Characterization of human very low density lipoproteins containing two electrophoretic populations: double pre-beta lipoproteinemia and primary dysbetalipoproteinemia

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Abstract Two discrete populations of very low density lipoproteins, with fast and slow pre-beta electrophoretic mobility, were found in 50% of normolipemic and 30% of hyperlipemic individuals selected at random. The two populations were isolated by preparative electrophoresis from five hyperlipemic subjects. The particles comprising the slow component were smaller than those of the fast component and the slow component contained a larger proportion of cholesteryl esters, free cholesterol, B-apoprotein, and arginine-rich apoprotein and a smaller proportion of triglycerides and the two most anionic apoproteins (R-glutamic acid and R-alanine). The properties of the slow component thus closely resemble those of "remnant" very low density lipoproteins that accumulate in blood plasma of functionally hepatectomized rats. The chemical composition of the slow component was also similar to that of the very low density lipoproteins with beta mobility found in primary dysbetalipoproteinemia. However, the proportion of cholesteryl esters and arginine-rich apoprotein was much higher in the latter. The arginine-rich apoprotein from very low density lipoproteins of most normolipemic and hyperlipemic subjects separates into three or four major bands upon isoelectric focusing electrophoresis in polyacrylamide gels, with pI varying from 5.57 to 6.03. In very low density lipoproteins from individuals with primary dysbetalipoproteinemia, this protein uniquely contains little or none of the two most cationic bands. The number of bands was constant in all subjects studied. The pattern was the same in very low density lipoproteins with fast and slow pre-beta mobility as well as in the beta and pre-beta components in primary dysbetalipoproteinemia. These results suggest that many individuals have "remnant" very low density lipoproteins in their plasma. However, the beta-migrating "remnant" that accumulated in large amounts in individuals with primary dysbetalipoproteinemia contains much more arginine-rich protein and this protein is structurally abnormal.

Supplementary key words arginine-rich apoprotein · cholesteryl esters · β-apoprotein · isoelectric focusing

Although it is recognized that the very low density lipoproteins (VLDL) of human blood plasma represent a heterogeneous population of particles, differing in size and in lipid and apoprotein composition (1–8), VLDL are usually found to migrate as a single band with pre-beta mobility upon electrophoresis in various supporting media (3, 7, 9). More than one pre-beta band has sometimes been observed with electrophoresis of both serum (9, 10) and VLDL (11). With most techniques, the extra pre-beta band in serum has proved to be the high density ("sinking") pre-beta lipoprotein (12, 13) that represents the Lp(a) lipoprotein (12, 14). More recently, we (15) and others (16–18) have demonstrated two electrophoretic populations in VLDL from many human sera. Two electrophoretic populations of VLDL are also observed in patients with primary dysbetalipoproteinemia, but the mobility of the more slowly migrating one is generally described as "beta" rather than "pre-beta" (3, 19). In the present research, we have isolated and characterized the two components of "double floating pre-beta lipoproteins" and have made additional observations on the prevalence of this variant in normolipemic and hyperlipemic individuals. We have also compared the properties of the slower component with those of the "beta"-VLDL of primary dysbetalipoproteinemia (3, 19, 20).

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Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; TMU, tetramethylurea; ARP, arginine-rich protein. Small molecular weight apoproteins: R-serine, apo C-I; R-glutamic acid, apo C-II; $R_{1'2'3'4'}$-alanine, apo C-III-0,1,2,3.

METHODS

Subjects

Two men from the Lipid Clinic at the University of California, San Francisco, whose VLDL had repeatedly contained two distinct electrophoretic components, and three hyperlipemic men in whom similar phenomena were observed during a health survey of a racially and occupationally heterogeneous population of 10,430 employees of a transportation company, were selected for detailed analysis. Their mean serum total cholesterol was 288 mg/dl (range 195–419) and mean serum triglycerides were 256 mg/dl (range 125–388). None of these individuals had xanthomata. One of the clinic patients was taking clofibrate, 1.0 g twice daily, and the other had insulin-independent diabetes mellitus. The other three took no hypolipidemic drugs. Four men and three women with primary dysbetalipoproteinemia, all of whom had xanthomata (planar, tuberous, or both) and characteristic beta-VLDL were also selected. Two of them were taking clofibrate. Their mean serum total cholesterol was 374 mg/dl (range 203–627) and mean serum triglycerides were 376 mg/dl (range 129–1170). The ratio of total cholesterol to triglycerides in their VLDL ranged from 0.49 to 1.08. All other subjects were participants in the health survey. The two patients from the Lipid Clinic were on diets restricted in saturated fats. The diets of the subjects of the health survey were usually self-selected and not controlled by us.

Blood sampling

Venous blood from the clinic patients was obtained in the morning, after the subjects had fasted for 9–15 hr, and was allowed to clot at room temperature for about 1 hr; it was then centrifuged at 1000 *g* for 20 min at room temperature. Blood from subjects of the health survey was obtained similarly, but was placed on ice 1 hr after it was obtained until it was centrifuged 2–4 hr later.

Separation of lipoproteins

VLDL ($d < 1.006$ g/ml) were separated (21) by ultracentrifugation at 12°C for 15–18 hr at 39,000 rpm in the 40.3 rotor of a Beckman preparative ultracentrifuge no more than 6 hr after the blood was drawn. For samples to be subjected to preparative electrophoresis or quantitative analysis of apoproteins, the VLDL were recentrifuged on the following day as before. Intermediate density lipoproteins (IDL, $1.006 < d < 1.019$ g/ml) were separated from the VLDL-free serum under the same conditions and purified by recentrifugation at the upper density limit. To eliminate the need for dialysis, D_2O in 0.15 M NaCl was used to increase the solvent density (0.143 ml per ml of VLDL-free serum).

Agarose electrophoresis

Serum and lipoprotein fractions were subjected to analytical agarose gel electrophoresis by a modification of Noble's method (10) that we have found to provide clearer and more consistent resolution of VLDL components with differing mobility. Serum or the lipoprotein fraction (250 μl) was added to 250 μl of warmed 1% agarose (Behringwerke, from Behring Diagnostics, Somerville, NJ) in 0.05 M barbital buffer, pH 8.6. The agarose was transferred to a microscopic slide cover glass and allowed to gel. A strip approximately 1 × 9 mm was cut out from the center of the cover glass and placed 4 cm from one edge of a 2.5 × 18 cm strip of Cronar separation film (E. I. Dupont de Nemours and Co., Wilmington, DE). The strip was then carefully covered with 4 ml of warm (~50°C) 0.5% agarose in 0.05 M barbital buffer, pH 8.6 containing 0.5% human serum albumin (USP, Cutter Laboratories, Berkeley, CA). After the agarose solution had solidified, a small hole was made by suction with a Pasteur pipet in the gel near one edge of the strip parallel to the sample. A few μl of 5% human serum albumin containing 0.5% bromphenol blue dye was added to the hole. Electrophoresis was for 90 min at 180–225 V (~7 mA per strip) or until the bromphenol blue–albumin marker had migrated approximately 3 cm. The dried strips were stained as described by Noble (10). R_f of lipoproteins was determined by scanning densitometry against the marker as reference.

Preparative agarose gel electrophoresis was performed in a polyethylene tray, 30 × 13.5 × 0.3 cm. Agarose was dissolved with heating in 0.05 M barbital buffer, pH 8.6 to make a 0.5% solution. A plastic rod (3 mm diameter, 29 cm long) was placed 4 cm from one end of the tray and approximately 125 ml of the agarose solution was poured into the tray. After 20 min, the rod was removed from the solidified gel. The resulting trough was filled with a mixture of 2.5 ml of VLDL (6–10 mg of protein) in 0.15 M NaCl and 0.5 ml of 1% agarose (50°C) in barbital buffer and allowed to gel. Electrophoresis was performed at 4°C in a chamber containing 0.05 M barbital, pH 8.6, in each tank with two thicknesses of Whatman chromatography paper wicks (W & R Balston, Ltd., Maidstone, Kent, England) (13 × 12 cm) extending onto 2 cm of the gel. A constant current of 150 mA was applied. After about 90 min, in favorable samples, two distinct, turbid bands were evident. The current was then interrupted and a section of gel about 5 mm in width was cut out just

ahead of and parallel to the leading band and filled with barbital buffer. The current was restarted and, when the leading band began to enter the trough, the current was again interrupted and the contained solution was removed with a Pasteur pipet. This process was repeated at 60 sec intervals until the leading band had been collected (usually 15–20 collections of about 1 ml).

The run was continued until the second band reached the edge of the trough. The current was interrupted and the content of the trough was discarded. The material in this band was then collected as before except that the current was increased to 200 mA between collections because of its reduced rate of migration. The two fractions were centrifuged at 1000 *g* for 10 min to remove fragments of agarose gel and dialyzed against 0.15 M NaCl–0.04% EDTA, pH 7.0 overnight. They were then concentrated by ultrafiltration through microporus membranes (UM 10, Amicon Corp., Lexington, MA) or by dialysis through cellulose membranes (Union Carbide Corp., Chicago, IL, pretreated by boiling in 2.5% $NaHCO_3$) under vacuum at 4°C.

Analyses

Portions of the concentrated fractions were subjected to analytical agarose gel electrophoresis to monitor the quality of the separation. Quantitative analyses of lipid components and total protein were performed as described elsewhere (22). Protein soluble in 4.2 M 1,1,3,3-tetramethylurea (TMU) was determined and content of B-apoprotein was estimated as protein insoluble in TMU (8, 23). Content of TMU-soluble proteins was determined by quantitative polyacrylamide gel electrophoresis in 8 M urea (8). For qualitative analysis of subunits of arginine-rich protein (ARP), portions of VLDL containing 50–300 μg of protein were delipidated in ethanol–ether 3:1 and ether (24). The protein was dissolved in 1% sodium decyl sulfate in 0.01 M Tris buffer and 20% sucrose, pH 8.2 and was subjected to isoelectric focusing electrophoresis in 7.5% polyacrylamide gels containing 8 M urea and 1.6% Ampholine (LKB Produkter, Bromma, Sweden), pH 3.5–7.0. The gels (8 × 0.7 cm) were prefocused for 30 min at 100 V. The upper electrolyte solution (0.02 M NaOH) was removed and samples of apolipoproteins (50–200 μg in 100–200 μl) were applied on the top of the gel followed by 100 μl of 1% Ampholine in 5% sucrose and the electrolyte solution. The lower chamber contained 0.01 M H_3PO_4. Focusing of 10–12 gels was at 10°C for 1 hr at 200 V and then for 3 hr at 400 V. Current was usually constant after 2 hr. The gels were stained with Coomassie blue as described by Malik and Berrie (25). The pI of apoprotein components was estimated from the pH of aqueous eluates of cut segments from unstained reference gels. Quantitative analysis of total cholesterol and triglycerides in VLDL, LDL (low density lipoproteins), and HDL (high density lipoproteins) was as described elsewhere (26). Diameters of VLDL fractions were measured (22) on electron photomicrographs of negatively stained preparations (27).

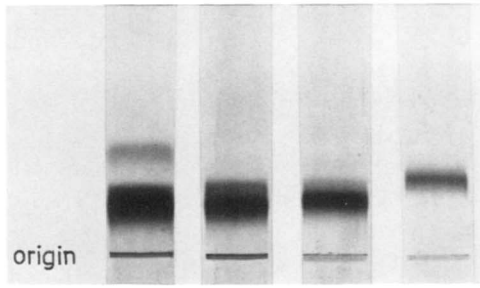

Fig. 1. Agarose gel electropherograms of serum and VLDL from a patient with primary dysbetalipoproteinemia. The beta and pre-beta components were separated by preparative agarose gel electrophoresis. From left: serum; VLDL; beta VLDL; pre-beta VLDL. Content (wt %) of components of unfractionated VLDL, beta fraction and pre-beta fraction, respectively, were: cholesteryl esters—28.5, 33.0, 19.5; triglycerides—40.9, 35.7, 50.3; cholesterol—10.3, 9.2, 8.1; phospholipids—13.3, 14.1, 13.4; B-protein—3.6, 4.6, 3.2; soluble proteins—3.5, 3.4, 5.4.

RESULTS

Evaluation of preparative agarose gel electrophoresis

To evaluate our method of preparative lipoprotein electrophoresis, beta and pre-beta components of VLDL from a patient with primary dysbetalipoproteinemia were separated and lipid and protein composition were determined (**Fig. 1**). The composi-

TABLE 1. Effect of preparative electrophoresis on the lipid and protein composition (wt %) of VLDL with a single pre-beta component

	Before Electrophoresis	After Electrophoresis
Total lipoprotein		
Cholesteryl esters	13.7 (6.10)[a]	13.1 (5.50)
Triglycerides	54.7 (4.70)	54.6 (5.85)
Cholesterol	6.01 (0.98)	5.93 (1.10)
Phospholipids	15.9 (1.69)	16.4 (0.36)
Proteins	9.50 (0.90)	9.86 (0.75)
Total protein		
B-protein	49.0 (5.00)	51.0 (5.57)
Arginine-rich protein	8.37 (1.76)	6.67 (0.80)[b]
R-serine	5.29 (2.34)	3.86 (2.83)[c]
R-Glutamic acid	8.73 (4.67)	9.58 (4.73)
R_2-Alanine	17.5 (6.10)	18.9 (4.36)
R_3-Alanine	10.8 (1.48)	11.7 (1.29)

[a] Mean and SD (n = 3).
[b] $P = 0.14$.
[c] $P < 0.05$.

tion of the two components was similar to that described previously when these components were separated by preparative electrophoresis in starch gel (19, 20). To determine the effect of the electrophoretic procedure on the composition of VLDL, samples from three patients with endogenous hyperlipemia who had a single pre-beta VLDL component on analytical agarose gel electrophoresis were subjected to preparative electrophoresis. Only a single band was visible in the preparative gels. As shown in **Table 1**, the proportion of the R-serine apoprotein as percent by weight of total protein in the material recovered after electrophoresis was reduced significantly. Content of ARP also fell consistently (mean reduction of 20%). The relative amounts of lipids and other protein components were not detectably altered.

Characterization of components of "double pre-beta" VLDL

The modified technique of agarose gel electrophoresis showed two distinct electrophoretic components in VLDL from many individuals in which the cholesterol–triglyceride ratio was well within the range of normal. When both components existed in appreciable concentration, they could sometimes be seen in electropherograms of whole serum (**Fig. 2**). The mobility of the slower component of separated VLDL was most variable, but it remained distinctly faster than that of beta (low density) lipoproteins. In some patients who had been categorized as "primary dysbetalipoproteinemia" on the basis of characteristic xanthomata and a high ratio of cholesterol to triglycerides in VLDL, the slower component had

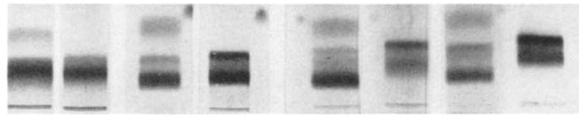

Fig. 2. Agarose gel electropherograms of serum and VLDL from four subjects. From left: serum and VLDL from a patient with primary dysbetalipoproteinemia who had beta VLDL and very little pre-beta component; serum and VLDL from a subject with primary dysbetalipoproteinemia who had widely separated beta and pre-beta components (R_f 0.35 and 0.58); serum and VLDL from a subject with well separated double pre-beta components (R_f 0.45 and 0.69); serum and VLDL from a subject with more narrowly separated double pre-beta components (R_f 0.48 and 0.68). In all cases but the one on the left, the mobility of the more rapidly migrating component is seen to be greater in VLDL than in serum (difference = 0.04–0.05 R_f units).

beta mobility but in most cases its mobility was slightly greater, overlapping that of beta lipoprotein in whole serum (Fig. 2). In most individuals with double pre-beta VLDL, the mobility of the slower component remained distinctly pre-beta, but it sometimes slightly overlapped that of beta lipoprotein in whole serum so that the distinction from beta VLDL of dysbetalipoproteinemia could not be made reliably. However, the average cholesterol–triglyceride ratio in VLDL was substantially lower in subjects with double pre-beta VLDL than in subjects with primary dysbetalipoproteinemia (**Table 2**).

When the concentration of both VLDL components was sufficient, the components could be separated by preparative agarose gel electrophoresis. The example shown in **Fig. 3** is from an individual who also had a "sinking" pre-beta lipoprotein and a substantial amount of IDL.

Analysis of the two components from subjects

TABLE 2. Lipid and protein composition (wt%) of VLDL fractions from subjects with two pre-beta components and of whole VLDL from patients with primary dysbetalipoproteinemia

	Double Pre-beta Lipoproteinemia				Primary Dysbetalipoproteinemia VLDL (n = 7)
	Unfractionated VLDL (d < 1.006) (n = 5)	Fast Pre-beta Fraction (n = 5)	Slow Pre-beta Fraction (n = 4)	1.006 < d < 1.019 Fraction (n = 5)	
Cholesteryl esters	14.6 (4.60)[a]	8.40 (3.67)	18.8 (5.31)[b]	29.3 (11.3)	28.0 (4.02)[f]
Triglycerides	51.7 (3.59)	60.6 (3.63)	41.5 (4.36)[b]	22.8 (9.44)[d]	39.3 (4.58)[f]
Free cholesterol	5.86 (0.71)	4.80 (0.63)	7.20 (0.73)[b]	9.42 (0.95)[e]	7.39 (1.50)[g]
Phospholipid	16.1 (1.13)	15.0 (0.89)	17.9 (2.18)[c]	18.9 (2.34)	17.0 (2.64)
TMU-soluble proteins	6.42 (1.31)	6.28 (0.98)	6.08 (0.90)	7.3 (3.25)	4.46 (0.92)[g]
TMU-insoluble proteins	5.30 (1.21)	4.90 (1.10)	8.60 (1.98)[b]	12.3 (2.43)	3.91 (1.08)[g]
Total cholesterol/triglycerides	0.29 (0.07)	0.16 (0.05)	0.45 (0.13)[b]	1.49 (0.99)	0.63 (0.13)[f]

[a] Mean and SD.
[b] Significantly different from fast pre-beta fraction $P < 0.01$.
[c] Significantly different from fast pre-beta fraction $P < 0.05$.
[d] Significantly different from slow pre-beta fraction $P < 0.01$.
[e] Significantly different from slow pre-beta fraction $P < 0.05$.
[f] Significantly different from unfractionated VLDL $P < 0.01$.
[g] Significantly different from unfractionated VLDL $P < 0.05$.

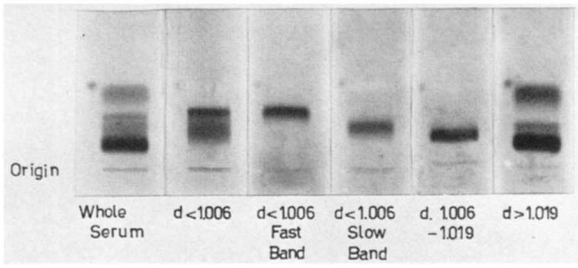

Fig. 3. Agarose gel electropherograms from a subject with two VLDL components, which were separated by preparative electrophoresis. This subject also had a substantial amount of IDL and a "sinking" pre-beta lipoprotein (gel on right).

with double pre-beta VLDL showed that both "core" and "surface" constituents of the particles differed: the slower component contained much more cholesteryl esters and less triglycerides (core constituents) and slightly more cholesterol and phospholipids as well as protein insoluble in TMU (surface constituents) (Table 2). As expected from these differences in composition (3), particles comprising the slower component were smaller than the more rapidly migrating ones (**Table 3**). The differences from the fast component were similar but more pronounced for IDL. Content of protein soluble in TMU was similar in the two VLDL components (Table 2), but its composition differed: the slower component contained significantly more ARP and less of the rapidly migrating C apoproteins (R-glumatic acid and the R-alanines) (**Table 4**). Content of R-serine and ARP was lower in both of the components separated by preparative agarose gel electrophoresis than in unfractionated VLDL. This presumably reflects loss of these components from the lipoproteins during electrophoresis (see Table 1). Except for the content of R-glutamic acid these differences in the slower component were similar, but less pronounced, than those observed in the unfractionated VLDL from patients with primary dysbetalipoproteinemia.

In three cases beta and pre-beta components of VLDL from patients with primary dysbetalipoproteinemia were analyzed. As reported previously (19, 20), the ratio of cholesterol to triglycerides was lower in the pre-beta component, but it was higher in each component than in corresponding fractions from subjects with double pre-beta VLDL (fast component: 0.31 vs. 0.16; slow component: 0.75 vs. 0.45). These differences were not statistically significant. The proportion of B-apoprotein as percent of total protein was lower in the pre-beta component from patients with primary dysbetalipoproteinemia than in the beta component (20). The differences were similar to those found in subjects with double pre-beta VLDL (fast component: 46.0 vs. 43.6; slow component: 62.0 vs. 61.8). However, the proportion of ARP was higher in both components from patients with primary dysbetalipoproteinemia (fast component: 13.3 vs. 5.5; slow component: 19.4 vs. 7.9% of total protein). The last of these differences was significant ($P < 0.05$).

Prevalence of "double pre-beta" VLDL

From these results it appeared that the presence of appreciable amounts of the slower VLDL component should be associated with an increased ratio of cholesterol to triglycerides in VLDL, analogous to the increased ratio found in individuals with primary dysbetalipoproteinemia. Partial confirmation of this hypothesis was provided from analysis of VLDL obtained as part of a health survey that included measurement of lipoprotein concentrations and distributions. The participation rate was 76%. Among 37 consecutive random samples (19 men and 18 women) from this population, 18 of the VLDL (from 9 men and 9 women) had two pre-beta components.[3] Among the 32 of these samples with serum triglyceride levels below 200 mg/dl, the ratio was higher in those with two components (0.278) than in those with a single one (0.255), but the difference was not statistically significant. Those with two components were significantly older. Among 47 consecutive samples (33 men and 14 women) that were analyzed because

[3] Only electropherograms in which two independent observers agreed that two components were present were so identified.

TABLE 3. Diameters (Å) of VLDL fractions from subjects with two pre-beta components

Subject	Unfractionated VLDL (d < 1.006)	Fast Pre-beta Fraction	Slow Pre-beta Fraction	1.006 < d < 1.019 Fraction
E.W.	392 (86)[a]	442 (85)	376 (61)[b]	280 (29)[c]
V.J.		426 (78)	333 (66)[b]	

[a] Mean and SD from measurements on electron photomicrographs of negatively stained preparations.
[b] Significantly different from fast pre-beta fraction $P < 0.01$.
[c] Significantly different from slow pre-beta fraction $P < 0.01$.

TABLE 4. Apoprotein composition (wt%) of VLDL fractions from subjects with two pre-beta components and of whole VLDL from patients with primary dysbetalipoproteinemia

	Double Pre-beta Lipoproteinemia				Primary Dysbeta-lipoproteinemia
	Unfractionated VLDL ($d < 1.006$) (n = 5)	Fast Pre-beta Fraction (n = 5)	Slow Pre-beta Fraction (n = 4)	$1.006 < d < 1.019$ Fraction (n = 5)	VLDL (n = 7)
B-protein	44.8 (6.96)[a]	43.6 (5.19)	61.8 (3.07)[b]	63.2 (14.0)	46.3 (7.90)
Arginine-rich protein	8.40 (1.81)	5.50 (1.60)	7.88 (0.67)[b]	13.5 (10.6)	22.5 (7.91)[d]
R-Serine	3.88 (2.20)	2.20 (1.25)	1.23 (0.46)	0.78 (0.54)	3.78 (2.67)
R-Glutamic acid	6.22 (1.72)	7.32 (2.11)	4.20 (1.30)[c]	2.05 (0.62)	6.02 (1.11)
R_2-Alanine	18.9 (3.15)	21.5 (2.24)	12.1 (1.67)	11.1 (3.82)	13.1 (2.81)[d]
R_3-Alanine	13.7 (2.02)	14.9 (2.49)	9.65 (1.23)	9.25 (3.84)	8.25 (1.89)[d]
R_4-Alanine	3.50 (1.82)	4.56 (1.76)	2.80 (0.83)	2.53 (1.39)	[e]

[a] Mean and SD.
[b] Significantly different from fast pre-beta fraction $P < 0.01$.
[c] Significantly different from fast pre-beta fraction $P < 0.05$.
[d] Significantly different from unfractionated VLDL $P < 0.01$.
[e] R_4-Alanine was not determined routinely when these analyses were performed.

serum triglyceride concentration exceeded 200 mg/dl, the ratio in 14 with two components was significantly higher (0.286) than in the 33 with a single one (0.216) (**Table 5**). No sex differences were found. Hyperlipidemic individuals with one component had higher serum triglyceride levels than those with two. However, the mean ratio was the same in 29 subjects with a single component whose serum triglyceride was less than 500 mg/dl as in the entire group of 33. Apoprotein composition of VLDL was determined in a subset of the hyperlipemic subjects (**Table 6**). VLDL with two components had significantly more B-apoprotein and less of the R-glu and R_2-ala apoproteins than those with a single component. In these samples the R_f of the single pre-beta VLDL was 0.58 ± 0.02 (mean ± SD); among those with two components the R_f values were 0.62 ± 0.04 and 0.44 ± 0.06.

Isoelectric focusing of ARP

To determine whether some individuals with double pre-beta VLDL might represent a forme-fruste of primary dysbetalipoproteinemia, the isoelectric focusing pattern of ARP was determined (28). In normolipemic individuals and in those with endogenous hyperlipemia who had single or double pre-beta VLDL, one of two patterns was observed (**Fig. 4**). About 80% of 70 normolipemic and hyperlipemic samples from a clinic and hospital population have shown three major components. The remainder contained four components (variant pattern). The four components from the subject whose apo-VLDL are shown in gel *f* of Fig. 4 were individually eluted from unstained gels. They reacted identically in immunodiffusion with rabbit antiserum to highly purified ARP from a subject with primary dysbetalipo-

TABLE 5. Comparison of VLDL from subjects with single and double pre-beta components (mean values ± SD)

			VLDL		
	Number	Age	Total Cholesterol	Triglycerides	Total Cholesterol / Triglycerides
		yr	*mg/dl*	*mg/dl*	
Normolipemic subjects					
Double pre-beta	18	45.6 (12.4)	15.0 (6.3)	53.4 (25.0)	0.278 (0.046)
Single pre-beta	14	34.6 (9.9)[c]	10.7 (4.6)[b]	41.3 (17.4)	0.255 (0.051)
Hyperlipemic subjects					
Double pre-beta	14	52.6 (9.7)	61.4 (27.2)	217 (85.9)	0.286 (0.073)
Single pre-beta	33	47.4 (9.8)	57.7 (33.9)	281 (147.5)	0.216 (0.045)[c]
Single pre-beta[a]	29	52.6 (9.7)	46.6 (15.1)[b]	222 (77.0)	0.217 (0.043)[c]

[a] Triglycerides 200–500 mg/dl.
[b] $P < 0.05$ (single vs. double).
[c] $P < 0.01$ (single vs. double).

TABLE 6. Apoprotein composition of VLDL in hyperlipemic subjects with single and double pre-beta components (% by weight)

	Single Pre-beta (n = 9)	Double Pre-beta (n = 9)
B-protein	37.5 (5.00)[a]	42.5 (1.65)[b]
Arginine-rich protein	9.72 (2.10)	11.5 (4.93)
R-serine	2.90 (1.46)	3.30 (1.65)
R-Glutamic acid	8.31 (2.46)	5.33 (1.58)[c]
R_2-Alanine	22.3 (4.51)	16.7 (3.63)[c]
R_3-Alanine	16.2 (2.42)	15.1 (3.32)
R_4-Alanine	3.52 (5.00)	5.80 (2.69)

[a] Mean and SD.
[b] $P < 0.02$.
[c] $P < 0.01$.

proteinemia.[4] As described by Utermann, Jaeschke, and Menzel (28), the pattern in patients with primary dysbetalipoproteinemia was distinct. These individuals lacked or had very small amounts of one or both of the components with the highest pI (bands 3 and 4) seen in subjects with other forms of hyperlipemia (Fig. 4). Of the remaining components, the most cationic one (band 2) invariably predominated (**Fig. 5**). The pattern did not differ in the two components of double pre-beta VLDL from two subjects, or in the beta and pre-beta components of two individuals with primary dysbetalipoproteinemia (**Fig. 6**). In one individual with normal serum lipid concentrations, whose VLDL had a high ratio of cholesterol to triglycerides (0.52), two pre-beta components, and a prominent ARP band in alkaline urea gel electrophoresis, the most common isoelectric focusing pattern of ARP (three bands) was present (**Fig. 7**).

DISCUSSION

In this research, we have characterized two electrophoretic components that are present in many individuals with normal as well as elevated concentrations of VLDL. In such individuals, mobility of the faster component is similar to that of the single electrophoretic component of VLDL found in other subjects and it varies less than that of the slower component. To isolate the two components by preparative electrophoresis, it was necessary to select samples in which they were well separated. Therefore, our observations on such samples may exaggerate the differences in their properties. It is well

[4] The amino acid composition of each of the four components from two individuals with the variant ARP pattern, determined after separation by preparative isoelectric focusing, has been found to resemble closely that of unfractionated ARP, with about 10 mol % of arginine (Kotite, L., J. P. Kane, and R. J. Havel, unpublished data).

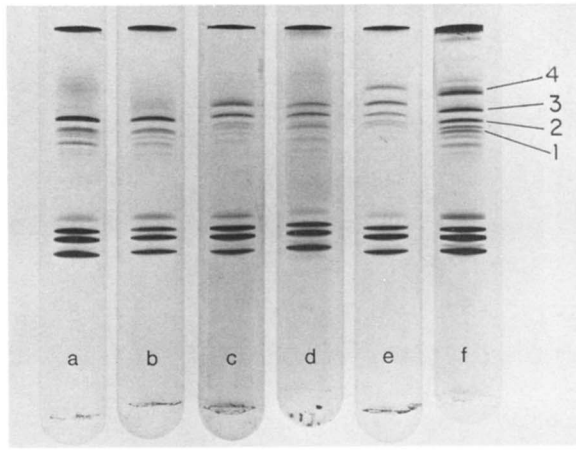

Fig. 4. Isoelectric focusing polyacrylamide gel electropherograms of apo-VLDL from six subjects. ARP is represented by the upper group of bands, numbered *1–4* (pI 5.57–6.03); band *1* is often split. Other minor bands of uncertain identity with pI < 5.57 are also evident in most cases. The anionic C-proteins comprise the lower group (pI 4.45–4.90). The B-protein remains at the point of application on the top of the gel. The gels *a* and *b* are from the patients with primary dysbetalipoproteinemia. Gels *c* and *d* demonstrate the most common pattern of ARP with three bands, seen in normolipemic and hyperlipemic subjects alike; note that the uppermost band (pI ≅ 5.87) is missing or greatly reduced in subjects with primary dysbetalipoproteinemia. Gels *e* and *f* show a third ARP pattern, in which still another more cationic component (band *4*) is present.

recognized that smaller VLDL have higher ratios of cholesteryl esters to triglycerides in their core (2, 3) and increased proportions of B-apoprotein and arginine-rich protein on their surface (4, 8). The average size of the slower electrophoretic component is smaller than that of the faster one (Table 3). However, the slower component evidently represents a separable electrophoretic species rather than part of a

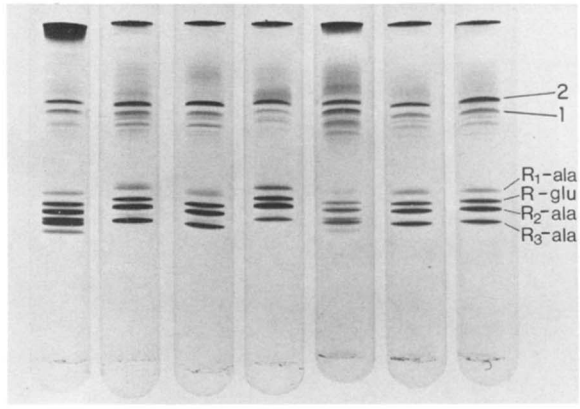

Fig. 5. Isoelectric focusing polyacrylamide gel electropherograms of apo-VLDL from seven patients with primary dysbetalipoproteinemia. Note that the most cationic of the two bands of ARP (pI ≅ 5.72) invariably predominates and that the one or two more cationic bands present in other individuals are not seen or are faint. Among the C-proteins, the intensity of the most cationic component (R_1-alanine, pI ≅ 4.90) is most variable.

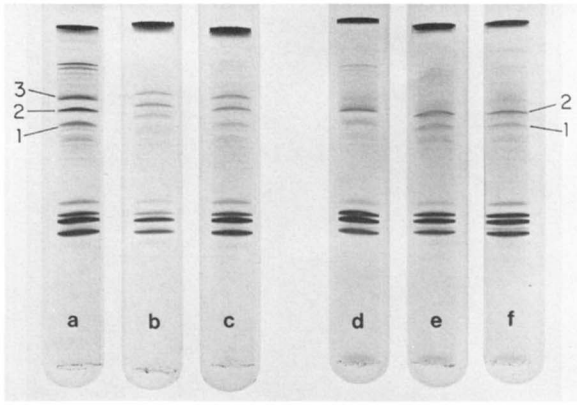

Fig. 6. Isoelectric focusing polyacrylamide gel electropherograms of apo-VLDL and its two electrophoretic components from two subjects. *Left group*: *a*, unfractionated VLDL; *b*, slowly migrating component; and *c*, rapidly migrating component from an individual with double pre-beta VLDL. *Right group*: *d*, unfractionated VLDL; *e*, beta component; and *f*, pre-beta component from a patient with primary dysbetalipoproteinemia. In each case the pattern of ARP in the unfractionated VLDL is preserved in the two electrophoretic fractions. The most cationic group of closely spaced bands in the gel on the far left represents albumin, present as a contaminant in this sample.

continuous spectrum of particles of varying size (7) (note that the diameters of the two components from subjects E.W. and V.J. in Table 3 overlap). The slower component does not appear to be produced by ultracentrifugation because two bands can be seen in whole serum when the concentration of each is sufficient. Ultracentrifugation does, however, result in increased mobility of the VLDL components relative to albumin and to LDL (Fig. 2). Possibly, this results from loss of the two soluble proteins of VLDL with the highest isoelectric points (R-serine and ARP) during centrifugation or during electrophoresis in the absence of other macromolecular components of serum. We have documented, by quantitative radioimmunoassay, that ARP is lost from rat VLDL during centrifugation.[5] It should be emphasized that the apparent particle size of single pre-beta VLDL was not altered by preparative electrophoresis, and that particles with slower mobility were not evident in the preparative gels. Therefore, it is most unlikely that the slower component is an electrophoretic artifact.

The reduced mobility of the slower component evidently is related to the loss of C-apoproteins relative to B-apoprotein and ARP. This change in protein composition and the increase in ratio of cholesteryl esters to triglycerides are remarkably similar to those that occur when "remnants" of VLDL are produced in functionally hepatectomized rats (22). These remnants also have reduced electrophoretic mobility and are smaller than the plasma VLDL from which they were produced. The present results are consistent with the concept that the slower component in human VLDL represents a remnant particle and therefore support the hypothesis that remnants are formed during the metabolism of human VLDL in extrahepatic tissues (29, 30). In rats, such remnants are rapidly taken up by the liver and it seems possible that the concentration of the slower component is related to differing efficiencies of hepatic metabolism of particles that are formed in all individuals.

Carlson and Ericson (16) found two pre-beta VLDL components in 35% of male and 25% of female subjects selected at random from healthy adults in Uppsala, Sweden. Hestrand and Vessby (18) found two components in 22% of 50-year-old men from the same population. In the present research, addition of VLDL in pregelled agarose to the electrophoretic medium decreased endosmotic transport during the period before electrophoresis was begun and thus sharpened the separation of the components. This may account for the higher prevalence of the phenomenon (50%) in our sample of an industrial population. It must be emphasized that the distinction between one and two components may be difficult and it cannot therefore be concluded that the two groups are distinct or homogeneous. Among hyperlipidemic subjects, the ratio of cholesterol to tri-

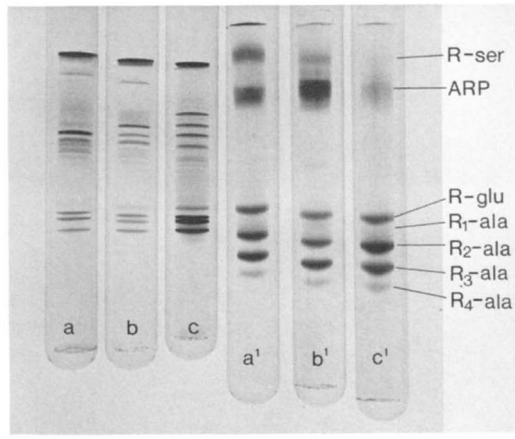

Fig. 7. Isoelectric focusing gel electropherograms (*left group*) and alkaline urea gel electropherograms (*right group*) of apoproteins of VLDL for three individuals. The uppermost band in alkaline urea gels (R-serine) does not focus between pH 3.5 and 7.0. Gels *a* and *a'*, apo-VLDL from a patient with primary dysbetalipoproteinemia. Gels *b* and *b'*, apo-VLDL from a normolipidemic subject with double pre-beta VLDL and an unusually large amount of ARP. Gels *c* and *c'*, apo-VLDL from a patient with single pre-beta VLDL and endogenous hyperlipemia (variant ARP pattern).

[5] Fainaru, M., R. J. Havel, and K. Imaizumi. *Biochem. Med.* Apoprotein content of plasma lipoproteins of the rat separated by gel chromatography or ultracentrifugation. **17:** 347–355.

glycerides was significantly higher in VLDL with two components. Although the concentration of serum triglycerides was higher in hyperlipemic individuals with one component, this did not account for the difference in the ratio (Table 5). These observations agree with previous reports (16, 18, 31, 32).

The differences between the physical and chemical properties of beta and pre-beta VLDL from patients with primary dysbetalipoproteinemia resemble those observed between the two components of individuals with double pre-beta VLDL, but they are more pronounced. The electrophoretic mobility of beta VLDL is usually slightly greater than that of LDL so that it cannot always be distinguished from the slower component of double pre-beta VLDL (Fig. 2). Others have drawn attention to the unreliability of beta VLDL in the diagnosis of primary dysbetalipoproteinemia (32, 33). In our hypertriglyceridemic subjects with double pre-beta VLDL (Table 5) only one had a ratio of cholesterol to triglycerides in VLDL exceeding 0.42, the minimum value proposed by Hazzard et al. for the diagnosis of primary dysbetalipoproteinemia when the triglyceride level exceeds 150 mg/dl (34). The lowest ratio that we have observed in 14 hyperlipemic individuals with dysbetalipoproteinemia is 0.49, supporting the usefulness of this measurement.

In normotriglyceridemic individuals, high cholesterol:triglyceride ratios may normally be observed in VLDL. In such subjects, determination of the subunit composition of ARP by isoelectric focusing of apo-VLDL may be of particular value in the diagnosis of dysbetalipoproteinemia. We have thus confirmed the diagnosis in three normotriglyceridemic individuals in whom we suspected dysbetalipoproteinemia because of a very high cholesterol:triglyceride ratio in VLDL.

The characteristic isoelectric focusing pattern of ARP was present in rapidly migrating pre-beta VLDL of individuals with primary dysbetalipoproteinemia, whose physical and chemical characteristics are otherwise normal (19, 20), as well as in the beta VLDL (Fig. 6). The pattern of ARP was also the same in the two components of double pre-beta VLDL. These observations suggest that the pattern in primary dysbetalipoproteinemia is not the result of altered metabolic events related to remnant formation, but rather is fundamental to the disorder. This interpretation is consistent with the fact that the content of ARP is much higher in VLDL from individuals with primary dysbetalipoproteinemia than in the presumed remnant population from individuals with double pre-beta VLDL (Table 4). Two observations are pertinent to the origin of the heterogeneity of ARP. First, the isoelectric focusing pattern of ARP in VLDL obtained from perfused rat livers cannot be distinguished from that of plasma VLDL (35). Second, during repeated observations for as long as one year, we have failed to observe any change in the number of components of ARP in human VLDL with each of the three patterns: "normal", "variant", and "primary dysbetalipoproteinemia". This constancy of the isoelectric focusing patterns of ARP should prove useful in establishing their prevalence and mode of inheritance. Analysis of the pattern in families may also help to determine whether there is a genetic relationship between any of the patterns and the phenomenon of double pre-beta VLDL.

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