Pre-β-Very Low Density Lipoproteins as Precursors of β-Very Low Density Lipoproteins

A Model for the Pathogenesis of Familial Dysbetalipoproteinemia (Type III Hyperlipoproteinemia)

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Abstract

The physical, chemical, and receptor binding properties of very low density lipoprotein (VLDL) fractions from familial dysbetalipoproteinemic (dys-β) subjects, homozygous for apolipoprotein (apo-) E2 (E2/2 phenotype), and subjects with the E3/3 phenotype were studied to gain insights into the pathogenesis of dysbetalipoproteinemia, a disorder characterized by the presence of β-VLDL in the plasma. Pre-β-VLDL from dys-β subjects were larger (27 vs. 17 × 10^6 D) and more triglyceride rich (68 vs. 43% dry weight) than β-VLDL. Pre-β-VLDL predominated in the S_f > 100 flotation fraction, whereas β-VLDL predominated in the S_f 20–60 fraction. Because lipolysis converts large VLDL (S_f > 100) in vivo to smaller, more cholesteryl ester-rich VLDL (S_f 20–60), it is likely that pre-β-VLDL are precursors of β-VLDL. Although β-VLDL were not found in type V hyperlipidemic E3/3 subjects, they were induced by intravenous heparinization, suggesting that lipolysis of pre-β-VLDL in vivo can result in β-VLDL formation. Similarly, heparinization of a dys-β subject produced more β-VLDL, at the expense of pre-β-VLDL. The pre-β-VLDL from normolipidemic and type V hyperlipidemic E3/3 subjects, respectively, had 90 and 280 times the affinity for the apo-B,E(LDL) receptor than did the pre-β-VLDL from dys-β subjects. Heparin-induced β-VLDL from type V hyperlipidemic subjects had a sixfold higher binding affinity than did heparin-induced β-VLDL from dys-β subjects. These data suggest that pre-β-VLDL from E2/2 subjects interact poorly with lipoprotein receptors in vivo, decreasing their receptor-mediated clearance and increasing their conversion to β-VLDL during lipolytic processing.

Introduction

Familial dysbetalipoproteinemia, also called type III hyperlipoproteinemia, is a hereditary disorder characterized by the presence of abnormal, cholesteryl ester-rich, β-migrating very low density lipoproteins (β-VLDL) in the plasma (1, 2). The disorder is strongly associated with homozygosity for apolipoprotein (apo-) E2, a mutant of apo-E. Although virtually all individuals homozygous for apo-E2 (E2/2 phenotype) have β-VLDL in their plasma, most are normolipidemic or hypolipidemic (3, 4). Only ~ 1% of E2/2 subjects develop overt hyperlipidemia, the result of marked elevation in β-VLDL. Most individuals with severe dysbetalipoproteinemia are affected by other hereditary or environmental factors that predispose them to hypertriglyceridemia (4, 5). Correction of hyperlipidemic factors, such as obesity or hypothyroidism, may completely reverse the elevated concentrations of triglyceride and cholesterol (5–7).

Apolipoprotein E was first linked to the pathogenesis of dysbetalipoproteinemia when it was identified as a prominent protein constituent of β-VLDL (8). Subsequently, apo-E was shown to be polymorphic by isoelectric focusing, and a particular isoform of apo-E was shown to be associated with type III hyperlipoproteinemia (9). This isoform displayed abnormally low uptake when complexed to phospholipids and perfused through the livers of estradiol-treated rats (10). Also, it was cleared from the plasma of dysbetalipoproteinemic (dys-β)[1] or normal subjects more slowly than normal apo-E (11).

The three common allelic forms of the human apo-E gene, E2, E3, and E4, give rise to three homozygous phenotypes (E2/2, E3/3, and E4/4) and three heterozygous phenotypes (E3/2, E3/4, and E4/2) (12, 13). Apolipoprotein E2 differs from apo-E3, the most common form, by a single amino acid, usually a substitution of cysteine for arginine at residue 158 (Arg_{158} → Cys) (13, 14). However, other rare substitutions also result in an apo-E2 phenotype (13, 15). In each case, purified apo-E2, when recombined with phospholipid, has lower apo-B,E(LDL) receptor binding affinity in vitro than does apo-E3. The binding affinity of apo-E3 ranges from 2.5- to 100-fold higher than that of the various apo-E2 mutants (for review, see references 16, 17). Dysbetalipoproteinemia has also been described in individuals with the E3/3, E3/2, and E2/1 phenotypes (15, 18–20). There is no obvious correlation between the magnitude of the binding defect expressed by the apo-E variants and the severity of dysbetalipoproteinemia.

Because apo-E mediates the interaction of VLDL with lipoprotein receptors in vitro (21–25), it is likely that apo-E is important for the receptor-mediated clearance of VLDL in vivo. During lipolytic catabolism of large VLDL (S_f 100–400), > 90% of the particles irreversibly leave the plasma, presumably by binding to lipoprotein receptors, before being converted to LDL (26, 27). In dys-β subjects, the clearance of a significant portion of VLDL with S_f 100–400 is delayed (26), a finding consistent with the hypothesis that defective interaction of apo-E with lipoprotein receptors contributes to dysbetalipoproteinemia. Delayed clearance of VLDL in dysbetalipoproteinemia involves lipoproteins of both hepatic and in-

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Abbreviations used in this paper: dys-β, familial dysbetalipoproteinemic; EC_{50}, the 50% effective concentration of competition; pre-β-VLDL, pre-β-migrating very low density lipoproteins; β-VLDL; β-VLDL, β-migrating very low density lipoproteins.

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testinal origin (28). Furthermore, the conversion of VLDL to LDL in dys-β subjects is delayed (29, 30). However, a problem confounding the interpretation of VLDL turnover studies in dysbetalipoproteinemia is the heterogeneity of the injected material. In particular, VLDL from dys-β subjects contain pre-β-VLDL, which are larger and more triglyceride rich than β-VLDL and may be precursors of β-VLDL (31–34). The kinetics of pre-β-VLDL and β-VLDL after intravenous administration of [^{14}C]palmitate are consistent with a precursor-product relationship (34). However, the in vivo kinetics of pre-β-VLDL and β-VLDL have not been studied separately using a nonexchangeable marker such as apo-B100 or apo-B48. Furthermore, no attempt has been made to include pre-β-VLDL and defective apo-E-mediated binding to receptors in a model of the pathogenesis of dysbetalipoproteinemia.

The present study was designed to examine the role of apo-E-mediated binding to lipoprotein receptors in the formation of β-VLDL. The physical, chemical, and receptor binding properties of pre-β-VLDL and β-VLDL were studied using dys-β subjects homozygous for apo-E2 (Arg_{158} → Cys) and normolipidemic or type V hyperlipidemic subjects homozygous for apo-E3. The data are incorporated into a model for the metabolism of $d < 1.006$ g/ml lipoproteins in dysbetalipoproteinemia in which the receptor-mediated catabolism of pre-β-VLDL, large, triglyceride-rich lipoproteins, is decreased as a result of their low affinity for lipoprotein receptors. This model proposes that decreased clearance of pre-β-VLDL from the plasma results in increased lipolytic conversion to smaller, more cholesteryl ester-rich β-VLDL.

Methods

Characteristics of subjects studied

Three dys-β (E2/2 phenotype) subjects, two type V hyperlipidemic (E3/3) subjects, and five normolipidemic (E3/3) subjects were studied (Table I). All three dys-β subjects have palmar and tuberous xanthomas and are homozygous for apo-E2(Arg_{158} → Cys) as determined by the method of Utermann et al. (35). The substitution in apo-E2 from dys-β subject D.R. is known to be Arg_{158} → Cys by protein sequencing (13). He has mild type II diabetes mellitus and was taking chlorpropamide (0.5 g) and gemfibrozil (0.6 g) twice daily at the time of this study. Subject S.B. is his sister. Subjects S.B. and N.K. did not take any medication for at least 1 mo before this study.

The type V hyperlipidemic subjects took no medication for at least 1 mo before the study. Subject E.T. has a history of gout. The sequence of the putative receptor binding region of his apo-E is identical to that of apo-E3 (Rall, S. C., Jr., personal communication). Subject C.F. had two episodes of pancreatitis before his lipid disorder was diagnosed. The normolipidemic controls were taking no medications that affect lipid concentrations. All subjects gave informed consent for the study; their participation was approved by the Human Research Committee at University of California, San Francisco.

Table I. Characteristics of Subjects Studied

Subject*	Age	Sex	Plasma cholesterol	Plasma triglyceride	Lipoprotein phenotype	Apo-E phenotype
			mg/dl	*mg/dl*		
D.R.	55	M	307	264	dys-β (type III)	E2/2
S.B.	58	F	336	339	dys-β (type III)	E2/2
N.K.	63	F	470	1480	dys-β (type III)	E2/2
E.T.	37	M	331	910	Type V	E3/3
C.F.	41	M	192	1350	Type V	E3/3
1	40	F	126	83	Normal	E3/3
2	39	M	145	56	Normal	E3/3
3	25	M	175	101	Normal	E3/3
4	57	F	197	117	Normal	E3/3
5	28	M	149	87	Normal	E3/3

* Subjects D.R., S.B., and N.K. were 49, 49, and 72%, respectively, above their ideal body weights. All other subjects were within 10% of their ideal body weight.

Lipoprotein preparation

Subjects fasted for 14 h before blood sampling. Blood samples were adjusted to contain 1 mg of EDTA (sodium salt)/ml and immediately placed on ice. Plasma was separated from the cells by centrifugation at 2,000 rpm for 15 min at 4°C. To prevent proteolytic degradation, plasma was adjusted to contain 10,000 U of aprotinin/liter (Mobray Chemical Corp., New York), 1 mM benzamidine, 1 mM PMSF, and 80 mg of gentamicin/liter (Sigma Chemical Co., St. Louis, MO) (36).

Intravenous heparin administration. Sterile heparin (100 IU/kg of body wt) (Elkins-Sinn, Cherry Hill, NJ) was administered by bolus injection into a cubital vein of the fasted subjects. Blood was removed by phlebotomy 1 h after injection and processed on the same day as described above.

Isolation of lipoproteins by ultracentrifugation. The $d < 1.006$ g/ml lipoproteins and LDL were isolated from plasma by ultracentrifugation as described (25). The $S_f > 100$, S_f 60–100, and S_f 20–60 flotation fractions were isolated according to the method of Lossow et al. (37).

Pevikon block electrophoresis. Lipoproteins were separated by electrophoresis using a Pevikon block (Mercer Consolidated Corp., Yonkers, NY) (38, 39). Human LDL were used as a reference for β-electrophoretic mobility for each Pevikon block. The cutting of the blocks was based on visual inspection; the pre-β-VLDL were faint yellow and were contiguous with the darker yellow of β-VLDL. Samples were concentrated under nitrogen gas using PM-30 membranes (Amicon Corp., Lexington, MA). The electrophoretic mobility of each fraction was confirmed using 1% agarose gels (Corning, Palo Alto, CA) in 0.05 M sodium barbital buffer, pH 8.6, for 35 min at 25°C and 90 V. Postheparinization samples required 1:16 dilution (vol/vol) with defatted BSA (100 mg/ml) because of their increased content of free fatty acids (40). Agarose gels were stained with fat red 7B (Corning Medical and Scientific, Medfield, MA).

Separation of VLDL by gel filtration chromatography. Whole plasma was dialyzed at 4°C in a buffer containing 0.01 M Tris, 0.15 M NaCl, and 0.001 M EDTA, pH 7.5. The dialyzed plasma was spun at 2,000 rpm for 10 min to remove clotted material, and 25 ml of the plasma was then applied to a 2.5- × 107-cm column of Sephacryl S-1000 (Pharmacia Fine Chemicals, Uppsala, Sweden) at a rate of 30 ml/h at 4°C. Fractions (5 ml) were collected and assayed for triglyceride and cholesterol content (see below). The electrophoretic mobility of lipoproteins in each fraction was determined using the 1% agarose gels. The triglyceride peak eluted ahead of the cholesterol peaks, and the leading half was divided into pools for use in receptor binding assays.

Lipoprotein and apolipoprotein characterization

Measurement of protein, cholesterol, triglyceride, and phospholipid was done three or four times with different aliquot sizes. Protein content was determined by the method of Lowry et al. (41) using BSA as the standard. Cholesterol and triglyceride contents were determined by a colorimetric enzymatic assay (Boehringer Mannheim Corp., Indianapolis, IN). Phospholipid content was estimated from the phosphorus content (42). Cholesteryl esters were measured by saponification in methanol/KOH and analyzed using a 10-m × 0.53-mm nonpacked, bonded, fused silica gas-liquid chromatography column coated with RSL-150 (No. 935110, Alltech Associates Inc., Deerfield, IL) at 250°C with nitrogen carrier gas flow rate of 11 ml/min. Stigmasterol was

added to each sample as an internal standard. The apolipoprotein content of the lipoproteins was determined by reducing samples in 0.05 M DTT followed by electrophoresis on 5–20% SDS-polyacrylamide gradient gels. The Coomassie-stained gels were scanned with a GS 300 transmittance/reflectance scanning densitometer (Hoefer Scientific Instruments, San Francisco, CA). Apolipoprotein E concentration was determined using a solid-phase RIA. The apo-E phenotypes were determined by isoelectric focusing on 6-cm, 5% polyacrylamide gels containing 8 M urea and 5% ampholyte (pH 4-6) (LKB, Bromma, Sweden). Lipoproteins were sized using a JEOL 100CX II electron microscope and phosphotungstic acid as a negative stain. The molecular weights of lipoproteins were estimated from their average molecular volume, assessed by measuring the diameters of 100 particles and using the partial specific volumes of the chemical constituents (43).

Fibroblast binding assays

Normal human fibroblasts were grown in 35-mm petri dishes (44). Lipoproteins were iodinated (specific activity 200–500 cpm/mg) by the iodine-monochloride method (45). The ability of $d < 1.006$ g/ml fractions at various concentrations to compete for the binding of ^{125}I-LDL at 2 μg of protein/ml at 4°C was determined in duplicate (44). Binding data were analyzed by nonlinear least squares curve fitting (Allfit) (46). All curves were fit simultaneously with their corresponding LDL standard curves. The upper and lower limits of the curves were shared for each curve fitting. The fitted curves are not shown. The average EC_{50} values presented are geometric means±standard deviations (47).

Monoclonal antibodies that specifically inhibit binding by apo-E (1D7) or apo-B100 (4G3) to the apo-B,E(LDL) receptor were generously provided by Drs. Y. Marcel and R. Milne, Clinical Research Institute of Montreal. The ability of 1D7 or 4G3 to inhibit the binding of ^{125}I-VLDL fractions to cultured human fibroblasts at 4°C was determined by incubating medium containing an excess of antibody and 1 μg of ^{125}I-VLDL/ml for 1 h at 25°C before use in the binding assays (25). Nonspecific binding was defined for each ^{125}I-labeled $d < 1.006$ g/ml lipoprotein fraction as the amount bound in the presence of 150- or 200-fold excess of the corresponding unlabeled lipoprotein fraction. All measurements were performed twice.

Results

Characterization of pre-β-VLDL and β-VLDL. The characteristics of the subjects studied are listed in Table I. The plasma cholesterol and triglyceride concentrations in the dys-β subjects ranged from modestly to markedly elevated. The size and the triglyceride content of both pre-β-VLDL and β-VLDL isolated by Pevikon block electrophoresis (Table II) increased with the severity of hyperlipidemia. The pre-β-VLDL had, on average, a 61% larger molecular weight and contained 147% more triglyceride, 68% less cholesteryl ester, and 60% more protein per particle than did the β-VLDL. On average, the pre-β-VLDL were less dense than the β-VLDL: 0.946 and 0.983 g/ml, respectively (as determined from their chemical compositions). In each dys-β subject the pre-β-VLDL had a wider size range than the β-VLDL, as indicated by the standard deviations for particle diameters (Table II).

The apolipoprotein content of the $d < 1.006$ g/ml fractions was estimated by scanning densitometry of SDS-polyacrylamide electrophoretic gels (Fig. 1). The relative amounts of apo-B100, apo-B48, apo-E, and total apo-C were determined (Table III). The pre-β-VLDL from dys-β subjects had lower apo-E to total apo-C ratios (E:C) than did β-VLDL, but slightly higher E:C ratios than did pre-β-VLDL from normolipidemic E3/3 subjects. The ratio of total apo-B (apo-B100 plus apo-B48) to apo-E plus apo-C (B:E + C) was lower in the pre-β-VLDL than in β-VLDL. Furthermore, the pre-β-VLDL were comparatively enriched in intestinal lipoproteins, as indicated by their higher apo-B48:apo-B100 ratio. The content of apo-E (% total protein) in pre-β-VLDL and β-VLDL from S.B. and D.R. was measured by RIA and averaged 11.5±0.03% and 23.3±3.7%, respectively. Albumin and other unidentified proteins were observed on SDS-polyacrylamide gels after electrophoresis of $d < 1.006$ g/ml lipoproteins isolated in a fixed angle rotor (Fig. 1) and were similar in all subjects. These proteins were not seen in lipoprotein fractions isolated in a swinging bucket rotor.

Characterization of $S_f > 100$, S_f 60–100, and S_f 20–60 fractions. Electrophoresis of the $S_f > 100$, S_f 60–100, and S_f 20–60 fractions from dys-β subject S.B. on 1% agarose revealed that the content of β-VLDL increased as the flotation rate decreased (Fig. 2). Isolation and sequential flotation of the

Table II. Composition (% Dry Weight) and Size of $d < 1.006$ Fractions*

Subject	Sample	Protein	Free cholesterol	Cholesteryl ester	Triglyceride	Phospholipid	Diameter‡	Molecular weight (×10⁶)
							nm	
Dys-β (E2/2)								
D.R.	Pre-β-VLDL	6.48±0.40	5.83±0.52	16.0±1.30	55.5±1.88	16.2±1.23	40.2±7.7	20.1
S.B.	Pre-β-VLDL	4.04±0.05	4.04±0.31	10.2±0.77	70.3±6.43	11.4±0.69	41.6±9.8	21.7
N.K.	Pre-β-VLDL	5.03±0.08	2.09±0.21	5.94±0.59	79.5±13.8	7.49±0.80	51.2±16.2	40.4
Normolipidemic (E3/3)								
1	Pre-β-VLDL	8.35±0.03	3.54±0.14	8.56±0.34	58.7±8.12	20.9±2.32	37.4±10.0	16.3
2	Pre-β-VLDL	7.22±0.08	3.37±0.14	6.99±0.30	63.4±7.13	19.1±0.87	41.7±6.9	19.1
Dys-β (E2/2)								
D.R.	β-VLDL	8.22±0.38	9.85±0.48	31.3±1.50	31.5±0.22	19.2±2.05	32.9±5.6	11.3
S.B.	β-VLDL	4.60±0.13	7.99±0.52	30.8±1.96	39.7±0.52	16.9±0.72	36.4±4.0	14.9
N.K.	β-VLDL	4.06±0.11	5.68±0.25	20.3±0.91	57.1±13.2	12.9±1.11	43.4±9.9	24.9
Dys-β (E2/2)								
S.B.	$S_f > 100$	3.14±0.10	6.01±0.49	25.8±2.21	50.3±1.64	14.8±1.80	51.5±13.9	41.6
S.B.	S_f 60–100	5.40±0.51	7.09±0.88	29.9±3.88	41.4±0.25	16.3±1.88	40.3±3.1	20.3
S.B.	S_f 20–60	9.50±0.69	10.7±0.54	32.3±1.60	27.3±0.40	20.1±0.94	28.6±2.9	7.4

* Percent dry weight±SD of three or four measurements. ‡ Diameter of 100 particles±SD.

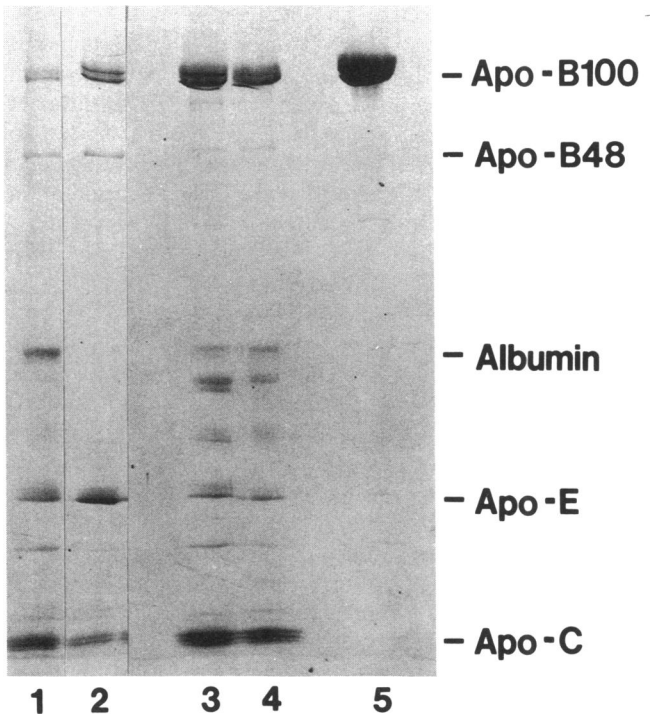

Figure 1. 5–15% SDS-PAGE of $d < 1.006$ g/ml fractions isolated using a fixed-angle rotor. Samples were reduced with 0.05 M DTT. Lanes *1* and *2*, pre-β-VLDL and β-VLDL from dys-β subject N.K., respectively. Lanes *3* and *4*, pre-β-VLDL from normolipidemic E3/3 subjects 1 and 2, respectively. Lane *5*, LDL from a normal subject.

pre-β-VLDL from dys-β subject S.B. showed that 48% of the protein was in the $S_f > 100$ fraction compared with 33 and 19% in S_f 60–100 and S_f 20–60 fractions, respectively. The distribution of β-VLDL was 15, 19, and 66%, respectively, in these fractions. Comparison of the agarose electrophoresis of the flotation fractions from all dys-β subjects suggested that the flotation rate of both pre-β-VLDL and β-VLDL increased as the degree of hyperlipidemia rose. The content of β-VLDL in the $S_f > 100$ fraction was greatest in the most hyperlipidemic subject, N.K. (data not shown).

The $S_f > 100$ fraction, which had the highest content of pre-β-VLDL, also had the highest triglyceride content (Table II). The S_f 20–60 fraction, in which β-VLDL predominated, was the most cholesteryl ester-rich. The apolipoprotein patterns of the $S_f > 100$, S_f 60–100, and S_f 20–60 flotation fractions from dys-β subject S.B., hyperlipidemic E3/3 subject E.T., and normolipidemic E3/3 subject 3 are shown in Fig. 3.

Table III. Scanning Densitometry of SDS-PAGE

Subject	Sample	E:C* ratio	B:E + C‡ ratio	B48:B100§ ratio
Dys-β (E2/2)				
N.K.	Pre-β-VLDL	0.46	0.24	0.49
N.K.	β-VLDL	1.10	1.10	0.18
Normolipidemic (E3/3)				
1	Pre-β-VLDL	0.34	1.10	—
2	Pre-β-VLDL	0.33	1.00	—
Dys-β (E2/2)				
S.B.	$S_f > 100$	0.76	0.56	—
S.B.	S_f 60–100	0.87	0.72	—
S.B.	S_f 20–60	0.96	1.10	—
Hyperlipidemic (E3/3)				
E.T.	$S_f > 100$	0.45	0.45	—
E.T.	S_f 60–100	0.45	0.72	—
E.T.	S_f 20–60	0.63	1.60	—
Normolipidemic (E3/3)				
3	$S_f > 100$	0.23	0.57	—
3	S_f 60–100	0.08	0.88	—
3	S_f 20–60	0.15	1.20	—

* Ratio of apo-E to total apo-C. ‡ Ratio of total apo-B (apo-B100 and apo-B48) to apo-E and apo-C. § Ratio of apo-B48 to apo-B100.

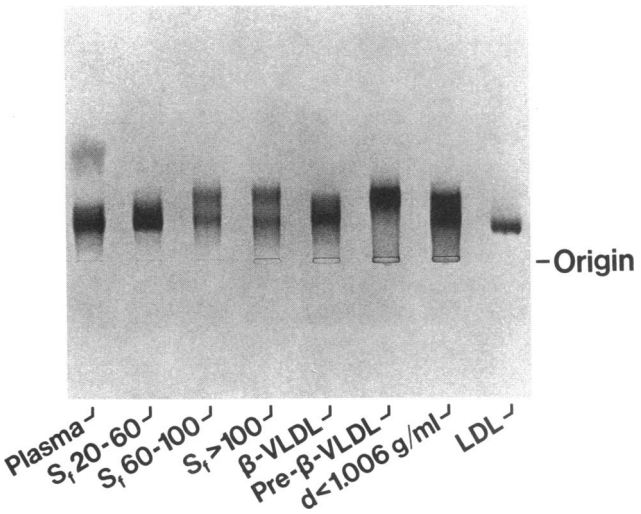

Figure 2. 1% agarose electrophoresis of $d < 1.006$ g/ml fractions from dys-β subject S.B. Samples were diluted with defatted BSA as described in Methods. The LDL in lane *8* were from a normal subject.

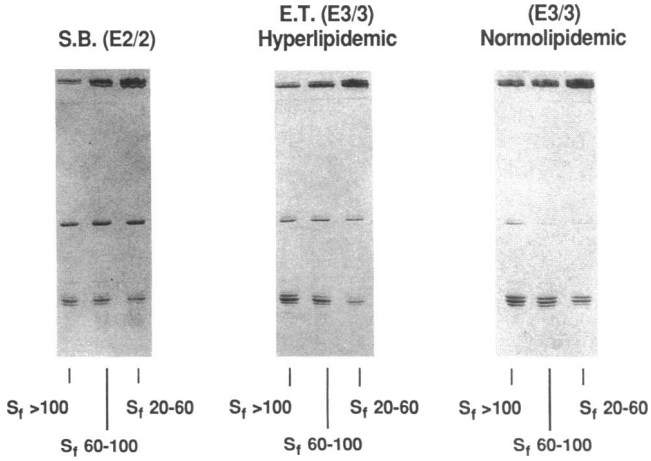

Figure 3. 5–20% SDS-PAGE of lipoproteins isolated in an SW41 rotor. Samples were reduced in 0.05 M DTT. (*Left panel*) $S_f > 100$, S_f 60–100, and S_f 20–60 from dys-β subject S.B. (*Center panel*) $S_f > 100$, S_f 60–100, and S_f 20–60 from hyperlipidemic E3/3 subject E.T. (*Right panel*) $S_f > 100$, S_f 60–100, and S_f 20–60 from normolipidemic E3/3 subject 3. The fractions from dys-β subject S.B. and hyperlipidemic subject E.T. were isolated from whole plasma. The fractions from subject 3 were isolated from the $d < 1.006$ g/ml supranatant using a fixed-angle rotor.

In the dys-β subject, the S_f 20–60 fraction, which had the greatest β-VLDL content, also had the greatest relative content of apo-E (Table III). All fractions from the dys-β subjects had more apo-E than the corresponding fractions from both normolipidemic and hyperlipidemic E3/3 subjects (Table III). The B:E + C ratios in all subjects were similar with respect to flotation rate. The apo-B48:apo-B100 ratios were highest in the S_f > 100 fraction in dys-β and E3/3 subjects (data not shown).

Effects of heparinization. Administration of heparin to the hyperlipidemic E3/3 subjects was associated with the formation of β-migrating lipoproteins with d < 1.006 g/ml. In the hyperlipidemic E3/3 subject E.T., there was a large decrease in plasma triglyceride (910 to 545 mg/dl) and a modest decrease in cholesterol (331 to 292 mg/dl) 1 h after heparinization. Total VLDL protein (S_f > 20) decreased 44% after heparin administration, concomitant with a redistribution of protein mass, predominantly to the S_f 20–60 fraction. The S_f 20–60 fraction increased from 36 to 82% of the total VLDL protein, and its cholesterol content (% dry weight) increased from 19 to 25% after heparin was injected.

Heparin administration to the dys-β subject D.R. resulted in a decrease in plasma triglyceride and cholesterol from 264 and 307 mg/dl to 117 and 268 mg/dl, respectively. A 71% decrease in total VLDL (S_f > 20) protein was associated with an increase in the S_f 20–60 fraction from 75 to 89% of the total VLDL protein. The cholesterol content of the S_f 20–60 fraction (% dry weight) increased from 34 to 42% after heparinization.

Binding of pre-β-VLDL and β-VLDL to the apo-B,E(LDL) receptor. The affinity of the d < 1.006 g/ml fractions for the apo-B,E(LDL) receptor of human fibroblasts was determined by their ability to compete for the binding of ^{125}I-LDL. A competition curve of ^{125}I-LDL versus unlabeled LDL was included as a control in each binding assay and as an indicator of interassay variation. The concentration of unlabeled LDL that resulted in 50% competition for the binding of ^{125}I-LDL (EC_{50}) was 2.54±0.37 μg of protein/ml (~ 4.5 nM) for 10 LDL preparations in 19 different assays. The competition curves of pre-β-VLDL from the dys-β, E3/3 hyperlipidemic, and E3/3 normolipidemic subjects are shown in Fig. 4. The pre-β-VLDL from the dys-β subjects displayed markedly impaired binding and did not completely compete for ^{125}I-LDL binding even at 1,000 μg of protein/ml. Because the binding affinities of pre-β-VLDL from the dys-β subjects were often too low to estimate using computer-assisted curve fitting, some affinities are reported as an EC_{50} greater than the highest concentration assayed. For example, the EC_{50} for pre-β-VLDL from dys-β subject D.R. was > 300 μg of protein/ml, whereas the EC_{50} values for pre-β-VLDL from dys-β subjects S.B. and N.K. could be estimated as 250 and 1,700 μg of protein/ml, respectively (Fig. 4 *A*). When expressed on a molar basis using the molecular weights from Table II, the EC_{50} values of pre-β-VLDL from D.R., S.B., and N.K. were > 230, 280, and 310 nM, respectively. The EC_{50} values for pre-β-VLDL from the hyperlipidemic E3/3 subjects E.T. and C.F. were 2.4 and 1.2 μg of protein/ml, respectively (Fig. 4 *B*). The EC_{50} measurements of pre-β-VLDL from the five normolipidemic E3/3 subjects varied between 2.5 and 18 μg of protein/ml (Fig. 4 *C*). In molar terms, using data from Table II, the respective EC_{50} values of pre-β-VLDL from normolipidemic E3/3 subjects 1 and 2 were 6.4 and 2.3 nM.

Compared with pre-β-VLDL, β-VLDL from the dys-β subjects D.R., S.B., and N.K. competed much more effectively for the binding of ^{125}I-LDL. Their respective EC_{50} values were 4.3, 14, and 3.8 μg of protein/ml or 4.6, 20, and 3.7 nM (data not shown).

The binding affinities of pre-β-VLDL and β-VLDL are summarized in Table IV. The degree of hyperlipidemia of these dys-β subjects varied considerably. In S.B. and N.K. the variation in hyperlipidemia resulted from a reduction in plasma lipids associated with dietary caloric restriction and weight loss. The details of these studies will be reported elsewhere (manuscript in preparation). Based on the mean EC_{50} values from Table IV, β-VLDL from the dys-β subjects had 62-fold greater affinity than their pre-β-VLDL. Pre-β-VLDL from normolipidemic and hyperlipidemic E3/3 subjects had 90- and 280-fold, respectively, greater affinity than pre-β-VLDL from dys-β subjects.

The Pevikon block of d < 1.006 g/ml lipoproteins from

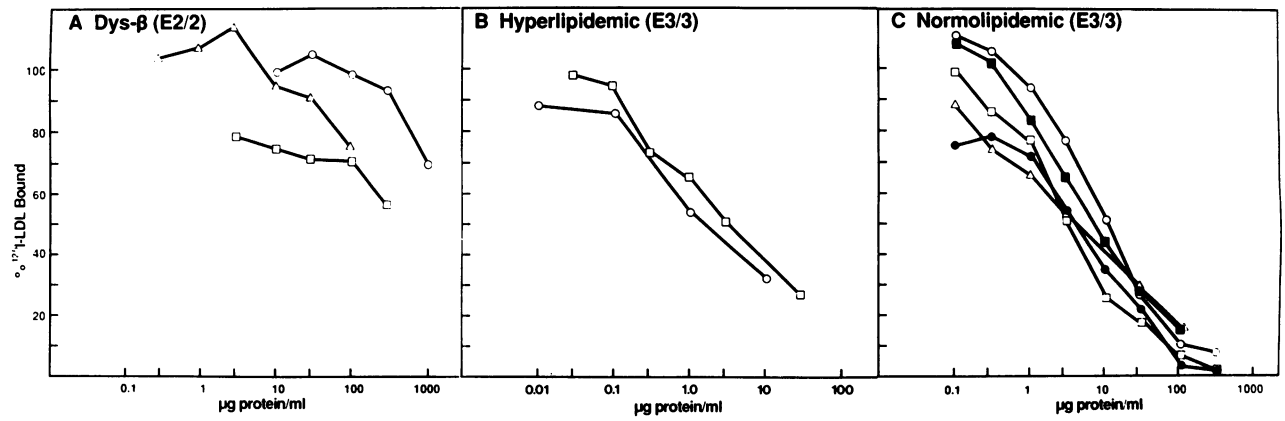

Figure 4. Competition of pre-β-VLDL for ^{125}I-LDL binding to cultured human fibroblasts at 4°C. The ability of lipoproteins at various concentrations to compete for the binding of ^{125}I-LDL at 2 μg of protein/ml was determined in duplicate. Fibroblasts were prepared as described under Methods. (*A*) (□), dys-β subject D.R.; (△), dys-β subject S.B.; (○), dys-β subject N.K. (*B*) (○), hyperlipidemic E3/3 subject C.F.; (□), hyperlipidemic E3/3 subject E.T. (*C*) Normolipidemic E3/3 subjects: (■), subject 1; (□), subject 2; (△), subject 3; (○), subject 4; (●), subject 5.

Table IV. Receptor Binding Affinities of d < 1.006 g/ml Fractions*

Subject	Pre-β-VLDL	β-VLDL	$S_f > 100$	S_f 60–100	S_f 20–60
Dys-β (E2/2)					
D.R.‡	290±63 (n = 3)	5.2±1.4 (n = 4)	—	—	—
	>300 (n = 1)				
S.B.‡	260±100 (n = 3)	13±5.9 (n = 6)	370±150 (n = 3)	62±16 (n = 5)	7.8±3.5 (n = 5)
	>300 (n = 3)		>100 (n = 2)		
N.K.‡	1,200±240 (n = 4)	7.0±2.9 (n = 8)	—	—	—
	>1,000 (n = 4)				
Mean	490±200 (n = 10)	8.0±3.3 (n = 18)			
Type V (E3/3)					
E.T.§	2.4 (n = 1)	—	2.6 (n = 2)	2.8 (n = 2)	2.0 (n = 2)
C.F.§	1.3 (n = 1)	—	4.0 (n = 2)	2.7 (n = 2)	1.3 (n = 2)
Mean	1.8 (n = 2)		3.2±0.41 (n = 4)	2.7±0.53 (n = 4)	1.6±0.41 (n = 4)
Normolipidemic (E3/3)					
1	8.7 (n = 1)		—	—	—
2	3.2 (n = 1)		—	—	—
3§	2.5 (n = 1)		5.6 (n = 1)	7.9 (n = 1)	5.1 (n = 1)
4	18 (n = 1)		—	—	—
5	4.1 (n = 1)		—	—	—
Mean	5.5±1.9 (n = 5)				

* EC_{50} expressed as micrograms of protein per milliliter±SD. Affinities that were too low to measure are reported as a EC_{50} greater than the highest concentration tested. Values in parentheses are the number of separate binding assays. ‡ Lipoproteins from subjects D.R., S.B., and N.K. were isolated on four, three, and five separate occasions, respectively. § Lipoproteins from subjects E.T., C.F., and 3 were isolated on two separate occasions.

dys-β subject D.R. was divided into four equal 1.5-cm fractions: from fraction 1, the most β-migrating, to fraction 4, the most pre-β-migrating. The cholesteryl ester content (percent dry weight) of fractions 1 through 4 was 41, 33, 14, and 6.8, whereas the triglyceride content (percent dry weight) was 25, 29, 46, and 54. Thus, the most β-migrating fraction was cholesteryl ester-rich and triglyceride-poor compared with the most pre-β-migrating fraction, and vice versa. The binding affinities varied directly with electrophoretic mobility: fractions 1, 2, and 3 had EC_{50} values of 3.5, 5.4, and 360 μg of protein/ml (Fig. 5). The affinity of fraction 4 was too low to measure.

Over 80% of the binding of both pre-β-VLDL and β-VLDL from dys-β subjects to the apo-B,E(LDL) receptor was due to apo-E. This was determined by the ability of an excess of the apo-E-specific monoclonal antibody 1D7 to inhibit the binding of ^{125}I-labeled $d < 1.006$ g/ml fractions to fibroblasts (Fig. 6). 1D7 inhibited ^{125}I-LDL binding by only 5% (Fig. 6). In contrast, the apo-B100-specific antibody 4G3 inhibited > 95% of ^{125}I-LDL binding, but < 25% of pre-β-VLDL and β-VLDL binding. The effect of 1D7 and 4G3 on the binding of ^{125}I-pre-β-VLDL from normolipidemic E3/3 subjects was similar to that found with the $d < 1.006$ g/ml fractions isolated from the dys-β subject N.K.

Binding of $S_f > 100$, S_f 60–100, and S_f 20–60 fractions to the apo-B,E(LDL) receptor. The receptor binding affinity of $d < 1.006$ g/ml lipoproteins from dys-β subjects varied directly with flotation rate. A representative pattern is shown in Fig. 7 *A*. The $S_f > 100$ fraction from S.B. had much lower affinity than that of the S_f 20–60 fraction (EC_{50} = 1,100 vs. 11 μg of protein/ml). The affinity of the S_f 60–100 fraction was 78 μg of protein/ml. In molar terms, using data from Table II, the respective EC_{50} values for the $S_f > 100$, S_f 60–100, and S_f 20–60 fractions were 840, 71, and 8.0 nM.

The receptor binding affinity of the $d < 1.006$ g/ml lipoproteins from E3/3 subjects varied only slightly with flotation

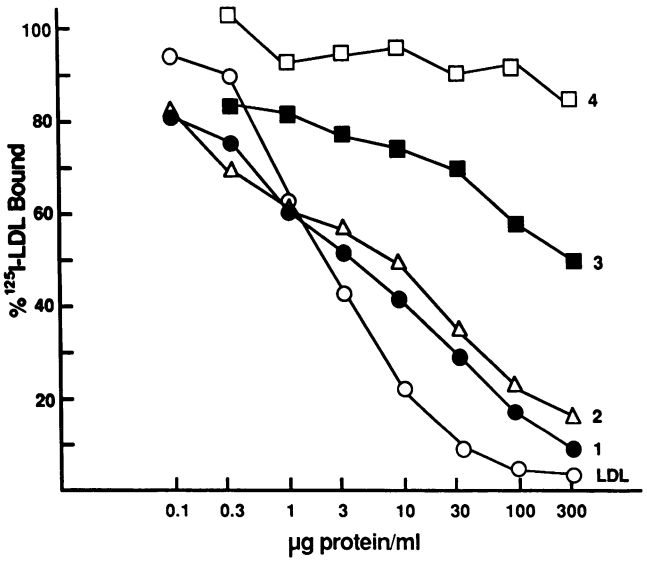

Figure 5. Competition of Pevikon block VLDL fractions from dys-β subject D.R. for ^{125}I-LDL binding to cultured human fibroblasts at 4°C. Four 1.5-cm fractions were taken: fraction 1 (●) was the most β-migrating, and fraction 4 (□) was the most pre-β-migrating. Low density lipoproteins were from a normal donor.

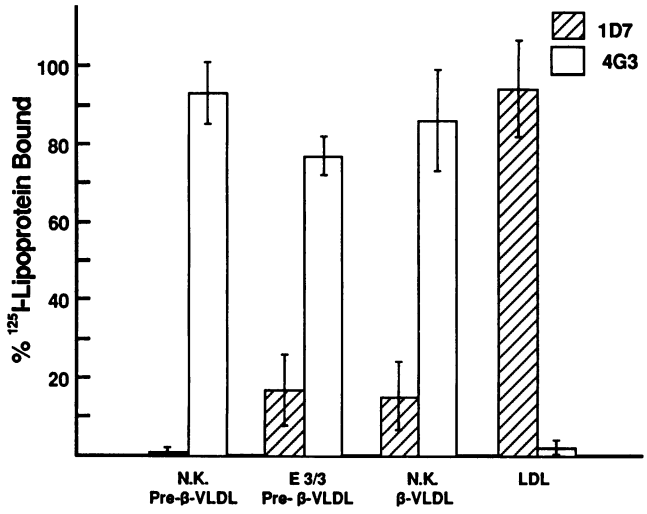

Figure 6. Competition of the apo-E-specific monoclonal antibody 1D7 or the apo-B100-specific monoclonal antibody 4G3 for ^{125}I-VLDL binding to cultured human fibroblasts at 4°C. The ^{125}I-VLDL fractions were incubated with an excess of either 1D7 or 4G3 for 1 h before the assay. The pre-β-VLDL and β-VLDL data are the mean of four separate preparations from dys-β subject N.K., the E3/3 pre-β-VLDL data are the mean of three different preparations from normolipidemic subjects 1 and 2, and LDL data are the mean of three different preparations from normal subjects. Standard error bars are indicated.

rate. The respective affinities of the $S_f > 100$, S_f 60–100, and S_f 20–60 fractions from the hyperlipidemic E3/3 subject E.T. were 3.0, 2.4, and 1.7 μg of protein/ml (120-, 23-, and 5-fold greater than corresponding fractions from the dys-β subject) (Fig. 7 *B*). On average, the affinities of the flotation fractions from the type V hyperlipidemic subjects were about threefold higher than those from the normolipidemic E3/3 subject 3 (Fig. 7 *C*). The LDL standard curves for Figs. 7 *B* and *C* are shown for comparison. The binding affinities of the flotation fractions are summarized in Table IV.

The contribution of apo-E to flotation fraction binding to the apo-B,E(LDL) receptor was analyzed as described above. In the hyperlipidemic E3/3 subject E.T., 1D7 inhibited 77% of ^{125}I-labeled $S_f > 100$ binding to cultured fibroblasts, but only 26% of ^{125}I-labeled S_f 20–60 binding (Fig. 8 *A*). In the dys-β S.B., 1D7 inhibited more than half of the binding of all flotation fractions (Fig. 8 *B*). Thus, receptor binding due to apo-E diminished as the flotation rate decreased in $d < 1.006$ g/ml lipoproteins from the hyperlipidemic E3/3 subject, but not in those from the dys-β subject.

Within the $S_f > 100$, S_f 60–100, and S_f 20–60 flotation fractions from dys-β subjects, receptor binding varied with electrophoretic mobility. These fractions from S.B. were isolated by Pevikon block electrophoresis and divided into their more β- and more pre-β-migrating portions. In each flotation fraction, lipoproteins in the more β-migrating portion were visibly smaller (by electron microscopy), had higher E:C ratios, and displayed higher receptor binding affinity (Fig. 9). The EC_{50} values for the more β-migrating portion and the more pre-β-migrating portion of the $S_f > 100$, S_f 60–100, and S_f 20–60 fractions were 70 vs. > 100, 20 vs. 430, and 5.3 vs. 38 μg of protein/ml, respectively. Pevikon block electrophoresis of flotation fractions from the hyperlipidemic E3/3 subject E.T. revealed a similar trend, but less than a twofold difference between the more β-migrating portion of the $S_f > 100$ and S_f 20–60 fractions and the more pre-β-migrating portion (data not shown).

As noted, β-migrating lipoproteins with $d < 1.006$ g/ml could be induced by the administration of intravenous heparin. These β-VLDL were found predominantly in the S_f 20–60 fraction. Heparinization raised the binding affinities of the S_f 20–60 flotation fractions from both hyperlipidemic E3/3 subject E.T. and dys-β subject D.R. 10-fold and 2-fold, respectively (data not shown). The heparin-induced β-VLDL from the E3/3 subject E.T. had six times the affinity of that from the dys-β subject D.R. (0.18 vs. 1.0 μg of protein/ml) (Fig. 10).

Since ultracentrifugation may remove apolipoproteins from lipoprotein particles and alter their biological properties, triglyceride-rich lipoproteins were isolated by gel filtration of whole plasma. The triglyceride-rich fractions eluted ahead of the cholesterol-rich fraction. The leading half of the triglyceride peak from the plasma of dys-β subject S.B. was divided into three pools, and their ability to compete for ^{125}I-LDL binding to the apo-B,E(LDL) receptor was assayed. Receptor binding affinity was lowest (EC_{50} = 580 μg of protein/ml) in the first

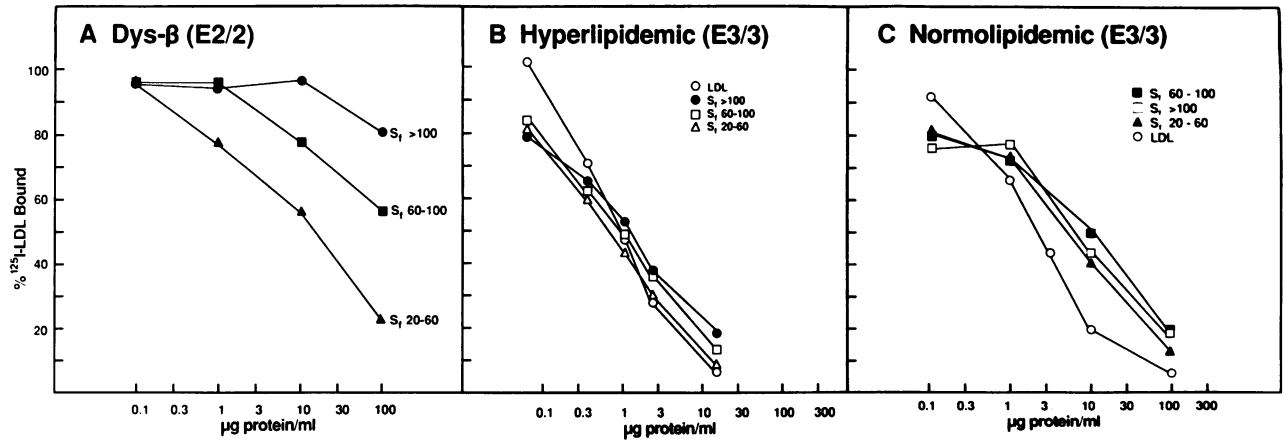

Figure 7. Competition of $S_f > 100$, S_f 60–100, and S_f 20–60 fractions for the binding of ^{125}I-LDL to cultured human fibroblasts at 4°C. (*A*) Dysbetalipoproteinemic subject S.B. (*B*) Hyperlipidemic E3/3 subject E.T.; the LDL were from a normal subject. (*C*) Normolipidemic E3/3 subject 3; the LDL were from a normal subject.

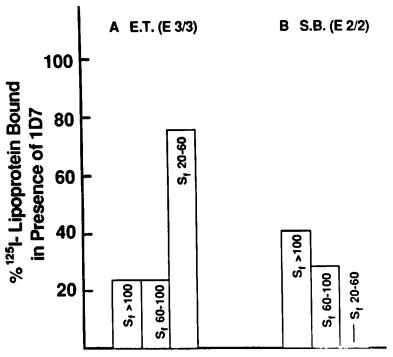

Figure 8. Competition of the apo-E-specific monoclonal antibody 1D7 for the binding of ^{125}I-labeled lipoprotein fractions to cultured human fibroblasts at 4°C. An excess of 1D7 was incubated with the ^{125}I-lipoproteins for 1 h before the assay. Data shown are the mean of duplicate determinations. (*A*) Hyperlipidemic E3/3 subject E.T. (*B*) Dysbetalipoproteinemic subject S.B.

pool (predominantly pre-β-VLDL) and increased over 50-fold, to 10 μg of protein/ml, in the third pool (predominantly β-VLDL) (data not shown). The triglyceride-rich fractions from the hyperlipidemic E3/3 subject E.T. were divided into two pools. There was only a small increment in binding affinity (about threefold), from 10 to 3.1 μg of protein/ml, between the first and the second pool. Although the apolipoprotein contents of the lipoproteins isolated by gel filtration and flotation were similar, as judged by SDS-polyacrylamide gel electrophoresis, the content of other proteins, particularly albumin, was greater in column-isolated lipoproteins (data not shown). However, with respect to their content of pre-β-VLDL and β-VLDL, the fractions isolated by gel filtration had binding affinities similar to those found in fractions isolated by Pevikon block electrophoresis or sequential ultracentrifugal flotation.

Discussion

Although mutations in apo-E that reduce receptor binding are strongly associated with dysbetalipoproteinemia, it is not clear how defective binding of apo-E leads to the formation and accumulation of β-VLDL. In the few well-characterized dys-β subjects with mutations in apo-E at different sites, there is no obvious correlation between the degree of defective receptor binding of mutant forms of apo-E and the severity of the associated dysbetalipoproteinemia (13, 48). Furthermore, it is well established that the vast majority of apo-E2 homozygotes are not hyperlipidemic (49–51). The E2/2 phenotype is usually due to the $Arg_{158} \rightarrow$ Cys mutation, and the apo-E binding is equally defective when isolated from hypolipidemic, normolipidemic, or hyperlipidemic individuals (14). The current study attempts to link the pathogenesis of dysbetalipoproteinemia to the physical, chemical, and receptor binding properties of $d < 1.006$ g/ml lipoproteins found in this disorder.

The pre-β-VLDL from dys-β subjects described here are triglyceride-rich particles of larger size and with higher average flotation rates than the more cholesteryl ester-rich β-VLDL (31). The content of the lipoprotein surface constituents (protein, free cholesterol, and phospholipid) increased as the particle size decreased (43), and the molecular weight of lipoproteins decreased progressively with decreased flotation rate (52).

All $d < 1.006$ g/ml fractions from the dys-β subjects were apo-E enriched compared with corresponding fractions from the E3/3 subjects. The ratio of apo-E to total apo-C (E:C) was always greater in β-VLDL and the S_f 20–60 fraction than in pre-β-VLDL and the $S_f > 100$ fraction in the dys-β subjects. The ratio of total apo-B to apo-E plus apo-C (B:E + C) was lowest in pre-β-VLDL and the $S_f > 100$ fraction. This suggests that apo-E and apo-C are lost, whereas apo-B is retained, during lipolytic conversion of pre-β-VLDL in the $S_f > 100$ fraction to β-VLDL in the S_f 20–60 fraction. The pre-β-VLDL and $S_f > 100$ particles were apo-B48 enriched compared with β-VLDL and S_f 20–60 particles, suggesting that intestinally derived lipoproteins have a larger average size and higher flotation rates than those of hepatic origin (53–55).

The size and triglyceride content of pre-β-VLDL and β-VLDL from the dys-β subjects increased with the severity of hyperlipidemia, suggesting that D.R., S.B., and N.K., respectively, exhibit progressive impairment in the ability to metabolize these large lipoproteins. On average, β-VLDL contained 68% more cholesteryl ester mass per particle than did pre-β-VLDL. This suggests that cholesteryl ester is acquired during

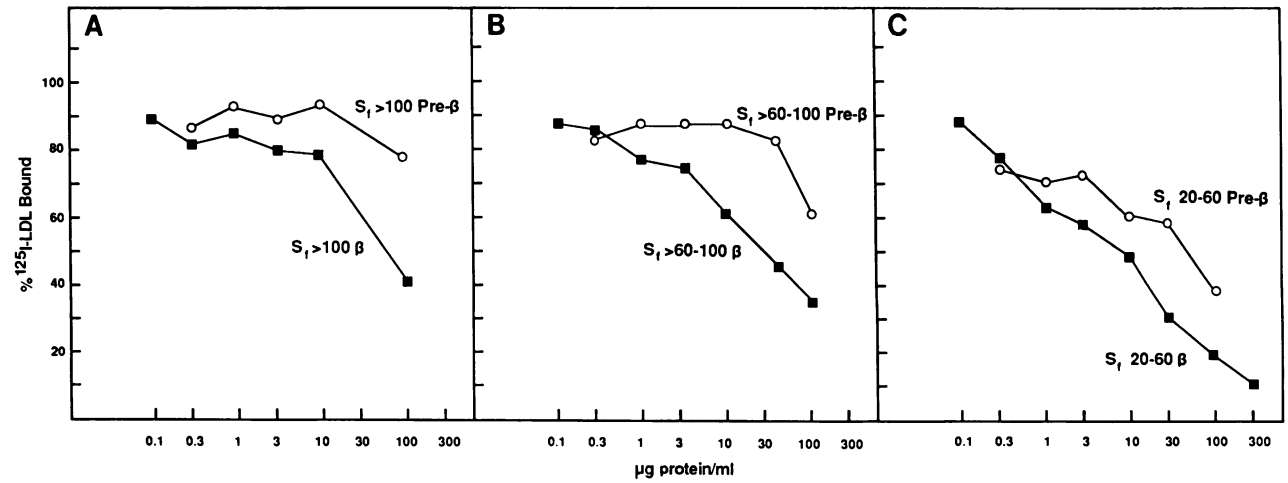

Figure 9. Competition of $S_f > 100$, S_f 60–100, and S_f 20–60 Pevikon block fractions from dys-β subject S.B. for ^{125}I-LDL binding to cultured human fibroblasts at 4°C. The flotation fractions were first isolated from whole plasma, then subjected to Pevikon block electrophoresis. The more β-migrating portion is designated β, and the more pre-β-migrating portion is designated pre-β.

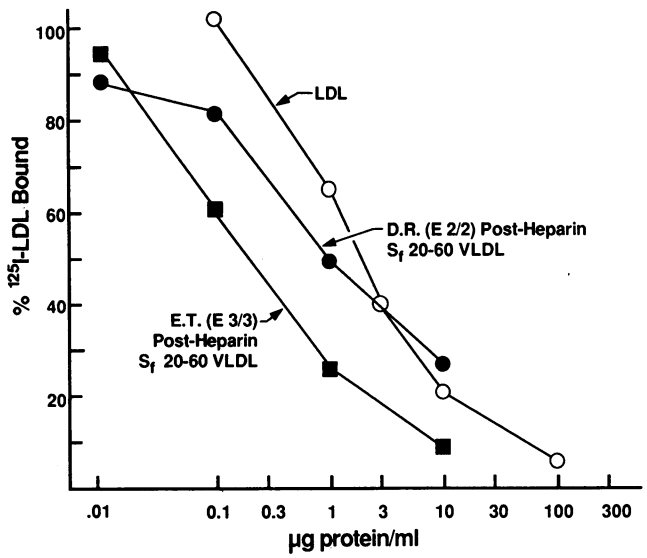

Figure 10. Competition of heparin-induced β-VLDL in the S_f 20–60 fraction for ^{125}I-LDL binding to cultured human fibroblasts at 4°C. Samples were isolated 1 h after the administration of intravenous heparin (100 IU/kg). The LDL were from a normal subject.

the metabolism of pre-β-VLDL. The activity of cholesteryl ester transfer protein increases during lipolysis in vitro, resulting in increased transfer of cholesteryl ester from high density lipoproteins to VLDL (56). Furthermore, this activity is increased in dysbetalipoproteinemia (57). The prolonged plasma residence time of VLDL in dysbetalipoproteinemia may also contribute to the acquisition of cholesteryl ester via cholesteryl ester transfer protein during the formation of β-VLDL (58).

Accelerated lipolysis induced by heparin results in the formation of β-migrating lipoproteins with $d < 1.006$ g/ml, at the expense of pre-β-VLDL, in both dys-β and hypertriglyceridemic subjects (32, 33). These observations suggest that lipolysis of pre-β-VLDL can result in the formation of β-VLDL even in subjects with normal apo-E. However, the heparin-induced β-VLDL are not identical to naturally occurring β-VLDL in E2/2 subjects.

The receptor binding affinity of $d < 1.006$ g/ml lipoproteins from dys-β subjects decreased with both decreasing electrophoretic mobility and flotation rate. The most β-migrating VLDL in the S_f 20–60 fraction had over 100-fold higher affinity than the most pre-β-migrating VLDL in the $S_f > 100$ fraction. The pre-β-VLDL from normolipidemic and type V hyperlipidemic subjects had 90- and 280-fold greater receptor binding affinity, respectively, than that of pre-β-VLDL from dys-β subjects. In all subjects, pre-β-VLDL obtained by sequential flotation or gel filtration of whole plasma displayed affinity similar to that of pre-β-VLDL isolated by ultracentrifugation in a fixed-angle rotor followed by Pevikon block electrophoresis.

Despite the low affinity of pre-β-VLDL from dys-β subjects for the apo-B,E(LDL) receptor, apo-E is responsible for most of the receptor binding. The apo-E-specific monoclonal antibody 1D7 inhibited the majority of the binding of both pre-β-VLDL and the $S_f > 100$ fraction from dys-β subjects. In contrast, the apo-B100-specific monoclonal antibody 4G3 had much less effect on the binding of pre-β-VLDL. In the type V hyperlipidemic subject, the ability of 1D7 to inhibit binding decreased as the flotation rate decreased from $S_f > 100$ to S_f 20–60. This observation confirms that apo-E becomes less important for binding as VLDL are converted from larger to smaller particles (23, 24). In contrast, in the dys-β subjects, apo-E was at least as important for the binding of small VLDL as it was for large VLDL. Thus, apo-E2 is the major determinant of binding for all $d < 1.006$ g/ml fractions studied in dys-β subjects.

The heparin-induced β-VLDL from both E2/2 and E3/3 subjects exhibited enhanced receptor binding affinity compared with corresponding lipoproteins studied before heparin administration. These findings are consistent with the enhancement of receptor binding affinity seen after in vitro lipolysis of VLDL (59, 60). The binding affinity of heparin-induced β-VLDL from the E3/3 subject was sixfold higher than that from the E2/2 subject and suggests that in the latter the defective receptor binding of the particle is due to abnormal apo-E.

The data demonstrate that the expression of apo-E2-mediated binding by the various lipoproteins in dysbetalipoproteinemia varies over 100-fold. The mechanism of the observed differences in the binding of VLDL fractions was not studied in detail. However, β-VLDL from dys-β subjects were enriched in apo-E and depleted of apo-C compared with pre-β-VLDL. This may contribute to the higher receptor binding affinity of β-VLDL. The pre-β-VLDL and β-VLDL also differ in size and lipid composition, both of which may be important determinants of receptor binding.

A model for the formation of β-VLDL in dysbetalipoproteinemia is shown in Fig. 11. In this model, $d < 1.006$ g/ml lipoproteins have either of two fates: irreversible loss from plasma by receptor-mediated endocytosis, or conversion to smaller lipoproteins by lipolysis. The width of the arrows connecting the compartments in Fig. 11 is proportional to the likelihood that the particle will be cleared from the plasma or be converted to a smaller particle during lipolysis. In E3/3

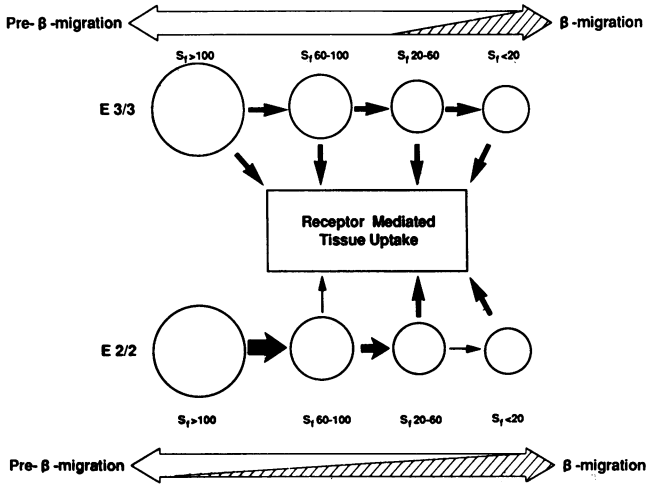

Figure 11. Model for the metabolism of $d < 1.006$ g/ml lipoproteins in apo-E2 and apo-E3 homozygotes. The width of the arrows connecting the various compartments is an indicator of the probability that the lipoproteins will be converted to smaller particles during lipolysis or undergo receptor-mediated tissue uptake. The hatched areas in the large arrows at the top and bottom are indicators of the relative content of pre-β-migrating and β-migrating lipoproteins in each compartment. See text for details.

subjects, the probabilities that $S_f > 100$, S_f 60–100, S_f 20–60, or $S_f < 20$ particles will undergo receptor-mediated clearance are assumed to be equal because their in vitro affinities for the apo-B,E(LDL) receptor are similar. This assumption is difficult to validate experimentally because receptor-mediated clearance and lipolytic conversion of lipoproteins occur simultaneously in vivo. However, a correlation between in vitro affinity and in vivo clearance is suggested by other studies in which chemical modifications that reduce the affinity of LDL or apo-E for the apo-B,E(LDL) receptor in vitro reduce their receptor-mediated clearance in vivo (61–64). In E3/3 subjects, the probabilities of receptor-mediated clearance and lipolytic conversion are assumed to be equal, so that 50% of the particles in each fraction will be cleared from plasma and 50% converted to smaller lipoproteins. This assignment of probability predicts that only 12% of the $S_f > 100$ particles will be converted to LDL in the $S_f < 20$ compartment; it is based on in vivo studies by Packard et al. (26) and Stalenhoef et al. (27) that show that ~ 90% of large VLDL irreversibly leave the plasma prior to conversion to LDL. Because LDL have in vitro receptor binding similar to that of the VLDL fractions from E3/3 subjects, their rates of receptor-mediated clearance are also assumed to be equal.

Since $S_f > 100$ lipoproteins from dys-β subjects interact poorly with the apo-B,E(LDL) receptor in vitro, it is likely that their in vivo receptor-mediated catabolism is slowed, resulting in increased conversion to smaller particles during lipolysis. Thus, the model shows all of the $S_f > 100$ compartment being converted to the S_f 60–100 compartment. Lipoproteins in S_f 60–100 still have defective receptor binding affinity compared with that in E3/3 subjects and, as a result, are converted primarily to the S_f 20–60 compartment. The receptor-mediated uptake of S_f 20–60 particles in E2/2 subjects and that in E3/3 subjects are equal in this model, based on their similar in vitro binding affinities.

The heparin-induced changes suggest that lipolysis of large pre-β-VLDL from both E3/3 and E2/2 subjects results in the formation of β-VLDL with enhanced binding affinity. However, the heparin-induced β-VLDL from E3/3 subjects had substantially higher receptor binding affinity than did LDL and thus are not likely to accumulate in plasma. Conversion of VLDL to LDL in dysbetalipoproteinemia is known to be delayed compared with normal VLDL based on in vivo studies (29, 30) and is indicated in the model by the thin arrow connecting the S_f 20–60 and $S_f < 20$ compartments. This delay may be due in part to the large mass of cholesteryl esters in β-VLDL that cannot be hydrolyzed during lipolysis. The β-VLDL reported here contain fivefold more cholesteryl ester per particle than do LDL (65).

Apolipoprotein B48 was detected in every $d < 1.006$ g/ml fraction studied from both E2/2 and E3/3 subjects. Although there was more apo-B48 in lipoproteins from E2/2 subjects than in corresponding lipoproteins from E3/3 subjects, the content of apo-B48 relative to apo-B100 was highest in the largest lipoproteins from all subjects. Perhaps the intestinally derived particles are larger than particles of hepatic origin upon secretion into the plasma. Since apo-E mediates the interaction of the largest lipoproteins from both E2/2 and E3/3 subjects with the apo-B,E(LDL) receptor, the larger size of apo-B48-containing particles may result in their clearance from plasma before being converted to LDL according to the scheme proposed in Fig. 11. However, the current study does not address the many other factors that may account for the differences in the metabolism of apo-B48 and apo-B100.

The proposed model may explain how hyperlipidemic factors, either environmental or genetic, exacerbate dysbetalipoproteinemia. The majority of type III hyperlipidemic subjects are obese and normalize their plasma triglyceride and cholesterol concentrations with diet alone (5, 6). In addition, familial type IV or combined hyperlipidemia may coexist with the inheritance of the E2/2 phenotype and result in severe type III hyperlipoproteinemia (4, 66–68). Although all E2/2 subjects have β-VLDL, the vast majority are not hyperlipidemic (49–51). The formation of β-VLDL, which have enhanced binding compared with pre-β-VLDL, compensates in part for the defective binding of apo-E2 and allows β-VLDL to undergo receptor-mediated catabolism. As a result, apo-E2-mediated binding is sufficient for the clearance of $d < 1.006$ g/ml lipoproteins in most E2/2 subjects. However, because of the defective binding of pre-β-VLDL, factors such as obesity or hereditary hypertriglyceridemia, which increase VLDL synthesis, can overwhelm the capacity for receptor-mediated clearance in E2/2 subjects. The pre-β-VLDL may not be cleared from the plasma by receptor-mediated endocytosis; they may first be converted to β-VLDL before they can interact with receptors.

In summary, the expression of apo-E-mediated binding of $d < 1.006$ g/ml lipoproteins from E2/2 and E3/3 subjects has been determined and incorporated into a model for the formation of β-VLDL in familial dysbetalipoproteinemia. Receptor binding mediated by apo-E2 is defective when compared with apo-E3-mediated binding in every lipoprotein studied, but most defective in pre-β-VLDL. The pre-β-VLDL appear to be precursors of β-VLDL in dysbetalipoproteinemia. The model predicts that receptor-mediated catabolism of pre-β-VLDL in vivo is impaired, leading to the formation of β-VLDL during lipolysis. No attempt has been made to treat apo-B100- and apo-B48-containing VLDL separately, although their catabolism in vivo may be different. No attempt has been made to consider the entry of lipoproteins directly into compartments other than the $S_f > 100$ fraction, or to take into account changes in the flotation rates of pre-β-VLDL and β-VLDL associated with varying degrees of hyperlipidemia. Nor is the effect of variations in the number of lipoprotein receptors in tissue considered. In addition, the model is not intended to describe lipoprotein catabolism in subjects with atypical forms of dysbetalipoproteinemia in which pre-β-VLDL and β-VLDL display much higher receptor binding affinities (69). Finally, the model is not quantitative. Rather, it incorporates available data into plausible hypotheses that can be tested in future investigations.

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