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, Naïg 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
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 deficient and WT cells (CD36 expression in the basal expression of the 2 receptors in PLTP-deficient and WT cells (n = 5), respectively, n.s.; SR-A △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 preβ-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 582.56 ± 113.88 ng/mL in nonsupplemented mice, P < 0.05; macrophages (mean ± SEM, n = 5): 2817 ± 4.90 ng/10⁶ cells in supplemented versus 919 ± 1.26 ng/10⁶ in nonsupplemented 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 cells (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-
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. 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.

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±SEM of n=7 WT, n=11 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±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±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-
opposed to PLTP-deficient bone marrow.21 Taken together, receptor deficient mice transplanted with PLTP-expressing as to increased atherosclerosis in irradiated LDL-

ted 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±SEM, n=6]: 18.9±4.1 ng/μg protein in supplemented versus 40.1±12.8 ng/μg 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-

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 μg/mL AcLDL. Results are expressed as ng cholesterol/μg protein and are the mean±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).

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,17,13–16,19,25 including macrophages.25 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.35 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 posttranscriptional 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 oxyesters. 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.39 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 al90 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 antiatherogenicity of PLTP depends on its site of expression and that interventions aimed at inhibiting PLTP should be restricted to the plasma protein.

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Disclosures
None.
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MATERIALS AND METHODS

Isolation of LDL - LDL (1.019-1.063 g/mL) was isolated from normolipidemic human plasma and stored at 4°C. One part of the LDL was radiolabeled by treatment with \[^{1}\alpha,2\alpha(n)\cdot^{3}\text{H}]\text{cholesterol}\) (Amersham Biosciences, Piscataway, NJ) and stored under N2 to be kept in their native form. A second part of the LDL was acetylated in the presence of acetic anhydride. A third part of the LDL was radiolabeled by treatment with \[^{1}\alpha,2\alpha(n)\cdot^{3}\text{H}]\text{cholesterol}\) then acetylated in the presence of acetic anhydride.

Collection of elicited peritoneal macrophages and culture conditions - Macrophages were harvested by peritoneal lavage with phosphate buffered saline (PBS) containing butylated hydroxytoluene (BHT) 5 days after intraperitoneal injection of 3% thioglycollate. No significant difference in average peritoneal macrophage counts appeared between wild-type (9.08 ± 1.46 millions/animal, n=7) and PLTP-deficient mice (8.41 ± 1.98 millions/animal, n=7). The cells were washed twice with PBS, collected by centrifugation and plated on 24-well plates (Greiner BioOne) at a cell density of 5.10^5 cells/mL in DMEM medium (Gibco Invitrogen, Germany) supplemented with 1% Nutridoma–SP (Roche) and penicillin-streptomycin. After 5h, the plates were washed three times with PBS and further incubated under various experimental conditions.

Macrophage cholesterol content – 5.10^5 wild-type and PLTP-deficient macrophages were placed in glass tubes. Lipids were extracted and analysed according to the Folch method (1).
**Cholesterol accumulation in macrophages**- In the radioisotopic assay, peritoneal macrophages were incubated for 2h, 4h, 6h, 24h or 48h at 37°C in the presence of $^{[3H]}$ CE-acetylated LDL ($^{[3H]}$CE-AcLDL) (80 µg/mL) or for 8h, 24h or 48h at 37°C in the presence of $^{[3H]}$CE-LDL (80 µg/mL). Media were collected and centrifuged to remove detached cells. Cells were washed and lysed in 0.1 M NaOH. Radioactivity was counted in cells and supernatants and cholesterol incorporation was expressed as the percentage of radioactivity recovered in cells/µg of cell protein.

In the non-radioisotopic assay, peritoneal macrophages were incubated for 8h, 24h or 48h with AcLDL (80 µg/mL). Lipids were extracted and cellular cholesterol was quantified by GC-MS as described above.

**Gene expression studies**- Total RNA was isolated from peritoneal macrophages and mRNA levels of the two scavenger receptors CD36 and scavenger receptor A (SR-A) were quantified by reverse transcription followed by real-time PCR using an ABI PRISM 7700 Sequence Detection System instrument (Applied Biosystems, Courtaboeuf, France). Reactions were performed using the Quantitect SYBR Green amplification kit (Qiagen S.A., Courtaboeuf, France) following the instructions provided by the manufacturer. 18S rRNA was used as a reference gene. Results were expressed as the crossing point deviation ($\Delta$CP) between target and reference genes.

**Cholesterol efflux**- Cholesterol efflux from peritoneal macrophages was measured using apoAI (A) or AcLDL (B) as acceptors. A. Peritoneal macrophages were loaded with 1 µCi/ml $^3$H-cholesterol and 80 µg/mL AcLDL in DMEM/2%BSA for 20h at 37°C. Cholesterol efflux was measured by incubation of the cells with DMEM/2% BSA
supplemented with 50 µg/mL purified, lipid-free apoAI. B. Peritoneal macrophages were loaded with 1 µCi/ml ³H-cholesterol in DMEM/2%BSA for 20h at 37°C. Cholesterol efflux was measured by incubation of the cells with DMEM/2% BSA supplemented with 80 µg/mL AcLDL.

Media were collected and cell debris removed. Cells were lysed in 0.1M sodium hydroxide. Radioactivity was determined in media and cell lysates by liquid scintillation counting. Cholesterol efflux was expressed as the percentage of radioactivity released into the medium relative to total radioactivity (cells+medium) and to the amount of cell proteins in each well. Specific apoAI- or AcLDL-mediated efflux is defined as the difference between the efflux in the presence of acceptor and 2% BSA minus the efflux in the presence of 2% BSA only.

**Alpha-Tocopherol quantification** – Alpha-tocopherol was extracted from plasma, erythrocytes and macrophages and quantified by high-pressure liquid chromatography as described previously (2). Tocol (Spiral, Couternon, France) was added as an internal standard before extraction. Tocopherol content was expressed per blood volume or per million cells for macrophages.

**Cellular hydroperoxides** – Cells were set in 96-well plates, incubated at 37°C for 5 hours, then washed twice with HBSS medium. Macrophage peroxide levels were determined by measuring the oxidation rate of dichlorofluorescein diacetate (DCFH-DA) using a Victor1420 multilabel counter, as previously described (3).

**Oxysterols** – The macrophages were washed twice in PBS and harvested. Oxysterol extraction and saponification were performed according to the procedure described
by Abo et al. (4). 19-hydroxycholesterol and epicoprostanol were used as internal standards for oxysterols and cholesterol, respectively.

**Oxidation of LDL by macrophages** – Peritoneal macrophages were incubated at a cell density of $5 \times 10^5$ cells/ml for 6h in RPMI 1640 containing 100 µg/ml LDL and 2.5 µmol/l copper. Supernatants were recovered and 0.2 mmol/l EDTA was added. One milliliter of 0.375% (w/v) thiobarbituric acid in 15% (v/v) trichloroacetic acid and 2% BHT were added to each sample and incubated at 100°C for 15 min. The absorbance was read at 535 nm. Results were expressed as nmol of malondialdehyde (MDA) in LDL per µg of cell protein.

**Lipid Staining** – The cells were washed with PBS, air dried, fixed with 4% paraformaldehyde for 3 minutes, stained with a saturated concentration of oil red O for 15 min and counterstained with Meyer’s Hemalun for 1 min.

**Protein Assay** – Proteins were measured in isolated lipoproteins and cell lysates by using the Bicinchoninic Acid assay kit (Pierce, Rockford, IL).


**LEGEND TO FIGURE I**

Figure I – [*[^3]H*]cholesterol efflux in wild-type and PLTP-deficient macrophages.

A. Peritoneal macrophages were loaded for 20 h with 80 µg/mL acetylated LDL and 1 µCi/mL [*[^3]H*]cholesterol. Efflux was measured after 2 h, 4 h, 6 h or 24 h incubation in the presence of 50 µg/mL apoA-I, or without addition (control). Cholesterol efflux was expressed as the percentage of radioactivity recovered in medium/µg cell protein. Values are mean±SEM of n=6 WT and n=6 PLTP-deficient mice.
B. Peritoneal macrophages were loaded for 20 h with 1 µCi/mL \[^3\text{H}\]cholesterol. Efflux was measured after 8 h or 24 h incubation in the presence of 80 µg/mL AcLDL, or without addition (control). Cholesterol efflux was expressed as the percentage of radioactivity recovered in medium/µg cell protein. Values are mean±SEM of n=4 WT and n=4 PLTP-deficient mice.