Plasma Phospholipid Transfer Protein

ADENOVIRUS-MEDIATED OVEREXPRESSION IN MICE LEADS TO DECREASED PLASMA HIGH DENSITY LIPOPROTEIN (HDL) AND ENHANCED HEPATIC UPTAKE OF PHOSPHOLIPIDS AND CHOLESTERYL ESTERS FROM HDL

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In vitro studies have shown that plasma phospholipid transfer protein (PLTP) converts isolated human high density lipoprotein-3 (HDL3) into larger HDL particles and generates lipid-poor apoA-I containing nascent HDL. To evaluate the role of PLTP in vivo we generated recombinant adenovirus vectors containing either human PLTP cDNA (rPLTP.AdV) or the reporter luciferase cDNA as a control. After intravenous infusion of 4 × 10⁷ plaque-forming units (low dose) and 4 × 10⁸ plaque-forming units (high dose) of rPLTP.AdV into mice, PLTP activity in plasma increased from base-line levels of 8.4 ± 0.2 to 108 ± 17 and from 8.9 ± 0.6 to 352 ± 31 μmol/ml/h, respectively, on day 4 (both p < 0.001). Thus, both low and high doses of rPLTP.AdV led to pronounced overexpression of human PLTP in mice. On day 4 after treatment, mice treated with low and high doses of rPLTP.AdV showed decreased LDL cholesterol (−54% and −91%) and apoA-I (−64% and −98%) (all p < 0.05). Kinetic studies revealed that the fractional catabolic rates of HDL labeled with [³H]phosphatidylethanolamine, [¹⁴C]phosphatidyldichlorohydrin ether, [³H]cholesterol ether, and ¹²⁵I-labeled mouse apoA-I were increased by 8.5, 8.7, 3.8, and 2.8-fold, respectively, in mice treated with low dose rPLTP.AdV (all p < 0.001). After injection of labeled HDL, mice treated with rPLTP.AdV showed an increased accumulation of labeled PC ether (+304%) and cholesteryl ether (+92%) in the liver (both p < 0.05). Two-dimensional gel electrophoresis of plasma 5 min after injection of HDL labeled with ¹²⁵I-apoA-I demonstrated increased levels of newly generated pre-β-HDL in mice overexpressing PLTP. In conclusion, HDL remodeling mediated by PLTP generates nascent, lipid-poor apoA-I in vivo and accelerates the hepatic uptake of HDL surface and core lipids in mice treated with rPLTP.AdV. Accelerated catabolism of HDL in mice overexpressing PLTP leads to low HDL levels. Our data indicate an important role for PLTP in modulating reverse cholesterol transport in vivo.

The rate of spontaneous transfer of phospholipids between circulating lipoproteins in human plasma is too slow to allow for the rapid intravascular remodeling of lipoproteins (1, 2). Thus, two specialized glycoproteins, cholesteryl ester transfer protein (CETP)¹ and phospholipid transfer protein (PLTP), are secreted into the circulation to facilitate the transfer of surface phospholipids between high density lipoproteins (HDL) and other lipoproteins and, potentially, between cells and lipoproteins (3, 4). Of the two plasma lipid transfer proteins, only CETP is capable of additionally catalyzing the transfer of neutral core lipids between HDL and other lipoproteins, whereas PLTP is not (5–7). However, lipid transfers between HDL subfractions mediated by PLTP may be just as important as lipid transfers between HDL and other lipoproteins (8, 9). Presumably, PLTP-mediated phospholipid transfer between HDL particles (9) converts isolated human HDL₃ into a population of larger HDL particles with concomitant loss of apoA-I from the particles (9–11). Thus, HDL remodeling mediated by PLTP may help to generate nascent HDL particles, which are considered crucial for the removal of cholesterol from cells (12), the first step in reverse cholesterol transport.

In the last 2 years, our understanding of the structure of PLTP has advanced considerably (13). The human PLTP gene contains 16 exons, spanning approximately 13 kilobase pairs on the long arm of chromosome 20 (14, 15). The cDNA is 1750 base pairs long and encodes a 17-amino acid signal peptide and a 476-amino acid mature protein (16). The molecular mass observed upon SDS-polyacrylamide gel electrophoresis of PLTP purified from human plasma is approximately 81 kDa (13), considerably higher than the protein mass predicted from the cDNA, i.e. 55 kDa (16), a difference presumably due to glycosylation. The cDNA of PLTP shows sequence homology to lipopolysaccharide-binding protein, bactericidal permeability-increasing protein, and CETP, which places PLTP in a gene family of lipid-binding proteins involved in host defense and plasma lipid transport (13, 16).

Despite these advances, the physiological role of PLTP in lipoprotein metabolism is still unclear. To investigate the in vivo role of PLTP in HDL metabolism and reverse cholesterol transport, we have used recombinant adenovirus to express human PLTP in mice, an animal lacking measurable activity of the second phospholipid transfer protein in plasma, i.e. CETP (4). We conclude that pronounced overexpression of PLTP in vivo (i) leads to shedding of nascent, lipid-poor apoA-I from mature HDL particles; (ii) increases the plasma clearance of phospholipids, cholesteryl esters, and apoA-I in HDL leading to

¹ The abbreviations used are: CETP, cholesteryl ester transfer protein; PLTP, phospholipid transfer protein; rPLTP.AdV, recombinant adenovirus containing human PLTP; rLucif.AdV, recombinant adenovirus containing the reporter gene luciferase; pfu, plaque-forming units; PC, phosphatidylcholine; PBS, phosphate-buffered saline; FPLC, fast protein liquid chromatography; [³H]PC, [³H]palmitoyl-phosphatidylcholine; HDL, high density lipoprotein(s); LDL, low density lipoprotein(s); VLDL, very low density lipoprotein(s).
low HDL levels; and (iii) enhances the hepatic uptake of phospholipids and cholesterol esters from HDL.

MATERIALS AND METHODS

Generation of Recombinant Adenovirus—A full-length human PLTP cDNA (18) was obtained by reverse transcriptase polymerase chain reaction using 5 μg of human placenta total RNA (CLONTECH, Palo Alto, CA) and oligonucleotide primers (5′-primer, AGGCTACCCGTTCCGGCCGCT; 3′-primer, GGTTAGAGGGGCGGCGACAGGC) and subcloned into a shuttle vector (pAd12PLPT) containing cytomegalo-

virus enhancer and promoter elements, an SV40 polyadenylation signal, and the E1 region of the human adenovirus (Ad5) (17). The 1.7-kilobase pair PLPT gene was sequenced using the Sanger dye-termin-

otide method (18), and the proper orientation was confirmed. Recombi-

nant E1-deleted adenovirus was generated after cotransfection of pAd12PLPT and pMM17 (Ad5 genome) in human embryonic kidney 293 cells (American Type Culture Collection, Rockville, MD) (19, 20). Recombi-

nent virus was grown in 293 cells and purified by cesium chloride density gradient ultracentrifugation. Purified virus was designated rPLPTAdv, titered, and diluted in 0.2% mouse albumin (Sigma) before infusion into the mice. Recombinant adenovirus containing the lucifer-

ase gene (21), i.e. rLucifAdv, was generated in an analogous way. An appro-

priate aliquot of the purified recombinant adenovirus containing either 4 × 10^9 plaque-forming units (pfu) or 4 × 10^9 pfu was infused into the saphenous vein of the mice on day 0 of the study.

Animals—Male C57Bl mice (4–7 months of age) were housed at the National Institutes of Health under protocols approved by the Animal Care and Use Committee of the NHLBI and fed a regular chow diet (NIH-07 chow diet 5% fat; Zeigler Brothers Inc., Gardners, PA). Blood samples from the retroorbital plexus were obtained from mice fasted for 4 h and anesthetized with methoxyflurane (Pitman-Moore, Mundelein, IL), placed into precooled tubes containing EDTA (final concentration 4 mM), and stored at -70 °C. Blood samples from the retroorbital plexus were obtained from mice fasted for 4 h and anesthetized with methoxyflurane (Pitman-Moore, Mundelein, IL), placed into precooled tubes containing EDTA (final concentration 4 mM), and stored at -70 °C.

Lipid, Lipoprotein, Apolipoprotein Quantitation, and Immunoblots—

Ten μl of plasma from fasting mice was diluted 1:50 with phosphate-

buffered saline (PBS), and total cholesterol, triglycerides, phospholi-

pids, and free cholesterol were determined using enzymatic kits from Sigma and Wako Chemicals (Richmond, VA) and the COBAS MIRA Plus automated chemistry analyzer (Roche Diagnostics Systems, Branchburg, NJ). HDL cholesterol was measured by dextran sulfate precipitation (Ciba-Corning, Oberlin, OH), as described previously (22). Protein concentrations were determined with the BCA protein reagent (Pierce). Lipoproteins were separated by electrophoresis on 7.5% polyacrylamide gel electrophoresis (2–36%). After blotting, membranes were de-

veloped with polyclonal antibodies raised in rabbits and purified mouse apoA-I and apoA-II as protein standards. Two-dimensional electrophoresis of plasma lipoproteins was performed as described previously (23).

Briefly, in the first dimension, lipoproteins were separated by electro-

phoresis on 0.7% agarose gels; subsequently, in the second dimension, sequential ultracentrifugation (1.063–1.21 g/ml) (26), and dialyzed exten-

sively (3). Briefly, plasma from male C57Bl mice was adjusted to a

density of 1.063 g/ml with KBr and centrifuged for 10 h at 95,000 rpm at 5 °C (26). The 1.063 infranatant was adjusted to a density of 1.21 g/ml with KBr and centrifuged for 10 h at 95,000 rpm at 5 °C (26). The 1.21 g/ml infranatant containing the HDL was analyzed against PBS. Fifty μCi of Cholpalmitoylphosphatidylcholine or Cholhexadecylcholine (both from NEN Life Science Products) were dried under nitrogen, dissolved in 95% ethanol, and incubated with the isolated HDL for 18 h at 37 °C. The labeled HDL were then reisolated at d = 1.063–1.21 g/ml and dialyzed extensively against PBS. Labeled HDL showed no appreciable degradation of apolipoproteins based on immunoblot analysis. Homogeneous labeling of HDL was ascertained by superimposing radioactivity and chemistry profiles obtained by fast protein liquid chromatography (FPLC). More than 98% of the label comigrated with dipalmitoyl-phosphatidylcholine on thin layer chromatography.

Preparation of HDL Labeled with 125I-Cholesteryl-palmitat-ether—

Labeled HDL was ascertained by superimposing radioactivity and chemistry profiles obtained by FPLC. More than 99.7% of the label comigrated with cholesteryl palmitate on thin layer chromatography. Less than 3.8% of the Cholhexa-

dicyclether was transferred from labeled HDL to LDL (LDL-CE:

LDL-Cmass ratio = 0.1) in 0.15 mM NaCl, 10 mM Tris, pH 7.4, during an 18-h incubation at 37 °C without the addition of CETP.

Preparation of HDL Labeled with 125I-Mouse ApoA-I—Lymphoplasma apoA-I was iodinated by a modification of the iodine monochlo-

ride method (28) as described previously (29). Approximately 0.5 mol of iodine was incorporated per mol of protein. Four μg of 125I-mouse apoA-I were added to 1 mg of labeled mouse HDL (250 μg of protein), isolated by sequential ultracentrifugation (1.063–1.21 g/ml) (26), and dialyzed exten-

sively against sterile PBS containing 0.01% EDTA at 4 °C. Radioactiv-

ity was quantitated in a Packard Cobra γ-counter (Packard Instru-

ment Co., Downers Grove, IL).

HDL Metabolic Studies—One million dpm of HDL labeled with

[3H]HC, [14C]Cholesterol-palmitat-ether, [3H]Cholesterol-palmitoyl-ether, or 5 μg of 125I-labeled mouse apoA-I was injected into the saphenous veins of mice that had been infused with 4 × 10^7 pfu of rPLPTAdv or rLucifAdv, respectively, 4 days previously. Plasma disappearance curves were generated by dividing the plasma radioactivity at each time point by the plasma radioactivity at the initial 1-min time point, which was considered among the study groups (all p > 0.4). The fractional catabolic rate was determined from the area under the plasma radioactivity curves using a multieponential curve fitting technique on the SAAM program (30). ApoA-I production rates were calculated from the following formula: production rate = (apoA-I plasma concentration × plasma volume) / fractional catabolic rate/weight.

After the 15-min blood sample was collected, a subset of mice injected
with [14C]dihexadecylether-PC or [3H]cholesterylpalmityl-ether-labeled HDL were perfused with cold 0.15 M NaCl, livers, heart, lung, spleen, kidneys, adrenals, and testes were harvested and extracted in 20 volumes of chloroform-methanol, 2:1 (v/v); phases were separated by the addition of water (21); and aliquots of the lower phase were counted in a Tri-Carb 2500 TR liquid scintillation counter (Packard Instrument Co.). Mean recoveries at 15 min were higher than 89% of injected counts for all groups with no statistical differences between the groups (both p > 0.7).

Statistical Analysis—Values are presented as means ± S.E. Comparisons between groups of mice treated with equivalent doses of rPLTP-AdV and rLucif-AdV, respectively, were made using Student’s t test for independent samples (two-tailed).

RESULTS
To investigate the in vivo role of PLTP, we utilized rAdV to express human PLTP as well as the reporter gene luciferase in male C57Bl/6 mice. Immunoblot analysis using rabbit anti-human PLTP IgG confirmed the presence of authentic PLTP (81 kDa) in the plasma of mice treated with rPLTP-AdV (Fig. 1A). Two different doses of recombinant adenovirus, a low dose corresponding to 4 × 10^5 pfu and a high dose corresponding to 4 × 10^6 pfu were studied. The radioassay utilized to quantitate PLTP expression has been previously demonstrated to be specific for PLTP (5, 11, 24, 25) and involves the transfer of [3H]PC from PC liposomes to HDL. On day 4 after infusion of low and high doses of rPLTP-AdV, phospholipid transfer activity in plasma was increased to 108 ± 17 and 352 ± 31 μmol/ml/h compared with mice receiving equivalent doses of rLucif-AdV (7.0 ± 0.96 and 8.3 ± 0.48 μmol/ml/h) (both p < 0.001) (Table I). Analysis of the time course of expression of PLTP in a subset of mice injected with high dose rPLTP-AdV revealed that PLTP activity in plasma peaked at day 4 and gradually decreased thereafter (Fig. 1A). However, even at day 15 after treatment, PLTP activity was still increased at 30.2 ± 1.68 μmol/ml/h compared with control mice (p < 0.001) (Fig. 1A). The time course of expression of the PLTP transgene is, thus, similar to that of several other transgenes delivered previously by the same vector (22, 32, 33). Thus, adenovirus-mediated delivery of the human PLTP cDNA primarily to the mouse liver leads to a significant, albeit transient, increase of biologically active PLTP in mouse plasma.

Table II summarizes the effects of PLTP overexpression on the plasma lipid and lipoprotein concentrations on day 4 after infusion of low and high doses of rPLTP-AdV compared with age- and sex-matched control mice receiving an equivalent amount of rLucif-AdV. In the low dose rPLTP-AdV group, cholesterol, triglycerides, phospholipids, HDL-cholesterol, apoA-I, and apoA-II decreased by 55, 48, 55, 54, 64, and 67%, respectively, after treatment (all p < 0.05) (Table II). In the high dose rPLTP-AdV group, cholesterol decreased by 27%, and triglycerides, phospholipids, HDL-cholesterol, apoA-I, and apoA-II decreased by 79, 45, 91, 98, and 100% after treatment (all p < 0.005) (Table II). Comparison of the time course of PLTP activity in plasma (Fig. 1A) and the time course of the decrease in HDL established a strong inverse relationship between the level of overexpression of PLTP at days 2–11 and the level of HDL cholesterol (Fig. 1B) and apoA-I (Fig. 1C). On day 15 after treatment, when PLTP activity in plasma was still increased at 30.2 ± 1.68 μmol/ml/h (p < 0.001), no changes in plasma lipids were detectable (data not shown). Examination of HDL subfractions by two-dimensional gel electrophoresis showed an almost complete loss of apolipoprotein HDL and, interestingly, an increase in pre-β-HDL on day 4 after treatment with rPLTP-AdV (Fig. 2). At baseline, FPLC showed that the lipoprotein profiles of the study groups were very similar and that HDL was the major lipoprotein (data not shown). In mice treated with low dose rPLTP-AdV, FPLC analysis on day 4 revealed a marked decrease in HDL phospholipids and cholesterol (Fig. 3, A and B). Mice treated with high dose rPLTP-AdV showed an even more pronounced decrease in HDL, and, additionally, an increase in VLDL phospholipids and cholesterol (Fig. 3, A and B). Immunoblot analysis of the VLDL indicated that the lipoprotein particles contained apoB-48, apoB-100, and apoE (data not shown).

To further investigate the metabolic basis of low HDL levels in mice overexpressing PLTP, we performed kinetic studies of HDL labeled in either the lipid or the protein moiety (Figs. 4–7). In these studies, we used PC and dihexadecylether-PC, amphiphatic lipids predicted to reside primarily in the HDL surface coat and cholesterylpalmityl-ether, a hydrophobic lipid

![Image](337x363 to 535x729)
predicted to partition primarily in the HDL core. Ether analogues of PC and cholesterylpalmitate were also used, since these lipids cannot be hydrolyzed in mammalian tissues. This property prevents resecretion of labeled PC and unesterified cholesterol after hydrolysis in tissues, thereby facilitating the interpretation of plasma clearance and organ distribution of the labeled lipids (34, 35). All three lipid labels and mouse apoA-I, utilized to trace the major HDL protein, were cleared from plasma much faster in mice overexpressing PLTP than in controls (Fig. 4, A–D). Fractional catabolic rates of dipalmitoyl-PC, dihexadecylether-PC, and cholesterylpalmitoyl-ether in HDL in mice treated with 4 × 10^7 pfu of either rPLTP.AdV or rLucif.AdV averaged 63.7 ± 3.46 versus 75.3 ± 0.48, 59.31 ± 3.46 versus 6.83 ± 0.48, and 21.71 ± 6.37 versus 5.7 ± 0.60 day⁻¹ (all p < 0.001; n = 3–4 mice/group). The fractional catabolic rate of apoA-I in mice treated with low dose rPLTP.AdV was also markedly increased compared with luciferase controls, averaging 6.12 ± 0.41 versus 2.19 ± 0.05 day⁻¹ (p < 0.001; n = 3); in contrast, apoA-I production rates were similar in both groups, averaging 32.9 ± 3.6 versus 41.3 ± 5.9 mg x kg⁻¹ x day⁻¹ (p > 0.29; n = 3 mice/group). The distribution of each of the labels among plasma lipoproteins after injection in mice treated with either rPLTP.AdV or rLucif.AdV were analyzed by FPLC (Fig. 5, A–D). In controls, the majority of radiolabeled PC (Fig. 5A) and dihexadecylether-PC (Fig. 5B) were still associated with HDL 15 min after injection; however, part of the radiolabeled lipids had already been transferred to LDL and VLDL. In mice treated with rPLTP.AdV, the percentage of PC and dihexadecylether-PC in HDL was decreased and the percentage of both labels in VLDL/LDL was increased, respectively (Fig. 5, A and B). In contrast to the distribution of phospholipids, most of the labeled cholesterylpalmitoyl-ether was still associated with HDL both in controls and in mice treated with rPLTP.AdV, respectively (Fig. 5C). In controls, mouse 125I-labeled apoA-I in plasma 5 min after injection of HDL labeled with mouse apoA-I was almost exclusively present in HDL (Fig. 5D). In mice injected with rPLTP.AdV, an additional peak of radiolabeled apoA-I in late FPLC fractions, corresponding to poorly lipitated apoA-I was observed in addition to the regular HDL peak (Fig. 5D). To determine whether the injection of apoA-I-labeled HDL into mice overexpressing PLTP would lead to increased levels of newly formed pre-β-HDL, we performed two-dimensional gel electrophoresis of mouse plasma 5 min after injection of 125I-apoA-I-labeled mouse HDL. Indeed, α-HDL were decreased and pre-β-HDL were markedly increased in mice treated with high dose rPLTP.AdV compared with controls (Fig. 6).

To evaluate which organs contributed to the accelerated removal of dihexadecylether-PC and cholesterylpalmitoyl-ether in HDL from the plasma compartment in mice overexpressing PLTP, we extracted these radiolabeled lipids from the livers and from heart, lung, spleen, kidney, adrenals, and testes of mice 15 min after the injection of labeled HDL. In mice injected with rPLTP.AdV, the percentages of dihexadecylether-PC and cholesterylpalmitoyl-ether in the liver were increased by 304 and 92% compared with controls (Fig. 7). All other organs together accounted for less than 6.2% of recovered counts in both groups.

**DISCUSSION**

Based on *in vitro* studies, PLTP has been proposed to have two major roles in HDL metabolism. First, partially purified PLTP enhances the transfer and exchange of phospholipids between VLDL and HDL during lipolysis and, thus, may serve in *vivo* to promote the net transfer of surface remnants of triglyceride-rich lipoproteins into HDL (2, 7, 36, 37). In addition, highly purified PLTP converts isolated HDL into a population of larger HDL particles with a concomitant loss of lipiddoor apoA-I (9–11, 38), which forms pre-β-HDL (39). Thus, PLTP may enhance the efflux of cholesterol and phospholipids from cellular membranes (39–41). In the present study, we use recombinant adenovirus expressing PLTP and luciferase to evaluate PLTP function *in vivo* in normal mice on a regular chow diet.

The concentrations of HDL cholesterol, apoA-I, and apoA-II in mice expressing human PLTP decreased by 54, 64, and 67% on the low dose and by 91, 98, and 100% on the high dose of rPLTP.AdV, respectively. Thus, both the lipid and apolipoprotein components of HDL, the major lipoprotein in mice, showed a proportionate dose-dependent decrease when PLTP activity in plasma was increased by 13- and 40-fold in mice treated with low and high dose rPLTP.AdV, respectively. Analysis of HDL subfractions by two-dimensional gel electrophoresis revealed an almost complete disappearance of α-migrating HDL and an increase in pre-β-migrating HDL particles in mice treated with rPLTP.AdV. Thus, our findings indicate that pronounced overexpression of PLTP in mice leads to a very marked decrease in HDL levels and an increase of pre-β-HDL relative to α-HDL.

To determine the metabolic basis of the low HDL levels in mice overexpressing PLTP, we performed kinetic studies using HDL labeled with PC, phosphatidylcholine ether (PC ether), cholesteryl ether, and apoA-I. Our studies demonstrate a markedly increased clearance of both HDL-PC and HDL-ether from plasma in the rPLTP.AdV study group, a result consistent with the findings of Pownall et al. in rats where HDL labeled with PC ether were removed from plasma more rapidly when partially purified human PLTP was injected simultaneously (42). In addition, HDL cholesteryl esters and apoA-I were also catabolized from plasma much more rapidly in the rPLTP.AdV group. We further studied the tissue sites of degradation of HDL lipids with nonhydrolyzable ether analogues of cholesteryl esters and phospholipids, which remain trapped in mammalian tissues after uptake (34, 35) and found that the increased clearance of HDL cholesteryl esters and phospholipids from plasma in mice treated with rPLTP.AdV was mainly due to increased uptake of these lipids by the liver. Thus, overexpression of PLTP significantly increases the clearance of HDL lipids and apolipoproteins from plasma in mice as well as enhances the uptake of HDL cholesteryl esters and phospholipids by the liver.

Many investigators believe that one potential mechanism by which HDL prevents atherogenesis is by mediating the process of reverse cholesterol transport. Increased levels of pre-β-HDL active in cholesterol efflux and accelerated delivery of HDL cholesteryl esters to the liver in mice overexpressing PLTP might suggest facilitated reverse cholesterol transport. How-

**Table I**

| rAdV          | PLTP Activity | Preinfusion | Postinfusion | µmol/mil/h |
|--------------|---------------|------------|-------------|------------|
| rPLTP.AdV    |               |            |             |            |
| Low dose (4 × 10^7 pfu) | 8.4 ± 0.20    | 108 ± 16.64 | p < 0.001, rPLTP.AdV low dose versus rLucif.AdV low dose. |
| High dose (4 × 10^7 pfu) | 8.9 ± 0.56    | 352 ± 30.56 | p < 0.001, rPLTP.AdV high dose versus rLucif.AdV high dose. |

* p < 0.001 (rPLTP.AdV low dose versus rLucif.AdV low dose).
already suffice to completely transfer surface remnants of triglycerides into HDL during lipolysis, and an increase in PLTP activity in rPLTP.AdV-treated mice would therefore not be expected to increase net phospholipid delivery to HDL.

Our findings provide support to some of the molecular mechanism(s) that have been proposed for PLTP function. Thus, according to a model of PLTP-mediated HDL conversion (9), PLTP effects net transfer of phospholipids from one subpopulation of HDL particles to another. This increases the surface pressure in the acceptor HDL particles, which promotes the shedding of apoA-I molecules from their surface coat. Detachment of apoA-I destabilizes the HDL particles and induces

![Pre-Infusion Post-Infusion](Image)

**FIG. 2.** Two-dimensional gel electrophoresis of mouse plasma before and on day 4 after infusion of 4 × 10⁷ plaque-forming units of rPLTP.AdV. Lipoproteins were separated by electrophoresis on 0.7% agarose gels (x axis); subsequently, lipoprotein size was determined by nondenaturing polyacrylamide gradient gel electrophoresis (2–36%) (y axis). Membranes were developed by anti-mouse apoA-I immunoblotting.

Table II

| Values represent means ± S.E. in mg/dl before and 4 days after infusion of either rPLTP.AdV (low dose, n = 8; high dose, n = 10) or rLucif.AdV (low dose, n = 9; high dose, n = 10) in male C57Bl mice. Groups of mice treated with equivalent doses of rPLTP.AdV and rLucif.AdV, respectively, were compared using Student's t test for independent samples (two-tailed). |
|---|---|---|---|---|---|---|
| AdV | Cholesterol | Triglycerides | Phospholipids | HDL-Cholesterol | ApoA-I | ApoA-II |
| Low dose rPLTP.AdV (4 × 10⁷ pfu) | 105 ± 4.2 | 99 ± 9.3 | 195 ± 7.4 | 70 ± 3.7 | 100 ± 8.2 | 11.0 ± 3.7 |
| Preinfusion | 47 ± 11.9° | 51 ± 6.8° | 88 ± 16.9° | 32 ± 8.8° | 36 ± 11.3° | 3.6 ± 2.3° |
| Postinfusion | 92 ± 5.2 | 80 ± 11.8 | 175 ± 9.2 | 70 ± 4.2 | 99.0 ± 9.9 | 6.2 ± 1.1 |
| Low dose rLucif.AdV (4 × 10⁷ pfu) | 106 ± 2.9 | 85 ± 4.2 | 190 ± 6.2 | 78 ± 3.9 | 113 ± 15 | 9.5 ± 1.6 |
| Preinfusion | 67 ± 13.5 | 17 ± 3.3° | 97 ± 16° | 6 ± 3.5° | 1.7 ± 1.1° | 0° |
| Postinfusion | 96 ± 4.4 | 76 ± 8.3 | 171 ± 9.6 | 66 ± 2.7 | 124.6 ± 13.9 | 8.6 ± 0.6 |
| High dose rPLTP.AdV (4 × 10⁸ pfu) | 92 ± 5.0 | 67 ± 4.9 | 163 ± 8.3 | 66 ± 7.0 | 138.2 ± 19.6 | 7.4 ± 1.7 |
| Preinfusion | 106 ± 5.1 | 105 ± 3.6 | 116 ± 2.3 | 116 ± 4.9 | 116 ± 10.1 | 116 ± 10.1 |
| Postinfusion | 109 ± 5.1 | 105 ± 3.6 | 116 ± 2.3 | 116 ± 4.9 | 116 ± 10.1 | 116 ± 10.1 |

Note: p < 0.001.

A p < 0.05.

<sup>c</sup> p < 0.005.

![A CHOLESTEROL](Image)

**FIG. 3.** Lipoprotein analysis by FPLC of pooled mouse plasma on day 4 after infusion of mice with 4 × 10⁷ pfu rPLTP.AdV (n = 2), 4 × 10⁸ pfu of rPLTP.AdV (n = 7), or 4 × 10⁷ pfu of rLucif.AdV (n = 7). Values represent cholesterol (A) and phospholipid (B) curves in mg/ml column eluate. Results of mice infused with 4 × 10⁷ pfu of rLucif.AdV (n = 9) were similar to the high dose luciferase group and were omitted for clarity of presentation. Relative elution positions of different lipoproteins on FPLC are indicated in each panel.
particle fusion, which leads to the generation of larger HDL particles. Our study provides the first in vivo evidence that, indeed, nascent lipid-poor apoA-I is generated during HDL remodeling by PLTP. As nascent, pre-β-HDL lack a core (12), it is not surprising that labeled cholesteryl ether was not found in the FPLC fractions where displaced, lipid-poor apoA-I eluted. Interestingly, larger sized HDL, the main end products of the HDL conversion process in vitro (9–11), were not readily detectable in vivo in our mice even when PLTP was overexpressed dramatically in the high dose rPLTP.AdV group. Similarly, in vitro incubation of pig plasma with PLTP generates larger HDL species, which, however, are not observed in normal pig plasma despite the presence of considerable PLTP activity (38). Lipolytic enzymes like hepatic lipase and/or cellular receptors may rapidly remodel these particles and/or remove them from the circulation. However, the rapid accumulation of PC ether in the liver clearly establishes that hydrolysis of the individual phospholipid molecules is not a prerequisite for uptake under our experimental conditions.

In the mice treated with high dose rPLTP.AdV, VLDL phospholipids and cholesterol were increased at the peak of PLTP overexpression on day 4 concomitant with the almost complete loss of HDL. PLTP accelerates the efflux of cell membrane

**FIG. 4.** Plasma kinetics of mouse HDL labeled with [3H]di-palmitoyl-phosphatidylcholine (A), [14C]dihexadecylether-PC (B), [3H]cholesteryl-palmitoyl-ether (C), and 125I-labeled mouse apoA-I (D) on day 4 after infusion of mice with 4 × 10⁷ plaque-forming units of rPLTP.AdV or rLucif.AdV, respectively. Values are percentages of remaining counts compared with the 1-min value (mean ± S.E.; n = 3–4 mice/group).

**FIG. 5.** FPLC analysis of the lipoprotein distribution of the radioactive label after injection of HDL labeled with [3H]di-palmitoyl-phosphatidylcholine (A), [14C]dihexadecylether-PC (B), [3H]cholesteryl-palmitoyl-ether (C), and 125I-labeled mouse apoA-I (D) on day 4 after infusion of mice with 4 × 10⁷ pfu of either rPLTP.AdV or rLucif.AdV. Values are presented as percentages of counts/fraction and were obtained by dividing the counts in individual FPLC fractions by the total amount of counts in the column eluate. Three to four mice were studied for each group, and the curve of a representative mouse is shown. PC (A), dihexadecylether-PC (B), and cholesteryl ether (C) distribution was examined 15 min after injection of labeled HDL; apoA-I distribution was examined 5 min after injection of labeled HDL. Relative elution positions of isolated lipoproteins on FPLC are indicated in each panel.
phospholipids and cholesterol (39, 40) and the transfer/exchange of vitamin E among VLDL, other lipoproteins, and cells (44), raising the possibility that increased VLDL phospholipids and cholesterol could derive from cellular membranes. Our metabolic studies indicate rapid transfer/exchange of injected, radiolabeled HDL phospholipids and phospholipids in apoB-containing lipoproteins in mice receiving rPLTP.AdV, confirming the results of previous in vitro experiments (2). We repeated these studies with radiolabeled PC ether, which in contrast to PC cannot be resecreted by the liver, to prove direct transfer/exchange from HDL to apoB-containing lipoproteins in plasma as opposed to an uptake/resecretion process of radiolabeled PC via the liver (45). In addition, increased delivery of HDL lipids to the liver in mice treated with rPLTP.AdV might be expected to modulate lipoprotein secretion by the liver.

During the preparation of this manuscript, Jiang et al. (46) reported the results of overexpression of human PLTP in transgenic mice. When plasma PLTP activity was increased by 29% in PLTP transgenic mice, no changes in lipoproteins were observed. Similarly, in this study, overexpression of PLTP to a plasma activity of 30 ± 1.7 μmol/ml/h on day 15 after adenovirus infusion failed to change plasma lipids. Hypothesizing a species-specific interaction of human PLTP with human apoA-I, Jiang et al. (46) observed a substantial increase of apoA-I in pre-β-HDL (56%) and a small increase in α-HDL (14%) in human PLTP/human apoA-I double transgenics overexpressing PLTP by 47% compared with human apoA-I transgenics. The metabolic mechanism(s) underlying these observations were not addressed (46). In mice treated with rPLTP.AdV, we also find markedly increased apoA-I in pre-β HDL, and our kinetic studies suggest that it is derived from mature HDL. In contrast to the small increase in α-HDL in the PLTP/apoA-I double-transgenic mice, however, α-HDL are markedly decreased in mice treated with rPLTP.AdV alone, a finding that was established to be due to an increase in HDL catabolism. Several important differences in experimental conditions including level, tissue pattern, and duration of PLTP expression, increased apoA-I pool size in the double transgenics, and the undesirable concomitant expression of human lysosomal protective protein in the transgenic mice (46) may account for some of these different results.

Some cautionary notes regarding the proposed role of PLTP in lipoprotein transport are appropriate. First, PLTP was overexpressed substantially, leading to a mean 13- and 40-fold increase in PLTP activity on day 4 in mice treated with low and high dose rPLTP.AdV, respectively, compared with base line; therefore, some of the effects may well exceed the physiological range. Second, human PLTP was overexpressed in mice. While it has been demonstrated that both human and mouse PLTP are able to convert mouse HDL to larger and smaller subspecies, respectively (47), human PLTP may differ from mouse PLTP in other functional aspects. Third, intravenous infusion of recombinant adenovirus leads to expression of the transgene primarily in the liver (22, 32, 33). While the liver presumable is an important source of circulating PLTP in mice, adipose tissue and lung probably contribute to plasma levels of PLTP (48). In addition, PLTP in humans and mice is expressed in a wide variety of other tissues (16, 47, 48). At present, it is unclear which effect, if any, this complex pattern of expression may have on the metabolic pathways of cholesterol and phospholipid metabolism. Fourth, duration of expression of PLTP was relatively short, which may limit counterregulatory responses, i.e. by the liver to the increased delivery of phospholipids and cholesteryl esters. While this is ideal for assessing the independent effect of PLTP on lipoprotein transport, counterregulatory responses may substantially alter the rates of sterol flux in long term studies. Finally, PLTP was expressed in mice, which, in contrast to humans, lack activity of the second lipid transfer protein in plasma, i.e. CETP (4). Because PLTP potentiates CETP action (7), high levels of PLTP may differ from mouse PLTP in other functional aspects. Third, intravenous infusion of recombinant adenovirus leads to expression of the transgene primarily in the liver (22, 32, 33). While the liver presumable is an important source of circulating PLTP in mice, adipose tissue and lung probably contribute to plasma levels of PLTP (48).

In summary, we have found the following. (i) Adenovirus-mediated expression of PLTP in vivo results in a marked decrease in HDL and, in mice receiving high dose rPLTP.AdV, an increase in apoB containing VLDL. (ii) PLTP significantly increases the plasma clearance of phospholipids, cholesteryl esters, and apoA-I in HDL. (iii) PLTP enhances the hepatic uptake of phospholipids and cholesteryl esters from HDL. (iv) Nascent, lipid-poor apoA-I, an efficient acceptor of cellular cholesterol, is regenerated during PLTP-mediated HDL remodeling in vivo. (v) PLTP may play a critical role in phospholipid metabolism and reverse cholesterol transport by modulating
cellular cholesterol efflux, intravascular remodeling of HDL, and delivery of cholesteryl esters to the liver.

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