

On the Lipoprotein Abnormality in Type III Hyperlipoproteinemia

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ABSTRACT Two lipoprotein species were isolated by starch block electrophoresis from the very low density lipoproteins (VLDL) (S_r 20–400) of patients with type III hyperlipoproteinemia. One of these, α_2 -VLDL, had a content of lipid and protein and physical characteristics similar to VLDL from normal subjects or patients with other forms of hyperglyceridemia. The other species, β -VLDL, contained more cholesterol and less triglyceride in relation to the protein, than normal VLDL. Only the apoprotein of low density lipoprotein was immunologically detectable in the β -VLDL; the proteins in the α_2 -VLDL reacted with antisera specific for low density lipoprotein and high density lipoprotein. The electrophoretic mobility of β -VLDL was similar to that of low density lipoprotein and significantly less than that of α_2 -VLDL. Isolated β -VLDL had a lesser mean flotation rate than α_2 -VLDL, but both α_2 - and β -VLDL were found throughout the S_r 20–400 flotation range.

The relative quantities of α_2 - and β -VLDL could be varied by changing the diet or by heparin administration. Most of the VLDL from type III patients on a high carbohydrate diet was in the α_2 -VLDL form. During fasting, α_2 -VLDL fell and β -VLDL increased becoming the predominant species of VLDL. Heparin-induced acceleration of triglyceride clearance also increased β -VLDL and decreased α_2 -VLDL. These findings suggest a precursor-product relationship between the α_2 - and β -forms of VLDL.

INTRODUCTION

Plasma very low density lipoproteins (VLDL) normally migrate in advance of low density lipoproteins (LDL)

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in most electrophoretic systems. On paper electrophoresis, VLDL¹ occupy a pre- β position (1, 2) and on starch block (3) they migrate as a fairly homogeneous band in the position of α_2 -globulins. The composition of VLDL isolated by electrophoresis or preparative ultracentrifugation is usually quite reproducible, consisting of about 10, 12, and 65% by weight of protein, cholesterol, and triglyceride, respectively (4, 5).

A group of patients has recently been identified with an aberrant form of VLDL. This VLDL has an unusual electrophoretic mobility, extending from the β - to the α_2 -zone on paper electrophoresis (2), and an abnormal composition, including significantly more cholesterol in proportion to triglyceride. The presence of such abnormal VLDL appears to be genetically determined and is associated with unique clinical features leading to its classification as type III hyperlipoproteinemia (2). The isolation from patients with this disorder of two distinct forms of VLDL, characterized by their electrophoretic mobility as β -VLDL and α_2 -VLDL, is described in this report.

METHODS

Patients

12 patients with type III, 5 with type IV hyperlipoproteinemia, and 5 normal volunteers (aged 19–26), classified according to previously published criteria (2), were studied as in-patients at the National Institutes of Health Clinical Center. Each was fed two different diets. The "balanced low cholesterol diet" contained approximately 20, 40, and 40% of calories, respectively as protein, carbohydrate, and fat (P/S ratio of about 2.0), and less than 300 mg cholesterol per day. The "high carbohydrate, low cholesterol diet" contained approximately 80% of calories as carbohydrate (7 gm carbohydrate/kg body weight), 20% as protein, and 1% as fat, and less than 200 mg cholesterol per day. The diets were usually fed for periods of 2–3 wk and adjusted to maintain constant body weight. Plasma triglyceride and

¹ Abbreviations used in this paper: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

cholesterol concentrations and lipoprotein paper electrophoretic patterns were determined on alternate days throughout the study. Detailed lipoprotein studies were usually performed when the lipid pattern had stabilized. The plasma triglyceride concentrations in some of the hyperglyceridemic subjects were rapidly reduced for experimental purposes by either of two methods; (a) complete fasting for up to 3 days; or (b) intravenous administration of 50 mg of sodium heparin.

Lipoprotein preparation

Ultracentrifugation. All blood samples were obtained from patients after a 14-hr overnight fast and collected in disodium EDTA (1 mg/ml). The plasma was separated from the red cells by centrifugation at 2500 rpm for 30 min at 4°C. The plasma was usually processed immediately but occasionally was stored at 4°C for a maximum of 2 days. If, after standing, the plasma from patients with type III hyperlipoproteinemia contained a "chylomicron" layer at the top, it was removed by a 25-min ultracentrifugation at 25,000 rpm ($1.03 \times 10^8 g \text{ min}$). The VLDL were isolated as the supernatant fraction after 16 hr of ultracentrifugation at 40,000 rpm ($1.01 \times 10^8 g \text{ min}$) in a solvent density of 1.006 (6). Ultracentrifugation was performed in a Spinco Model L2 ultracentrifuge (Beckman Spinco, Palo Alto, Calif.) using a 40-rotor head. The isolated VLDL fraction was centrifuged twice more through a 0.85% saline supernate to remove contaminants. The infranatant layer remaining after VLDL isolation was used for high density lipoprotein (HDL) and LDL markers in subsequent electrophoretic studies of the VLDL. LDL was prepared from the *d* 1.006 infranate by heparin-manganese precipitation (7). Ultracentrifugal subfractions of VLDL (S_r 100-400, S_r 60-100, and S_r 20-60) were prepared by the density gradient method of Lossow, Lindgren, Murchio, Stevens, and Jensen (8).

Electrophoresis. Preparative electrophoresis of the ultracentrifugally isolated lipoproteins were performed according to the method of Kunkel and Trautman (3). Potato starch washed with 10 volumes of distilled water and 20 volumes of barbital buffer (0.05 M, pH 8.6) per volume of starch was used to prepare a block with dimensions of $50 \times 20 \times 1$ cm. Electrophoresis was carried out in barbital buffer (0.05 M, pH 8.6) at a field strength of 10 v/cm for an average duration of 18 hr. Albumin labeled with bromphenol blue was added to each run and the mobilities of isolated HDL and LDL were also determined on a separate lane. At the conclusion of the electrophoresis, the starch support was cut transversely at 1 cm intervals. The lipoproteins were eluted from the starch twice with 2 volumes of buffer per volume of starch. Protein, cholesterol, triglyceride, and phospholipids were measured in the eluates of the VLDL electrophoretic fractions. When detailed studies of type III VLDL composition were performed, the eluates of the slower migrating VLDL peak were combined, as were the eluates of the faster migrating peak and the zone of overlap between the two discarded. From 60-75% of the VLDL protein and cholesterol applied was recovered. In a few studies, total lipid was directly extracted from the starch block segments (9), and the relative lipid composition was found to be similar to that of the respective buffer eluates. The eluates of the lane containing HDL and LDL were screened immunochemically to determine the positions of migration of these fractions.

Lipoprotein screening

Paper electrophoresis was performed on all plasma samples and the paper strips were stained for lipid. HDL, LDL, and VLDL concentrations were quantified by their cholesterol content (2). The VLDL were isolated in the ultracentrifuge as described above, and the HDL and LDL measured after heparin-manganese precipitation of the *d* 1.006 infranate fraction (7). The flotation patterns of the lipoproteins were obtained in a Beckman Model E analytical ultracentrifuge (Beckman Spinco, Palo Alto, Calif) by the method of deLalla and Gofman (10). These results were corrected for concentration and Johnston-Ogston effects by a computer program (11), and expressed as concentrations within 29 S_r 0-400 concentration intervals.

Chemical analysis

Proteins were determined by the method of Lowry, Rosebrough, Farr, and Randall (12), using bovine albumin to define the standard curve. Any turbidity in the samples was extracted by diethyl ether before reading.

Lipids were extracted from the lipoproteins by the procedure of Folch, Lees, and Sloane-Stanley (9). Cholesterol, cholesteryl esters, triglycerides, and phospholipids were isolated from the lipid extract after chromatography on a 1 mm silica gel G thick layer plate in 90:10:1, petroleum ether-diethyl ether-glacial acetic acid. The isolated phospholipids were further subfractionated by two dimensional thin-layer chromatography in a chloroform-methanol-water system (13). Mass determinations of cholesterol and triglyceride were obtained by automated techniques (14, 15). Phospholipid concentrations were determined by the lipid phosphorous method of Bartlett (16).

The fatty acid compositions of the isolated steryl esters, triglycerides, and phospholipids were determined after transmethylation with 2 ml of 2% H_2SO_4 in absolute methanol at 65°C for 14 hr (17). The fatty acid methyl esters were extracted from a methanol-water mixture with redistilled heptane and analyzed on a Barber-Colman gas-liquid chromatograph (Barber-Colman Co., Rockford, Ill.) fitted with a hydrogen flame detector and a 0.2 inch \times 8 ft column packed with 15% ethylene glycol succinate on 60-80 mesh chromosorb W. The column temperature was 180°C. The instrument and column were calibrated with NIH methyl ester standards E and F, and methyl oleate, linoleate, linolenate, and arachidonate standards were used as reference standards. Chromatography was continued for a period exceeding approximately twice the retention time of methyl arachidonate. Quantitation was based on the relative area of the fatty acid peaks as obtained by triangulation.

Immunochemistry

Partial delipidation of VLDL for immunochemical studies was accomplished by the diethyl ether technique of Avigan (18) and the heptane delipidation procedure of Gustafson (19). Immuno-electrophoresis, on 1% agarose with a barbital buffer system (pH 8.2, $\mu = 0.1$), and double diffusion analyses were made by conventional techniques (20, 21). The antisera to whole human serum, isolated HDL, LDL, and delipidated VLDL were prepared in rabbits; they have been previously characterized (22, 23).

RESULTS

Composition of VLDL

While on either balanced or high carbohydrate diets, VLDL from patients with type III hyperlipoproteinemia consistently was separated into two fractions by preparative starch block electrophoresis. These were a more rapidly migrating band which moved to the α_2 -position (α_2 -VLDL), and a slower β -migrating band (β -VLDL) (Fig. 1, bottom panel). The mobility of the latter band was the same as that of separately run LDL obtained from either type III patients or controls. The VLDL from both type IV patients and controls migrated to the α_2 region in a homogenous band (Fig. 1, top panel), with mobility identical with that of the rapidly migrating α_2 -VLDL fraction from type III patients.

Both the lipids and protein of the type III-VLDL were distributed in a bimodal fashion on the starch block (Fig. 1, bottom panel). The two VLDL peaks obtained from five type III patients were eluted, and the triglyceride, phospholipid, cholesterol, and protein content of the β -VLDL and α_2 -VLDL determined (Table I). The relative amounts of each component differed in the α_2 - and β -VLDL regions. Compared to the protein content, a much greater amount of cholesterol was consistently present in the β -VLDL fraction, and somewhat

more phospholipid was also found in some of the β -VLDL samples. The triglyceride content relative to protein was much greater in the α_2 -VLDL. This difference between α_2 - and β -VLDL composition was seen on both the balanced and high carbohydrate diets.

The composition of VLDL (α_2 -VLDL) from four patients with type IV hyperlipoproteinemia is shown in Table II. The relative proportions of its components were similar to those of normal VLDL (2, 5) and comparable to those of α_2 -VLDL obtained from patients with type III hyperlipoproteinemia. A slightly higher cholesterol content in the type III samples possibly reflects some contamination of the α_2 -VLDL with β -VLDL in the preparations from these patients. Neither the isolated VLDL of type IV or normal patients on balanced diets had detectable β -migrating material when evaluated on paper or starch block electrophoresis.

The lipids in the VLDL from four type III patients on a balanced diet were further analyzed. In both the α_2 - and β -VLDL fractions, about 60% of the total sterol was esterified and an average of 55% of the total lipid phosphorous was present as lecithin and 22% as sphingomyelin. The proportions of the remaining phospholipids were also similar for both fractions.

TABLE I
Composition of VLDL in Patients with Type III Hyperlipoproteinemia*

Patient†	Age	Sex	β -VLDL				α_2 -VLDL					
			PROT‡	PL	TG	CHOL	PROT	PL	TG	CHOL		
				<i>mg per 100 mg</i>					<i>mg per 100 mg</i>			
H. H.	61	F	13.7	18.5(1.4)¶	42.7(3.1)	25.1(1.8)	11.4	13.7(1.2)	64.8(5.7)	10.1(0.9)		
W. C.	1.	50	M	6.1	16.0(2.6)	39.8(6.5)	38.1(6.2)	5.2	11.1(2.1)	67.6(13.0)	16.1(3.1)	
				2.	7.4	15.2(2.1)	29.2(3.9)	48.2(6.5)	5.9	11.8(2.0)	53.8(9.1)	28.5(4.8)
J. T.	1.	45	M	6.4	22.5(3.5)	44.6(7.0)	26.5(4.1)	5.8	13.9(2.4)	65.7(11.3)	14.6(2.5)	
				2.	8.8	14.4(1.6)	52.8(6.0)	24.0(2.7)	8.4	13.3(1.6)	69.4(8.3)	8.9(1.1)
M. L.	1.	58	F	9.8	21.5(2.2)	29.2(3.0)	39.5(4.0)	8.7	15.2(1.7)	66.9(7.7)	9.2(1.1)	
				2.	12.3	22.5(1.8)	30.1(2.4)	35.1(2.9)	12.4	15.7(1.3)	65.4(5.3)	6.5(0.5)
M. G. R.	1.	33	F	9.3	19.3(2.1)	42.9(4.6)	28.5(3.1)	8.2	17.5(2.1)	54.3(6.6)	20.0(2.4)	
				2.	8.8	20.8(2.4)	41.5(4.7)	28.9(3.3)	8.6	22.5(2.6)	47.9(5.6)	21.0(2.4)
Mean			9.2	19.0(2.1)	39.2(4.3)	32.6(3.5)	7.5	15.0(2.0)	61.8(8.2)	15.0(2.0)		
±SE			0.8	1.0	2.7	2.7	1.1	1.02	1.73	2.14		

* All patients were on balanced diets at the time of study except for study J. T. 2 and M. G. R. 2 where a high carbohydrate diet was fed.

† 1 and 2 denote studies of type III VLDL samples obtained from the same patient on 2 different days.

‡ Abbreviations: Prot. = protein; PL = phospholipids, TG = triglycerides, and CHOL = cholesterol.

|| Standard error of the mean expressed as mg per 100 mg.

¶ Number in parenthesis represents ratio of respective lipid to protein.

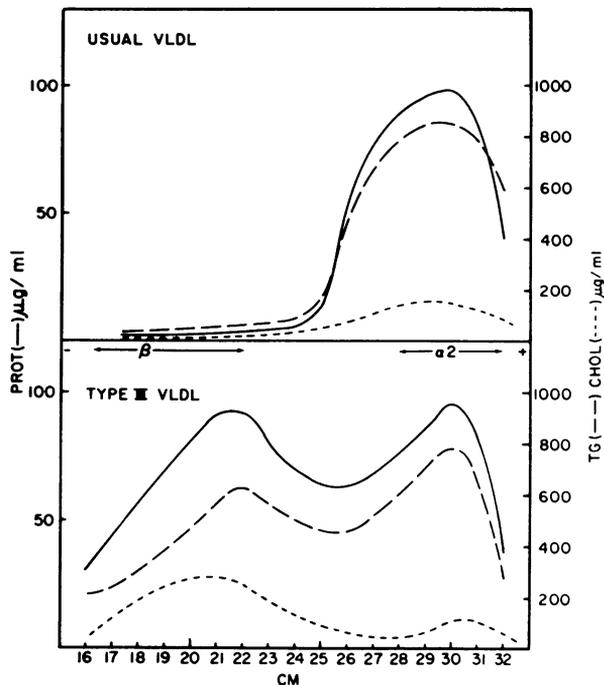


FIGURE 1 Starch block electrophoresis of VLDL from a patient with type IV hyperlipoproteinemia (top panel, usual VLDL) and VLDL from a Type III patient (bottom panel). Both were in-patients on a balanced diet. PROT, protein; TG, triglyceride; CHOL, cholesterol.

Ultracentrifugal subfractions of VLDL

The flotation properties of the two VLDL fractions isolated from plasma of a patient with type III by starch electrophoresis were examined in the analytical ultracentrifuge. The β -VLDL had a mean S_r° of 25 and the α_2 -VLDL a mean S_r° of 55.

The chemical composition of subfractions of VLDL representing S_r 20–60, S_r 60–100, and S_r 100–400 isolated from both type III and type IV plasma of patients

TABLE II
Percent Composition of Total VLDL in Patients with Familial Type IV Hyperlipoproteinemia*

Patient	Age	Sex	PROT†	PL	TG	CHOL
	(yr)			mg per 100 mg		
W. B.	48	M	7.4	10.9 (1.5)‡	70.8 (9.6)	10.9 (1.5)
S. K.	62	F	7.6	15.8 (2.1)	66.2 (8.7)	10.4 (1.4)
A. S.	57	M	8.5	12.4 (1.5)	67.8 (8.0)	11.3 (1.3)
H. W.	46	M	7.2	14.4 (2.0)	67.9 (9.4)	10.5 (1.5)
Mean			7.7	13.4 (1.7)	68.0 (8.8)	10.6 (1.4)
SE§ ±			0.29	1.08	0.99	0.50

* All patients were on balanced diets at the time of study except for S. K. who was on an outpatient *ad libitum* diet.

† Abbreviations same as in Table I.

‡ Standard error of the mean expressed as mg per 100 mg.

§ Number in parenthesis represents ratio of respective lipid to protein.

on a balanced diet is shown in Table III. In type III the relative cholesterol content of each of the subfractions were similar and significantly higher than that in the same subfractions from type IV VLDL. The relative glyceride content was less in the type III subfractions compared to type IV subfractions.

The electrophoretic mobilities of the ultracentrifugal subfractions from type III plasma were compared with the electrophoretic mobility of the unfractionated VLDL on a single starch block. Despite the differences in mean S_r represented by each subfraction, there was no difference in their migration. Each spanned the α_2 - and β -zones and each had peaks at about the same segments. The type IV subfractions and the parent type IV VLDL all had identical α_2 -mobilities.

Fatty acid composition

The fatty acid composition of phospholipids, triglycerides, and cholesteryl esters in the β - and α_2 -VLDL from four type III patients is shown in Table IV. Samples from two of the patients (C.H. and T.P.) represent fatty

TABLE III
Composition of Ultracentrifugal Subfractions of VLDL in Type III and Type IV Hyperlipoproteinemia*

	Protein		Phospholipid		Cholesterol		Triglyceride	
	Type III	Type IV	Type III	Type IV	Type III	Type IV	Type III	Type IV
	mg per 100 mg							
S_r 20–400	10	7	20	15	29	10	42	68
S_r 20–60	13	15	14	23	31	13	33	58
S_r 60–100	8	10	22	17	29	14	42	59
S_r 100–400	5	5	16	19	30	7	48	69

* All values represent the mean of duplicate determinations.

TABLE IV
Type III VLDL Fatty Acid Composition*

Patient	α_2 -VLDL			β -VLDL		
	TG	PL	CE†	TG	PL	CE
	* % of total fatty acids					
C. H.	14:0	2.0	3.3	2.4	1.6	2.8
	16:0	34.2	42.1	31.6	34.8	44.0
	16:1	7.3	5.6	8.4	6.0	5.0
	17:0		1.5		0.3	0.9
	18:0	2.7	12.0	3.6	3.4	8.4
	18:1	49.2	29.2	34.2	48.3	33.9
	18:2	4.6	6.3	19.7	5.6	5.1
T. P.	14:0	2.8	1.1	0.8	1.9	0.7
	16:0	45.3	51.8	23.8	42.1	54.9
	16:1	5.4	1.2	8.7	6.1	1.3
	17:0		0.7			
	18:0	5.1	22.5	1.5	5.6	24.1
	18:1	36.6	12.0	38.6	40.0	12.9
	18:2	4.8	10.8	26.7	4.3	6.0
B. H.	14:0	1.4	2.0	0.9	1.7	5.6
	16:0	25.0	36.9	9.7	24.5	55.8
	16:1	4.0	5.1	5.3	3.5	3.8
	18:0	5.6	9.6	2.1	7.3	22.3
	18:1	34.3	27.6	35.4	46.9	8.7
	18:2	30.0	18.0	46.7	16.2	3.7
	18:3					
P. F.	14:0	1.6	5.3	1.7	2.7	17.4
	16:0	23.1	27.7	10.7	23.3	24.3
	16:1	8.7	8.8	4.6	4.5	5.3
	18:0	2.4	11.3	1.2	6.2	13.1
	18:1	48.3	32.1	28.6	48.3	25.5
	18:2	13.3	14.8	53.2	13.3	6.1
	18:3	1.7			1.7	8.3

* Patients C. H. and T. P. were eating the balanced low cholesterol diet, while B. H. and P. F. were eating an *ad libitum* diet as out-patients. Each was maintaining body weight. All values are the mean of duplicate determinations.

† Abbreviations: CE = cholesteryl esters; others as in Table 1.

acids present after 1 wk of the balanced low cholesterol diet. No obvious differences in fatty acid composition of respective lipid classes from β - and α_2 -VLDL were apparent. In the VLDL obtained from two other out-patients who were eating an uncontrolled diet (B.H. and P.F.), the composition of the phospholipid fatty acids in the two VLDL fractions differed. Higher proportions of linoleate were present in the cholesteryl esters from both β - and α_2 -VLDL of these patients when compared to those on the hospital diet (Table IV).

Immunochemical properties

Ether and heptane delipidation of α_2 -VLDL separately isolated from control, type III and type IV plasmas yielded soluble proteins which reacted with specific antisera to either HDL or LDL. The respective apoproteins from each of the patient groups appeared immunochemically identical both by immunoelectrophoresis and double diffusion. Apoprotein obtained by similar delipidation of β -VLDL from type III plasmas reacted only with antiLDL sera. The apoLDL in this form of VLDL appeared immunochemically identical with normal apoLDL prepared by the same delipidation technique.

Variations in distribution of the type III VLDL fractions

The distribution of the total VLDL mass, in these type III patients, between α_2 - and β -VLDL forms, was alterable by several means. After a few days on the high carbohydrate diet, all the patients had an increase in total VLDL. In this "carbohydrate-induced" state proportionally more of the VLDL was in the α_2 -VLDL fraction

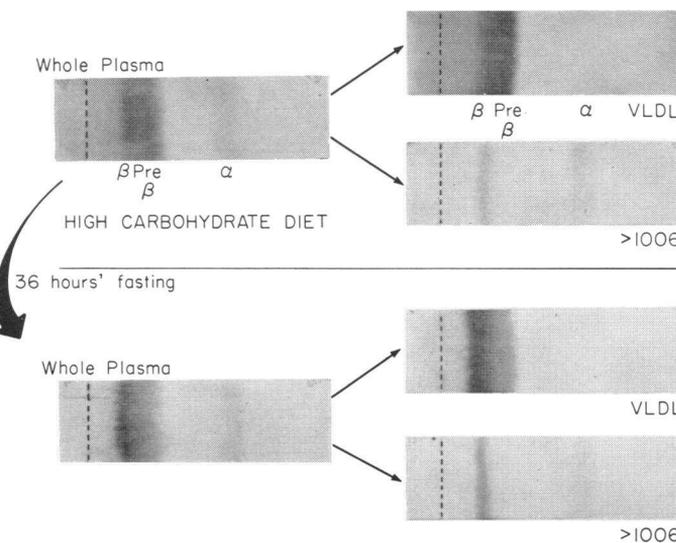


FIGURE 2 Alterations of type III VLDL induced by fasting. Top panel: Pre-fasting lipoprotein electrophoresis. Bottom panel: Lipoprotein electrophoresis 36 hr after fasting.

than there was when the patients were eating the balanced diet or fasting for several days (Fig. 2). Moreover, the ratio of triglyceride to cholesterol in the total VLDL after carbohydrate induction was close to the value (4 to 5) found in normal type IV VLDL. When the plasma triglyceride concentration was abruptly lowered by fasting, more of the VLDL began to migrate as β -VLDL (Fig. 2) and the ratio of triglyceride to cholesterol in whole VLDL approached unity, a ratio characteristic of isolated β -VLDL (Table I).

When the plasma triglyceride concentration was reduced by intravascular lipolysis following heparin injection, changes in VLDL were different for patients with types III and IV hyperlipoproteinemia. A greater loss of triglyceride than protein was observed in type III VLDL, and most of the residual VLDL had an electrophoretic mobility slower than preheparin VLDL (Fig. 3). This mobility was similar to that of the LDL in the patient's postheparin plasma. In contrast all components of the type IV VLDL fell proportionately in response to heparin (23) and the usual increase in VLDL mobility associated with lipolysis (24) was obtained.

Lipoprotein distributions in pre- and postheparin plasma were measured in the analytical ultracentrifuge

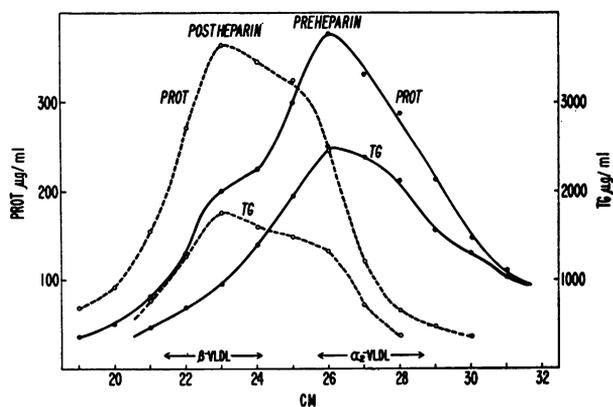


FIGURE 3 The electrophoretic mobility on starch of type III VLDL before and 2 hr after heparin administration.

for a type III and type IV patient on balanced diets (Fig. 4). 2 hr after heparin about 47% of the VLDL (S_r 20-400) was cleared from the plasma of the type IV and 31% from the type III patient. If some of this catabolized VLDL served as precursor to LDL, as has been proposed (25-27), calculations suggest that 20% of the cleared type III VLDL subsequently appeared as S_r

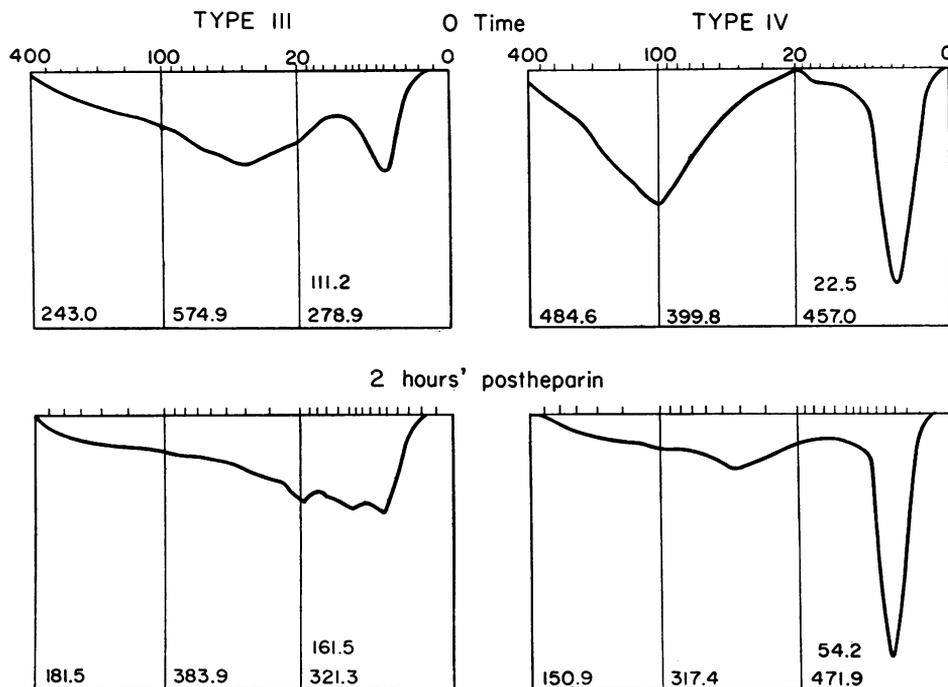


FIGURE 4 Ultracentrifugal patterns of plasma lipoproteins in type III and type IV hyperlipoproteinemia before and 2 hr after heparin administration. The numbers in the boxes under each ultracentrifugal pattern represent the absolute concentration of lipoprotein in mg/100 ml. In each box the three frames, from left to right represent the lipoproteins of S_r 100-400, 20-100, and 0-20. The numbers in the S_r 0-20 box represent the concentrations of S_r 0-12 (below) and 12-20 (above) lipoproteins.

12–20 lipoprotein in contrast to 8% of the cleared type IV VLDL.

DISCUSSION

Analysis of the plasma VLDL isolated from patients with type III hyperlipoproteinemia was undertaken to initiate an understanding of the still unknown metabolic defect in this intriguing disease. Two distinct populations of VLDL were isolated from the type III subjects by starch block electrophoresis. This was in contrast to the single form of VLDL found in normal subjects and type IV patients studied by the same method. The usual α_2 migrating form of VLDL (α_2 -VLDL) was accompanied in type III by a VLDL band having the mobility of beta lipoprotein (β -VLDL). These two VLDL forms also differed in lipid and protein composition as well as flotation properties.

The α_2 -VLDL from type III patients was essentially homogeneous and identical in all its physicochemical properties with VLDL from other subjects. The delipidated apoproteins of type III- α_2 -VLDL reacted with antisera prepared to normal HDL and LDL. Subsequent experiments in this laboratory have further demonstrated that the α_2 -VLDL in type III, as in other subjects, also contains three apoproteins, in quantities roughly similar to normal, that differ from the major apoproteins of LDL and HDL (28). Except for a slight increase in relative cholesterol content, attributed to minor contamination with β -VLDL, the lipid compositions of type III α_2 -VLDL and normal VLDL are similar.

The β -VLDL found in type III was quite different. In both electrophoretic mobility and apoprotein content it appeared identical with normal LDL. Subsequent, more detailed study of the apoprotein of β -VLDL has revealed it to be nearly all apoLDL with only traces of other apoproteins (28). β -VLDL differed from LDL only in its increased content of triglyceride, the latter undoubtedly accounting for the increased flotation rate of β -VLDL over that of normal LDL. When the relative proportions of lipids other than glyceride in β -VLDL are calculated, the ratio of cholesterol to protein is identical with that of LDL and the ratio of phospholipid to protein only slightly higher.

Although the estimated mean S_r of β -VLDL was 25, all density subfractions of VLDL prepared from type III plasma over the range of S_r 20–400 contained β -VLDL detected by starch block electrophoresis. Each such sample of β -VLDL had the same abnormal lipid composition. In normal subjects and patients with type IV hyperlipoproteinemia β -VLDL was not detected in any subfraction of VLDL, and VLDL of increasing S_r rates differed only by relative increases in triglyceride content.

Hazzard, Lindgren, and Bierman have recently re-

ported studies of the composition of VLDL in a patient with type III (29). They observed an abnormal proportion of cholesterol throughout the VLDL density spectrum, but found beta migrating VLDL only in the S_r 20–60 fraction of VLDL. They used paper electrophoresis to search for β -VLDL, a less sensitive test than the concentration of larger quantities of VLDL on a starch block, as used in the present studies.

Despite their differences in physical properties and composition, the α_2 - and β -VLDL in type III plasmas contained the same ratio of free to esterified cholesterol, similar proportions of major phospholipid classes, and similar fatty acid patterns for the respective lipids of the two VLDLs. These findings are compatible with the precursor-product relationship of α_2 -VLDL to β -VLDL suggested by several maneuvers capable of altering the relative concentrations of these two forms of VLDL in type III patients. When plasma triglyceride concentrations were increased by feeding diets high in carbohydrate content, the α_2 -VLDL increased. Following starvation or intravenous heparin administration the plasma triglycerides and total VLDL were acutely depressed and β -VLDL increased at the apparent expense of α_2 -VLDL.

The accumulation of β -VLDL in type III plasma and its persistence through wide swings in VLDL concentration is still unexplained. The anomaly appears to be genetically determined. VLDL having β -mobility is seen in one other form of dyslipoproteinemia, the homozygous state of Tangier disease. Here the retarded migration of VLDL has been attributed to absence of HDL apoproteins in the VLDL (22). The VLDL in Tangier disease is not enriched in cholesterol and has a normal mean S_r (22, 30).

The construction of possible explanations of the defect in type III is hampered by limited knowledge of the normal metabolism of VLDL and its relationship to other lipoprotein families, particular LDL. There is evidence that some, perhaps the bulk, of LDL in plasma could be derived from degradation of VLDL (25, 26). Such residual LDL would arise from VLDL through loss of glyceride and part of the apoprotein component. These events are believed to be dependent upon or closely related to the hydrolysis and removal of the glyceride that probably occurs at or within the vascular endothelium in many tissues and also possibly in hepatic parenchymal cells. Older studies support the "conversion" of protein-labeled VLDL to LDL (27) but at no stage in this process have lipoproteins with the unusual composition of the β -VLDL in type III been identified in plasma.

It is conceivable that the normal removal of VLDL does occur in discrete steps with lipolysis of different VLDL particles governed by separate mechanisms with

possibly one at a late stage of degradation being selectively faulty in the type III disorder. An alternative is the elaboration of abnormal VLDL in type III which cannot progress normally through otherwise intact mechanisms for removal and conversion to LDL. The studies presented here have failed to detect abnormalities in the LDL apoprotein associated with the β -VLDL, but other molecular changes have not been excluded by the analyses.

The evidence which favors α_2 -VLDL as precursor of the β -VLDL in type III does not completely exclude an independent origin of the latter and thus a different explanation for the type III defect. It is obvious that clarification of this problem will move in parallel with acquisition of more information about the normal processes of metabolism of both LDL and VLDL.

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