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Research Article

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Plasma Lipoproteins in Familial Lecithin:Cholesterol Acyltransferase Deficiency: Physical and Chemical Studies of Low and High Density Lipoproteins

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ABSTRACT Low density lipoproteins (LDL) and high density lipoproteins (HDL) from the plasma of patients with familial lecithin: cholesterol acyltransferase (LCAT) deficiency have been characterized by gel filtration, analytical ultracentrifugation, and gel electrophoresis, and their relative content of lipid and protein has been determined. The LDL of d 1.019–1.063 g/ml show marked heterogeneity. A subfraction of the LDL emerges from columns of 2% agarose gel with the void volume, has corrected flotation rates (S_{r}°) in the range of 20–400, and contains 4–10 times as much unesterified cholesterol, phosphatidylcholine, and triglyceride per mg protein as normal LDL. A major subfraction of the LDL emerges from the gel in the same general position as normal LDL, but exhibits somewhat higher flotation rates and contains 1.5–3 times as much unesterified cholesterol and phosphatidylcholine and 13 times as much triglyceride per mg protein. The HDL, shown to be heterogeneous in earlier studies, are mainly comprised of molecules which have flotation rates of $F_{1.20}$ 3–20, migrate in the α_1 – α_2 region on electrophoresis, and contain about 12 times as much unesterified cholesterol and 5 times as much phosphatidylcholine per mg protein as normal HDL. Smaller molecules are also detected, which have flotation rates of $F_{1.20}$ 0–3, migrate in the prealbumin region on electrophoresis, and contain only slightly more

unesterified cholesterol and phosphatidylcholine per mg protein than normal HDL.

INTRODUCTION

An inborn error of metabolism which affects plasma cholesteryl esters was recently discovered in Scandinavia (1–4). The disease is characterized by a deficiency of the plasma cholesterol esterifying enzyme, lecithin: cholesterol acyltransferase (LCAT),¹ and is called familial LCAT deficiency (5). Patients with this disease have corneal infiltration, anemia, and proteinuria as well as elevated concentrations of unesterified cholesterol and phosphatidylcholine and reduced concentrations of cholesteryl ester and lysophosphatidylcholine in plasma. All classes of lipoproteins are abnormal in lipid composition but appear to react with partially purified LCAT in the same way as normal lipoproteins (6). Evidence (5, 6) suggests that many of the lipoprotein abnormalities are directly caused by absence of the enzyme. Therefore, careful study of all these abnormalities may provide valuable information concerning the effect of the LCAT reaction on the structure and metabolism of normal plasma lipoproteins. Also correlative studies of the physical and chemical properties of the abnormal lipoproteins may provide important insight concerning our present methods of evaluating lipoprotein structure. The present study compares some physical and chemical properties of low density lipoproteins (LDL) and high density lipoproteins (HDL) from two patients with LCAT deficiency with normal lipoproteins from control groups.

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¹ Abbreviations used in this paper: HDL, high density lipoprotein(s); LCAT, lecithin: cholesterol acyltransferase; LDL, low density lipoprotein(s).

TABLE I
Composition of Low Fat-High Carbohydrate Diet

Food	Weight				Total calories
	Protein	Fat	Carbo- hydrate	Total	
			g		
Meritene*	74.3	0.55	131.4	225	821.3
Dextri-Maltose†	—	—	196.0	200	784.0
Dry gelatin	4.7	—	44.0	50	185.5
Banana	1.1	0.20	22.2	100	85.0
Water and flavor	—	—	—	900	—
	80.1	0.75	393.6	1475	1875.8

Soft drinks, beer, apples, oranges as directed to adjust daily caloric consumption.

* D. M. Doyle Pharmaceutical Co., Minneapolis, Minn.

† Mead Johnson & Co., Evansville, Ind.

Studies of the lipoproteins by electron microscopy are presented in an accompanying article (7).

METHODS

Two female patients previously described, A. R., age 36 yr (1, 2) and A. A., age 42 yr (4), were studied. Although some of the studies were performed while the patients were under no special form of treatment, most experiments (all those not specifically designated) were performed with plasma obtained from A. A. after she had ingested an essentially fat-free diet for 1 wk. The diet (Table I) was administered while she was an outpatient.³ During the dietary period, the concentration of triglyceride in the plasma decreased markedly, and the unesterified cholesterol and phosphatidylcholine in the plasma also decreased, but the plasma cholesteryl ester changed only slightly (Fig. 1). Most of the decrease in lipid concentration occurred in the fraction of $d < 1.006$ g/ml (Table II), but the concentra-

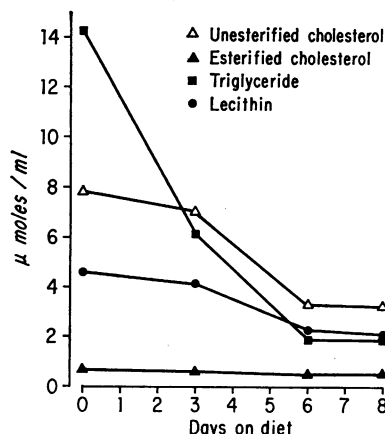


FIGURE 1 Effect of low fat-high carbohydrate diet on plasma lipids of A. A.

³The patient was under the care of Dr. S. Börsting, Kristiansund Hospital, Kristiansund, Norway, who performed the dietary experiments.

tions of triglyceride, unesterified cholesterol, phosphatidylcholine, sphingomyelin, and protein in the LDL also decreased. On the other hand, LDL cholesteryl ester appeared to increase. The unesterified cholesterol and phosphatidylcholine of the HDL also increased, but the cholesteryl ester decreased.

Control samples used for comparison of lipid:protein ratios were prepared from the plasma of six normal females, ages 22-35 yr, who were taking no medication. Data concerning their plasma lipid concentrations have already been reported (6). Plasma from the two patients and from the six normal controls was prepared and fractionated by preparative ultracentrifugation as described previously (6). Control samples for the analytical ultracentrifugation studies were prepared from 16 other normal females, ages 35-49 yr. These data have been described elsewhere (8).

LDL were subfractionated by gel filtration on 2% agarose gel (Bio-Gel A 50m, 100-200 mesh; Bio-Rad Labs, Richmond, Calif.) at 4-6°C by reverse flow filtration through 2.5 × 90 cm columns. The buffer was 0.01 M Tris-HCl-0.14 N NaCl-0.001 M EDTA, pH 7.4; the rate of flow was 10 ml/hr; and the volume of effluent collected per fraction was 5 ml. HDL were subfractionated by gel filtration on 4.5 × 150 cm or K9 (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) columns of Sephadex G200 (9). Lipoprotein fractions were concentrated by ultrafiltration through Diaflo filters (Amicon Corp., Lexington, Mass.).

Analytical ultracentrifugation of the plasma lipoproteins was performed by the method of Lindgren, Freeman, and Ewing, and graphic representations of lipoprotein distributions were prepared by their computer procedures (8). To facilitate comparison of shapes and flotation ranges of normal and abnormal lipoproteins, the patients' lipoprotein distributions were plotted to correspond with concentration values for the same density classes of lipoproteins in the normal control sample. Corrected flotation rates for LDL, described by S_r^0 values, are rates in Svedberg units for lipoproteins in a medium of d 1.063 g/ml (NaCl, 26°C, 52,640 rpm). Flotation rates for HDL, described by $F_{1.00}$ values, are rates in Svedbergs for lipoproteins in a medium of 1.20 g/ml (NaCl-NaBr, 26°C, 52,640 rpm). Both are corrected for concentration and Johnston-Ogston effects.

TABLE II
Effect of Low Fat Diet on Plasma Lipoprotein Lipids and Protein of A. A.

Lipoproteins	UC*	CE	PC	S	TG	Protein
d < 1.006						
Before diet	4.86	0.55	2.70	0.27	13.35	0.60
After diet	0.90	0.35	0.57	0.07	1.42	0.29
d 1.006-1.019						
Before diet	0.22	0.01	0.17	0.02	0.20	0.09
After diet	0.12	0.03	0.09	0.01	0.12	0.07
d 1.019-1.063						
Before diet	2.00	0.03	1.30	0.16	0.48	0.41
After diet	0.95	0.07	0.54	0.08	0.23	0.24
d > 1.063						
Before diet	0.54	0.06	0.47	0.07	0.25	
After diet	0.94	0.03	0.68	0.10	0.22	

* UC = unesterified cholesterol; CE = cholesteryl ester; PC = phosphatidylcholine; S = sphingomyelin; TG = triglyceride.

Electrophoresis was performed on paper according to the method of Lees and Hatch (10) or on 0.6% agarose (Bio-Rad Labs) as described by Noble (11). Lipoproteins were stained with Oil Red O, Sudan Black, or Amido Schwartz. In some experiments lipoprotein unesterified cholesterol was labeled with cholesterol-4-¹⁴C (Amersham/Searle Corp., Des Plaines, Ill.) as described by Avigan (12). After electrophoresis the paper or agarose gel was cut into 0.5-cm segments, and the radioactivity present in each segment was measured (1).

Immunodiffusion was performed according to the Ouchterlony technique (13). The anti- α - and anti- β -lipoprotein sera were purchased from Behringwerke, A. G. Marburg-Lahn, Germany.

Lipid analyses were performed as described previously (9). All analyses were done in duplicate or triplicate, and the values were averaged. The results of the measurements of cholesteryl ester are expressed as μ moles cholesterol/ml plasma or mg protein; the results of the measurements of phosphatidylcholine and sphingomyelin are expressed as μ moles lipid phosphorus, and the results of the measurements of triglyceride are expressed as μ moles triglyceride glycerol. Protein was analyzed by the method of Lowry as modified by Gustafson, Alaupovic, and Furman (14).

RESULTS

Low density lipoproteins. The patients' LDL of d 1.019–1.063 g/ml were unusual in that they were markedly heterogeneous in size, flotation distribution in the analytical ultracentrifuge, and composition. Some of the LDL apparently were so large that solutions containing them were visibly turbid. This turbidity could be readily observed in the region of the preparative tube associated with normal LDL after overnight ultracentrifugation of the native plasma. Fig. 2 illustrates this turbidity in the lower region of the preparative tube, just above the boundary of sedimenting plasma proteins.

The lipoprotein fraction of d 1.019–1.063 g/ml isolated from the plasma by preparative ultracentrifugation was also turbid. Additional evidence for the presence of large particles was obtained by filtering the lipoproteins of d 1.019–1.063 g/ml through columns containing beads of 2% agarose gel (Fig. 3). Large lipoproteins emerged at the void volume, whereas smaller lipoproteins emerged in the same general position as normal LDL of the same density.

The flotation distribution obtained upon analytical ultracentrifugation of the patients' LDL of d 1.019–1.063 g/ml (Fig. 4) was abnormal. The range of flotation rates (S_r^0 5–50) was much broader than that (S_r^0 0–12) of normal lipoproteins of the corresponding density range. In the experiment shown in Fig. 4, only 37% of the LDL appeared in the S_r^0 0–12 fraction; the rest had flotation rates of S_r^0 and greater. Note that despite the abnormal ultracentrifugal distribution, the flotation rate of the major LDL peak (mean rate S_r^0 9.4) was only somewhat greater than that of normal LDL (mean rate S_r^0 7.3).

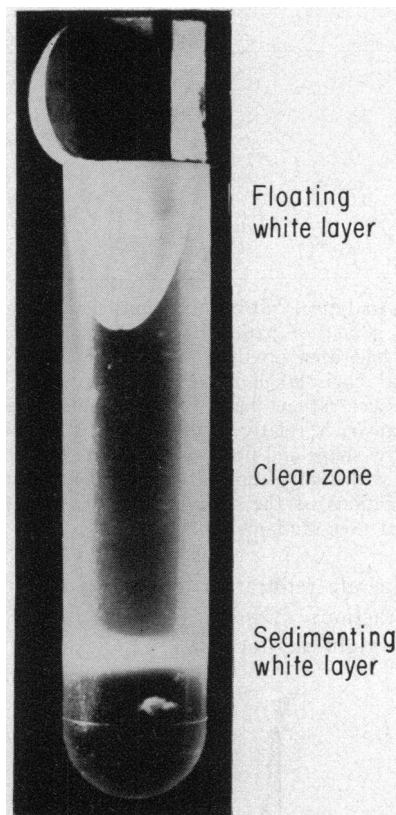


FIGURE 2 Preparative ultracentrifugation of plasma of A. A. Plasma obtained before A. A. was placed on low fat-high carbohydrate diet, was centrifuged at its native density in a Spinco 40.3 rotor at 40,000 rpm for 16 hr at 8°C. Similar sedimenting white layer obtained after diet.

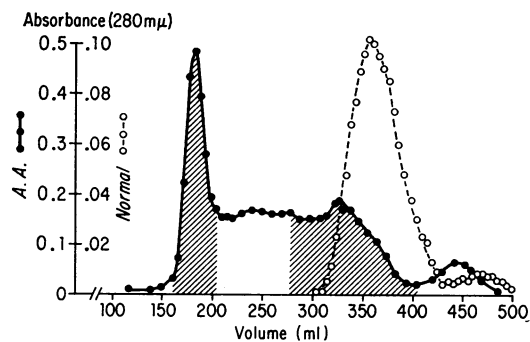


FIGURE 3 Filtration of LDL (d 1.019–1.063 g/ml) of A. A. through Bio-Gel A 50 m. 9 ml lipoprotein solution corresponding to approximately 54 ml plasma applied to column. Subfractions corresponding to the shaded areas were subsequently rechromatographed (see Figs. 5 and 6). Note that the large molecular weight subfraction emerged as a turbid solution at the void volume and that the associated "absorbance" at 280 $m\mu$ was mainly caused by light scattering. The position of normal lipoproteins of d 1.019–1.063 g/ml also is shown in the figure.

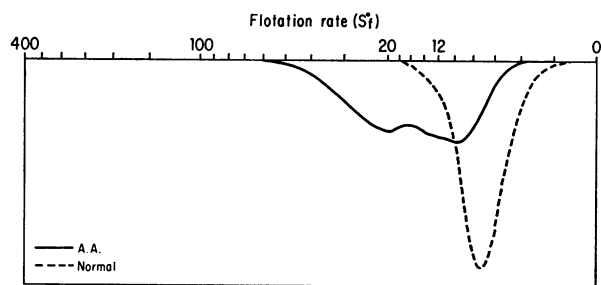


FIGURE 4 Analytical ultracentrifugation of the LDL (d 1.019–1.063 g/ml) of patient A. A. Plasma obtained after the patient had been on the low fat diet for 8 days. Data from normal LDL taken from a previous study (8). Note that the concentrations have been normalized so that the ordinate scale is a relative one only, thus permitting comparison of the shape and flotation range of the patient's LDL distribution with that of normal LDL, but not comparison of the concentrations of the abnormal and normal lipoproteins in the plasma (see Methods).

Upon separate refiltration on 2% agarose of the two LDL subfractions (shaded areas in Fig. 3) a large molecular weight subfraction (Fig. 5 A) and a smaller

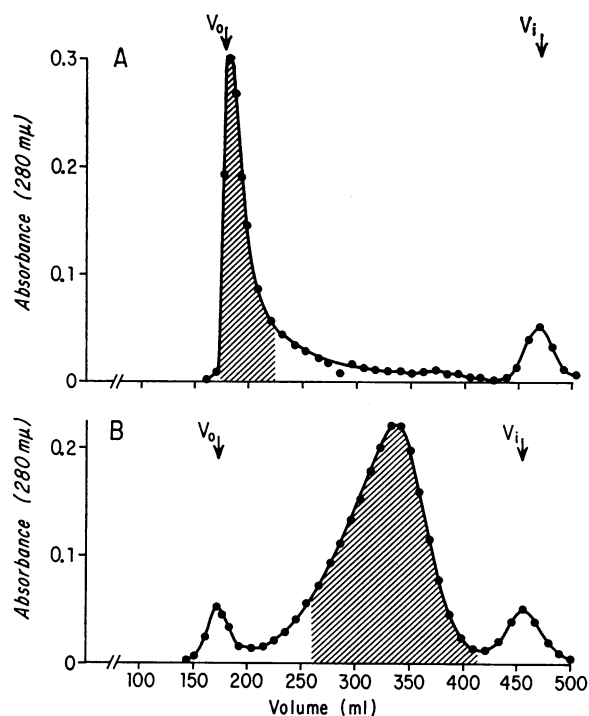


FIGURE 5 Refiltration of subfractions of the LDL of A. A. through separate columns of Bio-Gel A 50 m. Arrows indicate void (V_o) and inner (V_i) volumes of the columns. (A) Large molecular weight subfraction from experiment shown in Fig. 3 refiltered once. Lipoproteins corresponding to shaded area taken for further analysis. (B) Small molecular weight subfraction from experiment shown in Fig. 3 refiltered twice. Lipoproteins corresponding to shaded area taken for further analysis.

molecular weight subfraction (Fig. 5 B) were again obtained. In the large molecular weight subfraction there was little detectable material corresponding to the smaller molecular weight subfraction, whereas in the latter a small amount of large molecular weight material was detected even after two successive refiltrations. Both subfractions yielded some material, not examined further, that emerged with the inner volume of the columns.

Analytical ultracentrifugation of the two subfractions produced the results shown in Fig. 6. The larger molecular weight subfraction consisted of heterogeneous material which floated in the range of S_r^o 35–300, peaks being apparent at approximately S_r^o 82, S_r^o 134, and S_r^o 200. Note that the presence of the large amount of material of $S_r^o > 50$ would not have been predicted from the experiment shown in Fig. 4. The apparent discrepancy between the two experiments probably depends on the difference between the concentrations of large molecular weight LDL. The amount of the latter present in the experiment shown in Fig. 4 was probably too small to be detected in view of its low protein content. To obtain the results shown in Fig. 6, the large molecular weight LDL subfraction had to be concentrated many fold. The smaller molecular weight subfraction consisted of material in the range of S_r^o 2.5–35, 61% being in the range of S_r^o 0–12 and 39% being in the range of S_r^o 12–20; there was a nearly symmetrical peak at S_r^o 9.8. This flotation rate is slightly higher than that of the main peak (S_r^o 9.4) of the isolated LDL fraction shown in Fig. 4, which in turn is higher than that of the main LDL peak (S_r^o 7.4) obtained by centrifuging whole plasma. We have no explanation for this apparent shift.

Electrophoresis on agarose of the small molecular weight subfraction yielded a single band slightly behind the position normally occupied by LDL, whereas the large molecular weight subfraction remained at the application site. Although further experiments with the LDL of A. A. were not performed, experiments with lipoproteins from A. R. suggest that the failure of the large molecular weight subfraction to migrate was prob-

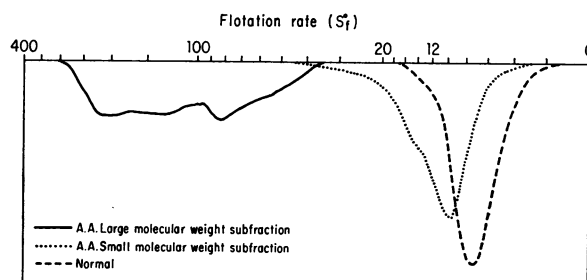


FIGURE 6 Analytical ultracentrifugation of normal LDL and subfractions of d 1.019–1.063 g/ml from the plasma of A. A. Subfractions correspond to the shaded areas shown in Fig. 5.

ably due to its large size. Thus, upon electrophoresis on paper the lipoproteins of d 1.019–1.063 g/ml of A. R. yielded only a single band irrespective of whether stained with Oil Red O or traced by labeled cholesterol (Fig. 7). This band had the same mobility as the small molecular weight subfraction of A. A.

Chemical analysis of the large and small molecular weight LDL subfractions of A. A. revealed that they differed from normal lipoproteins of the same density, from unfractionated lipoproteins of d 1.019–1.063 g/ml prepared before and after A. A. was put on the diet, and from each other (Table III). Both subfractions contained abnormally large amounts of unesterified cholesterol, phosphatidylcholine, and triglyceride per mg protein. The most striking abnormalities were the high content of unesterified cholesterol and phosphatidylcholine of the large molecular weight subfraction and the high content of triglyceride of the smaller molecular weight subfraction. Note that the latter not only contained much more triglyceride than the large molecular weight subfraction but also more than twice as much cholesteryl ester.

High density lipoproteins. The HDL of three patients with familial LCAT deficiency (A. A., A. R., M. R.) have already been shown (6) to be comprised of a major high molecular weight subfraction and a minor low molecular weight subfraction. Fig. 8 suggests that in A. A. on the low fat diet there was a shift in the size distribution of the HDL of the major subfraction. When A. A. was on an ordinary diet, the major HDL peak emerged essentially with the void volume of the column of Sephadex G200, whereas that obtained when she was on the low fat diet emerged later. Note that only very small amounts of smaller molecular weight HDL were present in both cases.

The heterogeneity indicated on gel filtration was also evident on analytical ultracentrifugation. Fig. 9 shows the flotation patterns of the HDL of A. A. before and 8 days after she was put on the low fat diet. Both pat-

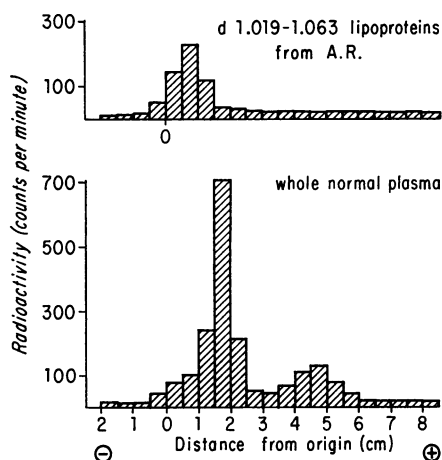


FIGURE 7 Electrophoresis on paper of LDL (d 1.019–1.063 g/ml) from A.R. and of lipoproteins in whole normal plasma. Cholesterol of lipoproteins was labeled with cholesterol-¹⁴C before electrophoresis. Origin of electrophoresis is marked 0, and ⊖ and ⊕ represent cathode and anode. The paper strips were dried and cut into segments of $\frac{1}{2}$ cm, starting at the origin. The distance of the segments from the origin is given on the abscissa, and the radioactivity of the segments is plotted on the ordinate. Note that the whole plasma sample yielded two peaks; the most anodic one corresponds to HDL or α_1 -lipoproteins, and the other corresponds to LDL or β -lipoproteins. The LDL of A. R. migrated slower than the normal LDL.

terns showed greater heterogeneity and higher mean flotation rates than normal female HDL, and these differences were accentuated in the HDL prepared when A. A. was on the diet. The HDL distribution consisted predominantly of components of $F_{1.20}$ 3.5 and greater. The postdiet sample showed a considerable amount of material in the $F_{1.20}$ 9–20 range not detected in the prediet distribution. In addition, the flotation rate of the major peak of the postdiet sample was $F_{1.20}$ 7.6, whereas that of the prediet sample was $F_{1.20}$ 4.8. In contrast, an average of 52% of the material of the normal HDL dis-

TABLE III
Lipid: Protein Ratios of d 1.019–1.063 LDL

	UC*	CE	PC	S	TG
	<i>μmoles lipid/mg protein</i>				
Normal controls (5)†	1.44 ± 0.105	1.77 ± 0.359	0.482 ± 0.053	0.261 ± 0.027	0.114 ± 0.014
A. A. before diet					
Whole fraction	4.91	0.06	3.14	0.38	0.93
A. A. after diet					
Whole fraction	3.95	0.31	2.24	0.32	0.91
Large molecular weight subfraction	11.05	0.22	4.96	0.62	0.48
Small molecular weight subfraction	2.31	0.59	1.40	0.18	1.44

* Abbreviations as in Table II.

† Values given in μ moles lipid/mg protein \pm sd.

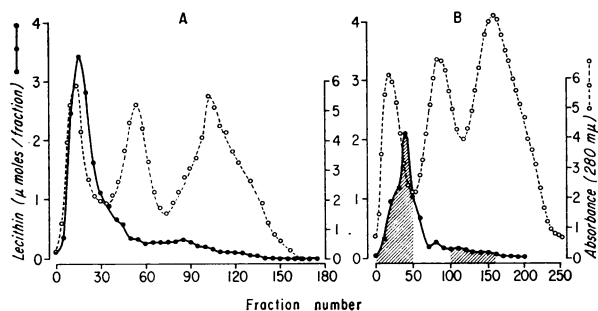


FIGURE 8 Gel filtration of the plasma proteins of $d > 1.063$ g/ml of A. A. (A) Plasma obtained when she was on her usual diet. Proteins of $d > 1.063$ g/ml from approximately 200 ml plasma filtered through "K9" column of Sephadex G200. Approximate volume per fraction of effluent was 20 ml. (B) Plasma obtained after 8 days on low fat-high carbohydrate diet. Proteins of $d > 1.063$ g/ml from 200 ml plasma filtered through Sephadex G200 as above. Approximate volume per fraction 15 ml. In both experiments the first absorbance peak emerged at the void volume of the column. Shaded areas correspond to subfractions refiltered in experiments shown in Fig. 10.

tribution was in the range of $F_{1.20}$ 0-3.5, the rest being in the range of $F_{1.20}$ 3.5-9, and the flotation rate of the major peak was $F_{1.20}$ 2.3.

When the HDL subfractions from the experiment shown in Fig. 8 B were purified by ultracentrifugation and chromatography on hydroxylapatite, and subsequently rechromatographed on columns of Sephadex G200, the results shown in Fig. 10 were obtained. Again, the large molecular weight subfraction emerged slightly after the void volume. Analytical ultracentrifugation of the two HDL subfractions from A. A. yielded the results shown in Fig. 11. The distribution of the large molecular weight HDL subfraction was similar to that of the parent HDL. 60% was in the range of $F_{1.20}$ 0-9, and the rest was in the range of $F_{1.20}$ 9-20. The rate of flotation of the major peak was $F_{1.20}$ 7.3, which compared favorably with that of the parent HDL. The small molecular weight HDL subfraction showed a distribution within the $F_{1.20}$ 0-3.5 range, with a major peak of $F_{1.20}$ 1.4.

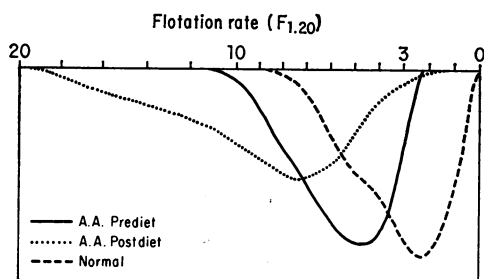


FIGURE 9 Analytical ultracentrifugation of the HDL of normal subjects and of A. A. before and after diet.

This material was not detected during analysis of the parent HDL, probably because of its extremely low concentration in the patient's plasma.

Fig. 12 shows the behavior of the two HDL subfractions on agarose gel electrophoresis. The small molecular weight subfraction had a mobility similar to that of prealbumin, whereas the large molecular weight subfraction migrated as a broad band in the α_1 - α_2 region. Note that electrophoresis on paper, agarose, or cellulose acetate of the corresponding subfraction of other patients with familial LCAT deficiency (on an ordinary diet) has always yielded a rather narrow band in the α_2 region.⁸ Both HDL subfractions from A. A. formed precipitin lines with anti- α -lipoprotein serum when tested by immunodiffusion. No reaction was detected with anti- β -lipoprotein serum.

Table IV compares the lipid:protein ratios of normal HDL, the unfractionated HDL of A. A. before and after the diet, and the two HDL subfractions obtained as shown in Fig. 10. The large molecular weight subfraction contained about 12 times as much unesterified cho-

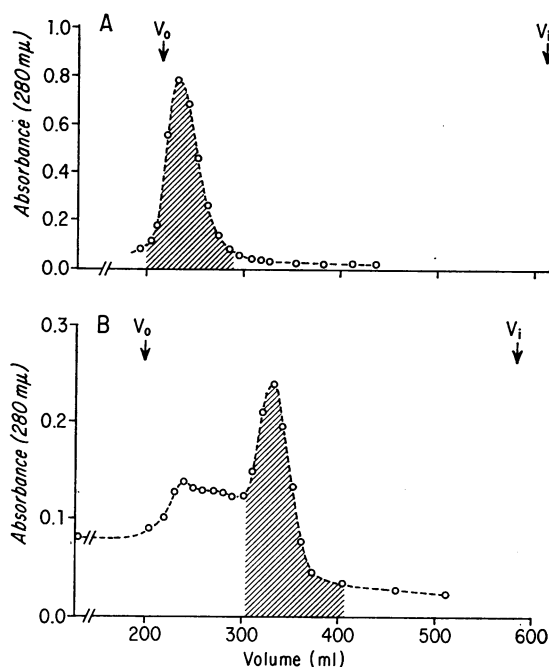


FIGURE 10 Refiltration of HDL subfractions from the plasma of A. A. on separate columns of Sephadex G200. Subfractions corresponding to shaded areas in Fig. 8 B were purified by preparative ultracentrifugation at d 1.25 g/ml, washed twice by ultracentrifugation, and subsequently filtered through hydroxylapatite (6). 10-ml solutions of the purified HDL were applied to 2.5×115 cm columns of Sephadex G200. Arrows indicate void (V_0) and inner (V_i) volumes of the columns. (A) Large molecular weight subfraction. (B) Small molecular weight subfraction.

⁸ Norum, K. R. Unpublished observations.

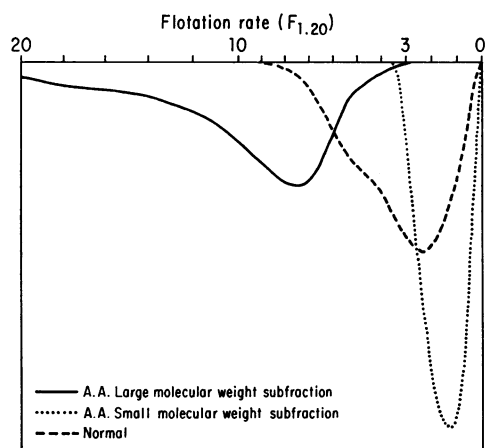


FIGURE 11 Analytical ultracentrifugation of normal HDL and HDL subfractions of the plasma of A. A. Subfractions prepared by gel filtration, preparative ultracentrifugation, and chromatography on hydroxylapatite (see Figs. 8 and 10).

lesterol and 5 times as much phosphatidylcholine as normal HDL, but the smaller molecular weight subfraction contained almost the same amount of these lipids per mg protein as normal HDL. Similar values have been obtained for the small molecular weight HDL of another patient with familial LCAT deficiency.⁴ Note that the triglyceride content of the smaller molecular weight subfraction was abnormally high, whereas the content of cholesteryl ester was abnormally low. On the other hand, the data in the table indicate that about 47% of the total weight of the subfraction was lipid. This figure corresponds closely with that found in normal HDL (15).

⁴ Unpublished experiments (Glomset, J. A., K. R. Norum, and W. King) have yielded the following values for the small molecular weight HDL of M. R., the sister of A. R. (1, 2): μ moles unesterified cholesterol/mg protein = 0.27; μ moles phosphatidylcholine/mg protein = 0.40.

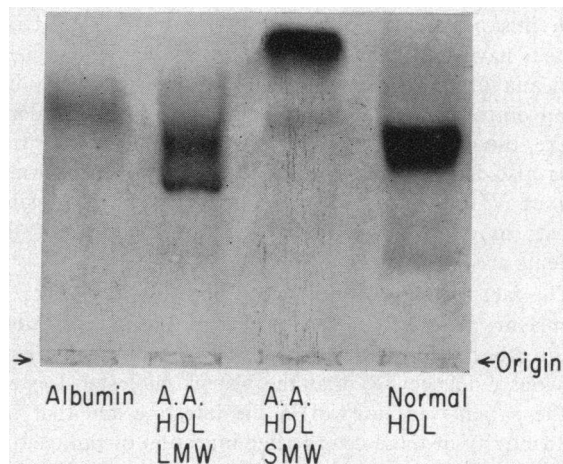


FIGURE 12 Electrophoresis on 0.6% agarose of large (LMW) and small (SMW) molecular weight subfractions of the HDL of A. A. Subfractions obtained as described in Fig. 10. Normal human HDL and albumin shown for comparison. Proteins were stained with Amido Schwarz.

DISCUSSION

The findings reported here primarily concern the properties of the abnormal LDL and HDL of two patients with familial LCAT deficiency. Most of the experiments were done with plasma obtained from patient A. A. after she had been on an essentially fat-free diet, and the changes which occurred during the dietary period deserve some initial comment. The hyperlipemia and plasma triglycerides of A. A. decreased markedly. Although we made no direct measurements of the concentrations of circulating chylomicrons, these changes suggest that they were initially high and decreased in the absence of dietary fat, i.e., that chylomicron clearance in A. A. may be defective despite the fact that lipolytic activity in her plasma is normal after injection of 1 mg heparin/kg body weight (4). The low concentration of LDL protein compared with that of normal subjects (16) is compatible

TABLE IV
Lipid: Protein Ratios of HDL

	UC*	CE	PC	S	TG
	μ moles lipid/mg protein				
Normal controls (6)†	0.268 \pm 0.057	0.703 \pm 0.102	0.475 \pm 0.046	0.088 \pm 0.016	0.025 \pm 0.005
A. A. before diet					
Whole fraction	1.86	0.029	1.48	0.22	0.39
A. A. after diet					
Whole fraction	1.92	0.014	1.50	0.23	0.18
Large molecular weight subfraction	3.33	—§	2.29	0.33	0.076
Small molecular weight subfraction	0.30	—§	0.67	0.04	0.089

* Abbreviations as in Table II.

† Values given in μ moles lipid/mg protein \pm SD.

§ Amount present in sample below reliable limit of detection.

with this possibility, since low concentrations of LDL protein have been found in patients with type I hyperlipidemia (16). However, since A. A. consumed the diet as an outpatient and we cannot be certain of isocaloric intake, the decreased lipemia and triglyceride concentrations also may have been caused by diminished formation of VLDL. Further investigations of the possible defects in chylomicron and VLDL metabolism of the patients are in progress.

The fact that the concentration of cholesteryl ester in the plasma decreased more slowly than the concentrations of the other lipids measured (Fig. 1) is compatible with previous evidence (1) that the plasma cholesteryl esters of the patients are formed in the intestine and that the radioactivity of these esters after ingestion of radioactive unesterified cholesterol, decreases more slowly than would be expected for the chylomicron cholesteryl esters of normal subjects (5).

Two other changes promoted by the diet are noteworthy. First, the concentration of LDL decreased, which supports the previous observation (6) that the concentrations of the lipoproteins of $d < 1.006$ g/ml and the LDL of the patients seem to be directly correlated. Second, the concentration of HDL increased, which suggests that an inverse correlation exists between the concentrations of $d < 1.006$ g/ml lipoproteins and the HDL. Similar correlations are present among the corresponding lipoproteins of normal subjects (15, 17).

We have previously shown (6) that the lipid compositions of the LDL and HDL of patients with familial LCAT deficiency are abnormal and that the HDL are heterogeneous with respect to size. Our present data show that the LDL also are heterogeneous with respect to size; a particularly striking finding is the presence of unusually large LDL with densities in the range of d 1.019–1.063 g/ml. The existence of these large particles *in vivo* is supported by the observation of turbidity in the VLDL-free infranatant after as mild a procedure as overnight ultracentrifugation of native plasma (Fig. 2) as well as by the gel filtration experiments (Figs. 3 and 5A). Both types of experiment suggest that the large LDL are probably not artifacts. However, some large particles may have been formed *in vitro* by the aggregation of smaller subunits since material which emerges from agarose columns with the void volume was observed even after two successive refiltrations of the smaller molecular weight LDL subfraction (Fig. 5). Also, further aggregation of the large particles *in vitro* has not been excluded and may partially explain the presence of rapidly floating material in the experiment shown in Fig. 6. The wide range of flotation rates (S_r ° 35–300) suggests a comparably wide distribution of particle sizes. This suggestion has been confirmed by electron micros-

copy (7) which has shown that most of the particles are in the range of 900–1200 Å in diameter.

The relative amount of the large molecular weight LDL subfraction can be calculated from the data in Fig. 3 and Tables II and III. About 5% of the protein, 20% of the unesterified cholesterol, and 15% of the phosphatidylcholine of the lipoproteins of d 1.019–1.063 g/ml appear to be present in this fraction. The ratio of unesterified cholesterol and phosphatidylcholine to protein is 8–10 times that of normal LDL, but ratios that are nearly as high can be calculated from data reported for the subfractions of normal VLDL (14). This is of interest because of the possibility that the large molecular weight LDL may be derived from VLDL. Although the peptide of the large molecular weight LDL subfraction remains to be identified, its ability to stabilize mixtures of unesterified cholesterol and phospholipid which resemble those of normal LDL is worth noting: the ratio of unesterified cholesterol to phosphatidylcholine is only somewhat lower than that in normal LDL, and the ratio of unesterified cholesterol to phosphatidylcholine + sphingomyelin is normal.

Ratios of unesterified cholesterol to protein and of phosphatidylcholine to protein of the smaller molecular weight LDL subfraction are much closer to those of normal LDL than are those of the large molecular weight subfraction. However, the contents of cholesteryl ester and triglyceride are markedly aberrant. Comparison of the contents of nonpolar "core" lipids of the small molecular weight LDL (cholesteryl ester + triglyceride = $0.59 + 1.44 = 2.03$ μ moles per mg protein) with those of the "core" lipids of normal LDL (cholesteryl ester + triglyceride = $1.77 + 0.11 = 1.88$ μ moles per mg protein) suggests an inverse relationship between LDL cholesteryl ester and triglyceride. The abnormally high ratio of triglyceride to cholesteryl ester in the small molecular weight LDL of A. A. probably can be ascribed to LCAT deficiency. Studies of the unfractionated lipoproteins of d 1.019–1.063 g/ml in A. A., A. R., and M. R. (6) have already shown that the cholesteryl ester content is increased, and the triglyceride content decreased by incubation with normal HDL previously incubated with LCAT.

Patients with obstructive jaundice have low plasma LCAT activity (18) and an abnormal LDL characterized by a very low content of cholesteryl ester and high contents of unesterified cholesterol and phospholipid (19–21). The possible relationship between this abnormal lipoprotein and the two plasma LDL subfractions found in familial LCAT deficiency needs to be clarified.

We have shown earlier (6) that the HDL of patients with familial LCAT deficiency are heterogeneous with respect to size and lipid composition. Our present findings indicate that the low concentration of HDL in the

plasma of A. A. was somewhat increased by the low fat diet. The concentration of HDL protein before the diet was about 15% of normal calculated on the basis of total concentration of HDL unesterified cholesterol (Table I; 6) and the ratio of unesterified cholesterol to protein (Table IV). The concentration of HDL protein calculated similarly after the diet was about 25% of normal. The large molecular weight HDL prepared after A. A. had been on the low fat diet appear to be smaller in size than the corresponding fraction prepared before she was put on the diet. This is of particular importance in view of the appearance of the large molecular weight HDL in electron micrographs (7). Thus, discs 150–200 Å in diameter are seen in stacks of varying length. Since the diameters of the discs are only slightly smaller than those of normal LDL and since our findings indicate that the HDL emerge from columns of Sephadex G200 or 2% agarose gel⁸ slightly after normal LDL, it seems likely that the stacking phenomenon is an artifact of the negative staining procedure and that the large molecular weight HDL probably circulate in the blood as single discs.

It can be calculated from the data in Table IV that about 70% of the large molecular weight HDL is lipid, whereas about 50% of normal (unfractionated) HDL is lipid. This high lipid content along with the large size of the HDL presumably explains the relatively high flotation rates observed in the experiment shown in Fig. 11. They may also account for the slower electrophoretic mobility (Fig. 12), although it is possible that the peptide of the large molecular weight HDL is abnormal. It should be noted that the immunodiffusion experiments suggest that at least some of the peptide is similar to that of normal HDL.

A striking abnormality of the small molecular weight HDL found in our present studies was its electrophoretic mobility, which was similar to that of prealbumin. Since we did not measure the content of unesterified fatty acid in the small molecular weight HDL fraction, we cannot rule out the possibility that the abnormally rapid mobility may have been caused by a high fatty acid content. However, none of the other lipoprotein subfractions had a mobility that was abnormally rapid, and there is no reason to believe that untoward lipolysis occurred either before or after the plasma was prepared. Whether a "prealbumin" HDL counterpart exists in normal plasma remains to be established. The possibility that such a fraction does exist is supported by the experiments of Dietrich (22) who fractionated normal plasma by starch block electrophoresis and found some material that stained with Sudan Black in the prealbumin

⁸ Norum, K. R., J. A. Glomset, A. V. Nichols, and T. Forte. Unpublished experiments.

region. It seems unlikely that the small molecular weight HDL are related to the tryptophan-rich prealbumin or α_1 -glycoprotein studied by others (23, 24) since the HDL subfraction floats at a density of 1.25 g/ml and forms precipitin line with anti- α_1 -lipoprotein serum.

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