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Primary Dysbetalipoproteinemia: Predominance of a Specific Apoprotein Species in Triglyceride-Rich Lipoproteins

(chylomicrons/very low-density lipoproteins/polyacrylamide gel electrophoresis/tetramethylurea)

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ABSTRACT **Lipoproteins of very low density that are unusually rich in cholesteryl esters accumulate in blood plasma in a characteristic primary form of human hyperlipoproteinemia. These lipoproteins, which are thought to be products of the initial catabolic step in the metabolism of normal triglyceride-rich lipoproteins, have beta rather than pre-beta mobility on electrophoresis, presumably because they have lost certain protein components from their surface. In this study, we have used polyacrylamide gel electrophoresis of apoprotein components that are soluble in tetramethylurea to show that the very low-density lipoprotein fraction of blood serum from seven patients with hyperlipoproteinemia contains unusually large amounts of an arginine-rich protein. Pre-beta migrating, very low-density lipoproteins separated from serum of post-absorptive patients and chylomicrons obtained after a fat-rich meal contain normal amounts of this arginine-rich protein, but beta-migrating, very low-density lipoproteins and chylomicron-like particles separated from serum of post-absorptive patients contain more than twice as much. These apparently partially degraded lipoproteins also contain more tetramethylurea-insoluble protein and smaller amounts of the other soluble protein components than their normal counterparts.**

Primary dysbetalipoproteinemia (Type III hyperlipoproteinemia, "floating beta" disease, xanthoma tuberosum) is an uncommon form of human hyperlipidemia characterized by accumulation in blood plasma of very low-density lipoproteins (VLDL) that have beta rather than pre-beta electrophoretic mobility (1). Like normal VLDL, these lipoproteins are polydisperse, spherical particles composed of a central droplet ("core") of nonpolar triglycerides and cholesteryl esters with a surface monolayer of polar lipid (phospholipids and cholesterol) and protein (2). Present evidence suggests that these particles are degradation products ("remnants") of normal triglyceride-rich lipoproteins that accumulate because of a catabolic defect. Consistent with this concept, apparently normal chylomicrons (3) and VLDL (4) also occur in plasma of affected subjects. The low triglyceride–cholesterol ratio in particles of all sizes in the post-absorptive state (2, 4, 5) can be explained by removal of triglyceride during the initial phases of catabolism. The reduced electrophoretic mobility has been explained by loss of a portion of the apoprotein since beta-migrating VLDL were found to react immunochemically only with antisera to the "B" apoprotein of low-density lipoproteins (LDL), whereas pre-beta VLDL from affected as well as unaffected subjects also react with antisera to high-density lipoproteins (HDL) (4). In addition, the beta-VLDL reportedly contain only traces of two of the "C" apoproteins that comprise a major fraction of the apoprotein of pre-beta VLDL (6). This observation is also in accord with the remnant character of these lipoproteins, since certain C-apoproteins are transferred to HDL during the catabolism of triglyceride-rich lipoproteins (7). In the present study, both chylomicron "remnants" and beta-migrating VLDL of subjects with dysbetalipoproteinemia have been found to contain greatly increased amounts of another polypeptide species normally present in triglyceride-rich lipoproteins.

Abbreviations: VLDL, LDL, and HDL, very low-density, low-density, and high-density lipoproteins, respectively; R-Ser, R-Glu, and R_2-Ala and R_3-Ala, apolipoprotein species with carboxyl-terminal serine, glutamic acid, and alanine (subscripts indicate polymorphic forms of R-Ala), respectively.

METHODS

The diagnosis of primary dysbetalipoproteinemia was made on the basis of primary hyperlipidemia characterized by beta-migrating, cholesterol-rich VLDL. Hypothyroidism was specifically excluded by measurement of serum thyroxine concentration. Healthy adults of both sexes served as controls.

Lipoproteins were separated by preparative ultracentrifugation (8) from blood serum obtained in the morning after the subjects had fasted 12–16 hr. All chemical analyses were performed on samples subjected to a second preparative ultracentrifugation under the same conditions or on subfractions of triglyceride-rich lipoproteins obtained by chromatography on 2% agarose gel (9). Chylomicrons were obtained 4 hr after ingestion of a meal containing 1.5 g of cream fat per kg of body weight (10), by centrifugation at 2×10^6 *g*-min in a 40.3 rotor of a Beckman model L ultracentrifuge at 12° and purified by chromatography on 2% agarose gel (9). Beta and pre-beta VLDL were separated by preparative electrophoresis in a starch block (11). Mobility of lipoproteins was determined by electrophoresis in agarose gel (12), and analyses for constituent lipids and protein were performed as described earlier (2). B-Apoprotein was determined as the difference between content of total protein and protein soluble in 4.2 M 1,1,3,3-tetramethylurea (13). Content of individual tetramethylurea-soluble polypeptide species was determined by electrophoresis in 7.5% polyacrylamide gels followed by standardized staining with Amido Schwarz and densitometric scanning (13). The amounts of protein in the individual bands were within the linear range of response of ab-

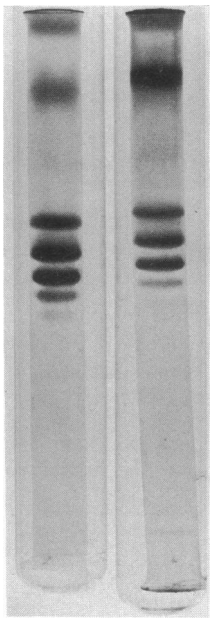

FIG. 1. Polyacrylamide disc gel patterns of tetramethylurea-soluble proteins of VLDL from a normolipidemic subject (*left*) and patient W.M. with dysbetalipoproteinemia (*right*). The slowest migrating band comigrates with authentic R-Ser and the three major fast-migrating bands comigrate with authentic R-Glu, R_2-Ala, and R_3-Ala in order of increasing mobility. The intermediate band typically predominates in patients with dysbetalipoproteinemia. About 120 μg of tetramethylurea-soluble protein was applied to each gel.

sorbance at 550 nm to concentration of pure samples of these proteins obtained by chromatography on DEAE-cellulose (14). Amino-acid composition was determined (15) on hydrolysates of individual bands from stained gels (16).

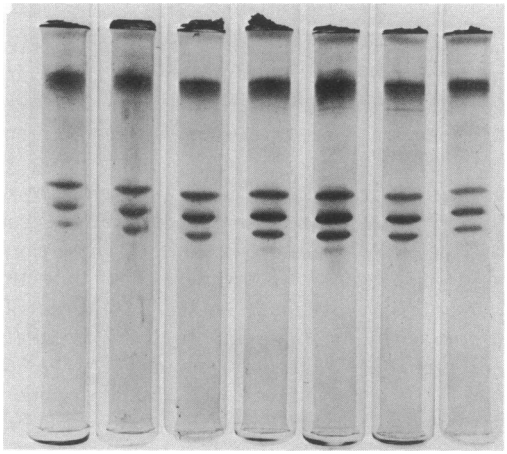

FIG. 2. Patterns of tetramethylurea-soluble proteins of subfractions of triglyceride-rich lipoproteins from patient G.N. From *left:* 6 subfractions eluted from columns of 2% agarose gel with approximate mean diameters of 800, 600, 500, 350, 310, and 280 Å, followed by density fraction 1.006–1.019 g/cm^3 (about 250 Å). Bands seen in the region of the arginine-rich protein account for 52, 44, 38, 37, 37, 37, and 47%, respectively, of the total densitometric band areas. Distinct pre-beta bands were evident on agarose gel electrophoresis of the third through the sixth fractions.

TABLE 1. *Composition of VLDL from normolipidemic subjects and patients with dysbetalipoproteinemia*

	Normolipidemia*		Dysbetalipoproteinemia†		*P* value
Percentage volume of polar constituents‡	29	(2.8)§	31	(4.9)	N.S.
Triglyceride/cholesteryl esters	4.9	(0.81)§	1.4	(0.40)	<0.001
Protein (weight %)	10.5	(0.7)§	7.9	(1.4)	<0.01
B-apoprotein (%)	40	(4.4)	50	(8.6)	<0.01
Tetramethylurea-soluble apoproteins					
(*% of densitometric area*)					
R-Ser	3.9	(2.1)	7.9	(4.5)	<0.05
Arg-rich	17	(2.4)	39	(12)	<0.001
R-Glu	20	(3.1)	15	(3.5)	<0.01
R_2-Ala	32	(2.6)	23	(5.8)	<0.01
R_3-Ala	27	(2.2)	14	(3.7)	<0.001

* Mean values for 10 subjects (5 female and 5 male) ± SD. No significant sex-related differences were observed.

† Mean values for 7 patients (3 female and 4 male) ± SD.

‡ Unesterified cholesterol + phospholipids + protein: an inverse function of particle diameter (2).

§ Values for 5 normal subjects reported in ref. 2.

RESULTS

The polyacrylamide gel pattern of tetramethylurea-soluble protein species of VLDL from patients with dysbetalipoproteinemia differed systematically from that of normal subjects and subjects with primary endogenous hyperlipemia. As shown in Fig. 1, the generally faint band that precedes the protein with carboxyl-terminal serine (R-Ser) in normal subjects and in hyperlipemic patients with VLDL of normal lipid composition predominated in patients with dysbetalipo-

TABLE 2. *Amino-acid composition of arginine-rich protein of VLDL (mol/10^3 mol of amino acids)*

Source	Patients with dysbetalipoproteinemia*			Hyperlipemic subjects†
	R.G.	L.A.	Mean	
Lysine	71	40	55	49
Histidine	16	7	11	13
Arginine	111	98	104	109
Aspartic acid	50	45	47	49
Threonine	32	42	37	39
Serine	45	57	51	56
Glutamic acid	228	242	235	240
Proline	20	22	21	28
Glycine	64	72	68	60
Alanine	125	125	125	111
Valine	57	61	59	70
Half-cystine	0	0	0	0
Methionine	11	29	20	25
Isoleucine	5	7	6	13
Leucine	123	127	125	112
Tyrosine	20	14	17	14
Phenylalanine	23	12	17	14

* Proteins separated on polyacrylamide gel.

† Proteins separated on DEAE-cellulose (ref. 17).

TABLE 3. *Composition of subfractions of very low-density lipoproteins of dyslipoproteinemic patient R.G.*

Sample	Percentage volume of polar constituents	Electrophoretic mobility	Triglyceride/cholesteryl esters	Protein (weight %)	B-Apoprotein (%)	Tetramethylurea-soluble apoproteins (% of densitometric area)				
						R-Ser	Arg-rich	R-Glu	R_2-Ala	R_3-Ala
I. Total VLDL	28	β + pre-β	1.05	7.6	35	15	37	12	20	16
Agarose gel fractions:										
1	16	Origin + smear to pre-β	1.34	2.4	26	19	48	12	10	11
2	25	β + pre-β	1.24	5.6	36	18	34	14	19	15
3	28	β + pre-β	1.17	7.5	47	13	37	13	20	17
4	35	β + slight pre-β	0.95	9.6	57	17	31	13	21	18
Density 1.006–1.019 g/cm³	45	β + trace pre-β	0.38	14.3	76	24	35	8	18	15
II. VLDL*	30	β + pre-β	0.97	9.3	48	—†	25	22	27	26
Starch block fractions:										
1	28	β	0.52	9.1	57	—	44	16	19	21
2	29	Pre-β	3.18	11.1	45	—	14	25	32	28

* Particles larger than 750-Å diameter removed by gel filtration.
† Insufficiently resolved for densitometry.

proteinemia. On the average, this protein accounted for 39% of the tetramethylurea-soluble protein in the patients as compared with 17% in normolipidemic individuals (Table 1). The relative contribution of tetramethylurea-insoluble B-apoprotein was higher and that of C-proteins was lower in the patients. However, the relative amounts of the components of C-proteins were comparable in the two groups.

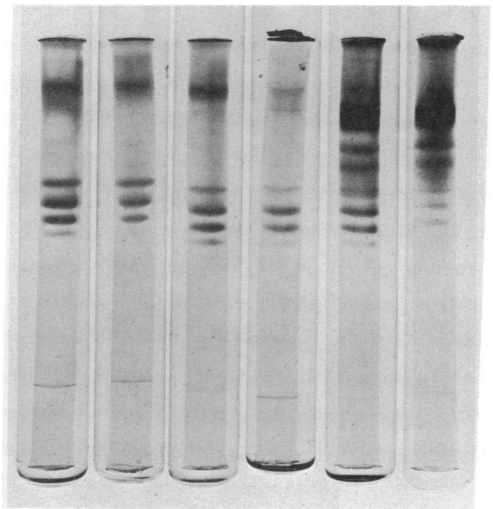

FIG. 3. Pattern of tetramethylurea-soluble proteins from lipoprotein fractions of patient L.An. From *left:* VLDL; beta-migrating VLDL separated by starch block electrophoresis; density fraction 1.006–1.019 g/cm³; LDL (density 1.019–1.063 g/cm³); HDL_2 (density 1.063–1.125 g/cm³); HDL_3 (density 1.125–1.121 g/cm³). A faint band in the region of the major apoprotein species of HDL can be seen in the soluble LDL-proteins, and a band in the region of the arginine-rich protein is evident in HDL_2. The relative amount of the fast-migrating C-proteins (R-Glu and the R-Alas) is greater in HDL_2 than in HDL_3.

The mobility in polyacrylamide gels of the predominant band was closely similar to that of the arginine-rich protein isolated consistently from VLDL by Shore and Shore, who found that it exists in three polymorphic forms with different elution volumes from columns of DEAE-cellulose (17). Polymorphism of the band was also evident in lightly loaded gels (Fig. 2). Its amino-acid composition, determined in two patients, closely resembled that of the arginine-rich protein (Table 2) and differed substantially from that of albumin, which has similar mobility in gels, and from the other tetramethylurea-soluble protein species of VLDL. The predominance of the arginine-rich protein was observed in all subfractions of triglyceride-rich lipoproteins, representing particles of diameters exceeding 800 Å down to about 350 Å (Fig. 2 and Table 3). The relative amount of arginine-rich protein in the tetramethylurea-soluble apoprotein of beta-migrating particles with density between 1.006 and 1.019 g/cm³, which exist in greatly increased quantity in this disorder, was equal to or greater than that in lipoproteins of density <1.006 g/cm³ (mean values 45% and 39%, respectively) (Fig. 3).* A similar pattern was observed in tetramethylurea-soluble proteins of LDL (density 1.019–1.063 g/cm³). A band in this region was also observed in proteins of HDL_2 and, sometimes, of HDL_3. As in healthy individuals,

* During the second preparative ultracentrifugation of VLDL at density 1.006 g/cm³, substantial amounts of lipoprotein often sediment. After subsequent flotation at density 1.019 or 1.063 g/cm³, these particles, which have beta-mobility on electrophoresis, resemble the density 1.006- to 1.019-g/ml fraction in lipid and subunit protein composition. The origin of this material is uncertain, but it should be noted that much less lipoprotein material sediments when VLDL from normolipidemic subjects or patients with other forms of hyperlipemia are similarly centrifuged.

TABLE 4. *Composition of chylomicron fractions from patients with dysbetalipoproteinemia and other subjects*

Source	Percentage volume of polar constituents	Triglycerides/ cholesteryl esters	Protein (weight %)	B-Apoprotein (%)	Tetramethylurea-soluble apoproteins (% of densitometric area) R-Ser	Arg-rich	R-Glu	R_2-Ala	R_3-Ala
I. Post-prandial*									
Normal subject									
J.G.	9	44	0.9	11	13	24	23	20	20
Endogenous hyperlipemia									
N.W.	10	34	2.5	—	7	23	21	28	21
Dysbetalipoproteinemia									
L.A.	10	21	2.5	16	7	22	23	29	19
R.G.	8	15	1.6	9	8	28	23	23	18
II. Post-absorptive dysbetalipoproteinemia									
R.G.	16	1.3	2.4	26	19	48	12	10	11
L.An.	14	3.3	2.6	34	12	66	8	10	4
G.N.	15	2.0	2.5	34	7	52	16	18	7

* 4 hr after ingestion of 1.5 g of cream fat per kg of body weight.

the fast-migrating species, R-Glu and the R-Alas, were present in greater proportion in HDL_2 than in HDL_3 (Fig. 3).

Most of the VLDL-subfractions of affected individuals contain two components of beta and pre-beta mobility (2, 4). The gel pattern of the pre-beta component was similar to that of normal subjects, whereas that of the beta component had greatly increased content of the arginine-rich protein (Fig. 4 and Table 3). Evidence for similar heterogeneity of particles in the size range of chylomicrons was obtained when chylomicrons were separated from serum obtained 4 hr after a fat-rich meal. As shown in Fig. 5 and Table 4, the relative amounts of B-apoprotein and the arginine-rich protein in chylomicrons obtained during active fat absorption from subjects with dysbetalipoproteinemia and from unaffected individuals were considerably lower than those of VLDL and of the largest group of particles (putative chylomicron remnants) separated from serum obtained in the post-absorptive state.

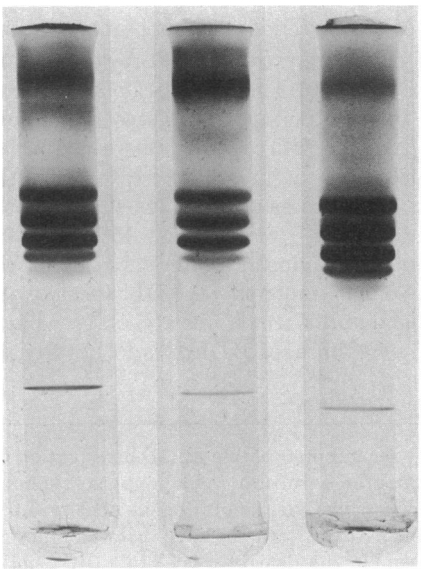

FIG. 4. Pattern of tetramethylurea-soluble proteins of electrophoretic subfractions of VLDL from patient R.G. From *left:* unfractionated VLDL; beta-migrating VLDL; pre-beta migrating VLDL. The densitometric areas obtained from these gels are given in Table 3.

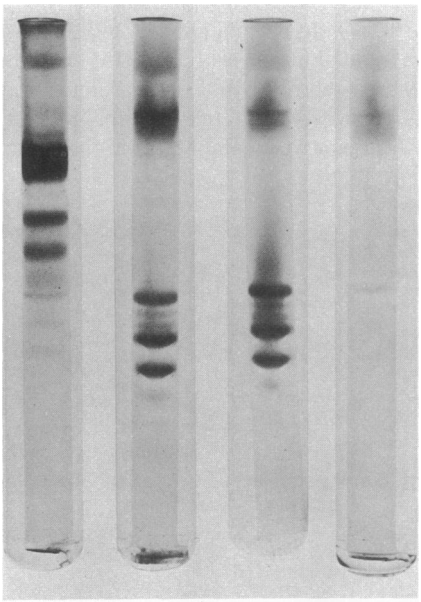

FIG. 5. Pattern of tetramethylurea-soluble proteins of lipoprotein fractions from patient L.A. From *left:* HDL_3; triglyceride-rich lipoproteins of density <1.006 g/cm³ from serum obtained 4 hr after a fat-rich meal; particles of diameters exceeding 750 Å that appeared in the void volume when these triglyceride-rich lipoproteins were subjected to chromatography on 2% agarose gel; particles larger than 750 Å separated similarly from lipoproteins of density <1.006 g/cm³ after an overnight fast. The band representing the arginine-rich protein predominates in the unfractionated triglyceride-rich lipoproteins and in the subfraction obtained after the overnight fast, but not in the subfraction obtained 4 hr after fat ingestion. A faint band in the region of the major apoprotein of HDL can be seen in the unfractionated triglyceride-rich lipoproteins (*second pattern from left*).

DISCUSSION

The characteristic pattern in polyacrylamide gels of tetramethylurea-soluble proteins of triglyceride-rich lipoproteins from patients with primary dysbetalipoproteinemia is consistent with other evidence that this group of patients conforms to a distinctive phenotypic pattern. The slower mobility of the VLDL that accumulate can be explained by a reduced proportion rather than a complete loss of C-proteins, together with a greatly increased content of the arginine-rich protein. The relationship of the accumulation of this polypeptide to the pathogenesis of the disorder is at present uncertain. Possibly, it represents a structural-protein abnormality. However, the observation that pre-beta VLDL and chylomicrons obtained during active fat absorption contain normal amounts of all major apoprotein species is consistent with the concept that newly secreted triglyceride-rich lipoproteins are unaltered and that the abnormally persisting large particles and beta-migrating VLDL constitute populations of partially degraded triglyceride-rich lipoproteins (remnants) from which most of the triglyceride has been removed through the action of lipoprotein lipase (2–4). The increased proportion of B-protein in beta-VLDL and chylomicron remnants may be explained by loss of component C species, consistent with other evidence that C-proteins are returned to HDL during this process, whereas the B-protein is retained, eventually to appear in LDL (7, 18). The increased proportion of the arginine-rich protein cannot be explained similarly, since the mean ratio of arginine-rich protein to B-protein was about 1:4 in VLDL from normolipidemic subjects and in pre-beta VLDL from the patients, and 1:2.5 in beta-VLDL. The data suggest that either the B-protein is also lost during the formation of remnants, or, more likely, that the arginine-rich protein is transferred to the surface of VLDL as the C-proteins are lost. The site from which such transfer might occur and a role for the arginine-rich protein in the catabolism of remnants of triglyceride-rich lipoproteins remain to be demonstrated.

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