Macrophage phospholipid transfer protein (PLTP) is atheroprotective in LDLr-/- mice with systemic PLTP deficiency

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Running Title: Macrophage-derived PLTP and atherosclerosis

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Abstract

Systemic phospholipid transfer protein (PLTP) is a recognized risk factor for coronary heart disease. In apolipoprotein E-deficient mice systemic PLTP deficiency is atheroprotective, whereas PLTP overexpression is pro-atherogenic. As expected, we also observed significantly smaller lesions ($P < 0.0001$) in hypercholesterolemic double mutant LDLr-/PLTP-/- mice compared to LDLr-/ mice expressing systemic PLTP. To assess the specific contribution of only macrophage-derived PLTP to atherosclerosis progression, bone-marrow transplantations were performed in LDLr-/- mice that also lacked systemic PLTP. Groups of double mutant PLTP-/LDLr-/ mice were irradiated with 1000 rad and injected with bone marrow (BM) cells collected from either PLTP-/ or wild type (WT) mice. When fed a high fat diet, BM cell expression of PLTP lowered plasma cholesterol of PLTP-/LDLr-/ mice from $878 \pm 220$ mg/dl to $617 \pm 183$ mg/dl, and raised the HDL cholesterol levels from $54 \pm 11$ mg/dl to $117 \pm 19$ mg/dl. This lowered total plasma cholesterol and raised HDL cholesterol contributed to the significantly smaller atherosclerotic lesions in both aortas and heart sinus valves observed in these mice. Thus, unlike total systemic PLTP, locally produced macrophage-derived PLTP beneficially alters lipoprotein metabolism and reduces lesion progression in hyperlipidemic mice.

Key Words: Phospholipid transfer protein; atherosclerosis; bone marrow transplant; macrophage; inflammation
Introduction

Elevated plasma phospholipid transfer protein (PLTP) levels are a risk factor for coronary heart disease (CHD) in both mice (1) and humans (2). Studies in mice show that overexpression of plasma PLTP activity decreases plasma HDL cholesterol levels (3-5). In apolipoprotein E-deficient (apoE-/-) mice, adenovirus-mediated overexpression of systemic PLTP increases lesion development with a concomitant decrease in HDL cholesterol, apolipoprotein Al (apoAI) levels and reduced protection against lipoprotein oxidation (6). However, recent epidemiological studies report an inverse correlation between plasma PLTP concentrations and the incidence of CHD (7). In addition, we recently demonstrated that PLTP expression by macrophages significantly reduces atherosclerosis in hypercholesterolemic male LDLr-/- mice (8). This is in contrast to a study by Vikstedt et al. (9) who reported that macrophage-derived PLTP is pro-atherogenic in female mice fed a Western-style diet.

Such complex and sometimes contradictory characteristics of PLTP are primarily attributed to its role in lipoprotein remodeling through its lipid transfer activities. It is particularly important in the regulation of high-density lipoprotein (HDL) populations, which have well-described anti-atherogenic properties (10,11). PLTP can increase lipoprotein susceptibility to harmful oxidative damage by altering vitamin E distribution (12); yet it can beneficially improve cellular cholesterol efflux from peripheral tissues by increasing the rate of reverse cholesterol transport (RCT) (13,14). In this latter pathway, HDL accepts excess
cholesterol from peripheral tissues and transports it to the liver for secretion into bile (15). An early event in RCT involves the transfer of free cholesterol and phospholipid from cell membranes to small lipid-poor or lipid-free apoAI (16). The lipid transfer activities of PLTP studied in vitro convert triglyceride-rich spherical HDL₃ into large (10.9 nm) and small (7.8 nm) particles with a concomitant release of the lipid-poor apoAI needed for efflux (17,18). The generation of these lipid-poor, preβ-migrating molecules (preβ-HDL) is a prerequisite for the transfer of cholesterol out of cells via the cell membrane-bound ATP-binding cassette A1 (ABCA1). Systemic overexpression of human PLTP in transgenic mice increases preβ-HDL generation, but decreases plasma HDL (19,20), suggesting a pro-atherogenic rather than an anti-atherogenic function of PLTP. However, we showed that PLTP expression in peripheral tissues alters lipoprotein metabolism, even when liver expression and plasma activity remain unchanged (8). In this previous study we examined the role of macrophage PLTP expression using bone-marrow transplantation (BMT) and demonstrated that macrophage-derived PLTP is atheroprotective in LDLr-/- irradiated recipient male mice that still express systemic PLTP. This suggests that the influence of PLTP on atherogenesis is highly dependent upon its site of expression (21). Our present studies extend these observations and examined the effect of macrophage-derived PLTP on lipoprotein metabolism and atherosclerotic lesion development in hypercholesterolemic LDLr-/- mice that lacked systemic PLTP.
Materials and methods

Animals and facilities

All mice in this study were on a C57Bl/6 background. PLTP-deficient mice were a kind gift from Drs X.-C. Jiang and A.R. Tall (Columbia University). Control C57Bl/6 mice were from the Scripps rodent facility and LDLr-/- mice from a colony maintained in-house with founder mice purchased from Jackson Laboratories (Bar Harbor). PLTP-/- mice were crossed with LDLr-/- mice to generate the double knockout, PLTP-/-LDLr-/- mice. After weaning, all mice were fed a chow diet (diet no. 5015; Harlan Teklad) until 8-9 weeks of age. Blood samples were collected by retro-orbital puncture using heparin-coated capillary tubes from fasted animals anesthetized with isoflurane. Blood was transferred to EDTA-coated tubes kept on ice. All procedures were performed in accordance with institutional guidelines.

Bone-marrow transplantation

Bone marrow transplantation was performed following methods described previously (22). Two cohorts of male PLTP-/-LDLr-/- mice 8-9 weeks of age were irradiated with a single dose of 1000 rad. Bone marrow (BM) cells extracted from tibias and femurs of age-matched PLTP-/- or C57Bl/6 mice were injected intravenously (2x10^6 cells/mouse) into recipient PLTP-/-LDLr-/- mice via the tail vein. The two recipient groups with 18 mice each will hereafter be designated PLTP-/- BMT and PLTP+/+ BMT mice. The diet of mice from the two BMT groups
was changed 3 weeks after BMT from a chow to an atherogenic, high-fat diet (HFD) containing 15.8% (w/w) fat, 1.25% (w/w) cholesterol, and no cholate (diet no. 94059; Harlan Teklad). All mice were fed the HFD for 16 weeks. Re-population of donor bone marrow in recipient PLTP-/-LDLr-/- mice was assessed by PCR using PLTP mRNA isolated from blood.

**Plasma lipids, apolipoprotein AI, PLTP activity, SAA and TNF levels**

Plasma was isolated from blood by centrifuging at 5,000 rpm for 5 min and stored at -80°C until use. Total plasma and HDL cholesterol levels were measured with a colorimetric kit (Thermo), with HDL cholesterol levels determined following precipitation of the VLDL and LDL fractions with phosphotungstic acid.

Phospholipid and triglyceride levels were determined using commercially available kits (Wako, Raichem). Specific sandwich ELISAs were used to measure mouse apoAI (23), serum amyloid A (SAA) (Biosource) and TNF alpha (Pharmingen) levels in plasma. Plasma PLTP activity was measured as previously described (23, 24).

**FPLC fractionation of plasma**

Plasma was fractionated by fast protein liquid chromatography (FPLC) using two Superdex 200 columns in series with 50 µl of pooled plasma from 5 mice per group applied to the column. Fractions (0.5 ml) were collected following elution of the columns with buffer containing 10 mM Tris, 1 mM EDTA and 150 mM NaCl.
(pH 7.4). Cholesterol distribution in the different lipoprotein fractions was determined using a fluorescent detection method (22).

**Lipid-poor/free apoAI analysis**

Levels of circulating lipid-poor/free apoAI in plasma were analyzed by native polyacrylamide gel electrophoresis. Pooled \((n=5)\) plasma samples diluted 1:3 in sample buffer (16% sucrose, 0.01% bromophenyl blue) were electrophoresed in non-denaturing, non-reducing 4-26% polyacrylamide gels at 110 V for 18 hrs at 4°C. Plasmas from non-BMT LDLr-/- and PLTP-/-LDLr-/- mice that were fed either the chow diet or the HFD for 8 weeks were examined. In PLTP-/-LDLr-/- BM recipients, lipid-poor/free apoAI levels were compared in plasma collected from the two BMT groups before and after BMT (chow diet), and again 8 weeks after consuming the HFD. Separated proteins were transferred to PVDF membranes and blotted with rabbit anti-mouse apoAI (Biodesign). Bound antibody was detected with an ECL chemiluminescent kit (Invitrogen). The density of the lipid-poor apoAI fraction was determined using AlphaErase FC software (Alphainnotech).

**Assessment of atherosclerosis**

Lesion size in the aortas and heart valves was measured to compare the extent of atherosclerosis in mice from the different groups following methods described elsewhere (25, 26). After consuming the HFD for 16 weeks the mice were euthanized and perfused with paraformaldehyde (4% PFA, 5% sucrose in PBS)
and the hearts and aortas extracted. *En face* lesion areas were assessed following staining of cleaned, cut open and pinned aortas with Sudan IV. Frozen sections of heart valves stained with oil-red O were examined to measure aortic valve lesion areas. Lesion quantification was achieved by averaging the area of fatty streaks in the valve cusps of four sections 40 µm apart. Statistical differences in mean heart valve lesion and aortic *en face* lesion area between mice in the BMT and non-BMT study groups were calculated using the Mann-Whitney *U* test for non-parametric data.
Results

PLTP deficiency in hypercholesterolemic LDLr-/- mice

As expected, total plasma cholesterol levels in LDLr-/- and double mutant PLTP-/-LDLr-/- mice increased considerably following a change in diet from chow to the atherogenic HFD (Fig. 1A). However, despite no differences in plasma cholesterol levels between the groups, the size of atherosclerotic lesions in the heart valve cusps of mice fed the HFD for 16 weeks was significantly higher in LDLr-/- mice that expressed systemic PLTP compared to PLTP-/-LDLr-/- mice (Fig. 1B). Similarly, mean lesion size measured in isolated en face aortas was greater in LDLr-/- mice (21.1 ± 5.9 %) than in PLTP-/-LDLr-/- mice (11.7 ± 4.3 %) (P < 0.0001). Although HDL cholesterol levels were lower in chow-fed PLTP-/-LDLr-/- mice, HDL cholesterol levels did not significantly differ between the two groups while they consumed the HFD (Table 1). Plasma triglyceride concentrations were consistently lower (P < 0.01) in LDLr-/- mice compared to LDLr-/-PLTP-/- mice. The majority of plasma cholesterol was present in the VLDL and LDL lipoprotein fractions as revealed by FPLC separation (Fig. 2A). These FPLC separations also suggested that a systemic deficiency of PLTP may lead to a wide range of large HDL. HDL analyzed by native polyacrylamide gel electrophoresis, showed that circulating lipid-poor/free apoAI was significantly greater in LDLr-/- mouse plasma compared to PLTP-/-LDLr-/- mouse plasma after 8 weeks of consuming the HFD (Fig. 2B,C).
The lipid transfer activity of plasma PLTP is known to affect lipoprotein remodeling in vivo (3-4), with gene expression up-regulated by oxysterols through the liver X receptor (LXR) (27). In LDLr-/- mice, plasma PLTP activity was not observed to differ from wild-type C57Bl/6 mice when they consumed the chow diet (Fig. 3A). However, a change in diet to the atherogenic HFD led to a significant increase in plasma PLTP activity in LDLr-/- mice.

Plasma SAA levels are strongly indicative of systemic inflammation (28) and have been positively correlated with hypercholesterolemia and increased atherosclerosis in mice (29). In the absence of any differences in total plasma cholesterol, LDLr-/- mice expressing systemic PLTP had higher plasma SAA levels than PLTP-/LDLr-/- mice fed the HFD (Fig. 3B). TNFα levels were higher but not significantly different (P = 0.236) in PLTP-expressing LDLr-/- mice compared to double mutant PLTP-/LDLr-/- mice (20.6 ± 10.6 and 14.7 ± 6.6 pg/ml respectively; n = 7).

**Macrophage-derived PLTP in PLTP-deficient LDLr-/- mice**

Successful reconstitution of hematopoietic cells from donor bone marrow following total body irradiation of PLTP-/LDLr-/- recipients was confirmed by PLTP mRNA expression in blood leukocytes (Fig. 4A). Four weeks after BMT, PLTP expression by bone marrow-derived cells increased total cholesterol levels in chow-fed mice compared to PLTP-/- BMT chimeras. However, while consuming the HFD, PLTP+/+ BMT chimeras experienced a significantly smaller increase in total plasma cholesterol levels than PLTP-/- BMT chimeras (Fig. 4B),
yet these same PLTP+/+ BMT chimeras had significantly elevated plasma HDL cholesterol levels (Table 1). Whereas plasma HDL phospholipid concentrations followed similar trends, plasma triglyceride levels in PLTP+/+ BMT chimeras decreased significantly compared to PLTP-/- BMT chimeras, 8 weeks after consuming the HFD.

Cholesterol distribution within the different lipoprotein species was influenced by bone marrow expression of PLTP. PLTP+/+ BMT chimeras displayed increased LDL cholesterol with a proportional decrease in VLDL cholesterol compared to PLTP-/- BMT mice (Fig. 5A). Each of the bone marrow transplanted groups displayed heterogeneous plasma HDL populations. In addition, apoAI in plasma was primarily associated with spherical alpha-migrating HDL at the expense of lipid-poor/free apoAI when these PLTP+/+ BMT mice consumed the HFD (Fig. 5B,C).

The contribution of macrophage-derived PLTP to circulating PLTP activity in PLTP+/+ BMT mice did not change following a change in diet (Fig. 6A). In addition, macrophage-specific PLTP expression in BMT recipients did not significantly alter either plasma SAA concentrations (Fig. 6B) or TNFα levels (10.6 ± 4.0 pg/ml in PLTP-/- BMT mice and 8.7 ± 2.9 pg/ml in PLTP+/+ BMT mice; P = 0.326; n = 7) when the mice were challenged with the atherogenic diet.

As expected from the lipoprotein changes, the influence of only PLTP expression on lesion development was remarkable: A 62.8 % reduction in lesions as measured in en face aortas and a 48.0 % reduction as measured in heart sinus valve lesion areas was observed (Fig. 7A,B). Thus, macrophage-derived
PLTP significantly inhibited atherosclerosis progression in mice that lacked systemic PLTP.
Discussion

Many studies have highlighted the physiologic significance of PLTP in the transfer of surface remnants from triglyceride-rich lipoproteins to HDL particles during lipolysis. PLTP-deficient mice show a defective transfer of phospholipids into HDL that results in the depletion of plasma HDL (30) and hypoalphalipoproteinemia (31). Similarly, a decrease in HDL is observed when the PLTP gene is overexpressed in transgenic mouse strains (3, 4), which, in hypercholesterolemic apoE-/- mice, results in increased atherosclerosis (6). We show that endogenous PLTP expression has a comparable pro-atherogenic effect in LDLr-/- mice when they are fed a HFD (Fig. 1B). This increased disease severity in LDLr-/- mice occurred despite comparable plasma cholesterol and HDL cholesterol levels and the presence of higher circulating levels of lipid-poor/free apoAI (Fig. 2B,C).

Elevated plasma triglyceride levels, like those observed in LDLr-/- mice (Table 1), occur during an acute-phase inflammatory reaction as a result of their interaction with several acute-phase proteins secreted by the liver (28) including SAA. LDLr-/- mice had higher plasma SAA protein levels than PLTP-/-LDLr-/- mice (Fig. 3B), thus the elevated levels of circulating lipid-poor/free apoAI in the LDLr-/- mice may have been the result of PLTP-mediated remodeling of HDL (4,17), which occurred in plasma as a result of greater hepatic synthesis of SAA-containing HDL at the expense of apoAI-only containing HDL (32). Although the role of plasma PLTP in this process is poorly understood, it is known that PLTP
can alter the anti-inflammatory properties of HDL, which may then impact the expression of important inflammatory cytokines (33).

Recent studies suggest that the actions of systemic PLTP on lipid metabolism may be considerably different from those regulated by PLTP expressed in peripheral tissues. We have previously shown using BMT that atheroprotection by macrophage-derived PLTP can be achieved in LDLr-/- mice even in the presence of systemic PLTP expression (8). In the present study we confirm that macrophage-derived PLTP alone has the opposite effect on atherosclerosis compared to a mouse model with systemic PLTP expression (Figs. 1B; 7A,B). These anti-atherogenic properties of macrophage PLTP may be the result of enhanced cellular interaction between PLTP and the ABCA1 transporter. This association has been shown to stabilize HDL binding to ABCA1 and increase the rate of cholesterol and phospholipid efflux (34,35). A second possibility is that locally produced PLTP increases the rate of RCT by enhancing the generation of preβ-HDL within the vessel intima (21). This hypothesis is supported by evidence that PLTP can generate preβ-HDL from spherical HDL in vitro (13), but has yet to be confirmed in vivo. It is key to point out that high levels of circulating lipid-poor/free apoAI similar to those observed in our LDLr-/- mice fed a HFD (Fig. 2B,C), may not be indicative of locally produced preβ-HDL levels. The anti-atherogenic effects of macrophage-derived PLTP in PLTP-/-/LDLr-/- recipient mice were observed despite a drop in circulating lipid-poor/free apoAI levels (Fig. 5B,C). These observations suggest that preβ-HDL generated locally within the lesion intima by macrophage-derived PLTP need not give rise to
plasma pre\(\beta\)-HDL. Instead, interstitial modeling of HDL near cell surfaces by PLTP and an increase in cellular cholesterol efflux through the association of lipid-poor apoAI with ABCA1 may lead to the local generation of cholesterol and phospholipid discoidal apoAI that in combination with ABCG1 and LCAT can give rise to spherical alpha-migrating HDL, which then enter the circulation to transport cholesterol back to the liver.

A recent study by Vikstedt et al. (9) using female LDLr-/- mice as recipients of PLTP-/- and PLTP+/+ bone marrow, reported that despite an increase in plasma pre\(\beta\)-HDL, a deficiency of macrophage-expressed PLTP decreased atherosclerosis. In this study, a Western-style diet (containing 0.25% cholesterol) and a shorter time exposure to that diet (9 weeks) was used. Combined with known sexual dimorphism in genes that regulate lipid metabolism (36), these results highlight the complexity of PLTP action on lesion development and the need to study lesion progression over time.

The anti-atherogenic properties of macrophage-derived PLTP observed in the present study may also be the result of other associated processes. We reported that PLTP influences \(\alpha\)-tocopherol distribution between lipoproteins and cells in the vascular wall. Cultured PLTP-deficient bone marrow-derived cells have lower vitamin E content than PLTP-expressing cells resulting in higher oxidative stress and increased LDL oxidation (8). Because oxidized LDL is a key factor in the initiation of lesion development, the beneficial properties of macrophage-derived PLTP observed in PLTP-/-LDLr-/- mouse recipients could
also be the result of combined lower oxidative stress limiting LDL uptake by macrophages (21).

This study demonstrates that macrophage-derived PLTP decreases plasma LDL, increases HDL cholesterol and has a strong anti-atherogenic effect in male LDLr/- mice that lack systemic PLTP when they are challenged with an atherogenic diet. This outcome did not mirror the effects of systemically expressed PLTP, which accelerated lesion progression. Further studies are necessary to understand how PLTP is directly involved in cholesterol efflux at the cell surface.
Acknowledgements

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References


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*Circulation*. **110**: 540-545.


FIGURE LEGENDS

FIGURE 1 – Total plasma cholesterol levels and atherosclerosis in LDLr-/- and double mutant PLTP-/-LDLr-/- mice. A: Total plasma cholesterol levels in PLTP-/-LDLr-/- (○; \( n = 17 \)) and LDLr-/- (△; \( n = 17 \)) mice before and after consuming the HFD. Values are means ± S.D. B: Lesion size in oil-red O-stained heart sinus valves sectioned from hearts dissected from mice that had consumed the HFD for 16 weeks. Closed dots on each graph represent the mean and S.D. of each data set.

FIGURE 2 – Plasma lipoprotein profiles and apoAI distribution among HDL fractions. A: Cholesterol distribution in plasmas from fasted LDLr-/- (△) and PLTP-/-LDLr-/- (●) mice, following size-fractionation on two Superdex 200 columns in series. The plasmas were collected from mice that had been feeding the HFD for 8 weeks. Elution patterns of the major lipoprotein species are indicated. B: Lipid-poor/free apoAI revealed by native 4-26% gradient gel electrophoresis of pooled plasma \( (n = 5) \) collected from mice fed chow and after consuming the HFD for 8 weeks. Twenty microliters of plasma, diluted 1:3 in sample buffer, was loaded per well. C: Corresponding apoAI band densities from HFD-fed mice from (B) assessed by scanning densitometry, expressed as fold change from densities in plasma from PLTP-/-LDLr-/- mice fed the HFD. Densities are representative of 4 gels.
FIGURE 3 – Plasma PLTP activity and SAA levels. A: Lipid transfer activity in plasma from LDLr-/- mice fed a chow diet and after consuming the HFD for 8 weeks, as measured by in vitro transfer of 14C[DPPC] from liposomes to HDL. PLTP activity in plasma from C57Bl/6 mice consuming a chow diet is shown for comparison. B: Serum amyloid A levels in plasma from PLTP-/-LDLr-/- and LDLr-/- mice that had been consuming the HFD for 12 weeks as measured by ELISA.

FIGURE 4 – Bone marrow transplantation studies. A: Successful reconstitution of PLTP-/- or PLTP+/+ donor bone marrow following BMT in PLTP-/-LDLr-/- recipients assessed by PCR using blood samples. B: Changes in total plasma cholesterol levels in PLTP-/-LDLr-/- mice that received PLTP-/- BM (○) or PLTP+/+ BM (△) before BMT, 4 weeks after consuming a chow diet and while consuming the HFD for 16 weeks. Significant differences (P < 0.05) are indicated (★).

FIGURE 5 – Plasma lipoprotein profiles and lipid-poor apoAI levels in PLTP-/-LDLr-/- BMT recipient mice. A: Cholesterol distribution among lipoprotein fractions separated by FPLC in double-mutant mice that had received either PLTP-/- (●) or PLTP+/+ (△) bone marrow. B: ApoAI size distribution in HDL fractions analyzed by 4-26% native gel electrophoresis using pooled plasma (n = 5) collected from PLTP-/-LDLr-/- mice before and after BMT and after consuming the HFD for 8 weeks. Twenty microliters of plasma diluted 1:3 in sample buffer was loaded per well. C: Quantification of lipid-poor/free apoAI levels
among the two BM recipient groups by scanning densitometry, expressed as fold change from densities in plasma from PLTP-/-BMT mice fed the HFD. Densities are representative of 4 scanned gels.

FIGURE 6 – Plasma PLTP activity and SAA levels in BM transplanted PLTP-/-LDLr-/- mice. A: Lipid transfer activity in plasma from PLTP-/-LDLr-/- mice that had received PLTP+/+ BM while consuming a chow diet and after consuming the HFD for 8 weeks. B: Serum amyloid A levels in plasma from PLTP-/-LDLr-/- mice that had received BM from either PLTP-/- or PLTP+/+ mice and that had been consuming the HFD for 12 weeks.

FIGURE 7 – Assessment of atherosclerosis. A: Lesions measured as percentage of en face aorta lesion area and lesion areas in heart valve sections in PLTP-/-LDLr-/- mice that received either PLTP-/- BM or PLTP+/+ BM, after consuming the HFD for 16 weeks. Closed dots on each graph represent the mean and S.D. of each data set. B: Representative aortas and heart sinus valve sections from the two BMT recipient mouse groups.
FIGURE 1

A

Plasma cholesterol (mg/dL) vs. Weeks fed a HFD

B

Heart sinus lesion area (µm²) vs. LDLr/-/ and PLTP/-/

P < 0.0001
**Figure 2**

(A) Cholesterol concentration (μg/dL) for different lipoprotein fractions: VLDL, LDL, and HDL, across various diets and genotypes.

(B) Fold change in lipid-poor/free apoAI levels in different genotypes and diets.

(C) Statistical analysis showing a significant difference in lipid-poor/free apoAI levels between PLTP−/− and LDLr−/− mice on a high-fat diet (HFD) compared to Chow diet (P < 0.01).

Legend:
- α-HDL
- Lipid-poor/free apoAI
- PLTP−/−
- LDLr−/−
FIGURE 3

A

Lipid transfer activity (µmol/ml/hr)

- C57Bl/6
- Chow
- HFD

P < 0.01

B

SAA levels (µg/ml)

- PLTP-/-
- LDLr-/-

P < 0.05
FIGURE 4

A

PLTP-/-BMT  PLTP+/+BMT

Knockout  WT

B

Plasma cholesterol (mg/dL)

BMT  HFD

Time (weeks)

FIGURE 4
FIGURE 5

A

Cholesterol conc (μg/dL)

Fraction

B

PLTP-/-BM

Pre-BMT

Post-BMT

HFD

PLTP+/+BM

Pre-BMT

Post-BMT

HFD

Kd

250

150

100

75

50

α-HDL

Lipid-poor/free apoAI

C

Fold change in lipid-poor/free apoAI levels

P<0.05

PLTP-/-BMT

PLTP+/+BMT
FIGURE 6

A

B

Lipid transfer activity (µmol/ml/hr)

SAA levels (µg/ml)

Chow HFD
PLTP+/+ BMT

PLTP-/ PLTP+/+
BM
En face lesion area (%)

Heart sinus lesion area (µm²)

PLTP-/-BMT

PLTP+/+BMT

P < 0.0001

FIGURE 7
TABLE 1

Changes in plasma lipids and apoAI levels in the different mouse groups when fed a chow or HFD. Values are means ±SD. Statistical differences at $P < 0.05$ (*) and $P < 0.01$ (**) .

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<td>168.8±55.9</td>
<td>205.5±59.2</td>
<td>325.2±154.4**</td>
<td>315.5±96.7**</td>
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<td>35.0±11.2</td>
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<td>100.9±18.6**</td>
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