Changes in the size of reconstituted high density lipoproteins during incubation with cholesteryl ester transfer protein: the role of apolipoproteins

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Abstract It has been reported previously that the particle size distribution of discoidal, reconstituted HDL (r-HDL) changes dramatically during incubation in vitro with cholesteryl ester transfer protein (CETP). The present study was undertaken in order to determine whether these changes are influenced by the apolipoprotein composition of the r-HDL. Two preparations of r-HDL that contained egg phosphatidylcholine (egg PC) and unesterified cholesterol (UC) but differed in their apolipoprotein composition were used for the study. One preparation contained apolipoprotein (apo) A-I only (A-I w/o A-II r-HDL) while the other contained apoA-I and apoA-II (A-I w A-II r-HDL). The Stokes' radius of the major population of particles in the (A-I w/o A-II) and (A-I w A-II) r-HDL was, respectively, 4.8 and 4.9 nm. When the (A-I w/o A-II) r-HDL were incubated with CETP, most of the particles of radius 4.8 nm were converted to populations of smaller and larger particles. The smaller particles had Stokes' radii of 4.3 and 3.9 nm. The radii of the larger particles ranged from 8.2 to 13.7 nm. When the (A-I w A-II) r-HDL were incubated with CETP larger particles (Stokes' radii=8.4–11.0 nm) appeared but there was minimal conversion to smaller particles. In addition, a significant proportion of the original (A-I w A-II) r-HDL of radius 4.9 nm was still present at the end of the incubation. These results are consistent with apoA-II inhibiting the conversion of r-HDL to small particles. It is concluded that the apolipoprotein content of r-HDL is an important determinant of the sizes of the particles that are formed during incubation with CETP.—**Rye, K-A., K. H. Garrety, and P. J. Barter.** Changes in the size of reconstituted high density lipoproteins during incubation with cholesteryl ester transfer protein: the role of apolipoproteins. *J. Lipid Res.* 1992. **33**: 215–224.

Supplementary key words reconstituted HDL • cholesteryl ester transfer protein • apolipoprotein A-I • apolipoprotein A-II

Plasma high density lipoproteins (HDL) comprise several populations of particles of distinct size, density, and composition (1). Recent studies have established that some subpopulations of HDL have specific functions with respect to the transport of cholesterol. For example, a minor population of small, pre-β-migrating HDL has been identified as the primary acceptor of unesterified cholesterol (UC) from peripheral tissues (2). Small HDL are also the principle substrates of lecithin:cholesterol acyltransferase (LCAT), the enzyme that esterifies cholesterol in plasma (3). These observations suggest that an understanding of the factors that regulate the particle size of HDL is extremely important.

Human plasma has been reported to contain a factor that promotes the conversion of isolated HDL_3 to populations of larger and smaller particles (4). It has recently been established that the cholesteryl ester transfer protein (CETP), a protein that mediates the transfer and exchange of cholesteryl esters and triacylglycerol between lipoprotein classes, is the major protein constituent in preparations of the putative HDL conversion factor (5). Further evidence that CETP regulates the particle size distribution of HDL has come from studies where changes to the size of HDL that were previously attributed to activity of the conversion factor were abolished when anti-CETP monoclonal antibodies were added to the incubation mixture (6).

CETP also converts reconstituted HDL (r-HDL), which contain neither cholesteryl esters nor triglyceride, to larger and smaller particles (7). R-HDL have several advantages over native lipoproteins for studying the regulation of CETP-mediated changes in the particle size of HDL. In the first instance, the composition of r-HDL can be tightly controlled. Furthermore, changes to the size of r-HDL are not complicated by redistributions of neutral lipids within or between lipoprotein fractions or by alterations to the partitioning

Abbreviations: HDL, high density lipoproteins; r-HDL, reconstituted HDL; LDL, low density lipoproteins; egg PC, egg yolk phosphatidylcholine; UC, unesterified cholesterol; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; TBS, Tris-buffered saline; (A-I w/o A-II) r-HDL, reconstituted HDL containing only apoA-I; (A-I w A-II) r-HDL, reconstituted HDL containing apoA-I and apoA-II.

of components between the surface and core of individual particles.

The present study investigates whether the CETP-mediated changes to the size of r-HDL are influenced by the apolipoprotein composition of the particles. This is an issue of considerable importance as human plasma contains two classes HDL that differ in their apolipoprotein composition: one contains only apolipoprotein (apo) A-I and is designated (A-I w/o A-II) HDL while the other contains apoA-I and apoA-II and is designated (A-I w A-II) HDL (8). At present it is not known whether these apolipoprotein-specific populations of HDL have distinct metabolic functions. This issue is addressed in the present study by asking the following question: does the replacement of a proportion of the apoA-I in r-HDL by apoA-II influence CETP-mediated changes in the particle size of the r-HDL?

MATERIALS AND METHODS

Isolation of HDL

HDL ($1.063 < d < 1.21$ g/ml) were isolated from human plasma by ultracentrifugation in a Beckman L8-70M ultracentrifuge. The 1.063 g/ml spin was carried out at a speed of 55,000 rpm for 17 h. The resulting infranatant was adjusted to a density of 1.21 g/ml and the HDL were isolated as the supernatant after 24 h of ultracentrifugation at a speed of 55,000 rpm. A 55.2 Ti rotor was used for these procedures. The HDL were then subjected to a wash spin at a speed of 50,000 rpm for 40 h in a 50 Ti rotor. All density adjustments were made by the addition of solid KBr (9). The plasma samples for this procedure were donated by the Red Cross Blood Bank at the Wollongong Hospital.

Isolation of apoA-I and apoA-II

Isolated HDL were exhaustively dialyzed against a 0.01 M ammonium bicarbonate solution and delipidated by well-established procedures (10). The resulting apoHDL were subjected, as previously described (11), to anion exchange chromatography on a column of Q Sepharose Fast Flow (Pharmacia LKB Biotechnology, Uppsala, Sweden). The apoA-I and apoA-II, each of which appeared as a single band after electrophoresis on a 10–15% SDS-polyacrylamide gradient gel, were lyophilized, stored at –20°C, and reconstituted as required.

Preparation of r-HDL

Egg PC, UC, and sodium cholate were purchased from Sigma (St Louis, MO) and used without further purification. (A-I w/o A-II) r-HDL were prepared by the cholate dialysis method (12). (A-I w A-II) r-HDL were prepared by adding apoA-II to (A-I w/o A-II) r-HDL at ambient temperature (11). Prior to use, the r-HDL preparations were subjected to ultracentrifugation at a density of 1.25 g/ml and gel permeation chromatography on a prepacked Superose 6 HR 10/30 column (Pharmacia LKB Biotechnology, Uppsala, Sweden). The details of these procedures have been described elsewhere (11).

Incubations

All incubations were carried out in 0.01 M Tris-buffered saline (pH 7.4) that contained 0.15 M NaCl, 0.005% (w/v) EDTA-NA_2, and 0.006% (w/v) NaN_3 (TBS). Samples were placed in stoppered plastic tubes in a shaking water bath that was maintained at 37°C. Nonincubated samples were stored at 4°C. Details of individual incubations are described in the legends to the figures. When the incubations were complete the samples were adjusted to a density of 1.25 g/ml with KBr (9) and the r-HDL were isolated as the supernatant by ultracentrifugation at 50,000 rpm for 40 h in a Beckman L8-70M ultracentrifuge. A 50 Ti rotor was used for this procedure. All ultracentrifugation procedures were carried out at 4°C.

Purification of CETP

CETP was isolated in a partially pure form from human plasma. The purification procedure has been described in detail elsewhere (5, 13). Briefly, 2 l of plasma was subjected to ammonium sulfate precipitation, ultracentrifugation, and chromatography on Phenyl Sepharose CL-4B (Pharmacia LKB Biotechnology, Uppsala, Sweden), CM-52 Cellulose (Whatman Biosystems, Ltd., Kent, England) and Mono Q (Pharmacia LKB Biotechnology, Uppsala, Sweden). After these procedures approximately 1.0 mg of protein was recovered in a volume of 4.0 ml. SDS-polyacrylamide gradient gel electrophoresis and silver staining revealed two bands with approximate molecular weights of 67,000 and 74,000. The activities of these preparations were assessed by their ability to transfer [^{3}H]cholesteryl esters from HDL_3 to LDL during a 3-h incubation at 37°C (14). After the incubation the LDL were precipitated by the addition of a heparin–$MnCl_2$ solution and the radioactivity remaining in the supernatant was determined (15). The results are expressed as transfer units, where a unit is the fraction of the [^{3}H]cholesteryl esters transferred from HDL_3 to LDL. The assay is linear as long as less than 40% of the [^{3}H]cholesteryl esters are transferred from HDL_3 to LDL. According to this assay system, human lipoprotein-deficient plasma contains approximately 10 transfer units/ml. The CETP isolated as described above

contained 128 transfer units/ml and was purified approximately 4,000-fold relative to human lipoprotein-deficient plasma.

Other methods

Particle size distributions of r-HDL were determined by electrophoresis on PAA 2/16 or 4/30 nondenaturing gradient gels (Pharmacia LKB Biotechnology, Uppsala, Sweden). Details of this procedure are described elsewhere (7). A Cobas-Fara Centrifugal Analyser (Roche Diagnostics, Zurich, Switzerland) was used to measure the concentrations of egg PC, UC, apoA-I, and apoA-II. Boehringer-Mannheim (Germany) enzymatic kits were used for the egg PC and UC assays. An immunoturbidometric assay was used to measure the concentrations of the apolipoproteins. Details of this procedure are described elsewhere (16).

RESULTS

Particle size distribution and stoichiometry of (A-I w/o A-II) and (A-I w A-II) r-HDL (Fig. 1, Table 1)

The particle size distributions of (A-I w/o A-II) and (A-I w A-II) r-HDL are shown in **Fig. 1**. The Stokes' radius of the major population of particles in the preparation of (A-I w/o A-II) r-HDL was 4.8 nm. Minor populations of smaller (Stokes' radius=4.5 and 4.0 nm) and larger (Stokes' radius=6.2 nm) particles were also present. The size of the (A-I w A-II) r-HDL was similar to that of the (A-I w/o A-II) r-HDL, with most of the particles having a Stokes' radius of 4.9 nm.

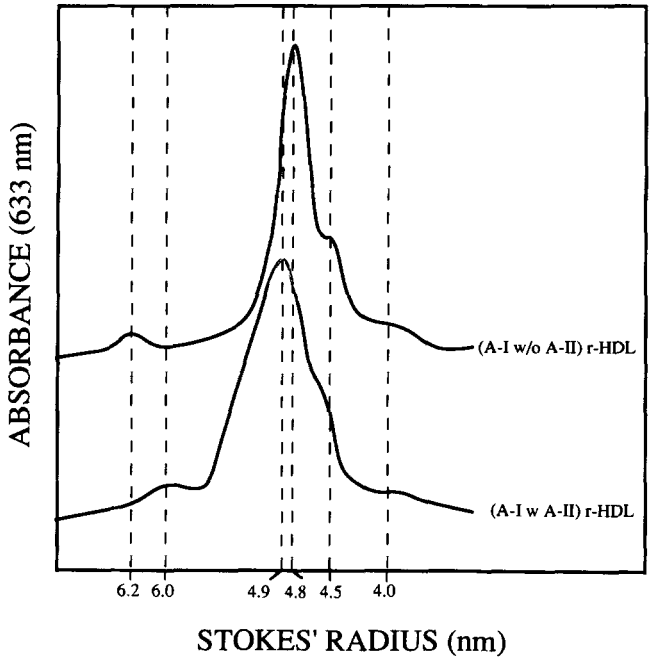

Fig. 1. Particle size distribution of (A-I w/o A-II) and (A-I w A-II) r-HDL. Aliquots of (A-I w/o A-II) and (A-I w A-II) r-HDL were electrophoresed on a pre-cast PAA 4/30 nondenaturing gradient gel. The profiles represent laser densitometric scans of the stained gel.

The stoichiometries of the (A-I w/o A-II) and (A-I w A-II) r-HDL preparations are shown in **Table 1.** The molar ratio of egg PC:apoA-I for the (A-I w/o A-II) r-HDL was 95:1. In the case of the (A-I w A-II) r-HDL, the respective molar ratios of egg PC:apoA-I and apoA-I:apoA-II were 176:1 and 1:1.5.

Particle size distribution of (A-I w/o A-II) and (A-I w A-II) r-HDL after incubation with CETP (Fig. 2)

(A-I w/o A-II) and (A-I w A-II) r-HDL were either maintained for 24 h at 4°C (profiles A and D) or incubated at 37°C in the absence (profiles B and E) or presence (profiles C and F) of CETP. When the incubations were complete the samples were reisolated by ultracentrifugation. Comparison of profiles A and D with those in Fig. 1 shows that ultracentrifugation does not affect the particle size distribution of r-HDL. Similarly, incubation in the presence of TBS does not alter the particle size distribution of (A-I w A-II) r-HDL (profile E). This was not the case when (A-I w/o A-II) r-HDL were incubated with TBS (profile B). Although the Stokes' radius of the major population of particles was unchanged at 4.8 nm, the particles of radius 4.5 nm became more prominent and additional populations of smaller (Stokes' radius=3.9 nm) and larger (Stokes' radii=6.2–8.2 nm) particles appeared. When CETP was present in the incubation mixture the (A-I w/o A-II) r-HDL were converted into two populations of smaller particles with Stokes' radii of 4.3 and 3.9 nm and several populations of larger particles with Stokes' radii ranging from 8.2 to 13.7 nm. The particle size of (A-I w A-II) r-HDL was, by contrast, much more resistant to change during incubation with CETP. The Stokes' radius of the major population of particles remained unchanged at 4.9 nm. However larger (Stokes' radius=8.4–11.0 nm) particles appeared and minor populations of smaller particles (Stokes' radius=4.5 and 4.0 nm) were also apparent.

TABLE 1. Stoichiometry and particle size distributions of (A-I w/o A-II) and (A-I w A-II) r-HDL

r-HDL	Stoichiometry Egg PC–UC–ApoA-I–ApoA-II	Stokes' Radius
	molar ratio	*nm*
A-I w/o A-II	95:8:1:0	4.8[a], 4.5
A-I w A-II	176:13:1:1.5	4.9[a], 4.5

(A-I w/o A-II) r-HDL were prepared by the cholate dialysis method. (A-I w A-II) r-HDL were prepared by displacing apoA-I from (A-I w/o A-II) r-HDL with apoA-II. Stokes' radii were determined by gradient gel electrophoresis. The concentrations of egg PC, UC apoA-I, and apoA-II were determined in duplicate as described in Materials and Methods. Stoichiometries were calculated from the mean concentrations of the individual components.
[a]Size of the major population of particles.

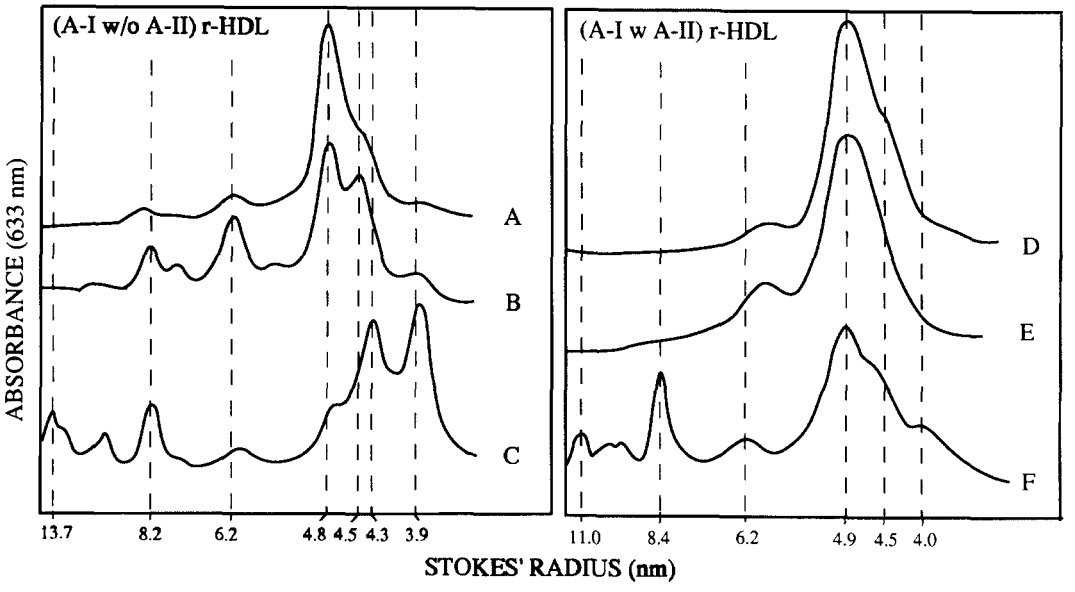

Fig. 2. Incubation of (A-I w/o A-II) and (A-I w A-II) r-HDL in the presence of CETP. Individual aliquots of (A-I w/o A-II) and (A-I w A-II) r-HDL that contained 2.0 mg of apolipoprotein were mixed with TBS (5.0 ml) or CETP (640 units of activity in a volume of 5.0 ml). Control samples were maintained at 4°C (profiles A and D) or incubated at 37°C (profiles B and E) for 24 h. The samples containing CETP (profiles C and F) were incubated at 37°C for 24 h. When the incubations were complete the samples were adjusted to a density of 1.25 g/ml and ultracentrifuged as described in Materials and Methods. The r-HDL were isolated as the supernatant and electrophoresed on PAA 4/30 gradient gels. The profiles represent laser densitometric scans of the stained gels.

Gel permeation chromatography of (A-I w/o A-II) and (A-I w A-II) r-HDL after incubation with CETP (Fig. 3)

After incubation with CETP the re-isolated (A-I w/o A-II) and (A-I w A-II) r-HDL were subjected to gel permeation chromatography. The mean column recoveries of egg PC, UC, and apoA-I for the (A-I w/o A-II) r-HDL were, respectively, 71%, 80%, and 95%. In the case of the (A-I w A-II) r-HDL, the recovery of each component was 100%. The elution profiles of the individual components are shown in **Fig. 3**. Panels A and D, respectively, represent the profiles for the (A-I w/o A-II) and (A-I w A-II) r-HDL that were maintained at 4°C. Panels B and E show the samples incubated at 37°C in the presence of TBS and panels C and F represent the samples incubated with CETP.

All the components in the nonincubated samples co-eluted in fractions 19–24 (panels A and D). Incubation in the presence of TBS did not effect the elution profile of the (A-I w A-II) r-HDL (panel E), but in the case of the (A-I w/o A-II) r-HDL a proportion of the egg PC, UC, and apoA-I eluted earlier, in fractions 16–19 (panel B). When the (A-I w/o A-II) r-HDL were incubated in the presence of CETP (panel C) approximately 34% of the egg PC, 15% of the UC, and 66% of the apoA-I were recovered in fractions 22–25. The remainder of these components eluted earlier, in fractions 8–19, in particles that were enriched in egg PC and UC and depleted of apoA-I. When the (A-I w A-II) r-HDL were incubated with CETP, 55% of the egg PC, 37% of the UC, 66% of the apoA-I, and 96% of the apoA-II were recovered in fractions 22–25. The remainder of the egg PC, UC, apoA-I, and apoA-II eluted in fractions 11–19 as components of larger particles that were enriched in egg PC and deficient in apoA-II (panel F). Selected fractions were combined as shown and the resulting samples were designated as pool I–pool VIb.

Particle size distribution of the pooled gel permeation chromatographic fractions (Fig. 4)

Comparison of the distribution of the particles in pool I and pool IV with profiles A and D in Fig. 2 shows that the size of r-HDL is not affected by gel permeation chromatography. The Stokes' radius of the major population of particles in the (A-I w/o A-II) r-HDL that had been maintained at 4°C (pool I) or incubated at 37°C with TBS (pool II) was 4.8 nm. These samples also contained minor populations of smaller particles with Stokes' radii of 4.5 and 3.9 nm. Pools IIIa, IIIb, and IIIc consisted of fractions from the incubation of (A-I w/o A-II) r-HDL with CETP. Pool IIIa contained low concentrations of particles with Stokes' radii ranging from 10.5–13.6 nm. Pool IIIb contained a single population of particles of radius 8.2 nm. Most of the particles in pool IIIc had a Stokes' radius of 3.9 nm. Minor populations of particles with radii of 4.5 and 4.8 nm were also present in this sample.

In the case of the (A-I w A-II) r-HDL, pool IV (nonincubated control) and pool V (incubated control)

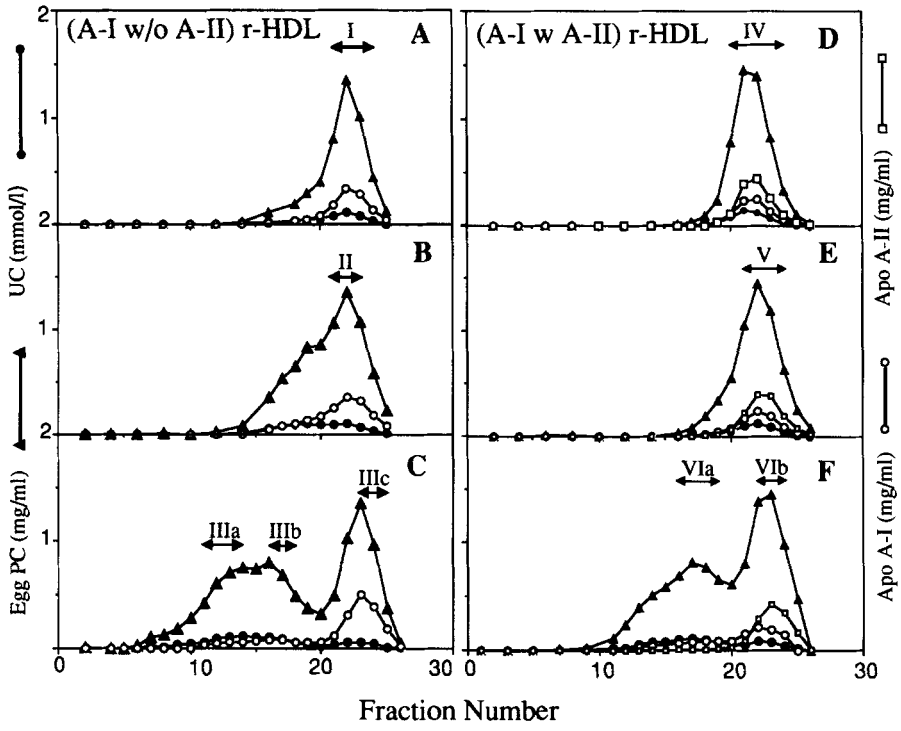

Fig. 3. Elution profiles of (A-I w/o A-II) and (A-I w A-II) r-HDL after incubation with CETP. The samples that were isolated as described in the legend to Fig. 2 were subjected to gel permeation chromatography on a prepacked Superose 6 HR 10/30 column that had been pre-equilibrated with TBS. The samples were eluted with TBS at a flow rate of 0.5 ml/min and 0.5-ml fractions were collected. The elution profiles of egg PC (▲—▲), UC (●—●), apoA-I (○—○), and apoA-II (□—□) are shown. Panels A and D represent the samples that were maintained at 4°C; panels B and E represent the samples that were incubated at 37°C for 24 h in the presence of TBS. Panels C and F represent the samples that were incubated at 37°C for 24 h in the presence of CETP. Individual fractions were combined into pools I-VIb.

contained particles of Stokes' radius 4.9 nm. When the (A-I w A-II) r-HDL were incubated with CETP, the resulting fractions were combined into two pools, VIa and VIb, which, respectively, contained particles with radii of 8.3 nm and 4.9 nm. The fractions containing particles with radii greater than 8.3 nm were also combined but were not processed further as the concentration of all the components was extremely low.

Stoichiometry of pooled samples I-VI (Table 2)

The stoichiometries of the (A-I w/o A-II) r-HDL that had been maintained at 4°C (pool I) or incubated at 37°C with TBS (pool II) were similar to those of the original (A-I w/o A-II) r-HDL (see Table 1). This confirms that the composition of r-HDL is not affected by ultracentrifugation or gel permeation chromatography. By contrast, the large particles in pool IIIa and pool IIIb that were formed during the incubation of (A-I w/o A-II) r-HDL with CETP were enriched in egg PC relative to apoA-I. The small conversion products in pool IIIc were, by contrast, depleted of phospholipid relative to the original (A-I w/o A-II) r-HDL.

The composition of the (A-I w A-II) r-HDL that had been maintained at 4°C (pool IV) or incubated at 37°C with TBS (pool V) was similar to that of the original r-HDL (see Table 1). The large particles in pool VIa that appeared during the incubation of (A-I w A-II) r-HDL with CETP were enriched in egg PC and deficient in apoA-II and similar in composition to the large (A-I w/o A-II) r-HDL conversion products in pool IIIb. The particles in pool VIb were enriched in apoA-II relative to the starting (A-I w A-II) r-HDL.

Incubation of CETP with (A-I w/o A-II) r-HDL that have been modified by the addition of increasing amounts of apoA-II (Table 3, Figs. 5 and 6)

This study was carried out in order to establish whether the CETP-mediated conversion of (A-I w/o A-II) r-HDL to small particles is influenced by the presence of (A-I w A-II) r-HDL in the incubation mixture. Increasing amounts of apoA-II were added to aliquots of (A-I w/o A-II) r-HDL such that the molar ratios of egg PC:apoA-I and apoA-I:apoA-II in the modified particles, respectively, increased and decreased (**Table 3**). This is consistent with apoA-II displacing apoA-I from an increasing number of the (A-I w/o A-II) r-HDL particles.

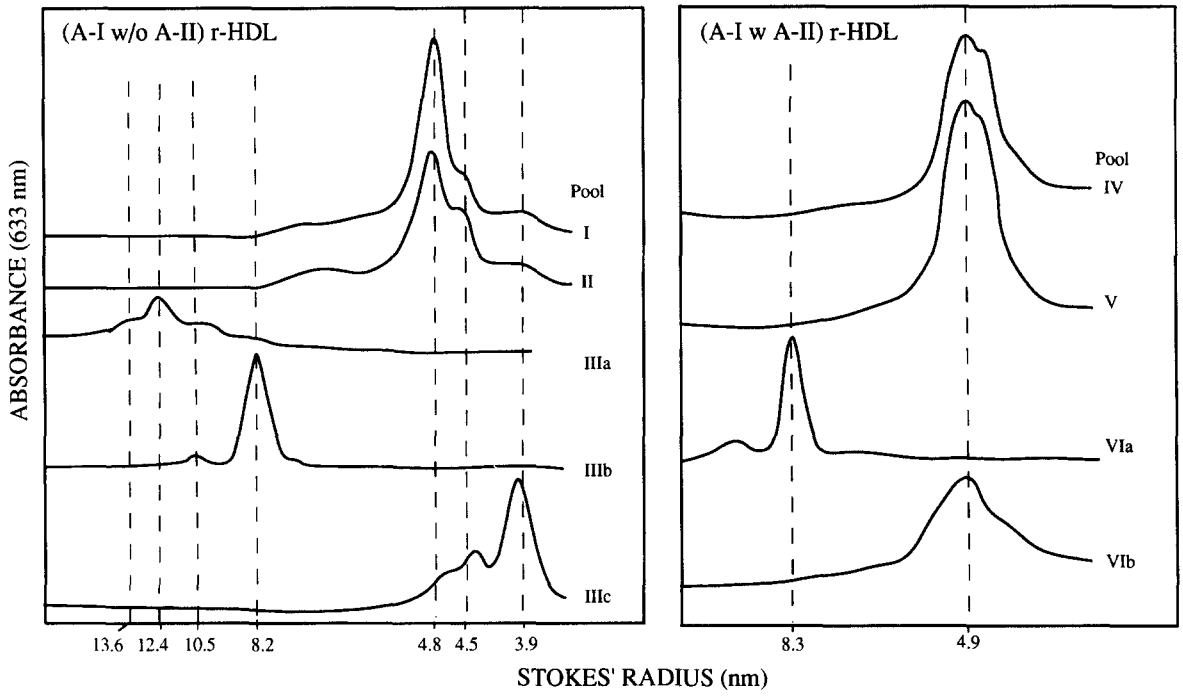

Fig. 4. Particle size distribution of the pooled fractions obtained by gel permeation chromatography. Aliquots of the pooled samples shown in Fig. 3 were electrophoresed on PAA 2/16 or 4/30 nondenaturing gradient gels. The profiles represent laser densitometric scans of the stained gels.

The r-HDL were either maintained at 4°C (**Fig. 5**, profiles A) or incubated for 24 h at 37°C in the presence of TBS (Fig. 5, profiles B) or CETP (Fig. 5, profiles C). The incubation mixtures all contained the same total concentration of apolipoproteins. Panel I shows the particle size distribution of the original (A-I w/o A-II) r-HDL. Panels II–IV, respectively, represent samples in which 23%, 36%, and 79% of the particles contained apoA-I as well as apoA-II. The Stokes' radius of the major population of particles in the (A-I w/o A-II) r-HDL was 4.8 nm (panel I, profile A). As the percentage of (A-I w A-II) r-HDL in the incubation mixture increased, larger particles of Stokes' radius 5.0 nm appeared (panels II–IV, profiles A).

The particle size distributions of r-HDL were not affected by incubation at 37°C with TBS (profiles B). This result is different from what is shown for (A-I w/o A-II) r-HDL in Fig. 2, profile B. However, it is consistent with what has been reported previously and reflects the relative resistance to changes in particle size

TABLE 2. Stoichiometry of (A-I w/o A-II) and (A-I w A-II) r-HDL after incubation in the presence and absence of CETP

r-HDL	Incubation Conditions	Fraction Number[a]	Pool Number[a]	Stoichiometry[b] Egg PC–UC–ApoA-I–ApoA-II
				molar ratio
A-I w/o A-II	4°C, –CETP	21-24	I	97:8:1:0
	37°C, –CETP	21-23	II	96:7:1:0
	37°C, +CETP	11-14	IIIa	324:46:1:0
		16-18	IIIb	216:24:1:0
		23-25	IIIc	65:3:1:
A-I w A-II	4°C, –CETP	21-24	IV	171:13:1:1.5
	37°C, –CETP	21-24	V	178:11:1:1.7
	37°C, +CETP	16-19	VIa	250:28:1:0.2
		22-24	VIb	194:10:1:2.3

(A-I w/o A-II) r-HDL and (A-I w A-II) r-HDL were maintained at 4°C in the absence of CETP or incubated at 37°C in the absence or presence of CETP. Details of the incubations are described in the legend to Fig. 2, and subsequent processing of the samples is described in the legends to Figs. 2 and 3. The stoichiometries of the pooled samples obtained after gel permeation chromatography are shown.

[a]From Fig. 3.

[b]The concentrations of individual components were determined in duplicate. The difference between the two values was less than 5%. Stoichiometries were calculated from the mean concentrations of the individual components.

TABLE 3. Progressive modification of (A-I w/o A-II) r-HDL by the addition of increasing amounts of apoA-II

ApoA-II Added	Stoichiometry[a] Egg PC–UC–ApoA-I–ApoA-II	Percentage of Particles with ApoA-II[b]
mg	*molar ratio*	*%*
0.00	87:5:1:0	0
0.25	97:8:1:0.26	23
0.50	102:7:1:0.44	36
1.00	193:10:1:1.3	79

Four aliquots of (A-I w/o A-II) r-HDL, each of which contained 2.0 mg of apoA-I, were mixed with 0.0, 0.25, 0.50, or 1.00 mg of apoA-II. TBS was added (if necessary) to adjust the volumes to 1.32 ml and the mixtures were maintained at room temperature for 10 min. The samples were then adjusted to a density of 1.25 g/ml and ultracentrifuged in a Beckman TL100 tabletop ultracentrifuge for 16 h at a speed of 100,000 rpm. A TLA 100.2 rotor was used for this procedure. The resulting supernatants were subjected to gel permeation chromatography as described in the legend to Fig. 3. The appropriate fractions were pooled and the concentrations of the individual components were determined in duplicate.

[a]Stoichiometries were calculated from the mean concentrations of the individual components.

[b]The percentage of particles containing apoA-II was calculated directly from the molar ratios of apoA-I:apoA-II assuming that only two categories of particles were present: (A-I w/o A-II) r-HDL, which contained two molecules of apoA-I, and (A-I w A-II) r-HDL, which contained one molecule of apoA-I and two molecules of apoA-II.

of r-HDL that contain a low concentration of UC (7).

When the unmodified (A-I w/o A-II) r-HDL were incubated with CETP, populations of small particles with Stokes' radii of 4.3 and 3.9 nm appeared (panel I, profile C). When 23% of the particles in the incubation mixture contained both apoA-I and apoA-II there was a significant decrease in conversion (panel II, profile C). When (A-I w A-II) r-HDL constituted 36% of the particles in the incubation mixture, conversion to small particles decreased still further (panel III, profile C). There was no evidence of conversion to small particles when 79% of the r-HDL contained both apoA-I and apoA-II (panel IV, profile C). The absence of large conversion products in these incubations reflects the low concentration of UC in the preparations and is consistent with what has been reported previously (7).

In order to establish whether conversion to small particles is directly related to the concentration of (A-I w/o A-II) r-HDL in the incubation mixtures, the area under the curve of each population of particles was determined from Fig. 5. The resulting values were expressed as a percentage of the total area under the curve and plotted as a function of the percentage of the particles in the incubation which contained apoA-II. The results are shown in **Fig. 6**. As the percentage of particles in the incubation mixture that contained both apoA-I and apoA-II increased, the conversion of 4.8 and 5.0 nm r-HDL to 4.3 and 3.9 nm r-HDL decreased.

DISCUSSION

CETP has been reported to convert both native and discoidal r-HDL into populations of small particles during incubation in vitro (4, 7). Although the physiological significance of these observations is not known, such conversion products may be comparable to the small, apoA-I-containing HDL that have been identified as the main plasma recipients of cellular cholesterol (2). In the case of native HDL, CETP-mediated conversion to small particles is regulated by the concentrations of other lipoproteins, such as VLDL and LDL, and by the concentration of non-esterified fatty acids (5, 17). The present study raises the possibility that the conversion of HDL to small particles may also be regulated by their apolipoprotein composition. Specifically, we have shown that the incorporation of increasing amounts of apoA-II into (A-I

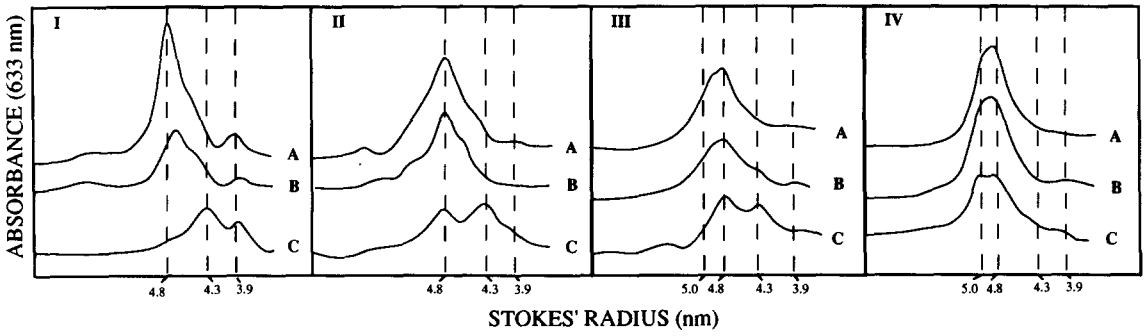

Fig. 5. Incubation of CETP with (A-I w/o A-II) r-HDL that have been modified by the addition of increasing amounts of apoA-II. (A-I w/o A-II) r-HDL were modified by the addition of increasing amounts of apoA-II and processed as described in the legend to Table 3. Aliquots of the resulting samples were mixed with TBS (0.08 ml) or CETP (10 units of activity in a volume of 0.08 ml). Each sample contained 0.035 mg of apolipoprotein in a final volume of 0.13 ml. The control samples were maintained at 4°C (profiles A) or incubated at 37°C (profiles B) for 24 h. The samples containing CETP were also incubated at 37°C for 24 h (profiles C). When the incubations were complete the samples were ultracentrifuged as described in the legend to Table 3 and particle size distributions were determined by electrophoresis on PAA 4/30 nondenaturing gradient gels. The figure shows laser densitometric scans of the stained gradient gels. Panel I shows the profiles for (A-I w/o A-II) r-HDL. Panels II–IV, respectively, represent preparations of r-HDL in which 23%, 36%, and 79% of the particles contained both apoA-I and apoA-II.

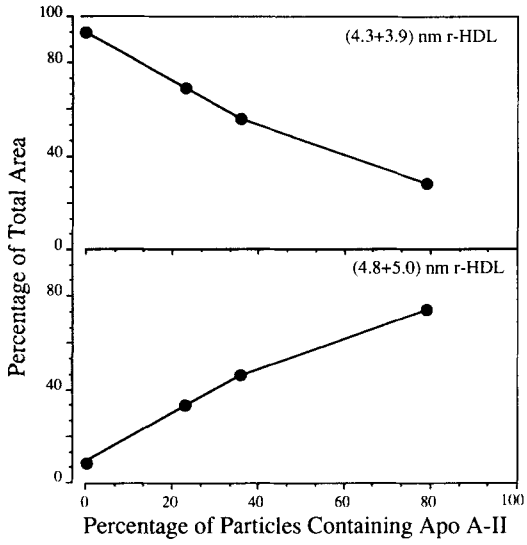

Fig. 6. Relationship between the conversion of (A-I w/o A-II) r-HDL to small particles and the percentage of particles in the incubation mixture that contain apoA-II. The gradient gel electrophoretic profiles in Fig. 5 were used to determine the areas under the curves of the (4.8 + 5.0) nm and (4.3 + 3.9) nm particles after incubation in the presence of TBS or CETP. The values were normalized and the areas of the control samples were subtracted from the areas of the samples that were incubated with CETP. The results were expressed as a percentage of the total area under the curve. The figure shows the changes in the areas of the (4.3 + 3.9) nm and (4.8 + 5.0) nm particles as a function of the percentage of the particles containing apoA-II in the incubation mixture. Peak areas were calculated as the product of the individual peak heights and the peak widths at half height.

w/o A-II) r-HDL is associated with a progressive decrease in the ability of CETP to convert the r-HDL to small particles. Given that the HDL of human plasma have been classified into two populations of particles, one of which contains A-I only while the other contains apoA-I and A-II (8), this finding is of potential physiological importance.

Although the present report does not directly address the mechanism by which apoA-II inhibits the CETP-mediated conversion of r-HDL to small particles, the results are consistent with apoA-II either *i*) increasing the stability of the r-HDL, or *ii*) inhibiting the interaction of CETP with the r-HDL. There is some evidence that the absence of small conversion products in the incubations of (A-I w A-II) r-HDL with CETP is related, at least in part, to the enhanced stability of the apoA-II-containing r-HDL particles. It has been shown previously (7), and confirmed in the present studies (Fig. 2), that (A-I w/o A-II) r-HDL that contain 8–10 mol% UC are spontaneously converted to larger and smaller particles during incubation in the absence of CETP. These changes in particle size are enhanced significantly when the incubation mixture contains CETP. When, by contrast, the r-HDL contain apoA-II, the spontaneous changes in particle size are abolished and the changes that occur during incubation with CETP are reduced significantly. It can therefore be argued that apoA-II stabilizes the r-HDL and renders the (A-I w A-II) r-HDL resistant to spontaneous as well as CETP-mediated changes in particle size.

The possibility that apoA-II may inhibit the interaction of CETP with r-HDL cannot be excluded by the present studies. However, it should be noted that CETP mediates the conversion of both (A-I w/o A-II) and (A-I w A-II) r-HDL to populations of large particles (Fig. 2). This suggests that CETP does interact to some extent with (A-I w A-II) r-HDL but that the presence of apoA-II in the r-HDL inhibits the conversion to small particles.

Comparison of the composition of the large and small conversion products with that of the original r-HDL affords an additional insight into the mechanism of the conversion process. The stoichiometry of the large conversion products (Table 2) is consistent with CETP promoting the fusion of several r-HDL particles. In order to determine how many r-HDL particles are required for fusion, the number of molecules of each component in the original r-HDL and their conversion products has been determined (Table 4). The original (A-I w/o A-II) r-HDL contained approximately two molecules of apoA-I and 200 molecules of egg PC per particle. The (A-I w A-II) r-HDL also contained approximately 200 molecules of egg PC as well as one molecule of apoA-I and two molecules of apoA-II per particle. Both preparations had molecular weights of about 200,000. The large conversion products, by contrast, contained approximately 600 molecules of egg PC and two or three molecules of apoA-I per particle. The molecular weight of these particles was about 560,000. This is consistent with the large conversion products arising from the fusion of three or more of the original r-HDL particles. Although the reason why fusion occurs is unclear, it is possible that CETP either mediates the process directly or modifies the structure of the r-HDL such that they become susceptible to fusion. In either case large, unstable complexes may result and the current data suggest that such complexes subsequently rearrange into populations of particles which may be larger, smaller or the same size as the original r-HDL.

The results in Table 4 are consistent with (A-I w/o A-II) r-HDL containing two molecules of apoA-I per particle and (A-I w A-II) r-HDL containing one molecule of apoA-I and two molecules of apoA-II per particle. In other words, the addition of apoA-II to (A-I w/o A-II) r-HDL appears to promote the replacement of one of the molecules of apoA-I from each (A-I w/o A-II) r-HDL particle with two molecules of apoA-II. It is possible, however, that apoA-II may displace all the apoA-I from a proportion of the (A-I w/o A-II) r-HDL, forming a population of apoA-II-only particles and

TABLE 4. Estimated number of component molecules in (A-I w/o A-II) and (A-I w A-II) r-HDL and their conversion products after incubation with CETP

r-HDL	Pool Number[a]	Stokes' Radius[b]	Stoichiometry[c]	Component	Number Component Molecules per Particle	Approximate Molecular Weight[d]
		nm				
(A-I w/o A-II)	I	4.8	97:8:1:0	Egg PC	176	192,000
				UC	14	
				apoA-I	1.7	
	II	4.8	96:7:1:0	Egg PC	173	195,000
				UC	13	
				apoA-I	1.9	
	IIIb	8.2	216:24:1:0	Egg PC	598	566,000
				UC	64	
				apoA-I	2.5	
	IIIc	3.9	65:3:1:0	Egg PC	107	131,000
				UC	5	
				apoA-I	1.6	
(A-I w A-II)	IV	4.9	171:13:1:1.5	Egg PC	180	201,000
				UC	14	
				apoA-I	1.0	
				apoA-II	1.5	
	V	4.9	178:11:1:1.7	Egg PC	178	204,000
				UC	11	
				apoA-I	1.0	
				apoA-II	1.8	
	VIa	8.3	250:28:1:0.2	Egg PC	575	561,000
				UC	64	
				apoA-I	2.6	
				apoA-II	0.6	
	VIb	4.9	194:10:1:2.3	Egg PC	175	201,000
				UC	9	
				apoA-I	0.9	
				apoA-II	2.0	

The number of component molecules in the pooled samples was determined from the stoichiometries in Table 2. The mol% and volume% of the individual components were calculated assuming the following partial specific volumes: egg PC, 0.970; UC, 0.968; apoA-I, 0.716, and apoA-II, 0.743 (18–20). The number of component molecules in the pooled sample was then determined from the proportion of the total particle volume occupied by each component. The volumes of the r-HDL were calculated assuming that particles with a Stokes' radius < 10.0 nm are discoidal (21) and that the width of an egg PC bilayer is 4.0 nm (22).

[a]From Fig. 3.
[b]From Fig. 4.
[c]From Table 2.
[d]Calculated as the sum of the individual components.

leaving a proportion of the (A-I w/o A-II) r-HDL intact. Two lines of evidence argue against this proposal. First, it has been reported that when an amount of apoA-II sufficient to displace all the apoA-I was added to (A-I w/o A-II) r-HDL, the radius of the resulting apoA-II-only particles was 3.9 nm (11). In the present study particles of this type were not observed when apoA-II was added to (A-I w/o A-II) r-HDL. Second, if a significant proportion of the original (A-I w/o A-II) r-HDL were still present after the addition of apoA-II, they should be converted into small particles during incubation with CETP. In the present study incubation of (A-I w A-II) r-HDL with CETP promoted minimal conversion to small particles (Figs. 2 and 5). Thus, the available evidence suggests that apoA-II displaces apoA-I from (A-I w/o A-II) r-HDL in a uniform manner to produce particles that consist mainly of one molecule of apoA-I and two molecules of apoA-II.

At present we do not know whether apoA-II influences either the stability of native HDL or their interactions with CETP. If the results presented herein reflect in any way the properties of the (A-I w/o A-II) and (A-I w A-II) HDL of human plasma, it is conceivable that CETP may convert (A-I w/o A-II) HDL more readily than (A-I w A-II) HDL to populations of small particles. There is some evidence that this may be the case. When native HDL are incubated in vitro in the presence of LDL, CETP, and nonesterified fatty acids, the HDL are converted to particles that are smaller than HDL_3 (17). The appearance of apoA-II in the small conversion products is delayed relative to that of apoA-I, suggesting that, as is the case with r-HDL, the CETP-mediated conversion of native (A-I w/o A-II) HDL to small particles may be achieved more readily than the conversion of HDL which contain both apoA-I and apoA-II.

In conclusion, this study provides evidence that apolipoproteins are important determinants of the particle size of r-HDL and raises the question of whether they play a hitherto unrecognized role in regulating the metabolism of HDL in plasma. The present results suggest that an investigation of the interaction of CETP with the native (A-I w/o A-II) and (A-I w A-II) HDL of human plasma is warranted.

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