Characterization of apolipoprotein E-rich high density lipoproteins in familial lecithin:cholesterol acyltransferase deficiency

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Abstract We have isolated and characterized a subfraction of high density lipoproteins, rich in apolipoprotein E, from the plasma of patients afflicted with familial lecithin: cholesterol acyltransferase deficiency. Prepared by successive ultracentrifugal flotation, affinity chromatography on heparin-agarose, and affinity chromatography on concanavalin A-agarose, the subfraction contained disc-shaped lipoproteins that measured 14–40 nm in diameter and 4.4–4.5 nm in thickness. The major components were apolipoprotein E, phosphatidylcholine, and unesterified cholesterol, though other apolipoproteins and lipids were present in small amounts. A second subfraction of high density lipoproteins, isolated during the chromatography, contained apolipoproteins A-I and A-II, but no apolipoprotein E. This subfraction included disc-shaped lipoproteins, 13–24 nm in diameter, as well as small round particles, 5.7 nm in diameter. Both subfractions contained similar proportions of total protein relative to lipid, similar amounts of unesterified cholesterol relative to phosphatidylcholine, and a similar distribution of phosphatidylcholine fatty acid.—**Mitchell, C. D., W. C. King, K. R. Applegate, T. Forte, J. A. Glomset, K. R. Norum, and E. Gjone.** Characterization of apolipoprotein E-rich high density lipoproteins in familial lecithin:cholesterol acyltransferase deficiency. *J. Lipid Res.* 1980. **21:** 625–634.

Supplementary key words electron microscopy · disc gel electrophoresis · gradient gel electrophoresis · nascent lipoproteins

Among the many plasma lipoprotein abnormalities associated with familial lecithin:cholesterol acyltransferase (LCAT) deficiency (1, 2) are several that involve the high density lipoproteins (HDL). Several years ago we showed (3, 4) that HDL from subjects with LCAT deficiency includes two subclasses of particles not ordinarily observed in normal human plasma. One subclass contains small round particles approximately 6 nm in diameter, whose sole apolipoprotein component is apolipoprotein A-I (apoA-I; 5). The second subclass is comprised of disc-shaped particles, 15–20 nm in diameter, that contain apoA-I, apolipoprotein A-II (apoA-II), and C apolipoproteins (apoC; 5). Utermann and colleagues (6, 7) more recently provided evidence for a third subclass of particles in HDL from LCAT-deficient plasma. They showed that HDL of d 1.063–1.125 g/ml includes disc-shaped particles and is rich in apolipoprotein E (apoE).

HDL containing apoE is of potential interest for two reasons. First, Hamilton et al. (8) and Felker et al. (9) have identified disc-shaped HDL, rich in apoE, in perfusates of rat livers, and have suggested that these HDL are nascent particles. Second, we have shown (10, 11) that incubation of plasma from patients with LCAT deficiency (patient plasma) with LCAT converts disc-shaped HDL to spherical HDL and leads to redistribution of apoE from HDL to VLDL. These observations suggest that action of LCAT on nascent apoE-rich-HDL in normal subjects may critically affect the metabolism of apoE in the plasma.

For these reasons it seemed important to purify and characterize patient apoE-rich-HDL to provide a basis for detailed studies of the role of similar HDL in the metabolism of normal human plasma lipoproteins. Experiments directed toward this goal are described in this study.

Abbreviations: apo, apolipoprotein; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; Con A, concanavalin A; EDTA, ethylenediaminetetraacetate; SDS, sodium dodecylsulfate; UC, unesterified cholesterol; CE, cholesteryl ester; PL, phospholipid; PC, phosphatidylcholine; OD, optical density; MW, molecular weight.

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MATERIALS AND METHODS

Patients and normal controls

Two female patients from different Scandinavian families were studied: M.R., aged 31, and A.A., aged 52. Their clinical features, laboratory findings, and plasma lipoproteins have been previously described (12–14). At the time of study, patient A.A. had hyperlipemia, and both patients had moderate proteinuria, with 0.5–1.5 mg protein/ml urine. They were otherwise in relatively good health, and were receiving no special form of therapy. In two instances, however, plasma was prepared after patients A.A. and M.R. had been given 16 g/day of cholestyramine for one month as part of another experiment. Total plasma cholesterol was unchanged in patient A.A. and gradually increased in patient M.R. during this treatment. The normal controls were females living in Seattle.

Preparation of plasma

Plasma from donors who had fasted overnight was prepared as described previously (15). Plasma from the normal controls was immediately made 2 mM in parachloromercuriphenyl sulfonic acid to inhibit LCAT activity.

Isolation and subfractionation of HDL

HDL (d = 1.063–1.250 g/ml) was isolated by sequential ultracentrifugal flotation (15). In this procedure, the plasma fraction of d > 1.063 g/ml and the two d = 1.063 g/ml KBr washes of the LDL were combined to prepare the HDL fraction. After recentrifugation twice at d = 1.250 g/ml and subsequent dialysis against 0.015 M NaCl, 0.001 M EDTA, 0.003 M NaN_3 (NaCl–EDTA–NaN_3), the HDL was subfractionated by affinity chromatography on heparin-agarose and concanavalin-A-agarose (con A-agarose) as described below.

Heparin (Sigma Chemical Co., St. Louis, MO) was coupled to Sepharose CL-2B (Pharmacia Fine Chemicals, Piscataway, NJ) that had been activated by treatment with cyanogen bromide (16). Six grams of Sigma H 3125 Heparin were mixed with 500 ml of activated Sepharose CL-2B for 24 hr at 4°C; then 1 M glycine was added for an additional 4 hr. After the product was washed with 2 M NaCl–0.005 M imidazole buffer (pH 6.5), it was stored in 0.1 M potassium phosphate buffer (pH 6.0) containing 0.2% NaN_3. For separation of HDL, 1.5 ml of packed heparin-agarose was used for each mg of HDL protein. Both the heparin-agarose and the HDL were equilibrated with 0.015 M NaCl in 0.005 M imidazole buffer (pH 6.5) before the HDL was applied to the column. After nonbound HDL had been completely eluted from the column, a gradient was begun with a limit buffer of 1–2 M NaCl in 0.005 M imidazole (pH 6.5). In a typical experiment, a mixing flask volume of 500 ml was used for a column bed volume of 60 ml. Elution of protein was monitored by measuring absorbance at 280 nm.

Affinity chromatography on Con-A-agarose was carried out by a modification of the method of McConathy and Alaupovic (17). Both sample and Concanavalin-A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) were equilibrated with a buffer containing 0.2 M NaCl, 0.1 M sodium acetate, 0.003 M $MgCl_2$, 0.003 M $MnCl_2$, and 0.003 M $CaCl_2$ (pH 6.8). Then the sample containing 18–21 mg protein was applied to a column containing a packed bed volume of 12 ml Con-A-Sepharose. After nonbound material had been eluted from the column, the same buffer with the addition of 0.2 M α-D-methylmannoside was used to elute bound fractions until no further material could be detected in the effluent. Final elution was made with 0.1 M EDTA. The eluted fractions were adjusted to a density of 1.25 g/ml with solid KBr, then centrifuged for 44 hr at 50,000 rpm in a 60 Ti rotor to separate lipoprotein from eluted free Con A.

Quantitative analysis of lipids and apolipoproteins

Quantitation of apoA-I, A-II and E was performed by densitometry of SDS gels. Samples were first extracted with ether, then boiled in 2% SDS for 5 min before being applied to sample wells in polyacrylamide gel slabs (a vertical slab gel apparatus, BioRad Model 220, BioRad Laboratories, Richmond, CA, was used throughout these studies). Laemmli's discontinuous SDS system (18) was modified to include 8 M urea in slabs of 15% polyacrylamide. After electrophoresis, gels were extracted overnight with 25% isopropanol–10% acetic acid to remove SDS, then stained 16–20 hr with 0.25% Coomassie Brilliant Blue R-250 in 45% methanol, 9% acetic acid (19). Gels were destained for 4–8 hr in 25% isopropanol–10% acetic acid, and further destained in 10% methanol–7% acetic acid until the backgrounds were clear. Quantitation of the apolipoproteins was done by densitometry using an Ortec gel scanner (Ortec, Inc., Oak Ridge, TN).

Secondary HDL and VLDL standards, at 4 to 5 protein levels, in duplicate, were analyzed in parallel with the unknowns on each slab. The standards were calibrated against pure apolipoproteins prepared from normal VLDL and HDL (20–22). Final purification of apo E was performed by preparative SDS gel electrophoresis in the system described above. Primary standards of pure apoA-I and A-II were prepared by a combination of gel filtration on Sepha-

dex G200 (Pharmacia Fine Chemicals, Piscataway, NJ) and chromatography on DEAE cellulose (Whatman DE52; Whatman Ltd., Springfield Mill Maidstone, Kent, England). The gel filtration was performed essentially as described by Scanu et al. (23) except that 6 M guanidine hydrochloride was substituted for urea. The DEAE chromatography was essentially that described by Shore and Shore (22). Quantitation of the primary standards was determined on the basis of amino acyl mass; corrections were made for 1/2 cystine and tryptophan where necessary. Replicates of each apolipoprotein, in amounts ranging from 1 to 10 μg, were electrophoresed and stained as described above, then scanned on the Ortec gel scanner. A linear response was obtained for amounts ranging from 1 to 8 μg for each apolipoprotein when the broad band green filter supplied with the instrument was used with a slit aperture of 5 × 0.5 mm. The coefficient of variation for replicate secondary standards was 4–7%.

Two-dimensional electrophoresis of HDL apolipoproteins was carried out by a modification of the system suggested by O'Farrell (24). Gradient gel electrophoresis of intact HDL was carried out on PAA 4/30 polyacrylamide gradient gels (Pharmacia Fine Chemicals, Piscataway, NJ), and was used to estimate the apparent hydrated particle diameter of intact HDL using the procedures suggested by Pharmacia. Pharmacia molecular weight standard mixtures were used for all calibrations; their hydrated diameters were determined from diffusion coefficients (25).

The methods for analysis of total unesterified cholesterol (UC), esterified cholesterol (CE), triglyceride (TG) and phospholipids (PL) have been described elsewhere (15). The fatty acid composition of HDL phosphatidylcholine (PC) was determined by analysis of material purified by thin-layer chromatography. The PC was eluted as described by Skipski (26) and methylated by heating to 80°C for 20 min in sodium methoxide (Applied Science, State College, PA). The methyl esters were separated on Silar 10 C on gas chrom Q (Applied Science, State College, PA) and analyzed in a model 5830 A Hewlett-Packard gas–liquid chromatograph.

For electron microscopy, samples were dialyzed against 0.13 M ammonium acetate buffer (pH 7.4) containing 345 μM EDTA and 124 μM merthiolate. Various fractions were examined by negative staining, as described previously (4). Size of particles was determined with a magnifying micrometer; in any given experiment, 75–150 free-standing particles were measured except where particles formed rouleaux, in which case the long axis of the particles was measured.

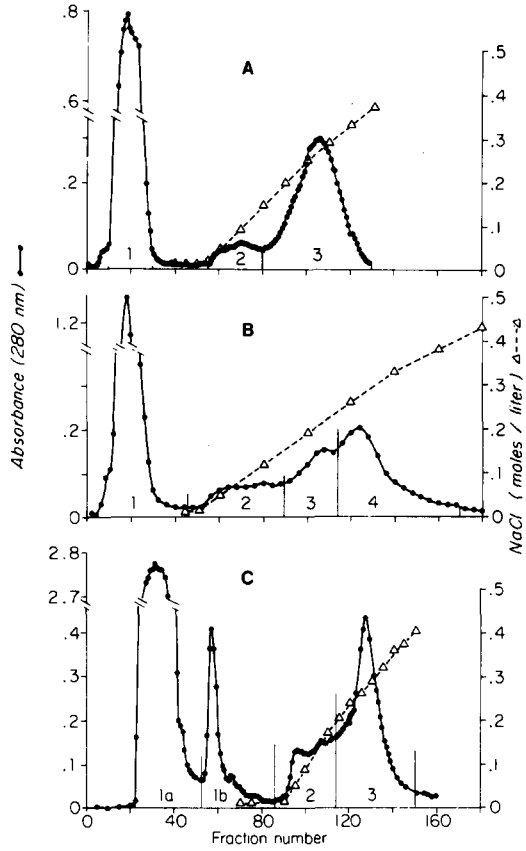

Fig. 1. Representative separations of HDL from LCAT-deficient patients and from a normal control on heparin-agarose columns (1.6 × 30 cm). HDL from patient M.R. (A), patient A.A. (B), and normal M.P. (C) were applied to heparin-agarose columns in 0.015 M NaCl, 0.005 M imidazole, pH 6.5. After subfraction 1 had been eluted, a gradient of NaCl in 0.005 M imidazole, pH 6.5, eluted the remaining subfractions. Recovery of total protein ranged from 81 to 90%. Most commonly, non-bound normal HDL was eluted with a slight trailing shoulder rather than the distinct second peak (Ib) obtained for M.P.

RESULTS

Chromatography on heparin-agarose

Characteristic patterns were obtained when the patients' HDL were chromatographed on heparin-agarose (**Fig. 1**). The distribution of total protein in the subfractions was remarkably reproducible for each patient over a period of 3 years, though several different preparations of heparin-agarose were employed. Subfraction 1, which passed through the column without adsorbing to it, contained 59.6% ± 2.8% (n, 5)[2] of the total protein in the case of patient M.R.; the corresponding value for patient A.A. was 54.9% ± 4.7% (n, 8). By contrast, the value for normal

[2] All values are reported with standard deviation, and the number of replicates is indicated in parentheses unless otherwise noted.

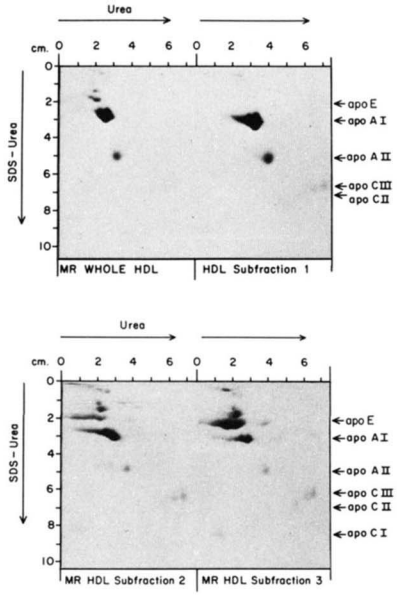

Fig. 2. Two-dimensional electrophoresis of HDL and HDL subfractions from patient M.R. HDL subfractions obtained by chromatography on heparin-agarose were treated with tetramethylurea, and the soluble apolipoproteins were electrophoresed first on a 7.5% polyacrylamide gel in 8 M urea, then on a 15% polyacrylamide gel containing 8 M urea, 0.1% SDS. Pure apoprotein standards were similarly analyzed to verify identification. The pattern of normal HDL is similar to that of M.R. HDL subfraction 1.

female HDL was 85% ± 10.8% (n, 7). The lipoproteins that adsorbed to the column were eluted over a range of 0.02 to 0.40 M NaCl. A small proportion of patient HDL (subfraction 2, Fig. 1) was eluted at the beginning of the gradient. The bulk of the adsorbed material emerged later (0.15–0.40 M NaCl), generally yielding a relatively symmetric peak in the case of patient M.R. and invariably yielding a peak with a preceding shoulder in the case of patient A.A. Adsorbed HDL from different normal subjects yielded a more variable elution pattern, occasionally with a relatively large amount of material in subfraction 2.

Quantitative analysis of the major apolipoproteins in the subfractions of the patients' HDL obtained by chromatography on heparin-agarose (representative experiments are described in Table 1) showed that subfraction 1 contained the bulk of the apoA-I and apoA-II but no detectable apoE or apoB.[3] Both apoE and apoB were selectively concentrated in the adsorbed subfractions, particularly in material that emerged at the highest concentrations of NaCl. This material also contained small amounts of apoA-I and apoA-II. Negligible amounts of other apolipoproteins, including apoC, were present in all subfractions as demonstrated by two-dimensional gel electrophoresis (**Fig. 2**).

Analysis of patient HDL subfractions by gradient gel electrophoresis (**Fig. 3**) showed that heparin-agarose subfraction 1 contained a mixture of discrete bands corresponding to particles ranging from 7.5 to 16 nm in apparent hydrated particle diameters.[4] The peak distribution differed markedly from that observed for normal HDL (Fig. 3A). The most strongly adsorbed subfractions contained lipoproteins with apparent particle diameter of >15 nm. The upper limits of the size range of strongly adsorbed material could not be determined adequately using this technique since much of the lipoprotein did not enter the gel. Similar gradient gels of the normal HDL subfractions (not shown) revealed particles with apparent hydrated particle diameters of 9.2 and 11.2 nm, similar to HDL_2 and HDL_3, in subfraction 1; material of about 10 to 12.5 nm diameter in subfraction 2; and

[3] Demonstrated by measurement of TMU-insoluble protein, by immunoassay, and by electrophoresis in SDS on 4% polyacrylamide gels.

[4] We found that disc-shaped particles do not migrate on gradient gels to the same position as spheres of equal diameter, but instead, migrate to approximately the position of a sphere of equal volume: $D_{app} \approx (1.5\ D^2T)^{1/3}$ where D and T are disc diameter and thickness.

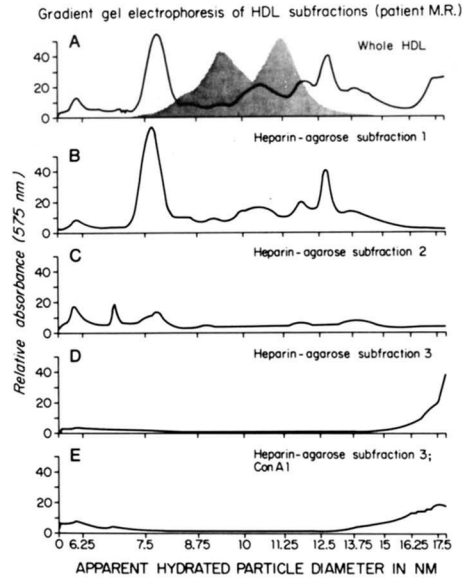

Fig. 3. Gradient gel electrophoresis of patient M.R.'s whole HDL and HDL subfractions. Samples of intact HDL and HDL subfractions (each 30 μg protein) were separated on precast polyacrylamide gradient gels (PAA 4/30) 16 hrs, 125 V, then stained with Coomassie R250 and scanned at 575 nm. In subfractions 2 and 3 (C–E) most of the lipoprotein was totally excluded from the gel. The stippled area in A represents the pattern of normal HDL in this system. High molecular weight standards from Pharmacia, applied to each gel in duplicate, were thyroglobulin, MW 669,000; Ferritin, MW 440,000; catalase, MW 232,000; lactate dehydrogenase, MW 140,000; and bovine serum albumin, MW 67,000.

material with diameters ranging from about 9.2 to >15 nm in subfraction 3.

Electron micrographs of negatively stained lipoproteins (**Fig. 4**) revealed that all subfractions of the patients' HDL contained discs similar to those described previously (4). The discs were of uniform thickness (4.4–4.5 nm), but discs that emerged in heparin-agarose subfraction 1 were somewhat smaller in diameter (13–24 nm) than those that emerged in the adsorbed subfractions (14–40 nm). In addition to disc-shaped lipoproteins, subfraction 1 also contained small round lipoproteins, 5.7 nm in diameter, similar to those described previously (4), whereas the adsorbed lipoproteins included round particles the size of normal LDL (21–22 nm). Electron micrographs of the subfractions of normal HDL (not shown) revealed round particles the size of HDL_2 and HDL_3 in subfraction 1; the subfractions of adsorbed lipoproteins contained heterogeneous, round particles (6.1–15.0 nm).

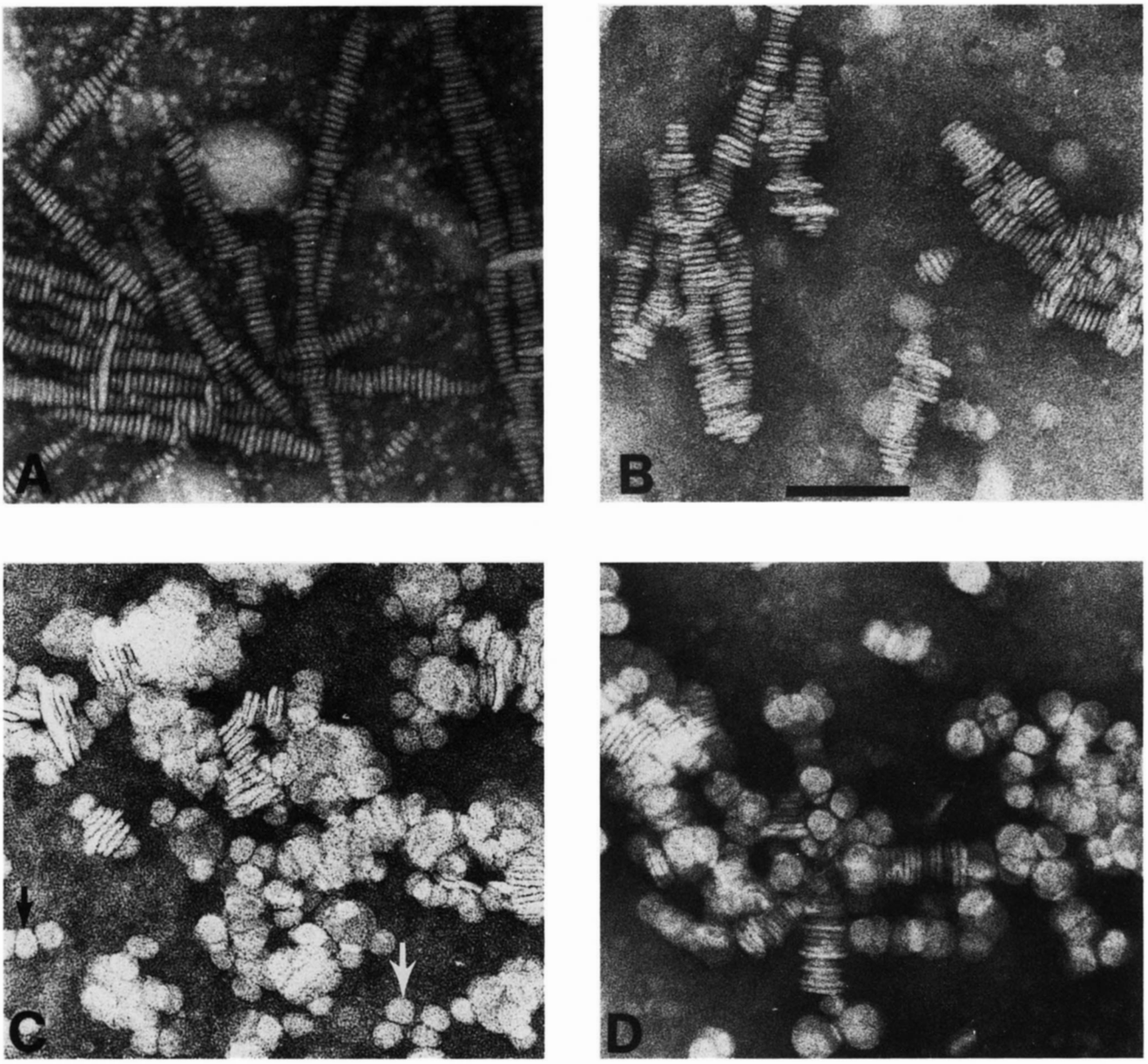

Fig. 4. Electron micrographs of negatively stained HDL subfractions from patient M.R. A., Heparin-agarose subfraction 1. Note small round particles in background which are missing in subfraction 2. B., Heparin-agarose subfraction 2. C., Heparin-agarose subfraction 3. Arrows indicate particles with the size and morphology of LDL. D., Heparin-agarose subfraction 3. Con A-1. The bar marker represents 100 nm and pertains to all micrographs.

Chromatography on Concanavalin-A-Sepharose

The demonstration by electron microscopy of both disc-shaped particles and LDL-like particles in the more strongly adsorbed subfractions of patient HDL indicated that these subfractions were still heterogeneous. We therefore chose affinity chromatography on Con-A-Sepharose to remove material containing apoB. This step further separated subfraction 3 of patient M.R. and subfractions 2–4 of patient A.A. into material not bound by the column (Con A-1), bound material that could be eluted by 0.2 M α-D-methylmannoside (Con A-2), and bound material eluted by 0.1 M Na EDTA, pH 7.4 (Con A-3). Recovery of protein ranged from 47.8% to 86.8%; recovery of UC ranged from 30.9% to 83.6%. Analysis of the apolipoprotein content of these subfractions, performed after recentrifuging the lipoproteins to remove contaminating Con A, showed that most of the apoE-rich-HDL had not become bound to the Con A-Sepharose (**Table 1**). This non-bound material, Con A-1, corresponded to 10–20% of the protein of the unfractionated HDL, whereas the bound material that was eluted by α-D-methylmannoside or EDTA contained little measurable apolipoprotein. Most of the apoB remained bound to Con A-Sepharose even after the 0.1 M EDTA elution.

Analysis of the apoE-rich-HDL, Con A-1, by two-dimensional electrophoresis (not shown) revealed a distribution of apolipoproteins similar to that observed in the parent subfractions obtained by chromatography on heparin-agarose (Fig. 2). Quantitative analysis of five different preparations of the apoE-rich-HDL (**Table 2**) showed a molar ratio of apoE to apoA-I ranging from 10.7 to 46.3, mean = 22.1. The molar ratio of UC to PL was 0.68 ± 0.23 (n, 5). The molar ratio of apoproteins to PL was 14.5 ± 3.0 (n, 5). Analysis of the corresponding apoA-I-rich fractions (heparin agarose subfractions 1) showed no apoE; the molar ratio of UC to PL = 0.69 ± 0.09 (n, 4). The apoprotein to PL ratio was 16.3 ± 5.9 (n, 4). The fatty acid distribution in PC isolated from apoE-HDL did not differ from that in PC isolated from the HDL in heparin agarose subfraction 1 (**Table 3**). The content of CE and TG and of the individual C apolipoproteins was too low for reliable estimation.

To obtain further information about some of the

TABLE 1. Composition of HDL and HDL subfractions[a]

Subject, HDL Subfraction	Total Protein	ApoE	ApoA-I	ApoA-II	UC	Total Lipid Phosphorus
	μg/ml plasma[b]	*μg amino acyl mass/ml plasma*			*μmol/ml plasma*	
Patient M.R.						
Unfractionated HDL	349	60	130	19	0.521	0.664
Heparin-agarose subfractions:						
1	219	0	117	19	0.257	0.431
2	16	4	4	1	0.029	0.031
3, Con A subfractions:						
1	34	40	3	1	0.090	0.087
2	13	4	1	0	0.026	0.027
3	8	5	1	1	0.010	0.018
Patient A.A.						
Unfractionated HDL	286	117	85	19	0.456	0.597
Heparin-agarose subfractions:						
1	155	0	63	13	0.189	0.298
2–4, Con A subfractions:						
1	64	85	5	2	0.141	0.182
2	7	4	0.4	0.1	0.018	0.022
3	7	0	1	0	0.001	0.001
Normal Female						
Unfractionated HDL	1575	35	1104	205	0.379	1.316
Heparin-agarose subfractions:						
1a	1360	9	908	170	0.292	1.133
1b	13	0	2	1	0.004	0.010
2, Con A subfractions:						
1	17	3	5	2	0.005	0.013
2	7	0	1	1	0.001	0.003
3, Con A subfractions:						
1	10	5	2	1	0.004	0.006
2	11	1	1	1	0.009	0.009

[a] HDL subfractionated by successive chromatography on heparin-agarose and Con-A-sepharose in three representative experiments.

[b] Analyzed by the method of Lowry (27), expressed on the basis of human serum albumin equivalents.

TABLE 2. Composition of apoE-HDL

Patient/Subfraction	ApoE[a]	ApoA-I[a]	ApoA-II[a]	UC[a]
	μmol/1000 μmol phospholipid			
M.R. Subfraction 3, Con A-1, 1977[b]	8.6	0.4	1.4	580
M.R. Subfraction 3, Con A-1, 1978	12.8	1.2	0.7	1034
A.A. Subfraction 3, Con A-1, 1977[b]	15.3	0.8	1.5	450
A.A. Subfraction 4, Con A-1, 1977[b]	13.9	0.3	0.9	578
A.A. Subfraction 2–4, Con A-1, 1978	13.0	1.0	0.6	775
Mean for Patients	12.7 ± 2.5	0.7 ± 0.4	1.0 ± 0.4	683 ± 228
Normal (MP) Subfraction 3, Con A-1	23.3	11.8	9.5	592

[a] Molecular weights assumed for apoE, apoA-I, apoA-II, and UC were 36,000, 28,000, 17,400 (28), and 387.

[b] From plasma obtained after 4 weeks administration of cholestyramine (manuscript in preparation). Neither the lipid concentration nor the UC/PL ratios differed in HDL heparin agarose subfractions 2–4 as a result of this treatment.

minor apolipoprotein components, we analyzed the SDS-solubilized apolipoproteins by electrophoresis on 10% polyacrylamide gels in the presence or absence of 5% mercaptoethanol. Minor components of approximately 48,000, 68,000 and 100,000 daltons, present in the purified apoE-rich-HDL from the patients' plasma, disappeared in the presence of mercaptoethanol. In addition, an apolipoprotein of about 48,000 daltons, which was present in the apoE-rich subfraction of the normal HDL, also disappeared in the presence of mercaptoethanol, while there was an increase in material present in the range of apoE.

Analysis of the patients' apoE-rich-HDL by gradient gel electrophoresis (heparin-agarose subfraction 3, Con A-1, Fig. 3) yielded results similar to those obtained for the parent subfraction from the heparin-agarose column. However, analysis of this HDL by electron microscopy (Fig. 4D) showed that the LDL-like particles had been removed. The apoE-rich-HDL of patient M.R. seemed to consist of disc-shaped particles, arrayed in stacks, with a thickness of 4.5 ± 0.4 nm and a diameter of 24.8 ± 5.2 if measured from the round profiles, or 28.0 ± 6.5 if measured as the long axis of stacked discs (**Fig. 5**). Corresponding measurements for the apoE-rich-HDL of patient A.A. were 4.4 ± 0.4 nm by 18.7 ± 2.5 nm (round profiles) or 18.9 ± 2.1 nm (long axis of stacked discs). ApoE-rich-HDL particles were morphologically very distinct from LpX particles. The dimensions of the latter (29, 30) are 50–60 nm long axis and 9–10 nm short axis. Electron microscopy of an apoE-rich subfraction of normal HDL, heparin agarose 3, Con A-1 (Table 1), showed no discs similar to those isolated from the patients' plasma. Instead, this normal subfraction contained round particles, diameter 11.4 ± 1.7, and occasional large, thin structures about 4 nm thick and 48.6 ± 22.7 nm in diameter.

DISCUSSION

The disc-shaped apoE-HDL that we have purified from the plasma of patients afflicted with familial LCAT deficiency has properties (**Table 4**) that are generally compatible with a model consisting of a bilayer of phospholipid and unesterified cholesterol surrounded by a protein rim (8). The actual disposition and packing of the protein are uncertain, however, since data are not yet available concerning the primary structure of apoE and the relative degree of helicity of apoE in these particles.

TABLE 3. Major fatty acids of the phosphatidylcholine in two distinct subfractions of HDL

Patient/Subfraction	16:0	18:0	18:1	18:2	20:4
	mole per cent[a]				
A.A. Subfraction 2–4, Con A-1, 1978 (apoE-rich)	28.8	18.0	19.5	23.0	8.6
A.A. Subfraction 1, 1978 (apo A-I-rich)	26.4	18.6	17.6	21.9	7.0
M.R. Subfraction 3, Con A-1, 1978 (apoE-rich)	29.5	19.6	19.6	22.8	7.8
M.R. Subfraction 1, 1978 (apoA-I-rich)	29.8	20.3	18.5	21.4	8.5

[a] Mean values from duplicate analyses.

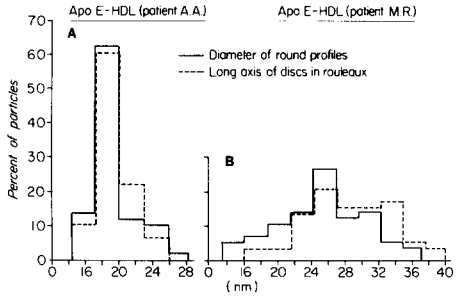

Fig. 5. Size distribution of apoE-HDL isolated from patients A.A. and M.R., 1978.

Comparison of the disc-shaped apoE-HDL isolated in the present experiments with similar particles studied under other circumstances is made difficult by the lack of complete compositional data. The apoE-rich HDL of d 1.063–1.125 g/ml studied by Utermann and coworkers (6, 7) was prepared from the plasma of patients of Sardinian origin afflicted with familial LCAT deficiency, and from the plasma of patients afflicted with secondary LCAT deficiency caused by liver disease. The HDL included disc-shaped particles, similar in size to the apoE-HDL described here, and contained slightly more apoE than apoA-I. However, the HDL was not purified further, the presence of disc-shaped particles that contained apoA-I but no apoE was not excluded, and no measurements of the lipid composition of the HDL were presented. Other investigators have more recently found evidence for the presence of apoE-HDL in the plasma of patients with cholestasis (33) or alcoholic hepatitis (34). Again, the particles from these patients have not yet been purified, preventing detailed comparisons.

More information is available concerning the "nascent" apoE-rich HDL that are released by the perfused rat liver (8, 9, 35). Hamilton et al. (8) and Felker et al. (9) isolated a fraction containing this HDL by ultracentrifugation of the perfusate at d 1.075–1.175 g/ml. The disc-shaped particles in this fraction had a mean thickness of 4.6 nm and a mean diameter of 19 nm. The main lipid components were phospholipid and UC, although CE and TG were also present. The ratio of apoE to apoA-I was 10:1 (9). These particles are thus very similar to those that we have isolated from LCAT-deficient plasma (see Table 4).

Several questions remain about the apoE-HDL of LCAT-deficient plasma. One concerns the relation between this HDL and apoE-rich HDL that circulate in patient plasma in vivo. We cannot entirely rule out the possibility that changes in particle composition and characteristics may have occurred during purification, although changes in content of apoE were probably not extensive. Unpublished experiments with Dr. Richard Havel have indicated that there is almost no release of apoE to the protein fraction of d

TABLE 4. Composition and characteristics of ApoE-HDL based on a disc-shaped model

Lipoprotein	Particle Dimensions[a] (nm)	Molecules per Particle[b] PL	UC	CE	TG	ApoE	ApoA-I	ApoA-II	Particle Weight (Daltons)	% Rim Volume[c]	% Protein Volume as α-Helix
A.A. Subfraction 2–4, Con A-1, 1978	D = 19.5 T = 4.4 ΔR = 1.5	569	441	n.d.	n.d.	7.4	0.6	0.3	892,000	27.1 23.6	96
M.R. Subfraction 3, Con A-1, 1978	D = 27.5 T = 4.5 ΔR = 1.5	1029	1064	n.d.	n.d.	13.2	1.2	0.7	1,716,000	18.7 22.1	77
Nascent HDL, perfused rat liver[d]	D = 19.1 T = 4.5 ΔR = 1.5	401	261	55	52	9.0	1.0	n.d.	843,000	28.9 31.6	77

[a] D is disc diameter, for mean particle volume; T is disc thickness as determined from electron micrographs; R is protein rim width chosen to approximate diameter of amphipathic helix (d = 1.5 nm).

[b] Calculated from chemical composition and particle dimensions, using following molecular weights and specific volumes (31): PL, 800, 0.970; UC, 386.6, 0.968; CE, 652.6, 1.004; TG, 885, 1.093; proteins, 35,000 for rat apoE (32), 0.705 specific volumes for all protein, others in Table 2.

[c] First value calculated from particle dimensions as $\% = 100[1 - ((D - 2\Delta R)^2/D^2)]$. Value in parentheses calculated from composition as 100 (Σ(protein volumes)/total volume).

[d] References 8 and 9.

Calculations were based on the assumption that the apoE-HDL consists of a disc-shaped bilayer of polar lipid surrounded by a rim of protein in a tightly packed amphipathic helix. Total volumes of component lipids and proteins per particle were calculated on the basis of compositional data (Table 2), reported specific volumes (31), and mean particle volumes based on electron microscopy (Fig. 5). Calculated values were used to compute number of component molecules per particle and particle molecular weight. Values for lipid volume and protein volume were compared with those computed for the model geometry assuming that the protein rim is one amphipathic helix in width (1.5 nm) and three amphipathic helices in thickness.

> 1.25 g/ml when patient HDL is centrifuged three times at 50,000 rpm for 44 hrs at this density in a Ti 60 rotor of a Beckman ultracentrifuge. The loss of apoE observed during chromatography of HDL (Table 1) included loss caused by removal of material containing both apoE and apoB.[5] In contrast to this, the content of apoA-I in apoE-HDL may have diminished during the centrifugation. We have shown previously (10) that large amounts of apoA-I are released to the bottom fraction of d > 1.25 g/ml when patient HDL is isolated by ultracentrifugal flotation.

Another question relates to the basis for the stable association between apoE and lipid in the disc-shaped HDL. Although little if any loss of apoE occurs during ultracentrifugation of the patients' disc-shaped HDL, we have shown elsewhere (11) that incubation of this HDL with LCAT leads both to the formation of spherical particles and to loss of apoE (11). Furthermore, experiments by other investigators (36)[6] have suggested that extensive loss of apoE from spherical HDL occurs during preparative ultracentrifugation of normal plasma from both rats and human beings. It seems, therefore, that apoE is more strongly associated with disc-shaped HDL than with spherical HDL. If so, a critical determinant of this strong association might be the hydrophobic surface contributed by the exposed fatty acid chains of rim phospholipids.

A final question relates to the origin of the various disc-shaped HDL obtained from LCAT-deficient plasma. The similarity of patient apoE-HDL to the apoE-HDL isolated from rat liver perfusates suggests that human apoE-HDL may be nascent HDL of hepatic origin. The origin of the apoA-I-rich discs in heparin-agarose subfraction 1, that coexist with the apoE-HDL discs, is less obvious. It is possible that the apoA-I-rich particles are nascent HDL of intestinal origin, since the HDL of rat intestinal lymph is rich in apoA-I and includes disc-shaped particles (37). However, Wu and Windmueller (38) have recently concluded that as much as one-half of the apoA-I in rat plasma HDL is derived from the liver. Therefore, some apoA-rich discs may also be of hepatic origin.

This investigation was supported by the Howard Hughes Medical Institute Laboratory, the Anders Jahre Foundation, grants HL10642, AG00299, RR00166 and HL18574 from the U.S. Public Health Service, and the Norwegian Research Council for Science and the Humanities.

Manuscript received 31 December 1979 and in revised form 25 March 1980.

[5] Mitchell, C. D., W. C. King, K. A. Applegate, T. Forte, J. A. Glomset, K. R. Norum, and E. Gjone. Unpublished data.

[6] Blum, C. Personal communication.

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