ApoA-II/apoA-I molar ratio in the HDL particle influences phospholipid transfer protein-mediated HDL interconversion

Pirkko J. Pussinen,[1] Matti Jauhiainen, and Christian Ehnholm

Department of Biochemistry, National Public Health Institute, Mannerheimintie 166, FIN-00300 Helsinki, Finland

Abstract Pig plasma phospholipid transfer protein (PLTP) facilitates interconversion of both human HDL_3 and pig HDL into HDL subpopulations of large and small particles. We recently suggested that there are two essential parts in the conversion mechanism, i.e., the release of apoA-I and phospholipids and the fusion of apoA-I-depleted unstable particles. Based on their mass composition and electrophoretic mobility in agarose gel, the released apoA-I-containing particles are similar to previously described preβ-HDL particles known as primary acceptors of peripheral cholesterol. The aim of this study was to determine whether the apolipoprotein composition of HDL regulates the PLTP-mediated conversion process. Pig HDL was incubated with increasing amounts of human apoA-II which incorporates into pig HDL with a concomitant release of apoA-I and some phospholipids. These hybrid pig HDL particles formed were isolated by a combination of ultracentrifugation at density 1.21 g/ml and gel filtration. The apoA-II/apoA-I molar ratios in the hybrid HDL particles ranged from 0.2 to 7.6 mol/mol. In the maximally modified HDL apoA-I was totally substituted by apoA-II. These particles were incubated in the presence of purified PLTP and the conversion products were isolated and characterized. Both the formation of large particles and the release of apoA-I were inhibited by increasing concentrations of apoA-II in the HDL particle. The hybrid HDL particles behaved similarly as native pig HDL as acceptors of phospholipid from PC-vesicles in the PLTP-assay. This study suggests that the apoA-II/apoA-I molar ratio in the HDL particle regulates PLTP-mediated HDL interconversion.—**Pussinen, P. J., M. Jauhiainen, and C. Ehnholm.** ApoA-II/apoA-I molar ratio in the HDL particle influences phospholipid transfer protein-mediated HDL interconversion, *J. Lipid Res.* 1997. **38:** 12–21.

Supplementary keywords phospholipid transfer protein (PLTP) • HDL subclasses • pig lipoproteins • apoA-I • apoA-II • interconversion of HDL

Numerous studies have shown an inverse correlation between plasma high density lipoprotein (HDL) cholesterol levels and the incidence and prevalence of atherosclerosis, suggesting that HDL protects against atherosclerosis (1). The molecular mechanism(s) underlying this protective effect is not known. It may, however, be related to the role of HDL in the reverse cholesterol transport, a process by which cholesterol is carried from peripheral cells to the liver for excretion. HDL exists in human plasma in multiple forms. Using different techniques, several populations of HDL that differ in density, composition, size, electrophoretic mobility, and apolipoprotein profile have been identified. Evidently these subpopulations have different metabolic functions (2, 3). It has been demonstrated, for instance, that the primary acceptors of cell membrane cholesterol in the reverse cholesterol transport are members of quantitatively minor HDL subclasses, i.e., $preβ_1$Lp-A-I (4) and γ-LpE (5). HDL composition and size, i.e., the distribution of HDL subpopulations, are regulated by the plasma lipid transfer proteins, cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP), as well as by enzymes, such as lecithin:cholesterol acyltransferase (LCAT), hepatic lipase (HL), and lipoprotein lipase (LPL).

The influence of CETP on the composition, size, and structure of HDL has been studied in detail (6). The role of apolipoproteins in CETP-mediated cholesterol ester transfer has also been reported by several investigators (7, 8). Another plasma protein, phospholipid transfer protein (PLTP), that promotes the conversion of HDL_3 to populations of larger and smaller particles has been recently isolated from human (9, 10) and pig (11) plasma. A similar HDL interconversion was also

Abbreviations: PLTP, phospholipid transfer protein; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; HL, hepatic lipase; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; PC, phosphatidylcholine; SM, sphingomyelin; D, dalton; HDL, high density lipoprotein; BHK, baby hamster kidney; PL, phospholipid; chol, cholesterol; TG, triglyceride; d, density.

[1]To whom correspondence should be addressed.

shown to be promoted by human PLTP which was expressed in a BHK cell line (12). The cDNA of human PLTP (13) predicts a protein sequence with 20% homology with CETP. Studies with reconstituted HDL particles indicate that in the HDL conversion mechanism there is an initial phospholipid transfer reaction followed by a release of apoA-I that finally causes the formation of particles that are prone to fuse (14).

In the present study we investigated whether the PLTP-mediated HDL interconversion is influenced by the apolipoprotein composition of the particles. This question is of physiological importance as human plasma contains two main populations of HDL that differ in apolipoprotein composition: one contains only apoA-I (A-I w/o A-II) while the other contains both apoA-I and apoA-II (A-I w A-II). Substantial evidence indicates that these subclasses differ in their metabolic behavior (15) and in their power to predict atherosclerosis (16).

Human HDL consists of two main populations, HDL_2 and HDL_3, while pig plasma contains only one population of HDL which resembles the human HDL_3 subfraction. Whereas human HDL contains two main apolipoproteins, apoA-I and apoA-II, the major pig HDL protein is apoA-I with no detectable apoA-II (17). In addition, functional CETP is nonexistent or minimally expressed in pig plasma (18, 19) which may be due to the presence of a lipid transfer inhibitor protein (LTIP) (20). Therefore the pig offers an attractive animal model for studying factors that regulate HDL interconversion.

Previous studies have demonstrated that apoA-II can rapidly displace apoA-I from HDL without significantly affecting the overall structural properties of the lipoprotein (21–23). In order to gain an understanding of the role of apolipoproteins on PLTP-mediated HDL conversion, we replaced a proportion of apoA-I in pig HDL with human apoA-II. Incubation of the modified HDL particles in the presence of PLTP showed that interconversion is gradually inhibited by increasing the concentration of apoA-II in the particle.

MATERIALS AND METHODS

Reagents

Egg phosphatidylcholine (PC) was from Sigma; 1-palmitoyl-2-[1-^{14}C]palmitoyl phosphatidylcholine (DPPC, specific activity, 55 mCi/mmol) was from Amersham, UK. Butyl-Toyopearl 650(M) and thin-layer chromatography silica plates were from Merck, Germany. Superose 6 HR gel filtration column, heparin-Sepharose CL-6B, CNBr-activated Sepharose CL-4B, and dextran sulfate were obtained from Pharmacia, Uppsala, Sweden. SeaKem ME agarose was from FMC Corporation, Rockland, ME, and DTSSP, 3,3′-dithiobis(sulfosuccinimidylpropionate) was from Pierce, Rockford, IL. Heparin (5000 units/ml) was from Medica, Helsinki, Finland. All chemicals were of analytical grade.

Isolation of lipoproteins

Human HDL_3 (1.125–1.21 g/ml) and pig HDL (1.087–1.21 g/ml) were isolated by sequential ultracentrifugation using solid KBr to adjust the density (24). The HDL fractions were washed by reflotation at their upper density 1.21 g/ml. These steps were carried out at a speed of 50,000 rpm in a Beckman Ti 50.2 rotor for 40 h. HDL was dialyzed against TBS buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl) and stored at 4°C.

Isolation of human apoA-I and apoA-II

Ultracentrifugally isolated HDL (1.063–1.21 g/ml) were made 6 M with respect to guanidinehydrochloride. ApoA-I and apoA-II were purified as described by Scanu (25). The fraction with apoA-II was delipidated overnight with ethanol–diethyl ether 3:1 (v/v) at −20°C in a volume ratio of 1:25 (26). The samples were subjected to ion-exchange chromatography on a DEAE-cellulose column (27). This protocol afforded homogeneous preparations of apoA-I and apoA-II that appeared as single bands on a 12.5% SDS-PAGE stained with Coomassie Brilliant Blue under non-reducing conditions. The purified apoproteins were dialyzed against 50 mM ammonium bicarbonate and lyophilized. The lyophilized proteins were dissolved in TBS and stored at −70°C.

Preparation of pig apoA-I antibody

Rabbits were immunized subcutaneously with 70 μg of pig apoA-I per injection emulsified in complete Freund's adjuvant (Gibco). Pig apoA-I was purified by the same method as human apoA-I (25) from ultracentrifugally isolated pig HDL (1.087–1.21 g/ml). Antiserum was collected 4 weeks after immunization, whereafter the rabbits received the same dose of the antigen with incomplete Freund's adjuvant. The immunoglobulin fraction was isolated from the antiserum by Protein A-Sepharose. The antibody was tested by immunoblotting pig serum and pig HDL, from which the antibody detects only a 28 kD band. The immunoglobulin fraction was stored in small aliquots at −20°C.

Purification of PLTP from pig plasma

The purfication was initiated from 1 liter of pig plasma stored at −70°C by precipitating it as described by Tu, Nishida, and Nishida (10). The purification of pig PLTP was performed as earlier (11) using hy-

drophobic chromatography on a Butyl-Toyopearl 650(M), affinity chromatography on a heparin-Sepharose column and a monoclonal anti-pig–PLTP column.

Preparation of apoA-II-modified pig HDL particles

Pig HDL (1.5 mg of protein) was incubated for 30 min in the presence of human apoA-II (0.2–2.0 mg) at room temperature in TBS (10 mM Tris-HCl, pH 7.4, 150 mM NaCl). The density of the samples was adjusted to 1.21 g/ml with solid KBr and the ultracentrifuge tubes were filled up to 3 ml with a solution having a density of 1.21 g/ml. The modified HDL particles were isolated by ultracentrifugation in a TL-100.3 rotor at a speed of 100,000 rpm for 16 at 10°C in a Optima TL-100 Tabletop Ultracentrifuge (Beckman). After centrifugation the tubes were divided into three 1-ml fractions from the top and the fractions were gel-filtered on a Superose 6 HR column with TBS at a flow rate of 0.5 ml/min. The chemical composition and the size of the particles in these three fractions were determined.

Incubation of apoA-II-modified pig HDL particles with PLTP

The incubations with PLTP were carried out essentially as described earlier (9) at +37°C in a shaking water bath for 40–48 h. The PLTP used in the incubation experiments was usually from the second step of the purification procedure, namely the heparin-Sepharose-bound fraction that was free of CETP, LCAT, and HL activity. The incubation mixture contained 0.1–0.7 mg of HDL total protein and the PLTP activity ranged from 500 to 1000 nmol/h. After incubation the tubes were placed on ice and the density of the samples was adjusted to 1.21 g/ml with solid KBr. The ultracentrifugation and gel filtration of the samples were performed as described above.

Analysis of HDL particle size

The HDL particle size was determined with native gradient gel electrophoresis (28) in 4–26% polyacrylamide gels (8.0 × 8.0 cm) prepared in our laboratory. High molecular weight electrophoresis calibration standards (Pharmacia) were used as molecular markers. The stained gels were scanned with the BioImage System (Millipore Co.)

Agarose gel electrophoresis of HDL

After incubation with pig PLTP the HDL subclasses were isolated by ultracentrifugation and gel filtration (**Fig. 1,** top), and their electrophoretic mobility was analyzed using 0.75% agarose gels. Thirty µl of the sample (50–100 µg of protein) was separated by electrophoresis at 4°C using a 50 mM barbital buffer, pH 8.6, until albumin as a marker had migrated about 7 cm from the origin. The proteins separated in the agarose gel were then transferred to the nitrocellulose membrane and detected by polyclonal anti-pig–apoA-I (Fig. 1, bottom).

Crosslinking of the HDL conversion products

The PLTP-mediated HDL conversion products were isolated as described above by ultracentrifugation and gel filtration. The crosslinker solution (DTSSP, stock 100 mM in 50 mM DMSO) was added in a final concentration of 1 mM to the lipoprotein particle solution (60 µg in PBS), followed by mixing and standing for 30 min at room temperature. The reaction was stopped by addition of loading buffer (1:1) used for reduced SDS-PAGE gels. After mixing, the reaction mixture was incubated for 15 min at room temperature. The 10-µg samples were electrophoresed on a 5–24% SDS-PAGE gradient gel and immunoblotted with polyclonal anti-pig–apoA-I (**Fig. 2**).

Phospholipid subclass analysis

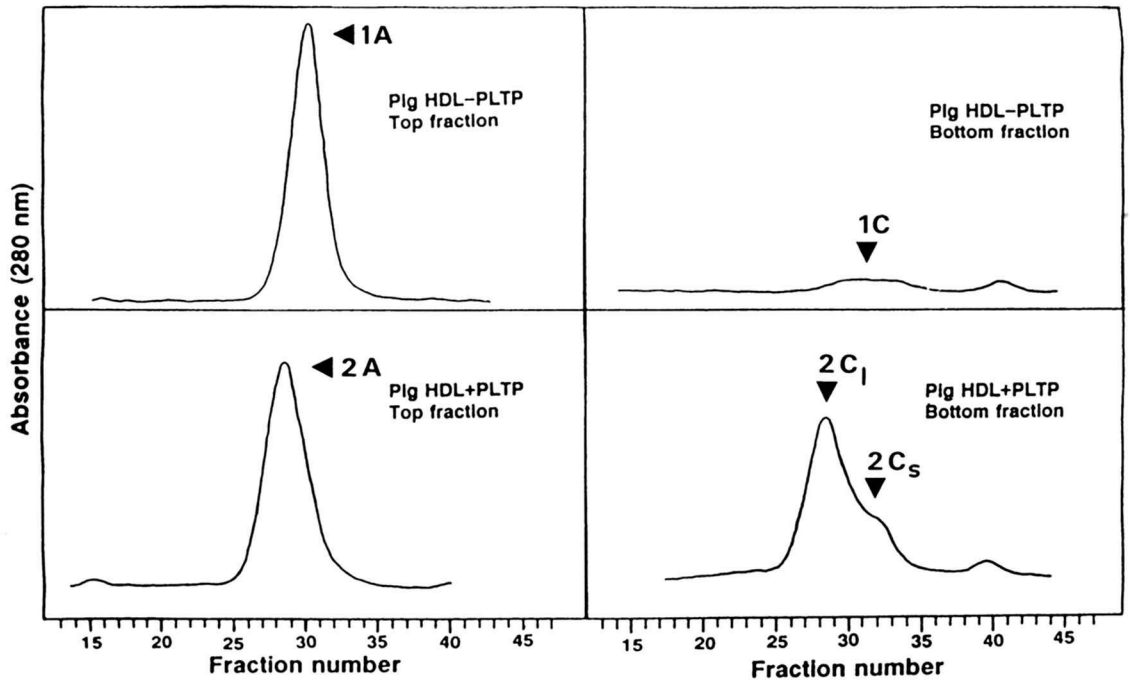
The lipids were extracted from 100-µl samples with a chloroform–methanol 2:1 (v/v) solution as described (29). The solvent was evaporated with nitrogen and the lipids were dissolved in chloroform and applied on a silica TLC-plate. The plate was developed in chloroform–methanol–acetic acid–water 75:45:12:6 (v/v) for about 45 min at room temperature. The lipids were visualized with iodine and the individual phospholipid spots were scraped and extracted from the silica as described (29). Total phosphorus was determined as described by Bartlett (30).

Other methods

Protein was determined by the method of Lowry et al. using human serum albumin as standard (31). Human apoA-I, apoA-II, and pig apoA-I were assayed by immunoturbidometry (32), and cholesterol, triglycerides, and phospholipids with enzymatic methods (33). CETP, LCAT, HL, and PLTP activities were analyzed as previously described (34-37). SDS-PAGE was run by the Laemmli method (38), and Western blotting was performed as described by Towbin, Staehelin, and Gordon (39).

RESULTS

Hybrid pig HDL particles containing increasing amounts of human apoA-II were prepared to study the effect of the apolipoprotein composition of HDL on PLTP-mediated HDL conversion. When human apoA-II was incubated with the pig HDL, apoA-II incorporated into the HDL particle, and apoA-I and some phos-



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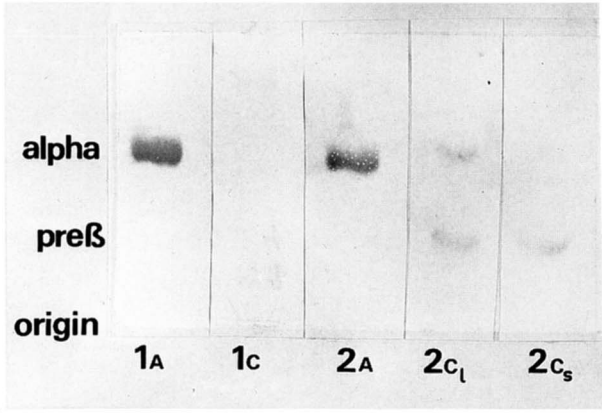

B

Fig. 1. A: Gel filtration chromatograms of pig HDL incubated in the absence and presence of PLTP. Pig HDL (1 mg of protein) was incubated without or with PLTP (800 nmol/h) for 48 h at 37°. The density of the samples was adjusted to 1.21 g/ml with solid KBr and the samples were ultracentrifuged. After ultracentrifugation the tubes were divided into top and bottom fractions that were applied on a Superose 6HR-column. The upper panel represents pig HDL incubated without PLTP, top (1A) and bottom (1C) fractions. The lower panel shows the gel filtration chromatograms of pig HDL incubated with PLTP, top (2A) and bottom ($2C_l$ and $2C_s$) fractions. For other experimental details, see Materials and Methods. B: Agarose gel electrophoresis of pig HDL. The electrophoretic mobility of HDL subclasses from Fig. 1A (1A, 1C, 2A, $2C_l$ and $2C_s$) after incubation in the absence and presence of PLTP was analyzed by 0.75% agarose gel electrophoresis. After electrophoresis the proteins were transferred to the nitrocellulose membrane and detected with polyclonal anti-pig apoA-I antibody. The fractions are labeled as in Fig. 1A.

pholipids were released. The two lipoproteins were then resolved by ultracentrifugation at d 1.21 g/ml. ApoA-II could be recovered in the HDL particle and was not found unassociated with HDL unless added in a great molar excess (apoA-II/HDL protein mass ratio $>$ 1.81/1.5 mg/mg). The amount of pig apoA-I released from HDL upon incubation increased as a function of apoA-II added. In the final hybrid HDL particles, one molecule of apoA-I was replaced by approximately two molecules of apoA-II (21). Some physicochemical properties of the hybrid HDL particles are shown in **Table 1.**

The addition of apoA-II to the particles resulted in only minor changes in the composition of the particles. The protein content increased somewhat while the cholesterol and phospholipid content decreased. The size of the particles, as determined by gradient gel electrophoresis, increased from 8.7 nm to 9.6 nm (10% increment) upon apoA-II incorporation (Table 1). In contrast, the proportions of apoA-I and apoA-II present in the particles changed dramatically upon incubation with apoA-II. The apoA-II/apoA-I molar ratios in the hybrid HDL particles were 0.2, 1.1, 2.1, and 7.6 mol/mol. In the hybrid HDL particles prepared by incubating 1.8 mg of apoA-II with 1.5 mg of pig HDL, no pig apoA-I could be detected by immunoturbidometry.

Prepared hybrid HDL particles were first tested as acceptors in the PLTP assay in which the amount of PC transferred from radioactively labeled PC-liposomes to acceptor particles by PLTP was measured. In these ex-

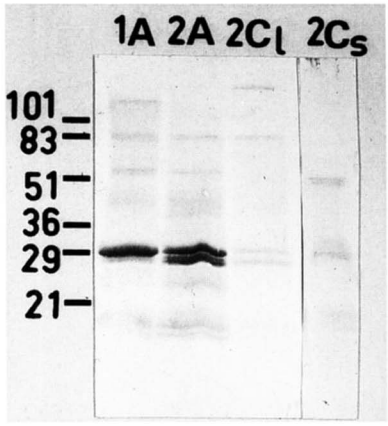

Fig. 2. The crosslinking of pig HDL. After incubation of pig HDL in the absence and presence of PLTP, the conversion products were isolated (see Fig. 1A), concentrated, and crosslinked with DTSSP. After crosslinking, 10-μg samples were electrophoresed on a 5–24% gradient SDS-PAGE and immunoblotted with polyclonal anti-pig apoA-I antibody. The samples are labeled as in Fig. 1A.

periments the hybrid HDL acceptor was added to the assay on the basis of its phospholipid content (110 μg/assay, 250 μg of protein/assay). As acceptors of phospholipid from PC-liposomes, the hybrid HDL particles behaved in a similar way as native pig HDL, although the spontaneous phospholipid transfer increased when the apoA-II/apoA-I molar ratio in the acceptor particle exceeded 7.6 mol/mol (**Fig. 3**).

To assess the effect of HDL apolipoprotein composition on HDL interconversion, pig HDL and the hybrid HDL particles were incubated in the presence of purified PLTP. After incubation the conversion products were isolated by ultracentrifugation and the particles were characterized. Upon centrifugation at a density of 1.21 g/ml, the large particles formed in the conversion reaction floated in the top fraction, while the two lipid-poor, denser HDL populations were recovered in the bottom fraction. These two populations were separated by gel filtration chromatography. The degree of HDL interconversion was monitored in two ways: as release of apoA-I, i.e., the formation of the small HDL particles ($d > 1.21$ g/ml), and as increase in the HDL particle size ($d < 1.21$ g/ml).

As the chemical composition of the large and small particles recovered in the bottom fraction ($d > 1.21$ g/ml) was quite similar, they were analyzed in detail. They both contained 86 ± 1% of apoA-I, 11 ± 2% of phospholipids, and trace amounts of free cholesterol. Both of these particles showed preβ-mobility in agarose gel electrophoresis (Fig. 1, bottom). The diameter of the small particles was 7.9 nm and that of the large particles 11.5 nm. To study how many molecules of apoA-I these particles contained, they were treated with a cross-linker (DTSSP) and analyzed by 5–24% SDS-PAGE (Fig. 2). The small HDL particles contained two and the large particles five apoA-I molecules per particle. The original population of pig HDL contained from two to four molecules of apoA-I per particle (**Table 2**). The fused large HDL particles with the density <1.21 g/ml contained from two to three molecules of apoA-I per particle (Table 2).

The phospholipid composition of pig HDL and the conversion products were determined by thin-layer chromatography. In the original pig HDL, the sphingomyelin/phosphatidylcholine (SM/PC) ratio was 0.25 which is close to that reported for human HDL_3 (40). After incubation in the presence of PLTP, the SM/PC ratios for the conversion products were as follows: large fusion particles ($d < 1.21$ g/ml) 0.19, small particles ($d > 1.21$ g/ml) 0.57, and large particles ($d > 1.21$ g/ml) 0.41 (Table 2).

PLTP mediated conversion of hybrid HDL particles that contained apoA-I, while the particles without apoA-I remained essentially unchanged. Native pig HDL was totally converted into three new populations of particles (Fig. 1, top). The formation of large fusion particles in the HDL conversion process can be seen in the gradient gel stained with Coomassie Blue (**Fig. 4**). Increasing amounts of apoA-II in the particle inhibited the conversion as shown by reduced enlargement of the particle.

TABLE 1. Chemical composition and size of pig HDL and the five hybrid HDL preparations

Sample	Composition (mass%)				A-II/A-I	Size
	Protein	PL	Chol	TG	*mol/mol*	*nm*
1. Pig HDL	47.6 ± 0.7	29.1 ± 1.9	20.9 ± 0.5	2.3 ± 0.7	0	8.7
2. Pig HDL + 0.2 mg A-II	49.7 ± 0.9	28.1 ± 2.3	20.1 ± 0.2	2.1 ± 1.1	0.2 ± 0.05	8.9
3. Pig HDL + 0.6 mg A-II	51.6 ± 1.3	27.0 ± 2.2	19.0 ± 0.2	2.3 ± 0.7	1.1 ± 0.07	9.3
4. Pig HDL + 1.0 mg A-II	53.9 ± 1.8	25.8 ± 2.5	18.2 ± 0.5	2.0 ± 1.0	2.1 ± 0.3	9.6
5. Pig HDL + 1.4 mg A-II	55.3 ± 1.9	24.8 ± 2.2	17.8 ± 0.3	1.9 ± 0.4	7.6 ± 1.4	9.6
6. Pig HDL + 1.8 mg A-II	57.5 ± 2.5	23.8 ± 2.9	16.9 ± 0.4	1.8 ± 0.8	—	9.5

The hybrid particles were produced by incubating 1.5 mg of pig HDL with increasing amounts of human apoA-II. In the particle size, the cv% varied between 0.5 and 1.3.

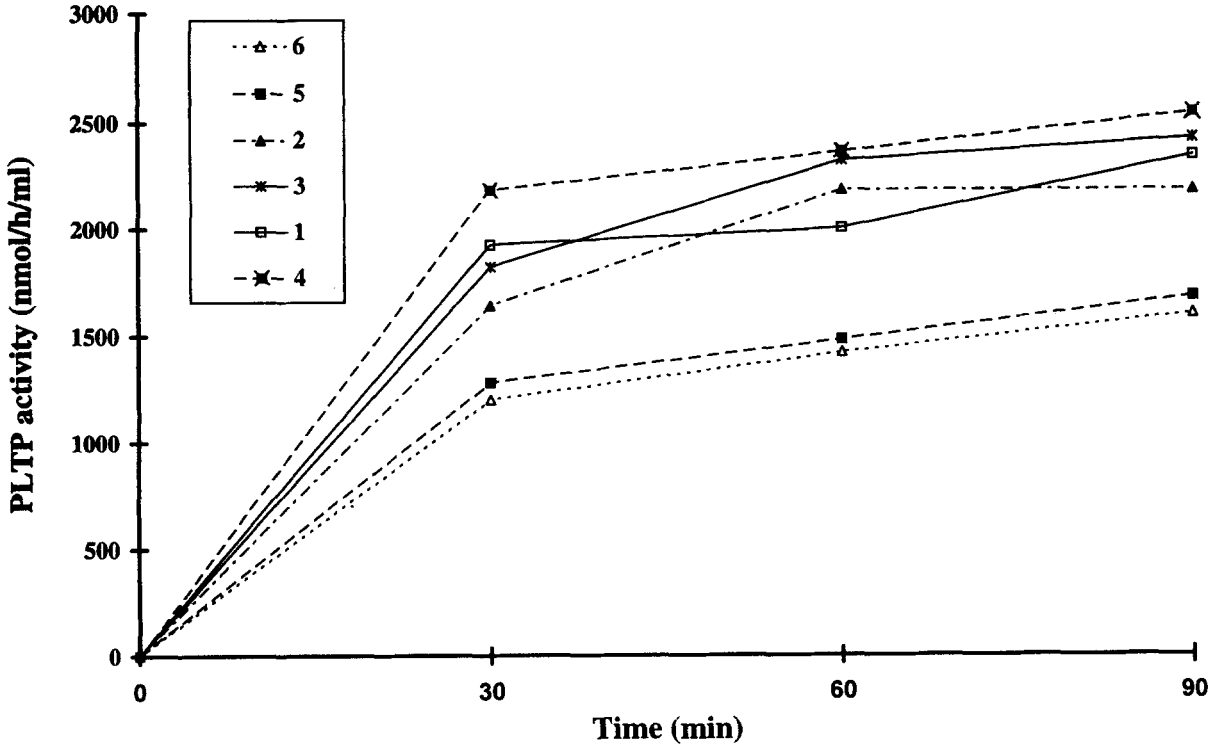

Fig. 3. The PLTP-mediated phospholipid transfer rates with hybrid HDL particles. Pig HDL and hybrid HDL particles were isolated and used as phospholipid acceptors in PLTP assay (110 μg of phospholipid, 250 μg of protein/assay) otherwise performed as described in Materials and Methods. The apoA-II/apoA-I molar ratios in the particles were 1, 0; 2, 0.2 mol/mol; 3, 1.1 mol/mol; 4, 2.1 mol/mol; 5, 7.6 mol/mol; 6, only apoA-II. The results shown are mean values of two experiments and four measurements; 1, 0 □; 2, 0.2 mol/mol ▲; 3, 1.1 mol/mol *; 4, 2.1 mol/mol ✖; 5, 7.6 mol/mol ■; 6, only apoA-II △.

The population of large particles formed also became more heterogeneous (**Table 3**). For example, when the apoA-II/apoA-I molar ratio in the particle was was 2.1 mol/mol, PLTP induced the formation of two populations of large fusion particles with diameters of 11.0 nm (50%) and 11.5 nm (50%).

The release of apoA-I can be seen in the gradient gel immunoblot with anti-pig–apoA-I antibody (**Fig. 5,** top). The formation of the small particles is also gradually reduced when apoA-II concentration in the particle increases. The immunoblot with anti-apoA-II antibody (Fig. 5, bottom) revealed the heterogeneity of the hybrid particles, although they were of the same size in the native gradient gel electrophoresis. After PLTP function there was a size change only in those hybrid particles that contained a small amount of apoA-II (apoA-II/apoA-I: 0.2 mol/mol) and the other particles remained unchanged.

After HDL interconversion the location of PLTP was determined by immunoblotting of the ultracentrifuged (d = 1.21 g/ml) and gel-filtered fractions with monoclonal anti-pig-PLTP antibody. PLTP was totally recovered in the bottom fraction (d > 1.21 g/ml) corresponding to a molecular weight of 70,000–120,000 D (Fig. 1A, fractions 32–35). A minor fraction of PLTP was detected in the position corresponding to a molecu-

TABLE 2. Chemical composition of pig HDL after incubation with PLTP

	Composition (mass%)					
Lipoprotein	Protein	PL	Chol	TG	SM/PC	A-I/Particle
Native HDL	50.3 ± 1.9	29.0 ± 1.8	18.6 ± 2.9	2.1 ± 0.2	0.25	2–4
Large fusion particles	49.5 ± 0.5	31.3 ± 1.2	16.2 ± 2.1	3.0 ± 0.4	0.19	2–3
Small particles	86.3 ± 0.9	11.6 ± 0.4	1.2 ± 0.1	—	0.57	2
Large particles (d > 1.21)	86.1 ± 0.7	11.8 ± 0.5	1.2 ± 0.1	—	0.41	5

The phospholipid subclass distribution was analyzed from two separate experiments and the apoA-I particle distribution from four separate experiments.

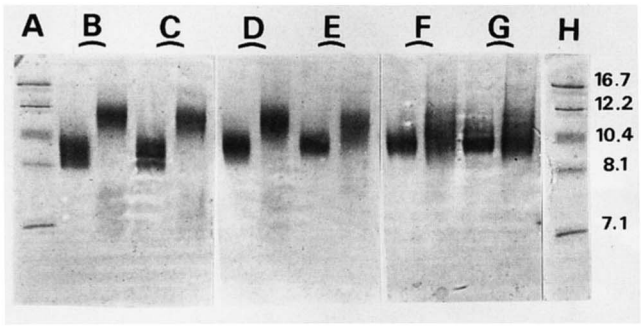

Fig. 4. Native gradient gel electrophoresis (4–26%) of apoA-II-modified pig HDL. The apoA-II-modified pig HDL particles were isolated by ultracentrifugation and gel filtration and incubated in the absence and presence of PLTP. In each pair of lanes the first lane displays the sample incubated in the absence of PLTP and the second lane in the presence of PLTP. ApoA-II concentration in the particle increases from left to right. The apoA-II/apoA-I molar ratios (mol/mol) are as follows. Lane A, globular protein molecular weight standard (nm); lane B, pig HDL, 0 mol/mol; lane C, hybrid HDL, 0.2 mol/mol; lane D, hybrid HDL, 1.1 mol/mol; lane E, hybrid HDL 2.1 mol/mol; lane F, hybrid HDL, 8.4 mol/mol; lane G, hybrid HDL, only apoA-II; lane H, standard (nm). The gel was stained with Coomassie Brilliant Blue.

lar weight of about 670,000 D (fractions 22–24). PLTP was located in this molecular weight position whether the incubated HDL sample was ultracentrifuged or not before gel filtration analysis. Nor did the apolipoprotein composition, i.e., apoA-II/apoA-I molar ratio, of the HDL particle used in the conversion reaction have an effect on the location of PLTP.

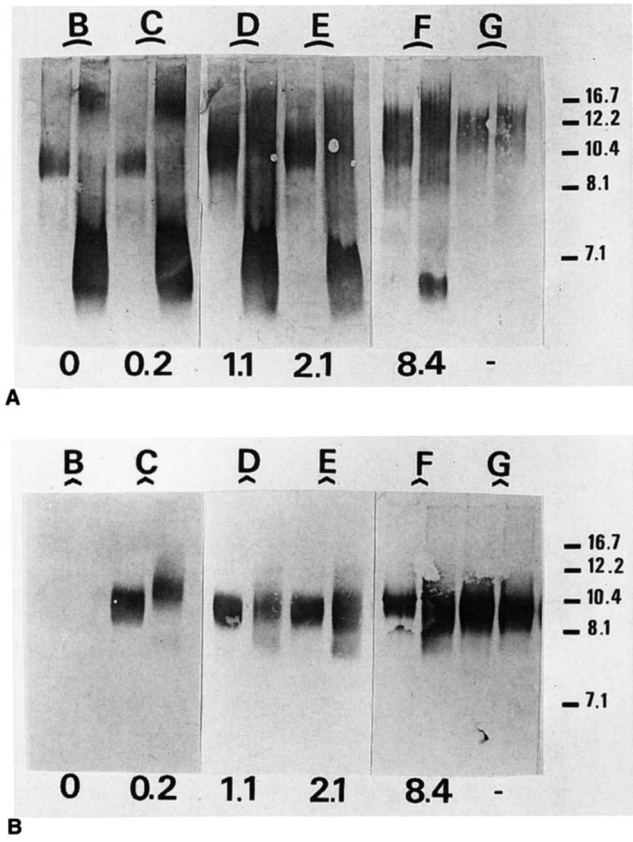

Fig. 5. Immunoblotting of the apoA-II-modified pig HDL isolated by native gradient gel electrophoresis. The gradient gel in Fig. 4 was run in triplicate and two were immunoblotted. The samples are the same as in Fig. 4. Panel A: the proteins were detected with anti-pig apoA-I antibody. Panel B: the proteins were detected with anti-human apoA-II antibody.

DISCUSSION

Although the physiological role of apoA-II has not yet been established, it is known to inhibit the LCAT reaction by displacing apoA-I, an activator protein of the enzyme, from the substrate lipid particles in vitro (41). Furthermore, inhibition of hepatic lipase by apoA-II is stronger than that of apoA-I, probably because of higher affinity of apoA-II for substrate lipid components (42). Controversial data also exist reporting that the lipolysis of triglycerides by hepatic lipase is higher in postprandial A-I/A-II–HDL_2 than in postprandial A-I–HDL_2 (43). These data clearly demonstrated that the

TABLE 3. Diameters of the hybrid HDL particles before and after PLTP incubation

Sample	Before	After
	nm	*nm*
1. Pig HDL	8.8	11.5 (100%)
2. Pig HDL + 0.2 mg A-II	8.9	11.2 (100%)
3. Pig HDL + 0.6 mg A-II	9.5	11.0 (36% ± 4) and 11.5 (64% ± 4)
4. Pig HDL + 1.0 mg A-II	9.7	11.0 (50% ± 9) and 11.5 (50% ± 9)
5. Pig HDL + 1.4 mg A-II	9.6	9.6 (70% ± 10) and 10.0 (30% ± 10)
6. Pig HDL + 1.8 mg A-II	9.5	9.3 (100%)

Numbers in parentheses show the percent distribution of different fusion particles ($d < 1.21$ g/ml). After PLTP-incubation, two other populations of particles with a constant diameter ($d > 1.21$ g/ml) also appeared, i.e., the large dense particles (11.5 nm) and preβ-particles (7.9 nm), which are not shown in the table. The hybrid particles were produced by incubating 1.5 mg of pig HDL with increasing amount of human apoA-II. The cv% in the particle sizes was 0.5–1.3.

major apolipoprotein composition in HDL, i.e., apoA-II/apoA-I molar ratio, affects the function of important enzymes in HDL metabolism.

Several studies have been published on the effect of HDL apolipoprotein composition on CETP activity. It is evident that cholesteryl ester transfer cannot be catalyzed by CETP in the absence of apolipoproteins (7). Furthermore, HDL apolipoproteins apoA-I and apoA-II may be important factors in modulating cholesteryl ester transfer rates in vivo independently of the lipid composition of the lipoprotein particle. It has been reported that the rate of cholesteryl esters transferred either from LDL toward HDL or in the opposite direction is considerably reduced as the apoA-II concentration of HDL_3 particles is increased. (8).

The aim of this study was to determine whether the protein composition of HDL had an effect on PLTP-mediated HDL conversion or phospholipid transfer. In order to answer this question we prepared hybrid particles from pig HDL that does not contain native apoA-II (17). ApoA-II, which binds lipids with higher affinity than apoA-I, displaces apoA-I completely from HDL (21, 22). In accordance with previous studies (21, 23), the substitution of apoA-II for apoA-I did not induce marked alterations in the lipid composition of HDL particles and was accompanied by only a slight increase in HDL total protein and particle size. These particles were then incubated with purified pig PLTP. In several previous studies with CETP similar experiments were performed using recombinant HDL particles with various amounts of apoA-I and apoA-II, or purified apolipoproteins were directly added to the experimental medium as free proteins (7, 44–46). In contrast, our report describes the effect of HDL apolipoproteins preincorporated into native HDL particles and, accordingly, the present HDL system more closely resembles physiological conditions.

After the apoA-II addition the HDL populations seemed to be rather homogeneous in their particle size (Table 1). The immunoblotting with anti-pig apoA-I or anti-apoA-II did not answer the question whether apoA-I and apoA-II are present in every particle in the same molar ratio. After PLTP treatment immunoblotting revealed two types of particles, i.e., those with apoA-II that remained unchanged and those with apoA-I that were converted.

The effect of increasing concentrations of apoA-II in the HDL particle on PLTP-mediated conversion was evident. Even a small amount of apoA-II (A-II/A-I molar ratio of 0.2 mol/mol) added to HDL inhibited the enlargement of the hybrid HDL particles and the amount of apoA-I released. The conversion was totally inhibited when the HDL particles were devoid of apoA-I.

It seems that the apoA-II/apoA-I molar ratio of HDL particles was not an important parameter in determining their ability to serve as acceptor lipoprotein substrates in the phospholipid transfer reaction. When we used the apoA-II-modified pig HDL particles in our PLTP assay as acceptors for the radiolabeled PC, the phospholipid transfer was linear only until 30 min and was not related to the A-II/A-I molar ratio in the HDL particle. As apoA-II has a higher affinity to lipid than apoA-I, the spontaneous phospholipid transfer was directly related to the apoA-II concentration in the acceptor particle.

A model for the PLTP-mediated HDL conversion mechanism has been presented recently proposing that the particle enlargement is due to fusion of apoA-I-depleted HDL (14). This study also reports that PLTP converts apoA-I recombinant-HDL but not apoA-II-rHDL, indicating that the presence of apoA-I is required for PLTP-mediated HDL fusion. This observation is confirmed in our study, although the suggested interaction of PLTP with apoA-I was not verified; we did not observe any difference in the location of PLTP after conversion whether the particle contained apoA-II or not. In the gel filtration PLTP eluted in the same position corresponding to its own molecular weight. In addition to the apolipoprotein content, neither ultracentrifugation nor incubation time had any effect on the PLTP location.

Although plasma LpA-I and LpA-I/A-II differ not only in their apolipoprotein content but also in their density, size, and lipid composition (47), the results of the present study suggest that modulation of the PLTP conversion activity by the A-II/A-I content of HDL particles is of physiological interest. Indeed, because the LpA-I/LpA-IA-II ratio (16) as well as LpA-II/(LpA-I + LpA-IA-II) ratio (48) have been shown to undergo significant physiological fluctuations, it is possible that HDL apolipoproteins may influence PLTP conversion activity in vivo.

The physiologically relevant observation of the present study is that increasing the amount of apoA-II in the HDL particle decreases the formation of apoA-I/phospholipid containing preβ-mobile HDL particles. These have been identified as primary acceptors of peripheral cell cholesterol in the reverse cholesterol transport (4). It has been suggested that LpA-I without A-II are more efficient acceptors of cholesterol than LpA-I with A-II (49, 50) and that the most avid acceptor of cholesterol from incubated fibroblasts is a plasma LpA-I-particle, slightly smaller than albumin and having a SM/PC ratio of about 0.6 (40). The small particles formed in PLTP-mediated HDL conversion fulfill these criteria. As a matter of fact, the most recent finding is that PLTP induces the generation of $preβ_1$-LpA-I particles, which take up cell-derived cholesterol (51).

The development of transgenic mice expressing human HDL apolipoproteins, lipolytic enzymes, and lipid transfer proteins has provided novel insights into lipoprotein structure, metabolism, and the role of these proteins in atherogenesis. Transgenic mice expressing human apoA-I have increased concentrations of HDL cholesterol and apoA-I and a human-like distribution of HDL into discrete HDL_2 and HDL_3 subclasses. Overexpression of apoA-I protects mice from diet-induced atherosclerosis (52). On the other hand, overexpression of apoA-II does not increase HDL cholesterol in mice, but their HDL particle size distribution is altered when apoA-II is expressed with apoA-I (53). The expression of apoA-II also diminishes the antiatherogenic effect of apoA-I (54). Thus the protein composition of HDL influences the remodelling of HDL and the metabolism of HDL subpopulations and may thereby affect the atherogenic potential of the particle.

Altogether, the data presented demonstrate that PLTP is an important factor participating in HDL subpopulation metabolism. PLTP produces initial cholesterol acceptors and thereby increases the capacity of the reverse cholesterol transport process. The data also demonstrate a crucial role of apoA-I in PLTP-mediated HDL interconversion.

We thank M.Sc. Jouko Sundvall and his group for performing chemical determinations and Ritva Keva and Ritva Nurmi for technical assistance. We also gratefully acknowledge Dr. Pedro Vidal-Marques for determining the phospholipid subclasses. This work was supported in part by a research grant from the Juho Vainio Foundation and by the Finnish Science Foundation.

Manuscript received 9 February 1996 and in revised form 2 October 1996.

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