Apolipoprotein A-I charge and conformation regulate the clearance of reconstituted high density lipoprotein in vivo

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Abstract **While low apolipoprotein A-I (apoA-I) levels are primarily associated with increased high density lipoprotein (HDL) fractional catabolic rate (FCR), the factors that regulate the clearance of HDL from the plasma are unclear. In this study, the effect of lipid composition of reconstituted HDL particles (LpA-I) on their rate of clearance from rabbit plasma has been investigated. Sonicated LpA-I containing 1 to 2 molecules of purified human apoA-I and 5 to 120 molecules of palmitoyl-oleoyl phosphatidylcholine (POPC) exhibit similar charge and plasma FCR to that for lipid free apoA-I, 2.8 pools/day. Inclusion of 1 molecule of apoA-II to an LpA-I complex increases the FCR to 3.5 pools/day, a value similar to that observed for exchanged-labeled HDL_3. In contrast, addition of 40 molecules of triglyceride, diglyceride, or cholesteryl ester to a sonicated LpA-I containing 120 moles of POPC and 2 molecules of apoA-I increases the negative charge of the particle and reduces the FCR to 1.8 pools/day. Discoidal LpA-I are the most positively charged lipoprotein particles and also have the fastest clearance rates, 4.5 pools/day. Immunochemical characterization of the different LpA-I particles shows that the exposure of an epitope at residues 98 to 121 of the apoA-I molecule is associated with an increased negative particle charge and a slower clearance from the plasma. We conclude that the charge and conformation of apoA-I are sensitive to the lipid composition of LpA-I and play a central role in regulating the clearance of these lipoproteins from plasma.**—Braschi, S., T. A-M. Neville, M-C. Vohl, and D. L. Sparks. **Apolipoprotein A-I charge and conformation regulate the clearance of reconstituted high density lipoprotein in vivo.** ***J. Lipid Res.*** **1999.** 40: **522–532.**

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The strong inverse correlation that has been demonstrated between plasma high density lipoprotein (HDL) concentrations, their major protein component (apolipoprotein A-I (apoA-I)), and the risk of coronary heart disease has generated much interest in elucidating the mechanisms that modulate their concentrations in vivo. Previous studies have established that accelerated apoA-I catabolism is the main metabolic predictor of HDL levels (1, 2); plasma apoA-I levels appear to be determined by the rate of apoA-I catabolism rather than by production (3). However, the factors that control the clearance of apoA-I from plasma have not been clearly delineated so far.

Previous works have suggested that HDL particle size is influenced by plasma triglyceride levels and triglyceride lipase activities (4) and may affect the catabolism of apoA-I (1, 2). These studies suggest that smaller apoA-I particles may have a shorter mean life span than larger ones (2, 5). Indeed, Brinton, Eisenberg, and Breslow (6) reported that 70% of the variability in apoA-I fractional catabolic rate (FCR) was due to variability in estimates of HDL size. It has been shown that triglyceride enrichment of HDL may promote lipolysis, which reduces their size (7) and may increase their catabolic rate (1, 8). Hypertriglyceridemic individuals with low plasma HDL levels have triglyceride-rich HDL and accelerated apoA-I FCR (1, 9). In vitro, the enrichment of HDL with triglycerides and their subsequent hydrolysis with lipoprotein lipase increases their elimination by isolated rabbit kidneys (5). As the hydrolysis of core triglycerides is known to promote the dissociation of apoA-I from HDL in vitro (10), it was proposed that hypertriglyceridemic individuals with low HDL may have an increased apoA-I FCR because they have an increased proportion of their apoA-I in a more loosely bound pool that can be more rapidly cleared by the kidney (5).

The determination of the specific properties that govern the metabolism of HDL in vivo has been hampered by the use of heterogeneous native HDL and different HDL labeling techniques, both of which give rise to highly vari-

Abbreviations: apo, apolipoprotein; CE, cholesteryl linoleate; CETP, cholesteryl ester transfer protein; DG, diolein; DMS, dimethyl suberimidate; FC, free (unesterified) cholesterol; FCR, fractional catabolic rate; GGE, gradient gel electrophoresis; GdnHCL, guanidine HCl; HDL, high density lipoproteins; HL, hepatic lipase; LCAT, lecithin: cholesterol acyltransferase; LpA-I, reconstituted HDL particles: Lp2A-I, reconstituted particles containing 2 molecules of apoA-I; Lp1A-I, reconstituted particles containing 1 molecule of apoA-I, LpA-I/A-II, reconstituted particles containing 2 molecules of apoA-I and 1 molecule of apoA-II; PL, phospholipid, POPC, palmitoyl-oleoyl phosphatidylcholine; TG, triolein.

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able results (11, 12). In order to determine how the biophysical properties of HDL may regulate their clearance from plasma, we have undertaken to use reconstituted LpA-I with well-defined biophysical characteristics. Both nuclear magnetic resonance and epitope expression studies show that slight changes in the LpA-I lipid composition have major effects on the conformation of apoA-I (13) and concomitantly on the charge and stability of the HDL particle (14–16). Studies with LpA-I have shown that apoA-I conformation is extremely sensitive to the neutral lipid content of sonicated particles and that the cholesteryl ester:triglyceride ratio in LpA-I complexes regulates the charge and stability of the particle (17). Investigations with these well-defined lipoprotein particles have also shown that apoA-I charge and conformation directly affect the in vitro metabolism of HDL (18–21). The activities of lecithin:cholesterol acyltransferase (LCAT) and hepatic lipase (HL) are affected by changes in both the charge and conformation of apoA-I (19, 20, 22). In addition, apoA-I physical properties also appear to affect the efflux of cholesterol from the plasma membrane of human fibroblasts (18). These observations suggest that the composition-dependent biophysical properties of HDL particles may control HDL metabolism in vivo.

The aim of this study is, therefore, to determine how HDL composition affects apoA-I charge and conformation and the clearance of this lipoprotein from rabbit plasma. We show that reconstituted sonicated LpA-I are cleared from rabbit plasma similarly to native HDL and that the LpA-I charge and apoA-I conformation are correlated to the rates of LpA-I clearance.

EXPERIMENTAL PROCEDURES

Materials

Cholesteryl linoleate (CE), triolein (TG), and diolein (DG) were purchased from Sigma Chemical Co. (St Louis, MO). 1-Palmitoyl 2-oleoyl phosphatidylcholine (POPC) was obtained from Avanti Polar Lipids (Birmingham, AL). The monoclonal antibody, 3G10, was produced by established protocols (23). All other reagents were analytical grade.

Purification of apoA-I and apoA-II

Human HDL (d 1.063–1.210 g/ml) was isolated from fresh plasma by sequential density gradient ultracentrifugation according to the procedure of Havel, Eder, and Bragdon (24). HDL was delipidated in chloroform:methanol as described (25). Purified apoA-I was isolated by size exclusion chromatography on a Sephacryl S-200 HR column (26) and apoA-II was purified by anion-exchange chromatography on Q-Sepharose (27). Both apoA-I and apoA-II were stored in lyophilized form at −80°C. Prior to use, they were resolubilized in 6 m guanidine HCl (Gdn HCl), 10 mm Tris, pH 7.2, and dialyzed extensively against 50 mm $NaPO_4$, pH 7.2 (PBS).

Iodination of apoA-I

Purified apoA-I was iodinated using Iodo-Beads (Pierce, Rockford, IL). Briefly, the beads were washed in PBS and incubated with 20 μl of ^{125}I (2 mCi of NaI) in 70 μl of PBS for 5 min at room temperature. One hundred fifty μg of apoA-I was added to the mixture and incubated at room temperature for 45 min with continuous agitation. In order to remove the unincorporated free iodine, the mixture was then passed through a desalting column (Excellulose GF5, Pierce, Rockford, IL) previously equilibrated with PBS BSA 1%, and the eluate was extensively dialyzed against PBS.

Preparation of ^{125}I-labeled LpA-I and LpA-I/A-II complexes

Reconstituted sonicated Lp2A-I, LpA-I/A-II, and Lp1A-I complexes were prepared by co-sonication of apoA-I, POPC and CE, DG, TG, or apolipoprotein A-II (apoA-II) (19). Briefly, specific amounts of lipids in chloroform (see Table 1 for concentrations) were dried down under nitrogen in a 12 × 75 mm glass tube, and 800 μl of PBS was added. The lipid–buffer mixture was successively sonicated under nitrogen for 1 min at constant output, incubated at 37°C for 30 min, and sonicated again for 5 min at 95% duty cycle under nitrogen. Cold apoA-I (1 mg of a 1.4 mg/ml phosphate solution) and ^{125}I-labeled apoA-I (50 μCi) were added to the lipid mixture and co-sonicated for 4 × 1 min at 90% duty cycle under nitrogen, with 1-min cooling periods between sonications. Discoidal Lp2A-I complexes were prepared using purified apoA-I and POPC according to the cholate dispersion:Bio-Bead removal technique previously described (15).

Determination of LpA-I and LpA-I/A-II physical and structural properties

The size and homogeneity of reconstituted particles were estimated by non-denaturing gradient gel electrophoresis on precast 8–25% gradient acrylamide gels (Pharmacia Biotech Phastgel, Baie d'Urfé, QC) or on 4–25% gradient acrylamide gels (Novex, San Diego, CA). Densitometric profiles were obtained by analyzing Coomassie Brilliant Blue G-stained gels on a Sharp JX 325 imaging densitometer. The mean apparent diameter was determined by comparison with protein standards (Pharmacia High Molecular Weight Protein Calibration Kit, Baie d'Urfé, QC) using a data analysis software (Onedscan, Scanalytics). The number of molecules of apoA-I per particle was determined by apoprotein cross-linking with dimethyl suberimidate as described by Swaney (28) and SDS-PAGE on 8–25% acrylamide gels to determine the extent of oligomer formation. Surface potential was calculated from electrophoresis on precast 0.5% agarose gels (Beckman, Paragon Lipo Kit) according to the method described by Sparks and Phillips (29). The free energy of unfolding of apoA-I on the surface of LpA-I complexes was calculated from the effect of GdnHCl concentration on the secondary structure of apoA-I in various LpA-I particles, as monitored by the changes in molar ellipticity at 222 nm. Aliquots of each complex (33 μg protein/mL buffer) were incubated with 0–6 m GdnHCl in phosphate buffer for 72 h at 4°C. The free energy of unfolding of apoA-I on the surface of LpA-I complexes (ΔG_D^o) was calculated as described previously (30).

Immunoreactivity of apoA-I on LpA-I and LpA-I/A-II complexes

Immunoreactivity measurements were determined from a competitive solid phase radioimmunometric assay similar to that previously described (23). Removawells (Immulon 2, Dynatech Laboratories, MA) were coated with 100 μl of HDL_3 (0.2 μg in 15 mm Na_2CO_3, 35 mm $NaHCO_3$, 0.02% NaN_3, pH 9.6), washed with 50 mm $NaPO_4$, 0.02% NaN_3, pH 7.2 (buffer A), and saturated with 0.5% gelatin (Bio-Rad Laboratories, Hercules, CA) in buffer A. Anti-apoA-I monoclonal antibody, 3G10 (at a predetermined dilution), was mixed with serial dilutions of LpA-I or LpA-I/A-II particles in 50 mm $NaPO_4$, 0.02% NaN_3, 0.05% Tween 20, 0.1% gelatin (buffer B) and transferred to the previously coated and saturated wells for 1 h incubation at room temperature. After

four washes with buffer B without gelatin, the removawells were incubated for 1 h with a ^{125}I-labeled anti-mouse IgG antibody diluted in buffer B. After four washes with buffer B, the well radioactivity was measured. The reactivity of the 3G10 was estimated by determining the particle concentration required for 50% inhibition of the maximal binding of 3G10 to the HDL_3-coated plate (ED_{50}). Results are expressed in μg/ml and are means of quadruplicate determinations.

Preparation of exchanged-labeled HDL_3

HDL_3 (d 1.18–1.21 g/ml) was isolated from fresh, fasted normolipidemic plasma by sequential density gradient ultracentrifugation. HDL_3 was then exchanged-labeled according to the method described by Horowitz et al. (5). Briefly, 30 μCi of iodinated apoA-I was incubated with 500 μg of HDL_3 at 37°C for 1 h. The HDL_3 was subsequently reisolated by ultracentrifugation at d 1.18 g/ml and d 1.21 g/ml, respectively, and dialyzed against PBS. The size and homogeneity of the HDL_3 fraction were estimated by non-denaturing gradient gel electrophoresis on precast 8–25% gradient acrylamide gels (Pharmacia Biotech Phastgel, Baie d'Urfé, QC).

In vivo metabolic studies

Twenty μCi (200 μg of apoA-I) of ^{125}I-labeled LpA-I or ^{125}I-labeled HDL_3 was injected into the marginal ear vein of a 4.5–5.5 kg male New Zealand White rabbit previously sedated (fentanyl citrate and fluanisone, 70 μg/kg and 2.2 μg/kg intramuscularly, respectively). Blood samples were drawn into tubes containing 0.1 mg/ml disodium EDTA from the opposite ear at 10 min, 1, 2, 4, 6, 12, 24, 30, 48, and 54 h. The isotope decay curves for both iodinated LpA-I and ^{125}I-exchanged-labeled HDL_3 were constructed by counting plasma radioactivity at each time point. The percent of injected dose was plotted as a function of time, using the 10-min sample as the zero time. In this study, each particle was injected two to three times into different rabbits, with different preparations of apoA-I, native HDL and LpA-I particles.

Kinetic modeling and statistical methods

Exponential decay curves for both ^{125}I-labeled LpA-I and ^{125}I exchanged-labeled HDL_3 were used to calculate the FCR. The curves were analyzed according to the two-pool model of Matthews (31). This model assumes the existence of an intravascular pool in dynamic equilibrium with an extravascular pool. According to this model, both new input and exit of apoA-I occur from the intravascular pool. Decay curves for each experiment were modeled separately with a biexponential equation:

$$y(t) = A_1 e^{-a_1(t)} + A_2 e^{-a2(t)}.$$

LpA-I FCR was determined from the area under the curve, as calculated from Matthews' equation (31):

$$FCR = 1/[(A_1/a_1 + A_2/a_2)/y(0)]$$

and expressed in pools/day.

Correlation coefficients were determined by the method of Spearman. $P \leq 0.05$ was regarded as statistically significant.

RESULTS

Effect of LpA-I composition on the lipoprotein structural properties

Reconstituted LpA-I and LpA-I/A-II particles were prepared by co-sonication to contain various amounts of POPC, CE, TG, and DG (see **Table 1** for compositions). Complexation of 5 to 120 molecules of POPC with apoA-I increased the hydrodynamic diameter of the molecule from approximately 6 to 7.9 nm, as evidenced by a retarded electrophoretic mobility on 8–25% gradient acrylamide gels. Consistent with that reported previously (18), chemical crosslinking of the poorly lipidated apoA-I complex (with 5 molecules of apoA-I) with dimethyl suberimidate (DMS) showed that the sonicated preparation contained only complexes having one molecule of apoA-I (Lp1A-I). Incorporation of 120 molecules of POPC into apoA-I caused the formation of a much larger Lp2A-I complex containing two molecules of apoA-I (Lp2A-I) (17,

TABLE 1. Composition, biophysical properties, and FCR of reconstituted and native HDL particles

Particle Composition POPC:?:ApoA-I[a]	Hydrodynamic Diameter[b]	Surface Potential[c]	Thermodynamic Stability $\Delta G_D{}^o$[d]	ED_{50} for Mab 3G10[e]	FCR[f]
mol:mol:mol	*±0.5 nm*	*±0.2 mV*	*±0.2 kcal/mol A-I*	*±0.4 μg/ml*	*±0.3 pools/day*
ApoA-I	5.6	−8.3	2.4	4.9	2.8
s 5:0:1	6.0	−8.5	0.9	3.4	2.7
s 120:0:2	7.9	−8.5	1.2	2.3	2.8
s 120:40:2 (CE)	8.1	−9.9	1.9	2.3	2.1
s 120:40:2 (TG)	7.7	−9.3	2.1	1.8	1.8
s 120:40:2 (DG)	7.5	−10.0	1.5	3.5	1.8
s 120:0:2 + ApoA-II	8.0	−8.9	ND	5.9	3.5
d 280:0:2	9.9	−7.8	3.0	5.1	4.5
HDL_3	9.2	−13.2	ND	ND	3.4

[a] 1-Palmitoyl 2-oleoyl phosphatidylcholine (POPC), cholesteryl linoleate (CE), triolein (TG), diolein (DG), apolipoprotein A-I (apoA-I), and apolipoprotein A-II (apoA-II) molar composition. Particles were prepared by sonication (s) or by a cholate dispersion/Bio-bead removal technique (d). Apoprotein stoichiometries were determined from SDS PAGE gels after crosslinking with dimethyl suberimidate. Values are representative of 3 different preparations of LpA-I.

[b] Particle diameters from non-denaturating gradient gel electrophoresis (±SD).

[c] Charge potential at the particle surface (±SD).

[d] Free energy of denaturation at zero guanidine HCl concentration (±SD).

[e] Particle concentration required to inhibit 50% of the maximal binding of 3G10 antibody by competitive radioimmunometric assay (ED_{50}) (±SD).

[f] Fractional catabolic rate (FCR) of the particle after injection in rabbits (±SD). FCR were calculated from the area under plasma decay curves according to Matthew's equation (31). Values are representative of two to three different clearance experiments with different preparations of each particle.

32, 33). Electrophoresis of Lp1A-I and Lp2A-I complexes in 0.5% agarose gels showed that the presence of a few molecules of POPC on apoA-I did not affect the electrophoretic mobilities of the particle and the negative surface potential as compared to lipid-free apoA-I (Table 1). As previously reported (17), inclusion of neutral lipids into the sonicated Lp2A-I complex had no significant effect on the particle hydrodynamic diameter, but decreased the negative particle surface potential. The inclusion of one molecule of apoA-II to a sonicated Lp2A-I complex had minimal effect on the hydrodynamic diameter of the particle, but significantly increased the negative charge of the particle. The discoidal Lp2A-I complex characterized in this study is similar to those evaluated previously (17). As compared to a sonicated Lp2A-I, discoidal Lp2A-I was considerably larger and less negatively charged.

Effect of LpA-I composition on the stability of apoA-I

The thermodynamic stability of apoA-I in various LpA-I complexes (ΔG_D^o) was calculated from the denaturation curves of LpA-I in the presence of GdnHCl (14). As indicated in Table 1, the thermodynamic stability of apoA-I on Lp1A-I was compromised in the presence of lipid. A few molecules of POPC reduced the ΔG_D^o for apoA-I by about 60%. However, further lipidation of apoA-I with POPC and formation of Lp2A-I increased the thermodynamic stability of apoA-I (Table 1). Inclusion of CE and TG increased the thermodynamic stability of apoA-I on sonicated Lp2A-I, while inclusion of DG had a similar effect, although to a lesser magnitude. As previously reported (17), apoA-I on a discoidal LpA-I particle appeared to be more resistant to GdnHCl denaturation than apoA-I on a sonicated particle.

Effect of LpA-I and LpA-I/A-II composition on the conformation of apoA-I

Differences in apoA-I immunoreactivity on various LpA-I and LpA-I/A-II complexes was assessed by competitive immunoassay. Preliminary comparison of apoA-I immunoreactivity on various LpA-I complexes with the FCR for similar particles showed that immunoreactivity of a central domain in apoA-I was specifically related to its plasma clearance. Because this domain, residues 98 to 121, represents the epitope of the monoclonal antibody 3G10, we evaluated the immunoreactivity of apoA-I for 3G10 on the reconstituted LpA-I prior to their injection into rabbits. Immunoreactivity, as indicated by an ED_{50} value, corresponds to the particle concentration required for 50% inhibition of the maximal binding of 3G10 to the HDL_3-coated plate and is inversely related to the 3G10 epitope exposure on the LpA-I and LpA-I/A-II complexes. As shown in Table 1, the exposure of 3G10 epitope was enhanced in poorly lipidated Lp1A-I particles, as compared to lipid-free apoA-I. Further lipidation of the Lp1A-I complex, with formation of an Lp2A-I complex, increased 3G10 epitope expression. These results are in accordance with earlier studies, which have shown that the exposure of residues 98–121 in apoA-I is increased when the molecule is associated with lipids (13, 16). Inclusion of CE into the Lp2A-I particle had no affect on the exposure of the 3G10 epitope, while inclusion of TG into the complex modified the conformation of apoA-I and increased the 3G10 epitope exposure. In contrast, the incorporation of DG or apoA-II into the LpA-I complex decreased the 3G10 epitope exposure, this effect being particularly pronounced with apoA-II. Similarly, 3G10 epitope exposure also appeared to be decreased in discs as compared to spheres (Table 1).

Plasma clearance of LpA-I and native HDL_3

LpA-I and LpA-I/A-II complexes were prepared from human apoA-I and their in vivo clearances from rabbit plasma were determined as indicated. The reproducibility of the method was good. Injection of the same particle in three different rabbits gave almost superimposable results (see FCR variability below). In preliminary experiments, we evaluated the ability of LpA-I made from human apoA-I to be remodeled in the plasma of a rabbit. Incubations with LpA-I prepared from human apoA-I showed that the human apolipoprotein is readily able to activate rabbit LCAT in vitro. To characterize LpA-I physical properties after their in vivo metabolism, we determined the electrophoretic mobility on 0.5% agarose gels of the different LpA-I complexes in rabbit plasma at various times between 10 min and 48 h after injection. Autoradiography of the agarose gels showed no modification of the spherical and discoidal LpA-I electrophoretic mobilities over 48 h, which indicates that the surface charge of the different particles was not modified in vivo (**Fig. 1**, upper panel). Similarly, analysis of LpA-I migration on non-denaturating gradient acrylamide gels by autoradiography showed that the size of the LpA-I particles was also not altered during the turnover period (Fig. 1, lower panel). Furthermore, the surface charge and size of LpA-I complexes were identical before and after injection.

Plasma clearance of exchanged-labeled native human HDL_3 was evaluated (**Fig. 2**) and the decay curves appear comparable to previously reported experiments (34). It is of note that sonicated LpA-I and LpA-I/A-II complexes exhibit plasma clearance curves similar to that for native HDL_3 particles. While the Lp2A-I was cleared slightly slower than the native HDL_3, addition of one molecule of apoA-II was associated with an increase in the rate of clearance of the particle from plasma (Fig. 2). Characterization of discoidal LpA-I complexes showed their rates of clearance from plasma to be very different than that for the sonicated particles. Discoidal LpA-I complexes were cleared extremely rapidly from the plasma of a rabbit, much faster than native lipoproteins or sonicated LpA-I (Fig. 2).

Plasma clearance rates were determined by fitting the decay curve data to a bi-exponential equation and then calculating the FCR from Matthews' equation (Table 1). Nearly identical results were obtained when the data were analyzed with the SAAM-II multi-exponential curve-fitting technique (Version 1.1 SAAM Institute, Inc., Seattle, WA), using a bicompartmental model. However, biexponential curve fits appeared statistically slightly better than those from SAAM II. FCR values obtained in this study are com-

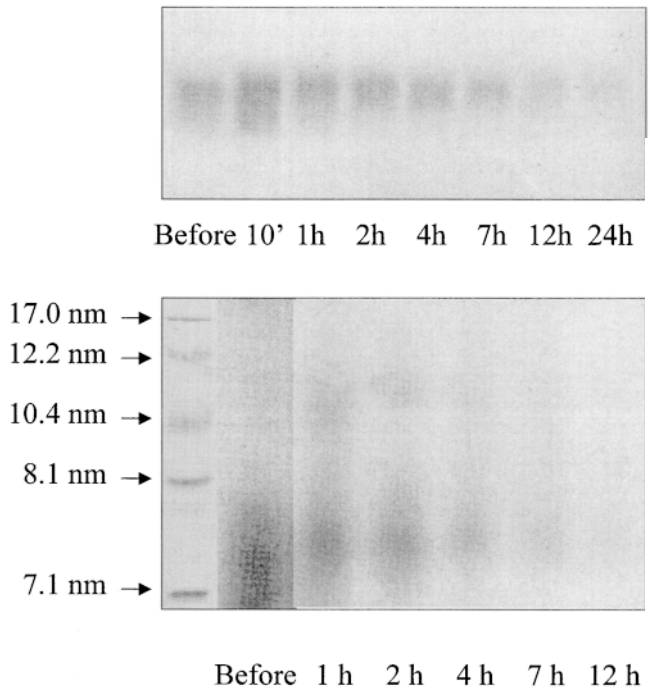

Fig. 1. LpA-I biophysical properties after injection in rabbit. Sonicated LpA-I prepared from ^{125}I-labeled apoA-I were injected into rabbits, and their in vivo remodeling was monitored over a 48-h period by electrophoresis on 0.5% agarose gels and 4–25% nondenaturating gradient acrylamide gels. Shown are autoradiographs of original agarose (upper panel) and acrylamide (bottom panel) gels, which exhibit electrophoretic profiles of a spherical 60:1 (POPC:apoA-I) LpA-I particle before injection and in the plasma of rabbit sampled at 10 min to 48 h after injection. Similar profiles were observed for both sonicated and discoidal LpA-I particles.

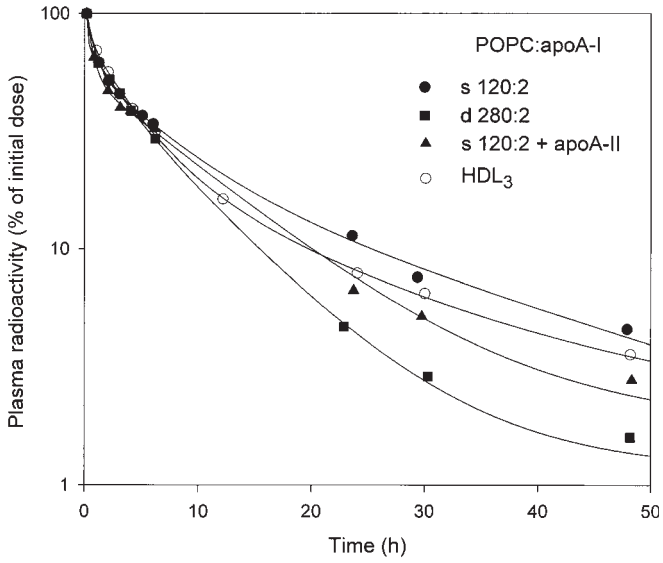

Fig. 2. Plasma decay curves of native high density lipoprotein (HDL) and reconstituted complexes. Sonicated and discoidal LpA-I prepared from ^{125}I-labeled apoA-I and exchange-labeled native HDL_3 were injected into rabbits and the rate of radioactivity decay in plasma was measured. Each curve is representative of two to three different clearance experiments with different preparations of each particle. The molar ratio of 1-palmitoyl 2-oleoyl phosphatidylcholine (POPC) and apolipoprotein A-I (apoA-I) is indicated for sonicated and discoidal reconstituted complexes containing 2 molecules of apoA-I (Lp2A-I) and sonicated reconstituted complexes containing 2 molecules of apoA-I and 1 molecule of apoA-II (LpA-I/A-II). Results are expressed as percentage of the initial radioactivity injected in plasma as a function of time.

parable to those of previous published works using similar protocols (34). As expected, FCR values closely reflect the clearance curves shown for the different particles. The inter-rabbit FCR variation (SD) observed for injections of the same particle in three different rabbits was approximately 0.3 pools/day. As indicated in Table 1, apoA-I on native HDL_3 exhibits a plasma FCR of 3.4 pools/day. While sonicated LpA-I has a slightly lower FCR, 2.8 pools/day, addition of apoA-II to the LpA-I complex increased the FCR of apoA-I to 3.5 pools/day, a value essentially identical to that for HDL_3. Of all the complexes studied, discoidal LpA-I were cleared the fastest and had the highest FCR value, 4.5 pools/day. As many studies suggest that the triglyceride and the cholesteryl ester content of HDL particles may affect HDL catabolism in vivo (1, 4), we prepared and characterized LpA-I complexes containing various neutral lipid constituents. As shown in **Fig. 3**, inclusion of TG, CE, or DG was associated with a decrease in the rate of clearance of the Lp2A-I particle from plasma. Furthermore, the decrease in clearance was of the same magnitude for the three neutral lipids studied. Indeed, as indicated in Table 1, FCR values for TG, CE, and DG LpA-I particles were similar, averaging approximately 1.9 pools/day, and much lower than that of LpA-I particles without neutral lipid, 2.8 pools/day. This result is consistent with recent reports of a decreased rate of clearance of triglyceride-enriched HDL in rabbits (34). The data may further explain observations of a decreased clearance of cholesteryl ester-enriched HDL in CETP-deficient patients (35) and an enhanced clearance of HDL particles in CETP-transgenic mice (36).

We also measured the in vivo plasma clearance of sonicated LpA-I complexes containing various amounts of POPC (**Fig. 4**). As compared to lipid-free apoA-I, the inclusion of 5 or 120 molecules of POPC did not affect the plasma clearance of sonicated LpA-I complexes. Indeed, FCR values for apoA-I, Lp1A-I with 5 molecules of POPC, and Lp2A-I with 120 molecules of POPC were almost identical, averaging 2.8 pools/day. Therefore, the POPC content of sonicated LpA-I may not be critical in determining their clearance from plasma.

Effect of LpA-I physical properties on their plasma clearance rates

In order to further understand how the physical properties may affect the clearance of LpA-I complexes from plasma, non-parametric Spearman correlation coefficients between LpA-I physical parameters and their FCR were determined. No significant relationship was found between the FCR of LpA-I particles and their size or stability (r = 0.4 and r = 0.2, respectively, n.s.). In contrast, as shown in **Fig. 5**, the exposure of residues 98 to 121 on the

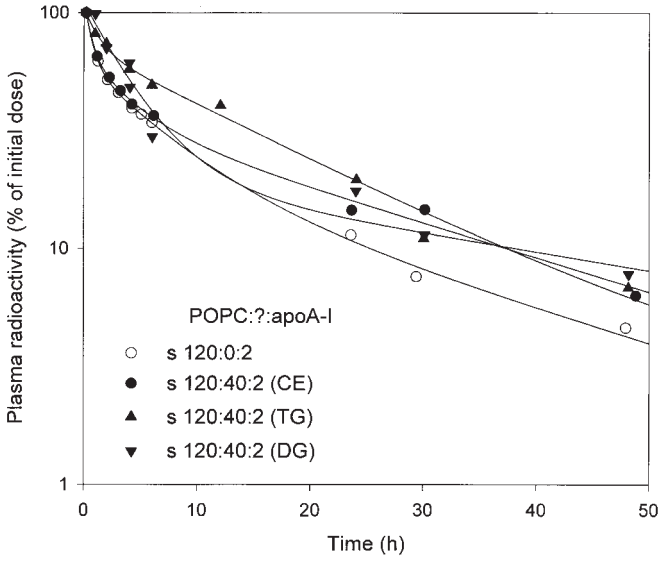

Fig. 3. Effect of the neutral lipid core on plasma decay curves of reconstituted complexes. Forty molecules of either cholesteryl ester (CE), triolein (TG), or diolein (DG) were included in reconstituted complexes containing 2 molecules of apolipoprotein A-I (apoA-I) and 120 molecules of 1-palmitoyl 2-oleoyl phosphatidylcholine (POPC) (Lp2A-I). Their plasma radioactivity decay curves in rabbits are indicated relative to that of an Lp2A-I particle devoid of neutral lipid. Each curve is representative of two to three different clearance experiments with different preparations of each particle. Results are expressed as percentage of the initial radioactivity injected in plasma as a function of time.

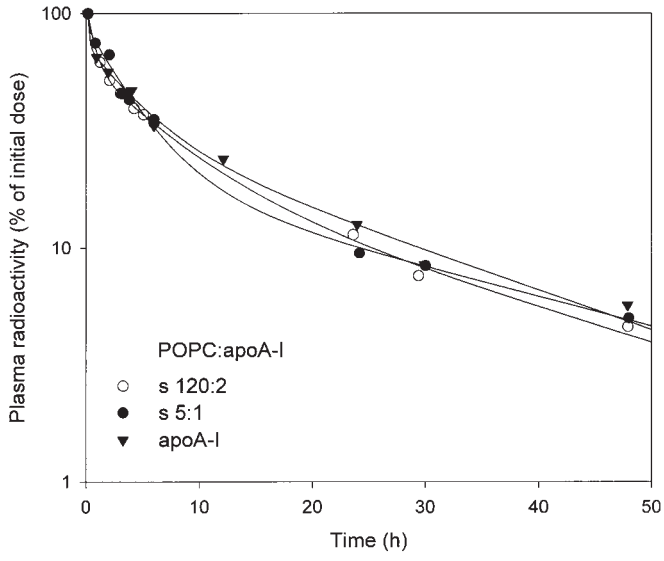

Fig. 4. Effect of the phospholipid content on the plasma decay curves of reconstituted complexes. Reconstituted complexes with either 1 molecule of apolipoprotein A-I (apoA-I) and 5 molecules of 1-palmitoyl 2-oleoyl phosphatidylcholine (POPC) (Lp1A-I) or 2 molecules of apoA-I and 120 molecules of POPC (Lp2A-I) were injected in rabbits. Their plasma decay curves are indicated relative to that of apoA-I. Each curve is representative of two to three different clearance experiments with different preparations of each particle. Results are expressed as percentage of the initial radioactivity injected in plasma as a function of time.

apoA-I molecule, as evaluated by 3G10 ED_{50} values for the different LpA-I complexes, was significantly related to a decreased rate of clearance from plasma ($r = 0.7$, $P = 0.05$). Similarly, a strong, significant correlation was identified between LpA-I surface potentials and their estimated FCR values ($r = -0.7$, $P < 0.04$) (**Fig. 6**). These results suggest that the apoA-I conformation and charge may affect the clearance of LpA-I particles from plasma.

DISCUSSION

This investigation has attempted to identify the factors that regulate HDL metabolism in vivo. We have utilized well-defined recombinant lipoprotein complexes, with which we can selectively manipulate many of their physicochemical properties. Plasma decay curves of sonicated LpA-I particles are similar to that of exchanged-labeled native human HDL_3 lipoproteins (Fig 1). In contrast, we show that discoidal LpA-I are cleared much faster from rabbit plasma. Different metabolic behavior appears to reflect differences in the physical properties of discoidal complexes compared to sonicated ones. Indeed, it is known that both charge and structure of apoA-I is dependent upon the kind of lipoprotein particle with which it is associated (30, 37–39). We have shown that conformational differences of the apoA-I molecule on a disc give rise to major changes in the negative surface charge and thermodynamic stability, relative to that observed for sonicated LpA-I (Table 1). This and other studies show that sonicated LpA-I are more structurally and functionally similar to native HDL than discoidal LpA-I (19). Our kinetic experiments provide further evidence that sonicated LpA-I complexes are metabolically comparable to native HDL.

Earlier studies in humans have reported conflicting results concerning the relative rates of clearance of native HDL_2 and HDL_3 from plasma. The clearance of large HDL has been reported to be either similar (40) or slower (41) than that of small HDL. Investigations in this laboratory have had similar conclusions; different preparations of HDL may have very unique and different clearance behaviors (data not shown). Differences in the rate of clearance of HDL_3 appear to be related to significant inter-patient differences in the composition of these lipoproteins. To determine which of the constituents may be important in HDL clearance we have systematically varied both the lipid and apolipoprotein composition of homogeneous LpA-I particles. As HDL_3 particles are known to contain 1 molecule of apoA-II, we evaluated the specific effect of apoA-II on HDL clearance. Inclusion of apoA-II in a reconstituted LpA-I particle is associated with a decrease of the particle negative surface charge (Table 1) and a marked increase in its rate of clearance from plasma (Fig. 1). If apoA-II can directly promote the clearance of HDL from the plasma, this observation supports the view that apoA-II has a pro-atherogenic propensity as suggested by recent studies (42–44). This finding, however,

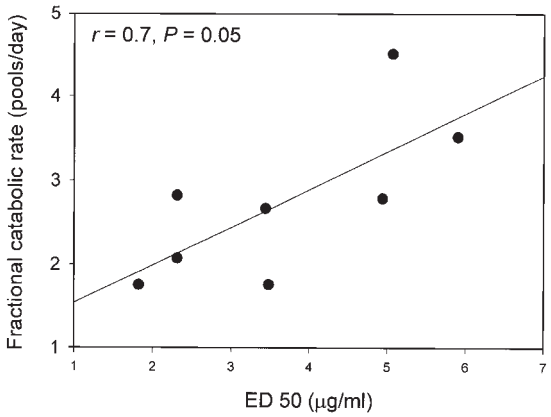

Fig. 5. Relationship between ED_{50} values for 3G10 and apoA-I fractional catabolic rate (FCR). ED_{50} represents the reconstituted complex concentration required to inhibit 50% of the maximal 3G10 binding on a HDL_3-coated plate in a radioimmunometric assay. 3G10 is a monoclonal antibody directed against residues 98–121 of the human apolipoprotein A-I molecule (23). A decrease in the ED_{50} value indicates an increase in the exposure of the 98–121 domain at the surface of the reconstituted Lp2A-I complex. FCR were calculated from the area under plasma decay curves of reconstituted complexes (see Table 1) using the equation of Matthew (31) and are expressed as pools/day ± 0.3 (SD), and plotted against ED_{50} values expressed in μg/ml ± 0.4 (SD). Correlation coefficients were determined by the method of Spearman.

does not appear to be consistent with that observed in humans, where it was shown that immunopurified LpA-I were cleared much faster from plasma than LpA-I/A-II (45). While this difference may reflect a unique metabolic behavior in the rabbit, an animal that could be considered hepatic lipase-deficient (46), results from human studies with native lipoproteins must also be viewed with caution. Native LpA-I/A-II and LpA-I HDL subclasses represent highly heterogeneous populations of particles that exhibit inter-patient compositional variation, but also differ in their physical (density, size and charge) and chemical properties (47). When interpreting these investigations it is difficult to know whether it was specifically apoA-II or some other factor that affected plasma clearance rates. We have made use of reconstituted particles with well-defined properties that can be selectively controlled. Therefore, our results show that an increase in the clearance of LpA-I/A-II, relative to LpA-I, is solely due to the presence of apoA-II. Changes in metabolic behavior appear to reflect a modification in the biophysical properties of the LpA-I particle induced by the presence of apoA-II. Addition of a molecule of apoA-II into the lipoprotein is associated with a change in particle charge and a major increase in the exposure of a central domain of the apoA-I molecule, located at residues 98 to 121 (Table 1).

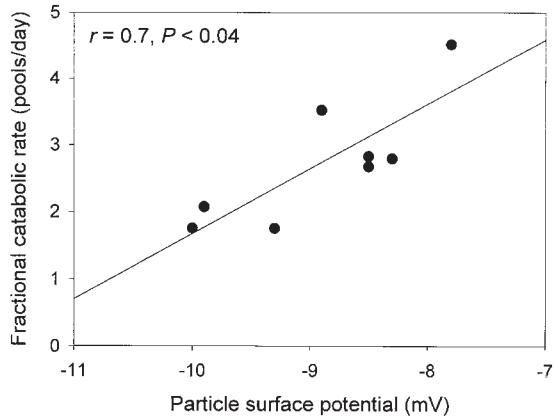

Fig. 6. Relationship between particle surface potential and fractional catabolic rate. Fractional catabolic rates were calculated from the area under plasma decay curves of reconstituted complexes (see Table 1 for characteristics) using the equation of Matthew (31) and are expressed in pools/day ± 0.3 (SD), and plotted against the particle surface potentials expressed in mV ± 0.2 (SD). Correlation coefficients were determined by the method of Spearman.

Inclusion of neutral lipids in reconstituted LpA-I also had distinct effects on apoA-I conformation and LpA-I physical properties (17, 30, 33, 37). Indeed, a decrease in the cholesteryl ester content in LpA-I particles is associated with a decrease in the amount of α-helices in apoA-I and a reduction in the lipoprotein structural stability. Such cholesteryl ester-depleted and triglyceride-enriched particles are very similar to abnormal HDL particles found in hypertriglyceridemic individuals with low plasma HDL cholesterol levels (48, 49). It is widely thought that these compositional abnormalities may affect plasma clearance of these lipoproteins in vivo (1, 5, 9). To test whether the kind of neutral lipid in HDL affects its rate of clearance from plasma, we prepared and injected into rabbits LpA-I containing either TG, DG, or CE. The presence of any core lipid was associated with an increase in the particle surface charge (Table 1) and a parallel decrease in the rate of clearance of the particle (Fig. 2). The delayed clearance of cholesteryl ester-containing LpA-I observed here appears consistent with previous investigations in rabbits, which showed that the overexpression of human LCAT in transgenic rabbits raises the cholesteryl ester content of HDL and decreases apoA-I catabolism from plasma (50). In addition, an increase in cholesteryl ester:triglyceride ratio in HDL, as observed in human genetic CETP deficiency, is also related to an increased HDL residence time and an elevation in plasma HDL levels (35).

While no data are available describing the effect of DG on the catabolic rate of LpA-I, our observation of a delayed clearance of TG-enriched particles is similar to that observed by Lewis et al. (34). Their recent study showed that large triglyceride-rich HDL were cleared markedly slower than small triglyceride-poor HDL in rabbits (34). Saku et al. (41) have also shown that large HDL are cleared more slowly than smaller particles in rabbits; however, in their study they characterized a subset of HDL containing only apoA-I. Increased HDL clearance rates in hypertriglyceridemic subjects actually appear to be associated with the formation of small TG-enriched HDL (48, 49). In general, HDL from hypertriglyceridemic patients are not only TG-enriched, but are usually very small and both core and surface lipid-poor (49). We have observed that the inclusion of very large amounts of TG in LpA-I complexes leads to the formation of very small TG-rich

Lp2A-I complexes that are depleted in POPC. These particles are much more positively charged and are rapidly cleared from rabbit plasma (S. Braschi and D. Sparks, unpublished data). Therefore, alterations in HDL-TG content may parallel changes in the amount of other HDL-constituents and together they may alter the physical properties of HDL from hypertriglyceridemic patients and promote their clearance from plasma.

ApoA-I catabolism in rabbits may be affected by the low levels of post-heparin hepatic lipase activity in rabbits, as compared to humans (46). A number of lines of evidence show that hepatic lipase (HL) plays an important role in HDL metabolism. Moderately increased triglyceride-rich HDL are found in individuals with genetic deficiency of HL (51). Similarly, the overexpression of HL in rabbits leads to a major reduction in their plasma HDL levels (52). In fact, it was shown that post-heparin HL activity is inversely related to HDL cholesterol levels in humans (53), and promotes the clearance of HDL particles (1, 9). It is therefore tempting to speculate that the low HL activity expressed in rabbits, as compared to humans, may uniquely affect the clearance of triglyceride-rich LpA-I in rabbits. Indeed, it was recently shown that HL hydrolyzes HDL phospholipids, diglycerides, and triglycerides and thereby alters the composition and charge of HDL (22). A deficiency of HL may also have an effect on the remodeling of HDL *in vivo*. Characterization of LpA-I physical properties after various times of in vivo incubation in rabbits showed that LpA-I charge and size were not modified. This was a surprising observation, however, we also saw no change in particle charge in turnovers with rabbit apoA-I and native HDL_3 and also in in vitro incubations of spherical LpA-I and rabbit or human plasma (data not shown). The data show that LpA-I clearance is not a function of the dissolution/remodeling of the LpA-I particle in vivo. It also suggests that HDL remodeling is a very slow process or a process that does not drastically alter the physical properties of HDL particles.

Several investigations have shown that the dissociation of apoA-I from HDL leads to the formation of a very small, lipid-poor HDL subclass that has been called preβ_1 HDL (54, 55). Some lines of evidence have suggested that this subclass may be cleared more rapidly from the plasma by renal filtration (56, 57). To investigate the effect of the lipidation of apoA-I on its clearance in vivo, we prepared and characterized sonicated Lp1A-I and Lp2A-I containing different amounts of POPC. No differences in the surface charge or FCR values were observed between LpA-I with either 5 or 120 molecules of POPC (Fig. 3). It is of note, however, that lipid-free apoA-I, Lp1A-I, and Lp2A-I were all cleared much faster than Lp2A-I containing CE, TG, or DG (Fig. 2). This result supports the hypothesis that free or poorly lipidated apoA-I would be more rapidly cleared from plasma than a neutral lipid-rich HDL particle (56, 57). Indeed, turnover studies conducted in individuals with low HDL cholesterol levels have shown that rates of HDL clearance are related to the concentration of poorly lipidated apoA-I in plasma (1, 2, 6). Similarly, in another study, the inhibition of lipoprotein lipase in monkeys led to the enrichment of HDL in triglycerides and consequently *i*) an increase in plasma concentration of lipid-poor apoA-I, *ii*) an increase in apoA-I catabolism, and *iii*) its concomitant accumulation in kidneys (54). Horowitz et al. (5) further demonstrated that the in vitro enrichment of HDL with triglycerides, followed by lipolysis, promoted the dissociation of apoA-I and its removal by perfused kidney cortex. It has been proposed therefore that small lipid-poor apoA-I molecules that are generated from unstable HDL (10), are susceptible to rapid clearance by the kidney (5, 56). This pathway may be partially responsible for the low plasma HDL levels in hyperlipidemic patients. Surprisingly, we found no relationship between the FCR of LpA-I particles and their size and stability in this study. This suggests that it is not LpA-I particle size or stability that regulates their catabolism in vivo, but rather that it is related structural and electrostatic properties that are important to this process.

In order to evaluate an association between apoA-I conformation and its in vivo metabolism, we have utilized immunochemical investigations with specific monoclonal antibodies to apoA-I. Early epitope expression studies using native lipoproteins have shown that slight changes in the HDL lipid composition produce large changes in apoA-I conformation, in particular in the exposure of a central domain composed of two adjacent antiparallel α-helices encompassing residues 99 to 143 (16). Residues 90 to 105 are well exposed on apoA-I at the surface of native HDL but are hidden on delipidated apoA-I (58). Similarly, we observed that the incorporation of a few phospholipid molecules in reconstituted LpA-I increases the exposure of residues 98 to 121. The exposure of this domain appears to play an important regulatory role in HDL metabolism. Indeed, studies suggest that it may act as a cholesterol binding domain (13, 32) and could be involved in various processes, such as LCAT activation (59) and cellular cholesterol efflux (60). In order to evaluate the relationship between the exposure of this apoA-I domain and HDL particle clearance in vivo, we determined ED_{50} values of the various LpA-I complexes for antibody 3G10 and compared them to particle FCR values. We identified a strong positive relationship between 3G10 ED_{50} values for the different LpA-I complexes and their FCR ($r = 0.7$, $P = 0.05$). This result indicates that the exposure of residues 98 to 121 on the apoA-I molecule is related to a decreased rate of clearance from plasma. The exposure of the 3G10 epitope also appears to be positively related to the particle surface potential; however, possibly due to the small number of particles characterized, this relationship was not statistically significant ($r = 0.4$, n.s.). In contrast, the exposure of other domains of the apoA-I molecule on the LpA-I complexes was not related to plasma clearance rates. For instance, no relationship was found between the exposure of residues 2 to 8, in the N-terminal domain of the apoA-I molecule, and the clearance rates of the particles (data not shown).

A number of investigations have shown that electrostatic interactions may be centrally important in the regulation of HDL metabolism. Studies have shown that the re-

modeling of HDL particles by the enzymes, LCAT, CETP, phospholipid transfer protein, and HL may be affected by HDL surface charge (19, 21, 22, 61). Similarly, class B type I scavenger receptor, SR-BI, which binds HDL and is involved in CE selective uptake from HDL, is also electrostatic sensitive and binds anionic phospholipids with a high affinity (62). The data suggest that interfacial interactions with HDL particles may be sensitive to the particle electrostatic properties. In order to elucidate how HDL charge may affect their metabolism in vivo, LpA-I surface charge was determined from their electrophoretic mobilities in 0.5% agarose and was then compared to the particle FCR values. Similar to that observed with 3G10 ED_{50} values, we also identified a strong positive relationship between the surface charge of the LpA-I particles and their clearance rates from plasma ($r = 0.7$, $P < 0.04$); the more positively charged LpA-I particles being cleared the fastest. HDL particle charge is primarily due to apoA-I and is sensitive to changes in the conformation of this apoprotein that result from different HDL compositions (33). About 10% of HDL charge is due to the direct effect of anionic lipids, such as phosphatidylinositol, which are present in the lipoprotein surface. The inclusion of 18 molecules of phosphatidylinositol in a sonicated Lp2A-I containing 120 molecules of POPC decreased the negative surface potential of the particle (from −8.5 mV to −11.2 mV) but did not decrease its clearance from plasma (results not shown). Similarly, HDL_3 plasma clearance was superimposable to that of LpA-I/A-II particle (Fig. 1), even though its surface potential was far more electronegative (−10.7 mV vs. −8.9 mV, respectively). As anionic lipids also contribute to the global charge of the HDL_3 particle (33), this suggests that the presence of charged lipids does not directly affect LpA-I clearance from plasma. Instead, it appears that it is an apoprotein conformation-dependent surface charge that regulates HDL clearance from plasma.

The tissue sites for HDL clearance from the circulation appear to differ for the different constituents of HDL. Most of apoA-I appears to be cleared by the kidneys (56), while selective uptake of HDL cholesteryl esters occurs mainly in the liver and the adrenals (63). Small HDL particles also appear able to equilibrate with the extravascular fluid space (64). In kidneys, apoA-I and small-sized HDL are filtered through the glomerulus, reabsorbed, and degraded by proximal tubular cells (8, 56, 65). A number of investigations conducted in humans and animals show that the kidney glomerular capillary wall exhibits charge selectivity (66, 67). The presence of negatively charged sites within the glomerular capillary wall hinders the filtration of anionic molecules on an electrostatic basis (66, 67). Therefore, it is conceivable that the delayed clearance of the more negatively charged LpA-I complexes in our study may be partially related to kidney glomerular filtration properties. It appears possible that the dissociation of apoA-I from LpA-I decreases the negative charge on apoA-I, promotes renal excretion, and thereby increases apoA-I clearance from plasma. On the contrary, highly electronegative HDL would have lower catabolic rates because their charge would inhibit renal clearance and instead promote intravascular particle remodeling by enzymes such as LCAT (59).

In conclusion, we have explored the relationship between the physicochemical properties of reconstituted LpA-I particles and their rate of clearance from plasma. We show that exposure of a central domain in apoA-I, residues 98 to 121, is related to both a decrease in the negative surface charge of LpA-I and a decrease in the catabolism of these particles. This study therefore shows that the apoA-I conformation-dependent charge properties of LpA-I govern their catabolism in vivo.

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