Phospholipid Transfer Protein Deficiency Protects Circulating Lipoproteins from Oxidation Due to the Enhanced Accumulation of Vitamin E*

Vitamin E is a lipophilic anti-oxidant that can prevent the oxidative damage of atherogenic lipoproteins. However, human trials with vitamin E have been disappointing, perhaps related to ineffective levels of vitamin E in atherogenic apoB-containing lipoproteins. Phospholipid transfer protein (PLTP) promotes vitamin E removal from atherogenic lipoproteins in vitro, and PLTP deficiency has recently been recognized as an anti-atherogenic state. To determine whether PLTP regulates lipoprotein vitamin E content in vivo, we measured α-tocopherol content and oxidation parameters of lipoproteins from PLTP-deficient mice in wild type, apoE-deficient, low density lipoprotein (LDL) receptor-deficient, or apoB/cholesterol ester transfer protein transgenic backgrounds. In all four backgrounds, the vitamin E content of very low density lipoprotein (VLDL) and/or LDL was significantly increased in PLTP-deficient mice, compared with controls with normal plasma PLTP activity. Moreover, PLTP deficiency produced a dramatic delay in generation of conjugated dienes in oxidized apoB-containing lipoproteins as well as markedly lower titers of plasma IgG autoantibodies to oxidized LDL. The addition of purified PLTP to deficient plasma lowered the vitamin E content of VLDL plus LDL and normalized the generation of conjugated dienes. The data show that PLTP regulates the bioavailability of vitamin E in atherogenic lipoproteins and suggest a novel strategy for achieving more effective concentrations of antioxidants in lipoproteins, independent of dietary supplementation.

The oxidation theory of atherogenesis has received wide support from a number of different lines of evidence (1, 2). In particular, treatment of hypercholesterolemic animals with a variety of potent synthetic anti-oxidants has resulted in inhibition of the progression of atherosclerosis (3). However, a direct relationship between the susceptibility of LDL to oxidation and the extent of atherosclerosis has not been found in all studies, and attempts to prevent atherogenesis by feeding diets enriched in "natural" anti-oxidants have provided mixed and sometimes disappointing results (2, 3). Recently, it was shown that feeding large doses of vitamin E to apoE-deficient mice decreased the progression of atherosclerosis (4, 5). However, with a few exceptions (6, 7), the administration of vitamin E in human trials has been negative (8–12). An important issue that has not been addressed in such studies is the actual concentrations of vitamin E in atherogenic lipoproteins. Recently, mice with α-tocopherol transfer protein deficiency were shown to have reduced vitamin E content in lipoproteins, and moderately increased susceptibility to atherosclerosis (13). However, little is known of the physiological mechanism regulating the turnover and levels of vitamin E in the plasma lipoproteins.

The plasma phospholipid transfer protein (PLTP) mediates both net transfer and exchange of phospholipids between lipoproteins (14). PLTP can also bind and transfer several other amphipathic lipids, including unesterified cholesterol, diacylglycerides, and lipopolysaccharides (15). PLTP has been shown in vitro to facilitate the transfer of vitamin E from VLDL to HDL (16, 17) and from lipoproteins into tissues (16, 17), but it is not known if PLTP regulates vitamin E levels in lipoproteins or tissues in vivo. PLTP knock-out (PLTP0) mice were recently shown to be resistant to atherosclerosis, in part related to decreased secretion and levels of apoB containing lipoproteins (18). The decreased secretion and levels of apoB lipoproteins was demonstrated by crossing the PLTP deficiency trait into apoE-deficient and apoB transgenic backgrounds. However, an anti-atherogenic effect of PLTP deficiency was also seen in LDL receptor knock-out mice, even though plasma levels of apoB lipoproteins were identical to controls. This indicates an additional anti-atherogenic mechanism of PLTP deficiency. In this study we have investigated the hypothesis that PLTP has a

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The abbreviations used are: LDL, low density lipoprotein; AAPH, 2′-azobis(2-amidinopropane)hydrochloride; apo, apolipoprotein; BLp, apoB-containing lipoprotein; CETP, cholesteryl ester transfer protein; CETPTg mouse, CETP-transgenic mouse; apoE0 mouse, apoE-deficient mouse; LDLR0 mouse, LDLR-deficient mouse; apOBtg mouse, apoB-transgenic mouse; Cu-LDL, copper-oxidized LDL; FPLC, fast protein liquid chromatography; HDL, high density lipoprotein; MDA-LDL, malondialdehyde-modified LDL; PLTP, phospholipid transfer protein; PLTP0 mouse, PLTP-deficient mouse; VLDL, very low density lipoprotein.
physiological role in transferring vitamin E between lipoproteins: this hypothesis predicts an increased content of vitamin E in apoB-containing lipoproteins in PLTP-deficient mice, and a decreased susceptibility to oxidation. Such findings would provide a plausible novel anti-atherogenic mechanism related to PLTP deficiency, beyond the effects of lowering Blp levels (18).

MATERIALS AND METHODS

Mice—PLTP knock-out (PLTP0) mice, back-crossed into the C57BL6 background (closed black crosses) were intercrossed with apoE-deficient (apoE0) mice (19–22), LDLR-deficient (LDLR0) mice (23), apoB-transgenic (apoBTg) mice (24, 25), and CETP-transgenic (CETPTg) mice (26), each in the C57BL6 background. ApoE0 mice were fed a Chow diet; LDLR0 and apoBTg/CETPTg mice were fed a Western-type diet containing 20% hydrogenated coconut oil and 0.5% cholesterol. These diets were not supplemented with vitamin E.

Lipid and Protein Measurements—Total cholesterol, phospholipids, and triglycerides were assayed by commercially available enzymatic kits, i.e. CHOD-PAD (Roche Molecular Biochemicals), PAP 150 (BioMérieux), and Triglyceride (Roche Diagnostic Systems-Hoffman-La Roche) kits, respectively. Total lipid was calculated as the sum of cholesterol, phospholipids, and triglycerides. Proteins were measured using a protein solution reagent (Protein Assay Reagent, Pierce) and the absorbance at 280 nm, 2.0–250-μm column (Macherey-Nagel, Düren, Germany) using 0.5 mg ammonium acetate in CH3OH as the eluant at a flow rate of 0.4 ml/min. Positive ion electrospray ionization-mass spectrometry was performed on an MSD 1100 mass spectrometer (Agilent Technology, Waldbronn, Germany). The voltages of the aperture and capillary were set up at 80 and 3500 V, respectively, and a 1.210 mass spectrometer (Agilent Technology, Waldbronn, Germany). The resulting infranatant was fractionated successively by hydrophobic interaction chromatography on a Phenyl-Sepharose CL-4B column (Amersham Biosciences) and by affinity chromatography on an Heparin-agarose column (Amersham Biosciences), yielding ~1000-fold purification of PLTP as compared with the plasma d > 1.21 g/ml fraction (33).

RESULTS

Effect of PLTP Deficiency on the Plasma Distribution and Arterial Content of Vitamin E—In Chow-fed mice in the C57Bl6 background, PLTP deficiency resulted in the redistribution of α-tocopherol among the plasma lipoproteins (Fig. 1). Although plasma levels of α-tocopherol did not differ significantly between PLTP0 and control mice (0.18 ± 0.01 versus 0.24 ± 0.02 mg/liter, respectively, NS), the α-tocopherol content of HDL was significantly reduced, and the α-tocopherol content of LDL was significantly increased in PLTP-deficient animals (Fig. 1, top panel). A 2-fold increase in the α-tocopherol to lipid ratio was observed in LDL from PLTP0 mice compared with wild type controls (Fig. 1, bottom panel), whereas no significant change was observed in the α-tocopherol to lipid ratio in HDL, reflecting the reduced levels of LDL in PLTP0 mice. The level of vitamin E was decreased in aorta, with α-tocopherol to artery weight ratios that were significantly lower in PLTP0 mice than in control mice of both sexes (Fig. 2). These findings show an essential role of PLTP in determining vitamin E levels in lipoproteins and vascular tissues. They are consistent with the hypothesis that PLTP transfers vitamin E from apoB-containing lipoproteins (BLPs) into HDL, and from lipoproteins into tissues (16, 17).

Effects of PLTP Deficiency on Plasma Levels and Distribution of Vitamin E in Hyperlipidemic Mice—To determine if accumulation of vitamin E in BLPS might contribute to the athero-protective effect of PLTP deficiency (18), we further investigated the effects of the PLTP deficiency trait on plasma α-tocopherol levels and lipoprotein distribution in hyperlipidemic plasmas of LDLR0, apoE0, and apoB/CETPTg backgrounds. Total plasma α-tocopherol levels were significantly higher in LDLR0/PLTP0 mice compared with LDLR0 mice (6.7 ± 0.9 versus 4.7 ± 0.7 mg/liter, respectively, p < 0.005). Moreover, lipoprotein analysis showed that concentrations of α-tocopherol were significantly increased in both VLDL and LDL but unchanged in HDL (Fig. 3). The increase in vitamin E in VLDL and LDL was demonstrated both as a plasma concentration and as a ratio to total lipids. Total α-tocopherol was dramatically increased in plasma of apoE0/PLTP0 mice compared with apoE0 mice (2.5 ± 0.4 versus 0.9 ± 0.2 mg/liter, respectively, p < 0.05), and there was a 5-fold increase in the per-oxidized bound antioxidants were then detected with either anti-mouse IgG or anti-mouse IgM antibodies coupled to alkaline phosphatase. Bound antibodies were finally detected in the presence of a chemiluminescent substrate, and results were expressed in relative light units per 100 ms (32).

Preparation of Human Plasma Phospholipid Transfer Protein—PLTP was isolated from fresh human plasma. All purifications steps were performed on an FPLC system (Amersham Biosciences) according to the sequential procedure previously described (33). Briefly, the d > 1.21 g/ml plasma fraction was isolated by a 48-h, 45,000 rpm ultracentrifugation step performed in a 50-Ti rotor. The resulting infranatant was fractionated successively by hydrophobic interaction chromatography on a Phenyl-Sepharose CL-4B column (Amersham Biosciences) and by affinity chromatography on an Heparin-agarose column (Amersham Biosciences), yielding ~1000-fold purification of PLTP as compared with the plasma d > 1.21 g/ml fraction (33).

Conjugated Diene Formation—LDL from LDLR0/PLTP0, LDLR0, apoBTg/CETPTg/PLTP0, and apoBTg/CETPTg mice were isolated by a two-step procedure: the Ultracentrifuge-isolated 1.006 < d < 1.063 fraction was passed through a Superose 6 column on an FPLC system (31), and 1-ml fractions containing only LDL were pooled. In the case of apoE0/PLTP0 and apoE0 mice, we used total apoB-containing particles that were Ultracentrifuge-isolated from total plasma as the d < 1.063 fraction. Isolated lipoproteins were oxidized at 37 °C in the presence of either copper sulfate (5 μM) or 2-azobis(2-aminodipropionyl)-hydrochloride (AAHP, Wako Pure Chemical Industries), and the formation of conjugated dienes was monitored at 234 nm over a 20-h period. Measurement of Anti-oxidized LDL Autoantibodies—Autoantibodies to epitopes of oxidized LDL were measured as described previously (32). Diluted plasma samples were added to microtiter wells coated with either malondialdehyde-modified LDL (MDA-LDL) or copper-oxidized LDL. Bound antibodies were then detected with either anti-mouse IgG or anti-mouse IgM antibodies coupled to alkaline phosphatase. Bound antibodies were finally detected in the presence of a chemiluminescent substrate, and results were expressed in relative light units per 100 ms (32).
Data (mean ± S.E.) are expressed in absolute α-tocopherol concentrations (top panel) or α-tocopherol to lipid ratio (bottom panel). Statistics were obtained by Mann-Whitney test.

3). This result suggests that CETP, although related to PLTP, does not substitute in transferring vitamin E out of apoB-containing lipoproteins (34). These studies show a major role of PLTP in determining the concentration of vitamin E in atherogenic lipoproteins.

Effect of PLTP Deficiency on Conjugated Diene Generation in Copper-oxidized ApoB-containing Lipoproteins—To establish whether the increased vitamin E content of atherogenic lipoproteins from PLTP-deficient animals rendered them less susceptible to oxidation, we isolated the VLDL plus LDL fraction and measured the generation of conjugated dienes in the presence of either copper sulfate (Fig. 4, a, c, and d) or AAPH (Fig. 5). The formation of conjugated dienes, monitored at 234 nm over a 20-h period, was remarkably delayed by PLTP deficiency in all genetic backgrounds (Fig. 4), and similar observations were made when lipoproteins were oxidized with either copper or AAPH (Fig. 4, b and d). The lag phase of conjugated diene formation in LDL particles was 45 + 15 min versus 150 + 30 min (LDLR0 and LDLR0/PLTP0 mice, respectively), 30 + 15 min versus 75 + 15 min (apoBTg/CETPTg and apoBTg/CETPTg/PLTP0 mice), and 45 + 15 min versus 180 + 30 min (VLDL±LDL particles from apoE0 and apoE0/PLTP0 mice). The differences were all highly significant (p < 0.001).

Effect of Exogenous, Purified PLTP on the Vitamin E Content and Oxidizability of ApoB-containing Lipoproteins from PLTP-deficient Mice—To confirm a direct role of PLTP in determining the distribution of α-tocopherol and the oxidizability of apoB-containing lipoproteins, plasma from LDLR0 or LDLR0/PLTP0 mice was incubated for 2 h at 37 °C in the presence or absence of purified exogenous PLTP. As observed previously, the oxidation susceptibility of LDL isolated from LDLR0/PLTP0 mice was markedly reduced compared with LDL from LDLR0 mice (Fig. 5). This difference was reversed by the addition of PLTP to the LDLR0/PLTP0 plasma. In parallel, the α-tocopherol content of the VLDL±LDL fraction from LDLR0/PLTP0 plasma was significantly reduced in the presence of PLTP, to levels similar to those observed in plasma from LDLR0 mice expressing normal levels of PLTP (Table I). Lag phase and α-tocopherol values did not vary significantly when LDLR0 samples were supplemented with PLTP, indicating that α-tocopherol was already equilibrated among the lipoproteins in mice expressing PLTP. The reversal of the abnormalities of vitamin E content and oxidizability of apoB-containing lipoproteins when PLTP-deficient plasmas were supplemented with purified PLTP proves that the observed effects (Figs. 3 and 4) are a direct consequence of PLTP action in plasma.

Effects of PLTP Deficiency on Circulating Levels of Anti-oxidized LDL Autoantibodies—Autoantibodies to epitopes of oxidized LDL are known to progressively rise over time in cholesterol-fed LDLR0 mice (35, 36), their titer correlates with the extent of atherosclerosis (32, 36, 37), and the baseline titer of autoantibodies to malondialdehyde-modified LDL (MDA-LDL), a model of oxidized LDL, is a predictive marker of atherosclerosis (35, 38). To determine if the increased vitamin E content of apoB-containing lipoproteins in PLTP-deficient animals might be associated with decreased oxidation of LDL in vivo, we measured the titer of IgG and IgM autoantibodies against MDA-LDL and copper-oxidized LDL (Cu-LDL). In each of the three hyperlipidemic backgrounds, PLTP deficiency was accompanied by a significant reduction (50–81%) in the titer of IgG autoantibodies, using either MDA-LDL or Cu-LDL as model epitopes of oxidized LDL (Fig. 6). Such a drop in the autoantibody titer in PLTP0 animals was not systematically observed with the IgM isotype. Indeed, although the IgM titer was significantly reduced in apoE0/PLTP0 mice, titers were increased or unchanged in LDLR0 and apoBTg/CETPTg backgrounds (Fig. 6).

**DISCUSSION**

We have previously shown that PLTP deficiency provides protection against atherosclerosis in apoE0 and apoBTg/CETPTg mice, due in part to decreased hepatic production of apoB and decreased plasma levels of atherogenic lipoproteins (18). However, PLTP deficiency also conferred protection in LDLR0 mice even though apoB levels were not decreased, suggesting that PLTP deficiency had other anti-atherogenic properties. The present study demonstrates a novel in vivo role
of PLTP in determining the concentration of vitamin E in BLps and suggests that increased vitamin E in BLps represents an additional mechanism by which PLTP deficiency protects against atherosclerosis in mice. PLTP deficiency led to an increased concentration of vitamin E in VLDL and/or LDL, and the magnitude of the effect on the vitamin E to lipid ratio varied from an increase of 70% in LDLR0 mice to 500% in apoE0 mice. In normolipidemic mice there was also a decrease in vitamin E content in HDL and aorta. These results are consistent with a physiological role of PLTP in transferring vitamin E from VLDL to HDL and then into tissues. The accumulation of vitamin E in BLps in PLTP-deficient mice was the most consistent and dramatic effect, and it was associated with a marked reduction in susceptibility of these particles to oxidative modification. This provides a cogent explanation for the previously observed anti-atherogenic effect of PLTP deficiency in LDL receptor KO mice, in which there was no change in BLp levels. Although the magnitude of the effect of vitamin
FIG. 5. Effect of the supplementation of total plasma with purified PLTP on the vitamin E content of LDL. Pooled plasmas (200 µl) were incubated for 2 h at 37 °C in the absence or in the presence of purified PLTP (10 µg of a partially purified, 2.5 µg/ml PLTP preparation). The LDL-containing fraction from each of them was isolated, and the formation of conjugated dienes was monitored at 234 nm over a 20-h period. Each value corresponds to mean ± S.D. of three to four determinations, each of them conducted with pooled plasmas from three to four animals.

**TABLE I**

| Mice          | Treatment  | α-Tocopherol (µg/ml) | α-Tocopherol (µg/ml) |
|---------------|------------|----------------------|----------------------|
| LDLR0         | No addition | 5.92 ± 0.10          | 5.92 ± 0.10          |
| LDLR0/PLTP0   | No addition | 6.48 ± 0.11          | 6.48 ± 0.11          |

*Significantly different from LDLR0, no addition, LDLR0, + PLTP, and LDLR0/PLTP0, + PLTP, p < 0.01 in all cases by analysis of variance.

E accumulation on atherosclerosis appears to be only moderate (5, 6), our findings illustrate the important principle that the concentration of anti-oxidants in the relevant BLps is determined by factors acting beyond the dietary intake. In addition to the reduced secretion of BLps, they identify an additional anti-atherogenic mechanism that can be anticipated from PLTP inhibition and further support the idea that PLTP inhibitors or combined PLTP/CETP inhibitors may have a role as anti-atherogenic drugs (18, 39).

The role of different genes in the absorption, transport, and tissue uptake of dietary α-tocopherol (the ingested form of vitamin E with the greatest biological activity) has been elucidated by various genetic deficiency states. Thus, intestinal BLp assembly is essential for vitamin E absorption, as patients with abetalipoproteinemia become deficient in vitamin E (40, 41). Following delivery of vitamin E in chylomicrons to the liver, vitamin E is incorporated into VLDL for hepatic secretion; the key role of α-tocopherol transfer protein in this process is illustrated by human and murine genetic deficiency states (40–49). Based on in vitro studies, PLTP had been proposed to mediate the transfer of vitamin E from VLDL into HDL and from lipoproteins into tissues. The present study in PLTP-deficient mice provides direct evidence for an essential role of PLTP in these processes in vivo, and the most consistent finding in PLTP deficiency was the significant increase in the vitamin E content of VLDL and/or LDL. The plasma α-tocopherol transfer activity clearly reflects a specific property of PLTP. The involvement of the related plasma cholesteryl ester transfer protein (CETP) in this process was ruled out by demonstrating a similar vitamin E enrichment of BLps in apoB/CETP-Tg mice with PLTP deficiency. Reconstitution of PLTP in PLTP-deficient plasma indicated that the major effects of PLTP on vitamin E distribution in lipoproteins likely reflect a direct action in plasma. However, the incorporation of vitamin E into HDL and tissues still occurred in the absence of PLTP, indicating additional mechanisms of vitamin E transport. In this regard, both lipoprotein lipase (44) and the cellular LDL receptor (45) were shown to contribute to the uptake of α-tocopherol by peripheral cells. In addition, the recent demonstration that ABCA1 can promote efflux of vitamin E from cells (46) suggests that hepatic ABCA1 could represent an alternative pathway for incorporation of vitamin E into HDL in the liver.

Recent studies have shown that PLTP deficiency reduces atherosclerosis in all three of the commonly used, atherosclerosis-susceptible hyperlipidemic mouse models (18). In part this was related to reduced secretion and levels of apoB-containing lipoproteins, but this could not explain reduced atherosclerosis in LDLR0/PLTP0 mice, where VLDL and LDL levels were unchanged. In the light of recent studies (18), high levels of vitamin E in apoB-containing lipoproteins from PLTP-deficient animals are a plausible mechanism to explain decreased atherosclerosis in LDLR0/PLTP0 mice, and increased vitamin E levels in BLps would likely contribute to decreased atherosclerosis in other mouse models. This is especially likely in the apoE0/PLTP0 mice, where a profound reduction in atherosclerosis was observed (18) and vitamin E content in VLDL was increased 5-fold (Fig. 3). Interestingly, quantitatively similar increases in the vitamin E content of apoB-containing lipoproteins and moderate decreases in atherosclerosis susceptibility were reported in apoE0 mice fed vitamin E-supplemented diets (4, 5). In these studies, changes in atherosclerosis were of similar magnitude to the apoB-independent, atheroprotective effects of PLTP deficiency (18). Although the relative decrease in atherosclerosis in LDLR0/PLTP0 mice was statistically significant only at the earlier time point (18), similar temporal effects of transgene expression have been repeatedly observed in mouse atherosclerosis studies, and differences in atherosclerosis in mice deficient in lymphocytes (47), MCP-1 receptor (48), or overexpressing apoA-I (49) were much more marked at earlier than later time points. One interpretation is that vitamin E is more important for the rate of lesion initiation, than for the rate of lesion progression.

Consistent with the evidence that the increased content of vitamin E helped to retard atherosclerosis through reduction in the generation of oxidized LDL, we noted a decrease in IgG autoantibody titers to both MDA-LDL and Cu-LDL. A close correlation between such antibody titers and both the extent of lesion formation and the level of oxidized LDL has been previously observed (32, 50, 51). The changes in titers of IgM autoantibodies were more variable, presumably reflecting the
admixture of both T cell-dependent as well as non-T cell-dependent "natural" antibodies (52).

The oxidation theory of atherogenesis had its origins in an attempt to understand the mechanisms by which LDL could promote macrophage foam cell formation, because native LDL is not taken up in sufficient amounts to make foam cells (1). Oxidative modification of LDL facilitates its uptake into macrophages by scavenger receptors, such as SR-A and CD36 (53, 54), and facilitates aggregation and uptake by additional pathways (55). The existence of oxidized epitopes in atherosclerotic lesions, as well as studies with CD36 and SRA knock-out mice, have generally supported an important role of oxidative modification in atherogenesis.

Most animal studies with potent lipophilic anti-oxidants, such as probucol, have consistently shown a protective effect of these agents against atherosclerosis (reviewed in Ref. 3). However, the results of intervention studies with less potent vitamin anti-oxidants, such as vitamin E, have provided mixed results. In one study of vitamin E-fed apoE KO mice, the mice were dramatically protected from lesions formation; the plasma levels of vitamin E correlated inversely with the extent of atherosclerosis and with the urinary excretion, plasma, and arterial levels of F2 isoprostanes that are decomposition products of lipid peroxidation (4). In contrast, most of the human studies, which have used lower doses of anti-oxidants, have been negative (3). Although there have been two small trials suggesting a benefit of vitamin E administration (6, 7), there are five trials using vitamin E that have been negative (8–12). A potential shortcoming of the human studies is that doses of vitamin E may have been too low to be effective, and equally important, that susceptible individuals may not have been studied. For example, Meagher et al. (56) have recently shown that even high doses of vitamin E did not reduce isoprostane levels in healthy subjects. In contrast, vitamin E supplemen-

![Fig. 6. Effect of PLTP deficiency on the circulating levels of anti-oxidized LDL autoantibodies. IgG (left panels) and IgM (right panels) levels of anti-oxidized LDL autoantibodies were determined by using malondialdehyde-modified LDL (upper panels) or copper-oxidized LDL (lower panels) as models of oxidized LDL. Data are mean ± S.E. of n = 4 animals. *, p < 0.05 versus PLTP+/+; Mann-Whitney test.]
tation to subjects undergoing hemodialysis, a condition known to be associated with enhanced oxidative stress, was associated with a 50–70% reduction in cardiovascular events (7). These observations emphasize the lack of knowledge of determinants of the bioavailability of anti-oxidants in relevant sites such as the apolipoprotein B-containing lipoproteins. The present study indicates that PLTP represents one such factor determining vitamin E concentration in BLPs. It is interesting to note that PLTP deficiency was athero-protective, even though vitamin E contents were moderately reduced in aorta of PLTP$0^{-}$ mice. This finding indicates a major role of anti-oxidant concentration in BLPs in determining atherosclerosis. Our data suggest that PLTP could play a role in the discordance observed between the susceptibility of LDL to oxidation \textit{ex vivo} and the dietary intake of vitamin E (57). For instance, no significant relationship was noted between the dietary intake and plasma concentration of vitamin E in type 2 diabetics (58), a population with increased PLTP levels (59, 60), decreased vitamin E content of atherogenic apoB lipoproteins and a resultant decrease in their susceptibility to oxidative modification. If PLTP plays a similar important role in humans, then PLTP inhibition may be a novel strategy to decrease atherosclerosis.

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