Characterization of lipoprotein in a kindred with familial hypercholesterolemia

Wolfgang Patsch, Richard Ostlund, Ilmar Kuisk, Richard Levy, and Gustav Schonfeld

Lipid Research Center, Departments of Preventive Medicine and Medicine, Washington University, St. Louis, MO

Abstract To study possible consequences of decreased numbers of cellular LDL receptors on plasma lipoproteins, we characterized the low density and high density lipoproteins in fasting plasmas of a kindred with receptor-defective hypercholesterolemia. The flotation rates ($S^0_{f\,1.063}$) of the major LDL populations, determined by analytic ultracentrifugation, ranged from 4.7 to 7.4; densities (d_{20}) ranged from 1.0348 to 1.0402 g/ml, and minimum molecular weights ranged from 2.5 to 3.5×10^6. On rate zonal ultracentrifugation, the major populations of LDL isolated from individual members of this kindred could be divided into fast and slow floating varieties. Fast floating LDL had a molecular weight $>3.15 \times 10^6$, slow floating, $<2.85 \times 10^6$. Both fast and slow floating LDL were found among affected members of the kindred. From molecular weights and chemical compositions, the numbers of molecules of lipid components per LDL particle were calculated. Numbers of phospholipid, free cholesterol, and cholesteryl ester molecules were each strongly correlated with the molecular weights of the LDL particles. Thus, the differences in mass of LDL resulted from alterations primarily of the phospholipid, free cholesterol, and cholesteryl ester contents per particle, whereas the amounts of protein and triglyceride per particle were relatively constant. An important and consistent finding of this study is that the LDL of members of the kindred affected with familial hypercholesterolemia (FH) differed from LDL of unaffected members by containing more molecules of cholesteryl ester and less triglyceride, even when LDL were matched for molecular weight. Thus, FH per se affected the core lipid composition of LDL. The mechanisms responsible for the change are unknown. Analysis of the distribution of LDL masses in this pedigree is compatible with genetic factors having some influence on LDL mass, but the great overlap of LDL mass between affected and nonaffected subjects implies that, whatever genetic or other factors limit LDL mass, these factors remain operative in FH. "Hepatic" apoB (B-100, B-74, and B-26) comprised 96% of the protein moiety in all subjects, while "intestinal" apoB (B-48) was not found in any of the LDL preparations. Therefore, LDL of both normal and affected members probably is derived from hepatic lipoproteins. HDL-cholesterol was low in children with FH, but it was also low in an unaffected child and there was no correlation between the presence or absence of HDL_2 and FH status. There appeared to be a tendency toward lower LDL-cholesterol in those affected subjects whose plasma contained HDL_2. However, this suggestive inverse relationship between LDL and HDL_2 needs confirmation.—**Patsch, W., R. Ostlund, I. Kuisk, R. Levy, and G. Schonfeld.** Characterization of lipoprotein in a kindred with familial hypercholesterolemia. *J. Lipid Res.* 1982. **23:** 1196–1205.

Supplementary key words low density lipoprotein • high density lipoprotein • apoB, E receptors

The familial hypercholesterolemias (FH) are distinct disorders characterized clinically by xanthomata, arcus corneae, and premature atherosclerosis. The diseases are inherited as autosomal dominant traits that demonstrate gene dosage effects. In individuals who inherit two mutant genes (homozygous) the diseases are more severe than in subjects who inherit only one mutant gene (heterozygous) (1). Biochemical markers of this disorder are deficiencies in cell surface receptors, which recognize apoproteins B and E and which mediate cellular uptake of LDL and thus regulate endogenous cholesterol synthesis (2, 3). Malfunction of the LDL (apoB, E) receptor pathway leads to accumulation of LDL in the plasma of these patients (2, 3). It has been suggested that increased residence time of LDL in the plasma of affected individuals may lead to changes of this lipoprotein class allowing them to be taken up by macrophages. This "scavenger pathway" (4) is believed to be related to the development of lipid foam cells and atherosclerosis. Although the significance of prolonged residence time for LDL-cell interactions is still largely speculative, workers have reported what they considered to be structural changes of LDL in FH. LDL has been found to be cholesterol-enriched and triglyceride-poor (5, 6), and in one study, the peak flotation rate of FH-LDL has been found to be higher than in normals (6), implying decreased density, increased size, or both. However, these results are difficult to assess, since there is considerable heterogeneity of LDL in normal subjects, although size, flotation rate, and composition

Abbreviations: FH, familial hypercholesterolemia; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; PL, phospholipid; TG, triglyceride; CE, cholesteryl ester; FC, free cholesterol; chol, cholesterol; TMU, tetramethylurea; SDS, sodium dodecyl sulfate; S^0_f, flotation rate in aqueous NaBr solution of density 1.063 g/ml at 20°C, corrected for concentration dependence, expressed in Svedberg units (10^{-13}s); apoB, major apoprotein of VLDL and LDL.

within any one subject are remarkably constant (7). Apart from possible abnormalities in LDL, other lipoprotein classes could be abnormal too in FH. Kwiterovich, Fredrickson, and Levy (8) found significantly lower levels of HDL-cholesterol in children with FH when compared with their unaffected siblings, and HDL-cholesterol in their parents also was lower than in unaffected controls. Low HDL-cholesterol levels have also been reported in a recent study on hypercholesterolemic patients in South Africa (9). In order to assess any possible consequences of altered LDL receptor function in FH on plasma lipoproteins, the levels and some physicochemical properties of LDL and HDL were studied in the affected members of a kindred with FH. Unaffected family members similarly studied, served as controls.

METHODS

After 12–14 hr of fasting blood was collected in tubes containing 1 mg/ml EDTA and plasma was separated by low speed centrifugation. Na-azide was added (1 mg/ml of plasma) and analyses were performed within 72 hr. Plasma triglyceride and cholesterol levels, and lipoprotein cholesterol were determined by standard procedures (10). Plasma lipoproteins were further analyzed by zonal ultracentrifugation. To separate VLDL and LDL, zonal ultracentrifugation was carried out at 42,000 rpm at 15°C for 140 min using a Ti-14 rotor and a Beckman L2-65B ultracentrifuge. A linear density gradient spanning the density of 1.00 to 1.30 g/ml was used (11). The zonal rotor effluent was monitored continuously at 280 nm prior to collecting 10-ml fractions. Appropriate fractions were pooled and concentrated using a Millipore cell equipped with a Pellicon membrane (PTGC 04710) and operated at 5–12 psi. In the experiments conducted to assess the flotation properties of LDL in different family members, LDL of one family member (subject III_2) was isolated by preparative ultracentrifugation between density 1.025 and 1.050 g/ml (12), and labeled with iodine monochloride (13, 14) to a specific activity of ~100 cpm/ng of protein. Labeling did not affect the flotation rates within the limits of detectability when compared with native LDL (**Fig. 1**). This figure also demonstrates that zonal flotation rates were independent of the amount of LDL loaded into the rotor over the range of 100 mg LDL (native plasma) to 10 μg (^{125}I-labeled LDL).

Density of LDL was determined by equilibrium centrifugation in the zonal rotor for 43 hr at 42,000 rpm and 20°C, using a linear NaBr gradient in the density range of 1.00 to 1.10 g/ml (15). Densities in effluent fractions were measured by a precision densimeter

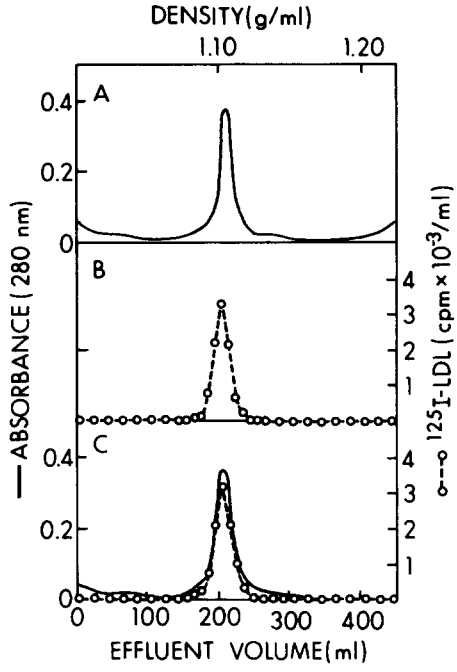

Fig. 1. Elution profiles of the LDL-region on zonal ultracentrifugation profiles of 4 ml of hypercholesterolemic plasma, A; ~10 μg ^{125}I-labeled LDL isolated from the same plasma, B; 4 ml of plasma plus ~10 μg ^{125}I-labeled LDL, C.

DMA-45 (Mettler-Paar, Graz, Austria). Flotation rate measurements were performed in a Beckman Model E ultracentrifuge using an AN-D rotor, Schlieren optics, and double sector cells. Measurements were done at 52,000 rpm, 20°C, and a density of 1.063 g/ml (NaBr). Solutions contained 1 mM NaN_3, 0.3 mM EDTA, pH 7.6. Lipoprotein concentration was 5 mg/ml. Flotation rates were corrected for concentration (16). The minimal molecular weight of LDL was calculated by the formula (17)

$$\text{Molecular weight} = 9 \cdot (2)^{1/2} \cdot \frac{N \cdot \pi}{\bar{v}} \frac{\eta \cdot S_f^0 \cdot \bar{v}^{3/2}}{1 - \bar{v}\rho}$$

where N is Avogadro's number, η the viscosity of the buffer, S_f^0 the flotation coefficient corrected for concentration dependence, $\bar{v}$ the partial specific volume, which was calculated from the inverse of the density obtained in zonal equilibrium ultracentrifugation (18), and ρ the density of the solvent (17).

Lipoprotein fractions were analyzed for protein (19) using albumin as standard, phospholipid (20), triglyceride (Triglyceride Kit, Boehringer, Mannheim, Germany), and free and esterified cholesterol (21). (The zonal fractions analyzed are indicated in Fig. 3.) Protein concentration was multiplied by a factor of 0.77 to correct for differences between the chromgenicity of apoLDL and albumin (22, 23). The number of molecules of constituent per LDL particle was calculated

TABLE 1. Plasma lipid and lipoprotein cholesterol levels in kindred

Subject	Age	Sex	Triglyceride		Cholesterol			
			Plasma	VLDL	Plasma	VLDL	LDL	HDL
					mg/dl			
I_1	54	f	138	39	414	18	308[a]	62
I_2	57	m	76	19	204	3	133	70
I_6	65	m	146	106	177	12	115	35
I_7	59	f	175	98	536	21	496[a]	28[b]
I_8	63	m	156	64	232	12	166	49
I_9	55	m	288	149	250	65	150	35
II_1	13	m	75	31	200	9	126	51
II_2	21	f	106	57	388	12	272[a]	39
II_3	22	m	197	89	175	18	78	56
II_4	24	f	156	35	204	8	113	45
II_5	31	f	72	22	218	4	170[a]	42
II_7	33	f	59	24	327	4	258[a]	65
II_8	33	m	128	75	422	20	362[a]	35
II_9	38	m	76	36	331	11	279[a]	37
II_{10}	39	f	53	27	179	2	119	57
II_{11}	28	f	40	9	186	3	126	58
III_1	5	f	100	45	200	3	120	48
III_2	6	m	104	15	564	6	512[a]	31[b]
III_3	1	m	222	17	960	23	893[a]	23[b]
III_4	14	m	49	19	247	0	208[a]	26[b]
III_5	16	m	99	68	138	8	102	31[b]

[a] Above the 95th percentile of sex- and age-matched controls (31).
[b] Below the 5th percentile cut point of sex- and age-matched controls (31).
No subjects received any lipid-lowering drug treatment for at least 6 weeks prior to the study. Subject III_2 has been on a plasmaphoresis program for 2 years.

from the particle weight and the chemical composition by assuming a component molecular weight of 775 for PL, 387 for cholesterol, 650 for CE, and 850 for TG.

To study apoprotein composition of LDL and possible heterogeneity of apoB, blood was drawn from eight subjects of the kindred on the same date into tubes containing 1 mg/ml EDTA and chilled on ice. Plasmas were separated immediately, gentamycin sulfate (0.1 mg/ml), chloramphenicol (0.05 mg/ml), and NaN_3 (0.02%) were added; all plasma samples were processed and analyzed together. LDL was isolated without delay between densities 1.025 and 1.05 g/ml by sequential ultracentrifugation in the same 40.3 rotor, and dialyzed against 1 mM EDTA saline, pH 7.6. Aliquots were delipidated twice with 50 volumes of ethanol–diethylether 3:1 (v:v) for 20 hr at 4°C, followed by an ether wash. Electrophoresis was done in tubes and slabs using 10% polyacrylamide gels (24) and 3% polyacrylamide gels (25), respectively. In the latter, albumin, cross-linked with dimethyl suberidimate (26), and fibronectin, purified from human plasma by affinity chromatography (27), were used as molecular weight markers. The apoB content of LDL was also measured by the method of Kane et al. (28) using tetramethylurea (TMU).

HDL were isolated by zonal ultracentrifugation (11, 29). Twenty-ml fractions were collected and protein content was measured in each fraction by a modification (30) of the procedure of Lowry et al. (19).

RESULTS

The plasma lipid and lipoprotein cholesterol levels of the kindred are given in **Table 1.** Using LDL-cholesterol levels above the age- and sex-specific 95th percentiles as cut points for diagnosis (31), eight heterozygotes were identified in the kindred. The two children (III_2 and III_3) were identified earlier (32, 33) as receptor-defective homozygotes according to the criteria of Goldstein and Brown (2). The family tree is shown in **Fig. 2** and is consistent with the dominant inheritance of the disease with a gene dosage effect.

LDL's of several members of the kindred were analyzed by zonal rate flotation using the ^{125}I-labeled LDL internal standard, shown in **Fig. 3.** In all but one subject (II_{10}), LDL appeared as one major population. However, in several individuals small shoulders on the leading or trailing edges of the major peak were found, consistent with a previous report on the heterogeneity of LDL (34). The major peaks and small shoulders were reproducible on re-analysis of the same plasma samples. Two groups of major LDL populations could be distinguished in this kindred based on the elution volumes

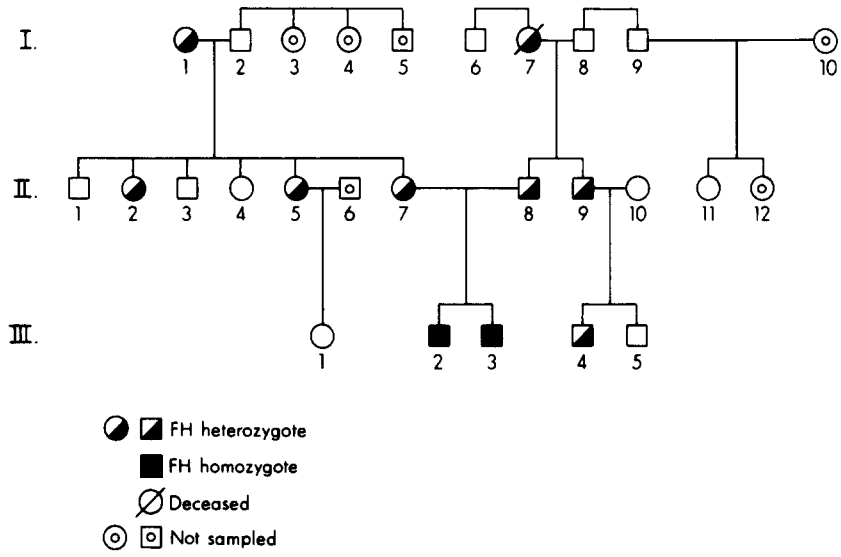

Fig. 2. Pedigree of the L-P kindred.

of LDL: a fast floating LDL population, with peaks eluting at 210 to 220 ml (which coincided with the elution volume of the ^{125}I-labeled LDL internal standard), and a slower floating population with peak elution volume of 230 to 250 ml. No LDL had peak elution volumes between 220 and 230 ml in this kindred. The LDL's of the two homozygous boys (III_2 and III_3) belonged to the faster floating species, as did the LDL's of the heterozygotes on the mother's side (I_1, II_2, II_5, and II_7). On the father's side, all heterozygotes possessed LDL of the slower floating variety (II_8, II_9, III_4). Heterozygotes on the mother's side were all female, whereas heterozygotes on the father's side were all males. The chemical compositions of these lipoproteins in terms of mass are given in **Table 2.**

To relate the observed zonal flotation behavior to molecular weight, LDL of subject II_7, representing a fast floating LDL, and LDL from subject II_8, representing a slow floating LDL, were subjected to analytical ultracentrifugation (**Fig. 4**) and equilibrium ultracentrifugation in the zonal rotor (**Fig. 5**). Flotation rates ($S^0_{f\,1.063}$) of these two LDL preparations were 7.40 and 5.05, respectively; buoyant densities (d_{20}) were 1.0348 g/ml, and 1.0374 g/ml. Minimum calculated molecular weights were 3.5×10^6, and 2.7×10^6. A third LDL preparation from a subject with endogenous hypertriglyceridemia was also analyzed. The zonal peak elution volume of this latter LDL preparation was 265 ml. $S^0_{f\,1.063}$ was 3.85, buoyant density 1.0426 g/ml, and calculated minimal molecular weight 2.25×10^6. The

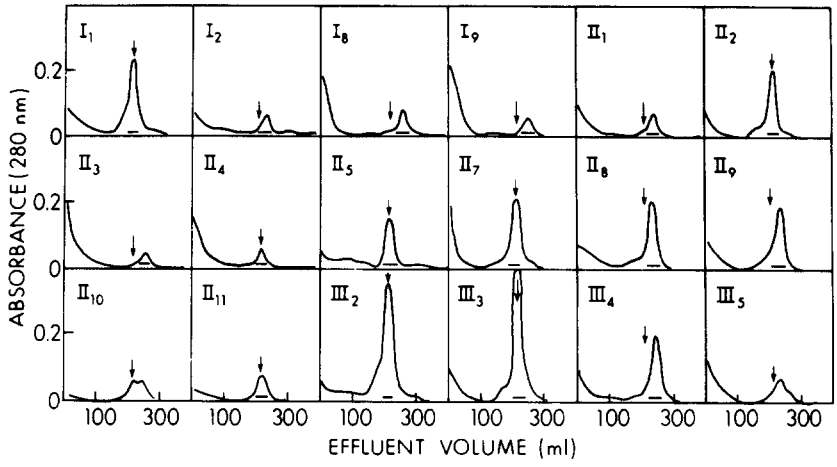

Fig. 3. Characterization of the LDL-region of the L-P kindred by zonal ultracentrifugation. Direction of flotation is from right to left. Four ml of plasma were centrifuged in the presence of 10 μg ^{125}I-labeled LDL, used as internal standard. Arrows refer to the peak of radioactivity in the effluent volume. Horizontal bars under major LDL peaks indicate fractions that were pooled for further analysis.

TABLE 2. Chemical composition of LDL in kindred

Subject	Protein	PL	TG	FC	CE
			% of mass		
Unaffected					
I_2	18.5	26.0	5.6	10.7	39.3
I_8	20.2	25.5	7.2	8.1	39.0
I_9	22.5	22.4	7.9	7.9	39.2
II_1	19.1	25.1	4.2	12.3	39.3
II_3	20.5	25.2	8.1	9.7	36.5
II_4[a]	16.4	24.8	5.5	13.0	40.3
II_{11}[a]	17.6	24.6	4.6	11.1	42.0
	19.2 ± 2.0	24.8 ± 1.2	6.1 ± 1.6	10.4 ± 1.9	39.3 ± 1.6
Affected					
I_1[a]	17.4	26.5	3.9	10.1	42.1
II_2[a]	17.5	24.0	2.7	10.6	45.2
II_5[a]	17.2	25.0	3.7	12.3	41.7
II_7[a]	17.0	24.7	2.6	11.3	44.2
II_8	19.3	24.3	3.4	10.0	42.8
II_9	20.1	23.9	3.7	10.1	42.1
III_2[a]	17.2	23.6	2.4	11.6	45.2
III_3[a]	16.8	23.6	3.3	11.9	44.5
III_4	19.6	23.3	3.2	10.4	43.5
	18.0 ± 1.3	24.3 ± 1.0	3.2 ± 0.5[b]	10.9 ± 0.8	43.4 ± 1.4[b]

[a] Fast floating LDL; see Fig. 3 for zonal fractions that were analyzed.
[b] $P < 0.01$.

above three LDL preparations were used to calibrate the zonal rotor over the effluent volumes of interest (**Fig. 6**). Minimum molecular weights for each of the major LDL preparations were then calculated from the zonal elution volumes (**Table 3**). Calculated molecular weights and the compositional data (see Table 2) were then used to compute the number of individual constituents per LDL particle (Table 3). On the average, LDL of affected subjects were less dense, contained slightly more protein and more CE, and less TG. Protein and TG were rather constant per particle over the entire molecular weight range, whereas there were significant positive correlations between the numbers of FC, CE, and PL molecules/particle and the calculated molecular weights of the particles both in normal and affected

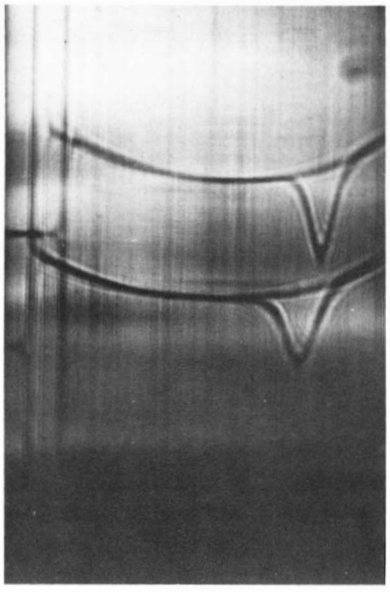

Fig. 4. Schlieren photographs of LDL-II_7 (bottom) and LDL-II_8 (top). LDL was isolated by zonal rate flotation. Direction of flotation is from right to left. Photographs were taken 34 min after reaching full speed (52,000 rpm). Solvent density was 1.063 g/ml, temperature 20°C, and lipoprotein concentration 5 mg/ml.

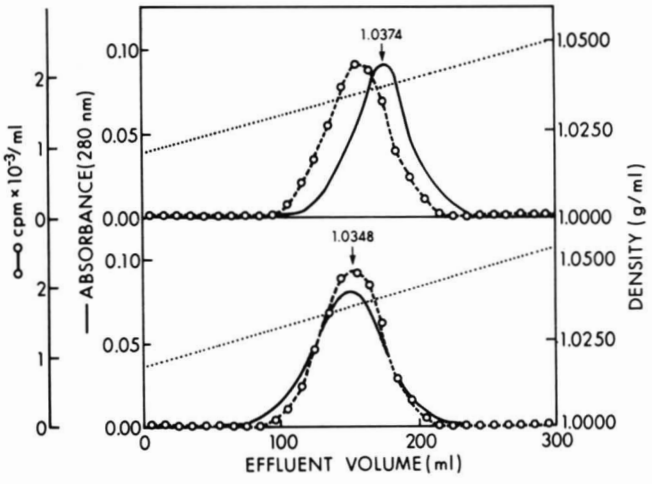

Fig. 5. Equilibrium centrifugation of LDL-II_8 (top) and LDL-II_7 (bottom) in the zonal rotor in the presence of iodinated ^{125}I-labeled LDL (from subject III_2).

TABLE 3. Molecular weights and chemical constituents of LDL

Subject	Molecular Weight	Protein	PL	TG	FC	CE
	10^{-6}	Dalton × 10^{-3}		moles/mol LDL		
Unaffected						
I_2	2.85	527	956	187	788	1722
I_8	2.55	514	839	216	533	1529
I_9	2.45	550	708	227	500	1477
II_1	2.7	516	874	133	858	1632
II_3	2.45	502	796	233	614	1375
II_4[a]	3.3	541	1056	213	1108	2046
II_{11}[a]	3.3	580	1044	178	946	2132
III_5	2.85	n.d.	n.d.	n.d.	n.d.	n.d.
	2.8 ± 0.34	533 ± 26	896 ± 129	198 ± 38	764 ± 231	1702 ± 288
Affected						
I_1[a]	3.3	574	1128	151	861	2137
II_2[a]	3.15	551	975	100	863	2190
II_5[a]	3.3	567	1064	143	1049	2117
II_7	3.5	595	1115	107	1022	2380
II_8	2.7	521	846	108	698	1778
II_9	2.85	572	878	124	744	1846
III_2[a]	3.4	584	1035	97	1019	2364
III_3[a]	3.4	571	1035	132	1045	2328
III_4	2.85	558	856	107	766	1907
	3.16 ± 0.219[b]	566 ± 21[b]	992 ± 109	119 ± 20[c]	896 ± 140	2116 ± 227[c]

[a] Fast floating, results represent mean ± 1 SD.
[b] $P < 0.05$; [c] $P < 0.01$.

individuals (**Table 4** and **Fig. 7**), suggesting that the increased particle weights were due to increases in cholesterol and PL. When the LDL of FH and unaffected subjects were matched for molecular weight, the LDL of FH subjects contained more CE and less TG (Table 3 and Fig. 7).

The protein moieties of LDL were further characterized by their solubilities in tetramethylurea and by sodium dodecyl sulfate gel electrophoresis. In all LDL preparations tested more than 96% of the protein moiety was insoluble in TMU. ApoB subspecies were analyzed on 3% SDS-gels according to Kane et al. (25) (**Fig. 8**). In all LDL, the predominant band was B-100. No B-48 protein was found and the proportion of B-47 plus B-26 varied. SDS electrophoresis in 10% acrylamide gels did not reveal any bands, other than apoB species, when gels were loaded with three times the usual amounts of protein (**Fig. 9**).

HDL-cholesterol levels were below the 5th percentile

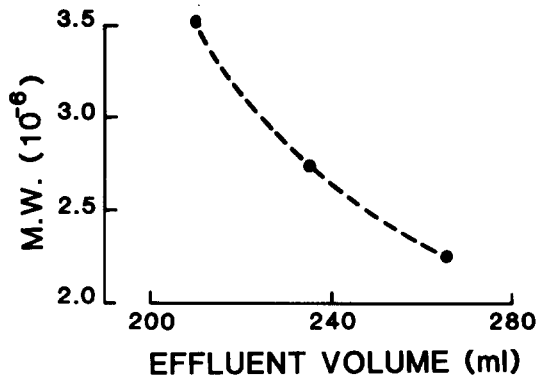

Fig. 6. Calibrations of zonal rotor elution volumes vs. molecular weight of LDL. Zonal elution profile peaks of major LDL populations of subjects II_7, II_8, and an unrelated subject with hypertriglyceridemia are plotted from left to right along the abscissa. The molecular weights of these preparations obtained by analytic ultracentrifugation are plotted on the ordinate.

TABLE 4. Correlations between LDL molecular weight and LDL components

Component	Linear Regression Equation	r	P
Unaffected			
Protein	y = 47.0 × +401.2	0.656	<0.2
PL	y = 334.1 × −38.9	0.956	<0.001
TG	y = 28.6 × +278.2	0.302	<0.5
FC	y = 557.9 × −798.2	0.913	<0.01
CE	y = 770.1 × −454.3	0.990	<0.001
Affected			
Protein	y = 56.5 × +387.1	0.773	<0.02
PL	y = 350.8× −116.5	0.931	<0.001
TG	y = 13.4 × +76.5	0.199	<0.5
FC	y = 450.3 × −527.2	0.930	<0.001
CE	y = 751.6 × −259.4	0.960	<0.001

Data are those from Fig. 7. *P* value denotes statistical significance of Pearson correlation coefficients.

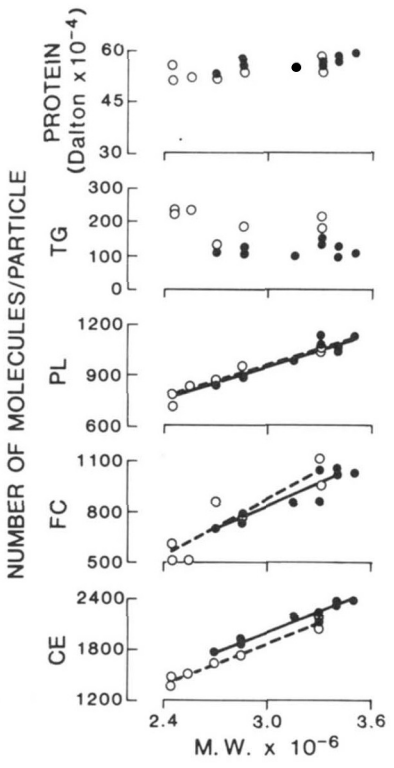

Fig. 7. Correlations between components of the LDL particle and LDL molecular weight. Each point is a single LDL preparation. Solid dots and lines are from FH subjects; circles and dashed lines are from unaffected relatives. Linear regression lines, Pearson correlation coefficients, and *P* values are provided in Table 4.

of age- and sex-matched controls (31) in subjects I_7, III_2, III_3, III_4, and III_5. To characterize HDL further, zonal ultracentrifugation was performed. The distribution of HDL subfractions, expressed as percentage of total HDL protein recovered, is shown in **Fig. 10.** HDL_2 was absent in subjects II_8, III_2, III_3, and III_5, but present in the heterozygotes II_9 and III_4 in small, but significant amounts. In subjects where HDL_2 was absent, the major HDL population had flotation properties similar to HDL_{3D}, described recently (29).

DISCUSSION

The aim of this work was to characterize some properties of LDL and HDL in the plasmas of subjects with receptor-defective FH in order to ascertain whether, as a consequence of decreased apoB, E cellular receptor numbers, altered populations of lipoproteins accumulate in plasma. The availability of a kindred gave us the unusual opportunity of comparing the lipoprotein populations of affected and unaffected relatives. To our knowledge similar comparisons have not been reported. Consistent with a recent study on human LDL (34), the LDL's in all members of this kindred (except II_{10}) appeared as single major populations with smaller populations on the leading or trailing edges of the major peaks. These smaller fractions (reproducibly present in both normal and affected individuals) were not further characterized in this study.

Calculation of the molecular weight of the LDL from the zonal rotor elution volume by means of a calibration curve revealed an overlapping range of values for LDL molecular weights in affected and normal subjects. Since frictional ratios of LDL preparations were not determined, the molecular weight of LDL may have been underestimated. Nevertheless, the range of calculated values in this study compares favorably with data obtained by others (7, 18). The availability of LDL masses allowed for the calculation of average compositions of the major LDL populations in terms of numbers of molecules of each component per particle. Using these data, the number of molecules of FC, CE, and PL contained in a LDL particle were directly related to LDL

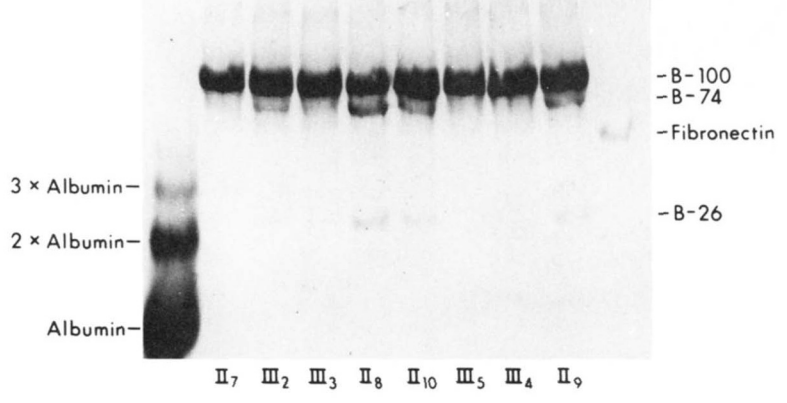

Fig. 8. Characterization of apoLDL in various subjects of the kindred by SDS-electrophoresis in 3% polyacrylamide gels according to Kane et al. (25). Anode is at bottom of figure. Cross-linked albumin (far left) and fibronectin (far right) were used as standards.

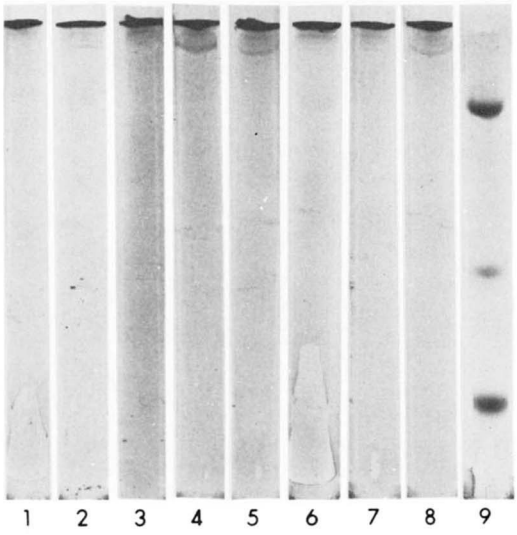

Fig. 9. Characterization of apoLDL from various members of the kindred by SDS-electrophoresis in 10% polyacrylamide gels. Anode is at bottom of figure. Samples from left to right (numbers 1–8) were taken from subjects II_7, II_2, III_3, II_8, II_{10}, III_5, III_4, and II_9. Molecular weight markers in gel #9 are bovine serum albumin, chymo-trypsinogen, and cytochrome C, from top to bottom. B-100 apparently did not enter the gels but the smaller forms B-74 and B-26 appear within 4 mm of the top. Compare with Fig. 8.

particle weight. No clear-cut relationship existed between LDL particle weight and LDL protein. This suggests that given amounts of protein were associated with varying amounts of lipids and that elevated LDL mass was due to a higher abundance of FC, CE, and PL molecules per LDL particle. Indeed, the slightly higher protein content in FH-LDL could have resulted from differences in frictional ratios or from slight errors in determination of molecular weight. Matching of LDL of comparable masses isolated from the plasmas of normal and FH subjects revealed that LDL isolated from FH patients contained more CE and less TG than LDL of unaffected individuals. These FH-related differences in LDL composition may be due to differences in LDL assembly or may reflect the effects of prolonged residence time in plasma (35) on lipoprotein lipolysis and lipid transfer within the plasma. Whatever the mechanism, the altered compositions may influence the thermotropic properties of LDL, which could be of importance in LDL catabolism, atherogenesis, or lesion reversibility (36, 37).

Apoprotein analysis of LDL revealed the B-100 protein to be the major apoB species in all subjects studied. Since no B-48 protein, which seems to originate in the intestine, was present, the major LDL population in the fasting state appears to be derived from lipoproteins secreted by the liver in both the normal and FH subjects of this kindred.

Fisher et al. (7) have proposed that genetic mechanisms control LDL mass. The distribution of the masses of the major LDL population in this kindred are consistent with genetic influences determining LDL mass. It should be noted that all female heterozygotes were confined to one side of the family tree (the family of subject II_7) and all had the faster floating or less dense

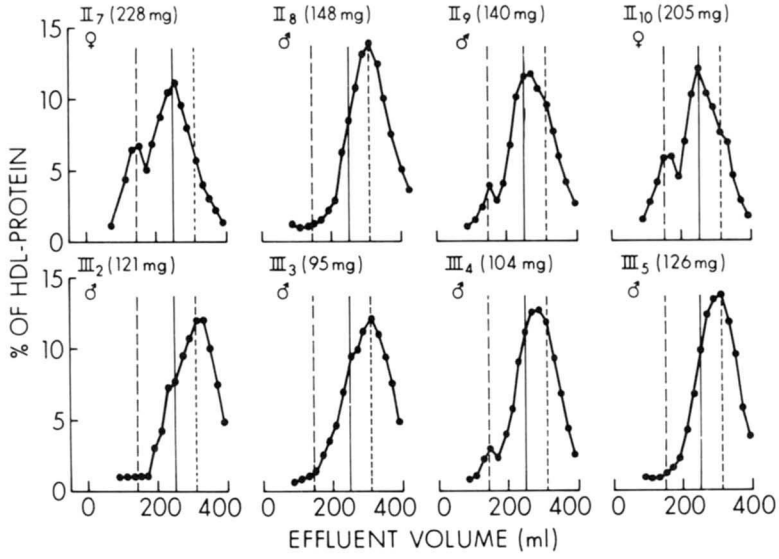

Fig. 10. Characterization of the HDL region by zonal rate flotation. After ultracentrifugation, 20-ml fractions were collected and assayed for protein (30). Results are expressed as percentage of HDL protein recovered between 100 and 400 ml of the zonal rates effluent. HDL-protein (mg/100 ml plasma) is given on top of each panel in parentheses. The peak elution volumes of HDL_2 (– – –), HDL_{3L} (——), and HDL_{3D} (---) as defined previously (29) are indicated for ease of comparison.

LDL as the major LDL population. On the other hand, the male heterozygotes appearing on the other side of the pedigree (the family of subject II_8) had the slower floating, more dense, LDL species. Thus, sex could be a determining factor controlling LDL size in this kindred, but the small sample size precludes any definitive conclusions. Nevertheless, a perusal of the pedigree is compatible with the conclusion that FH per se may not affect whatever mechanisms govern LDL mass. The apparently small effects of FH on LDL mass differ strongly from the effects of high cholesterol diets on the LDL populations of some nonhuman primates (38). In the latter, LDL mass may increase greatly, depending upon the degree of hypercholesterolemia attained.

Analyses of HDL-cholesterol revealed levels in the lower 5th percentile in subjects I_7 and III_2–III_5. Since subject III_5 was unaffected and the others had FH, low levels of HDL-cholesterol segregated separately from FH. Subfractionation of HDL by zonal ultracentrifugation provided further insight into possible relationships between HDL subpopulations and FH. HDL_2 was absent in II_8, III_2, and III_3, affected individuals, and III_5, a normolipemic male, and present in small but significant quantities in subjects II_9 and III_4, both heterozygotes from FH. Thus, the presence of HDL_2 did not correlate with the presence or absence of FH. However, it is possible that the level of HDL_2 may modulate the concentrations of LDL-cholesterol in plasma. For example, the lipoprotein profiles in the brothers II_8 and II_9, both affected, differed from each other. LDL-cholesterol levels in II_8 were 360 mg/dl, HDL-cholesterol was 35 mg/dl, and HDL_2 was absent. LDL-cholesterol in II_9 was 279 mg/dl, HDL-cholesterol was 37 mg/dl, and HDL_2 was present. Thus, the lower LDL-cholesterol levels were associated with the presence of HDL_2, suggesting that HDL_2 may be of aid in clearing plasma cholesterol through pathways not dependent on the LDL receptor.

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