



HAL
open science

Cholesterol Accumulation Is Increased in Macrophages of Phospholipid Transfer Protein-Deficient Mice

Nicolas Ogier, Alexis Klein, Valérie Deckert, Anne Athias, Ginette Bessède, Naig Le Guern, Laurent Lagrost, Catherine M Desrumaux

► **To cite this version:**

Nicolas Ogier, Alexis Klein, Valérie Deckert, Anne Athias, Ginette Bessède, et al.. Cholesterol Accumulation Is Increased in Macrophages of Phospholipid Transfer Protein-Deficient Mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 2007, 27, pp.2407 - 2412. 10.1161/atvbaha.107.151753 . hal-04200513

HAL Id: hal-04200513

<https://hal.science/hal-04200513>

Submitted on 8 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Cholesterol Accumulation Is Increased in Macrophages of Phospholipid Transfer Protein-Deficient Mice

Normalization by Dietary Alpha-Tocopherol Supplementation

Nicolas Ogier, Alexis Klein, Valérie Deckert, Anne Athias, Ginette Bessède, Naig Le Guern, Laurent Lagrost, Catherine Desrumaux

Objective—Phospholipid transfer protein (PLTP) is a multifunctional, extracellular lipid transport protein that plays a major role in lipoprotein metabolism and atherosclerosis. Recent *in vivo* studies suggested that unlike systemic PLTP, macrophage-derived PLTP would be antiatherogenic. The present study aimed at characterizing the atheroprotective properties of macrophage-derived PLTP.

Methods and Results—Peritoneal macrophages were isolated from PLTP-deficient and wild-type mice and their biochemical characteristics were compared. It is shown that macrophages isolated from PLTP-deficient mice have increased basal cholesterol content and accumulate more cholesterol in the presence of LDL compared with wild-type cells. Cholesterol parameters in macrophages of PLTP-deficient mice were normalized by dietary α -tocopherol supplementation.

Conclusions—The antiatherogenic properties of macrophage-derived PLTP are related at least in part to its ability to reduce cholesterol accumulation in macrophages through changes in the α -tocopherol content and oxidative status of the cells. (*Arterioscler Thromb Vasc Biol.* 2007;27:2407-2412.)

Key Words: phospholipid transfer protein ■ vitamin E ■ tocopherol ■ macrophage

Phospholipid transfer protein (PLTP) is a multifunctional, extracellular lipid transport protein that plays a major role in lipoprotein metabolism and atherosclerosis.¹⁻⁴ In addition to phospholipids, PLTP transfers several amphipatic compounds including unesterified cholesterol, lipopolysaccharides, diacylglycerides, and α -tocopherol, the main isomer of vitamin E.⁵⁻⁷ PLTP is produced by a variety of tissues and secreted by macrophages, the hallmark cells of atherosclerotic lesions.⁸⁻¹¹ Several recent studies indicated that PLTP not only affects plasma lipid and lipoprotein profiles, but also modulates cell physiology.¹²⁻¹⁶ To date, the question of the pro- or antiatherogenicity of PLTP has not been fully elucidated. On the one hand, recent *in vivo* studies indicated that systemic PLTP deficiency is atheroprotective in different strains of hypercholesterolemic mice, and sustained overexpression of plasma PLTP in PLTP transgenic or bone marrow-transplanted mice leads to an increased risk of atherosclerosis.¹⁷⁻²¹ In further support of the proatherogenic potential of plasma PLTP *in vivo*, a positive correlation between PLTP activity and the risk of coronary artery disease was observed in humans.²² On the other hand, recent observations from our group²³ as well as from Liu et al²⁴ indicated that the onset of atherosclerosis is significantly delayed in low density lipoprotein-receptor-deficient and apoE-

deficient mice that were reconstituted with PLTP-expressing compared with PLTP-deficient (PLTP^{-/-}) bone marrow cells. In these experiments, PLTP expression was mostly restricted to macrophages with only minimal modification of plasma PLTP level, suggesting that unlike systemic PLTP, macrophage-derived PLTP would be atheroprotective *in vivo*. In other words, the consequences of PLTP on atherosclerosis in experimental models would result from a combination of antiatherogenic effect of macrophage PLTP and proatherogenic effect of systemic plasma PLTP. To assess selectively the impact of macrophage-derived PLTP on cell properties or cholesterol accumulation, peritoneal macrophages were isolated from PLTP-deficient and wild-type (WT) mice, and their biochemical characteristics were compared in the present study. It is shown that macrophages isolated from PLTP-deficient mice have increased basal cholesterol content and accumulate more cholesterol in the presence of LDL compared with WT cells. Dietary α -tocopherol supplementation to PLTP-deficient mice allowed us to demonstrate that the proatherogenic potential of macrophages isolated from PLTP-deficient mice is related at least in part to reduced α -tocopherol content and increased oxidative stress in these cells. Together with observations made in PLTP-deficient and PLTP-transgenic mice, the present findings suggest that the

Original received April 20, 2007; final version accepted August 1, 2007.

From INSERM U866, Centre de Recherche INSERM, IFR Santé-STIC, Faculté de Médecine, Université de Bourgogne, Dijon, France.

Correspondence to Dr Catherine Desrumaux/Dr Laurent Lagrost, INSERM U866 - Faculté de Médecine, 7, Bd Jeanne d'Arc BP 87900, 21079 DIJON Cedex, France. E-mail Catherine.Desrumaux@u-bourgogne.fr or Laurent.Lagrost@u-bourgogne.fr

© 2007 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.107.151753

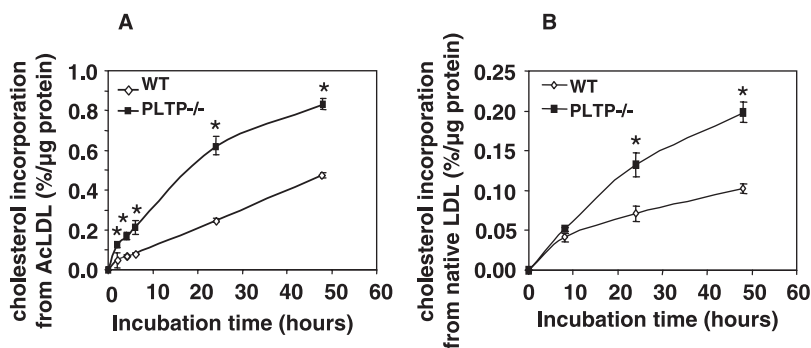


Figure 1. Cholesterol accumulation in WT and PLTP-deficient macrophages. Peritoneal macrophages were incubated for 2 hours, 4 hours, 6 hours, 24 hours, or 48 hours with [³H]CE-AcLDL (A) or freshly isolated, native [³H]CE-LDL (B; 80 μg protein/mL). Cholesterol accumulation was expressed as the percentage of radioactivity recovered in cells/μg cell protein. Values are mean ± SEM of n=6 WT and n=6 PLTP-deficient mice (A) or n=4 WT and n=4 PLTP-deficient mice (B). **P*<0.005 vs WT (Mann-Whitney *U* test).

pro- or antiatherogenicity of PLTP depends on its site of expression.

Materials and Methods

Mice

All mice were on an homogeneous C57Bl/6 background. PLTP-deficient mice were a kind gift from Drs X.-C. Jiang and A.R. Tall (Columbia University, New York). The mice were matched for sex and age and fed either a standard chow diet or an α -tocopherol-supplemented (800 mg/kg) chow diet (2 months) before harvest. Blood samples were collected from fasted animals by retro-orbital puncture using heparin-coated capillary tubes. All procedures were performed in accordance with institutional guidelines.

Statistical Analysis

Results are expressed as mean ± SEM. The statistical significance of differences between data means was determined with the nonparametric Mann-Whitney *U* test.

Supplemental Materials and Methods (collection of elicited peritoneal macrophage, culture conditions and phenotypic characterization) are available online at <http://atvb.ahajournals.org>.

Results

Cholesterol Accumulation in Macrophages Isolated From PLTP-Deficient and WT Mice

Cholesterol accumulation was measured in macrophages isolated from PLTP-deficient and WT mice after 2-hour, 4-hour, 6-hour, 24-hour, or 48-hour incubation in the presence of radiolabeled AcLDL. As shown in Figure 1A, both short- and long-term exposure to AcLDL resulted in significantly higher cholesterol accumulation in macrophages from PLTP-deficient mice compared with those from WT mice. When freshly isolated, native LDL were used instead of acetylated LDL to promote cholesterol accumulation, again a significantly higher accumulation of cholesterol was observed in macrophages from PLTP-deficient mice as compared with WT cells (Figure 1B).

Scavenger Receptor Expression in Macrophages Isolated From PLTP-Deficient and WT Mice

SR-A and CD36 mRNA quantification indicated no difference in the basal expression of the 2 receptors in PLTP-deficient and WT cells (CD36 Δ CP: 3.75±2.05 and 3.05±1.96 for PLTP-deficient and WT cells (n=5), respectively, n.s.; SR-A Δ CP: 4.45±2.77 and 4.35±2.55 for PLTP-deficient and WT cells (n=5), respectively, n.s.). When cells were incubated in the presence of AcLDL, again no difference in the expression level of the 2 receptors between

PLTP-deficient and WT cells was observed (results not shown).

Cholesterol Efflux in Macrophages Isolated From PLTP-Deficient and WT Mice

Several in vitro studies led to demonstrate that PLTP can promote cholesterol efflux from macrophages both through the generation of pre β -HDL cholesterol acceptors and through the stabilization of ABCA1 and enhancement of cellular cholesterol efflux. To assess whether under our experimental conditions cholesterol efflux is impaired by lack of PLTP, cholesterol-loaded peritoneal macrophages were incubated in the presence of lipid-free apoAI or acetylated LDL as cholesterol acceptors. As shown in supplemental Figure I, we observed no difference in cholesterol efflux from WT and PLTP-deficient elicited peritoneal macrophages toward lipid-free apoAI or acetylated LDL.

Effect of Dietary α -Tocopherol Supplementation on α -Tocopherol Content in Plasma, Erythrocytes, and Macrophages of PLTP-Deficient Mice

When PLTP-deficient mice were fed an α -tocopherol-enriched diet for 2 months, a significant increase in the α -tocopherol content of plasma, red blood cells, as well as of peritoneal macrophages was observed (plasma (mean ± SEM, n=5): 6.51±2.28 mg/L in supplemented versus 0.87±0.28 mg/L in nonsupplemented PLTP-deficient mice, *P*<0.0001; red blood cells (mean ± SEM, n=7): 1165.23±102.52 ng/mL in supplemented versus 582.36±113.88 ng/mL in nonsupplemented mice, *P*<0.05; macrophages (mean ± SEM, n=5): 28.17±4.90 ng/10⁶ cells in supplemented versus 9.19±1.26 ng/10⁶ in non-supplemented mice, *P*<0.02).

Effect of Dietary α -Tocopherol Supplementation on Oxidative Stress in Macrophages

Previous results from our group indicated that oxidative stress, as measured by DCFH-DA oxidation rate, is significantly increased in macrophages isolated from PLTP-deficient mice compared with WT cells²³ (Figure 2A).

In the present study, we moreover observed a significant, 2-fold increase in the oxysterol content of macrophages isolated from PLTP-deficient mice compared with WT cells (Figure 2B).

After dietary α -tocopherol supplementation, oxidative stress (as measured by DCFH-DA oxidation rate) and cellular oxysterols were significantly reduced in macrophages iso-

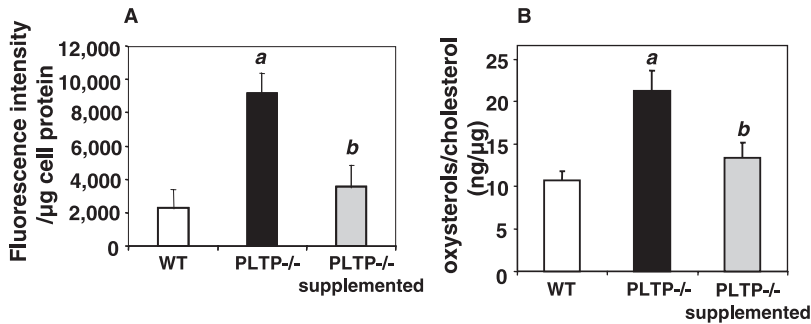


Figure 2. Effect of dietary α -tocopherol supplementation on oxidative stress in peritoneal macrophages from PLTP-deficient mice. A, Intracellular hydroperoxides were quantified by fluorimetry using DCFH-DA. Values are expressed as fluorescence intensity/ μg cell protein. B, Levels of intracellular oxysterols were determined by GC-MS. Values are expressed as ng/ μg cholesterol. All values are mean \pm SEM of $n=13$ WT, $n=11$ PLTP-deficient, and $n=7$ α -tocopherol-supplemented/PLTP-deficient mice. *a*, $P<0.05$ vs WT; *b*, $P<0.05$ vs PLTP^{-/-} (Mann-Whitney *U* test).

lated from PLTP-deficient mice, reaching values found in WT macrophages (Figure 2A and 2B).

Effect of Dietary α -Tocopherol Supplementation on LDL Oxidation by Macrophages

The ability of macrophages from PLTP-deficient mice to oxidize LDL is significantly higher than that of WT cells.²³ As shown in Figure 3, dietary α -tocopherol supplementation led to a significant decrease in LDL oxidation by macrophages from PLTP-deficient mice, with values returning to those observed in WT cells.

Effect of Dietary α -Tocopherol Supplementation on Cholesterol Accumulation in Macrophages

As shown in Figure 4, dietary α -tocopherol supplementation led to a significant decrease in cholesterol accumulation in macrophages from PLTP-deficient mice. After 24-hour incubation with labeled AcLDL, cholesterol accumulation in the supplemented group was less than half that in the nonsupplemented group (% influx/ μg cell protein [mean \pm SEM, $n=7$]: 0.212 ± 0.021 versus 0.462 ± 0.070 %/ μg cell protein in supplemented and nonsupplemented groups, respectively; $P<0.005$) and was not significantly different from that observed with WT macrophages (0.163 ± 0.028 %/ μg cell protein).

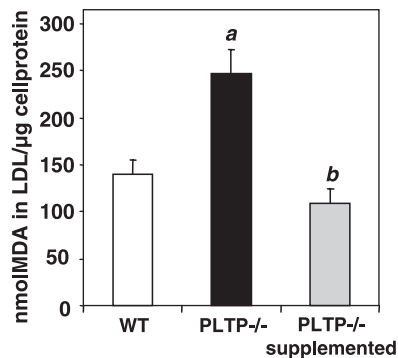


Figure 3. Effect of dietary α -tocopherol supplementation on LDL oxidation by macrophages from PLTP-deficient mice. LDL oxidation was determined by measurement of TBARS. Results are expressed as nanomoles of malondialdehyde in LDL per microgram of cell protein and are mean \pm SEM of $n=13$ WT, $n=11$ PLTP-deficient, and $n=7$ α -tocopherol-supplemented/PLTP-deficient mice. *a*, $P<0.05$ vs WT; *b*, $P<0.005$ vs PLTP^{-/-} (Mann-Whitney *U* test).

Effect of PLTP Deficiency and Dietary α -Tocopherol Supplementation on Cholesterol Content in Macrophages

Additional experiments were conducted to determine the impact of PLTP deficiency and α -tocopherol supplementation on cholesterol content in macrophages. As shown in Figure 5A, cholesterol levels in PLTP-deficient macrophages were about twice that of WT macrophages under basal conditions (total cholesterol [mean \pm SEM, $n=6$]: 21.0 ± 1.3 and 11.8 ± 0.7 ng/ μg protein in PLTP-deficient and WT cells, respectively; $P<0.02$). Esterified cholesterol, but not free cholesterol content, was significantly higher in PLTP-deficient compared with WT cells (Figure 5A). Cholesterol levels were markedly increased in both PLTP-deficient and WT cells on incubation with AcLDL. In accordance with observations made with the radioisotopic assay (Figure 1), we observed a much greater cholesterol (mainly esterified cholesterol) enrichment in PLTP-deficient compared with WT cells on incubation with AcLDL (cholesterol mass increment versus basal values [mean \pm SEM, $n=6$]: 40.1 ± 12.8 ng/ μg versus 17.3 ± 2.9 ng/ μg protein, respectively; $P<0.02$; Figure 5B and 5C). Dietary α -tocopherol supplementation led to a significant decrease in basal cholesterol content in PLTP-deficient macrophages, with values returning to those measured in WT cells (Figure 5A). In accordance with observations made with the radioisotopic assay (Figure 4), cholesterol enrichment was significantly reduced in the α -tocopherol-supplemented group compared with the nonsupple-

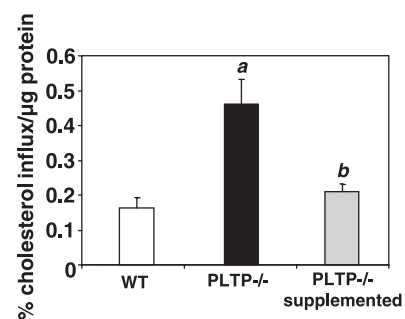


Figure 4. Effect of dietary α -tocopherol supplementation on cholesterol accumulation in macrophages from PLTP-deficient mice. Peritoneal macrophages were incubated for 24 hours with $80 \mu\text{g}$ protein/mL of [^3H]CE-AcLDL. Cholesterol influx was expressed as the percentage of radioactivity recovered in cells/ μg cell protein. Values are the mean \pm SEM of $n=7$ mice in each group. *a*, $P<0.005$ vs WT; *b*, $P<0.05$ vs PLTP^{-/-} (Mann-Whitney *U* test).

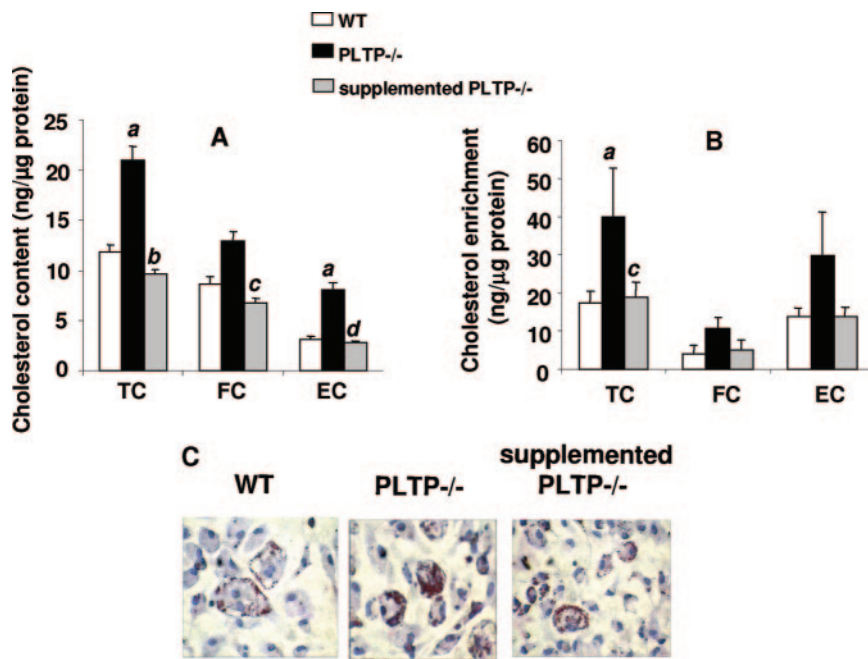


Figure 5. Cholesterol content of macrophages isolated from wild-type, PLTP-deficient, and α -tocopherol-supplemented/PLTP-deficient mice. **A**, Basal cholesterol content of freshly isolated peritoneal macrophages. **B**, Cholesterol enrichment (increment in cholesterol content) of macrophages after a 24-hour incubation with 80 μ g/mL AcLDL. Results are expressed as ng cholesterol/ μ g protein and are the mean \pm SEM of $n=6$ mice in each group. **C**, Oil Red O staining of mouse macrophages after incubation with AcLDL, showing increased lipid content in macrophages from PLTP-deficient mice compared with WT cells, and normalization after dietary α -tocopherol supplementation. TC indicates total cholesterol; FC, free cholesterol; EC, esterified cholesterol. *a*, $P<0.02$ vs WT; *b*, $P<0.005$ vs PLTP^{-/-}; *c*, $P<0.05$ vs PLTP^{-/-}; *d*, $P<0.02$ vs PLTP^{-/-} (Mann-Whitney *U* test).

mented group after 24-hour incubation with AcLDL, and was not significantly different from values measured in WT cells (cholesterol mass increment versus basal values [mean \pm SEM, $n=6$]: 18.9 \pm 4.1 ng/ μ g protein in supplemented versus 40.1 \pm 12.8 ng/ μ g in non-supplemented PLTP-deficient mice, $P<0.05$; Figure 5B and 5C).

Discussion

Although PLTP was initially identified as a phospholipid transfer protein that can modify high density lipoprotein (HDL) structure and composition, its biological function recently appeared to be much more complex. Over the past few years, several studies have demonstrated that PLTP is expressed in a variety of tissues, and that beyond its role in the intravascular compartment, PLTP can affect the function of various organs, including liver,¹³ brain,¹⁴ and testis.¹⁵ Recent *in vivo* studies indicated that systemic PLTP deficiency is atheroprotective in different strains of hypercholesterolemic mice, and sustained overexpression of plasma PLTP in PLTP transgenic or bone marrow transplanted mice leads to an increased risk of atherosclerosis.^{17–21} PLTP is highly expressed in macrophages, ie, essential cellular actors in atherogenesis, and it is present in the intima of atherosclerotic arteries.^{9–11} Recent observations from our group as well as from Liu et al indicated that the onset of atherosclerosis is significantly delayed in irradiated LDL-receptor deficient and apoE-deficient mice that were reconstituted with PLTP-expressing compared with PLTP-deficient bone marrow-derived cells.^{23,24} In these studies, alteration in PLTP expression level was mostly restricted to macrophages, with only minimal changes in systemic PLTP level. In contrast, Vikstedt et al reported a marked increase in plasma PLTP level together with increased atherosclerosis in irradiated LDL-receptor deficient mice transplanted with PLTP-expressing as opposed to PLTP-deficient bone marrow.²¹ Taken together, these observations suggested that the pro- or antiatherogenic-

ity of PLTP may actually depend on its site of expression. To test this hypothesis, the impact of macrophage-derived PLTP on cell properties and cholesterol accumulation was addressed in the present study in a plasma PLTP-free context. It is shown that endogenous PLTP deficiency leads to increased cholesterol content of macrophages, and cholesterol enrichment in the presence of modified LDL is markedly increased in cells from PLTP-deficient compared with WT mice. To assess further the impact of PLTP deficiency on cholesterol accumulation in macrophages, cholesterol uptake by peritoneal macrophages from wild-type and PLTP-deficient mice was subsequently measured in the presence of freshly isolated, native LDL. Again, and as observed earlier with acetylated LDL, a markedly higher cholesterol accumulation was observed in peritoneal macrophages from PLTP-deficient mice as compared with wild-type cells after long-term incubation with LDL particles. Because only native LDL were used in these experiments, their nonregulated uptake through scavenger receptors must have required their oxidation by macrophages in the incubation mixtures. We previously demonstrated that this key event indeed takes place in the experimental medium with a stronger oxidative potential of PLTP-deficient macrophages as compared with wild-type macrophages.²³ Thus, PLTP arises as a previously unrecognized factor that can modulate cholesterol accumulation in macrophages, a crucial step in the initiation of atherosclerosis, and increased LDL oxidation may contribute to increased cholesterol accumulation in PLTP-deficient macrophages.

The role of PLTP in α -tocopherol metabolism *in vivo* has been the subject of intense research over the past few years, and high α -tocopherol requirements were proven to be dependent in part on PLTP in several tissues,^{7,13–16,19,25} including macrophages.²³ α -tocopherol is the principal and most potent lipid soluble antioxidant in plasma and LDL. Beyond its antioxidant properties, α -tocopherol also displays

nonantioxidant properties that may affect the biological functions of various cell types including monocyte-macrophages.^{26,27} In particular, several studies have addressed the effect of α -tocopherol on foam cell formation. Although some studies showed no effect of α -tocopherol on the accumulation of cholesteryl ester in macrophages,^{28–30} most of these studies demonstrated beneficial effects with regard to foam cell formation. For instance, Suzukawa et al reported that enrichment of J774 cells with α -tocopherol inhibited cholesteryl ester formation,³¹ and Shige et al reported that pretreatment with α -tocopherol significantly reduced the uptake of modified LDL and suppressed acyl cholesterol acyl transferase (ACAT) activity, resulting in reduced cholesterol esterification.³² Devaraj et al reported that α -tocopherol enrichment of macrophages significantly decreased cholesterol ester accumulation induced by oxidized LDL and acetylated LDL.³³ Iuliano et al showed that injection of autologous radiolabeled native LDL into 7 patients with carotid stenosis resulted in cholesterol accumulation in the foam cells of the atherosclerotic plaques.³⁴ Also, in 3 patients treated for 4 weeks with vitamin E (900 mg/d), they observed an almost complete suppression of radiolabeled LDL uptake by macrophages.³⁴ In the present study, we sought to determine whether altered α -tocopherol content in macrophages isolated from PLTP-deficient mice might affect their ability to accumulate cholesterol. We demonstrated that dietary α -tocopherol supplementation leads to a significant reduction of cholesterol accumulation in macrophages of PLTP-deficient mice, with values returning to those measured in wild-type cells. Both the antioxidant and nonantioxidant properties of α -tocopherol might be relevant in relating the α -tocopherol deficit in macrophages of PLTP-deficient mice to accelerated foam cell formation. Although several *in vitro* studies suggested that α -tocopherol may downregulate SR-A and CD36 activities through both transcriptional and post-transcriptional mechanisms,^{33,35,36} no evidence for the effect of α -tocopherol on scavenger receptor activity has been reported *in vivo*. Our results showed no difference in scavenger receptor expression in macrophages from PLTP-deficient and WT mice. Thus, increased cholesterol uptake by macrophages of PLTP-deficient mice compared with WT cells would not rely on altered expression of classical scavenger receptors.

Another hypothesis is that α -tocopherol may affect cholesterol accumulation through its antioxidative properties. Indeed, Suzukawa et al and Devaraj et al showed that high doses of α -tocopherol significantly reduced oxidation of LDL by J774 macrophages.^{31,33} In addition, previous work from our group showed that α -tocopherol levels are reduced in PLTP-deficient macrophages, and they are associated with increased oxidative status, an established major contributing factor to atherosclerosis.²³ Finally, the ability of macrophages from PLTP-deficient mice to oxidize LDL is significantly increased compared with that of wild-type cells.²³ In the present study, we brought the first evidence for a direct link between the reduction in α -tocopherol content and the increase in oxidative properties of macrophages. We found that α -tocopherol supplementation leads to a significant reduction in macrophage oxidative status, as assessed by DCFH-DA

oxidation rate and cellular oxysterols. After α -tocopherol supplementation, both parameters returned to values close to those measured in WT cells. In addition, the ability of macrophages to oxidize LDL was markedly reduced after α -tocopherol supplementation. Taken together, these observations suggest that *in vivo*, α -tocopherol can modulate macrophage oxidative status, thus decreasing their ability to generate oxidized LDL and reducing cholesterol accumulation.

It is worthy of note that α -tocopherol-independent mechanisms may also contribute to the atheroprotective effect of macrophage-derived PLTP. In particular, PLTP may be one component of the ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux pathway in macrophages.³⁷ Lee-Rueckert et al demonstrated that macrophage-derived PLTP can act as a rate-limiting factor of ABCA1-mediated cellular cholesterol efflux when ABCA1 is not upregulated by cAMP treatment.³⁸ In a recent study, Liu et al demonstrated that the atheroprotective potential of macrophage PLTP is related at least in part to its ability to increase apoE secretion by these cells.²⁴ It is worthy of note that the above mentioned studies were conducted with resident peritoneal macrophages. In the present study, we observed no alteration in cholesterol efflux from elicited peritoneal macrophages of PLTP-deficient mice, either to ApoAI or to acetylated LDL. A potential explanation to these contradictory findings is that the contribution of endogenous PLTP to cholesterol efflux is blunted by responses induced by the inflammatory thioglycollate exposure of macrophages. In support of the latter view, previous observations from our group²³ as well as from Cao et al³⁹ reported no alteration in cholesterol efflux from elicited peritoneal macrophages of PLTP-deficient mice as compared with WT cells.

In conclusion, we have shown in the present study that PLTP can modulate cholesterol accumulation in macrophages, ie, a crucial step in atherosclerosis. In a PLTP deficiency context, macrophages contain lower amounts of α -tocopherol and their oxidative state is increased, which results in increased oxidation of LDL and its internalization via the scavenger receptor pathway. Increased cholesterol accumulation in macrophages of PLTP-deficient mice seems to occur independently of any modulation of scavenger receptors expression. These findings indicate that the pro- or anti-atherogenicity of PLTP depends on its site of expression and that interventions aimed at inhibiting PLTP should be restricted to the plasma protein.

Acknowledgments

We thank Philip Bastable for critical reading of this manuscript.

Sources of Funding

This study was supported by INSERM, the Conseil Régional de Bourgogne, the Université de Bourgogne, the Fondation de France, and the Agence Nationale de la Recherche. Nicolas Ogier is the recipient of a fellowship from the Ministère de l'Éducation Nationale, de la Recherche et des Technologies.

Disclosures

None.

References

- Huuskonen J, Ehnholm C. Phospholipid transfer protein in lipid metabolism. *Curr Opin Lipidol*. 2000;11:285–289.
- Van Tol A. Phospholipid transfer protein. *Curr Opin Lipidol*. 2002;13:135–139.
- Jiang XC. The effect of phospholipid transfer protein on lipoprotein metabolism and atherosclerosis. *Front Biosci*. 2002;7:d1634–1641.
- Stein O, Stein Y. Lipid transfer proteins and atherosclerosis. *Atherosclerosis*. 2005;178:217–230.
- Lagrost L, Desrumaux C, Masson D, Deckert V, Gambert P. Structure and function of the plasma phospholipid transfer protein. *Curr Opin Lipidol*. 1998;9:203–209.
- Kostner GM, Oetl K, Jauhiainen M, Ehnholm C, Esterbauer H, Dieplinger H. Human plasma phospholipid transfer protein accelerates exchange/transfer of alpha-tocopherol between lipoproteins and cells. *Biochem J*. 1995;305:659–667.
- Desrumaux C, Deckert V, Athias A, Masson D, Lizard G, Palleau V, Gambert P, Lagrost L. Plasma phospholipid transfer protein prevents vascular endothelium dysfunction by delivering alpha-tocopherol to endothelial cells. *FASEB J*. 1999;13:883–892.
- Albers JJ, Wolfbauer G, Cheung MC, Day JR, Ching AF, Lok S, Tu AY. Functional expression of human and mouse plasma phospholipid transfer protein: effect of recombinant and plasma PLTP on HDL subspecies. *Biochim Biophys Acta*. 1995;1258:27–34.
- Desrumaux CM, Mak PA, Boisvert WA, Masson D, Stupack D, Jauhiainen M, Ehnholm C, Curtiss LK. Phospholipid transfer protein is present in human atherosclerotic lesions and is expressed by macrophages and foam cells. *J Lipid Res*. 2003;44:1453–1461.
- Laffitte BA, Joseph SB, Chen M, Castrillo A, Repa J, Mangelsdorf D, Tontonoz P. The phospholipid transfer protein gene is a liver X receptor target expressed by macrophages in atherosclerotic lesions. *Mol Cell Biol*. 2003;23:2182–2191.
- O'Brien KD, Vuletic S, McDonald TO, Wolfbauer G, Lewis K, Tu AY, Marcovina S, Wight TN, Chait A, Albers JJ. Cell-associated and extracellular phospholipid transfer protein in human coronary atherosclerosis. *Circulation*. 2003;108:270–274.
- Jiang XC, D'Armiento J, Mallampalli RK, Mar J, Yan SF, Lin M. Expression of plasma phospholipid transfer protein mRNA in normal and emphysematous lungs and regulation by hypoxia. *J Biol Chem*. 1998;273:15714–15718.
- Jiang XC, Li Z, Liu R, Yang XP, Pan M, Lagrost L, Fisher EA, Williams KJ. Phospholipid transfer protein deficiency impairs apolipoprotein B secretion from hepatocytes by stimulating a proteolytic pathway through a relative deficiency of vitamin E and an increase in intracellular oxidants. *J Biol Chem*. 2005;280:18336–18340.
- Desrumaux C, Risold PY, Schroeder H, Deckert V, Masson D, Athias A, Laplanche H, Le Guern N, Blache D, Jiang XC, Tall AR, Desor D, Lagrost L. Phospholipid transfer protein (PLTP) deficiency reduces brain vitamin E content and increases anxiety in mice. *FASEB J*. 2005;19:296–297.
- Drouineaud V, Lagrost L, Klein A, Desrumaux C, Le Guern N, Athias A, Ménétrier F, Moiroux P, Sagot P, Jimenez C, Masson D, Deckert V. Phospholipid transfer protein (PLTP) deficiency reduces sperm motility and impairs fertility of mouse males. *FASEB J*. 2006;20:794–796.
- Klein A, Deckert V, Schneider M, Dutrillaux F, Hammann A, Athias A, Le Guern N, Pais de Barros J.P., Desrumaux C., Masson D., Jiang X.C., Lagrost L. Alpha-tocopherol modulates phosphatidylserine externalization in erythrocytes: relevance in phospholipid transfer protein-deficient mice. *Arterioscler. Thromb. Vasc. Biol*. 2006; 26:2160–2167.
- Jiang XC, Qin S, Qiao C, Kawano K, Lin M, Skold A, Xiao X, Tall AR. Apolipoprotein B secretion and atherosclerosis are decreased in mice with phospholipid transfer protein deficiency. *Nat Med*. 2001;7:847–852.
- Van Haperen R, van Tol A, van Gent T, Scheek L, Visser P, van der Kamp A, Grosveld F, de Crom R. Increased risk of atherosclerosis by elevated plasma levels of phospholipid transfer protein. *J Biol Chem*. 2002;277:48933–48934.
- Yang XP, Yan D, Qiao C, Liu RJ, Chen JG, Li J, Schneider M, Lagrost L, Xiao X, Jiang XC. Increased atherosclerotic lesions and lipoprotein oxidizability in apolipoprotein E-null mice overexpressing plasma phospholipid transfer protein. *Arterioscler Thromb Vasc Biol*. 2003;23:1601–1607.
- van Tol A, Jauhiainen M, de Crom R, Ehnholm C. Role of phospholipid transfer protein in high density lipoprotein metabolism: insights from studies in transgenic mice. *Int. J. Tissue React*. 2000;22:79–84.
- Vikstedt R, Ye D, Metso J, Hildebrand RB, Van Berkel TJ, Ehnholm C, Jauhiainen M, Van Eck M. Macrophage phospholipid transfer protein contributes significantly to total plasma phospholipid transfer activity and its deficiency leads to diminished atherosclerosis lesion development. *Arterioscler Thromb Vasc Biol*. 2007;27:578–586.
- Schlitt A, Bickel C, Thumma P, Blankenberg S, Rupprecht HJ, Meyer J, Jiang XC. High plasma phospholipid transfer protein levels as a risk factor for coronary artery disease. *Arterioscler. Thromb. Vasc. Biol*. 2003;23:1857–1862.
- Valenta DT, Ogier N, Bradshaw G, Black AS, Bonnet DJ, Lagrost L, Curtiss LK, Desrumaux CM. Atheroprotective potential of macrophage-derived phospholipid transfer protein in low density lipoprotein receptor-deficient mice is overcome by apoAI overexpression. *Arterioscler. Thromb. Vasc. Biol*. 2006;26:1572–1578.
- Liu R, Hojjati MR, Devlin CM, Hansen IH, Jiang XC. Macrophage phospholipid transfer protein deficiency and apoE secretion. Impact on mouse plasma cholesterol levels and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol*. 2007;27:190–196.
- Jiang XC, Tall AR, Qin S, Lin M, Schneider M, Lalanne F, Deckert V, Desrumaux C, Athias A, Witztum JL, Lagrost L. Phospholipid transfer protein deficiency protects circulating lipoproteins from oxidation due to the enhanced accumulation of vitamin E. *J Biol Chem*. 2002;277:31850–31856.
- Devaraj S, Harris A, Jialal I. Modulation of monocyte-macrophage function with alpha-tocopherol: implications for atherosclerosis. *Nutr Rev*. 2002;60:8–14.
- Del Rio M, Ruedas G, Medina S, Victor VM, De la Fuente M. Improvement by several antioxidants of macrophage function in vitro. *Life Sci*. 1998;63:871–881.
- Van der Schroeff JG, Havekes L, Weerheim AM, Emeis JJ, Vermeer BJ. Suppression of cholesteryl ester accumulation in cultured human monocyte-derived macrophages by lipoxygenase inhibitors. *Biochem Biophys Res Commun*. 1985;127:366–372.
- Asmis R, Llorente VC, Gey KF. Prevention of cholesteryl ester accumulation in P388D1 macrophage-like cells by increased cellular vitamin E depends on species of extracellular cholesterol. Conventional heterologous non-human cell cultures are poor models of human atherosclerotic foam cell formation. *Eur J Biochem*. 1995;233:171–178.
- Asmis R, Jek J. Vitamin E supplementation of human macrophages prevents neither foam cell formation nor increased susceptibility of foam cells to lysis by oxidized LDL. *Arterioscler. Thromb. Vasc. Biol*. 2000;20:2078–2086.
- Suzukawa M, Abbey M, Clifton P, Nestel PJ. Effects of supplementing with vitamin E on the uptake of low density lipoprotein and the stimulation of cholesteryl ester formation in macrophages. *Atherosclerosis*. 1994;110:77–86.
- Shige H, Ishikawa T, Suzukawa M, Nishiwaki M, Yamashita T, Nakajima K, Ito T, Higashi K, Ayaori M, Yonemura A, Nestel P, Nakamura H. Vitamin E reduces cholesterol esterification and uptake of acetylated low density lipoprotein in macrophages. *Lipids*. 1998;33:1169–1175.
- Devaraj S, Hugou I, Jialal I. Alpha tocopherol decreases CD36 expression in human monocyte-derived macrophages. *J Lipid Res*. 2001;42:521–527.
- Iuliano L, Mauriello A, Sbarigia E, Spagnoli LG, Violi F. Radiolabeled native low density lipoprotein injected into patients with carotid stenosis accumulates in macrophages of atherosclerotic plaque: effect of vitamin E supplementation. *Circulation*. 2000;101:1249–1254.
- Teupser D, Thiery J, Seidel D. Alpha-tocopherol down-regulates scavenger receptor activity in macrophages. *Atherosclerosis*. 1999;144:109–115.
- Ricciarelli R, Zingg JM, Azzi A. Vitamin E reduces the uptake of oxidized LDL by inhibiting CD36 scavenger receptor expression in cultured aortic smooth muscle cells. *Circulation*. 2000;102:82–87.
- Oram JF, Wolfbauer G, Vaughan AM, Tang C, Albers JJ. Phospholipid transfer protein interacts with and stabilizes ATP-binding cassette transporter A1 and enhances cholesterol efflux from cells. *J Biol Chem*. 2003;278:52379–52385.
- Lee-Rueckert M, Vikstedt R, Metso J, Ehnholm C, Kovanen PT, Jauhiainen M. Absence of endogenous phospholipid transfer protein (PLTP) impairs ABCA1-dependent efflux of cholesterol from macrophage foam cells. *J Lipid Res*. 2006;47:1725–1732.
- Cao G, Beyer TP, Yang XP, Schmidt RJ, Zhang Y, Bensch WR, Kauffman RF, Gao H, Lyang Y, Eacho PI, Jiang XC. Phospholipid transfer protein is regulated by liver X receptors in vivo. *J Biol Chem*. 2002;277:39561–39565.