Phospholipid transfer protein deficiency ameliorates diet-induced hypercholesterolemia and inflammation in mice

Lorraine Shelly, Lori Royer, Thomas Sand, Heather Jensen, and Yi Luo[1]

Department of Cardiovascular, Metabolic, & Endocrine Diseases, Pfizer Global Research Division, Pfizer, Inc., Groton, CT 06340

Abstract Phospholipid transfer protein (PLTP) facilitates the transfer of phospholipids from triglyceride-rich lipoproteins into HDL. PLTP has been shown to be an important factor in lipoprotein metabolism and atherogenesis. Here, we report that chronic high-fat, high-cholesterol diet feeding markedly increased plasma cholesterol levels in C57BL/6 mice. PLTP deficiency attenuated diet-induced hypercholesterolemia by dramatically reducing apolipoprotein E-rich lipoproteins (−88%) and, to a lesser extent, LDL (−40%) and HDL (−35%). Increased biliary cholesterol secretion, indicated by increased hepatic ABCG5/ABCG8 gene expression, and decreased intestinal cholesterol absorption may contribute to the lower plasma cholesterol in PLTP-deficient mice. The expression of proinflammatory genes (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) is reduced in aorta of PLTP knockout mice compared with wild-type mice fed either a chow or a high-cholesterol diet. Furthermore, plasma interleukin-6 levels are significantly lower in PLTP-deficient mice, indicating reduced systemic inflammation. These data suggest that PLTP appears to play a proatherogenic role in diet-induced hyperlipidemic mice.—Shelly, L., L. Royer, T. Sand, H. Jensen, and Y. Luo. **Phospholipid transfer protein deficiency ameliorates diet-induced hypercholesterolemia and inflammation in mice.** ***J. Lipid Res.*** **2008.** 49: **773–781.**

Supplementary key words ATP binding cassette transporter G5 • ATP binding cassette transporter G8 • cholesterol absorption

Plasma phospholipid transfer protein (PLTP) plays an important role in the metabolism of lipoproteins (1). PLTP belongs to the family of lipid transfer/lipopolysaccharide binding proteins, including cholesteryl ester transfer protein, lipopolysaccharide binding protein, and bactericidal permeability-increasing protein (2, 3). It has been shown that PLTP facilitates the transfer and exchange of phospholipids between VLDL and HDL (4). It also transfers phospholipids between HDL particles that result in the conversion of HDL_3 into larger and smaller HDL particles (5, 6). PLTP can also bind several other amphipathic molecules, including α-tocopherol, diacylglycerides, cerebrosides, and lipopolysaccharides (7). Several clinical studies suggest that high plasma PLTP activity is a risk factor for coronary artery disease and a determinant of carotid intima-media thickness in type 2 diabetes mellitus (8, 9).

Studies using genetically modified mice strongly suggest that PLTP functions as a proatherogenic factor (10–12). PLTP deficiency causes a marked decrease in HDL lipids and apolipoprotein A-I (apoA-I), as a result of higher catabolism, in both chow-fed and 2 week Western diet-fed mice (13, 14). The absence of PLTP in hyperlipidemic apoE-deficient and human apoB transgenic mouse strains results in reduced production in plasma levels of apoB-containing lipoproteins, mostly LDL (10). Atherosclerotic lesion areas are also decreased in PLTP knockout mice in the LDL receptor- or apoE-deficient or apoB transgenic background, despite decreased HDL. Furthermore, reduced lipoprotein oxidation and improved anti-inflammatory properties of HDL in PLTP knockout mice may also contribute to the antiatherogenic potential in these mouse models (15, 16). The proatherogenic role of PLTP is further supported by results that demonstrate increased PLTP expression in hyperlipidemic mouse models, which show increased susceptibility to atherosclerosis (11, 12).

The newly revealed function of PLTP in regulating apoB-containing lipoprotein levels is based on studies in hyperlipidemic apoE knockout or apoB transgenic mice. Both models have an overwhelming accumulation of

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Abbreviations: apoA-I, apolipoprotein A-I; FPLC, fast-protein liquid chromatography; HFHC, high-fat, high-cholesterol; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; PLTP, phospholipid transfer protein; SR-BI, scavenger receptor class B type I; VCAM-1, vascular cell adhesion molecule-1.

[1] To whom correspondence should be addressed.
e-mail: yi.luo@pfizer.com

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plasma apoB-containing lipoproteins. It has not been studied in detail how PLTP regulates lipid metabolism in hyperlipidemic mice with no defects in lipoprotein production or clearance. In this study, we analyzed the effects of PLTP deficiency on lipoprotein metabolism in high-fat, high-cholesterol (HFHC) diet-induced hyperlipidemic mice. Atherosclerosis is a chronic inflammatory disease in which early atherogenic events include increased expression of vascular adhesion molecules and chemoattractants followed by increased adhesion of monocytes and lymphocytes (17). We analyzed plasma levels of the proinflammatory factor interleukin-6 (IL-6) and the expression of genes encoding vascular adhesion molecules in aorta to assess inflammation and atherosclerosis potential in these mice.

MATERIALS AND METHODS

Animals

Male PLTP knockout mice in the C57BL6 background and control C57BL6 mice were obtained from Jackson Laboratory. Mice were maintained on a 12 h light/dark cycle and fed rodent chow diet (5K50; LabDiet) or A-IN-76A Western diet (TestDiet; 20% fat, 0.15% cholesterol, and 34% sucrose). The study protocol was approved by the Institutional Animal Care and Use Committee. All animals received humane treatment according to the criteria stated by the National Academy of Sciences National Research Council.

Lipid measurement and lipoprotein isolation

Blood was collected from mice fasted for 6 h. Total cholesterol, free cholesterol, triglyceride, and phospholipids in plasma or lipoproteins were assayed by enzymatic methods (Wako Pure Chemical Industries, Ltd.). Cholesteryl ester concentration was calculated by subtracting the amount of free cholesterol from total cholesterol. Lipoprotein profiles were analyzed by fast-protein liquid chromatography (FPLC). Pooled mouse plasma (200 μl) from 10 mice was loaded onto a Superose 6 column (Pharmacia LKB Biotechnology, Piscataway, NJ) and eluted with lipoprotein separation buffer (154 mM NaCl, 1 mM EDTA, and 0.02% NaN_3) as described previously (18).

Apolipoprotein analysis

For Western blot analysis of apoB, apoE, apoA-IV, and apoA-I in FPLC fractions, equal volumes of pooled FPLC fractions were separated by SDS-PAGE followed by transfer to a nitrocellulose membrane and immunoblotted using anti-mouse apoA-I antibody, anti-mouse apoE antibody, and anti-mouse apoB antibody (Biodesign). Anti-mouse apoA-IV antibody was provided by Dr. Charles L. Bisgaier (Esperion). Concentrated pooled fractions were assayed for total cholesterol, phospholipids, and free cholesterol by enzymatic methods (Wako) and for apoB and apoA-I by ELISA.

Triglyceride, apoE, and apoB production in mice

Apolipoprotein secretion in Western diet-fed mice was determined based on serum accumulation of triglyceride after injection with tyloxapol (Triton WR-1339) (Sigma Chemicals) to inhibit lipolysis and lipoprotein clearance from the circulation (19, 20). Four wild-type C57BL6 mice and four PLTP knockout mice were fasted for 4 h, and then 500 mg/kg tyloxapol was injected into the tail vein. Retro-orbital blood samples were collected before and 2 h after tyloxapol injection. ApoE protein levels were measured by Western blot using 1 μl of plasma. The immunoblot chemiluminescence image was captured, scanned, and quantitated with a Lumi-Imager F1 (Roche). ApoB levels were measured by ELISA. Baseline triglyceride or protein levels were subtracted from the 2 h value in each of the mice, and triglyceride and apoB production rates were calculated as mg/kg/h, assuming the plasma volume to be 3.5% of the body weight. ApoE production rate was calculated as chemiluminescence units/kg/h. The data are expressed as percentage of wild-type controls.

Gene expression analysis

RNA was isolated from tissues using the Qiagen RNeasy kit as described by the manufacturer and then subjected to reverse transcription to synthesize cDNA using the Reverse Transcription kit (Applied Biosystems). Real-time quantitative PCR analysis was performed using TaqMan mix and an ABI-7700HT Sequence Detection System (Applied Biosystems). Expression was normalized to cyclophilin controls.

Intestinal absorption of cholesterol

Cholesterol absorption was determined by a fecal dual-isotope ratio method in male age-matched wild-type and PLTP-deficient mice on chow or HFHC diet for 10 weeks. Individually housed mice were dosed intragastrically with a bolus of 150 μl of corn oil containing 5 μCi of [^{14}C]cholesterol (American Radiolabeled Chemicals, Inc.) and 5 μCi of [5,6-^{3}H]sitostanol (American Radiolabeled Chemicals, Inc.) and then returned to fresh cages. Feces were collected for 3 days. Feces were dried and ground. Feces powder was extracted with chloroform-methanol (2:1), and the ratio of ^{14}C to ^{3}H in each sample was calculated. The percentage of cholesterol absorption was expressed as percentage of administered dose absorbed using the following formula:

$$\%\ \text{cholesterol absorption} = \{1 - [\text{fecal } (^{14}\text{C}/^{3}\text{H})]/[\text{administered } (^{14}\text{C}/^{3}\text{H})]\} \times 100$$

Plasma IL-6 levels

Plasma IL-6 concentration was measured using the Quantikine immunoassay kit for mouse IL-6 according to the enclosed kit directions (R&D Systems).

RESULTS

Effects of PLTP deficiency on plasma lipids and lipoproteins

To understand how PLTP deficiency regulates lipid metabolism in diet-induced hyperlipidemia, PLTP-deficient mice (n = 11) and control wild-type C57BL6 mice (n = 10) were subjected to a HFHC diet challenge for 10 weeks. Wild-type mice developed hypercholesterolemia, as plasma total cholesterol increased by ~2-fold after diet feeding. Although total plasma cholesterol also increased in diet-challenged PLTP knockout mice compared with chow-fed mice, total cholesterol levels were much lower in PLTP knockout mice than in control wild-type mice (**Table 1**). The distribution of lipoproteins in hyperlipidemic mice was analyzed by FPLC of pooled plasma from hyperlipidemic mice (**Fig. 1A**). ApoA-I and apoB levels in FPLC fractions were analyzed by ELISA, and their distributions were used to determine LDL and HDL fractions. In wild-

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TABLE 1. Plasma lipid analysis in wild-type and PLTP knockout mice on chow and HFHC diet

	Chow Diet		HFHC Diet	
Mouse	Total Cholesterol	Triglyceride	Total Cholesterol	Triglyceride
	mg/dl			
Wild type	110 ± 12[a]	80 ± 24	197 ± 33[a]	109 ± 21
PLTP knockout	40 ± 4	62 ± 12	101 ± 26	133 ± 32

HFHC, high-fat, high-cholesterol; PLTP, phospholipid transfer protein. Values are means ± SD of 10–11 animals per group.

[a] $P < 0.005$, PLTP knockout versus wild-type mice.

type C57BL/6 mice, HFHC feeding not only increased cholesterol levels in apoB-containing lipoproteins (fractions 10–16) but markedly increased cholesterol in fractions 17–19 as well, which had low levels of apoB (Fig. 1).

To further analyze the lipids and protein composition in these lipoprotein fractions, we pooled fractions 6–9 as VLDL, 10–16 as LDL, 20–25 as HDL, and 17–19 as a separate category based on apoB and apoA-I distribution. Apolipoproteins in these pooled fractions were analyzed by immunoblot (Fig. 1B). Fractions 17–19 were heterogeneous, and the major apolipoproteins were apoE proteins. Besides apoE-rich particles, fractions 17–19 also contained small amounts of apoB-containing small LDL and apoA-I-containing large HDL, which appeared to be greater in wild-type mice than in PLTP knockout mice, probably as a result of the higher LDL and HDL in wild-type mice (Fig. 1, **Table 2**). In PLTP-deficient mice, both apoE and cholesterol levels were reduced dramatically in apoE-rich lipoprotein fractions, implying a decreased number of particles (Fig. 1B, Table 2). ApoB and cholesterol levels in LDL fractions were also reduced in PLTP knockout mice by 30% and 40%, respectively (Table 2). VLDL cholesterol and apoB levels, which constitute <10% of total cholesterol and apoB, were higher in mice lacking PLTP (Table 1, Fig. 1B). HDL cholesterol was also lower in PLTP knockout mice than in wild-type mice, a phenotype also observed in chow-fed mice, which is consistent with previous reports (13, 14). We also observed that apoA-IV distribution was partially shifted to apoB-containing VLDL and LDL (fractions 6–16) in PLTP-deficient mice (Fig. 1B).

ApoE and apoB production in Western diet-fed mice was determined based on serum accumulation of VLDL-triglyceride after injection with Triton WR-1339 (tyloxapol) to inhibit lipolysis and lipoprotein clearance from the circulation (19, 20). ApoB and triglyceride production rates were similar in wild-type and PLTP knockout mice. There was no significant difference in apoE production either (**Fig. 2**), suggesting that the marked decrease of apoE-rich lipoproteins in PLTP knockout mice was probably attributable to increased clearance.

Effects of PLTP deficiency on hepatic lipids and gene expression

Body and liver weights of hyperlipidemic wild-type and PLTP-deficient mice were similar (data not shown). Liver total cholesterol and triglyceride levels were increased upon HFHC feeding. However, there were no significant differences in the levels of total cholesterol, phospholipids, and triglyceride when PLTP-deficient and wild-type control mice were compared (**Table 3**). We analyzed the expression of the genes encoding enzymes involved in lipid metabolism, such as fatty acid synthase, stearoyl-CoA desaturase, and HMG-CoA reductase. No differences were observed when wild-type and PLTP knockout mice were compared (data not shown). We also analyzed the expression of the genes involved in cholesterol transport, such as ABCA1, ABCG1, ABCG5, and ABCG8. The expression of ABCG5 and ABCG8 was induced by the HFHC diet. Importantly, PLTP-deficient mice exhibited significantly higher expression levels of ABCG5/G8 than wild-type mice fed either a chow diet or a HFHC diet (**Fig. 3A**). The expression of the gene encoding cholesterol 7α-hydroxylase appeared to be higher in PLTP knockout mice than in wild-type mice under chow-fed conditions but not under HFHC-fed conditions (Fig. 3A).

Cholesterol absorption

Because ABCG5/G8 genes are also expressed in intestine and have been shown to be involved in cholesterol absorption (21), we analyzed the intestinal expression of these genes in HFHC diet-induced mice. The results showed that the expression levels of both ABCG5 and ABCG8 were higher in the intestine of PLTP-deficient mice than in wild-type control mice (Fig. 3B). There was no significant difference in intestinal cholesterol content between wild-type and PLTP knockout mice (data not shown). We then measured fractional absorption of cholesterol by the dual-isotope ratio method in the PLTP knockout and wild-type mice. The intestinal cholesterol absorption was mildly reduced by ∼10% in chow-fed PLTP knockout mice (**Fig. 4A**). After 14 weeks of HFHC diet feeding, intestinal cholesterol absorption was reduced significantly by 23% in PLTP knockout mice compared with heterozygous and wild-type control mice (Fig. 4B).

Effects of PLTP deficiency on aortic and systemic inflammation

It has been reported that PLTP deficiency results in a reduction of atherosclerosis lesion areas in apoE knockout, apoB transgenic, and LDL receptor knockout mice (10). Atherosclerosis is a chronic inflammatory disease in which early atherogenic events include increased expression of vascular adhesion molecules and chemoattractants, followed by increased adhesion of monocytes and lymphocytes (17). Increased intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) expression has been shown to be associated with or to predict atherosclerosis (22–24). To assess the atherogenic potential of the diet-induced hyperlipidemic wild-type and PLTP-deficient mice, we analyzed the expression of proatherogenic VCAM-1 and ICAM-1 in aorta. The expression of both ICAM-1 and VCAM-1 was markedly induced by the HFHC diet in wild-type mice (**Fig. 5**). However, ICAM-1 and VCAM-1 expression was not induced significantly by the HFHC diet in PLTP knockout mice (Fig. 5). Consequently, ICAM-1 and VCAM-1 expression

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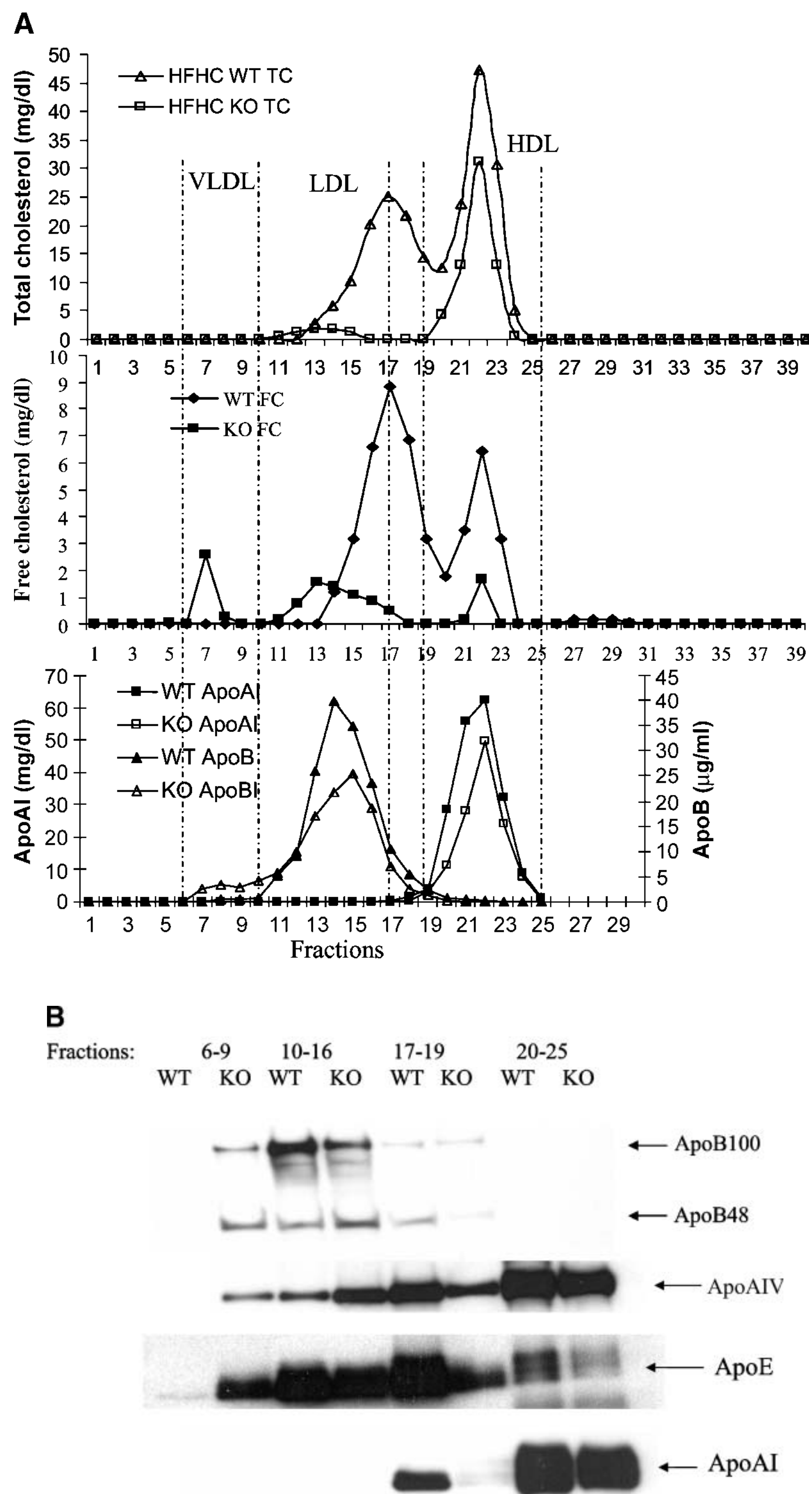

Fig. 1. A: Fast-protein liquid chromatography (FPLC) profile of plasma lipoprotein total cholesterol (TC), free cholesterol (FC), apolipoprotein B (apoB), and apoA-I distribution in male mice fed a high-fat, high-cholesterol (HFHC) diet. Pooled plasma (200 μl) from 10 mice was fractionated by FPLC as described in Materials and Methods. Total cholesterol, free cholesterol, apoB, and apoA-I levels were determined and plotted as a function of FPLC fractions. The fractions containing lipoproteins are indicated. B: Immunoblot analysis of apolipoprotein levels in FPLC fractions from HFHC diet-fed mice. FPLC fractions 6–9, 10–16, 17–19, and 20–25 were pooled and subjected to immunoblot analysis for apoB, apoA-I, apoA-IV, and apoE as described in Materials and Methods. Fractions 17–19 are enriched in apoE-containing particles. KO, knockout; WT, wild type.

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TABLE 2. Lipoprotein lipid analysis in wild-type and PLTP knockout mice fed a HFHC diet

Lipoprotein	Total Cholesterol	Phospholipid	Free Cholesterol	ApoB	ApoA-I
VLDL					
Wild type	1.49	0.25	0.00	1.00	ND
PLTP knockout	9.84	11.97	2.80	9.00	ND
LDL					
Wild type	44.24	26.58	10.95	139.30	ND
PLTP knockout	26.10	28.93	5.80	101.70	ND
ApoE-rich lipoproteins					
Wild type	32.97	21.39	18.80	18.20	6.43
PLTP knockout	3.78	2.44	0.5	11	3.68
HDL					
Wild type	101.75	83.91	14.80	ND	189.18
PLTP knockout	47.68	56.99	1.80	ND	121.34

ApoA-I, apolipoprotein A-I. Plasma from wild-type and PLTP knockout mice (n = 10) were pooled, and lipoproteins were separated by fast-protein liquid chromatography. Fractions corresponding to VLDL (fractions 6–9), LDL (fractions 10–16), apoE-rich lipoproteins (fractions 17–19), and HDL (fractions 20–25) were pooled and concentrated. Total cholesterol, phospholipids, triglyceride, and free cholesterol were measured by enzymatic methods (assay coefficient of variation < 10%). ApoB and apoA-I were measured by ELISA. Lipids and apoA-I values are presented as mg/dl, and apoB values are presented as μg/ml. ND, not detectible.

was significantly lower in PLTP knockout mice than in wild-type control mice fed a HFHC diet (Fig. 5). Reduced expression of these adhesion molecules has been shown to protect against atherosclerosis in mice (25, 26). Furthermore, decreased systemic inflammation in PLTP-deficient mice was demonstrated by significantly reduced plasma IL-6 levels in PLTP knockout mice compared with wild-type mice fed a HFHC diet (**Fig. 6**). These data imply that PLTP deficiency reduces atherogenic factors in diet-induced hyperlipidemic mice.

DISCUSSION

In the present study, we analyzed the effects of PLTP deficiency on lipid metabolism in HFHC diet-induced hyperlipidemic mice. Previous reports showed that PLTP regulates plasma HDL cholesterol, LDL cholesterol, or apoB levels in apoE-deficient and apoB overproduction mice (10). Here, we show that PLTP deficiency attenuated diet-induced hypercholesterolemia (Fig. 1). Lipoprotein profiles were analyzed by FPLC, and fractions were pooled based on major apolipoproteins. Because of the nature of FPLC separation, it should be noted that these pooled fractions were not homogenous lipoproteins and were defined based on the major lipoprotein components. We observed that besides lower cholesterol in HDL fractions (−35%), as reported previously (13, 14), PLTP deficiency resulted in marked reduction of cholesterol in apoE-rich lipoproteins (−88%) and to a lesser extent in apoB-containing LDL (−40%). Although VLDL levels were low and constituted <10% of total cholesterol, we observed that VLDL levels were higher in mice lacking PLTP. PLTP transfers phospholipids from VLDL to HDL (4), and blockage of this process in PLTP-deficient mice may cause hypocatabolism of VLDL and hypercatabolism of HDL, resulting in the accumulation of VLDL and the reduction of HDL (4, 27).

Upon HFHC diet feeding, the dramatic reduction of apoE-rich lipoproteins in PLTP-deficient mice may be attributable to increased catabolism, because no decrease in apoE production was observed between wild-type and PLTP knockout mice (Fig. 2). High-cholesterol diet-induced apoE-rich lipoprotein was shown to be functionally similar to LDL by binding to LDL receptor and to be cleared in mice by LDL receptor (28, 29). Hepatic expression of Niemann-Pick C1-Like 1, a target of ezetimibe,

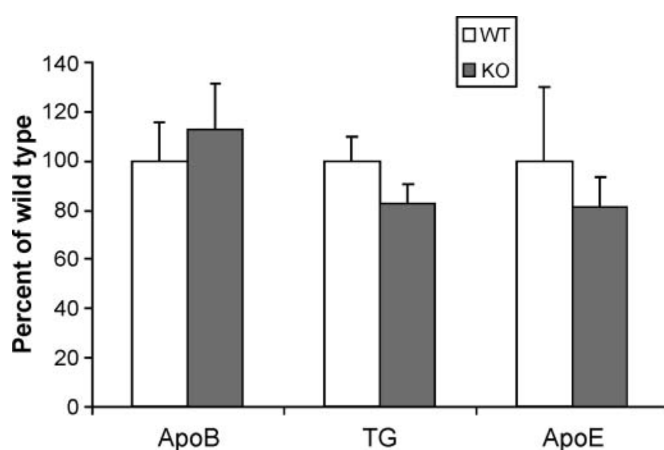

Fig. 2. Production of triglyceride (TG), apoE, and apoB in mouse. Apolipoprotein production in Western diet-fed mouse was determined based on serum accumulation of lipoproteins after injection with tyloxapol. Retro-orbital blood samples were collected before and 2 h after tyloxapol (500 mg/kg) injection. Proteins and triglyceride were quantitated, and production rates were calculated as described in Materials and Methods. The data are expressed as percentage of wild-type control (n = 4). Values are presented as means ± SD. KO, knockout; WT, wild type.

TABLE 3. Liver lipid levels

Diet and Animal	Total Cholesterol	Phospholipid	Triglyceride
		mg/g liver	
Wild-type, chow	2.69 ± 0.20	17.83 ± 1.22	13.27 ± 2.18
PLTP-deficient, chow	2.80 ± 0.11	19.11 ± 0.90	15.93 ± 2.11
Wild-type, HFHC	4.75 ± 0.31	17.06 ± 0.83	27.98 ± 1.52
PLTP-deficient, HFHC	4.89 ± 0.17	19.09 ± 0.96	29.20 ± 1.91

Liver lipids were extracted and contents were determined as described in Materials and Methods. n = 10–11 in each group.

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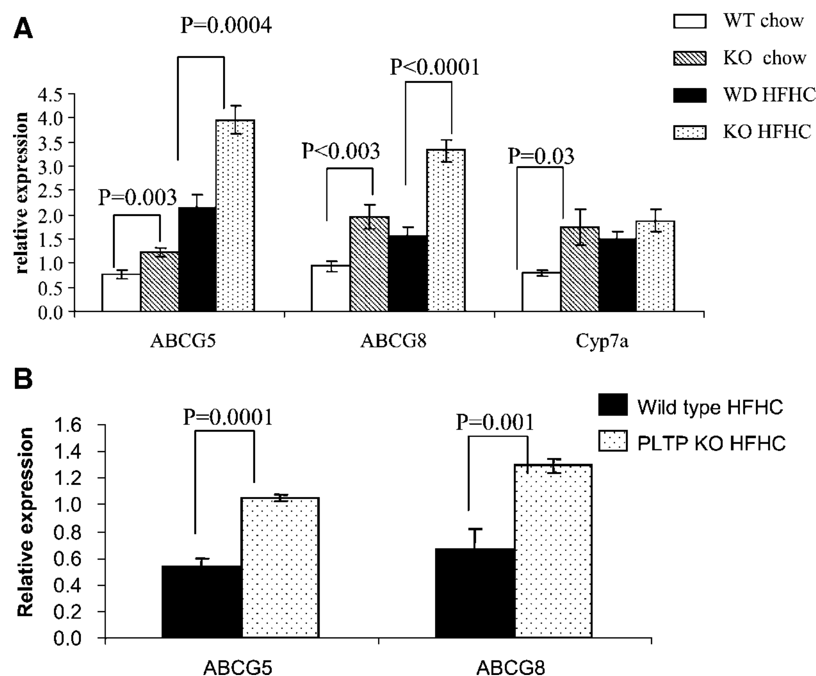

Fig. 3. Real-time PCR analysis of ABCG5 and ABCG8 gene expression in mice of different genotypes fed a chow or HFHC diet. A: Liver ABCG5/G8 expression in mice on a chow or HFHC diet. B: Intestinal ABCG5/G8 expression in mice on a HFHC diet. Duodenum and jejunum sections of each animal were combined to prepare RNA. Liver or intestinal RNA samples from individual animals were subjected to real-time PCR analysis for gene expression. Values are presented as means ± SEM (n = 10–11). KO, knockout; PLTP, phospholipid transfer protein; WT, wild type.

markedly increased apoE-rich lipoproteins (30). It has been reported that apoE-rich lipoproteins, with size distribution between LDL and HDL, accumulate in scavenger receptor class B type I (SR-BI)-deficient mice (31, 32). Increased apoE-rich particles have been shown to be associated with dysfunctional HDL and accelerated atherosclerosis (31, 33). A study in human suggests that HDL from subjects with coronary artery disease was selectively enriched in apoE (34). ApoE-enriched lipoproteins from diet-induced wild-type mice may be proatherogenic.

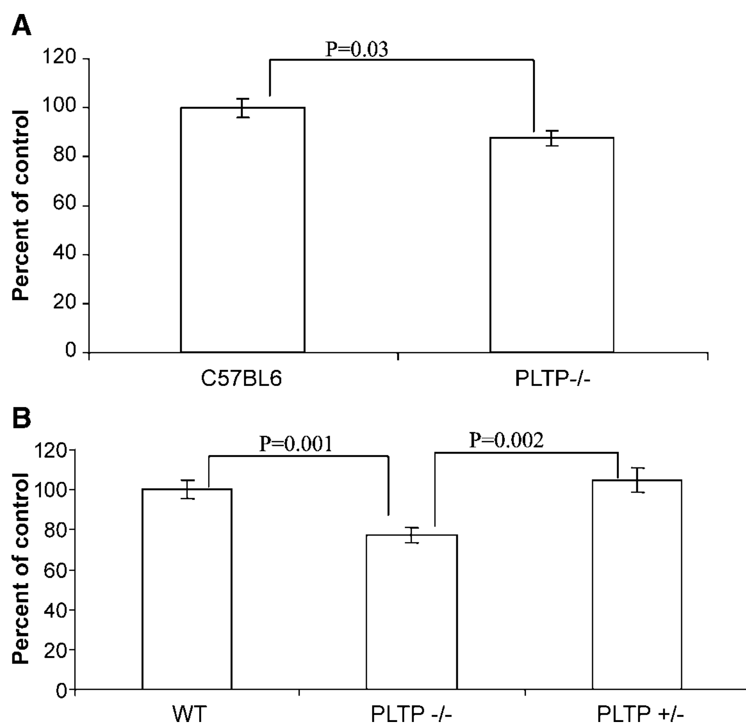

Fig. 4. Cholesterol absorption was determined in age-matched male mice fed either a chow (A) or HFHC (B) diet for 10 weeks as described in Materials and Methods. Values are presented as means ± SEM (n = 10–11). WT, wild type.

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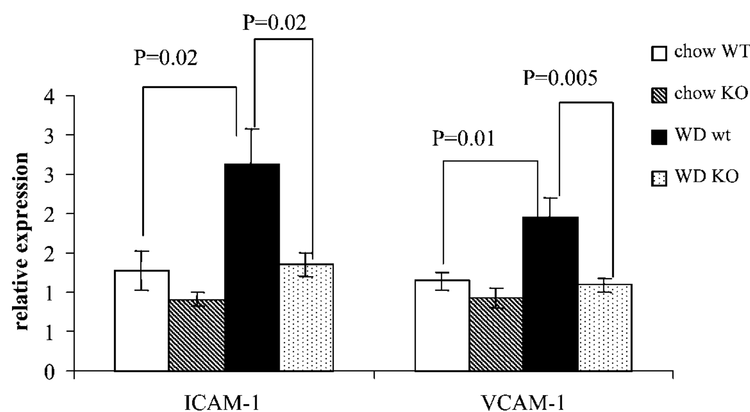

Fig. 5. Real-time PCR analysis of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) gene expression in aorta from mice fed a chow or HFHC diet. The expression levels of ICAM-1 and VCAM-1 genes are higher in wild-type control (WT) than in PLTP knockout (KO) mice. Values are presented as means ± SEM (n = 10 for wild-type mice and n = 11 for knockout mice). WD, Western diet.

Increased ICAM-1 and VCAM-1 expression has been suggested to be associated with or to predict atherosclerotic lesions (22–24). Reduced expression of these adhesion molecules has been shown to protect against atherosclerosis in mice (25, 26). We observed that the expression of proatherogenic ICAM-1 and VCAM-1 was reduced in the aorta of PLTP knockout mice compared with wild-type mice fed a high-cholesterol diet. Plasma IL-6 levels were decreased significantly in PLTP-deficient mice compared with wild-type control mice, implying reduced systemic inflammation in the absence of PLTP in mice (Fig. 6). Reduced aortic and systemic inflammation indicates that PLTP-deficient mice may have decreased atherogenic potential. Our data support previous reports that PLTP deficiency decreases atherosclerosis and that PLTP is proatherogenic in mice (10–12).

In HFHC diet-fed mice, the reduced inflammation in PLTP-deficient mice may be secondary to decreased plasma cholesterol in apoE-rich lipoproteins and LDL. Increased LDL/apoE-rich lipoproteins in LDL receptor-deficient mice are associated with increased inflammation and atherosclerosis (28, 29, 35, 36). It has been reported that there are higher levels of vitamin E on LDL in PLTP-deficient mice that lead to a decrease in oxidized LDL production (15). We also observed an increase in apoA-IV in LDL fractions (fractions 10–16) in PLTP-deficient mice (Fig. 1B). ApoA-IV has been shown to reduce LDL oxidation and atherosclerosis (37, 38). Thus, apoA-IV-enriched LDL might have antioxidation and antiatherogenic properties. Oxidized LDL has been reported to induce inflammation (39, 40). Although HDL cholesterol levels are lower in PLTP-deficient mice than in wild-type mice, HDL from PLTP knockout mice appears to possess improved anti-inflammatory properties, which may also contribute to reduced systemic inflammation in PLTP-deficient mice (16).

It is not known whether PLTP deficiency per se will contribute to the decrease in inflammation. PLTP may also be directly involved in innate immunity, which is suggested by increased PLTP expression during macrophage differentiation (data not shown). Macrophage-expressed PLTP contributes ~20% of plasma PLTP activity (41, 42). The role of macrophage-derived PLTP in atherosclerosis was studied by bone marrow transplantation (41–43). The results were controversial because of a reduction of apoE protein in macrophages deficient in PLTP and the use of different recipient mice. Recently, increased PLTP activity was shown to be associated with the inflammatory marker C-reactive protein in patients with cardiovascular disease (44).

Our studies indicate that there might be several mechanisms that contribute to the reduction of plasma cholesterol in PLTP knockout mice fed a HFHC diet. PLTP has been shown to regulate apoB-containing lipoprotein secretion from hepatocytes (10). In HFHC diet-fed mice, we did not observe changes in apoB production rate, measured by the Triton WR-1339 method (Fig. 2), implying that the reduced plasma apoB-containing lipoproteins are probably attributable to increased clearance. The reduction of apoE-rich lipoproteins in PLTP knockout mice may also be attributable to increased clearance, because there was no difference in apoE production com-

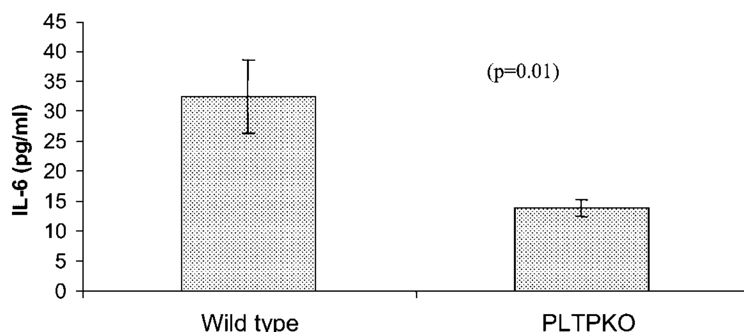

Fig. 6. Plasma interleukin-6 (IL-6) levels are significantly lower in PLTP-deficient (PLPTKO) mice than in wild-type control mice fed a HFHC diet. Plasma IL-6 concentrations were determined as stated in Materials and Methods. Values are presented as means ± SEM (n = 10 for wild-type mice and n = 11 for knockout mice).

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pared with that in control mice. It appears that SR-BI is not involved in this mechanism, because we did not detect any changes in SR-BI protein levels in the liver or intestine of control versus PLTP knockout mice (data not shown). ABCG5/G8 play important roles in hepatobiliary cholesterol secretion, and increased ABCG5/G8 expression results in increased biliary cholesterol efflux (45, 46). Transgenic expression of ABCG5/G8 in LDL receptor-deficient mice was associated with lower HFHC diet-induced LDL/apoE particles, plasma cholesterol, and atherosclerosis lesions (47).

We observed that the expression of hepatic ABCG5/G8 genes was higher in PLTP-deficient mice (Fig. 3), suggesting that there might be an increase in biliary cholesterol secretion in PLTP-deficient mice, which will consequently reduce lipoprotein secretion to plasma. Intestinal ABCG5/G8 has been suggested to be involved in cholesterol absorption (48). A fractional cholesterol absorption study showed that PLTP-deficient mice have reduced cholesterol absorption compared with wild-type-control mice and that the reduction is more pronounced in HFHC diet-fed mice (Fig. 4). Increased intestinal ABCG5/G8 gene expression may contribute to the reduction of cholesterol absorption (Fig. 4). It is possible that PLTP in enterocytes is involved in assembling cholesterol into apoB-containing particles and regulating cholesterol absorption. It is also possible that PLTP deficiency affects the phospholipid composition of subcellular organelles, such as the plasma membrane, and alters the conformation of membrane proteins involved in cholesterol absorption, resulting in reduced assimilation of cholesterol by enterocytes. The possibility that the reduced cholesterol absorption is secondary to the increase in biliary cholesterol output could not be ruled out. During the review of this article, Liu et al. (49), using a different method to measure cholesterol absorption, also showed that PLTP-deficient mice have reduced cholesterol absorption. The aforementioned mechanisms may contribute to the decreased apoE-rich lipoproteins in the absence of PLTP; however, we cannot exclude the possibility that a lack of phospholipid transfer to apoE particles may also cause the high clearance of these particles.

The mechanisms that mediate the increased expression of ABCG5/G8 in PLTP-deficient mice are not known. The HFHC diet increased ABCG5/G8 expression (Fig. 3A), consistent with a previous report that they are liver X receptor target genes (50). The expression of other liver X receptor target genes, such as ABCA1, ABCG1, fatty acid synthase, and stearoyl-CoA desaturase-1, was also increased upon high-cholesterol diet feeding (data not shown). However, the expression levels of these genes was similar in wild-type and PLTP knockout mice (data not shown). Intestinal ABCA1 and ABCG1 gene expression also remained unchanged upon PLTP deletion (data not shown). We did not observe any difference in liver total cholesterol or free cholesterol levels between wild-type and PLTP knockout mice. These data indicate that the liver X receptor pathway may not be involved in the increased ABCG5/G8 expression in PLTP-deficient mice. We are currently investigating the mechanisms involved in the regulation of ABCG5/G8 by PLTP.

This study showed that PLTP deficiency decreases plasma cholesterol and intestinal cholesterol absorption in HFHC diet-induced hyperlipidemic mice. Our results also suggest that PLTP-deficient mice have lower systemic and aortic inflammation. These data imply that PLTP deficiency reduces atherogenic factors and that PLTP appears to be proatherogenic in diet-induced hyperlipidemic mice.

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