Abnormal Phospholipid Composition Impairs HDL Biogenesis and Maturation in Mice Lacking Abca1[†]

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ABSTRACT: Recent studies have demonstrated that the ATP-binding cassette transporter A1 (ABCA1) facilitates the efflux of phospholipids and cholesterol to apoprotein acceptors, leading to the synthesis of HDL. The purpose of this study was to determine the changes in the lipoprotein fractions in Abca1-deficient mice and study the mechanisms responsible for the low levels of HDL when ABCA1 is absent. Plasma phospholipid concentration was decreased by more than 75%, mostly due to a reduction of phosphatidylcholine (PC) in HDL. Abca1$^{-/-}$ HDL represents less than 2% of wild-type levels and is smaller and enriched in phospholipids (11.2-fold more than HDL from controls). Compared to wild-type littermates, Abca1$^{-/-}$ HDL had a 4-fold increase in PC, whereas lysophosphatidylcholine (LPC) (125-fold), sphingomyelin (SPH) (49-fold), and phosphatidylethanolamine (PE) (18-fold) showed even higher increases. As a consequence, the ratios of LPC/PC, SPH/PC, PE/PC, and phosphatidylinositol + phosphatidylserine (PI+PS)/PC were all much higher in HDL from Abca1$^{-/-}$, compared to wild-type HDL. Plasma phospholipid transfer protein (PLTP) and lecithin cholesterol acyltransferase (LCAT) activities were decreased by more than 80%, suggesting that the maturation of HDL is affected. To test this hypothesis, plasma from Abca1$^{-/-}$ mice was incubated with CHO cells that are known to express high levels of ABCA1 with the intent of restoring the flux of phospholipid and cholesterol onto apoAI. Compared to native plasma, no change in maturation of HDL was observed. In contrast, a 220% increase in the formation of mature HDL was observed when ABCA1 function and LCAT activities were restored. Taken together, these observations suggest that ABCA1 is necessary for the adequate lipidation of apoAI, which enables the interaction with LCAT and subsequent maturation.

ATP-binding cassette transporter A1 (ABCA1)[1] is a transporter that is crucial for the synthesis of high-density lipoproteins (HDL). Since its discovery, the relationship between expression, lipid efflux and HDL synthesis has been a subject of intense investigation. ABCA1 is present in all tissues and cell lines examined to date (*1*, *2*), and its expression is regulated by intracellular cholesterol levels, particularly in macrophages (*1*). In addition, ABCA1 activity is increased by protein kinases (*3*), reduced by IFNγ (*4*) and geranylgeranyl pyrophosphate (*5*), and modulated at the transcriptional level by cAMP (*2*, *6*, *7*) and agonists of the nuclear receptor LxR (*7*−*10*). From its primary sequence, ABCA1 appears to be a pore-forming protein consisting of 6 + 6 transmembrane domains connected by a hydrophobic segment (*11*). ABCA1 facilitates the efflux of phospholipid, mostly phosphatidylcholine (PC), toward nascent apolipoproteins, which can then accept cholesterol forming a lipid-poor particle, triggering the maturation and formation of HDL. Several apolipoproteins mediate phospholipid and cholesterol efflux by the ABCA1 pathway. Apolipoprotein AI (apoAI), the most abundant and most studied apoprotein on HDL, shows the greatest ability to efflux cholesterol (*2*, *12*); however, all exchangeable apolipoproteins such as AII, AIV, C-I, C-II, C-III, and E promote ABCA1 dependent efflux. The physiological relevance and contribution of various apoproteins to efflux and synthesis of HDL remain unclear. It is likely, however, that the overall level of lipid efflux depends on the relative concentration of apoproteins in the extracellular fluid rather than the plasma compartment and is determined by the rate of synthesis, catabolism, and cycles of dissociation and reassociation with lipoproteins in the plasma compartment. Studies in patients with Tangier Disease (*13*−*15*) and mice lacking ABCA1 (*16*, *17*) demonstrated a critical role of ABCA1 in the generation of HDL. In these models, plasma levels of HDL are virtually undetectable, implying that the lipidation of apolipoproteins

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[1] Abbreviations: ABCA1, ATP-binding cassette transporter A1; ApoAI, apolipoprotein A1; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); LCAT, lecithin:cholesterol acyltransferase; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PLTP, phospholipid transfer protein; SPH, sphingomyelin.

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at the plasma membrane of cells expressing ABCA1 is the first and foremost important step in the biogenesis of HDL. It has been proposed that when ABCA1 is absent or not functional, apoproteins do not acquire cellular lipids necessary to form nascent particles; thus, the transport of lipids among plasma lipoproteins does not occur, and apoAI-containing particles are cleared rapidly from the circulation (*18*, *19*). Although a significant amount of cholesterol and phospholipids present in HDL is derived from triglyceride-rich lipoproteins (*20*), plasma apoAI and HDL levels remain unchanged in ABCA1-deficient mice fed either a chow or a high-fat and high-cholesterol diet (*16*), indicating that cholesterol and phospholipid from apoB-containing lipoproteins are incapable of substituting for cellular lipids. Although the relative contribution of the various biochemical processes leading to the synthesis and maturation of HDL is not well understood, the phenotype observed in mice lacking ABCA1 suggests that several processes involved in the maturation of HDL are affected. In this study, we have used mice lacking Abca1 to characterize the changes in the lipoproteins and determine the mechanisms responsible for the low levels of HDL when ABCA1 is absent. The findings from these studies indicate that the absence of ABCA1 leads to profound changes in the phospholipid composition of HDL that alter their metabolic stability and maturation.

MATERIALS AND METHODS

Animals. ABCA1 deficient (Abca1$^{-/-}$) mice were created in DBA1 lac/J background as described previously (*16*). Abca1$^{-/-}$ and control littermates were maintained on a 12-hour light/dark cycle and fed a chow diet. All animal experiments were performed according to protocols approved by the institutional animal care and use committee.

Plasma Lipids, Lipoprotein Analysis, and Characterization. All phenotypic characterizations were performed in Abca1$^{+/+}$, Abca1$^{+/-}$, and Abca1$^{-/-}$ littermates fed a chow diet. Plasma was isolated from blood collected from the retro-orbital plexus and spun at 3000 rpm for 15 min at 4 °C. HDL was separated from non-HDL lipoproteins by dextran sulfate precipitation as described previously (*16*). Individual lipoprotein subclasses were isolated from 200 μL pooled plasma from the three different genotypes using two tandem Superose 6 columns (Pharmacia LKB Biotechnology, Piscataway, NJ) as previously described (*16*). Fractions corresponding to VLDL, LDL, and HDL were pooled and concentrated using Slide-A-Lyzer concentrating solution (Pierce, Rockford, IL). Cholesterol, phospholipids, and triglycerides were determined using enzymatic colorimetric assays (Wako Biochemicals, Osaka, Japan). ApoAI and apoB were determined by ELISA (*21*).

Further characterization of the HDL species in Abca1$^{-/-}$ and wild-type mice was carried out by two-dimensional gradient gel electrophoresis (*22*). In short, the first-dimension gradient gel electrophoresis was run on a 0.75% (wt/vol) agarose in 50 mM barbital buffer on Gelbond (FMC, Rockville, ME) and subsequently run on a 3−16% polyacrylamide gradient gel in 25 mM tris-glycine buffer (pH 8.3). Electrophoresis was carried out for 4.5 h. Plasma proteins were transferred to NitroPlus transfer membranes and then immunoreacted with a rabbit polyclonal antibody to apoAI.

Analysis of Phospholipid Species in Plasma and Lipoprotein Fractions. The total lipids present in either plasma or lipoprotein fractions were extracted as described by Bligh and Dyer (*23*). Phospholipids were separated on silica gel TLC plate, using chloroform:methanol:acetic acid:0.15 M NaCl (60:30:10:3 v/v). The lipids were visualized by exposure to iodine vapors, and spots corresponding to the various phospholipid standards were scraped from the plate. The phosphorus content in each spot was determined by the modified Bartlett procedure (*24*) using KH_2PO_4 as standard. In certain experiments, the HDL fraction was passed through a protein G (HiTrap Protein G HP (Amersham Pharmacia Biotech AB, Uppsala, Sweden) cross-linked to rabbit antiserum to mouse albumin (ICN, Costa Mesa, CA) to remove the albumin. This procedure removed over 80% of the albumin present in the HDL fraction. HDL lipid composition was not affected, as demonstrated by the cholesterol to apoAI ratios (7.5 and 6.8 before and after the column, respectively). Removal of albumin from HDL was also accomplished by using a combination of ultracentrifugation and fast protein liquid chromatography. Plasma lipoproteins from either Abca1$^{-/-}$ or wild-type mice were obtained by ultracentrifugation at $\delta \leq 1.25$ in a SW41 rotor at 40 000 rpm for 60 h at 15 °C. Potassium bromide was used for density adjustments. Isolated lipoproteins were extensively dialyzed against 0.9% NaCl, 1 mM EDTA, and 0.02% sodium azide to remove the KBr and then applied onto 2 tandem Superose 6 columns to isolate individual lipoprotein classes. Fractions corresponding to the HDL fractions were pooled and assayed for LPC and apoAI. Apoprotein composition was examined by using a 4−12% polyacrylamide gradient gel followed by silver staining using the Proteo Silver Staining Kit (Sigma Chemical Co., St. Louis, MO) following manufacturer instructions.

Determination of Lecithin:Cholesterol Acyltransferase (LCAT) Activities. LCAT activity was measured as the rate of synthesis of ^{3}[H]-cholesteryl esters from unilamellar vesicles prepared with a French pressure cell and activated with human apoAI (Sigma Chemical Co) to form discoidal synthetic lipoproteins (*25*). 1,2-[^{3}H]Cholesterol (New England Nuclear, Boston, MA) was purified by TLC on silica gel plates developed in cyclohexane/ethyl acetate (60:40, v/v). Liposomes contained egg lecithin (800 μg/mL), unesterified cholesterol (100 μg/mL, specific activity 5×10^5 cpm/μg), and apoAI (100 μg/mL). Each assay mixture contained 50 μL of the dispersed lipid, an equal volume of recrystallized human albumin (15%, w/v) (Sigma Chemical Co) in buffer containing 60 mM phosphate at pH 7.4, 198 μL 150 mM NaCl, and 2 μL mouse plasma in a total assay volume of 300 μL. LCAT activities were measured over 20 min at 37 °C. The reaction was stopped by adding 900 μL chloroform/methanol/H_2O (4:4:1, v/v) and was lipid-extracted. After separation of the phases, the content of [^{3}H]-cholesteryl ester radioactivity in the organic phase was determined by TLC on silica gel plates developed in hexane/diethyl ether/acetic acid (83:16:1, v/v). Cholesteryl ester radioactivity was determined by liquid scintillation counting. LCAT activity was linear up to 30 min of incubation at 37 °C and independent of the concentration of plasma lipoproteins. Therefore, a decrease in the esterification rate corresponds to a decreased level of LCAT protein.

LCAT-Mediated Lipoprotein Cholesterol Esterification. Blood was drawn from wild-type and Abca1$^{-/-}$ mice in tubes containing lithium heparin and plasma was obtained by centrifugation at 3000 rpm for 15 min at 4 °C. LCAT activity was immediately inhibited by adding 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) to a final concentration of 1.5 mM. Cholesterol esterification in plasma was determined by the method described by Stokke and Norum (*26*). In short, the incubation mixture containing 36 μL of DTNB-treated plasma and 9 μL of albumin-stabilized emulsion of 1,2-[^{3}H]-cholesterol (7000−10 000 cpm/μL) was incubated at 37 °C for 4 h to allow tracer equilibration with endogenous lipoprotein cholesterol. The enzyme was reactivated by adding β-mercaptoethanol to a final concentration of 10 mM, and synthesis of lipoprotein-derived cholesterol was monitored for 30 min at 37 °C. The rate of cholesterol esterification is linear up to 60 min. ^{3}H-Cholesteryl esters were separated by TLC as described earlier (*25*) and radioactivity measured by a liquid scintillation counter.

Determination of Phospholipid Transfer Protein (PLTP) Activity. Plasma PLTP activities were assayed in duplicate, by measuring the transfer of [^{3}H]-dipalmitoyl phosphatidylcholine (DPPC) and [^{14}C]-sphingomyelin (SPH) from liposomes to HDL, as previously described (*27*). In this system, the measured PLTP activity varies linearly with the amount of plasma added to the incubation mixture (about 1 μL). Activity is measured in the presence of excess exogenous HDL and expressed as % of reference pool plasma, which is included in each run. The PLTP activity of the reference plasma is virtually identical for labeled DPPC and SPH (15.0 and 14.5 μmol/mL plasma/h, respectively), and the within-run variation coefficient of the assay is 3.5%. All samples were analyzed using the same batch of substrates.

Isolation of Mouse Hepatocytes. Mouse hepatocytes were isolated using a modification of the method described by Sattler et al. (*28*). In short, mice were anesthetized by intraperitoneal injection of Nembutal and placed on a heating pad. The portal vein was ligated using a 25 3/4G-butterfly needle, the vena cava cannulated through the right atrium, and the inferior vena cava ligated above the kidneys. The liver was perfused with oxygenated (95% O_2, 5% CO_2) Hanks balanced salt solution (HBSS) without Mg^{2+} or Ca^{2+} for 15 min at 7 mL/min rate. Subsequently, the perfusion was continued at 5 mL/min with 0.1% collagenase type IV (Sigma) in HBSS supplemented with 2 mM calcium chloride for 20−30 min. The perfused liver was placed in oxygenated HBSS media containing collagenase for an additional 10 min with constant agitation. Cells were filtered through a 70-micrometer cell strainer (BD Biosciences) and spun at 1000 rpm for 5 min. Cell suspension was resuspended in DMEM/F12 containing 1% bovine serum albumin, and an equal volume of a solution containing nine parts Percoll (Sigma) and one-part 10X Hanks buffer pH 7.5 was added. Following a 15-minute spin at 1500 rpm, the pellet was resuspended in DMEM/F12 media containing 10% fetal bovine serum. Cell viability was determined by Trypan blue exclusion, and the hepatocytes were plated at 600 000 cells/well in collagen coated six well plates (BD Biosciences) for 4−6 h prior to the start of the experiments. Cells were incubated at 37 °C in the presence of DMEM/F12 and 10% fetal bovine serum, 2 mM glutamine, and 40 μg/mL gentamicin, and the accumulation of apoAI in the culture medium was determined over a 16 h period by ELISA as previously described (*21*).

Role of Cellular Cholesterol Efflux, LCAT, and PLTP in the Maturation of HDL from Abca1$^{-/-}$ Mice. Blood was obtained from Abca1$^{-/-}$ mice and collected into ice-cooled tubes containing streptokinase (300 IU/mL final concentration) as anticoagulant. Plasma was obtained by centrifugation at 3000 rpm for 15 min at 4 °C diluted into DMEM/F-12 at a concentration of 60% and then incubated for 6 h at 37 °C with either wild-type or LCAT-expressing CHO cells grown in DMEM/F-12 medium containing 10% fetal calf serum and 40 μg/mL gentamicin. CHO cells expressing human LCAT were generated by transfecting a pBK CMV vector (Stratagene, Cedar Creek, TX) containing the entire human LCAT gene ligated into XhoI/SmaI sites into CHO K1 cells (ATCC, CCL 61, Rockville, MD). LCAT activity was determined as the rate of synthesis of ^{3}H-cholesteryl esters from apoAI-activated unilamellar vesicles. LCAT activity in the culture medium was 15.3 nmol CE formed/mL/h after 6 h at 37 °C. To assess the role of PLTP in HDL synthesis and maturation, purified human PLTP (*29*) (specific activity: 80 μmol/mL/h) was added to the plasma to a final activity of 250 nmol/h/mL and incubated with either wild-type or LCAT expressing cells as described above. An additional experiment was performed in which human PLTP was added to the plasma to a final activity of 26 000 nmol/h/mL to reach similar activity levels to those found in mice or human plasma (*30*, *31*). Culture media were collected after 6 h at 37 °C and HDL species separated immediately by two-dimensional gradient gel electrophoresis and transferred onto nitrocellulose membranes. Apo AI-containing HDL species were visualized by using a rabbit polyclonal antibody to mouse apoAI. The proportion of apoAI among the preβ and α-HDL species was determined by quantitative scanning using a phosphoimager. Formation of mature HDL was determined by measuring the increase in apoAI in the α-HDL region and expressed as the percent increase in the apoAI within the α-HDL region compared to plasma incubated in the absence of cells.

RNA Isolation and Northern Blot Analysis. Total liver and intestinal RNA was isolated by using TRISOLV reagent. After extraction, RNA was precipitated with 2-propanol, and its integrity was assessed by agarose gel electrophoresis. Five micrograms of RNA was separated in a 1% agarose gel containing 2.2 M formaldehyde and transferred to Nylon membranes, crossed-linked, and hybridized to a 278 bp apoAI cDNA (residues 161 to 436 in murine apoAI cDNA) and an exon 6 murine LCAT cDNA probes using the rapid hybridization system (Amersham Corp, Arlington Heights, IL) according to the manufacture's conditions. To verify equal loading, the probes were stripped, and the membranes were rehybridized with a mouse 18S RNA probe (Ambion, Austin, TX).

Statistical Analysis. The results are expressed as means ± SD. The statistical significance of the differences between the groups was estimated by the Student's *t*-test.

RESULTS

Plasma Lipids and Lipoprotein Analysis. Plasma triglycerides, cholesterol, HDL-cholesterol, and apoAI levels in Abca1 homozygous, heterozygous, and wild-type control

Table 1: Total Cholesterol, HDL Cholesterol, Triglycerides, and ApoAI Levels in Wild-type, Heterozygous, and Homozygous Abca1 Mice[a]

mice	triglycerides	total cholesterol	HDL cholesterol	apoAI
+/+	164.5 ± 52.5	66.3 ± 6.7	38.5 ± 4.0	70.7 ± 5.3
+/−	167.8 ± 37.6	33.3 ± 1.9[a]	23.1 ± 3.2[a]	41.5 ± 7.0[a]
−/−	139.3 ± 40.1	6.3 ± 4.5[b]	4.1 ± 3.0[b]	1.1 ± 0.2[b]

[a] Blood was collected from the retro orbital plexus and spun at 3000 rpm for 15 min at 4 °C to isolate plasma. The total cholesterol, triglycerides, and HDL cholesterol were determined by enzymatic method and expressed as mg/dl. HDL was separated from apoB containing lipoproteins by dextran sulfate precipitation. ApoAI was measured by ELISA and expressed as mg/dL of plasma. Values are means ± SD based on analysis of individual mouse plasma and represent 6−10 animals per group. [b] $p < 0.01$. [c] $p < 0.001$ compared to wild-type littermates.

littermates are shown in Table 1. Consistent with previous studies (*16*), Abca1-deficient mice were severely hypocholesterolemic and had a near complete absence of HDL. Plasma levels of LDL were also reduced in mice lacking Abca1. The distribution of cholesterol, phospholipids, and apoAI among plasma lipoproteins is shown following separation of lipoprotein classes by FPLC (Figure 1) and demonstrates a gene-related dose-dependent decrease in HDL and apoAI with almost undetectable levels in Abca1$^{-/-}$.

Secretion of ApoAI by Primary Hepatocytes. To investigate whether the low levels of HDL and apoAI observed in mice lacking Abca1 resulted from an impairment in apoAI production by the liver, hepatocytes were isolated from Abca1$^{-/-}$ (*n*: 4) and control littermates (*n*: 4), plated on collagen coated plates, and the accumulation of apoAI in the culture medium was determined over a 16 h period by ELISA. As shown in Figure 2A, no difference was found in the amount of apoAI secreted by hepatocytes isolated from the two genotypes. Further support for this hypothesis comes from the cellular levels of apoAI mRNA in the two groups of mice. Liver apoAI mRNA levels were not decreased but rather slightly increased in Abca1$^{-/-}$ mice (Figure 2B, lanes A−D and E−I for wild type and Abca1$^{-/-}$, respectively) compared to wild-type littermates (ApoAI/18S RNA 0.4 ± 0.1 (*n*: 4) and 0.7 ± 0.2 (*n*: 5) for wild-type and Abca1$^{-/-}$ mice, respectively). Since human and rodent intestines synthesize apoAI (*32*, *33*) and contribute to circulating levels of HDL, intestinal apoAI mRNA levels were also examined in homozygous Abca1-deficient mice (*n*: 5) and control littermates (*n*: 4), shown in Figure 2C (lanes A to E and F to I for wild type and Abca1$^{-/-}$, respectively). No difference was found between genotypes as demonstrated by the apoAI/18S RNA ratios (0.98 ± 0.19 and 0.74 ± 0.07 for wild type and Abca1$^{-/-}$, respectively). Although the secretion of apoAI by enterocytes has not been determined, the intestinal mRNA levels suggest that the secretion of apoAI is not impaired. Therefore, these studies strongly suggest that the low levels of apoAI in Abca1$^{-/-}$ mice cannot be attributed to changes in apoAI production.

HDL Characterization and Lipid Composition. HDL from Abca1$^{-/-}$, which represents less than 2% of wild-type levels, is distributed among several populations located within the preβ size interval (Figure 3) described for human plasma (*34*). When plasma was run in a two-dimensional gradient gel to equilibrium, no apoAI with a molecular weight corresponding to free apoAI was observed, suggesting the

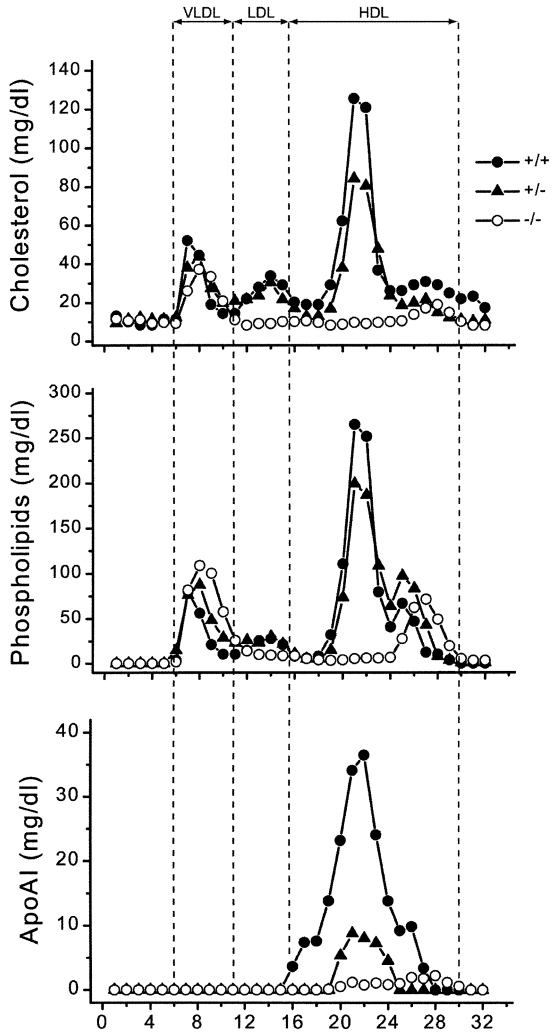

FIGURE 1: Plasma lipoprotein cholesterol, phospholipid, and apoAI distribution in mice of different genotypes. Two-hundred microliters of pooled plasma from the three genotypes was fractionated by FPLC as described under Materials and Methods. Cholesterol, phospholipids, and apoAI were determined and plotted as a function of FPLC fractions. The position at which known lipoproteins eluted from the column is indicated.

absence of nonlipidated forms of apoAI. Although these results do not rule out a poorly lipidated form of apoAI, they do suggest that all the apoAI migrating in the preβ position have the same electrophoretic characteristics as preβ species present in wild-type mice. Also, no detectable HDL within the α HDL size interval is present in mice lacking Abca1, suggesting a defect on the ability of lipid poor apoAI to develop into mature HDL species. As apoAI secretion is not affected by the absence of Abca1 and several studies have suggested that ABCA1 facilitates the transport of phospholipids and cholesterol, we sought to examine whether the composition of the residual HDL particles was altered in mice lacking Abca1 compared to heterozygous and wild-type mice. Therefore, pooled plasma samples from all three genotypes were fractionated by FPLC, and fractions corresponding to HDL were pooled and concentrated to determine their lipid composition. Abca1$^{-/-}$ HDL are smaller in size than wild-type and heterozygous HDL (Figure 1). The phospholipid composition is significantly different from wild-type HDL or preβ-HDL (*35*). Compared to HDL isolated from control littermates, the HDL from Abca1$^{+/-}$ and Abca1$^{-/-}$ contained

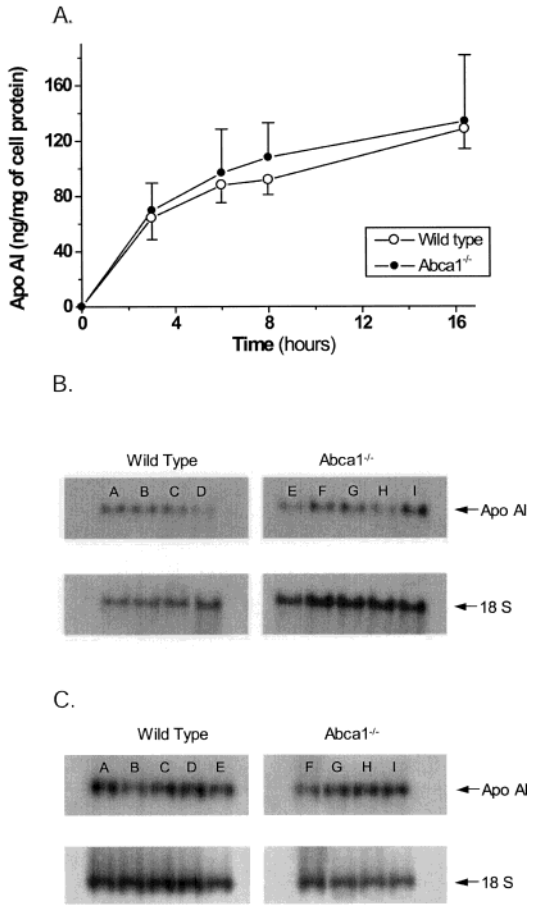

FIGURE 2: ApoAI secretion and mRNA levels. (A) Secretion of apoAI from primary hepatocytes isolated from either Abca1$^{-/-}$ (closed circles) or wild-type littermates (open circles) as described under Materials and Methods. (B) Liver apoAI mRNA levels in wild-type (lanes A to D) and Abca1$^{-/-}$ (lanes E to I) mice. Total RNA was isolated from liver, electrophoresed, transferred to a Nylon membrane, and hybridized to mouse apoAI or 18S probes. (C) Intestinal apoAI mRNA levels in wild-type and Abca1$^{-/-}$ mice. Total intestinal RNA was isolated from small intestine of wild-type (lanes A−E) and Abca1$^{-/-}$ (lanes F−I) deficient mice, electrophoresed in 1% agarose gels as described under Materials and Methods. To verify for equal loading, the probes were stripped and the membranes rehybridized with a murine 18S RNA probe.

more phospholipids per μg of apoAI (2- and 11.2-fold respectively, Table 2). In addition, the lipid composition also revealed an increased percentage of triglycerides in HDL isolated from homozygous Abca1-deficient mice. These findings are consistent with previous studies published on the composition of HDL isolated from patients with Tangier disease (*18*) and support the presence of a lipid core as previously demonstrated for preβ_1-HDL particles isolated from follicular fluid (*35*). No apoB was detected.

Phospholipid Composition of Plasma and Lipoprotein Fractions Isolated from Mice from the Three Different Genotypes. Although the preceding findings demonstrate the presence of phospholipids associated with apoAI, the composition and proportion of different phospholipid species in HDL isolated from Abca1$^{-/-}$ is unknown. Therefore, HDL was isolated from mice from the three different genotypes by FPLC and concentrated, and the phospholipid composition was determined by silica gel TLC and lipid phosphorus estimation. The distribution of phospholipid subclasses within HDL isolated from Abca1$^{-/-}$, Abca1$^{+/-}$, and wild-type mice are shown in Table 3.

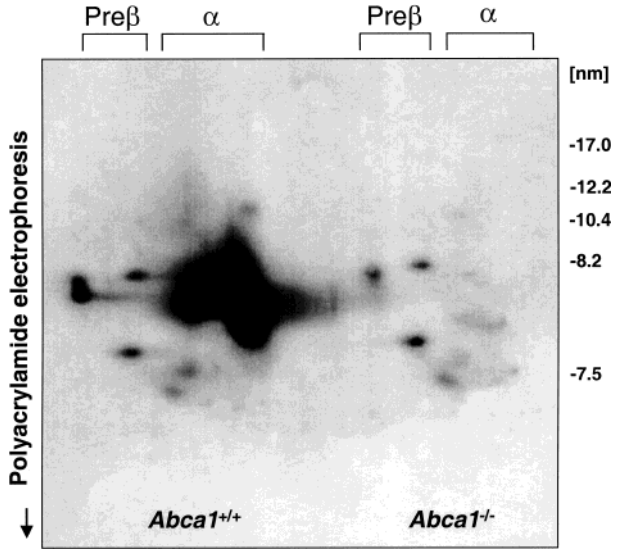

FIGURE 3: Distribution of apoAI in plasma from Abca1$^{-/-}$ and wild-type mice. Wild-type (Abca1$^{+/+}$) and Abca1$^{-/-}$ plasma was electrophoresed in a two-dimensional gradient gel electrophoresis as described under Materials and Methods. Plasma proteins were transferred to nitrocellulose membranes and apoAI was visualized with anti-mouse apoAI antibodies.

Table 2: HDL Lipid Composition in +/+, +/−, and −/− Abca1 Mice[a]

mice	TG	PL	TC	apoAI	PL/apoAI
+/+	0.45 (0.7%)	30.3 (49.5%)	30.5 (49.8%)	54	21
+/−	0.40 (1.0%)	21.6 (52.5%)	19.1 (46.5%)	19.5	43
−/−	0.40 (28.2%)	0.46 (32.9%)	0.56 (38.9%)	0.075	235

[a] Pooled plasma samples from controls and Abca1 mutant mice were used to isolate HDL by FPLC as described under Materials and Methods. Fractions containing the HDL were pooled and concentrated. Lipids and apoAI concentrations were determined as described under Experimental Procedures and expressed as mg/dL. TG, triglycerides; PL, phospholipids; TC, total cholesterol; %, percentage composition relative to the sum of TG, PL, and TC values. PL/apoAI is expressed as molar ratio.

Table 3: Phospholipid Composition of Abca1$^{+/+}$, Abca1$^{+/-}$, and Abca1$^{-/-}$ HDL[a]

phospholipid	+/+	+/−	−/−
LPC	0.08 ± 0.02	0.20 ± 0.09	10.05 ± 5.93[b]
SPH	0.03 ± 0.02	0.03 ± 0.01	1.47 ± 0.87[b]
PC	0.27 ± 0.08	0.33 ± 0.05	1.12 ± 0.23[c]
PI + PS	0.04 ± 0.02	0.04 ± 0.04	0.30 ± 0.51[b]
PE	0.03 ± 0.01	0.03 ± 0.02	0.93 ± 0.48[b]
Total	0.44 ± 0.13	0.63 ± 0.10	13.86 ± 7.52
LPC/PC	0.33 ± 0.19	0.61 ± 0.23	8.49 ± 3.24[b,d]
SPH/PC	0.10 ± 0.05	0.10 ± 0.04	0.85 ± 0.40[b,d]
PE/PC	0.06 ± 0.05	0.23 ± 0.24	6.98 ± 6.25
LPC/PE	6.79 ± 6.32	7.96 ± 6.62	10.87 ± 6.35
SPH/PE	2.15 ± 1.66	1.20 ± 0.52	1.26 ± 0.61
PI + PS/PC	0.11 ± 0.11	0.12 ± 0.11	0.22 ± 0.37

[a] HDL was isolated from pooled plasma (6−12 mice) by FPLC. Phospholipids and apoAI were determined as described under Materials and Methods. Values are expressed as nmol/μg apoAI from three independent experiments on pooled plasma. [b] $p < 0.05$. [c] $p < 0.005$ compared to wild type. [d] $p < 0.05$.

As expected, there was a dramatic decrease in plasma levels of HDL phospholipids in Abca1$^{-/-}$ mice, compared to heterozygous and wild-type littermates. However, when expressed as nanomoles per microgram of apo AI, HDL from Abca1$^{-/-}$ mice showed a significant increase in all phos-

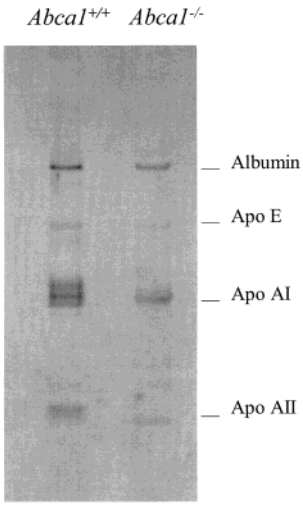

FIGURE 4: SDS-PAGE analysis of HDL isolated from Abca1$^{-/-}$ and Abca1$^{+/+}$ mice. Plasma lipoproteins ($\delta \leq 1.25$) were separated by preparative ultracentrifugation, individual lipoprotein subclasses were fractionated by FPLC, and fractions corresponding to HDL were pooled. Abca1$^{-/-}$ and Abca1$^{+/+}$ HDL were electrophoresed on a 4−12% SDS-polyacrylamide gradient gel. Proteins were detected by Silver staining using a Proteo Silver Staining kit as described by the manufacturer.

pholipid classes. Furthermore, the major phospholipid in HDL from Abca1$^{-/-}$ mice was lysophosphatidylcholine (LPC), whereas phosphatidylcholine (PC) was the major phospholipid in both wild-type and heterozygous HDL. Since HDL isolated by FPLC coelutes with albumin and most of the LPC present in plasma is bound to albumin (*36*), one possible explanation of the preceding results is the contamination of HDL fraction with albumin. Therefore, an additional experiment was performed in which albumin was removed by passing the HDL through a protein G column cross-linked with rabbit antisera to mouse albumin. This procedure removed over 80% of the albumin present in the HDL fraction and did not affect the lipid composition of HDL as reflected by the cholesterol/apoAI ratios (7.5 and 6.8 for HDL before and after column, respectively). The resulting HDL fraction was reassayed for phospholipid content. Although the total amount of LPC was decreased, the LPC/apo AI ratio in HDL did not change confirming the increased amount of LPC in HDL. Additional experiments were performed to confirm the increased levels of LPC observed in HDL isolated from Abca1$^{-/-}$ mice. Total lipoproteins ($\delta \leq 1.25$) were isolated by ultracentrifugation from Abca1$^{-/-}$ and control littermates and then separated in individual lipoprotein subclasses by FPLC. Fractions containing HDL were pooled, characterized by SDS-PAGE, and assayed for phospholipid content. As shown in Figure 4, albumin levels are very low and identical between wild-type and Abca1$^{-/-}$ HDL. LPC levels, expressed as nanomoles per microgram of apoAI, were 16-fold higher in HDL isolated from Abca1$^{-/-}$ mice when compared to wild-type littermates (3.0 nmol/μg apoAI vs 0.19 nmol/μg apoAI for Abca1$^{-/-}$ and wild type, respectively), confirming the increased in LPC in Abca1$^{-/-}$ mice. Compared to wild-type littermates, there was a 4-fold increase in PC in HDL isolated from Abca1$^{-/-}$ mice, whereas LPC (125-fold), sphingomyelin (SPH) (49-fold), and phosphatidylcholine (PE) (18-fold) showed even higher increases. As a consequence, the ratios of LPC/PC, SPH/PC, phosphatidylethanolamine (PE)/PC, and phosphatidylinositol + phosphatidylserine (PI + PS)/PC were all much higher in HDL from Abca1$^{-/-}$ mice, compared to HDL isolated from wild-type and Abca1 heterozygous mice. In general, the changes observed in the phospholipid composition of HDL from heterozygous Abca1 were insignificant, except for a decrease in PC and an increase in LPC/PC ratio (Table 3). Taken together, these findings suggest that one normal allele of Abca1 is sufficient to maintain HDL phospholipid composition similar to that of wild-type mice.

Table 4: Plasma Phospholipid Composition in Abca1$^{-/-}$, Abca1$^{+/-}$, and Abca1$^{+/+}$ Mice[a]

phospholipid	+/+	+/−	−/−
LPC	0.35 ± 0.10	0.32 ± 0.03	0.16 ± 0.01[b,c]
SPH	0.15 ± 0.07	0.11 ± 0.03	0.04 ± 0.01[d,c]
PC	1.06 ± 0.29	0.75 ± 0.19	0.14 ± 0.04[b,c]
PI + PS	0.15 ± 0.03	0.12 ± 0.02	0.05 ± 0.01[b,c]
PE	0.12 ± 0.03	0.12 ± 0.02	0.07 ± 0.02[d,e]
Total	1.83 ± 0.36	1.42 ± 0.24	0.47 ± 0.07[b,c]
LPC/PC	0.34 ± 0.13	0.46 ± 0.13	1.28 ± 0.27[b,c]
SPH/PC	0.15 ± 0.09	0.14 ± 0.03	0.34 ± 0.13[d,c]
PE/PC	0.12 ± 0.04	0.17 ± 0.06	0.56 ± 0.09[b,c]
LPC/PE	3.17 ± 1.76	2.92 ± 0.85	2.29 ± 0.39
SPH/PE	1.40 ± 0.85	0.97 ± 0.41	0.60 ± 0.19
PI + PS/PC	0.15 ± 0.04	0.17 ± 0.03	0.36 ± 0.04[b,c]

[a] Plasma from controls (*n*: 7), Abca1$^{+/-}$ (*n*: 7) and Abca1$^{-/-}$ (*n*: 5) mice were used to determine phospholipid species as described under Materials and Methods. Values are expressed as nmol/mL plasma and are mean ± SD (*n*: 7). [b] $p < 0.005$ compared to wild-type. [c] $p < 0.005$ compared to Abca1$^{+/-}$ mice. [d] $p < 0.05$. [e] $p < 0.05$.

Table 5: VLDL Phospholipid Composition in Abca1$^{-/-}$, Abca1$^{+/-}$, and Abca1$^{+/+}$ Mice

phospholipid	+/+	+/−	−/−
LPC	3.11 ± 2.51	2.14 ± 0.70	1.21 ± 0.41
SPH	3.83 ± 1.47	3.59 ± 3.28	1.43 ± 0.33
PC	15.97 ± 7.01	13.92 ± 4.36	22.36 ± 2.54
PI + PS	3.96 ± 3.33	1.83 ± 0.76	2.45 ± 0.74
PE	4.53 ± 2.59	1.95 ± 1.27	1.50 ± 0.03
Total	31.38 ± 16.50	23.43 ± 8.70	28.94 ± 3.88
LPC/PC	0.18 ± 0.09	0.16 ± 0.05	0.05 ± 0.01[a,b]
SPH/PC	0.24 ± 0.06	0.25 ± 0.21	0.06 ± 0.01[c]
PE/PC	0.27 ± 0.11	0.14 ± 0.07	0.07 ± 0.01[a]
SPH/PE	0.96 ± 0.31	1.70 ± 0.51	0.96 ± 0.22
PI + PS/PC	0.22 ± 0.11	0.13 ± 0.05	0.11 ± 0.02

[a] Phospholipid species was determined in FPLC isolated VLDL fractions as described under Materials and Methods. Values are expressed as nmol/μg of apoB and are mean ± SD (*n*: 7). [a] $p < 0.05$. [b] $p < 0.05$ compared to Abca1$^{+/-}$ mice. [c] $p < 0.005$ compared to wild-type.

To determine whether the abnormalities in the composition of phospholipids are specific for HDL or due to a general alteration in phospholipid synthesis and transport, we analyzed the phospholipid composition in whole plasma (Table 4) and VLDL fraction (Table 5). LPC is the major phospholipid present in plasma of Abca1$^{-/-}$ mice, while PC is the major phospholipid in wild-type and heterozygous mice. The total phospholipid concentration in plasma was decreased by 75% in Abca1$^{-/-}$ mice, but only modestly in Abca1$^{+/-}$ mice. The decrease in the latter was mostly due to a reduction in PC, with no significant changes in other phospholipids. On the other hand, all phospholipid classes decreased in Abca1$^{-/-}$ mice, with the decrease in PC concentration being more pronounced than that of others. As observed in isolated HDL, the LPC/PC, SPH/PC, PE/PC, and PI + PS/PC ratios were all significantly higher in

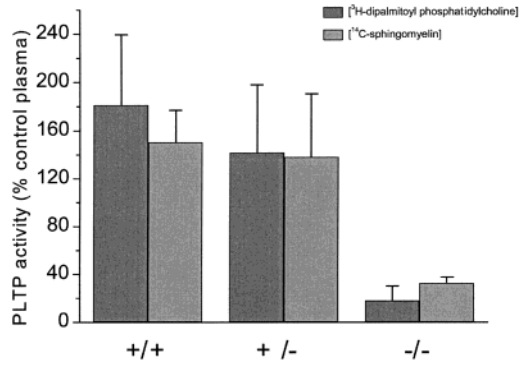

FIGURE 5: PLTP activities in plasma from Abca1$^{-/-}$, Abca1$^{+/-}$, and Abca1$^{+/+}$ mice. [^{3}H]-DPPC and [^{3}H]-SPH transfer from liposomes to HDL as described under Materials and Methods. Transfer activity is measured in the presence of excess exogenous HDL and expressed as % of reference pool plasma (15.0 and 14.5 μmol/mL/h, for DPPC and SPH, respectively), which is included in each run.

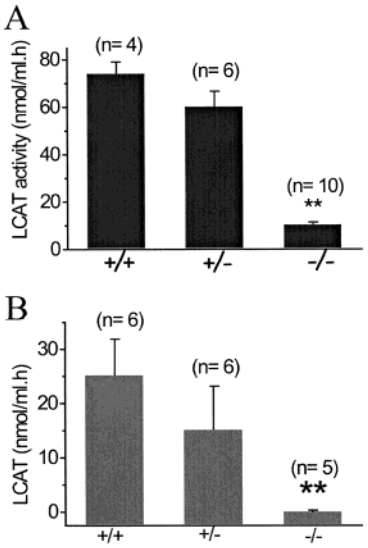

FIGURE 6: LCAT activities in plasma from Abca1$^{-/-}$, Abca1$^{+/-}$, and Abca1$^{+/+}$ mice. (A) LCAT-mediated esterification of lipoprotein-derived cholesterol. Plasma was obtained from mice from different genotypes, and LCAT activity was immediately inhibited by DTNB. Lipoprotein cholesterol was labeled with an albumin-stabilized emulsion of 1,2-[^{3}H]-cholesterol as described under Materials and Methods. LCAT activity was reactivated by adding β-mercaptoethanol, and the synthesis of [^{3}H]-lipoprotein-derived cholesteryl ester was determined for 30 min at 37° C. Lipids were extracted, and the content of [^{3}H]-labeled cholesteryl ester in the organic phase was determined by TLC as described under Materials and Methods. (B) Plasma LCAT activities were measured as the rate of synthesis of [^{3}H] cholesteryl esters from unilamellar vesicles prepared by French press cell and activated with apoAI as described under Materials and Methods.

plasma of Abca1$^{-/-}$ mice compared to wild-type mice. In the heterozygotes, only the LPC/PC ratio showed a significant increase. These results therefore suggest that the PC concentration of plasma is disproportionately affected by the absence of ABCA1.

In contrast to HDL, the VLDL fraction from Abca1$^{-/-}$ mice did not show a decrease in the total phospholipid (Table 5). However, there was a significant decrease in SPH and an increase in PC compared to VLDL isolated from heterozygous and wild-type mice. Furthermore, LPC, which was the major phospholipid in the HDL fraction, was a minor component in VLDL from Abca1$^{-/-}$ mice. Interestingly, the ratios of LPC/PC, SPH/PC, and PE/PC all showed a significant decrease in VLDL isolated from mice lacking Abca1, while these ratios were all increased in HDL. These results thus show that the deficiency of ABCA1 differentially affects the composition of various lipoproteins, with a more pronounced effect on HDL.

Plasma PLTP Activities in Abca1$^{-/-}$, Abca1$^{+/-}$, and Wild-Type Mice. Several lines of evidence demonstrate that the transfer of phospholipids from VLDL to HDL as well as within the HDL fraction plays an important role in the remodeling and maintenance of HDL levels (*20*, *37*, *38*). Since the Abca1$^{-/-}$ mouse has virtually no HDL and its phospholipid composition is significantly altered, we investigated whether plasma PLTP activity levels are affected in Abca1$^{-/-}$ mice. PLTP activities measured in plasma samples from mice from the three different genotypes are shown in Figure 5. Compared to wild-type littermates and Abca1 heterozygous mice, the transfer of either [^{3}H]DPPC or [^{14}C]-SPH from liposomes to exogenously added HDL, is markedly reduced in Abca1$^{-/-}$ mice (79% and 78% respectively, $p < 0.001$), consistent with a decrease in the transfer of both major plasma phospholipid classes onto HDL.

LCAT Activities in Abca1$^{-/-}$, Abca1$^{+/-}$, and Abca1$^{+/+}$ Mice. The esterification of cholesterol by LCAT is a key step in the maturation of nascent HDL species (*34*), as it facilitates the formation of a lipid core in nascent HDL, which then develops into mature HDL. The phospholipid composition of Abca1$^{-/-}$ HDL suggests that the high SPH/PC ratio could affect their reactivity with LCAT. Consistent with this hypothesis, the esterification of cholesterol is virtually absent in Abca1$^{-/-}$ mice (less than 0.5% compared to controls, 0.13 ± 0.11 nmol mL^{-1} h^{-1} (n: 6) versus 25.19 ± 6.7 nmol mL^{-1} h^{-1} (n: 6), $p = 0.008$), while Abca1$^{+/-}$ have a 40% decrease compared to controls (Figure 6B). Since sphingomyelin is known to compete with PC for binding to the active site of LCAT (*39*) and inhibit the binding of LCAT to HDL (*40*), we estimated whether the plasma concentration of LCAT was also decreased in Abca1$^{-/-}$ mice by using a proteoliposome assay. This measurement is independent of the concentration of plasma lipoproteins and proportional to the amount of LCAT protein (*25*). LCAT was markedly decreased in Abca1$^{-/-}$ mice when compared to wild-type littermates (15% of the levels found in controls, $p < 0.005$], while Abca1$^{+/-}$ was not significantly different than control littermates (Figure 6A). Liver LCAT mRNA levels were not different between genotypes (data not shown), suggesting a normal synthesis and secretion of LCAT protein.

Role of LCAT and PLTP on HDL Biogenesis. The absence of mature HDL implies that the biogenesis of HDL is impaired as consequence of the absence of ABCA1. The initial step in the biogenesis of HDL is thought to involve the ABCA1-mediated transfer of phospholipid and cholesterol onto apoAI, resulting in the formation of nascent preβ-HDL species (*41*). Subsequent interaction and activities of LCAT and PLTP transform these particles into mature spherical α-migrating HDL species (*38*, *42*). Therefore, we performed an in vitro study to assess whether the absence of ABCA1 alone or in combination with defective PLTP or LCAT activities is responsible for the low levels and the absence of mature HDL in Abca1$^{-/-}$ mice. The synthesis of mature HDL was determined by measuring the formation

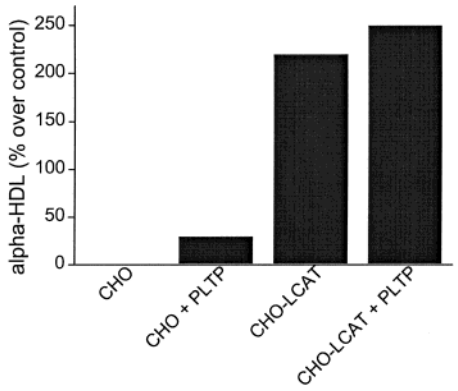

FIGURE 7: Effect of ABCA1, LCAT, and PLTP on the biogenesis of HDL. Plasma from Abca1$^{-/-}$ plasma was incubated with either wild-type CHO cells or CHO cells expressing LCAT alone or in combination with purified PLTP for 6 h at 37 °C as described under Materials and Methods. HDL species were immediately electrophoresed in a two-dimensional gradient gel electrophoresis. Proteins were transferred onto nitrocellulose membranes and apoAI containing HDL species identified by using a polyclonal rabbit antibody to murine apoAI. Formation of mature HDL species was expressed as % increases over Abca1$^{-/-}$ plasma incubated with CHO cells. The figure shown is a representative experiment from three independent experiments.

of α-migrating HDL species by two-dimensional gradient gel electrophoresis and compared to native plasma incubated in the absence of cells. No increase in α-migrating HDL occurred when Abca1$^{-/-}$ plasma was incubated with LCAT alone or in combination with PLTP in the absence of CHO cells. In this experiment, plasma from Abca1$^{-/-}$ mice was incubated with CHO cells that are known to express high levels of ABCA1 (*2*) with the intent of restoring the flux of phospholipid and cholesterol onto ApoAI. Compared to native plasma (Figure 7), no changes in α-HDL were observed, suggesting that the normalization of ABCA1-mediated lipidation was insufficient to induce the maturation of HDL. In contrast, a 220% increase in the formation of α-HDL was observed when ABCA1 and LCAT activities were restored by incubating Abca1$^{-/-}$ plasma with CHO cells expressing LCAT. LCAT activity in Abca1$^{-/-}$ plasma was initially 0.1 ± 0.1 nmol CE formed mL^{-1} h^{-1} in Abca1$^{-/-}$ plasma and increased to 15.3 nmol CE formed mL^{-1} h^{-1} after 6 h incubation with CHO cells expressing LCAT reaching activity levels similar to those found in wild-type mice (Figure 5B). The addition of PLTP alone or in combination with LCAT did not result in a significant additional increase in the proportion of α-migrating HDL. An additional experiment was performed in which PLTP was used at a final activity of 26 000 nmol/mL/h to emulate plasma activities in mice or humans (*30*, *31*), and no additional increase in mature HDL was observed (data not shown). Taken together, these findings suggest that ABCA1 is necessary for the adequate lipidation of apoAI, which enables the interaction with LCAT and subsequent formation of HDL.

DISCUSSION

ABCA1 plays a crucial role in the synthesis of HDL. ABCA1 is present in all tissues and facilitates the efflux of phospholipids toward nascent apolipoproteins, which can then accept cholesterol forming a lipid-poor particle that triggers the maturation and formation of HDL. Animal models with either natural mutations or targeted inactivation of ABCA1 have confirmed the importance of ABCA1 and raised several important questions regarding the key biochemical processes responsible for the maturation of nascent HDL. In this study, we have used Abca1-deficient mice to extend our characterization of the changes in the plasma lipoproteins and understand the mechanism/s determining the low levels of HDL when ABCA1 is absent. Several novel findings resulted from this study. HDL from Abca1$^{-/-}$ mice represents less than 2% of wild-type levels and is small and enriched in phospholipids compared to wild-type mice HDL. Their phospholipid composition is significantly different from other HDL or preβ-HDL (*35*) and characterized by increased ratios of LPC/PC, SPH/PC, PE/PC, and PI + PS/PC as a consequence of a disproportionate decrease in PC. Cell culture experiments demonstrated that PC is the major phospholipid species released onto apoAI (*43*, *44*) by ABCA1 suggesting that the abnormal phospholipid composition of Abca1$^{-/-}$ HDL may reflects the specificity of the ABCA1 toward PC. In addition, changes in phospholipid transfer activity as observed in Abca1$^{-/-}$ mice may contribute, at least in part, to the abnormal phospholipid composition of HDL. The transfer rates of PC, the major phospholipid species in plasma lipoproteins, is decreased by 80% in Abca1$^{-/-}$ compared to wild-type mice, suggesting that the transfer of PC from VLDL to HDL is markedly impaired contributing to the abnormal low levels of PC observed in Abca1$^{-/-}$ HDL.

The increase of LPC in Abca1$^{-/-}$ HDL mice is intriguing. Since LPC is carried by albumin in normal plasma (*37*), we decided to remove the albumin by immunoaffinity columns. Although a small amount of LPC (~20%) was removed by this procedure, the LPC/apoAI ratio remained higher in Abca1$^{-/-}$ HDL when compared to wild type. These findings are also confirmed in HDL isolated by the combination of ultrancentrifugation and FPLC techniques. Following these procedures, HDL contains negligible amounts of albumin indicating the LPC is indeed associated to HDL, as previously suggested in the literature (*45*). In addition, LPC was the major phospholipid component in the whole plasma from Abca1$^{-/-}$ mice, indicating that the relative concentration of LPC is increased in ABCA1 deficiency. The major source of LPC in plasma is the LCAT reaction (*45*). However, this activity is decreased drastically in the homozygous mice, and therefore could not account for the increase in HDL LPC. It is also highly unlikely that the LPC is generated in vitro after the collection of the sample, since all plasma samples were treated similarly, and the increase in LPC is found only in homozygous plasma. It is also of interest to note that VLDL LPC is actually lower than in controls, indicating a specific effect on HDL composition. LPC is secreted by the liver directly into plasma (*46*), suggesting that this pathway could be stimulated in the absence of ABCA1. Preliminary experiments show that peritoneal macrophages from homozygous mice secrete relatively more LPC than control macrophages (results not shown), supporting an increased secretion of LPC from peripheral cells and probably liver in the absence of ABCA1. The consequence of increased levels of LPC in plasma and HDL are unknown. However, it is likely that the physical structure and stability of HDL is significantly altered and, thus, contributes to the low levels of HDL observed in Abca1$^{-/-}$ mice. LPC potentiates the

mitogenic activity of murine and human macrophages and may contribute to the tissue accumulation of macrophages observed in Abca1$^{-/-}$ mice. Further studies are required to evaluate the metabolic consequences of increased levels of LPC in plasma and HDL in Abca1$^{-/-}$ mice.

The synthesis and maturation of plasma HDL requires the interaction of many plasma proteins and cellular receptors. In vivo, apoAI synthesized by the liver and intestine or recycling from the plasma compartment becomes lipidated by ABCA1 on the surface of cells leading to the formation of lipid-poor, nascent HDL particles, also known as preβ-HDLs, which are transformed into large-sized HDL particles with α-mobility by the action of LCAT (*25*, *42*). Subsequent maturation and formation of larger HDL species is dependent upon the interaction and continual remodeling induced by various plasma proteins and surface receptors, resulting in the structural heterogeneity of circulating HDL (*47*). Recent studies have confirmed that the first and foremost important step in the biogenesis of HDL is the interaction of apoAI with ABCA1 on the cell surface. Since apoAI synthesis is not affected in Abca1$^{-/-}$ mice, our studies suggest that the defective lipidation of apoAI leads to the formation of abnormal HDL precursors that cannot be transformed into large and mature HDL species. Several evidences are in support of this hypothesis. The composition of phospholipids in HDL is abnormally high in SPH and low in PC, resulting in a SPH/PC ratio known to affect the binding and interaction of LCAT with its substrates (*39*, *40*). In addition, the increase in the concentration of LPC would further inhibit the LCAT reaction. In agreement with this hypothesis, plasma LCAT activity and concentration are drastically reduced in Abca1$^{-/-}$ mice despite normal levels of liver mRNA. Since esterification of cholesterol by LCAT is responsible to trigger the maturation of HDL and no detectable mature HDL species with α-mobility are present in Abca1$^{-/-}$ mice, we hypothesized that the conversion of preβ-HDL into α-HDL is impaired in Abca1$^{-/-}$ mice. Consistent with this hypothesis, our in vitro studies demonstrate that the generation of α-HDL can be restored in Abca1$^{-/-}$ when ABCA1 function and LCAT are restored. In addition, our previous in vivo studies in which the presence of transplanted wild-type macrophages in Abca1$^{-/-}$ mice was sufficient to generate α-migrating HDL (*48*) also support our hypothesis and suggest that the adequate lipidation of apoAI by ABCA1 enables the interaction of nascent HDL with LCAT and subsequent maturation.

Plasma PLTP activity toward both PC and SPH is decreased by about 80% in Abca1$^{-/-}$ mice, suggesting a decrease in the transfer of all phospholipid species onto apoAI. This is likely due to a decrease in PLTP mass or activity because PLTP mRNA is increased in Abca1$^{-/-}$ mice (*16*). In several animal species, PLTP was found to be associated with HDL sized lipoproteins (*27*), and lowering of HDL could stimulate its degradation. Whether the transfer of phospholipids by PLTP is necessary to the initial step on HDL synthesis remains unclear. The phenotype of PLTP deficient mice (*20*) as well as in vitro studies (*49*, *50*) demonstrated that this transfer protein is essential to remodel and maintain HDL levels. Our in vitro studies suggest that the addition of PLTP alone or in combination with LCAT does not induce the formation of any additional large-sized α-HDL and suggest that the transfer of most phospholipids to HDL occurs after the transformation of preβ into α-HDL. The phenotype observed in LCAT or PLTP-deficient mice suggest that LCAT is the primary enzyme responsible for the transformation of preβ into α-HDL. LCAT deficiency in humans (*51*) and mice (*52*) leads to the accumulation of discoidal HDL particles with preβ-mobility, while vesicular lipoproteins within the IDL/LDL fraction are present in PLTP-deficient mice (*20*). Alternatively, the absence of mature HDL following incubation with PLTP may be explained by the low levels of LDL present in these mice (*16*), leading to a decrease in transfer of phospholipids onto HDL.

Taken together, the findings from these studies suggest that the absence of ABCA1 results in profound changes in the phospholipid composition of HDL that alter their biosynthesis and maturation. In addition to a decrease in plasma activities of LCAT and PLTP as important mechanism/s for the failure of HDL to mature, small HDL filter rapidly in kidneys as demonstrated in patients with Tangier Disease (*18*, *19*). This process, which is independent of the change in lipid composition of nascent HDL, is also likely to contribute to the low levels of HDL in the Abca1-deficient mice.

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