Phospholipid transfer protein gene knock-out mice have low high density lipoprotein levels, due to hypercatabolism, and accumulate apoA-IV-rich lamellar lipoproteins

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Abstract Phospholipid transfer protein gene knock-out (*Pltp* KO) mice have defective transfer of very low density lipoprotein (VLDL) phospholipids into high density lipoprotein (HDL) and markedly decreased HDL levels (Jiang et al. 1999. *J. Clin. Invest.* 103: 907–914). These animals also accumulated VLDL- and LDL-sized lipoproteins on a high saturated fat diet. The goals of this study were to further characterize the abnormal lipoproteins of *Pltp* KO mice and to determine the mechanisms responsible for low HDL levels. A lipoprotein fraction enriched in lamellar structures was isolated from the low density lipoprotein (LDL) region and was shown to be phospholipid- and free cholesterol-rich and to have apoA-IV (55%) and apoE (25%) as major apolipoproteins. The lamellar lipoproteins accumulating in these mice probably represent surface material derived from triglyceride-rich lipoproteins (TRL). The HDL was found to be protein-rich (primarily apoA-I) and specifically depleted in phosphatidylcholine (PC) (28% in wild-type mice (WT) vs. 15% in *Pltp* KO mice, $P < 0.001$). Unexpectedly, turnover studies using autologous HDL revealed a profound 4-fold increase in the catabolism of HDL protein and cholesteryl ester in *Pltp* KO mice compared to wild-type, with minor differences in synthesis rates. In contrast, injection of WT mouse HDL into *Pltp* KO mice showed only a 2-fold increase in fractional catabolism. Reminiscent of the defect in Tangier disease, the failure of transfer of PC from TRL into the HDL fraction results in dramatic hypercatabolism of HDL. These results suggest that defective phospholipid transfer from TRL into HDL, arising from decreased lipolysis or decreased PLTP activity, could lead to hypoalphalipoproteinemia characterized by hypercatabolism of HDL protein.—Qin, S., K. Kawano, C. Bruce, M. Lin, C. Bisgaier, A. R. Tall, and X-c. Jiang. **Phospholipid transfer protein gene knock-out mice have low high density lipoprotein levels, due to hypercatabolism, and accumulate apoA-IV-rich lamellar lipoproteins. *J. Lipid Res.* 2000.** 41: **269–276.**

Supplementary key words HDL • lipoproteins • apolipoproteins • phospholipids • fractional catabolic rate

Plasma PLTP circulates bound to HDL and mediates both net transfer and exchange of phospholipids among different lipoproteins. In vitro evidence has suggested that PLTP plays an important role in lipoprotein interconversion and remodeling. During lipolysis of VLDL, partially purified PLTP affected both the transfer and exchange of phospholipid between these particles and HDL (1). The PLTP gene belongs to a family that includes cholesteryl ester transfer protein, lipopolysaccharide binding protein, and bactericidal permeability increasing protein (2) PLTP can also cause conversion of HDL_3 to large and smaller particles in a time- and concentration-dependent fashion (3). Furthermore, PLTP activity on HDL can modulate the activities of LCAT and CETP in vitro (4, 5). There is also accumulating in vivo evidence that PLTP has a central role in lipoprotein metabolism. In PLTP transgenic mice, PLTP overexpression increases the influx of phospholipid, and secondarily of cholesterol, into HDL, leading to an increase in potentially antiatherogenic preβ-HDL particles (6–8).

Recently, we have developed PLTP gene knock-out (*Pltp* KO) mice. Our initial characterization of these animals indicates that: *1*) PLTP is the major agent facilitating transfer of VLDL phospholipid into HDL, *2*) reduced plasma PLTP activity causes markedly decreased HDL lipid and apolipoprotein, demonstrating the importance of transfer of surface components of triglyceride-rich lipoproteins in the maintenance of HDL levels, and *3*) on a high saturated fat diet, PLTP deficiency causes accumulation of lamellar lipoproteins, which are isolated in the IDL/LDL region by FPLC and in HDL by ultracentrifugation (9). In the previous study the centrifugally isolated HDL fraction

Abbreviations: apo, apolipoprotein; FPLC, fast protein liquid chromatography; KO, knock-out; PLTP, phospholipid transfer protein; *Pltp*, phospholipid transfer protein gene; TRL, triglyceride-rich lipoproteins; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; FCR, fractional catabolic rate; SM, sphingomyelin; CE, cholesteryl oleyl ether.

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contained both spherical HDL and lamellar lipoproteins, and the lamellar lipoprotein fraction isolated by FPLC was contaminated with VLDL and LDL (9). In the present study we have characterized purified HDL and lamellar lipoprotein fractions, and then carried out lipoprotein turnover studies. Our results indicate that inhibition of phospholipid transfer from apoB-containing particles to HDL in *Pltp* KO mice leads to changes in HDL composition and altered metabolic stability of these particles.

MATERIALS AND METHODS

Animals and diets used in this study

All phenotypic characterizations were performed with wild-type (WT) and *Pltp* KO homozygous littermates after backcrossing to C57BL/6. The N_4 generation (93% C57BL/6 and 7% 129 genetic background), 10–12 weeks old, was used for analysis. Female mice were used in all the studies. Two diets were used: Purina Rodent Chow (no. 5001) with or without 20% hydrogenated coconut oil and 0.15% cholesterol (Research Diets Inc. New Brunswick, NJ, USA).

Lipids and lipoproteins measurements

Fasting plasma was collected for lipoprotein isolation and lipid measurement. Total cholesterol, free cholesterol, and phospholipids in plasma and lipoproteins were assayed by enzymatic methods (Wako Pure Chemical Industries Ltd., Osaka, Japan). Cholesteryl ester concentration was calculated by subtracting the free cholesterol from total plasma cholesterol.

Isolation of phospholipid/free cholesterol/apoA-IV-rich particles

The PL/apoA-IV-rich particles were isolated from the plasma of *Pltp* KO mice fed the high saturated fat diet by ultracentrifugation followed by fast protein liquid chromatography (FPLC). Briefly, 500 μl of pooled *Pltp* KO mouse plasma (n = 5) was adjusted to density 1.063 g/ml and placed in a 5-ml tube; 4.5 ml of d 1.063 g/ml NaBr solution was overlaid on the plasma. The tube was sealed and spun at 98,000 rpm for 4 h in an Optima TL ultracentrifuge (Beckman, Brea, CA). The tube was sliced, the top 0.8 ml solution (VLDL and LDL) was discarded, and the bottom 4.2 ml solution was adjusted to density 1.21 g/ml. The tube was spun at 98,000 rpm for 12 h and then sliced. The top 0.8 ml of solution, which contains apoA-IV-rich particles and HDL, was desalted and concentrated to a volume of 220 μl with a Centricon 30 (Pharmacia). The final solution was loaded onto a Superose 6 column (Pharmacia) and eluted with 0.1 m Tris-HCl (pH 7.5) at a constant flow rate of 0.35 ml/min. The apoA-IV-rich particles were located in the LDL size-range fractions (fractions 16–19).

Western blot of apoA-IV, apoA-I, and apoE

To analyze the apolipoprotein composition, the isolated apoA-IV-rich particles (20 μl, corresponding to 50 μl plasma) were run on 4–20% gradient SDS-PAGE gels under reducing conditions. The proteins were then transferred to a nitrocellulose membrane (Bio-Rad Laboratories) and immunoblotted using a polyclonal anti-mouse apoA-I antibody, a polyclonal anti-mouse apoE antibody (Biodesign), and a polyclonal anti-rat apoA-IV antibody that cross-reacts with mouse apoA-IV. The signal was detected by chemiluminescence using the ECL kit (Amersham). The proportion of apolipoproteins among the particles was determined by quantitative scanning using a Phosphorimager (Molecular Dynamics). Each apolipoprotein band was individually quantitated by a customized box that isolated one band from another.

In vivo turnover studies

Except where otherwise noted, HDL was purified first by ultracentrifugation and then by gel filtration to exclude the apoA-IV-containing lipoprotein species described here. Isolated HDL was labeled with ^{125}I (Pierce Chemical Company, Rockford, IL), and [^{3}H]cholesteryl oleyl ether (CE) (NEN, Boston, MA) as described previously (10). For heterologous lipoprotein studies, *Pltp* KO and WT mice were injected intravenously in the femoral vein with WT HDL labeled with ^{125}I and [^{3}H]cholesteryl oleyl ether (1.1×10^6 and 0.95×10^6 cpm, respectively). For autologous lipoprotein studies, *Pltp* KO and WT mice were injected intraveously with their own HDL labeled with ^{125}I or [^{3}H]CE (3×10^6 and 2×10^6 cpm, respectively). After injection, blood (70 μl) was taken from the tail vein at 0.17, 0.5, 1, 2, 4, 8, and 24 h for determination of radioactivity. The fractional catabolic rates (FCR) for protein and lipid were calculated from the decay curves of ^{125}I and [^{3}H]CE radioactivity in whole plasma according to the Matthews method (11). The production rates were calculated by multiplying the FCR by the plasma pool and dividing by the body weight (12).

Electron Microscopy

Negative-stain electron microscopy was done as described (9).

Statistical analysis

Differences between groups were tested by Student's *t* test. Data are presented as mean ± SD.

RESULTS

Previously we showed that, on a high saturated fat diet, there was an increase in phospholipid (210%), free cholesterol (66%), and cholesteryl ester (40%) without change in apoB levels in the centrifugally isolated VLDL/LDL of *Pltp* KO mice (9). The excessive accumulation of phospholipids and free cholesterol suggested the presence of

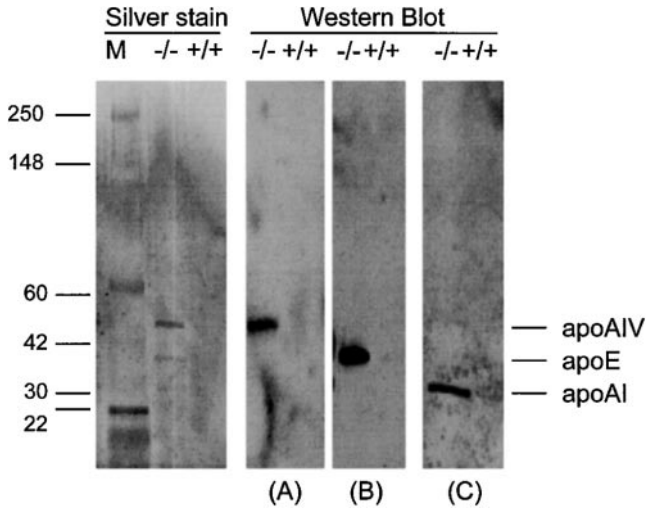

Fig. 1. Protein composition of lamellar lipoprotein fraction. Particles were isolated by ultracentrifugation followed by FPLC. Proteins were separated by SDS, 4–20% gradient polyacrylamide gel electropheresis under reducing conditions, and immunoblotted with a polyclonal anti-rat-apoA-IV antibody which can cross-react with mouse apoA-IV (A), a polyclonal anti-mouse apoE antibody (B), or a polyclonal anti-mouse apoA-I antibody (C), respectively.

lamellar lipoproteins in the non-HDL fraction and this was confirmed by negative stain electron microscopy (9). When isolated by ultracentrifugation, the lamellar particles were found in the HDL density range (9). Taking advantage of their physical characteristics, i.e, HDL density and IDL/LDL size (9), we isolated these lipoproteins in relatively pure form, using a combination of preparative ultracentrifugation followed by FPLC. Compositional analysis showed that these particles are composed of PC (50 ± 3%), SM (11 ± 1%), free cholesterol (17 ± 2%), cholesteryl ester (11 ± 2%), and protein (9 ± 1%). The protein composition was determined by SDS 4–20% gradient-PAGE run under reducing conditions. A major band (about 55% of total protein) with the size of apoA-IV and two minor bands (25 and 15%) with the sizes of apoE and apoA-I were visible (**Fig. 1**, left panel). Western blotting with specific antibodies confirmed that these proteins were indeed apoA-IV, apoE, and apoA-I (Fig. 1, right panel). To confirm that apoA-IV was enriched in these particles, we carried out Western blot analysis of FPLC fractions of the plasma of *Pltp* KO and WT mice, using anti-apoA-IV polyclonal antibody. This revealed an apoA-IV-containing lipoprotein particle accumulating in the IDL/LDL size-range fractions in FPLC (**Fig. 2**), where lamellar particles were observed (9).

Using negative-stain electron microscopy, these apoA-IV-rich particles appeared as a mixture of spherical and lamellar structures with a diameter of 40–50 nm (**Fig. 3**). Discoidal structures, some in stacks, were visible edge-on. The thickness of most of the stacked disks was 4–5 nm, although some were thicker, suggesting collapsed vesicles. Other round structures may represent vesicles or discs en face or spherical lipoprotein. However, the low content of neutral lipid in their fraction suggests a paucity of neutral lipid core-containing particles.

To characterize the metabolism of these apoA-IV-rich particles, they were labeled with ^{125}I and injected into *Pltp* KO mice (n = 5). We found that the fractional catabolic rate (FCR) for the protein associated with these particles was 0.20 ± 0.02 pools/h and the half life was about 45 min (**Fig. 4**). This was similar to the FCR of HDL protein in the *Pltp* KO mice (see below).

To determine the HDL composition, we pooled plasma from 5 *Pltp* KO and 5 wild-type (WT) mice, respectively, and took the HDL region from the FPLC profile, then we spun up the HDL at density 1.21 g/ml. The results from

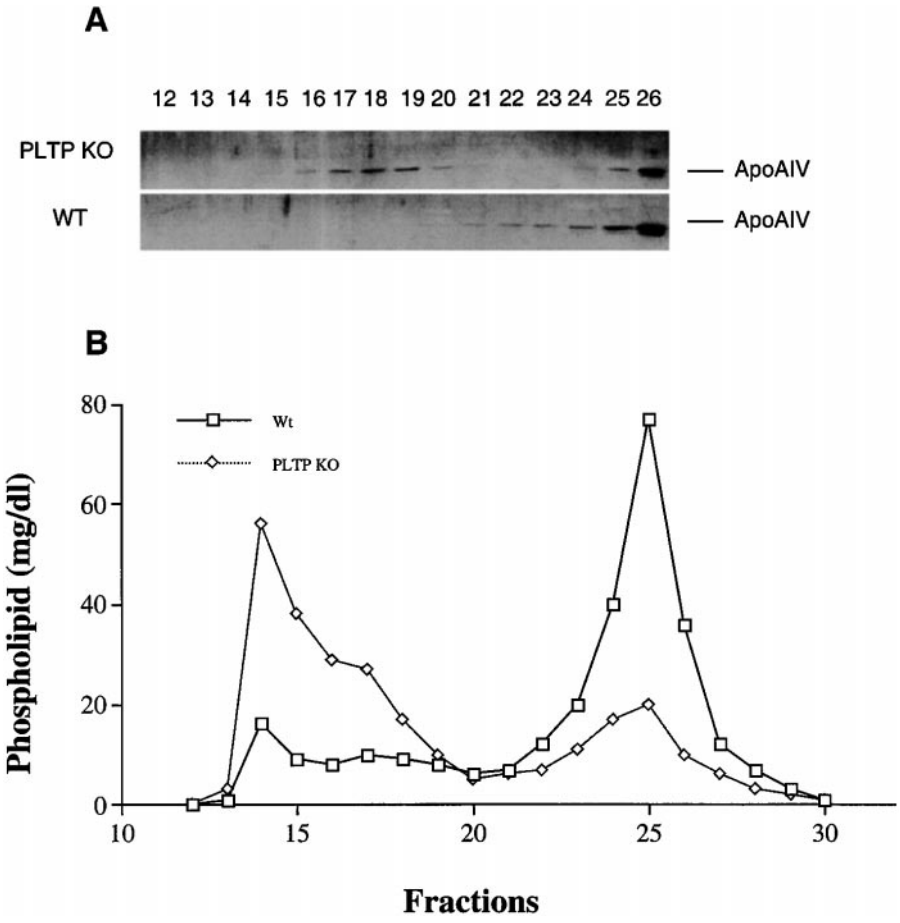

Fig. 2. ApoA-IV-rich particles accumulate in *Pltp* KO mouse plasma on a high saturated fat diet. A 200-μl aliquot of pooled plasma (from 6 animals) was fractionated on a Superose 6B column (1 ml/fraction). The indicated fractions were concentrated 10-fold, and 20 μl of solution was analyzed by Western blotting. A) Immunoblot of apoA-IV in each FPLC fraction; B) phospholipid distribution in each FPLC fraction.

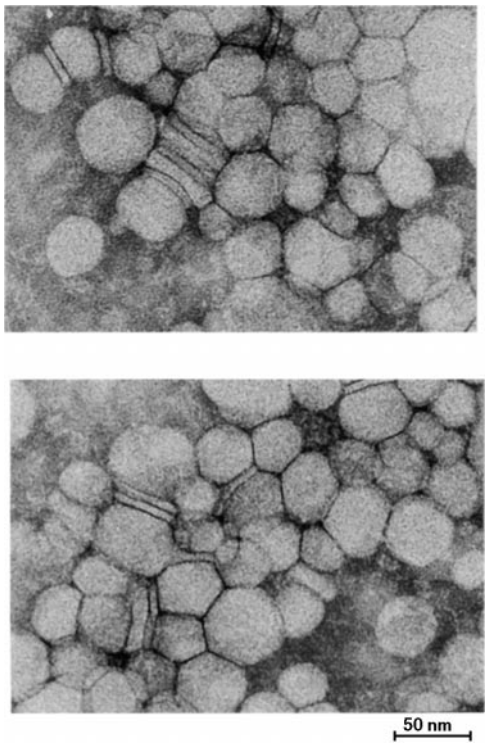

Fig. 3. Negative-stain electron microscopy of apoA-IV-rich particles purified from *Pltp* KO mouse plasma, pooled from 5 animals. Two representative views are shown.

three independent experiments showed that 58 ± 6% of the HDL mass of *Pltp* KO mice is protein, 42 ± 3% of the particle mass is lipid, while HDL particles from WT mice contain 41 ± 3% protein and 59 ± 5% lipid (**Table 1**). As shown in **Fig. 5**, the major HDL protein was apoA-I in both WT and *Pltp* KO mice. Among the lipids, it is notable that the HDL of *Pltp* KO mice is specifically deficient in phosphatidylcholine (PC), while the percentage composition of other lipids is not changed (Table 1). We also measured lipid and protein composition of the centifugally isolated VLDL and LDL of *Pltp* KO mice (i.e. without lamellar lipoproteins) and found that both lipoproteins are specifically depleted in sphingomyelin (SM) (Table 1). Thus, the PC/SM ratio was higher in VLDL and LDL and lower in HDL of PLTP KO animals compared to WT mice.

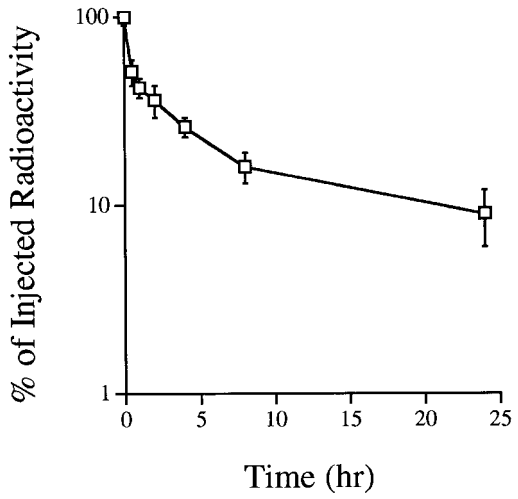

Fig. 4. Radioactivity decay curves for ^{125}I-labeled apoA-IV-rich particles. *Pltp* KO mice (n = 5) were injected intravenously with ^{125}I-labeled apoA-IV-rich particles, then 70 μl of blood was collected from the tail vein at the indicated time points; 35 μl of plasma was used for radioactivity measurement.

Our previous work revealed that in *Pltp* KO mice there is a marked 85% reduction in apoA-I levels (9). To understand the mechanism for this dramatic change, we carried out HDL turnover studies in PLTP KO and WT mice, using both autologous and heterologous HDL. We initially hypothesized that in *Pltp* KO mice the inhibition of phospholipid and apolipoprotein transfer from triglyceride-rich lipoprotein (TRL) particles into HDL would result in a predominant HDL synthetic defect.

In autologous turnover studies, *Pltp* KO and WT mice were injected with their own HDL, doubly labeled by [^{3}H]CE incorporation and protein iodination with ^{125}I. In this experiment, the FCRs of [^{3}H]CE-HDL, ^{125}I-labeled HDL and ^{125}I-labeled apoA-I in *Pltp* KO mice were about four times greater than for WT mice (**Fig. 6, Table 2**). Based on the FCR, body weight, and pool size, we calculated the production rates of HDL-CE and HDL-apoA-I for both WT and *Pltp* KO mice (**Table 3**), and found the difference to be insignificant.

In the heterologous turnover studies, HDL from WT mice was isolated and doubly labeled by incorporating [^{3}H]CE into its lipids and radioiodination of its proteins with ^{125}I. When this doubly labeled HDL from WT mice was injected into WT and *Pltp* KO mice, we found that the FCRs for both the [^{3}H]CE and ^{125}I-labeled apolipoprotein in *Pltp* KO were about twice those in WT mice (**Fig. 7, Table 4**). We isolated ^{125}I-labeled apolipoproteins in HDL from mouse plasma at each time point by ultracentrifugation and SDS-PAGE. The proportion of apolipoproteins among the particles was determined by quantitative scanning using a Phosphorimager (Molecular Dynamics). The FCRs of all apolipoproteins showed similar changes (Table 4).

DISCUSSION

In this study we have extended our characterization of the plasma lipoprotein changes in *Pltp* KO mice. The major novel finding was that low HDL in these animals arises from dramatically increased catabolism of HDL protein, possibly related to a decrease in PC content of HDL. Also, lamellar lipoproteins accumulating in these animals were found to be enriched in apoA-IV and apoE. Such particles could influence atherogenesis in these mice.

Low HDL in PLTP-deficient mice was found to be due primarily to a marked increase in catabolism of HDL particles, with similarly increased catabolism for both HDL proteins and CE. The increased catabolism is unlikely to be solely secondary to a decrease in HDL pool size as HDL from WT mice was catabolized more slowly than HDL from

TABLE 1. Lipid and protein composition in *Pltp* KO and wild-type (WT) mice lipoproteins

	SM	PC	FC	CE	TG	Protein	PC/SM	CE/FC	PL/TC	Protein/Lipid
				%						
VLDL										
WT	7.0 ± 0.2	29 ± 0.4	8.9 ± 0.4	25 ± 2.2	24 ± 4.2	6.6 ± 0.9	4.1 ± 0.2	2.8 ± 0.3	0.9 ± 0.1	0.07 ± 0.02
Pltp KO	3.6 ± 0.3	29 ± 1.0	9.9 ± 0.2	27 ± 3.9	23 ± 3.6	6.5 ± 0.3	8.1 ± 0.5	2.7 ± 0.6	1.0 ± 0.2	0.07 ± 0.03
P value	<0.001	NS	NS	NS	NS	NS	<0.001	NS	NS	NS
LDL										
WT	6.2 ± 0.1	29 ± 0.2	8.6 ± 0.3	30 ± 3.2	6.0 ± 0.5	21 ± 1.6	4.7 ± 0.2	3.5 ± 0.3	0.9 ± 0.2	0.27 ± 0.02
Pltp KO	4.8 ± 0.3	28 ± 1.0	8.8 ± 0.2	28 ± 3.3	6.6 ± 0.6	24 ± 0.3	5.8 ± 0.5	3.2 ± 0.1	0.9 ± 0.1	0.31 ± 0.05
P value	<0.05	NS	NS	NS	NS	NS	<0.02	NS	NS	NS
HDL										
WT	5.6 ± 0.7	28 ± 3.5	4.9 ± 0.6	17 ± 2.0	3.9 ± 0.3	41 ± 3.4	4.9 ± 0.9	3.5 ± 0.3	1.6 ± 0.3	0.7 ± 0.4
Pltp KO	4.5 ± 0.5	15 ± 2.5	4.2 ± 0.2	15 ± 1.9	3.4 ± 0.2	58 ± 5.6	3.4 ± 0.5	3.6 ± 0.6	1.0 ± 0.2	1.4 ± 0.8
P value	NS	<0.001	NS	NS	NS	<0.001	<0.02	NS	<0.05	<0.001

SM, sphingomyelin; PC, phosphatidylcholine; FC, free cholesterol; CE, cholesteryl ester; TC, total cholesterol; TG, triglyceride. In order to exclude the apoA-IV-containing particles from HDL of *Pltp* KO mice, we took the HDL region from the FPLC profile and spun it at density 1.21 g/ml. Other lipoproteins were purified by ultracentrifugation only.

Pltp KO mice when injected into *Pltp* KO mice (Table 4). Also, apoA-I KO mice do not show increased HDL catabolism despite a profoundly reduced HDL pool size (13). This indicates that a defect in the HDL per se is at least partly responsible for its increased catabolism. Compositional analysis indicated that the HDL of *Pltp* KO mice was enriched in protein and specifically depleted in PC. These changes presumably reflect the defect in the transfer of PC from TRL into HDL (9). The abnormal composition of HDL, and possible associated changes in physical properties, likely lead to hypercatabolism of apoA-I and HDL particles. An intriguing question is the mechanism of increased catabolism of these PC-depleted HDL particles.

These findings are reminiscent of the defect in Tangier disease, where extremely low HDL levels result from increased catabolism, independent of HDL pool size (14). In Tangier disease, a defect in the ATP-binding cassette transporter, ABC1, appears to cause a defect in transfer of

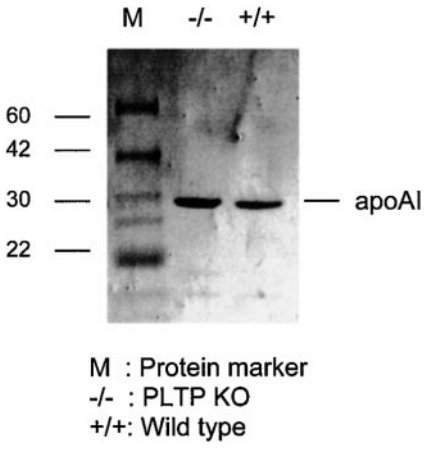

Fig. 5. SDS-PAGE analysis of apolipoproteins from WT and *Pltp* KO mouse HDL. HDL was isolated by FPLC followed by ultracentrifugation at d 1.21 g/ml. HDL containing equal amounts of cholesterol was loaded on a 4–20% SDS-polyacrylamide gradient gel and the apolipoproteins were stained by Coomassie Brilliant Blue.

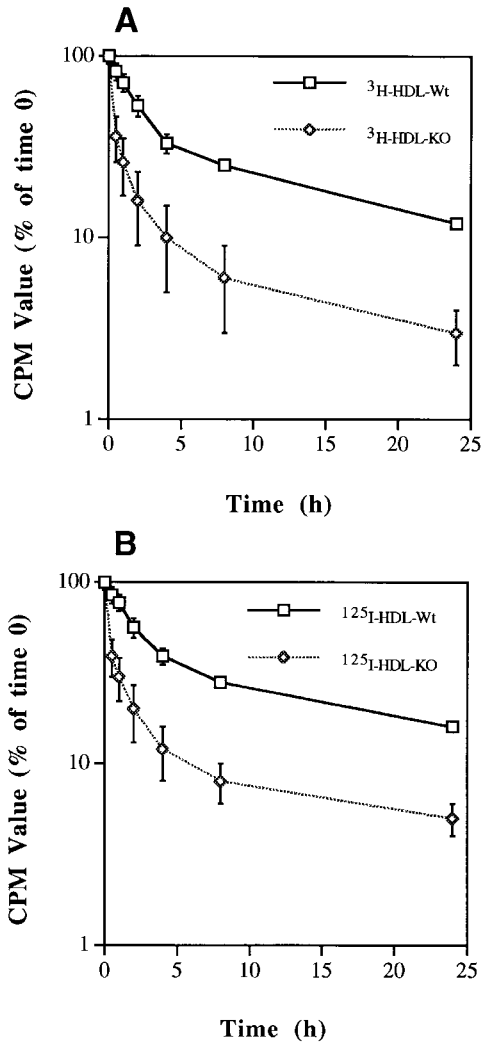

Fig. 6. Plasma fractional catabolic rates for ^{125}I- and [^{3}H]CE-labeled HDL in mice (autologous HDL). *Pltp* KO (n = 5) and WT mice (n = 5) were injected intraveously (femoral vein) with their own HDL labeled with ^{125}I and [^{3}H]CE. Blood (70 μl) was collected from the tail vein at 10 min, 0.5, 1, 2, 4, 8, and 24 h for determination of radioactivity. The values are mean ± SD.

TABLE 2. Plasma fractional catabolic rates for ^{125}I-labeled and [^{3}H]-cholesteryl oleyl ether-labeled HDL in mice (autologous)

Mice	[^{3}H]CE-HDL	^{125}I-labeled HDL	^{125}I-labeled ApoA-I
WT	0.096 ± 0.021	0.071 ± 0.012	0.080 ± 0.016
Pltp KO	0.361 ± 0.039	0.286 ± 0.052	0.296 ± 0.036
P value	<0.0001	<0.0001	<0.0001

Pltp KO and wild-type (WT) mice were injected intravenously (femoral vein) with their own HDL labeled with ^{125}I and [^{3}H]CE. Blood was collected periodically from mice over 24 h after injection of labeled HDL. The fractional catabolic rates (FCR) for protein and lipid were calculated from the decay curves of ^{125}I and [^{3}H]cholesteryl ester radioactivity in whole plasma according to the Matthews method (11). The values are means ± SD and significance was determined by Student's *t*-test.

TABLE 3. HDL-CE and HDL-apoA-I production rates in wild-type (WT) and *Pltp* KO mice (autologous)

Mice	HDL-CE	HDL-ApoA-I
	μg/g/h	
WT	13.3 ± 1.8	8.5 ± 1.2
Pltp KO	12.9 ± 1.3	8.1 ± 1.6

The production rates were calculated by multiplying the FCR (Table 2) by the plasma pool and dividing by the body weight (12). The value are means ± SD, and significance was determined by Student's *t*-test.

cellular phospholipids and cholesterol onto apoA-I (15–17). This may interfere with the interaction of apoA-I with the plasma membrane (18), and could also result in defective formation of nascent HDL in the liver. As pointed out by Young and Fielding (19), the defect in Tangier disease is more severe than in PLTP deficiency. This could indicate that the ABC1 molecule is involved in an earlier step during the biogenesis of HDL, forming nascent HDL particles which then develop into mature HDL particles as a result of PLTP and LCAT activities (**Fig. 8**).

There are some interesting similarities in the HDL changes of *Pltp* KO mice and those seen in humans or mice with abetalipoproteinemia (20–22). In human abetalipoproteinemia, HDL levels are moderately reduced, and the HDL has an increased SM/PC ratio (20). Moreover, low HDL reflects, in part, increased catabolism of HDL protein (23). In mice with liver-specific knock-out of microsomal triglyceride transfer protein, HDL cholesterol levels are reduced by about 50% (21). These similarities

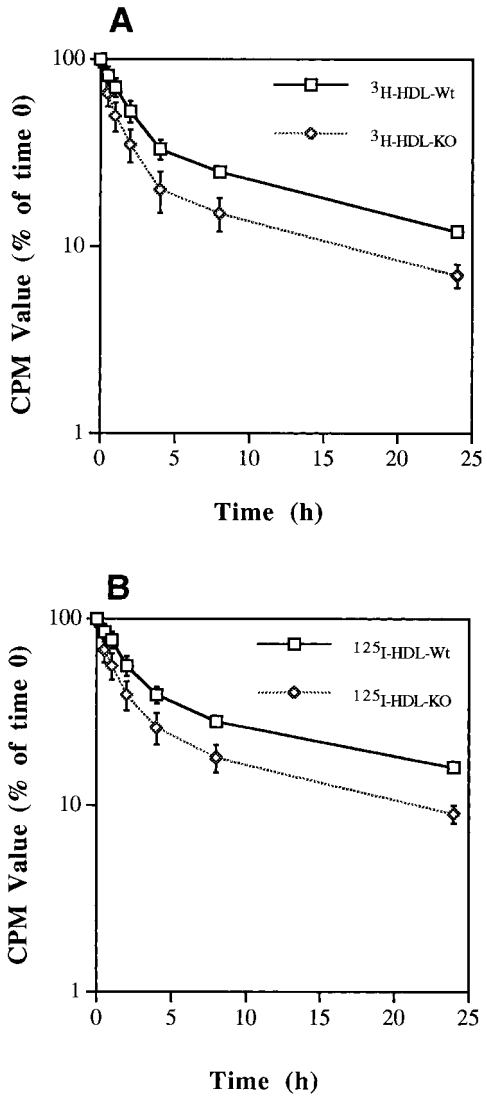

Fig. 7. Plasma fractional catabolic rates for ^{125}I- and [^{3}H]CE-labeled HDL from WT mice (heterologous HDL). *Pltp* KO (n = 5) and WT mice (n = 5) were injected intravenously (femoral vein) with WT HDL labeled with ^{125}I and [^{3}H]CE. Blood (70 μl) was collected from the tail vein at 10 min, 0.5, 1, 2, 4, 8, and 24 h for determination of radioactivity.The values are mean ± SD.

to the PLTP deficiency state suggest that the major source of phospholipids transferred by PLTP is derived from apoB-containing lipoproteins. However, PLTP is highly ex-

TABLE 4. Plasma fractional catabolic rates for ^{125}I-labeled and [^{3}H]cholesteryl oleyl ether-labeled HDL in mice (heterologous)

Mice	^{125}I-labeled ApoA-I	^{125}I-labeled ApoA-II	^{125}I-labeled-ApoC	^{125}I-labeled HDL	[^{3}H]CE-HDL
			pools/h		
WT	0.082 ± 0.008	0.077 ± 0.005	0.071 ± 0.008	0.072 ± 0.010	0.109 ± 0.02
Pltp KO	0.201 ± 0.019	0.195 ± 0.021	0.175 ± 0.025	0.166 ± 0.031	0.205 ± 0.028
P value	<0.001	<0.001	<0.001	<0.001	<0.001

Pltp KO and WT mice were injected intravenously (femoral vein) with WT HDL labeled with ^{125}I and [^{3}H]CE. Blood was collected periodically from mice over 24 h after injection of labeled HDL. The FCRs for protein and lipid were calculated from the decay curves of ^{125}I and [^{3}H]CE radioactivity in whole plasma according to the Matthews method (11). The values are means ± SD and significance was determined by Student's *t*-test.

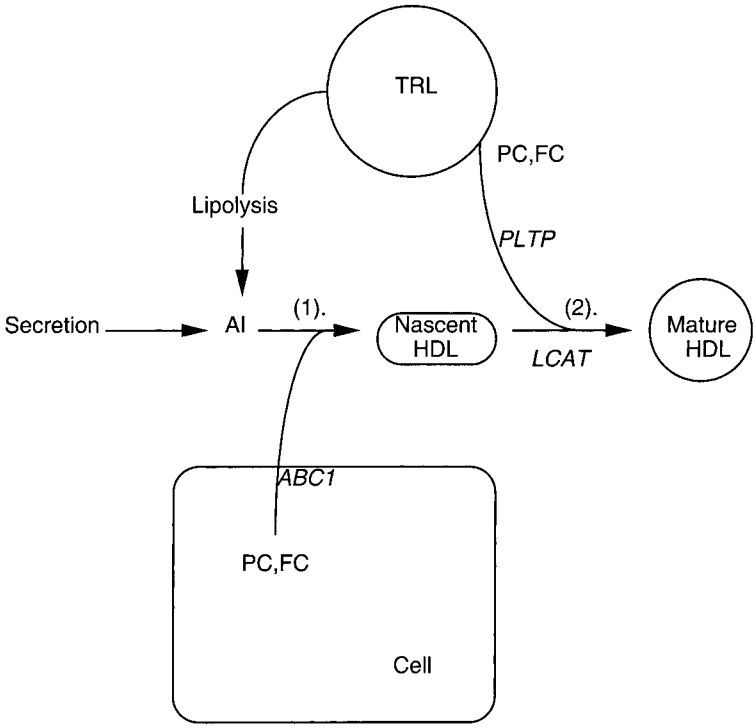

Fig. 8. A hypothetical scheme for the role of PLTP in the formation of mature HDL particles. In step 1, phospholipids and free cholesterol, effluxed from cells as a result of ABC1 activity, combine with apoA-I, generating nascent HDL. In step 2, nascent HDL acquires additional phospholipids and free cholesterol as a result of transfer from triglyceride-rich lipoprotein (TRL) by PLTP, and is acted on by LCAT, generating mature HDL. PC, phosphatidycholine; FC, free cholesterol; ABC1, ATP-binding cassette transporter 1; LCAT, lecithin:cholesterol acyltransferase; TRL, triglyceride-rich lipoproteins; AI, apolipoprotein A-I.

pressed in several peripheral tissues such as lung and adipose tissue and PLTP has been reported to promote cellular phospholipid efflux to HDL (24). This could represent an additional source of phospholipids transferred into HDL by PLTP.

Human subjects with low HDL levels typically have increased catabolism of HDL protein. A significant subset of these individuals have mutations in ABC1 without having features of classic Tangier disease (15). The present results suggest that hypoalphalipoproteinemia associated with increased catabolism of HDL protein could also result from defective transfer of phospholipids from TRL into HDL. Theoretically, this could result from defects in the PLTP gene (though none have yet been found) or from defective lipolysis. There are several common LPL variants that are associated with hypertriglyceridemia and decreased HDL levels (25). Defective phospholipid transfer secondary to the lipolytic defect could cause low HDL due to increased catabolism of HDL particles, as in the PLTP-deficient mouse. This is a mechanism distinct from the currently held view that increased core lipid exchange mediated by CETP leads to low HDL in hypertriglyceridemia (26).

Once freed of lamellar particles, the VLDL and LDL were found to be specifically deficient in SM (Table 1). This is interesting because nascent VLDL isolated from the rat Golgi is deficient in SM (27). Thus, it is possible that SM enters the lipoprotein system predominantly through HDL (e.g., from the interaction of apoA-I with plasma membrane (18)), and is transferred to VLDL and LDL by PLTP. SM may be an important component of atherogenic lipoproteins related to its interaction with sphingomyelinase in the arterial wall (28).

Another striking feature of the PLTP-deficient mice fed a high saturated fat diet was the accumulation of lamellar lipoproteins, enriched in PC, free cholesterol, apoA-IV, and apoE. These particles presumably represent surface material derived from TRL that fails to be incorporated into HDL as a result of PLTP deficiency. It is notable that these particles are only present in mice fed a high saturated fat, coconut oil-based diet, and are not seen in mice fed chow (9) or a milk fat-based Western diet (unpublished). The enrichment with apoA-IV could indicate a specific role of this apolipoprotein in the phospholipid transfer process, as suggested by some previous experiments (29). It is more likely, however, that apoA-IV is attracted to the surface material by mass action, or that the apoA-IV particles are surface components detached from triglyceride-rich particles that have lost core volume after lipoprotein lipase activity. Recently, Bergeron et al. (30) have demonstrated the accumulation of lamellar lipoproteins enriched with apoA-IV as a major constituent in compound *Hl* KO/*Apoe* KO mice. These workers described signet ring structures which we did not observe in our studies, even though the lamellar fraction did contain significant amounts of neutral lipid. Bergeron et al.(30) speculated that these structures might represent TRL remnants that circulated for an extended period allowing depletion of core lipid by selective uptake in the liver. Interestingly, the accumulation of these particles in *Hl* KO/*Apoe* KO mice was associated with an anti-atherogenic effect. Thus, it will be of interest to determine whether the lamellar lipoprotein fraction influences atherogenesis in the *Pltp* KO mice.

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