLTP activity in plasma have been associated with several conditions that are also related to an increased risk of atherosclerosis development. An increased PLTP activity has been reported in individuals with diabetes mellitus (both type 1 and type 2), metabolic syndrome and obesitas [5–8]. In addition, some studies have shown a relationship between a higher PLTP activity and an increased risk of coronary artery disease [9,10]. More recently PLTP was identified as a marker for cardiovascular disease in a large genetic study [11]. However, there is still debate on the mechanism by which PLTP can be atherogenic in humans. Well-controlled studies in experimental animals maybe helpful to shed more light on this issue.

PLTP deficient mice have a lowered susceptibility for atherosclerosis, while PLTP transgenic mice have more atherosclerosis [4,12]. However, animal studies have not been unequivocal either, because both PLTP deficient and PLTP transgenic mice have lowered HDL levels [4,12]. Most likely, the mechanism involved is different in these two models: in PLTP deficient mice the decreased level of

* Corresponding author. Department of Cell Biology & Genetics, Erasmus University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands. Tel.: +31 10 7043459; fax: +31 10 7044743.
E-mail address: m.decrom@erasmusmc.nl (R. de Crom).

0021-9150
http://dx.doi.org/10.1016/j.atherosclerosis.2012.11.022

atherosclerosis has been attributed to a decreased production of very low density lipoproteins (VLDL) by the liver, maybe further deteriorated by a decreased lipoprotein susceptibility to oxidation and reduced cholesterol absorption [2], while in PLTP transgenic mice, the lowered HDL level has been held responsible [12,13]. Also, conflicting results have been reported in bone marrow transplantation studies using PLTP deficient mice, aiming at studying the role of bone marrow derived cells in the process of atherogenesis.

In previous work from our group, we invariably found that elevated PLTP expression in transgenic mice, resulting in increased PLTP activity in plasma, causes an increased susceptibility for diet-induced atherosclerosis. We also performed bone marrow transplantation experiments and found a similar relationship between PLTP activity and atherosclerosis [13]. However, all of our studies have been performed in mice that were susceptible to atherosclerosis because of LDL-receptor deficiency. In PLTP deficient mice, the outcome of atherosclerosis susceptibility studies was shown to be dependent on the mouse model used: the results in LDL-receptor deficient mice appeared to differ from the results obtained in apolipoprotein (apo) E deficient or apoB transgenic mice [4]. Therefore, in the present set of experiments, we crossed our PLTP transgenic mice with apo E deficient mice, the other commonly used mouse model for atherosclerosis development. We also performed bone marrow transplantation studies with these animals to produce more clarity on the atherogenic potential of PLTP in bone marrow derived cells.

2. Materials and methods

2.1. Mice

ApoE deficient (ApoE-KO) mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and are in C57BL6/J background. PLTP transgenic (PLTPtg) mice were generated in our laboratory and have been described before [12]. The animals used in the present study are derived from the P4 line and were crossed for more than 15 generations into C57BL6/J background. The animals express the human PLTP gene driven by the autologous human PLTP promoter. Animals received water and food ad libitum. Food consisted of regular chow or, for the atherosclerosis experiments, of high fat high cholesterol diet containing 40% (w/w) sucrose, 15% (w/w) cocoa butter, and 0.25% (w/w) cholesterol (diet W; Hope Farms, Woerden, The Netherlands). All animals used for experiments were male. Groups consisted of $\geq$9 animals. There were three groups of mice in the experiments described here: group 1 consisted of PLTPtg and PLTPtg/ApoE-KO mice that were subjected to atherosclerosis experiments without bone marrow transplantations (see below), group 2 consisted of animals that were subjected to bone marrow transplantation experiments in which the recipient mice were ApoE-KO mice while the donor mice were either wild type control mice or PLTPtg mice (i.e., in both cases ApoE proficient), while group 3 consisted of animals that were subjected to bone marrow transplantation experiments in which the recipient mice were ApoE-KO mice while the donor mice were either also ApoE-KO mice or ApoE-KO/PLTPtg mice (i.e., in both cases ApoE deficient). All of the procedures in this study were approved by the committee on animals experiments and are in accordance with national and institutional guidelines.

2.2. Bone marrow transplantation experiments

Experiments were performed essentially as described before [13]. Acceptor mice received a split dose of in total 900 rad of γ-irradiation from a ^{137}Cs source with an interval of 3 h. Bone marrow transplants were derived from donor mice by collecting cells from tibia and femurs. Five million cells were injected per animal via the tail vein. Acceptor mice were fed chow diet and water containing 0.16% Neomycin. Starting at nine weeks after transplantation, animals received the atherogenic diet for another 21 weeks (group 2) or eight weeks (group 3), before the level of atherosclerosis was determined.

2.3. Atherosclerosis

Animals were sacrificed and in situ fixation was performed by perfusing 4% (v/v) formaldehyde via the heart. Atherosclerosis was quantified in sections from the aortic root [12,13].

2.4. Plasma PLTP activity, lipids and lipoproteins

Quantification of the activity of plasma PLTP, levels of triglycerides, cholesterol and lipoproteins in plasma was performed as described before [12,13].

2.5. Statistics

All values are expressed as mean $\pm$ S.E. Statistical analyses are by Mann–Whitney tests to compare groups and paired *t*-tests to compare time points within a group. Correlations are tested by linear regression. All tests were performed in Stata 12.0 (College Station, TX USA).

3. Results

In the first set of experiments, we studied the impact of elevated expression of PLTP on diet-induced atherosclerosis in mice deficient for apoE. As shown in Fig. 1A, PLTP activity in plasma is about 3-fold higher in PLTP transgenic mice (PLTPtg) than in control mice. After feeding the animals a high fat, high cholesterol (HFHC) diet for 8 weeks, PLTP activity is approximately 40% higher in both groups. Plasma triglyceride levels are moderately increased in PLTPtg mice, while the HFHC diet resulted in a decrease (Fig. 1C). Total and non-HDL plasma cholesterol is $\pm$40% lower in PLTPtg animals than in controls, both before and after feeding the animals the HFHC diet, while HDL cholesterol is extremely low in PLTPtg animals and is not affected by the diet regime (Fig. 1B, D, E). The level of atherosclerosis is considerably higher in PLTPtg mice (approximately 2.5-fold; Fig. 1F, G, H), in spite of the lowered level of total plasma cholesterol. Probably the very low level of HDL cholesterol is causing this effect.

Subsequently, we performed a series of experiments with bone marrow transplantations in which we used PLTPtg or wild type control mice, so both with apoE expression, as donors and apoE deficient mice as acceptors. We observed that plasma PLTP activity was substantially increased after transplantation with bone marrow from PLTPtg mice, as compared to animals transplanted with bone marrow from wild type mice (Fig. 2A). This effect was found both before and after feeding the mice an HFHC diet. The diet itself also induced an increase in PLTP activity, as was observed in apoE deficient mice (Fig. 1) and in other mice in previous work from our group [13]. Triglycerides, total plasma cholesterol and non-HDL cholesterol are strongly decreased after 9 weeks following bone marrow transplantation (Fig. 2B–D), which is very likely caused by the introduction of apo E by the bone marrow derived cells. The HFHC diet does not affect plasma triglycerides, nor is there any difference in plasma triglycerides between animals transplanted with wild type cells or with PLTPtg cells (Fig. 2C). After the diet period there is again a rise in total cholesterol, but this does not result in a difference in plasma cholesterol between the group transplanted with wild type cells and the group transplanted with PLTPtg cells (Fig. 2B) and only in a small difference in non-HDL cholesterol (Fig. 2D). In contrast, HDL cholesterol is strongly

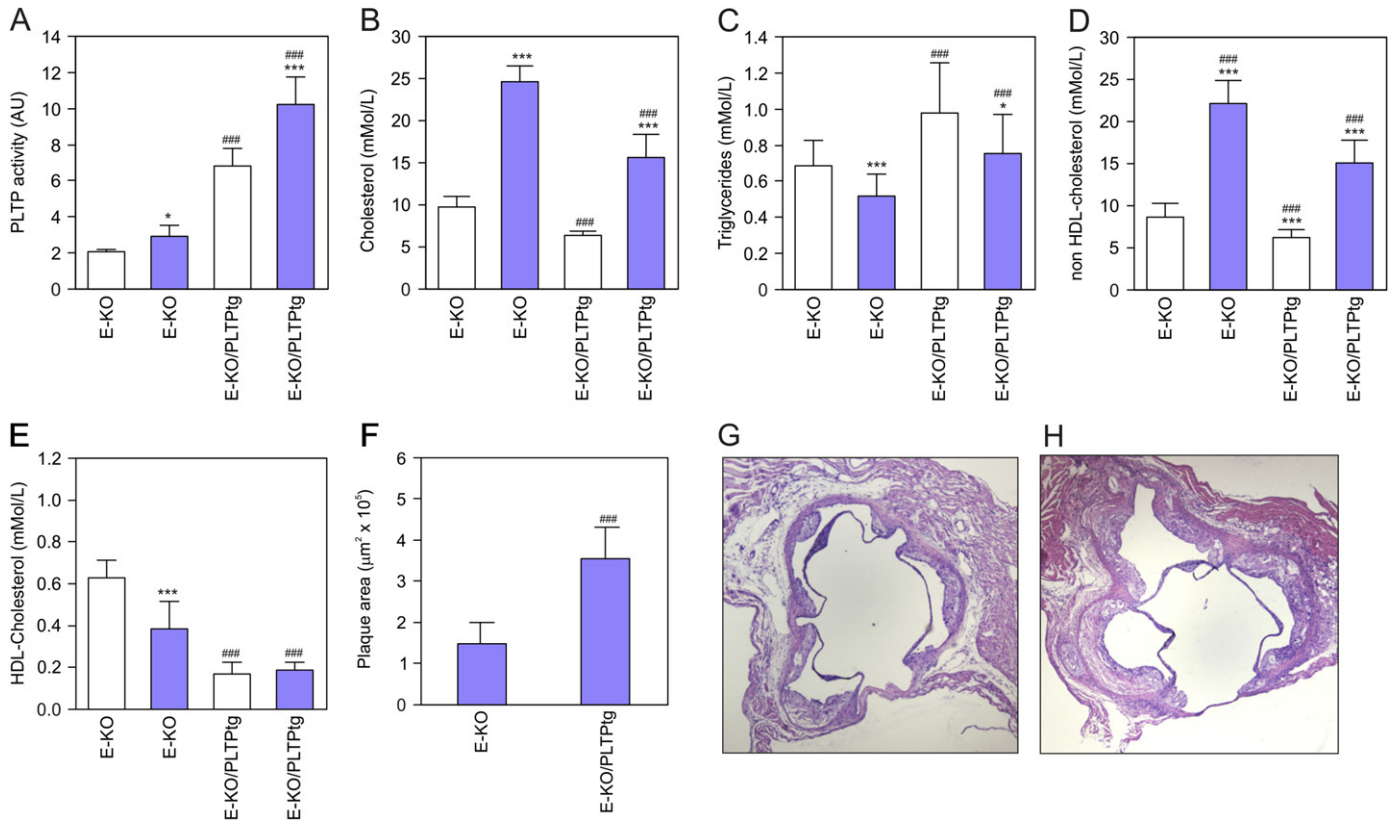

Fig. 1. Plasma PLTP activity levels (A), total cholesterol (B), triglycerides (C), non-HDL cholesterol (D), HDL cholesterol (E) and atherosclerotic lesion size (F) were measured in apoE deficient mice (E-KO) and in PLTP transgenic mice in an apoE deficient background (E-KO/PLTPtg) before (white bars) and after 8 weeks of a high fat, high cholesterol diet (blue bars). $^{*}p < 0.05$, $^{***}p < 0.001$ versus 0 weeks (same genotype) $^{###}p < 0.001$ versus apoE deficient mice (same diet). Representative photomicrographs of atherosclerotic lesions are provided in G (E-KO) and H (E-KO/PLTPtg). See Methods for details, where this group is referred to as "group 1". (The reader is referred to the web version of this article for full color figures)

decreased in the animals that were transplanted with PLTPtg bone marrow cells (Fig. 2E), while atherosclerosis is markedly enhanced (by a factor of 5) in this group (Fig. 2F, G, H). So, also in this set of experiments, elevated PLTP appears to result in a strong induction of diet-induced atherosclerosis that coincides with lowered levels of plasma HDL.

Because of the strong effect of apoE that was reintroduced in the set of experiments described above, we decided to perform a third set of experiments with a similar setup but with apoE deficient donor mice. Here too, PLTP activity was increased following transplantation with PLTPtg bone marrow cells (Fig. 3A). Plasma triglycerides are not affected by either diet or genotype of the bone marrow cells used for transplantation (Fig. 3C). On a chow diet, there are no, or only marginal, differences in total and non-HDL plasma cholesterol between the group that was transplanted with control cells (ApoE-KO) or with cells with the PLTP transgene (PLTPtg/ApoE-KO) (Fig. 3B, D). HDL cholesterol is lower in the mice transplanted with PLTP expressing cells (Fig. 3E). After feeding the animals the high fat, high cholesterol diet for 8 weeks, total and non-HDL cholesterol is decreased in the PLTPtg/ApoE-KO group and HDL cholesterol even more so. Atherosclerosis was about 75% higher in the animals transplanted with PTLP expressing cells (Fig. 3F, G, H).

4. Discussion

The most important results of the present study are: 1) As previously demonstrated for low density lipoprotein receptor deficient mice, in apoE deficient mice an elevated expression of PLTP results in an increased PLTP activity in plasma, a decreased total plasma cholesterol (which is mainly caused by a decrease in non-HDL cholesterol) and a strongly decreased HDL cholesterol level in plasma. This again results in increased atherosclerosis. 2) After transplantation with bone marrow from mice with elevated PLTP expression and a normal expression of apoE as donors and apoE deficient mice as acceptors, the total cholesterol level is strongly decreased, despite the HFHC diet. On the other hand, HDL cholesterol is much lower and atherosclerosis substantially increased compared with animals that received wild type bone marrow cells. 3) After transplantation with bone marrow from mice with elevated PLTP expression but with deficient apoE expression, the plasma levels of total cholesterol are strongly elevated after feeding the animals a HFHC diet, although to a lesser extent than in mice that were submitted to transplantation with bone marrow from mice with *normal* (i.e., wild type) PLTP expression but with deficient apoE expression. HDL cholesterol again is strongly decreased and atherosclerosis is 75% higher in animals that received cells with increased PLTP expression. In conclusion: in all the models with apoE deficiency, elevated PLTP expression causes higher levels of diet-induced atherosclerosis coinciding with decreased plasma levels of HDL cholesterol.

Obviously, coinciding observations do not constitute proof of a causal relationship. It would be interesting to study if a statistically significant correlation exists between the levels of plasma PLTP activity, HDL cholesterol and atherosclerotic plaque size within experimental groups. Because of the strong variation between individual animals, much larger groups would be needed, however. If animals with and without elevated PLTP expression, or animals

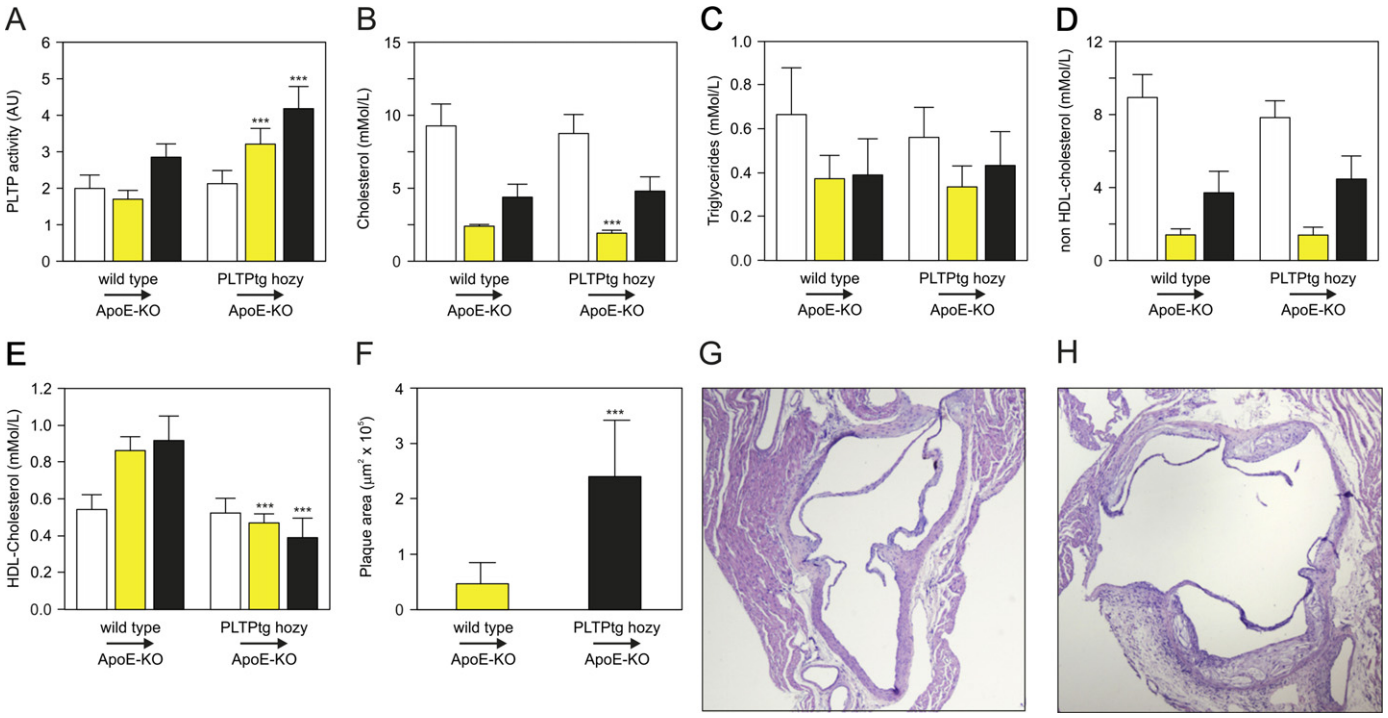

Fig. 2. Plasma PLTP activity levels (A), total cholesterol (B), triglycerides (C), non-HDL cholesterol (D), HDL cholesterol (E) and atherosclerotic lesion size (F) were measured in mice at the moment of the start of the bone marrow transplantation (white bars), after 9 weeks or recovery following the bone marrow transplantation procedure (yellow bars) and after 21 weeks of feeding the animals a high fat, high cholesterol diet following the nine weeks recovery period (black bars). Donor animals were either wild type (wt) or homozygous PLTP transgenic mice (PLTPtg hozy). Recipient mice were apoE deficient animals (ApoE-KO). ***$p < 0.001$ versus wild type → apoE deficient mice (same time point). Representative photomicrographs of atherosclerotic lesions are provided in G (wild type → ApoE-KO) and H (PLTPtg hozy → ApoE-KO). See Methods for details, where this group is referred to as "group 2". (The reader is referred to the web version of this article for full color figures)

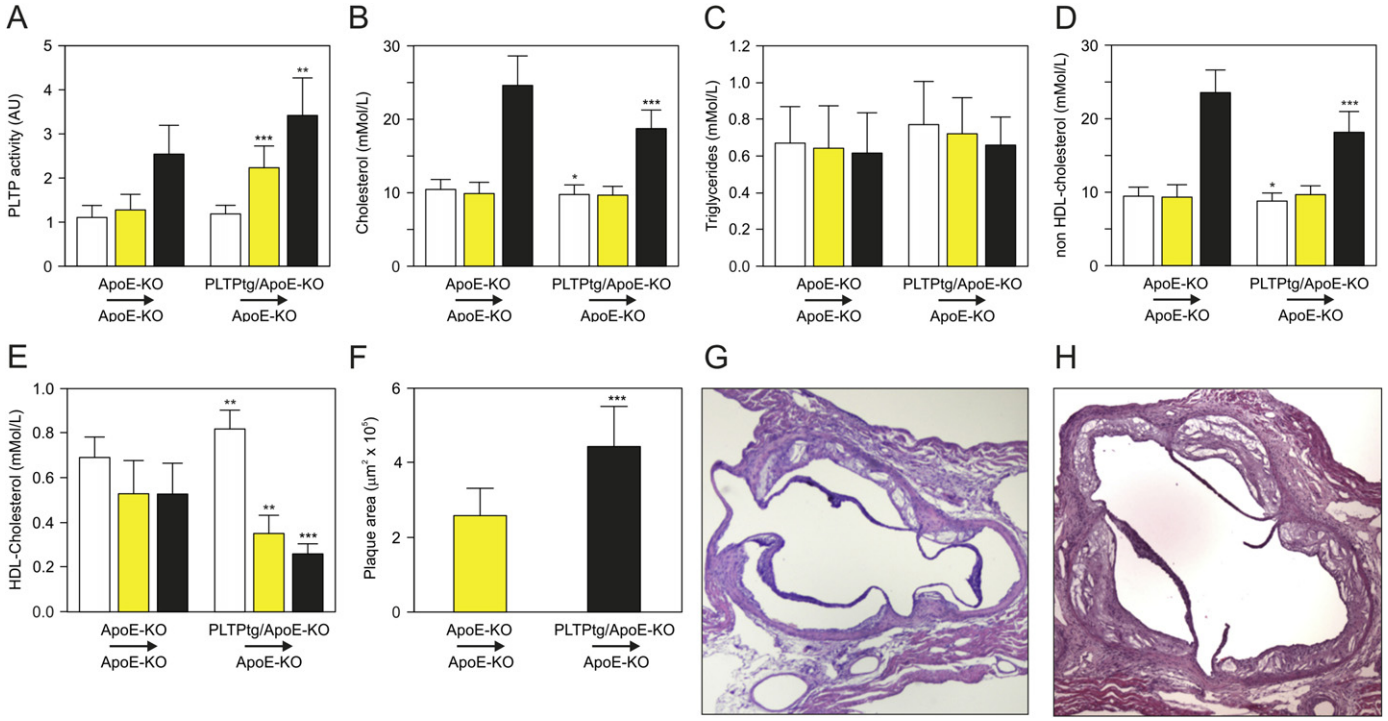

Fig. 3. Plasma PLTP activity levels (A), total cholesterol (B), triglycerides (C), non-HDL cholesterol (D), HDL cholesterol (E) and atherosclerotic lesion size (F) were measured in mice at the moment of the start of the bone marrow transplantation (white bars), after 9 weeks or recovery following the bone marrow transplantation procedure (yellow bars) and after 8 weeks of feeding the animals a high fat, high cholesterol diet following the nine weeks recovery period (black bars). Donor animals were either apoE deficient (ApoE-KO) or apoE deficient/PLTP transgenic mice (PLTPtg/ApoE-KO), recipient mice were apoE deficient animals (ApoE-KO). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus ApoE-KO → ApoE-KO mice (same time point). Representative photomicrographs of atherosclerotic lesions are provided in G (ApoE-KO → ApoE-KO) and H (PLTPtg/ApoE-KO → ApoE-KO). See Methods for details, where this group is referred to as "group 3". (The reader is referred to the web version of this article for full color figures)

treated with cells with and without elevated PLTP expression, are taken together, as they have been compared in Figs. 1–3 respectively, strong correlations between these parameters can be found, but not in the separate groups, even though tendencies are surely present in several of these groups (results not shown).

The reintroduction of apoE, even if only in bone marrow derived cells, has strong effects on plasma lipids and the susceptibility to diet-induced atherosclerosis. This has been demonstrated previously [14,15], leading to the conclusion that apoE has critical roles in the protection against atherosclerosis. The mechanism appears to be complex, as there are several functions of apoE that might contribute to its anti-atherogenic properties. These include the role of apoE in reverse cholesterol transport [16], as well as its anti-inflammatory, antiproliferative and immunomodulatory properties [17]. The present study shows that PLTP from bone marrow derived cells has its effects on HDL and atherosclerosis both in the absence and presence of macrophage apoE, indicating that these effects are not apoE dependent.

The expression or deficiency of either apoE or PLTP in bone marrow derived cells both have strong effects on lipoprotein levels in the plasma compartment. Both apoE and PLTP are secreted by macrophages, which probably explains these effects. In the case of apoE, the effects are unexpectedly high because more than 90% of ApoE is derived from the liver [18] and therefore macrophages have only a modest contribution to the normal levels of plasma apoE. It has been reported in apoE deficient mice that have been reconstituted with apoE expressing bone marrow cells, plasma levels of apoE are approximately 10% of the normal values [18]. Additional studies have shown that even (much) lower levels of plasma apoE still have clear effects on plasma lipids, suggesting that there is an excess of plasma apoE under normal conditions [19,20]. In the case of PLTP, we previously showed PLTP production and secretion by macrophages from both wild type and PLTPtg mice [13]. In the present study, plasma PLTP activity in animals transplanted with bone marrow from PLTPtg mice was 50–80% higher than in animals transplanted with wild type bone marrow. This modestly elevated PLTP activity caused profound effects on plasma total cholesterol and HDL cholesterol levels. Apparently, the elevation of PLTP in this model exceeds a threshold level. It was shown in a previous study that 30% elevation of PLTP did not result in any significant effects on plasma lipoproteins [21].

PLTP overexpression in apoE deficient mice was also studied by Yang et al. [22] in an adenovirus-associated virus mediated system. In this model of acute overexpression, increased atherosclerosis was also found to coincide with decreased HDL levels in plasma. They did not observe any differences in non-HDL cholesterol levels, but did find an increase in lipoprotein oxidizability. In our study we did not test the antioxidative protection of apolipoprotein B (apoB) containing lipoproteins, because we observed decreased levels of non-HDL cholesterol. However, the possibility that even the decreased levels of apoB containing lipoproteins are more atherogenic because their antioxidative protection capabilities are dramatically decreased, cannot be excluded.

In a recent study, the effect of liver-specific PLTP expression in a PLTP deficient background was examined [23]. In this model, a strong increase in non-HDL cholesterol was observed without any effect on HDL cholesterol. This work shows independent effects by PLTP on apoB containing lipoproteins and HDL, demonstrating the complexity of the role of PLTP in lipid and lipoprotein metabolism. The relative contribution of these effects to atherogenesis might well be dependent on the experimental model used.

The effect of PLTP in bone marrow derived cells on the development of atherosclerosis has also been studied in PLTP deficient mice by two independent groups [24,25]. Surprisingly, these studies showed strikingly different outcomes. Valenta et al. found that PLTP deficiency in macrophages results in higher levels of diet-induced atherosclerosis, while Vikstedt et al. reported decreased atherosclerosis. It is not easy to find a satisfactory solution for this apparent discrepancy, although there are several differences in the experimental setup of both studies. Some of these are probably more relevant than others, as we discussed before [13]. Already in an early stage, PLTP deficient mice have yielded puzzling results: even though HDL levels were decreased in PLTP knockout mice, diet-induced atherosclerosis was lower [4]. This was initially explained by a reduction of apoB containing lipoproteins. However, this effect was observed in apoE knockout mice, but not in LDLR knockout mice. It is therefore of interest to see that the effects of elevated PLTP expression as described in the previous and in the present studies from our group, are observed both in LDLR knockout mice and in apoE knockout mice.

A new animal model was recently developed to study the role of PLTP in atherosclerosis [26]. In these animals, PLTP transgenic rabbits, the human PLTP cDNA was driven by the human eF1-α promoter. This resulted in a widespread tissue expression and a marked elevation of plasma PLTP activity. Importantly, diet-induced atherosclerosis was increased in these animals as compared to nontransgenic littermates. So, this new model provides further evidence for a pro-atherogenic potential of PLTP.

The role of PLTP in atherosclerosis in humans has long been under debate [27]. Higher plasma PLTP levels were found in humans with conditions associated with an increased risk for artery disease, including obesitas and diabetes mellitus [2,8]. Variation in plasma PLTP activity in humans is at least in part caused by genetic factors [28]. Recently, a large number of individuals from five studies were analyzed by Vergeer et al. [11]. They investigated genetic variation at the PLTP locus and found an association with decreased plasma PLTP activity and a reduced risk of cardiovascular disease. Although the effect is probably too small to allow predicting cardiovascular disease based on PLTP SNPs, PLTP might prove an interesting target for (pharmacological) inhibition [11,29,30]. Our present and earlier findings in mice substantiate this notion.

References

[1] Albers JJ, Vuletic S, Cheung MC. Role of plasma phospholipid transfer protein in lipid and lipoprotein metabolism. Biochim Biophys Acta 2011; 1821:345–57.
[2] Masson D, Jiang XC, Lagrost L, Tall AR. The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis. J Lipid Res 2009;50: S201–6.
[3] Jiang XC, Bruce C, Mar J, et al. Targeted mutation of plasma phospholipid transfer protein gene markedly reduces high-density lipoprotein levels. J Clin Invest 1999;103:907–14.
[4] Jiang XC, Qin S, Qiao C, et al. Apolipoprotein B secretion and atherosclerosis are decreased in mice with phospholipid-transfer protein deficiency. Nat Med 2001;7:847–52.
[5] Colhoun HM, Taskinen MR, Otvos JD, Van Den Berg P, O'Connor J, Van Tol A. Relationship of phospholipid transfer protein activity to HDL and apolipoprotein B-containing lipoproteins in subjects with and without type 1 diabetes. Diabetes 2002;51:3300–5.
[6] De Vries R, Dallinga-Thie GM, Smit AJ, Wolffenbuttel BH, van Tol A, Dullaart RP. Elevated plasma phospholipid transfer protein activity is a determinant of carotid intima-media thickness in type 2 diabetes mellitus. Diabetologia 2006;49:398–404.
[7] Riemens S, van Tol A, Sluiter W, Dullaart R. Elevated plasma cholesteryl ester transfer in NIDDM: relationships with apolipoprotein B-containing lipoproteins and phospholipid transfer protein. Atherosclerosis 1998;140:71–9.
[8] Van Tol A. Phospholipid transfer protein. Curr Opin Lipidol 2002;13:135–9.
[9] Schlitt A, Bickel C, Thumma P, et al. High plasma phospholipid transfer protein levels as a risk factor for coronary artery disease. Arterioscler Thromb Vasc Biol 2003;23:1857–62.
[10] Schlitt A, Blankenberg S, Bickel C, et al. PLTP activity is a risk factor for subsequent cardiovascular events in CAD patients under statin therapy: the AtheroGene study. J Lipid Res 2009;50:723–9.
[11] Vergeer M, Boekholdt SM, Sandhu MS, et al. Genetic variation at the phospholipid transfer protein locus affects its activity and high-density lipoprotein size and is a novel marker of cardiovascular disease susceptibility. Circulation 2010;122:470–7.

[12] Van Haperen R, Van Tol A, Van Gent T, et al. Increased risk of atherosclerosis by elevated plasma levels of phospholipid transfer protein. J Biol Chem 2002; 277:48938–43.
[13] Van Haperen R, Samyn H, Moerland M, et al. Elevated expression of phospholipid transfer protein in bone marrow derived cells causes atherosclerosis. PLoS One 2008;3:e2255.
[14] Linton MF, Atkinson JB, Fazio S. Prevention of atherosclerosis in apolipoprotein E-deficient mice by bone marrow transplantation. Science 1995;267: 1034–7.
[15] Mahley RW, Rail Jr SC. Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 2000;1:507–37.
[16] Zanotti I, Pedrelli M, Poti F, et al. Macrophage, but not systemic, apolipoprotein E is necessary for macrophage reverse cholesterol transport in vivo. Arterioscler Thromb Vasc Biol 2011;31:74–80.
[17] Kockx M, Jessup W, Kritharides L. Regulation of endogenous apolipoprotein E secretion by macrophages. Arterioscler Thromb Vasc Biol 2008;28:1060–7.
[18] Linton MF, Fazio S. Macrophages, lipoprotein metabolism, and atherosclerosis: insights from murine bone marrow transplantation studies. Curr Opin Lipidol 1999;10:97–105.
[19] Hasty AH, Linton MF, Swift LL, Fazio S. Determination of the lower threshold of apolipoprotein E resulting in remnant lipoprotein clearance. J Lipid Res 1999;40:1529–38.
[20] Van Eck M, Herijgers N, Yates J, et al. Bone marrow transplantation in apolipoprotein E-deficient mice. Effect of ApoE gene dosage on serum lipid concentrations, (beta)VLDL catabolism, and atherosclerosis. Arterioscler Thromb Vasc Biol 1997;17:3117–26.
[21] Jiang X, Francone OL, Bruce C, et al. 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 1996;98:2373–80.
[22] Yang XP, Yan D, Qiao C, et al. Increased atherosclerotic lesions in apoE mice with plasma phospholipid transfer protein overexpression. Arterioscler Thromb Vasc Biol 2003;23:1601–7.
[23] Yazdanyar A, Jiang XC. Liver phospholipid transfer protein (PLTP) expression with a PLTP-null background promotes very low-density lipoprotein production in mice. Hepatology 2012;56:576–84.
[24] Valenta DT, Ogier N, Bradshaw G, et al. Atheroprotective potential of macrophage-derived phospholipid transfer protein in low-density lipoprotein receptor-deficient mice is overcome by apolipoprotein AI overexpression. Arterioscler Thromb Vasc Biol 2006;26:1572–8.
[25] Vikstedt R, Ye D, Metso J, et al. Macrophage phospholipid transfer protein contributes significantly to total plasma phospholipid transfer activity and its deficiency leads to diminished atherosclerotic lesion development. Arterioscler Thromb Vasc Biol 2007;27:578–86.
[26] Masson D, Deckert V, Gautier T, et al. Worsening of diet-induced atherosclerosis in a new model of transgenic rabbit expressing the human plasma phospholipid transfer protein. Arterioscler Thromb Vasc Biol 2011;31:766–74.
[27] Quintao EC, Cazita PM. Lipid transfer proteins: past, present and perspectives. Atherosclerosis 2010;209:1–9.
[28] Jarvik GP, Rajagopalan R, Rosenthal EA, et al. Genetic and nongenetic sources of variation in phospholipid transfer protein activity. J Lipid Res 2010;51:983–90.
[29] Lagor WR, Rader DJ. Phospholipidation of HDL–how much is too much? Clin Chem 2011;57:4–6.
[30] Luo Y, Shelly L, Sand T, et al. Pharmacologic inhibition of phospholipid transfer protein activity reduces apolipoprotein-B secretion from hepatocytes. J Pharmacol Exp Ther 2010;332:1100–6.