Distribution of cholesterol and apolipoprotein A-I and A-II in human high density lipoprotein subfractions separated by CsCl equilibrium gradient centrifugation: evidence for HDL subpopulations with differing A-I/A-II molar ratios

Marian C. Cheung and John J. Albers[1]

Division of Metabolism, Endocrinology and Gerontology, Department of Medicine and Northwest Lipid Research Clinic, Harborview Medical Center, University of Washington School of Medicine, Seattle, WA 98104

Abstract The purpose of this experiment was to characterize the high density lipoproteins (HDL) as a function of hydrated density. HDL was subfractionated on the basis of hydrated density by CsCl density gradient centrifugation of whole serum or the d 1.063–1.25 g/ml HDL fraction isolated from three men and three women. Apolipoprotein A-I and A-II quantitation by radial immunodiffusion showed that the A-I/A-II ratio varied with the lipoprotein hydrated density. The A-I/A-II molar ratio of HDL lipoproteins banding between d 1.106 and 1.150 g/ml was nearly constant at 2.2 ± 0.2. In the density range 1.151–1.25 g/ml the A-I/A-II ratio increased as the density increased. On the other hand, in the density range between 1.077 and 1.105 the A-I/A-II ratio increased as the density decreased, ranging from 2.8 ± 0.5 for the d 1.093–1.105 g/ml fraction to 5.6 ± 1.3 for the d 1.077–1.082 g/ml fraction. The d 1.063–1.076 g/ml fraction and the d 1.077–1.082 g/ml fractions had comparable A-I/A-II ratios. Serum and the d 1.063–1.25 g/ml HDL fraction exhibited similar trends. The cholesterol/(A-I + A-II) ratio decreased as the density increased in all 12 samples (six serum and six HDL) examined. Gradient gel electrophoresis of the density gradient fractions showed that as the density increased from 1.063 to 1.200 g/ml the apparent molecular weight decreased from 3.9×10^5 to 1.1×10^5. HDL subfractions with the same hydrated densities had comparable molecular weights and A-I/A-II and cholesterol/(A-I + A-II) ratios when isolated from men or women. HDL contains subpopulations that differ in the A-I/A-II molar ratio.—**Cheung, M. C., and J. J. Albers.** Distribution of cholesterol and apolipoprotein A-I and A-II in human high density lipoprotein subfractions separated by CsCl equilibrium gradient centrifugation: evidence for HDL subpopulations with differing A-I/A-II molar ratios. *J. Lipid Res.* 1979. **20:** 200–207.

Supplementary key words radial immunodiffusion · gradient gel electrophoresis

High density lipoproteins (HDL) contain two major polypeptides, apolipoproteins A-I and A-II (1, 2). Most (ca. 90%) HDL particles contain both A-I and A-II whereas some HDL particles appear to contain only A-I (3). HDL subfractionation by rate zonal ultracentrifugation has suggested that the molar ratio of A-I/A-II is much higher in the lighter density HDL_2 fraction than in the heavier density HDL_3 fraction (9:1 compared to 2:1) (4). It has been reported that both the HDL_2 and HDL_3 subfractions isolated by preparative ultracentrifugation have an A-I/A-II molar ratio of 2:1 (5). Recently we showed that the A-I/A-II molar ratio in the d 1.063–1.10 g/ml HDL fraction isolated by preparative ultracentrifugation was significantly higher than that observed in the d 1.10–1.21 g/ml fraction (3–4:1 compared to 2:1) (6). In this investigation we wanted to further characterize the structural heterogeneity of HDL isolated from men and women. In order to subfractionate HDL on the basis of hydrated density we applied CsCl equilibrium density gradient ultracentrifugation to both serum and the d 1.063–1.25 g/ml fraction. We report on the distribution of cholesterol and apolipoproteins A-I and A-II in serum and HDL subfractionated by equilibrium gradient ultracentrifugation and show that the A-I/A-II ratio and cholesterol/(A-I + A-II) ratio vary with the hydrated density of the HDL particle. Furthermore, we demonstrate that HDL subfractions with comparable hydrated densities have similar compositions whether isolated from men or women.

Abbreviations: HDL, high density lipoprotein(s); A-I and A-II, apolipoproteins A-I and A-II, respectively; LCAT, lecithin:cholesterol acyltransferase.

[1] Send reprint requests to Dr. John J. Albers, Northwest Lipid Research Clinic, Harborview Medical Center, 325 Ninth Ave., Seattle, WA 98104.

METHODS

Serum samples

Blood was drawn from the antecubital vein into Vacutainer tubes from six healthy normolipidemic adult volunteers, consisting of three males of ages 23, 52, and 29 and three females of ages 50, 55, and 33. Prior to drawing the blood, all subjects had fasted overnight for 12–14 hr. Upon separation of serum at 4°C by low speed centrifugation, 0.5 g/l NaN_3, 0.1 g/l Merthiolate, and 10^{-3} M disodium ethylenediaminetetraacetic acid (EDTA) (pH 7.4) were promptly added. An aliquot of the serum was used for HDL preparation on the same day. The remaining portion was stored at 4°C for 3 days and then subjected to CsCl gradient centrifugation along with the HDL preparation isolated from the same serum.

Preparation of HDL

HDL of d 1.063–1.25 g/ml was isolated from serum by sequential ultracentrifugation. (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 up to 1.21 g/ml). Four-ml aliquots of fresh serum were adjusted to the nonprotein solvent density of 1.063 g/ml with solid KBr, overlayered with 2 ml of KBr solution of d 1.063 g/ml containing 0.5 g/l NaN_3, 0.1 g/l Merthiolate, and 10^{-3} M EDTA (pH 7.4), and centrifuged in a Beckman 40.3 Ti rotor at 39,000 rpm at 16°C for 26 hr. The top 2.5 ml containing the d < 1.063 fraction was carefully aspirated with a fine-tipped Pasteur pipet and discarded. The bottom 3.5 ml containing the d > 1.063 g/ml fraction was adjusted to density 1.25 g/ml by solid KBr and overlayered with 2 ml of KBr solution of d 1.25 g/ml containing NaN_3, Merthiolate, and EDTA as previously described and centrifuged in a Beckman 40.3 Ti rotor at 39,000 rpm at 16°C for 48 hr. The top 2.0 ml containing the d 1.063–1.25 g/ml fraction was carefully aspirated and saved for equilibrium gradient centrifugation.

Equilibrium gradient centrifugation

Serum and HDL for CsCl gradient centrifugation were dialyzed exhaustively against three changes of four l of 10^{-3} M EDTA, pH 7.4, overnight and adjusted to density 1.110 g/ml by solid CsCl. The CsCl gradient consisted of the following: 2.0 ml of d 1.130 g/ml, 2.0 ml of d 1.120 g/ml, 3.9 ml of serum or HDL of solvent density 1.110 g/ml, 3.9 ml of d 1.100 g/ml and 1.0 ml of d 1.090 g/ml. All CsCl solutions were made in 10^{-3} M EDTA (pH 7.4) and their densities were checked by refractometry (Abbe-3L refractometer, Bausch and Lomb, Rochester, NY), and carefully layered in the above order. In control tubes, the serum or HDL was replaced by CsCl solution of d 1.110 g/ml. These gradients were centrifuged in a Beckman SW 41 rotor at 40,000 rpm at 16°C for 72 hr. Each centrifugal run consisted of the following six tubes: two controls, two sera (one each from a male and a female), and the HDL isolated from each of the respective sera.

After centrifugation, 35 fractions of 0.36 ml each were aspirated, starting from the bottom of the tube, by a peristaltic pump and collected in 10×75 mm disposable glass tubes. Each of these fractions (numbered 1–35) was monitored by absorption at 280 nm and refractometry and the results were plotted. Based on the density distribution of the controls, fractions were pooled and immediately dialyzed against 0.15 M NaCl with 10^{-3} M EDTA, 0.5 g/l NaN_3, and 0.1 g/l Merthiolate (pH 7.4). Pooled fractions intended for lipid and apolipoprotein analyses were lyophilized. Fractions for gradient gel electrophoresis were stored at 4°C.

Cholesterol and apolipoprotein A-I and A-II analyses

The lyophilized materials from equilibrium gradient centrifugation were redissolved in appropriate volumes of 4.2 M 1,1,3,3-tetramethylurea (TMU, Burdick and Jackson Laboratories, Inc., Muskegon, MI), 1 mM Tris-HCl buffer (pH 8.0), 6.4 M ultrapure urea (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, NY) mixture for immunochemical analyses of apoA-I and A-II as previously described (6, 7). Cholesterol of the same fractions was analyzed by the Auto-Analyzer II (Technicon Corp., Tarrytown, NY) according to Lipid Research Clinic procedures (8). Under the conditions used for sample analysis, the TMU–Tris–urea mixture was shown not to interfere with the cholesterol analysis. Serum high density lipoprotein levels were estimated by heparin–Mn^{2+} precipitation of very low density (VLDL) and low density lipoproteins (LDL) and subsequent cholesterol analysis of the supernate (8).

Gradient gel electrophoresis

Gradient gel electrophoresis was performed in a Pharmacia GE-4 electrophoresis apparatus with precasted PAA 4/30 gradient gels (Pharmacia Fine Chemicals, Uppsala, Sweden). Approximately 50–100 μg of the HDL fraction or standard proteins were loaded on the gel using sample applicators of larger capacity than the ones supplied by Pharmacia. Electrophoresis was performed in Tris–borate buffers (pH 8.35) at 125 V at 4°C for 20 hr as described by the manufacturer. Gels were stained in Coomassie

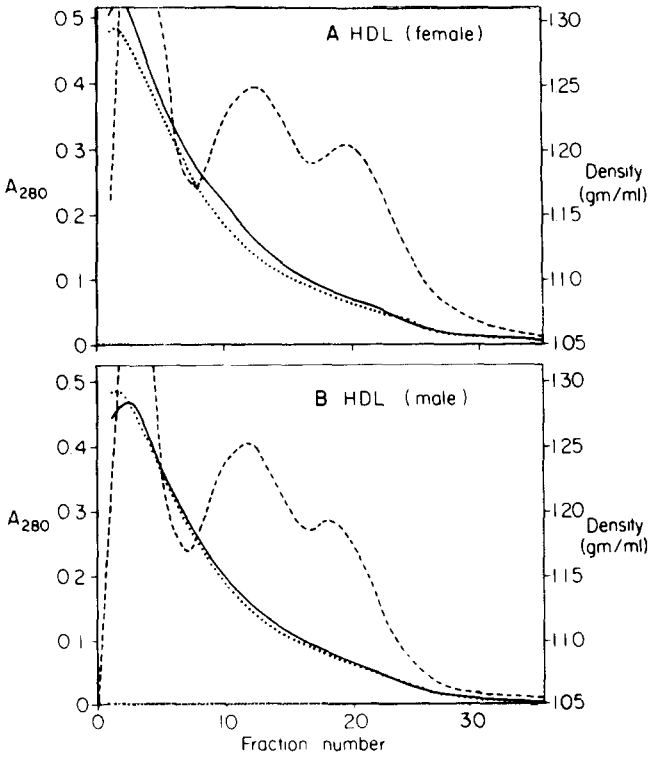

Fig. 1. Absorption and density profiles of HDL at the end of a 72-hr CsCl gradient centrifugation run. (*A*) HDL isolated from a 50-yr-old healthy female (subject 4) with an HDL cholesterol of 59 mg/dl. (*B*) HDL isolated from a 23-year-old healthy male (subject 1) with an HDL cholesterol of 54 mg/ml. (– – –) Denotes absorption at 280 nm, (——) denotes density of each HDL fraction calculated from the refractive index of the fraction, (· · ·) denotes density of the corresponding fraction in the control tubes.

brilliant blue G250–perchloric acid solution (9) for a minimum of 2 hr and transferred to 5% acetic acid. Migration distance of each stained protein band was measured as the distance between the top of the gel and the middle of the stained band. Migration distances of protein standards were plotted against the logarithms of their molecular weights to create standard curves from which molecular weights of the HDL bands were estimated. The protein standards initially used were: bovine thyroglobulin, horse spleen apoferritin, bovine liver catalase, human albumin, and ovalbumin (Sigma Chemical Co., St. Louis, MO). In later studies we used the High Molecular Weight (HMW) Calibration Kit (hog thyroglobulin, horse spleen ferritin, bovine liver catalase, bovine heart lactate dehydrogenase, and bovine serum albumin) of Pharmacia Fine Chemicals.

RESULTS

To establish the conditions in which the distribution of lipoprotein particles along the gradient reached equilibrium, we initially layered the HDL fraction either in the middle or the bottom of our 5-layer gradient and centrifuged at 40,000 rpm at 16°C for 24, 48, or 72 hr. At the end of each run, the absorption and density profiles of each tube were compared. The absorption and density profiles of the HDL initially layered in the middle and bottom were comparable to each other only after 72 hr of centrifugation.

The density gradient absorption profile of HDL isolated from the serum of either a female or a male was bimodal (**Fig. 1**). One absorption maximum was observed in the density range of 1.12–1.13 g/ml and the second maximum occurred at approximately d 1.08–1.09 g/ml. The high level of absorption (A280) at d > 1.18 g/ml was due to albumin in the HDL preparations which ranged from approximately 1 mg/dl for the d 1.063–1.076 g/ml fraction to approximately 30 mg/dl for the d 1.151–1.200 g/ml fractions as determined by radial immunodiffusion studies with rabbit anti-human serum albumin. The ratio of protein in the lighter density range (d 1.063–1.15 g/ml) to that in the heavier density range (d 1.15–1.21 g/ml) was greater in the sample isolated from the female. Due to the presence of albumin, the bimodal absorption profile was not evident when serum was applied to the density gradient (not shown).

The 35 fractions of each gradient tube were selectively pooled according to their densities and analyzed for A-I, A-II, and cholesterol (**Figs. 2** and **3**). To estimate the precision of the analyses of the gradient fractions, eight aliquots of an HDL sample were carried through the dialysis, lyophilization, and dissolving steps and were analyzed for apolipoproteins and cholesterol. The total coefficient of variation for sample handling and A-I or A-II analysis was 16%, whereas the coefficient of variation of the cholesterol analysis was 4%. The distribution of A-I, A-II, and cholesterol along the density gradient from the HDL preparations was quite similar to that obtained with serum preparations. Cholesterol contents of the serum fraction of d < 1.063 g/ml were not analyzed because of the presence of low density lipoproteins (LDL) and very low density lipoproteins (VLDL). Both serum and HDL had no detectable A-II (<0.2 mg/dl) at d > 1.25 g/ml and very little A-II (<1 mg/dl) in the d 1.21–1.25 g/ml region. Results of density gradient centrifugation of six different sera indicated that the d 1.21–1.25 g/ml fraction and the d > 1.25 g/ml fraction contained approximately 8% and 12%, respectively, of the total serum A-I. Gradient centrifugation of HDL fraction resulted in 5% and 9% of the total A-I in the d 1.21–1.25 g/ml and d > 1.25 g/ml fractions. Apoprotein and cholesterol distribu-

tions were similar for samples obtained from males and females.

The A-I/A-II ratio varied with the lipoprotein hydrated density (**Figs. 4** and **5**). Between d 1.106–1.150 g/ml the A-I/A-II weight ratio was relatively constant at approximately 3.6 (2.2 molar ratio) for HDL samples from males or females. The A-I/A-II ratio increased as the density increased for lipoprotein in the density range of 1.151–1.25 g/ml. However, in the density range of d 1.077–1.105 g/ml the A-I/A-II ratio increased as the density decreased. Similar A-I/A-II ratio distributions were obtained with serum samples. The cholesterol/(A-I + A-II) ratio decreased as density increased.

Analysis of the A-I, A-II, and cholesterol levels from density gradient centrifugation of HDL isolated from two other males and two other females gave results very similar to those illustrated with the previous samples (**Table 1**). The A-I/A-II molar ratio of the d 1.106–1.118 g/ml fraction was 2.2. ± 0.3, nearly identical to that of the d 1.119–1.150 g/ml fraction (2.2 ± 0.2). The HDL fraction of d 1.151–1.200 g/ml from all six subjects had a higher molar ratio than

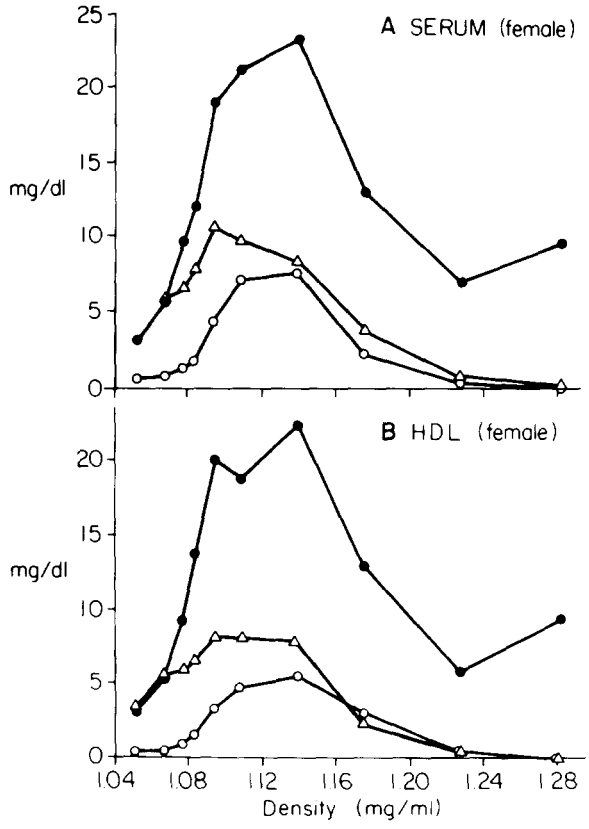

Fig. 2. Cholesterol, A-I, and A-II contents of each density fraction after (*A*) serum of a female (subject 4) and (*B*) HDL of the same female underwent a 72-hr CsCl gradient centrifugation. Symbols are: (△) cholesterol, (●) A-I, (○) A-II.

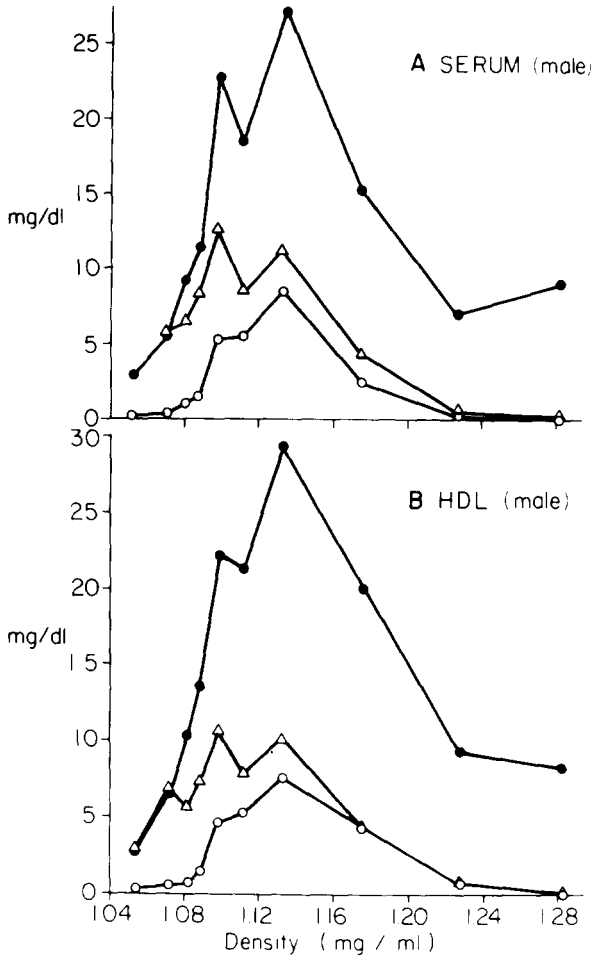

Fig. 3. Cholesterol, A-I, and A-II contents of each density fraction after (*A*) serum of a male (subject 1) and (*B*) HDL of the same male underwent a 72-hr CsCl gradient centrifugation. Symbols are: (△) cholesterol, (●) A-I, (○) A-II.

the d 1.106–1.15 g/ml lipoproteins with a mean ± SD of 2.6 ± 0.2. On the other hand, in the density range between 1.077–1.105 g/ml the A-I/A-II ratio increased as the density decreased in all HDL samples examined. The mean ± SD for the A-I/A-II molar ratio for the d 1.093–1.105 g/ml, d 1.083–1.092 g/ml, and d 1.077–1.082 g/ml fractions was 2.8 ± 0.5, 4.5 ± 1.1, and 5.6 ± 1.3, respectively. The A-I/A-II molar ratio of the d 1.063–1.076 g/ml fraction was 5.5 ± 1.3. HDL subfractions with the same hydrated density had similar A-I/A-II ratios when isolated from men or women. Serum and the d 1.063–1.25 g/ml HDL fraction revealed similar trends in the A-I/A-II ratio as a function of density. However, serum samples consistently gave higher ratios for the d 1.151–1.200 g/ml fraction but lower ratios for fractions of d < 1.106. Though the cholesterol/(A-I + A-II) ratio decreased as the density increased in all subjects examined, HDL subfractions with the same hydrated

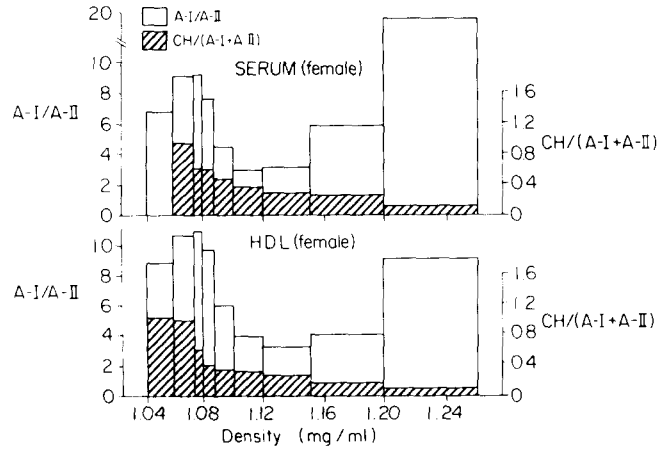

Fig. 4. A-I/A-II and cholesterol/(A-I + A-II) ratios of serum and HDL subfractions from a female (subject 4) at the end of a 72-hr CsCl gradient centrifugation.

density generally had comparable cholesterol/(A-I + A-II) ratios.

HDL lipoprotein separated by gradient electrophoresis demonstrated an inverse relationship between hydrated density and particle size (**Fig. 6**). When the migration distances of the protein standards in each gradient gel were plotted against the logarithm of their molecular weights, linear plots were obtained. Therefore, the apparent molecular weights of the lipoprotein particles were calculated from this standard line. As the density increased from 1.063 to 1.20 g/ml, the molecular weights of the particles decreased from 3.9×10^5 to 1.1×10^5. HDL subfractions with comparable hydrated densities had similar apparent molecular weights whether isolated from males or females. HDL subfractions obtained from

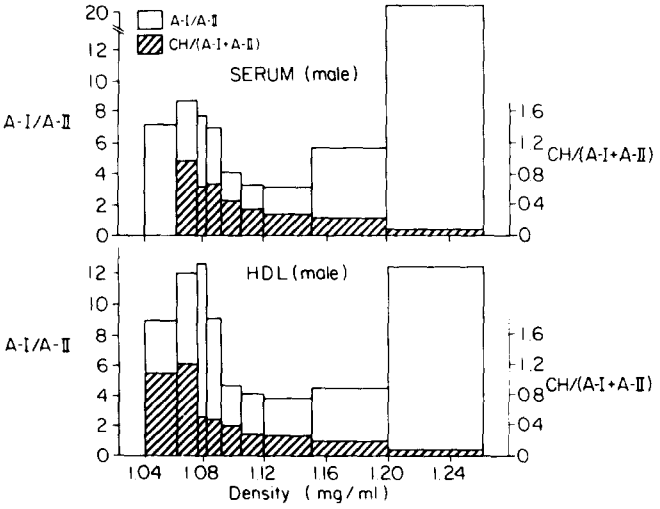

Fig. 5. A-I/A-II and cholesterol/(A-I + A-II) ratios of serum and HDL subfractions from a male (subject 1) at the end of a 72-hr CsCl gradient centrifugation.

density gradient centrifugation of serum was grossly contaminated with albumin. Thus, we did not determine the molecular weights of HDL subfractions from serum.

DISCUSSION

In our initial experiments, serum or HDL was layered either on CsCl, NaBr–NaCl, or a sucrose gradient and was placed either in the middle or bottom of the tubes to establish conditions for equilibrium distribution of lipoproteins. Our data indicated that, in a SW 41 rotor at 40,000 rpm at 15°C, equilibrium banding conditions were attained within 72 hr with CsCl, but not with sucrose or NaBr–NaCl. Centrifugation of HDL for 72 or 96 hr in CsCl gave identical banding profiles based on absorbance measurements at 280 nm. Therefore, the hydrated density of each lipoprotein subfraction could be considered equal to its banding position density.

The A-I/A-II molar ratios of the lipoproteins banding between d 1.106 and 1.150 g/ml from both males and females were relatively constant at approximately 2.2. This observation is consistent with our previous report (6) which indicated that the mean A-I/A-II molar ratio of the d 1.10–1.21 g/ml fraction was 2.2 ± 0.1 for men and 2.3 ± 0.1 for women. Furthermore, these data are consistent with the observations of Kostner et al. (4) and Freidberg and Reynolds (5) who suggested that the molar ratio of the HDL_3 fraction was approximately 2:1 but is inconsistent with the 1:1 molar ratio reported for HDL_3 by Curry, Alaupovic, and Suenram (10). While Curry et al. (10) and Freidberg and Reynolds (5) suggested that the two major HDL subclasses, HDL_2 and HDL_3, have similar A-I/A-II ratios, we found that the lighter density fractions of HDL (d 1.063–1.092 g/ml) have an A-I/A-II ratio twice to thrice that of the d 1.106–1.200 g/ml fraction. Similar observations have been reported by Kostner et al. (4) for HDL subfractions isolated by rate zonal ultracentrifugation.

Since it can be argued that some of the observed heterogeneity of HDL as a function of hydrated density may, in part, be due to alteration of HDL during preparative ultracentrifugation, it was important to confirm that serum and HDL had comparable heterogeneity of A-I/A-II and HDL cholesterol/(A-I + A-II) ratios as a function of density. While some redistribution of polypeptides and lipids may occur during equilibrium gradient centrifugation, it should be noted that HDL subpopulations with differing polypeptide compositions and hydrated densities are also isolated by a shorter 24-hr rate zonal centrifuga-

TABLE 1. Apparent molecular weight, A-I/A-II and cholesterol/(A-I + A-II) ratios in HDL and serum subfractions isolated by density gradient centrifugation

	Density of Subfraction	10^{-5} × Mol Wt[a]		A-I/A-II Molar Ratio[b]						10^2 × CH/(A-I + A-II) Wt Ratio					
				HDL			Serum			HDL			Serum		
	g/ml														
Subject # (Male)		1	2	1	2	3	1	2	3	1	2	3	1	2	3
	1.151–1.200	1.1	1.3	2.7	2.8	2.3	3.6	3.4	3.6	18	23	21	26	30	28
	1.119–1.150	1.4	1.5	2.3	2.3	2.0	2.0	2.6	1.7	27	36	32	31	30	29
	1.106–1.118	1.9	2.1	2.5	2.4	2.0	2.1	2.3	1.8	29	43	43	35	37	35
	1.093–1.105	2.5	2.4	2.8	2.3	2.9	2.6	2.5	2.0	39	45	39	45	46	36
	1.083–1.092	2.9	3.0	5.5	3.2	4.4	4.3	2.2	2.6	48	53	59	65	58	55
	1.077–1.082	3.2	3.4	7.7	5.1	4.3	4.8	4.7	2.3	50	73	65	63	42	61
	1.063–1.076	3.6	3.8	7.4	5.5	4.0	5.4	4.5	2.3	100	62	52	97	33	143
Subject # (Female)		4	5	4	5	6	4	5	6	4	5	6	4	5	6
	1.151–1.200	1.2	1.3	2.5	2.8	2.6	3.6	3.4	5.4	15	22	19	25	30	24
	1.119–1.150	1.4	1.5	2.1	2.4	2.0	1.9	2.3	2.2	27	32	31	27	31	30
	1.106–1.118	2.0	1.9	2.4	2.2	1.8	1.8	2.3	2.0	34	43	37	35	34	35
	1.093–1.105	2.6	2.3	3.7	2.4	2.7	2.8	2.2	2.5	35	43	36	45	38	36
	1.083–1.092	3.0	3.0	6.0	3.5	4.4	3.7	2.8	3.0	41	51	50	58	53	47
	1.077–1.082	3.3	3.3	6.7	5.3	4.6	4.1	4.2	2.8	59	60	63	60	54	60
	1.063–1.076	3.6	3.9	6.5	4.9	4.5	4.0	4.7	3.1	99	57	64	93	44	75

[a] Molecular weight was not determined on subjects three and six.
[b] A-I mol wt 28,300; A-II mol wt 17,400.

tion method (4). Each of the six serum samples had higher A-I/A-II ratios than their respective HDL samples in the d 1.151–1.25 g/ml region, suggesting that some A-I is readily dissociated from HDL during centrifugation as reported in other studies (3, 4, 11). Interestingly, serum samples had lower A-I/A-II ratios than their respective HDL samples in the d 1.063–1.105 g/ml region. Furthermore, two HDL preparations (one each from a male and a female serum) that had been centrifuged twice at d 1.25 g/ml prior to density gradient centrifugation had a lower A-I/A-II ratio in the d 1.15–1.25 g/ml region but a higher A-I/A-II ratio in the d 1.063–1.105 g/ml region than HDL centrifuged only once at d 1.25 g/ml. These observations strongly suggest that both preparative and density gradient centrifugation alter the polypeptide distribution of high density lipoproteins. It is possible that some dissociated A-I binds or forms HDL particles with lower hydrated densities than those from which they were derived. The observation that some A-I (2% of the total A-I) is found in the d < 1.063 g/ml fraction of the CsCl gradient of HDL of d 1.063–1.25 g/ml supports this latter hypothesis. Contrasting with the results with A-I, the d > 1.25 g/ml fraction had no detectable A-II whether HDL or serum was layered on the gradient, indicating that A-II binds more tightly than A-I to the HDL particle. Additional studies are needed to clarify the mechanism of the apoprotein redistribution upon centrifugation.

While the gradient gel electrophoresis system appears to give evidence that lipoprotein particle size varies with hydrated density, extrapolation of particle size to absolute molecular weight must be interpreted with caution until the gradient gel approach to molecular weight determination of proteins of hydrated densities less than 1.35 g/ml is validated. However, it should be pointed out that Anderson et al. (12), using a similar gradient gel electrophoresis system, showed that the estimation of molecular weight of HDL particles by this method agreed well with that obtained by

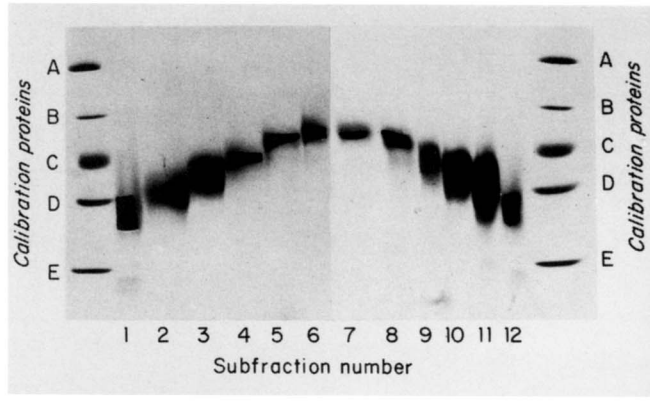

Fig. 6. Gradient gel electrophoresis of HDL subfractions isolated by CsCl gradient ultracentrifugation. Left, fractions 1–6, HDL subfractions from a female (subject 5). Right, fractions 7–12, HDL subfractions from a male, (subject 2). Fractions 1 and 12, d 1.151–1.200 g/ml; 2 and 11, d 1.119–1.150 g/ml; 3 and 10, d 1.106–1.118 g/ml; 4 and 9, d 1.093–1.105 g/ml; 5 and 8, d 1.083–1.092 g/ml; 6 and 7, d 1.077–1.082 g/ml. The calibration proteins are: *A*, thyroglobulin (mol. wt. 669,000); *B*, ferritin (mol. wt. 440,000); *C*, catalase (mol. wt. 232,000); *D*, lactate dehydrogenase (mol. wt. 140,000); and *E*, bovine serum albumin (mol. wt. 67,000).

electron microscopy and analytical ultracentrifugation.

An A-I/A-II molar ratio of slightly greater than 2:1 for the d 1.119–1.150 g/ml subfraction, coupled with estimation of molecular weight of approximately 1.45 $\times 10^5$, is consistent with the interpretation that the majority of HDL particles of this density range have two A-I polypeptides and one A-II polypeptide (dimer). An A-I/A-II ratio somewhat greater than 2:1 could readily be explained if this fraction also contained a minor fraction of particles with three A-I polypeptides and one A-II polypeptide and/or some particles with two or three A-I polypeptides without A-II. The mean A-I/A-II ratio of the d 1.151–1.200 g/ml fraction of 2.5 can be most likely accounted for by particles with two A-I's and one A-II and particles with two or three A-I's without A-II. Since the A-I/A-II molar ratio is significantly higher in the d 1.063–1.090 g/ml region it is likely that the particles in this region have more than three A-I polypeptides to each A-II polypeptide or, alternatively, a major proportion of particles have A-I without A-II. While Albers and Aladjem (3) have already given immunochemical evidence for HDL particles containing A-I without A-II in both the d 1.063–1.125 g/ml and d 1.125–1.21 g/ml density ranges, it remains to be determined whether the proportion of particles having A-I without A-II varies with the hydrated density. Clearly, any model of HDL structure must consider numerous subpopulations with differing polypeptide compositions. The functional significance of HDL subclasses defined on the basis of the molar ratio of A-I/A-II remains to be determined.

Recently, Anderson et al. (12) reported the presence of three major components in HDL with mean molecular weights and hydrated densities as follows: I, 4.1 $\times 10^5$ and d 1.090 g/ml; II, 2.6 $\times 10^5$ and d 1.110 g/ml; III, 1.8 $\times 10^5$ and d 1.145 g/ml. We observed two absorption maxima rather than three. One component at d 1.13 g/ml with an apparent average molecular weight of approximately 1.6 $\times 10^5$ closely resembles component III and most closely corresponds to the $F_{1.20}$ 0–3.5 HDL_3 subclass. Assuming that this HDL subpopulation contains 55% protein and one of the C apolipoproteins (C-I, C-II, or C-III, molecular weight range 7,000–10,000) per HDL particle, then the molecular weight and apolipoprotein analysis is consistent with the structural model of two A-I's and one A-II polypeptide in each of these particles. The other component at d 1.08–1.09 g/ml with an apparent average molecular weight of approximately 3.2 $\times 10^5$ most closely resembles component I and the $F_{1.20}$ 3.5–9.0 HDL_2 subclass. We did not observe an absorption maximum at d 1.110 g/ml corresponding to the subclass II of Anderson et al. (12); however, the conditions of the density gradient ultracentrifugation were quite different in the two studies. Anderson et al. used a NaCl–NaBr salt gradient in a fixed angle rotor whereas we used a CsCl gradient in a swinging bucket rotor. These differences in methodology may have accounted for the differences in results.

HDL newly secreted by the liver is quite different from that normally observed in plasma (13). Presumably, nascent HDL is converted to plasma HDL through the action of lecithin:cholesterol acyltransferase (LCAT) (13). We did not add specific LCAT inhibitor to the serum but immediately processed the samples in such a manner as to minimize the action of LCAT. The specific role of LCAT in the production of HDL subpopulations remains to be clarified. Recently, evidence that A-I is synthesized by the rat and human intestine (14, 15) suggests that chylomicron catabolism may also play a role in the production of HDL subpopulations. Our studies were performed with fasting normolipidemic subjects without chylomicronemia. Thus, the contribution of the intestine to the HDL heterogeneity would likely be minimal.

These studies were supported by Public Health Service Contract N01-HV-2157L, Lipid Metabolism Branch of NIH.

Manuscript received 27 February 1978; accepted 16 August 1978.

REFERENCES

1. Shore, V., and B. Shore. 1968. Some physical and chemical studies on two polypeptide components of high-density lipoproteins of human serum. *Biochemistry.* **7:** 3396–3403.
2. Scanu, A., J. Toth, C. Edelstein, S. Koga, and E. Stiller. 1969. Fractionation of human serum high density lipoprotein in urea solutions. Evidence for polypeptide heterogeneity. *Biochemistry.* **8:** 3309–3316.
3. Albers, J. J., and F. Aladjem. 1971. Precipitation of I-125 labeled lipoproteins with specific polypeptide antisera. Evidence for two populations with differing polypeptide compositions in human high density lipoproteins. *Biochemistry.* **10:** 3436–3442.
4. Kostner, G. M., J. R. Patsch, S. Sailer, H. Braunsteiner, and A. Holasek. 1974. Polypeptide distribution of the main lipoprotein density classes separated from human plasma by rate zonal ultracentrifugation. *Eur. J. Biochem.* **45:** 611–621.
5. Friedberg, S. J., and J. A. Reynolds. 1976. The molar ratio of the two major polypeptide components of human high density lipoprotein. *J. Biol. Chem.* **251:** 4005–4009.
6. Cheung, M. C., and J. J. Albers. 1977. The measurement of apolipoprotein A-I and A-II levels in men and women by immunoassay. *J. Clin. Invest.* **60:** 43–50.

7. Albers, J. J., P. W. Wahl, V. G. Cabana, W. R. Hazzard, and J. J. Hoover. 1976. Quantitation of apolipoprotein A-I of human plasma high density lipoprotein. *Metabolism.* **25:** 633–644.
8. Manual of Laboratory Operations, Lipid Research Clinics Program, Vol. 1. 1974. Lipid and lipoprotein analysis. DHEW Pub. N. (NIH) 75-628.
9. Holbrook, I. B., and A. G. Leaver. 1976. A procedure to increase the sensitivity of staining by Coomassie brilliant blue G250–perchloric acid solution. *Anal. Biochem.* **75:** 634–636.
10. Curry, M. D., P. Alaupovic, and A. Suenram. 1976. Determination of apolipoprotein A and its constitutive A-I and A-II polypeptides by separate electroimmunoassay. *Clin. Chem.* **22:** 315–322.
11. Levy, R. I., and D. S. Fredrickson. 1965. Heterogeneity of plasma high density lipoproteins. *J. Clin. Invest.* **44:** 426–441.
12. Anderson, D. W., A. V. Nichols, T. M. Forte, and F. T. Lindgren. 1977. Particle distribution of human high density lipoproteins. *Biochim. Biophys. Acta.* **493:** 55–68.
13. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667–680.
14. Glickman, R. M., and P. H. R. Green. 1977. The intestine as a source of apolipoprotein A-I. *Proc. Natl. Acad. Sci. USA.* **74:** 2569–2573.
15. Rachmilewitz, D., J. J. Albers, D. R. Saunders, and M. Fainaru. 1978. Apoprotein synthesis by the duodenojejunal mucosa. *Gastroenterology.* **75:** 677–682.