Heterogeneity of human very low density lipoproteins by gel filtration chromatography

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Abstract Very low density lipoproteins were separated by gel filtration on Sepharose 4B. A decrease in mean particle diameter and flotation rate was seen with increasing elution volumes. The smaller lipoproteins had relatively more protein and phospholipid and less triglyceride than the larger ones. No differences were noted in the relative contents of the various phospholipids or partial glycerides between small and large lipoproteins. Fatty acid patterns of triglycerides and cholesteryl esters were also similar for the various lipoproteins. Relatively more lecithin containing linoleoyl acyl groups was found in smaller lipoproteins of some subjects. More of the protein of smaller lipoproteins was apo-LDL protein. Apo-HDL peptide was lost from the very low density lipoprotein as a consequence of the gel filtration.

Supplementary key words lipoprotein heterogeneity · gel filtration

HUMAN very low density lipoproteins (VLDL, S_f 20–400) are heterogeneous with respect to both physical characteristics and chemical compositions (1). Somewhat more homogeneous VLDL fractions have been isolated by ultracentrifugation (2) and gel filtration (3), and their physical properties and gross chemical compositions have been evaluated. These studies have demonstrated that the slower floating, smaller sized VLDL contain proportionately more protein and phospholipid and less triglyceride than the larger lipoproteins. This increase in the relative content of protein and phospholipid in particles with increased surface-to-volume ratios supports the suggestion by Gustafson (4) that phospholipid and protein are surface components of these lipoproteins, possibly influencing their stability.

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

Aside from their gross lipid and protein content, the compositions of different sized VLDL have not been closely defined. An understanding of the compositional heterogeneity of this lipoprotein spectrum may provide insight into factors stabilizing these lipoproteins as well as details of their catabolism. The relative increment of a particular lipid or protein in small as contrasted with large VLDL may indicate its localization on the surface of the lipoprotein and its potential role in lipoprotein stability. If small VLDL are products of large particle catabolism as previously suggested (4, 5), a comparison of compositional differences between the two might indicate substrate preferences for plasma VLDL clearance.

This study describes the heterogeneity of human VLDL; a gel filtration system has been used to isolate homogeneous fractions of different sizes. VLDL were obtained from patients with normal levels of this lipoprotein and from patients with elevated concentrations (type IV hyperlipoproteinemia). Physical properties of the column fractions were monitored by electron microscopy, analytical ultracentrifugation, and agarose electrophoresis. Detailed lipid compositions of these fractions were determined by combined thin-layer and gas–liquid chromatography. The protein contents were studied by sodium dodecyl sulfate–polyacrylamide electrophoresis in combination with immunodiffusion.

MATERIALS AND METHODS

Lipoprotein preparation

Blood was collected from humans with normal and with elevated plasma VLDL concentrations (type IV hyperlipoproteinemia) in disodium ethylenediaminetetraacetic acid (1 mg/ml). All subjects were sampled after a 14-hr fast. The plasma was separated from the red

cells by centrifugation at 2500 rpm for 20 min in a Lourdes LRA refrigerated centrifuge at 8°C. Lipoproteins were isolated by a modification of the method described by Havel, Eder, and Bragdon (6). Chylomicrons were removed from the plasma of each subject by ultracentrifugation at the native density of plasma for 25 min at 20,000 rpm (1×10^6 *g*-min) in a Spinco model L-2 ultracentrifuge using a 40 rotor. VLDL (S_f 20–400) were isolated from the chylomicron-free plasma by ultracentrifugation at 40,000 rpm for 18 hr (1×10^8 *g*-min) at 1.006 and 8°C. The VLDL were washed through saline twice by a similar ultracentrifugation and were dialyzed against 200 vol of 0.03 M Tris, 0.12 M NaCl, pH 7.4, prior to its application to the column. The VLDL were uncontaminated by plasma protein as judged by immunoelectrophoresis and immunodiffusion using antisera to human plasma proteins. LDL were prepared from the chylomicron- and VLDL-free plasma by ultracentrifugation at 40,000 rpm for 22 hr at d 1.063. Centrifugation was repeated if the LDL was determined to be contaminated as judged by immunoelectrophoresis. The immunochemically pure LDL was dialyzed against column buffer prior to gel filtration. Human albumin, obtained from Hyland Laboratories, Costa Mesa, Calif., was also dialyzed prior to gel filtration.

Gel filtration

Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) gel was used for the separation. The Sepharose was washed twice with 0.03 M Tris, 0.12 M NaCl, pH 7.4, using 10 vol/vol of the gel. The column (100 cm × 2.5 cm I.D.) was packed to a height of 75 cm and was run in descending fashion at 4°C at a flow rate of 0.15 ml/min. Tris–NaCl buffer equivalent to twice the total column volume was run through the column, and the sample was then applied in a volume less than 4 ml using a syringe attached to a three-way stopcock which in turn was connected to the flow adapter of the column. The sample was eluted from the column with the same Tris-NaCl buffer. The exclusion volume for chylomicrons (1000–3000 Å) in this system was observed to be 80–95 ml. The total volume of the column was determined (from the elution of glucose) to be 370 ml. 5- to 10-ml column fractions were collected and monitored at 280 and 660 nm in a Beckman DU spectrophotometer. Some of the column fractions were concentrated by pressure ultrafiltration through an Amicon UM 10 filter (Amicon Corp., Lexington, Mass.) and separated on the same column.

Analytical ultracentrifugation

Aliquots of the column fractions were occasionally directly evaluated in the analytical ultracentrifuge, but it was frequently necessary to concentrate these fractions by pressure ultrafiltration prior to analysis. The samples were run against a solvent blank in a Beckman model E analytical ultracentrifuge using a double-sectored cell, similar to the technique of DeLalla and Gofman (7). The density of the sample was adjusted to 1.063 by addition of KBr, and the centrifugation was performed at a rotor speed of 52,000 rpm. The flotation velocity was determined from the conventional relationship (8).

Electrophoresis

Aliquots of whole VLDL and separated fractions were electrophoresed on 1.2% agarose by a modification of the technique of Noble (9). Other aliquots were delipidated at 4°C by three extractions with ethanol–diethyl ether 3:1, using 50 vol of solvent/vol of lipoprotein. The peptides were then washed twice with 50 ml of diethyl ether. The delipidated peptides were dried under nitrogen, dissolved in 1% sodium dodecyl sulfate, 1% mercaptoethanol in 0.01 M Tris buffer, pH 7.0, and incubated at 37°C for 2 hr according to the procedure of Weber and Osborn (10). They were then dialyzed against 0.01 M Tris buffer, pH 7.0, containing 0.1% mercaptoethanol and 0.1% sodium dodecyl sulfate. A 10% polyacrylamide gel was prepared using the same buffer system, and the delipidated peptides were electrophoresed at 85 ma for 4 hr. The gels were stained with Coomassie blue and destained in 10% acetic acid at 45°C. The stained gels were then scanned at 520 nm by a Gilford scanning attachment to a Beckman DU spectrometer. The relative amounts of the various peptides were determined from the scans by planimetry. Although the color developed was linear for different concentrations of total VLDL apoprotein, the relationship of color yield to peptide mass was unknown for each individual peptide. The relative peptide compositions described were obtained from the color yields.

Immunochemistry

Immunoelectrophoresis and immunodiffusion were performed by a micromethod (11). Antisera to human HDL, LDL, and VLDL were prepared in rabbits. The antiserum to HDL was specific for the major 28,000 mol wt apo-HDL peptide, whereas antisera to LDL and VLDL reacted with a large (250,000 mol wt) apoprotein common to both. Antiserum (prepared in a goat) to whole human serum proteins was purchased from Hyland Laboratories.

Chemical analysis

Protein concentrations in native and delipidated lipoproteins were determined by the method of Lowry et al. (12), using human albumin as a standard. Turbidity was removed from the samples by extraction with ether. The lipids were extracted from the whole VLDL and from the column fractions by the method of Folch, Lees, and

Sloane Stanley (12), and the chloroform phase was dried under nitrogen and stored in chloroform–methanol 1:1 at −20°C until analyzed. Aliquots were removed from this extract for determination of triglyceride, cholesterol, and phospholipid. These procedures were performed by the techniques of Kessler and Lederer (14), Abell et al. (15), and Bartlett (16), respectively. Other aliquots of the extract were chromatographed on silica gel G thin-layer plates, using petroleum ether–diethyl ether–glacial acetic acid 80:20:1 for the separation of neutral lipids, chloroform–methanol–ammonia–water 50:25:3:1 for phospholipid separations, and dichloroethane–methanol 98:2 for separation of partial glycerides. The chromatography in each system was performed with known standards and the lipid bands were identified after a brief exposure of the plate to iodine vapor. The bands of gel containing lipid were scraped from the plate and the lipids were eluted from the silicic acid with chloroform–methanol 1:1. Analyses were carried out as indicated above.

Some of the lipid fractions were transmethylated for gas–liquid chromatography by a method similar to that of Karmen, Whyte, and Goodman (17). The lipid was dissolved in absolute methanol containing 2% sulfuric acid and was incubated at 60°C overnight. Methyl esters were extracted from the methanol mixture by partitioning into heptane after the addition of redistilled heptane and water. The methyl esters were analyzed on a Research Specialties gas–liquid chromatograph, equipped with a coiled 6-ft column packed with 10% EGSS-X on 100–120 mesh Gas-Chrom P. The column was standardized with a series of known fatty acid standards purchased from Applied Science Laboratories, State College, Pa. The fatty acid peaks were triangulated and the relative fatty acid compositions were calculated.

Electron microscopy

The sizes of the total heterogeneous VLDL and the lipoprotein in the column fractions were studied by electron microscopy. The specimens were negatively stained with 2% silicotungstic acid (pH 6.5) and viewed with an AEI electron microscope. The diameters of 100–500 particles were determined for each column fraction, and the mean diameter of the particles and the standard deviation were calculated.

RESULTS

The 280-nm elution patterns of ultracentrifugally purified VLDL separated on a Sepharose 4B column differed among patients. Individuals with higher plasma triglyceride concentrations, reflecting larger amounts of plasma VLDL, appeared to have proportionately more of this lipoprotein in earlier elution volumes. The 280-nm absorbance, however, is a function not only of the concentration but also of the light-scattering effects of these turbid dispersions. When actual lipoprotein concentration, obtained by summing the content of all lipid and protein components of the column fractions, was plotted against the elution volume, this difference in lipoprotein distribution between individuals with elevated (patient G) and normal (patient Ma) levels of VLDL was somewhat less pronounced, though still apparent (Fig. 1). Each of the two patients with high plasma VLDL concentrations (type IV hyperlipoproteinemia) had proportionally more VLDL at earlier elution volumes when compared with normals.

The reseparation of column fractions usually resulted in elution volumes identical with those originally observed. This was not true, however, for the early VLDL eluates which were eluted just after chylomicrons (i.e., elution volume of 100–120 ml). A significant amount of this lipoprotein was distributed in later eluates when these fractions were rechromatographed. However, electron microscopy and analytical ultracentrifugation of these early column fractions indicated a greater size heterogeneity than any of the later column eluates.

Electron microscopic study of the column fractions (Fig. 2) documented both a progressive decrease in mean particle diameter with increasing elution volume and a greater homogeneity of the column fractions in comparison with the parent VLDL. Mean particle diameters of VLDL column fractions ranged from 672 Å at early elution volumes to 263 Å for later eluates (Table 1). As would be anticipated, the mean flotation rates also decreased with increasing elution volume, ranging from 343

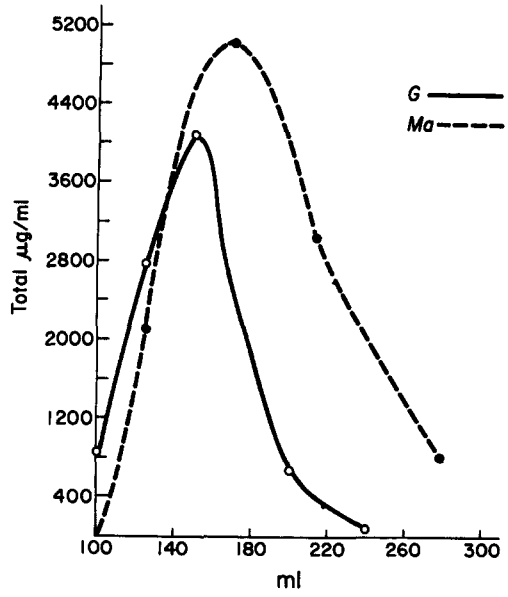

FIG. 1. Elution patterns of subjects G (elevated VLDL) and Ma (normal VLDL) expressed as μg of total lipoprotein per ml of eluate. 248 mg of G and 375 mg of Ma were applied.

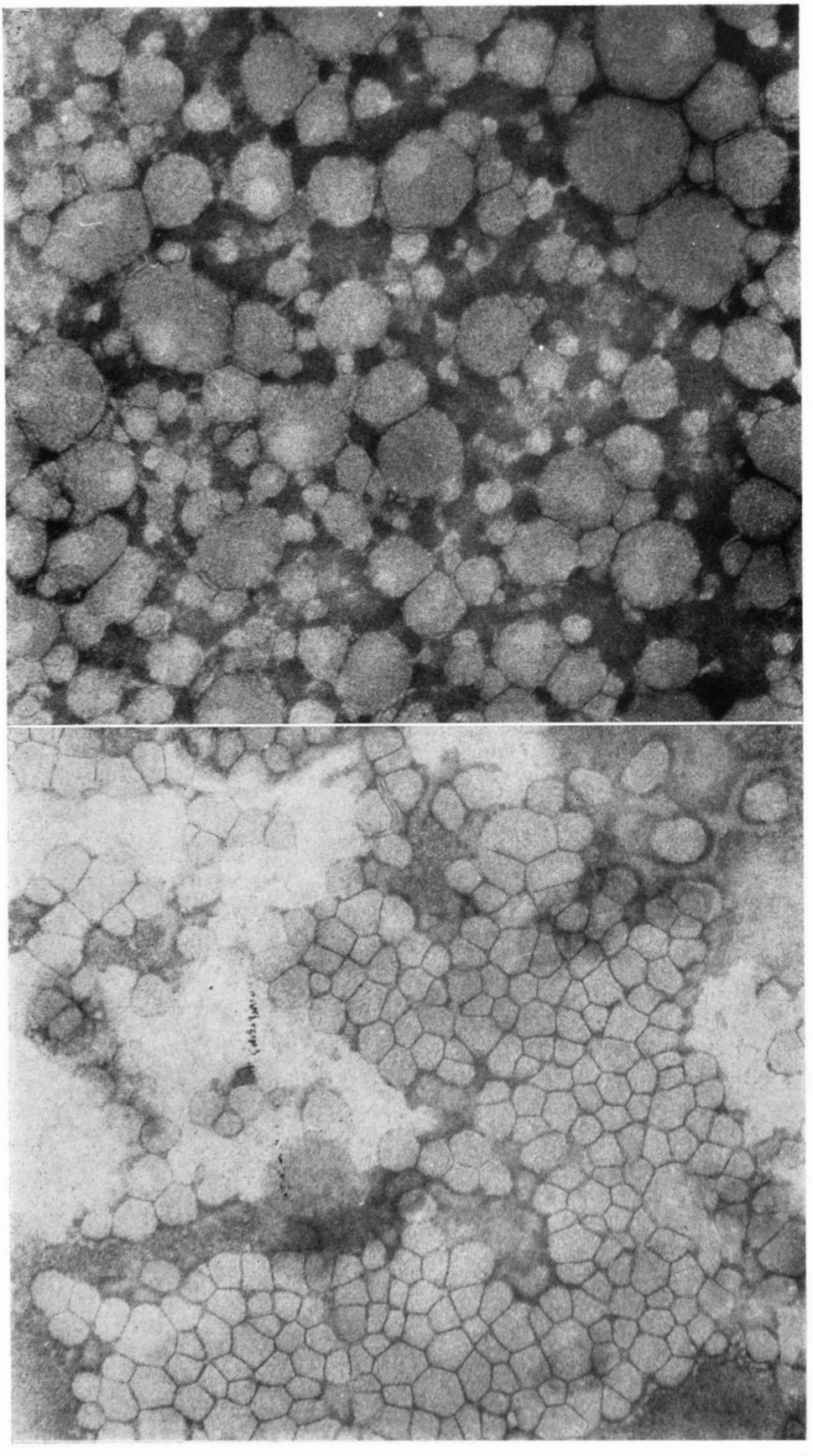

FIG. 2. Electron micrographs of negatively stained VLDL from the following column fractions: (top) 115 ml and (bottom) 175 ml. × 80,000.

TABLE 1. Mean diameters of VLDL column fractions[a]

Subject	Elution Volume	Mean Diameter[b]
	ml	*Å*
G	121	614 ± 142
	181	476 ± 76
	211	263 ± 48
M	100	672 ± 196
	143	460 ± 88
	179	286 ± 51
H	132	667 ± 121
	159	468 ± 65
	195	386 ± 52

[a] Diameters determined by electron microscopy as described in text.
[b] ± SD.

for larger diameter fractions down to 39 for smaller diameter VLDL. The flotation patterns demonstrated a greater homogeneity for the chromatographed lipoproteins in comparison with the parent material. The electrophoretic mobilities of these VLDL fractions in an agarose system were greatest for the fractions containing smaller diameter particles.

Sepharose filtration of VLDL from the same individual obtained at different times (Table 2, Ma, 9/70 and 12/70) demonstrated somewhat similar compositions for analogous elution volumes. A consistent trend in the percentage compositions of lipids and protein was noted in each study as the eluted fractions progressed from larger to smaller diameter VLDL (Table 2). The larger VLDL contained relatively more triglyceride and less phospholipid and protein than the smaller lipoproteins. Similar trends were not observed for the relative cholesterol contents of different sized VLDL. The relative free cholesterol content appeared to increase progressively with decreasing lipoprotein diameter in only one of the subjects studied (Table 3, G). However, this individual had less total cholesterol in his smaller VLDL (Table 2, G), which would indicate that there was no real progressive increase in free sterol of these smaller lipoproteins. The phospholipid compositions of the different sized VLDL fractions indicated no clear trend toward a predominating phospholipid class in the smaller lipoproteins having more surface per unit volume (Table 4). The relative contents of the major phospholipid groups were similar for the different sized lipoprotein fractions of each study. Two VLDL studies (Table 4, D and M) revealed proportionately more lysolecithin in larger diameter lipoproteins, but this was not a consistent observation. Partial glyceride contents were determined for the column fractions (Table 5). Aside from more diglyceride in the larger diameter VLDL of two patients (Table 5, D and H), the partial glyceride distributions were similar for the VLDL of different sizes.

TABLE 2. Percentage compositions of VLDL column fractions[a]

Subject	Elution Volume	Protein	Cholesterol	Phospholipid	Triglyceride
	ml	*% of total weight*			
Mo[b]	Whole VLDL	6.4	7.3	19.8	66.5
	144	5.8	4.5	19.6	70.2
	164	6.6	5.4	20.9	67.1
	182	7.1	10.1	20.6	62.3
	201	8.8	9.4	27.1	54.7
	219	7.4	6.4	28.8	57.4
G	101	4.0	9.9	17.7	68.3
	154	4.6	8.3	14.4	72.7
	201	11.1	5.2	18.3	65.5
N	109	5.2	9.8	11.9	73.0
	128	6.3	9.0	14.1	70.6
	158	8.1	10.2	15.8	65.9
	170	9.8	9.9	14.2	66.1
	200	12.0	10.1	18.2	59.8
	212	12.1	10.7	18.9	58.2
Ma 9/70	Whole VLDL	10.8	14.7	14.2	62.4
	131	9.0	16.4	10.0	64.7
	167	10.3	13.3	11.9	64.4
	203	12.6	14.5	19.4	53.4
	216	14.9	12.9	36.1	36.1
Ma 12/70	125	8.4	11.1	18.8	61.7
	170	12.1	11.9	22.7	53.3
	212	13.2	14.7	27.8	44.3
H	Whole VLDL	6.4	13.2	16.1	64.4
	105	6.3	13.7	12.1	67.9
	132	6.2	13.8	15.5	64.3
	159	8.2	15.6	17.5	58.8
	195	8.6	17.1	19.5	54.8
	231	14.3	16.9	25.5	43.3

[a] Molecular sieve chromatography on Sepharose 4B as described in text.
[b] The following plasma triglyceride values in mg/dl were found for each of the subjects at the time of study: Mo, 115; G, 754; N, 136; Ma 9/70, 140; and H, 520.

Palmitate and oleate were the only triglyceride fatty acids of the two VLDL samples studied (Table 6). No consistent trend in triglyceride fatty acid was noted for the various sized VLDL of either study. The cholesteryl ester fatty acid compositions were also similar for the different sized lipoproteins (Table 7). Lecithin fatty acid patterns changed with decreasing VLDL size in three subjects (Table 8, D, N, and H). A decrease in the relative palmitate content along with a reciprocal increase in linoleate was found in these studies. No significant change in lecithin fatty acid content was observed for the VLDL fractions of the other subject (Table 8, G).

Virtually all of the VLDL was recovered in each of the separations before 250 ml, the elution volume for LDL. However, in every VLDL study a "trail" of material absorbing at 280 nm was observed between 250 and 330 ml. This "trail" amounted to no more than 2% of the lipoprotein mass applied to the column and was 75% protein, 20% phospholipid, and 5% free cholesterol. No

TABLE 3. Free and esterified cholesterol of VLDL column fractions[a]

Subject							
N	Elution volume (ml)	109	128	158	170	200	212
	% Ester	47.9	44.8	40.6	39.1	42.6	40.5
	% Free	52.1	55.2	59.4	60.9	57.4	59.5
Ma 9/70	Elution volume (ml)	Whole VLDL	131	167	203	216	
	% Ester	53.1	51.9	50.7	51.7	52.0	
	% Free	46.9	48.1	49.3	48.3	48.0	
Ma 3/70	Elution volume (ml)	125	170	212			
	% Ester	40.9	40.6	38.7			
	% Free	59.1	59.4	61.3			
H	Elution volume (ml)	Whole VLDL	105	132	159	195	231
	% Ester	52.8	53.5	53.4	52.1	56.1	50.2
	% Free	47.2	46.5	46.6	47.9	43.9	49.8
G	Elution volume (ml)	104	129	153	177	201	
	% Ester	94.0	82.7	76.9	69.0	64.7	
	% Free	6.0	17.3	23.1	31.0	35.3	
D	Elution volume (ml)	121	145	180	208		
	% Ester	78.7	64.6	68.1	68.5		
	% Free	21.3	35.4	31.9	31.5		

[a] Molecular sieve chromatography on Sepharose 4B as described in text.

TABLE 4. Relative phospholipid content of VLDL column fractions[a]

Subject							
D	Elution volume (ml)	105	121	145	180	208	236
				% of total lipid P			
	LL[b]	7.1	3.3	1.4	1.5	2.4	2.6
	Sph	19.6	18.4	14.2	16.5	18.6	16.5
	L	65.9	72.4	74.9	74.5	72.6	73.9
	PE	7.4	6.0	9.5	7.5	6.3	6.9
G	Elution volume (ml)	104	129	153	177	201	237
				% of total lipid P			
	LL	2.4	2.3	1.5	1.6	2.6	2.2
	Sph	20.1	18.5	16.4	14.7	16.1	15.6
	L	72.6	74.2	77.7	78.9	76.6	77.3
	PE	4.9	5.0	4.4	4.8	4.7	4.9
H	Elution volume (ml)	Whole VLDL	105	132	159	195	231
				% of total lipid P			
	LL	2.1			4.9	5.8	5.8
	Sph	14.1	10.7	17.8	15.5	14.2	14.2
	L	77.7	82.6	76.2	74.4	72.8	72.3
	PE	6.1	6.6	6.0	5.2	7.2	7.8
M	Elution volume (ml)	125	170	212			
				% of total lipid P			
	LL	7.4	3.5	4.0			
	Sph	12.2	7.2	11.6			
	L	75.6	84.7	80.1			
	PE	4.8	4.6	4.3			

[a] Molecular chromatography on Sepharose 4B as described in text.
[b] LL, lysolecithin; Sph, sphingomyelin; L, lecithin; PE, phosphatidylethanolamine.

cholesteryl ester or triglyceride was found, and the relative phospholipid composition appeared to be similar to that of the VLDL column fractions.

A relative increment of VLDL protein was consistently seen in lipoproteins of smaller size (Table 2). However, the relative content of peptides comprising this protein was not identical for each column fraction. Delipidated peptides of whole VLDL and of the column fractions were electrophoresed in a sodium dodecyl sulfate–polyacrylamide system reported to give a linear relationship between peptide mobility and the logarithm of molecular weight (10). The peptide patterns on this polyacrylamide system were confirmed by chromatography on a guanidine–Sepharose system (18). Six major peptide bands were consistently detected in immunochemically pure VLDL which had been delipidated

TABLE 5. Relative glyceride content of VLDL column fractions[a]

Subject							
G	Elution volume (ml)		104	129	153	201	237
				% of total glycerides			
	MG[b]		1.2	1.9	3.1	0.9	1.1
	DG		9.7	8.5	8.9	7.9	8.8
	TG		89.1	89.6	88.0	91.2	90.1
D	Elution volume (ml)	Whole VLDL	121	145	180	208	236
				% of total glycerides			
	MG	0.6	1.2	0.5	0.6	1.6	1.9
	DG	5.2	11.7	5.0	5.4	5.9	7.3
	TG	94.3	87.1	94.5	94.0	92.5	90.8
H	Elution volume (ml)	Whole VLDL	105	132	159	195	231
				% of total glycerides			
	MG	0.8	2.0	0.4	0.4	0.6	1.6
	DG	5.9	11.5	3.3	3.0	4.9	6.1
	TG	93.3	86.5	96.3	98.6	94.5	92.3
M	Elution volume (ml)		125	170	212		
				% of total glycerides			
	MG		1.0	0.4	0.8		
	DG		3.4	2.6	3.6		
	TG		95.6	97.0	95.6		

[a] Gel filtration on Sepharose 4B as described in text.
[b] MG, monoglyceride; DG, diglyceride; TG, triglyceride.

TABLE 6. Fatty acid composition of triglycerides of VLDL column fractions

Subject	Elution Volume	16:0[a]	18:1[a]
	ml	*% of total fatty acids*	
G	104	45.5	54.5
	129	43.2	56.9
	153	40.3	59.7
	177	38.4	61.6
	201	40.2	59.8
	237	46.3	53.7
D	121	43.5	56.5
	145	42.5	57.5
	180	40.8	59.2
	208	42.2	57.8

[a] 16:0 is palmitic acid and 18:1 is oleic acid.

TABLE 7. Fatty acid composition of cholesteryl ester of VLDL column fractions

Subject	Elution Volume	16:0[a]	16:1	18:1	18:2
	ml		*% of total fatty acids*		
G	129	29.1	5.6	41.8	23.5
	153	25.5	3.8	45.5	25.3
	177	24.4	5.5	43.8	26.3
	201	28.0	5.8	39.1	27.0
	237	32.2	4.6	40.2	22.9
D	121	27.2		32.6	40.1
	145	22.0		39.5	38.5
	180	19.2		33.0	47.8
	208	17.8		34.3	47.9
	236	28.4		31.9	39.8

[a] 16:0, 16:1, 18:1, and 18:2 are palmitic, palmitoleic, oleic, and linoleic acids, respectively.

TABLE 8. Fatty acid composition of lecithin of VLDL column fractions

Subject	Elution Volume	16:0	18:0[a]	18:1	18:2
	ml		*% of total fatty acids*		
G	104	58.4	27.4	11.1	3.1
	129	50.2	27.4	15.4	7.1
	153	54.1	25.6	13.7	6.6
	177	52.1	25.0	14.8	8.2
	201	52.0	30.6	12.9	4.5
	237	50.0	30.8	15.0	4.3
D	105	46.5	24.0	17.2	12.2
	121	48.6	20.4	16.7	14.2
	145	39.2	20.7	17.5	22.6
	180	36.2	25.1	18.1	20.7
	208	38.7	26.0	15.1	20.2
N	128	40.4	15.2	32.4	11.9
	158	33.4	24.6	31.7	10.2
	170	32.6	23.4	17.9	26.1
	200	30.7	26.3	17.6	25.5
	212	30.7	23.3	20.8	25.2
H	105	50.9	22.1	17.7	9.3
	132	37.1	23.4	20.0	19.5
	159	44.7	19.3	16.1	19.9
	195	43.5	21.8	15.6	19.1

[a] 18:0 is stearic acid.

(Fig. 3). A peptide migrating ahead of band 3 in this figure was not observed in some of the other VLDL studies. Three of these peptides have been identified. Band 1 migrated exactly as did apo-LDL and gave an identity line by Ouchterlony analysis with native LDL and sodium dodecyl sulfate-solubilized apo-LDL using antisera which reacted with the 250,000 mol wt apopro-

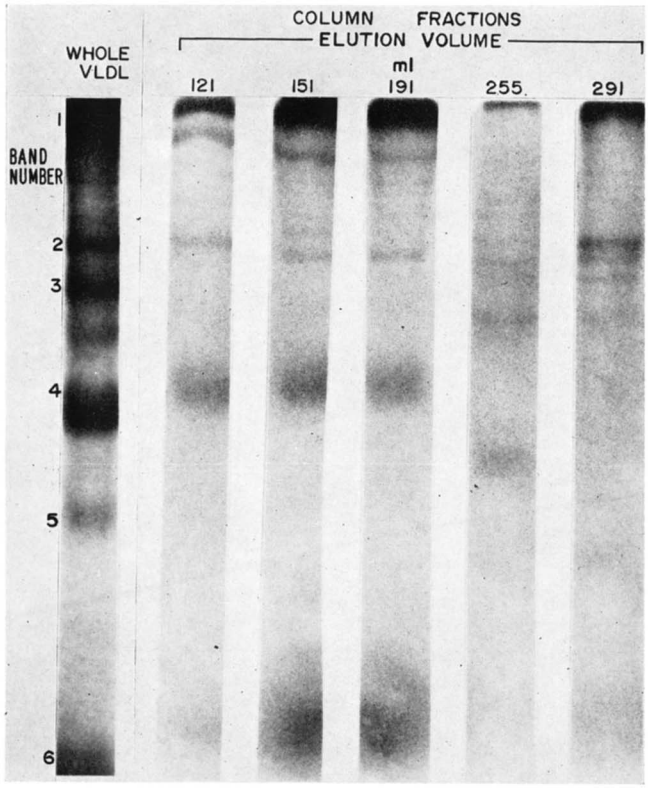

FIG. 3. Polyacrylamide gel electrophoresis of sodium dodecyl sulfate-solubilized, delipidated whole VLDL and column fractions.

tein of LDL. Band 2 migrated in the same position as serum albumin (i.e., mol wt of 68,000), but could not be further identified immunochemically. Two bands (Fig. 3, bands 3 and 4) are also presently unidentified. Band 5 migrated identically with the 28,000 mol wt peptide of HDL and reacted with the anti-HDL antibody, which reacts only with the 28,000 mol wt major HDL apoprotein. This apo-HDL molecular weight determination, although twice that obtained by Shore and Shore (19) from amino acid data, is identical with the value obtained by Scanu et al. (20) using the same technique. Band 6 was somewhat diffuse, spanning a molecular weight range of 8000–15,000. Because of its molecular size this band was felt to be a combination of the recently described low molecular weight VLDL peptides (21).

A consistent difference in peptide pattern was seen for the different diameter VLDL of each of the six studies where this was evaluated. Bands 1 and 6 were the major peptides of each of the VLDL column fractions (Table 9). Relatively more band 1 or apo-LDL was apparent in smaller sized particles when the gels of each of the studies were scanned and quantitated. Almost all of the 3rd and 5th (apo-HDL) peptide bands and much of the 2nd band were lost from the VLDL as a consequence of gel filtration. These were found to be among the major peptide constituents of the VLDL "trail." A small fraction of the other peptides was also lost from the VLDL during gel filtration.

TABLE 9. Relative content of individual peptides in VLDL column fractions (subject H)[a]

Elution Volume	Peptide Band Number 1	2	3	4	5	6
ml						
105	38.8[b]	6.7		7.6		47.0
130	39.8	5.7	0.2	8.8		45.5
160	42.4	5.2	0.3	8.6		43.5
195	48.9	4.5		7.6		38.9
225	57.0	6.8		7.2		28.9
280[c]	21.5	41.2	5.2	8.7	2.8	20.8
Whole VLDL	42.6	7.5	1.2	10.3	0.5	38.5

[a] Relative peptide contents obtained from color yield. See text for description of peptide band identification.
[b] Values are means of duplicate determinations.
[c] Column fraction from the VLDL "trail" eluted after VLDL (see text).

DISCUSSION

VLDL heterogeneity has previously been evaluated by both ultracentrifugal (2, 22) and gel filtration techniques (3). The ultracentrifugal studies of Gustafson, Alaupovic, and Furman (2) and of Lossow et al. (22) showed a relative increment of VLDL phospholipid and protein in smaller diameter, slower floating fractions. The results obtained by Sata et al. (3) from gel filtration studies similarly suggest relative phospholipid increments in smaller particles. However, neither the sizes nor the flotation properties of the column fractions were characterized in their study. The data presented here indicate that separations of different sized VLDL with differing flotation properties are achieved by the gel filtration technique.

Similar to previous observations (2, 22), the smaller VLDL in this study contained proportionately more protein and phospholipid and less triglyceride than larger diameter lipoproteins. The compositional range of VLDL is somewhat broader for this series of patients than those previously described. The larger contents of protein and phospholipid in smaller VLDL suggest a surface localization for those substances, and a probable role in stabilizing the lipid dispersion. Gustafson (4) has indicated that sufficient phospholipid, protein, and free sterol are present in all the VLDL subfractions to loosely cover the surface with a monomolecular layer. Using the molecular areas for cholesterol and lecithin of Lecuyer and Dervichian (23), similar calculations with the present data indicate that more than enough protein, free cholesterol, and phospholipid are present on these VLDL fractions for a 20-Å molecular surface coat.

Aside from the relative content of triglyceride and phospholipid, detailed lipid analysis of the different sized VLDL revealed no striking heterogeneity. The similar relative amounts of each of the phospholipids on different sized VLDL suggests that no single phospholipid class disproportionately occupies the surface layer. If the precursor–product relationship of the large and small VLDL is considered, this finding does not support a preferential loss of a particular phospholipid during catabolism. The change in lecithin fatty acid composition seen in the smaller VLDL of three studies possibly reflects a preferential catabolic loss of lecithin containing a high proportion of palmitate as opposed to lecithin with a high proportion of linoleate. Alternatively, this relatively greater "linoleyl lecithin" content in smaller VLDL may indicate an enrichment of this lecithin species in the surface layer of the lipoproteins. Because it was observed (24) that the surface coat of chylomicrons contains only the free sterol, it was anticipated that the smaller VLDL having relatively more surface coat would reveal a progressive increment in sterol similar to what was observed for phospholipids. However, no clear cut increase in relative amounts of free cholesterol was seen for the smaller sized VLDL. This may be a reflection of the lecithin–cholesterol acyltransferase activity which has been proposed to accompany the metabolism of these particles (25).

Triglyceride fatty acid compositions were not significantly different for the different sized VLDL. This would indicate that lipoprotein lipase has no pronounced specificity for either of the two triglyceride fatty acids. The similar cholesteryl ester fatty acid compositions of large and small VLDL also suggest a relatively random plasma clearance of this lipid. The finding of similar relative partial glyceride contents in smaller lipoproteins would suggest that either the lipolysis is fairly complete or the partial glycerides are lost to other lipoprotein groups during plasma triglyceride clearance.

Although the spectrum of size and composition in VLDL is determined to some extent by the catabolic process (4, 5), it has been noted that VLDL of different sizes are synthesized by the perfused rat liver (26). The hepatic assembly of this lipoprotein may be a major determinant of its plasma heterogeneity. It has been suggested (27) that VLDL is a micellar aggregate. If VLDL were solubilized by a readily dissociating amphipath, analogous to the micellar dispersion found in bile, it would be anticipated that this amphipath would be lost from the lipid aggregate during gel filtration with a resulting change in the physical properties of the aggregate. This is seen when the mixed micelles of bile are chromatographed on a gel filtration system.[1] The bile acids are lost from the micelle and a coarser lamellar dispersion of phospholipid and cholesterol is recovered. The absence of a similar phenomenon when VLDL are chromatographed does not support a micellar solubilization analogous to the biliary micelle for the lipid of these lipoproteins.

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[1] Quarfordt, S. H. Unpublished data.

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