Distribution of high density lipoprotein particles with different apoprotein composition: particles with A-I and A-II and particles with A-I but no A-II

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Abstract High density lipoproteins (HDL) were subfractionated by equilibrium CsCl gradient centrifugation of the d 1.063–1.21 g/ml HDL fraction isolated from two men and two women. The various HDL subfractions were analyzed for their apoproteins (apo) A-I, A-II, B, D, and E and the major lipid contents. ApoA-I and A-II were found throughout the density gradient with the maximum concentration between the d 1.105 and 1.120 g/ml fractions. ApoE was found in all HDL fractions with the higher concentration in the lower density fractions. Conversely, the concentration of apoD increased as the density of the HDL fraction increased. Each density subfraction underwent quantitative precipitation with anti-A-I and anti-A-II immunoglobulin. Essentially all A-II in all density subfractions was precipitated with either immunoglobulin. Particles from each density subfraction precipitated with anti-A-II immunoglobulin had an A-I/A-II molar ratio of approximately 2.0 (range 1.9–2.3). However, particles precipitated with anti-A-I immunoglobulin had A-I/A-II molar ratios identical to the A-I/A-II ratio of the subfraction (range 2.1–7.1). The subfractions (d 1.105–1.149 g/ml fractions) with A-I/A-II molar ratios of about 2 had the least proportion of A-I in particles containing A-I but not A-II. Conversely, the subfractions (d 1.063–1.075 g/ml fractions) with the highest A-I/A-II molar ratio had the greatest proportion of apoA-I in particles containing A-I but not A-II. These data indicate that HDL contains at least two types of particles: particles with both A-I and A-II in a 2:1 molar ratio, and particles containing A-I but no A-II. The variation in A-I/A-II ratio observed in different HDL density subfractions was due to the different proportions of these two types of particles.—**Cheung, M. C., and J. J. Albers**. Distribution of high density lipoprotein particles with different apoprotein composition: particles with A-I and A-II and particles with A-I but no A-II. *J. Lipid Res.* 1982. **23:** 747–753.

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High density lipoproteins (HDL) have been shown to contain numerous chemically and immunochemically distinct polypeptides referred to as apolipoproteins (apo) A-I, A-II, B, C-I, C-II, C-III, D, E, and F (1–5). ApoA-I and A-II represent approximately 80–90% of the total protein content of HDL, while the other seven apoproteins make up the rest of the 10–20%. Kostner and Alaupovic (2) demonstrated by immunochemical techniques that not all apoproteins are found in the same HDL particles. Instead, some particles in HDL contained apoA as their protein component; others contained apoB or apoC as their protein component. Albers and Aladjem (16) showed that most HDL particles contain apoA-I and apoA-II. However, about 10% of HDL particles contain apoA-I without A-II. By using radial immunodiffusion assay, we found that the A-I/A-II ratio in the d 1.063–1.105 g/ml fraction was higher than that obtained in the d 1.105–1.21 g/ml HDL fraction (7). A similar observation on HDL_2 and HDL_3 fractions prepared by zonal ultracentrifugation had also been reported by Kostner et al. (8). Using equilibrium CsCl gradient centrifugation, we showed that HDL subfractions vary in molecular size and apoA-I/A-II ratio as a function of particle density (9). In order to understand the particle composition of the various HDL subfractions, we studied the apoA-I, A-II, B, D, and E, and the major lipid contents of each density subfraction. We also performed quantitative immunoprecipitation on samples from each subfraction. The following data show that these subfractions contained two different kinds of particles in varying proportions: particles with both apoA-I and A-II in a 2:1 molar ratio, and particles with apoA-I but no apoA-II.

METHODS

Serum samples

Blood for isolation of HDL was collected from the antecubital vein of four healthy normolipidemic adult volunteers (two males and two females) after an overnight (12–14 hr) fast. The subjects were chosen for hav-

Abbreviations: HDL, high density lipoprotein(s); apo, apoprotein(s).

ing a relatively high level of HDL cholesterol (males: 72 and 54 mg/dl, respectively; females: 63 and 65 mg/dl, respectively) to ensure that there would be sufficient quantities of the lower density HDL subfraction for the various apoprotein and lipid analyses. Serum was separated at 4°C by low speed centrifugation in a refrigerated centrifuge, and sodium azide, Merthiolate, and disodium ethylene diaminetetraacetic acid (EDTA) were promptly added to final concentrations of 0.5 g/1, 0.1 g/1, and 1 mM, respectively. The serum was used for HDL preparation on the same day.

Preparation of HDL

HDL of d 1.063–1.25 g/ml were isolated from serum by sequential ultracentrifugation in a Beckman 40.3 rotor at 39,000 rpm at 16°C for 26 hr at density 1.063 g/ml, and for 48 hr at density 1.25 g/ml as described (9). The upper density of 1.25 g/ml was chosen instead of the conventional 1.21 g/ml to maximize recovery of lipoproteins of density 1.21 g/ml. The HDL thus obtained was dialyzed exhaustively against three changes of 4 liters of 1 mM EDTA, pH 7.4, and adjusted to density 1.110 g/ml by solid CsCl for equilibrium gradient centrifugation.

Equilibrium gradient centrifugation

Equilibrium CsCl gradient centrifugation was performed in a Beckman SW41 rotor at 40,000 rpm at 16°C for 72 hr as described (9). After centrifugation, fractions were collected and their density and absorption at 280 nm were monitored. The fractions were pooled according to their density and immediately dialyzed against 1 mM EDTA, pH 7.4, with 0.15 M NaCl, 0.5 g/1 NaN_3, and 0.1 g/1 Merthiolate for apoprotein and lipid analysis, and for quantitative immunoprecipitation study.

Apoprotein and lipid analyses

Apoproteins A-I, A-II, B, D, and E and albumin (HSA) of each density pool were analyzed by radioimmunoassay or radial immunodiffusion assay (4, 7, 10, 11). Cholesterol and triglyceride were analyzed by the AutoAnalyzer II according to the Lipid Research Clinic procedures (12). Free cholesterol and cholesteryl ester were quantitated by the enzymic method as described by Nagasaki and Akanuma (13), and phospholipid was quantitated by the method of Bartlett (14).

Quantitative immunoprecipitation study

Rabbit anti-A-I and goat anti-A-II immunoglobulins prepared by the modified method of Sober and Peterson (15) were used for the quantitative immunoprecipitation study. Rabbit anti-A-I immunoglobulin produced a single precipitin arc of identity between whole serum and purified apoA-I, but did not react with apoA-II, B, C-I, C-II, C-III, D, E, or HSA. Goat anti-A-II immunoglobulin produced a single precipitin arc of identity between whole serum and purified apoA-II, but did not react with purified apoA-I, the other apoproteins, or HSA. Prior to immunoprecipitation, the immunoglobulins were titered for their ability to precipitate 30 μg of d 1.090–1.21 g/ml HDL containing tracer quantities of ^{125}I-labeled HDL (d 1.090–1.21 g/ml). The quantity of immunoglobulin capable of precipitating the maximum amount of HDL under such conditions was used for quantitative immunoprecipitation study.

Immunoprecipitation was performed by adding anti-A-I or anti-A-II immunoglobulin to duplicate aliquots of the various density pools. Density fractions of three of the four subjects (one male and two females) underwent quantitative immunoprecipitation. Each mixture was incubated at 37°C for 1 hr and then at 4°C overnight. This incubation time gave comparable results to 48 hr incubation at 4°C in a subset of samples in this study. The precipitates were separated by low speed centrifugation at 4°C, washed once with cold saline, delipidated with ether–ethanol (3:1), and analyzed for their apoA-I and A-II contents. The supernates obtained from immunoprecipitation of the density fractions from one male and one female subject were also delipidated and analyzed for A-I and A-II contents. In order to estimate and compare recovery in each step of the experiment, a tracer quantity of ^{125}I-labeled HDL was added to each pool prior to immunoprecipitation. To verify that the anti-A-I or anti-A-II immunoglobulin present in the precipitate did not significantly interfere in the subsequent immunoassay for A-I or A-II, six portions of an HDL fraction (d 1.090–1.21 g/ml) was precipitated by anti-A-I and another six portions by anti-A-II immunoglobulin. After correcting for the actual percentage of HDL precipitated as determined by the ^{125}I-labeled HDL tracer and for the losses by the delipidation step, the amount of A-I found in the precipitate formed with anti-A-I immunoglobulin was 98 ± 3% of the expected value, while the amount of A-II found in the precipitate formed with anti-A-II immunoglobulin was 97 ± 4% of the expected value.

RESULTS

The establishment of equilibrium centrifugation conditions and the estimation of the precision of analysis of various lipid and apoprotein measurement of the gradient fractions have been presented in our earlier publication (9). In the quantitative precipitation experiments, we used an amount of anti-A-I and anti-A-II immunoglobulin that precipitated the maximum amount of A-I and A-II, respectively, in the fractions of each density pool

used for precipitation. Hence, when a tracer quantity of ^{125}I-labeled HDL was added to each density pool prior to precipitation, a comparable amount of ^{125}I-labeled HDL was precipitated. The inclusion of tracer ^{125}I-labeled HDL also enabled us to estimate our recovey in the delipidation of immunoprecipitates and supernates for apoA-I and A-II analysis. For the 112 immunoprecipitates and supernates delipidated and analyzed, recovery in the delipidation process was 90% ± 3%.

After equilibrium CsCl centrifugation, the salt gradient ranged from density 1.055 to 1.290 g/ml. Fractions with density between 1.063 and 1.20 g/ml from each gradient tube were selectively pooled according to their density for various analyses. The mean and range of the concentrations of apoA-I and A-II of each density fraction of the four subjects are shown in **Table 1**. The recovery of apoA-I and A-II from the starting material was 88 ± 3% and 90 ± 3%, respectively. The distribution of apoA-I, A-II, D, and E in HDL subfractions from a female subject along the density gradient is shown in **Fig. 1**. The distributions are similar in the two males and the other female subject. ApoA-I and A-II were found throughout the density gradient with their concentrations reaching a maximum between d 1.105 and 1.120 g/ml. ApoE was found in all HDL fractions, most of it being in the lighter density fractions. On the other hand, the concentration of apoD increased as the density of the HDL fraction increased. Albumin (6–10 mg/dl) was found between d 1.120 and 1.20 g/ml, while apoB (1–6 mg/dl) could only be detected in fractions of density less than 1.105 g/ml. These apoB-containing fractions also reacted with anti-Lp(a) sera.

The lipid profiles of the major lipids of the CsCl density gradient fractions are shown in **Table 2**. There was little variation in lipid composition within the d 1.063–1.20 g/ml range. Fifty percent or more of the total lipid content in each gradient fraction was phospholipid, with the d 1.105–1.120 g/ml fraction containing the highest percentage (54–58%) in both males and females. Cholesteryl ester averaged 37% and 41% of the lipids of the gradient fractions of the male and female subjects, respectively. Unesterified cholesterol represented 3–10% and 3–6%, while triglyceride represented 2–3% and 2–6% of the lipids in the density gradient fractions of the male and female subjects, respectively.

TABLE 1. ApoA-I and A-II concentration of HDL subfractions isolated by CsCl gradient centrifugation

Density of Subfraction	ApoA-I		ApoA-II	
	Mean[a]	Range	Mean[a]	Range
g/ml	*mg/dl*		*mg/dl*	
1.149–1.200	10.7	(9.1–12.9)	3.3	(2.6–4.1)
1.120–1.149	20.0	(15.0–28.3)	7.0	(4.9–10.2)
1.105–1.120	20.8	(16.6–23.4)	6.5	(5.1–8.5)
1.093–1.105	16.0	(14.2–20.3)	4.8	(3.2–5.8)
1.083–1.093	12.2	(8.8–17.7)	3.0	(2.4–3.3)
1.075–1.083	12.4	(8.0–20.6)	1.7	(1.4–1.9)
1.063–1.075	7.0	(3.7–10.9)	0.9	(0.5–1.2)

[a] Mean of four subjects.

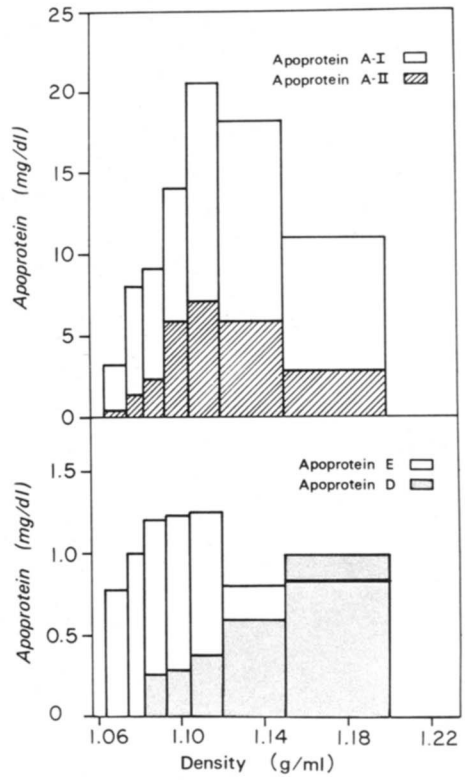

Fig. 1. A representative distribution of apolipoproteins A-I, A-II, D, and E in HDL subfractions at the end of a 72-hr CsCl gradient centrifugation. This sample was from a female subject with an HDL cholesterol level of 63 mg/dl. The concentration of apolipoprotein D in the highest density fraction (d 1.149–1.200 g/ml) is represented by the total height of the bar. The height of the bar up to the dark horizontal line is the concentration of apolipoprotein E.

Fractions from the density gradient of three of the four subjects (one male and two female) underwent quantitative immunoprecipitation. The apoA-I/A-II molar ratios of particles precipitated with anti-A-I immunoglobulin ranged from 2.1 to 7.1 (**Table 3**). Fractions from a given narrow density range had similar A-I/A-II ratios for each of the subjects. However, particles precipitated with anti-A-II immunoglobulin had an apo A-I/A-II molar ratio of approximately 2.0 (range 1.9–2.3) regardless of the density of the subfraction. Hence anti-A-II immunoglobulin failed to precipitate all apoA-I from the density subfractions. This suggested that there existed in the density subfractions particles containing apoA-I without apoA-II. The proportion of these particles in each density subfraction varied with the hydrated density of the subfraction (**Fig. 2**). The subfractions with an

TABLE 2. Lipid composition of HDL subfractions isolated by CsCl gradient centrifugation

Sex	Density of Subfraction	% of Total Lipid[a]				Lipid Ratio (Molar)[b]		
		UC	CE	PL	TG	UC/PL	UC/TC	CE/TG
	g/ml							
Male	1.149–1.200	6.4	36.8	54.5	2.5	0.23	0.22	9.1
(N = 2)	1.120–1.149	3.6	37.0	55.2	4.3	0.13	0.14	11.5
	1.105–1.120	2.6	34.5	58.4	4.4	0.09	0.12	11.0
	1.093–1.105	3.5	37.1	54.0	5.5	0.13	0.14	9.2
	1.083–1.093	5.0	38.3	51.7	5.0	0.19	0.18	10.3
	1.075–1.083	6.3	38.6	49.7	5.4	0.25	0.21	9.4
	1.063–1.075	10.2	35.8	49.9	4.2	0.40	0.32	12.4
Female	1.149–1.200	6.4	42.1	51.6	0	0.25	0.20	
(N = 2)	1.120–1.149	3.4	41.8	53.4	2.1	0.13	0.12	26.2
	1.105–1.120	3.3	39.8	54.3	2.6	0.12	0.12	20.0
	1.093–1.105	3.7	40.4	53.1	2.8	0.13	0.13	19.7
	1.083–1.093	4.0	40.6	52.7	2.8	0.15	0.14	19.8
	1.075–1.083	4.8	40.4	51.7	3.2	0.18	0.17	16.7
	1.063–1.075	5.3	41.5	50.5	2.8	0.20	0.18	20.4

[a] Abbreviations: UC, unesterified cholesterol; CE, cholesteryl ester; PL, phospholipid; TG, triglyceride; TC, total cholesterol.
[b] Molecular weights used for calculation: UC, 387; CE, 665; PL, 775; TG, 850.

apoA-I/A-II molar ratio of about 2:1, the d 1.093–1.149 g/ml fractions, had the least proportion of apoA-I in particles containing A-I but not A-II. Conversely, the subfractions with the highest A-I/A-II molar ratio, the d 1.063–1.075 g/ml fraction, had the greatest proportion of apoA-I in particles containing apoA-I but not apoA-II. The proportions of apoA-I in particles containing apoA-I but no apoA-II were not related to the level of HDL material in the subfractions.

The supernates obtained from the immunoprecipitation of the density fractions from one male and one female were also analyzed for apoA-I and A-II contents. Of the 14 supernates analyzed, 7.7 ± 2.5% of the total apoA-I remained in the supernates of samples precipitated with anti-A-I, indicating that the percentage of A-I remaining in the supernates of samples precipitated with anti-A-I was the same regardless of the subfraction precipitated. However, the percentage of apoA-I in the supernates of anti-A-II precipitates varied with the hydrated density of the fraction. As much as 60% of apoA-I remained in the supernates of the anti-A-II precipitates of the subfractions of density 1.063–1.082 g/ml, the fractions with the highest apoA-I/A-II ratio. The mean percentage of apoA-II in the supernates of samples precipitated with either anti-A-I or anti-A-II were similar (anti-A-I supernates: 14.5%; anti-A-II supernates: 13.4%). This indicated that anti-A-I and anti-A-II immunoglobulins precipitated similar amounts of A-II in the various density subfractions. Some of the apoA-I and apoA-II detected in the supernates probably represented soluble antigen-antibody complexes which failed to precipitate by direct precipitation, as demonstrated by Albers and Aladjem (6).

TABLE 3. A-I/A-II molar ratio in HDL particles precipitated with specific anti-A-I or anti-A-II immunoglobulin

Density of Subfraction	A-I/A-II Ratio in HDL Particles Precipitated with Anti-A-I IgG[a]		A-I/A-II Ratio in HDL Particles Precipitated with Anti-A-II IgG[a]	
	Mean	Range	Mean	Range
g/ml				
1.149–1.200	3.3	(2.8–3.9)	2.1	(1.7–2.6)
1.120–1.149	2.4	(2.0–2.8)	2.2	(1.8–2.6)
1.105–1.120	2.2	(2.0–2.3)	2.0	(1.8–2.2)
1.093–1.105	2.1	(1.8–2.3)	1.9	(1.7–2.0)
1.083–1.093	3.4	(2.6–4.7)	1.9	(1.3–2.4)
1.075–1.083	5.3	(4.9–6.2)	2.3	(1.7–3.0)
1.063–1.075	7.1	(6.5–7.9)	2.3	(2.3–2.4)

[a] Mean of three subjects

DISCUSSION

The CsCl HDL subfractions of the four normolipidemic subjects had apoA-I and A-II distributions similar to those reported earlier (9). A-I and A-II were found throughout the d 1.063–1.20 g/ml density range, with most of these apoproteins within the d 1.105–1.149 g/ml density region. The A-I/A-II molar ratio of these four subjects also remained relatively constant at 2 to 1 between 1.093–1.20 g/ml and increased as the density of the fraction decreased from 1.093 to 1.063 g/ml, or increased beyond d 1.20 g/ml. As expected, apoB could only be detected in lipoprotein fractions with d < 1.105 g/ml. The majority of the apoB was probably associated

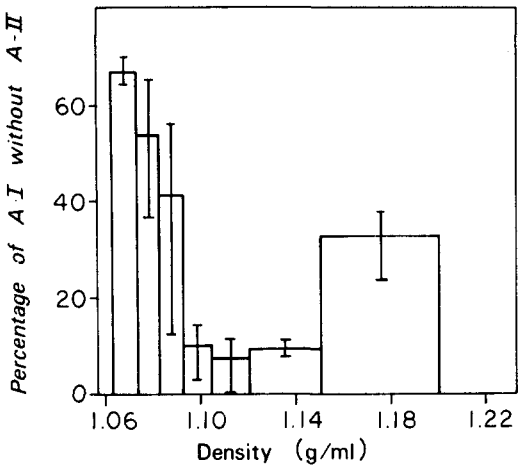

Fig. 2. Mean and range percentage of apoprotein A-I without apoprotein A-II in HDL subfractions from one male and two female subjects after CsCl gradient ultracentrifugation.

with the Lp(a) lipoprotein which has a hydrated density of 1.050–1.100 g/ml (16). We were not able to detect apoD in HDL subfractions with density less than 1.075 g/ml. Above that density, the concentration of apoD increased as the density of the HDL subfraction increased. This is consistent with a report indicating that HDL_3 contains twice as much apoD as does HDL_2 (17). The distribution of apoE within the d 1.063–1.21 g/ml lipoprotein region was opposite to that of apoD. ApoE was found in all HDL subfractions but most of it was in the lighter density subfractions. This agrees with the observation of Blum, Aron, and Sciacca (18) that the apoE content of the d 1.063–1.125 g/ml lipoprotein fraction from normolipidemics was higher than that of the d 1.125–1.21 g/ml lipoprotein fraction. The content of albumin in the lipoprotein fractions was also estimated. We found that albumin occurred only in lipoprotein fractions of density greater than 1.120 g/ml and decreased from 10 mg/dl in the d 1.149–1.20 g/ml fraction to less than 6 mg/dl in the d 1.120–1.149 g/ml fraction. This albumin concentration represented approximately 5% and 2% of the protein of these two HDL subfractions, respectively. Our preliminary study with the distribution of the enzyme lecithin:cholesterol acyltransferase (LCAT) in these HDL subfractions indicated that, as with apoD, the concentration of LCAT increased as the density of the HDL subfraction increased.

The lipid composition of HDL_2 (d 1.063–1.125 g/ml) and HDL_3 (d 1.125–1.21 g/ml), the two major HDL subclasses, had been studied many years ago by several groups of investigators (19–21). Recently, Anderson et al. (22) and Patsch et al. (23) have also analyzed the lipid composition of their HDL subfractions obtained by NaBr-NaCl density gradient ultracentrifugation and zonal ultracentrifugation, respectively. In general, these studies show that the percentage of lipid in HDL subclasses increases as the density of the subclasses decreases. We observed the same trend in our CsCl density gradient HDL subfractions. The percentage of lipid in our HDL subfraction from both male and female plasma ranged from 45% for the d 1.149–1.200 g/ml fraction to 70% for the d 1.063–1.075 g/ml fraction. We found that, in general, the composition of cholesteryl ester, triglyceride, and phospholipid of each of our CsCl density subfractions remained relatively constant throughout our HDL subfractions. This is consistent with the finding of Skipski et al. (21) and Kuksis et al. (24), but differs from that of Anderson et al. (22) who found considerably more phospholipid in their lower density HDL subfractions than in their higher density HDL subfractions. The proportion of phospholipid in their lighter density subfractions was about 20–30% higher than our finding although their phospholipid content in the higher density subfractions agreed with those of our present study. As found by other investigators (22, 24), the percentage of unesterified cholesterol was higher in our lighter HDL density subfractions (d 1.063–1.093 g/ml) than in our heavier HDL density subfractions (d 1.093–1.149 g/ml). Consequently, the ratios of unesterified cholesterol to phospholipid and unesterified cholesterol to total cholesterol were higher in the lighter HDL density subfractions than in the heavier HDL density subfractions (Table 2).

We have also performed quantitative precipitation on the density gradient fractions using a combination of anti-A-I and anti-A-II immunoglobulin. The apoA-I/A-II ratio of particles precipitated by a combination of anti-A-I and anti-A-II immunoglobulin was similar to the HDL subfraction and to that precipitated by anti-A-I immunoglobulin alone. This indicated that anti-A-I immunoglobulin and anti-A-II immunoglobulin precipitated comparable quantities of apoA-II from the HDL subfraction, suggesting that HDL particles containing apoA-II also contain apoA-I. Preliminary experiments have been performed to isolate and characterize particles containing both apoA-I and A-II, and particles containing apoA-I but no apoA-II. Gel diffusion studies using antisera specific for albumin, apoB, and apoD showed that both particles reacted with antisera specific for albumin and apoD while neither reacted with anti-apoB serum. Particles containing apoA-I and A-II also reacted with anti-E serum.

The presence of HDL subclasses with differing apoA-I/A-II ratios not only has been demonstrated in our laboratory (7, 9) but by other investigators as well (8, 23). These differences in apoA-I/A-II ratios can be due to several possibilities. First, HDL may contain particles with apoA-I and A-II at differing molar ratios. Second, HDL subfractions may contain particles with apoA-I and A-II at a fixed molar ratio, and varying proportions

of particles with A-I but no A-II or A-II but no A-I. Third, HDL subfractions may contain particles with apoA-I and A-II at different molar ratios and varying proportions of particles with only apoA-I or A-II. In this study, we have attempted to look at these possibilities by the quantitative precipitation technique. Albers and Aladjem have given immunochemical evidence of HDL particles containing apoA-I without apoA-II in each of the major HDL subclasses (6). Kostner et al. (25) identified an α lipoprotein fraction which reacted with anti-A-I but not anti-A-II sera. Borresen and Berg (26) have suggested the existence of "free" apoA-I in serum using radial immunodiffusion and cross-immunoelectrophoresis. Our quantitative precipitation study indicates that HDL contains at least two subpopulations: particles with both apoA-I and A-II usually in a 2:1 molar ratio regardless of the hydrated density of the particle, and particles with apoA-I but no A-II. The variation in apoA-I/A-II ratio observed in different HDL density subfractions therefore appears to be primarily due to the different proportions of these two types of particles. We have no evidence of HDL with particles containing apoA-II but no apoA-I in normal subjects. These observations are of fundamental importance to our understanding of HDL structure.

On the basis of the apoA-I concentration of each subfraction and the quantitative precipitation data, we estimated that 24% of apoA-I in the d 1.063–1.20 g/ml CsCl gradient fractions is found in particles without apoA-II. We do not know if a similar percentage exists in serum. An earlier study suggested that approximately 10% of the HDL particles contain apoA-I without apoA-II (6). However, that value was estimated from specific activity of the radiolabeled apoproteins and no apoA-I measurements were made. Furthermore, the two subjects in this study had lower HDL levels than the four subjects in the current study. We have performed preliminary experiments to fractionate from serum the HDL particles containing apoA-I but not apoA-II using immunoaffinity chromatography. Our data indicate that approximately 25% of the apoA-I in the serum is not associated with apoA-II in subjects with elevatd HDL levels. This estimate may not be representative of the quantity of the particles in the serum of all subjects. It is likely that the proportion of particles containing apoA-I without A-II varies with the serum HDL levels and subjects with high HDL levels may have a greater proportion of these particles. Since the HDL fractions we studied had undergone CsCl gradient centrifugation in addition to several days of ultracentrifugation, it is possible that some of the A-I without A-II particles found in the subfractions were formed during these centrifugation procedures. In the present study we cannot distinguish between apoA-I particles in the serum prior to centrifugation and apoA-I dissociated from HDL particles during centrifugation. Early studies consistently showed that some apoA-I is dissociated from HDL during preparative ultracentrifugation (6, 20). Therefore, the apparent increase in the proportion of HDL particles with apoA-I but no apoA-II in the higher density range, e.g., d 1.149–1.200 g/ml may be an artifact of ultracentrifugation. In performing quantitative precipitation, the samples were incubated at 37°C for 1 hr. Such incubation could have led to changes in HDL particle composition. However, immunochemical studies of fresh whole plasma have clearly demonstrated the presence of particles containing apoA-I but no apoA-II in the absence of ultracentrifugation (6, 26, 27) or 37°C incubation (26, 27, and a subset of samples in this study).

Recently, several reports have presented evidence indicating that apoA-I and A-II are removed from plasma at different rates and that there may be at least two plasma pools of apoA-I that are kinetically distinct (28–31). The observed differences in apoA-I and A-II removal could be readily explained if the HDL particles containing apoA-I but no A-II were metabolized at a faster rate than particles containing apoA-I and A-II.

From our present knowledge of apoA-I and A-II synthesis, and the metabolic pathways of the various classes of lipoproteins, it is possible to speculate that the particles containing apoA-I but no A-II have a different origin than particles containing both apoA-I and A-II. The former may be products of chylomicron catabolism, while the latter are secreted by the liver. Another possible mechanism of formation of particles containing apoA-I but no A-II can be postulated from recent publications of Ritter and Scanu (32) and Kwok, Dawson, and Ritter (33), who demonstrated that when HDL_3 was exposed to either human skin fibroblasts or human circulating leukocytes there was a selective loss of apoA-II.

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