Properties of an apolipoprotein E-enriched fraction of triglyceride-rich lipoproteins isolated from human blood plasma with a monoclonal antibody to apolipoprotein B-100

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Abstract A monoclonal antibody to apolipoprotein (apo) B-100 (JI-H) with unique binding properties has been used to separate a population of triglyceride-rich lipoproteins from blood plasma of normotriglyceridemic individuals and patients with various forms of hypertriglyceridemia. This antibody fails to recognize an apoE-rich population of very low density lipoproteins (VLDL) containing apoB-100 as well as all triglyceride-rich lipoproteins containing apoB-48, but it binds other VLDL that contain apoE and almost all lipoproteins that contain apoB-100, but no apoE. The unbound triglyceride-rich lipoproteins separated by ultracentrifugation after separation from plasma by immunoaffinity chromatography contained 10–13% of the apoB of triglyceride-rich lipoproteins from three normotriglyceridemic individuals, 10–29% of that from five patients with endogenous hypertriglyceridemia, 40–48% of that from three patients with familial dysbetablipoproteinemia, and 65% of that from a patient with lipoprotein lipase deficiency. In all cases, the unbound triglyceride-rich lipoproteins contained more molecules of apoE and cholesteryl esters per particle than those that were bound to monoclonal antibody JI-H, and they were generally depleted of C apolipoproteins. These properties resemble those described for partially catabolized remnants of chylomicrons and VLDL. The affinity of the unbound lipoproteins for the low density lipoprotein (LDL) receptor varied widely, and closely resembled that of the total triglyceride-rich lipoproteins from individual subjects. Our results demonstrate that remnant-like chylomicrons and a population of remnant-like VLDL can be isolated and quantified in blood plasma obtained in the postabsorptive state from normotriglyceridemic and hypertriglyceridemic individuals alike.—**Campos, E., K. Nakajima, A. Tanaka, and R. J. Havel.** Properties of an apolipoprotein E-enriched fraction of triglyceride-rich lipoproteins isolated from human blood plasma with a monoclonal antibody to apolipoprotein B-100. *J. Lipid Res.* 1992. **33:** 369–380.

Supplementary key words chylomicrons • remnant lipoproteins • apolipoprotein B-48 • dysbetalipoproteinemia • hypertriglyceridemia • lipoprotein lipase

Plasma very low density lipoproteins (VLDL) are heterogeneous in origin, size, and composition. Their main protein components are apolipoproteins B-100, E, and the three C apolipoproteins. VLDL apolipoprotein (apo) B is thought to be the sole or major source of apoB in LDL, and current evidence suggests that its conformation differs in these two lipoproteins. Thus, the accessibility of regions of apoB in human VLDL to monoclonal antibodies and proteases differs from that of apoB in LDL (1, 2). The other apolipoproteins modulate the interaction of VLDL with lipolytic enzymes and cell receptors and thus influence the metabolism of VLDL lipids as well as that of the particles themselves (3).

In the postabsorptive state, VLDL (defined as lipoproteins that float in the ultracentrifuge at a density of 1.006 g/ml) are derived predominantly from the liver, and these particles contain a single molecule of apoB-100. Some particles of comparably low density are found in normal individuals that almost certainly are derived from the small intestine, and these contain apoB-48.

The first methods used to isolate VLDL subfractions relied mainly upon physical differences (size, hydrated density, and charge) (4–6). Such methods yield subfractions of lipoproteins that are still metabolically heterogeneous. Shelbourne and Quarfordt (7) showed that VLDL can be separated into two fractions on the basis of their affinity for heparin. The unbound fraction contained much less apoE than the bound fraction. Yamada et al. (8–10) separated rabbit VLDL by anti-apoE immunoaffinity chromatography into "B"

Abbreviations: VLDL, very low density lipoprotein; apo, apolipoprotein; LDL, low density lipoprotein; Mab, monoclonal antibody; IDL, intermediate density lipoprotein; TMU, tetramethyl urea; CE, cholesteryl ester; LPL, lipoprotein lipase.

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and "B,E" particles, and showed that the presence of apoE on VLDL particles influenced their removal from the blood and conversion to LDL.

VLDL from normolipidemic humans bind poorly to LDL receptors, whether or not they contain apoE (11). Large VLDL particles from many hypertriglyceridemic individuals, however, are readily taken up by lipoprotein receptors, and this uptake is mediated by apoE (12). These and other observations (13, 14) indicate that apoE exists in different conformations on the surface of VLDL and can be accessible or inaccessible to receptors.

In the current research, we have used a monoclonal antibody (Mab) to apoB (JI-H) to separate a population of VLDL that is enriched in apoE. We have characterized the physical, chemical, and receptor-binding properties of this population, in which the epitope for Mab JI-H is not expressed, in normolipidemic subjects and in patients with exogenous and endogenous hypertriglyceridemia and familial dysbetalipoproteinemia. We show that the properties of the particles that fail to bind to Mab JI-H resemble those of VLDL and chylomicron remnants.

METHODS

Subjects

We studied eight patients with various forms of primary hyperlipoproteinemia (one with lipoprotein lipase deficiency, four with primary endogenous hyperlipemia, three with familial dysbetalipoproteinemia), one patient with endogenous hyperlipemia secondary to lipodystrophy of limbs and trunk (Köbberling-Dunnigan Syndrome (15)), and three normolipidemic subjects (**Table 1**). They fasted overnight before blood was taken by venipuncture into EDTA (2 mM) containing protease inhibitors (16) and immediately cooled in ice. Plasma was separated by centrifugation (2000 *g*, 30 min, 4°C).

Immunoaffinity chromatography and ultracentrifugation

Monoclonal antibody JI-H, raised in a Balb/c mouse immunized with normal human LDL, was obtained by standard techniques (17), and produced routinely by injecting 5×10^6 hybridoma cells into pristane-primed adult Balb/c mice. The immunoglobulin fraction from ascites fluid was coupled to CNBr-activated Sepharose 4B (Pharmacia) at a ratio of 10 mg/ml, according to the recommendations of the manufacturer. Ten-ml immunoaffinity columns (1.5 × 6.0 cm) were routinely used and maintained at 4°C. Before use, the columns were conditioned with normal human plasma to reduce nonspecific binding and eluted with 3 M NaSCN, pH 7.4. Finally, the columns were washed extensively with 0.15 M NaCl containing 1.3 mM EDTA, pH 7.4 (EDTA/saline), and benzamidine (0.3 mg/ml). The capacity was determined by applying increasing amounts of plasma from normolipidemic subjects (0.25–3.0 mg apoB), and measuring the total amount of unbound apoB by immunonephelometry with a commercial polyclonal antiserum (International Immunology Corp., Murietta, CA) (18). ApoB in the unbound fraction comprised 5–6% of total apoB when 0.25–2.0 mg of apoB was applied. Therefore, the capacity of the column was considered to be 2 mg apoB. Plasma samples (0.5–1.0 ml) were routinely applied to the column, and the unbound fraction was eluted at 15 ml/h for 1 h, then at 30 ml/h for 1 h. Bound lipoproteins were then eluted at a rate of 30 ml/h with 25 ml of 3 M NaSCN containing bovine serum albumin (1 mg/ml) and benzamidine (0.3 mg/ml) at pH 7.4. Lipoproteins in the bound fraction were immediately dialyzed against EDTA/saline, pH 7.4, and the column was washed with 65 ml of the same buffer containing benzamidine (0.3 mg/ml). To ascertain that the capacity of the column had not been exceeded, we measured apoB in the infranate solution after flotation of triglyceride-rich lipoproteins present in the unbound fraction, as described below. Less than 4% of the apoB of IDL and LDL failed to bind to the column.

The same coupling procedure was used to couple the immunoglobulin fraction (19) of rabbit antiserum to human apoE (20) to CNBr-activated Sepharose. Immunoaffinity columns (1.5 × 9 cm) containing ~100 mg of IgG were constructed and maintained at 10°C. Their capacity was found to be 50 μg of apoE, as determined by radioimmunoassay (20). Unbound and bound fractions were eluted as described above. Unbound and dialyzed bound fractions from both immunoaffinity columns were concentrated in a micro protein dialysis/concentrator (Biomolecular Dynamics, Beaverton, OR) with dialysis membrane PA-10. VLDL were isolated from these concentrated fractions by discontinuous density gradient ultracentrifugation in an SW 41 swinging bucket rotor by a modification of the method of Redgrave, Roberts, and West (21). A solution of NaCl of density 1.2015 g/ml was used to adjust plasma to densities 1.035 and 1.063 g/ml. A discontinuous gradient was formed by carefully layering 4 ml of plasma (ρ = 1.0315 g/ml) above 4 ml plasma (ρ = 1.063 g/ml) in a cellulose nitrate tube. Finally, the tube was filled with 4.0 ml of distilled water. The samples were centrifuged at 40,000 rpm for 22–24 h at 12°C. After centrifugation, chylomicrons and VLDL, layered at the top of the tube, were collected after slicing the tube 1.0 cm below the meniscus, and the tube wall was rinsed with EDTA/saline, pH 7.4. In some ex-

TABLE 1. Lipids and apoproteins unbound to Mab JI-H in plasma and VLDL

Group Age/Sex	Cholesterol	Triglycerides	ApoB	ApoE	ApoE Phenotype	VLDL-Cholesterol Unbound Fraction		VLDL-ApoB Unbound Fraction	
						% of Total Plasma Cholesterol	% of VLDL Cholesterol	% of Total Plasma ApoB	% of VLDL ApoB
	mg/dl								
Normotriglyceridemia									
29/M	172	63	63	2.9	3/3	< 1	13	< 1	13
65/M	264	93	107	3.5	3/3	< 1	24	< 1	10
38/F	145	51	63	4.1	3/3	< 1	20	< 1	10
Endogenous Hypertriglyceridemia									
38/M	230	218	98	6.2	3/3	5	30	3	29
66/M	260	349	119	13.0	3/2	6	18	3	10
49/F[a]	321	444	144	16.3	3/3	13	43	5	27
74/F	398	362	197	14.4	3/3	4	30	4	28
45/M	334	468	169	11.2	3/3	9	32	5	28
Familial dysbetalipoproteinemia									
60/M[b]	210	163	53	35.8	2/2	25	56	10	48
73/M	173	177	55	9.8	2/2	10	42	8	37
64/M	296	297	110	20.2	2/2	13	40	5	30
Lipoprotein lipase deficiency									
57/M	338	1550	105	19.7	4/3	61	81	30	65

[a]Patient with lipodystrophy.
[b]Patient with E2/2 (arg_{145}→cys).

periments, VLDL were also separated from fresh plasma on a 2 × 90-cm column of 4% agarose (Bio-Gel A-15 m, Bio-Rad) (22).

Analysis of lipids and proteins

Total cholesterol, free cholesterol, and triglycerides were determined by enzymatic assays (23, 24) with an automated procedure on the Cobas (Mira) Chemistry System (Roche). Total phospholipids were determined as lipid phosphorus (25). Protein was determined by the method of Lowry et al. (26), with bovine serum albumin as standard. ApoE was quantified with a polyclonal antiserum raised in rabbits by radioimmunoassay (20). ApoB was estimated as protein not soluble in tetramethyl urea (TMU) (27) in fractions from hyperlipoproteinemic subjects. Alternatively, in fractions from normolipidemic subjects, apoB was determined by immunonephelometry after hydrolysis of lipoprotein triglycerides with lipoprotein lipase from cows' milk (28). ApoC was estimated by difference, assuming the total protein of VLDL is composed of apoBs, apoE, and apoCs.

Slab gel electrophoresis was carried out in 3–15% linear gradient of polyacrylamide in Tris buffer (pH 6.8) in the presence of 3% SDS (29). Samples were first mixed with SDS, glycerol, sodium mercaptoacetate, dithiothreitol, and bromophenol blue at final concentrations of 2%, 10%, 1%, 0.05 M, and 0.003%, respectively. The gel was stained overnight in 0.25% Coomassie Blue R250 in methanol–acetic acid–water 5:1:5 (v/v). Isoelectric focusing tube gel electrophoresis was carried out in 8 M urea, 1.6% (w/v) ampholine (pH range 3.5–7.0) as described (6).

Immunoblotting

After migration in SDS gel slabs, the separated bands were transferred electrophoretically to nitrocellulose sheets at 200 mA for 20–24 h and processed as described (30). Mab JI-H (10 μg/ml) and polyclonal LDL antisera (1:500) were used. Bound antibodies were detected with ^{125}I-labeled goat anti-mouse serum or ^{125}I-labeled protein A.

Analytical agarose gel electrophoresis was carried out as described (6). The diameter of lipoprotein particles was measured in electron photomicrographs of negative stains (31), by a semiautomated method utilizing a magnetic digitizer (32).

LDL receptor-binding assay

Binding of lipoprotein fractions to rat liver endosomal membranes was carried out as previously described (33). Briefly, endosomal membranes from estradiol-treated rats (34) containing 8–10 μg protein were added to 100 μl of buffer (100 mM NaCl–0.5 mM $CaCl_2$–50 mM Tris-HCl–2 mg bovine serum albumin) in the absence or presence of 30 mM EDTA, pH 7.5.

Fifty µl of ^{125}I-labeled lipoproteins was added at the concentrations indicated in the figure legends, and the tubes were incubated for 60 min at 4°C. Bound and unbound ligands were separated by centrifugation in a Beckman 42.2 rotor (35). Lipoproteins were labeled with ^{125}I by a modification (36) of the iodine monochloride method of McFarlane (37).

RESULTS

Presence of an apoE-rich population in human VLDL

Initial studies showed that VLDL from normotriglyceridemic subjects contain an apoE-enriched population unreactive with Mab JI-H. In three such individuals, this population contained 19 ± 5.6% of total VLDL cholesterol and 11.0 ± 1.7% of total VLDL apoB (Table 1). The mass ratio of apoB/apoE was 0.42 ± 0.15, whereas this ratio was 0.13 ± 0.061 in total VLDL. Total B,E particles contained, by contrast, 59.7 ± 7.4% of total VLDL cholesterol. In patients with various forms of hypertriglyceridemia, the unbound population contained a larger fraction of the cholesterol and apoB in VLDL (Table 1), but likewise represented only a portion of total B,E particles. In all cases, more of the VLDL-cholesterol than apoB was found in the unbound fraction (Table 1).

Physical characteristics of unbound and bound fractions

The electrophoretic mobility of the unbound fraction tended to be lower than that of the bound fraction (**Fig. 1**). Lipoprotein particles comprising the slower component were on average larger than the more rapidly migrating ones (**Table 2**). Electron photomicrographs of negatively stained preparations from one patient are shown in **Fig. 2**, together with the distribution of particle diameters. In the unbound fraction, the diameter of 95% of the particles ranged from 300 to 720 Å, with a skewed distribution towards particles of larger diameter. The bound fraction had a more restricted distribution of size, with 95% of the particles ranging from 260 to 640 Å.

Chemical characteristics of unbound and bound fractions

As expected from the differences in size (5), the sum of the mass of surface components, as weight percent (phospholipids, free cholesterol and protein), was higher in the bound fraction, and the sum of core components (cholesteryl ester and triglycerides) was lower. This finding applied to all hyperlipoproteinemic patients, and was most pronounced in the patient with lipoprotein lipase deficiency (Table 2).

Although the overall lipid composition of the two fractions was similar, there was a distinct difference in the mass ratio of cholesteryl esters to apoB (CE/B); this ratio was higher in the unbound fraction than in the bound fraction in all subjects studied (Table 2). The difference was most pronounced in the patient with LPL deficiency and least pronounced in the patients with endogenous hypertriglyceridemia and the normolipidemic subjects. The CE/B ratio in both fractions from patients with dysbetalipoproteinemia was much higher than in those with endogenous hypertriglyceridemia and the normolipidemic subjects.

The apolipoprotein composition of unbound and bound fractions also differed. The percentage of total protein as apoB was lower in the unbound fraction than in the bound fraction from normal subjects (20.8 vs. 28.8). Our immunonephelometric assay of apoB used in the normolipidemic subjects probably underestimated apoB (in these subjects low concentration of apoB did not permit analysis of apoB by TMU precipitation).[2] This difference was less pronounced in the patient with lipoprotein lipase deficiency (42.8 vs. 50.4) and in the patients with endogenous hyper-

[2]In five samples of VLDL in which apoB was estimated by the TMU and nephelometric methods, the value by nephelometry averaged 95% of that obtained with the TMU method (range 85–100%).

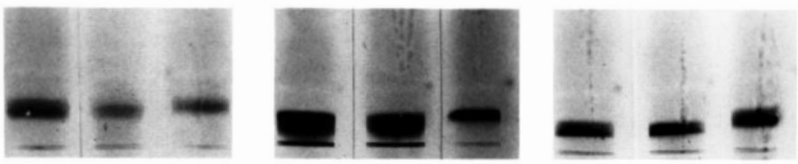

Fig. 1. Agarose electropherograms of VLDL and its two subfractions from three individuals. Lanes 1–3: patient with endogenous hypertriglyceridemia; lanes 4–6: patient with lipoprotein lipase deficiency; lanes 7–9: patient with dysbetalipoproteinemia. In each case, left lane shows total VLDL, middle lane shows Mab JI-H unbound subfraction, and right lane shows Mab JI-H bound subfraction.

TABLE 2. Composition and size of VLDL unbound and bound to Mab JI-H

	Normolipidemia (n = 3)		Endogenous Hypertriglyceridemia (n = 5)		Familial Dysbetalipoproteinemia (n = 3)		Lipoprotein Lipase Deficiency (n = 1)	
	Unbound	Bound	Unbound	Bound	Unbound	Bound	Unbound	Bound
				weight %				
Cholesteryl ester (CE)	5.5 ± 0.95	6.4 ± 1.8	11.0 ± 2.5	14.2 ± 3.2	23.3 ± 6.4	22.2 ± 5.3	10.5	11.5
Triglyceride	60.8 ± 2.8	54.4 ± 3.2	54.3 ± 2.5	46.1 ± 3.7	40.9 ± 7.6	38.1 ± 5.4	66.0	48.8
Cholesterol	6.1 ± 1.5	7.0 ± 1.1	6.9 ± 1.8	8.0 ± 0.95	8.2 ± 1.7	8.3 ± 1.6	5.2	6.3
Phospholipids	17.2 ± 0.7	17.9 ± 0.5	19.6 ± 2.2	20.4 ± 1.8	20.5 ± 3.6	21.1 ± 3.2	14.1	22.1
Protein	10.4 ± 1.1	14.3 ± 1.4[a]	8.2 ± 1.3	11.4 ± 1.2[a]	7.1 ± 1.4	10.3 ± 1.0[b]	4.2	11.3
Median particle diameter (Å)	495[c]	390	417 ± 57[d]	342 ± 28	432[e]	377	576	403
Mass ratios								
CE:apoB	2.6 ± 0.13[f]	1.7 ± 0.7[f]	3.2 ± 1.3	2.3 ± 0.36	7.3 ± 2.9	5.1 ± 2.3[b]	6.0	2.0
ApoE:apoB	0.42 ± 0.15	0.14 ± 0.11[b]	0.24 ± 0.11	0.13 ± 0.045[b]	0.34 ± 0.13	0.13 ± 0.052[b]	0.30	0.072
ApoE:apoC	0.13 ± 0.067	0.059 ± 0.011	0.12 ± 0.14	0.12 ± 0.14	0.44 ± 0.29	0.13 ± 0.10[b]	0.28	0.080

Values given as mean ± SD.
[a] $P < 0.02$.
[b] $P < 0.05$.
[c] n = 1.
[d] n = 3.
[e] n = 2.
[f] ApoB measured by immunonephelometry.

triglyceridemia (43.9 vs. 53.5), and no difference was found in the patients with dysbetalipoproteinemia (46.5 vs. 44.7).

SDS-gel electrophoretograms of apolipoproteins from total, unbound and bound VLDL are shown in **Fig. 3** and **Fig. 4.** ApoB-48 was consistently found in

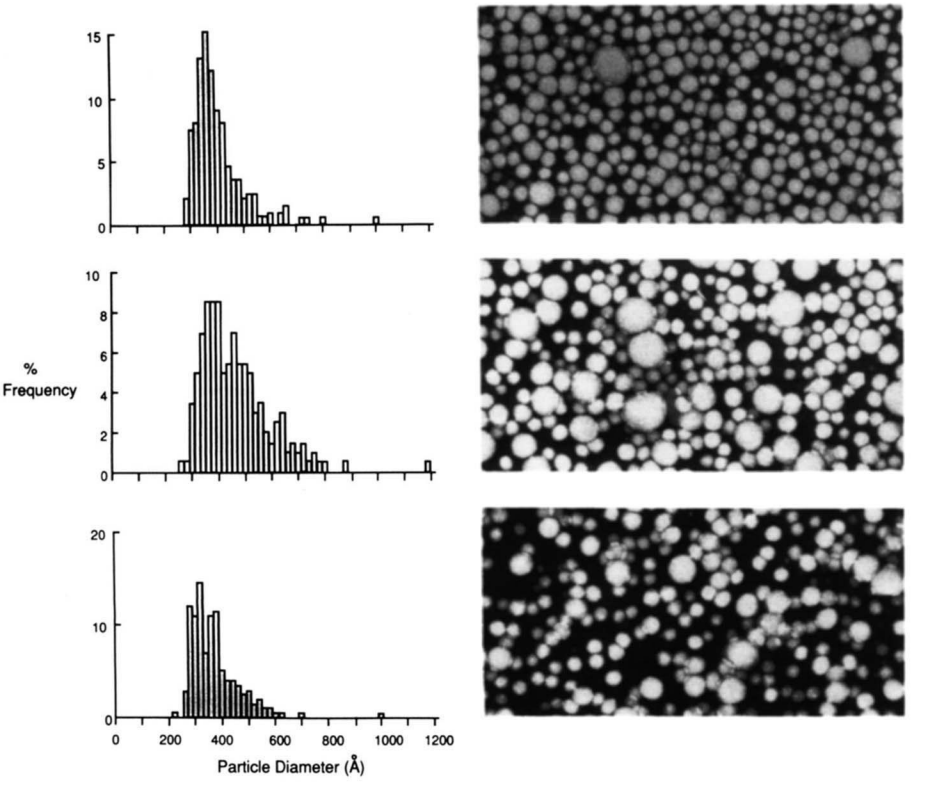

Fig. 2. Distribution of particle diameters of VLDL and its two Mab JI-H fractions from a patient with dysbetalipoproteinemia. Top: total VLDL; middle: unbound fraction; bottom: bound fraction. Left: distribution of particle diameters. Right: corresponding electron photomicrographs.

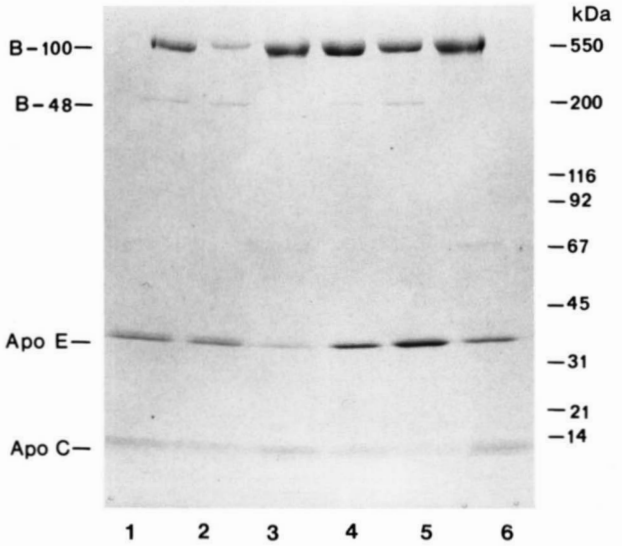

Fig. 3. Three–15% SDS-polyacrylamide gel electrophoretograms of 40 μg of proteins of VLDL and its two Mab JI-H fractions from a patient with lipoprotein lipase deficiency (lanes 1–3) and a patient with dysbetalipoproteinemia (lanes 4–6). In each case, left lane shows total VLDL, middle lane shows Mab JI-H unbound subfraction, and right lane shows Mab JI-H bound subfraction. Position of marker proteins is indicated at right margin.

total VLDL and unbound fractions of all subjects and was absent in the bound fraction. Both fractions contained apoB-100. Thus, the unbound VLDL were enriched in lipoproteins of intestinal origin. Immunoblotting of the unbound and bound fractions with Mab JI-H gave no signal at the apoB-48 position, but both fractions yielded signals for apoB-100 (results not shown). Thus, Mab JI-H does not react with apoB-48 and selectively fails to bind to a portion of apoB-100 in VLDL particles.

The particles in the unbound fraction contained significantly more apoE, as expressed by the mass ratio of apoE/apoB (Table 2). ApoE/apoB (apoB-48 + apoB-100) was on average threefold higher in the unbound fraction from normolipidemic subjects. This difference was more pronounced in the patient with lipoprotein lipase deficiency and less pronounced in the other groups. The apoE/apoC mass ratio was three- to fourfold higher in the unbound fraction from the patient with lipoprotein lipase deficiency and the dysbetalipoproteinemic patients, and twofold higher in the normolipidemic subjects, but it did not differ in the two fractions from the patients with endogenous hypertriglyceridemia (Table 2). The SDS and isoelectric focusing gel electrophoretograms (Figs. 3, 4, and **Fig. 5**) of unbound and bound fractions confirmed these values, and the latter showed a similar distribution of apoC-II and apoC-III in the two fractions.

To confirm that the apoE-enriched fraction of VLDL contains only B,E particles, we passed the unbound VLDL over an anti-apoE immunoaffinity column. In two experiments, one of which is shown in **Fig. 6**, virtually all lipoproteins of $\rho < 1.006$ g/ml were bound by the anti-apoE column. To evaluate the effect of ultracentrifugation on the amount of apoE found in the VLDL unbound to Mab JI-H, a portion of the un-

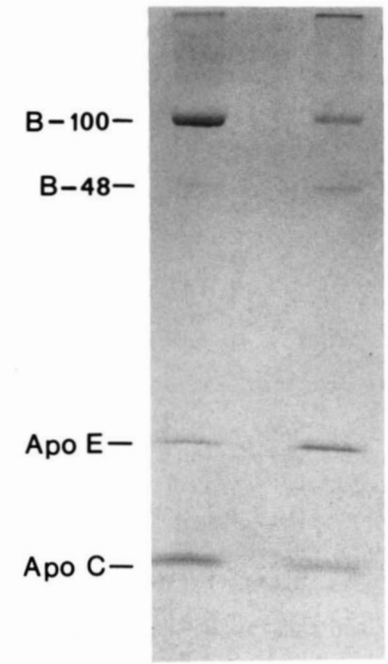

Fig. 4. SDS-polyacrylamide gel electropherograms of 25 μg of proteins of total VLDL (left) and its Mab JI-H unbound subfraction (right) from a normolipidemic subject.

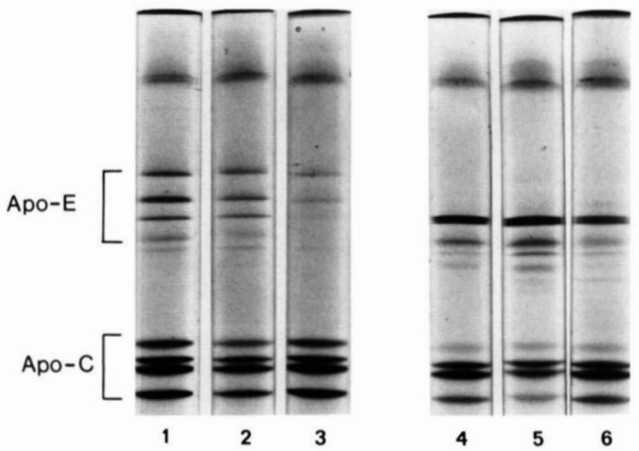

Fig. 5. Isoelectric focusing polyacrylamide gel electropherograms of 40 μg of apoVLDL and its two Mab JI-H fractions from a patient with lipoprotein lipase deficiency (lanes 1–3; apoE phenotype 4/3), and a patient with dysbetalipoproteinemia (lanes 4–6; apoE phenotype 2/2). In each case, left lane shows total VLDL, middle lane shows Mab JI-H unbound subfraction, and right lane shows Mab JI-H bound subfraction.

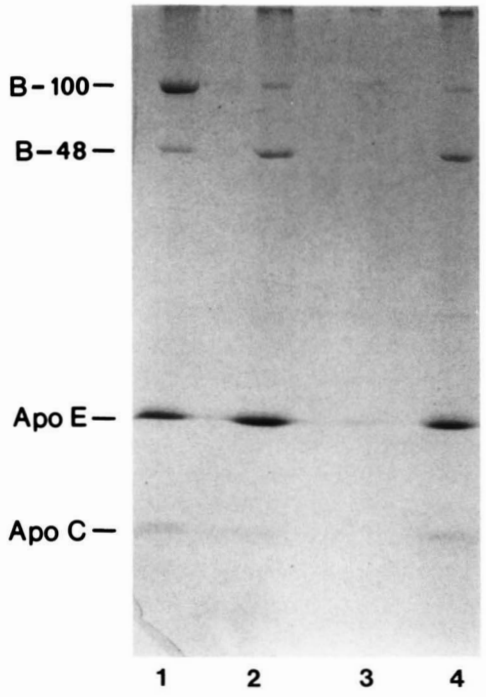

Fig. 6. SDS-polyacrylamide gel electropherograms of VLDL and its subfractions from a patient with dysbetalipoproteinemia. Lane 1: total VLDL (30 μg protein); lane 2: Mab JI-H unbound subfraction (30 μg protein); lane 3: material from Mab JI-H unbound subfraction that remained unbound after passage through anti-apoE immunoaffinity column; lane 4: same material that bound to anti-apoE immunoaffinity column.

bound fraction of plasma from four patients with endogenous hypertriglyceridemia was ultracentrifuged, and another portion was applied to a column of 4% agarose gel. The fraction containing triglyceride-rich lipoproteins was taken for chemical analysis. There was no systematic difference in the lipid composition of the total VLDL separated by ultracentrifugation or gel chromatography. The mean apoE/apoB ratio of VLDL separated by ultracentrifugation was 0.433 (SD 0.165) and that of the VLDL separated by gel chromatography was 0.384 (SD 0.226). Thus, there was no evidence for loss of apoE during ultracentrifugation of the apoE-enriched fraction.

The apoE/apoB ratio of Mab JI-H unbound particles was compared with that of total B,E particles in VLDL of three individuals (**Table 3**). In two subjects, one normolipidemic and the other with familial dysbetalipoproteinemia, the ratio was higher in the Mab JI-H unbound particles; in the third subject, with endogenous hypertriglyceridemia, it was similar to that of total B,E particles.

Binding of VLDL fractions to the LDL receptors

Specific (EDTA-sensitive) binding of Mab JI-H unbound VLDL to endosomal LDL receptors was studied in three subjects: one normolipidemic, one hypertriglyceridemic, and one dysbetalipoproteinemic. Data from one subject are shown in **Fig. 7.** A tendency to saturation of the binding isotherm in the presence of 30 mM EDTA suggested the presence of a saturable EDTA-resistant site in endosomal membranes, as described by Jäckle, Brady, and Havel (33). The results are summarized in **Table 4** and compared with the receptor-binding properties of LDL. Normolipidemic VLDL bound to the LDL receptor with low affinity (K_d = 30 μg/ml), and hypertriglyceridemic VLDL bound with much higher affinity (K_d = 4.9 μl/ml), when compared with LDL (K_d = 9.1 μg/ml). VLDL from the dysbetalipoproteinemic patient had the highest affinity (K_d = 2.3 μg/ml). In previous studies, VLDL from this patient with E-2 (arg_{145}→cys) bound to liver membranes with high affinity and were taken up at a normal rate by perfused rat livers from estradiol-treated rats (38, 39).

DISCUSSION

We have shown that Mab JI-H recognizes apoB-100 in most VLDL particles in normolipidemic subjects and those with endogenous hypertriglyceridemia, as well as almost all IDL and LDL, but it fails to recognize a minor fraction of VLDL, unusually rich in apoE. The consistent failure of Mab JI-H to bind particles containing apoB-48 suggests that its epitope is situated beyond the C-terminus of apoB-48. Virtually none of the particles unbound by Mab JI-H (those containing apoB-48 as well as those containing apoB-100) lack apoE. The unbound population is also enriched in cholesteryl esters and is depleted of C apolipoproteins when compared with those VLDL particles that bind Mab JI-H. These characteristics are consistent with

TABLE 3. Comparison of apoE/apoB mass ratios of VLDL unreactive with Mab JI-H and total B,E particles in VLDL

Subject	% of VLDL ApoB	ApoE/ApoB
Normolipidemia		
Total VLDL		0.15
Fraction unbound to Mab JI-H	16	0.47
Fraction bound to polyclonal anti-apoE	40	0.33
Endogenous hypertriglyceridemia		
Total VLDL		0.27
Fraction unbound to Mab JI-H	26	0.35
Fraction bound to polyclonal anti-apoE	82	0.33[a]
Dysbetalipoproteinemia		
Total VLDL		0.55
Fraction unbound to Mab JI-H	25	1.1
Fraction bound to polyclonal anti-apoE	58	0.84[a]

[a]Ratio calculated from percent of B,E particles in total VLDL.

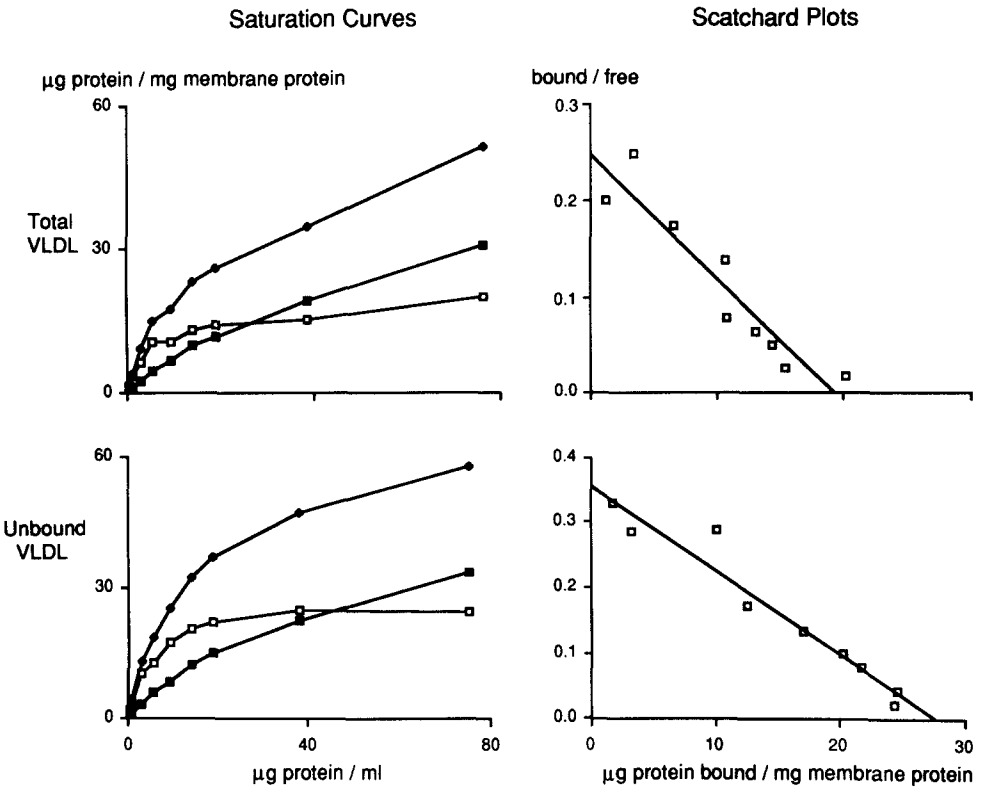

Fig. 7. Binding of total VLDL and Mab JI-H unbound VLDL from a patient with endogenous hypertriglyceridemia to the LDL receptor on endosomal membranes. Left total (♦), EDTA-resistant (■), and EDTA-sensitive (□) binding; right: Scatchard plots of specific (EDTA-sensitive) binding data. Values are means of duplicate samples.

those described for remnants of triglyceride-rich lipoproteins in the rat (40). The current observations are also consistent with those described for human VLDL populations separated by other methods, especially lipoprotein electrophoresis. Pagnan et al. (6) described two populations of VLDL in some normotriglyceridemic as well as hypertriglyceridemic individuals, separated by preparative agarose gel electrophoresis. The chemical properties of the slowly migrating population resemble those of the VLDL unbound by Mab JI-H, although slow prebeta VLDL were on average smaller than the more rapidly migrating fraction. The larger species probably did not migrate into the agarose gel. Two such populations were also observed in patients with familial dysbetalipoproteinemia by Pagnan et al. (6) and, in earlier studies, Sata, Havel, and Jones (5) and Havel and Kane (41) described three populations of VLDL. The largest species failed to migrate in agarose gel electrophoresis, the species of intermediate size had prebeta mobility, and the smallest species had beta mobility. These species were considered to represent chylomicron remnants, normal VLDL, and VLDL remnants, respectively. The electrophoretic properties of the unbound VLDL described in the current study are likewise consistent with the presence of chylomicron and VLDL remnants in familial dysbetalipoproteinemia, whereas the bound species had prebeta mobility (Fig. 1). Following the identification of apoB-48 as a marker of chylomicrons, Fainaru et al. (42) and Kane et al. (43) confirmed that large triglyceride-rich particles in the plasma of patients with familial dysbetalipoproteinemia contain considerable amounts of apoB-48, and Milne et al. (44) used Mabs against apoB-100 to separate and characterize VLDL subfractions containing only apoB-48 or apoB-100 from patients with this disorder. They found that the particles containing apoB-48 were enriched in apoE relative to C apolipoproteins when compared with particles containing apoB-100. In the current research, we have found that remnant-like chylomicron and VLDL particles are also generally present in the triglyceride-rich plasma lipoproteins of postabsorptive, normolipidemic individuals as well as those with other forms of hypertriglyceridemia, although the fraction of VLDL particles that fails to bind Mab JI-H tended to be larger in the latter.

The method that we have used to separate the unbound lipoproteins excludes the possibility that they are generated during ultracentrifugation. We isolated

TABLE 4. LDL receptor-binding properties of lipoprotein fractions

Subject	Total VLDL		VLDL Unreactive with Mab JI-H		LDL	
	K_d[a]	B_{max}[b]	K_d	B_{max}	K_d	B_{max}
Normolipidemia	30	63	34	46		
Endogenous hypertriglyceridemia	4.9	19	4.8	27		
Dysbetalipoproteinemia[c]	2.3	8.9	2.5	8.5		
Normolipidemia[d]					9.1 ± 2.2	26.8 ± 6.0

[a]Micrograms of protein/ml.
[b]Micrograms of protein/mg of membrane protein.
[c]Patient with E2/2 (arg_{145}→cys).
[d]Mean ± SD; n = 3.

these particles directly from freshly obtained blood plasma, and only separated the triglyceride-rich lipoproteins by ultracentrifugation thereafter. This approach has been used previously in studies of VLDL heterogeneity in rabbits (8) and humans (45). We have also shown that ultracentrifugation does not materially alter the content of apoE in the unbound VLDL of patients with endogenous hypertriglyceridemia.

Why is apoB-100 in the unbound lipoproteins not recognized by Mab JI-H? The only clue provided by our studies lies in their differing composition, with enrichment of the particle surface in apoE. However, populations with widely varying numbers of apoE molecules failed to bind to the antibody (Table 2).

Additional studies, including enrichment of VLDL with apoE (46, 47) may help address this question, although it is possible that differences in lipid composition of the lipoprotein surface affect the accessibility of the epitope recognized by Mab JI-H. The failure of those particles containing apoB-48 to bind Mab JI-H reflects the location of the epitope beyond the C-terminus of apoB-48; thus, nascent chylomicrons as well as chylomicron remnants, if present in plasma, will fail to bind to this antibody. Indeed, we found that all particles containing apoB-48 from a patient with lipoprotein lipase deficiency remained unbound (Fig. 3). These particles were also enriched in cholesteryl esters and apoE. The former can be explained by the long residence time of chylomicrons in this disorder (48). The high content of apoE may reflect the larger than average particle size of the unbound species in the patient studied (Table 2) or the production of remnant-like particles by the action of hepatic lipase on smaller hepatogenous VLDL (49).

Remnant-like lipoproteins are defined in this study, as in earlier studies of human VLDL (6, 41), by their physical and chemical properties. In rats, chylomicron and VLDL remnants have been isolated from the blood of functionally hepatectomized rats (40). Such particles are taken up very rapidly into the liver by receptor-dependent mechanisms involving apoE (46, 50). In the case of chylomicron remnants, rapid hepatic uptake has also been deduced from the removal of labeled lipid components of chylomicrons by the liver of dogs and sheep (51). It is unlikely that the unbound triglyceride-rich lipoproteins isolated in the current study are subject to such efficient uptake, at least in the hypertriglyceridemic patients. Absent evidence for rapid turnover, we thus designate them as "remnant-like," based upon their physical and chemical properties.

Our binding studies, although limited, suggest that the unbound lipoproteins, despite their enrichment in apoE, do not necessarily have high affinity for lipoprotein receptors that recognize this protein. Indeed, the apparent dissociation constant (K_d) for the LDL receptor in hepatocytic endosomes closely resembled that of the parent VLDL from which they were derived. The K_d values, however, varied widely and were unrelated to the average content of apoE in the unbound lipoproteins. Although interpretation of these data is not simple, several points can be made. *1*) The apparent K_d of a mixed population of particles will tend to be dominated by those with the highest affinity (52); *2*) even in our unbound population, particles with widely differing affinity may be present; and *3*) as shown by Bradley and Gianturco (12) and Krul et al. (53), the conformation of apoE may differ remarkably in populations of VLDL from different individuals. Our data indicate that the varying affinity of such populations applies as well to those B,E particles that are particularly rich in apoE. As observed by Bradley et al. (13) and Krul, Tikkanen, and Schonfeld (14), the affinity is likely to be considerably higher in VLDL from hypertriglyceridemic individuals, although in additional studies, we have found the affinity to vary widely. The apoE (arg_{145}→cys) from our patient with familial dysbetalipoproteinemia is known from earlier studies to have only moderately reduced affinity for the LDL

receptor as compared with normal apoE (38, 39), yet particles resembling chylomicron and VLDL remnants accumulate in large amounts in this individual.

The affinity of VLDL for lipoprotein receptors can be increased substantially by enriching the particles in apoE (47, 54) and such enrichment can increase the rate of catabolism of VLDL in rabbits (55, 56). Recent studies in rats have raised the possibility that the uptake of chylomicron remnants may require addition of apoE shown to exist on the surface of hepatocytes (57, 58). Thus, there is increasing evidence that a large number of apoE molecules on triglyceride-rich particles may not be sufficient for rapid hepatic uptake, particularly if the apoE is not in a suitable conformation. Such a phenomenon could account for the presence of appreciable concentrations of remnant-like particles in human VLDL.

Mab JI-H appears to be a potentially useful reagent to isolate a population of remnant-like hepatogenous VLDL as well as particles derived from lymph chylomicrons from plasma. By combining Mab JI-H with a Mab to apoA-I on a solid support, it has been possible to devise a simple method to separate remnant-like lipoproteins from the great bulk of plasma lipoproteins quantitatively (59). This combined reagent should facilitate analysis of the concentration of these remnant-like particles in various disorders of plasma lipid transport and evaluation of the relationship between the concentration of these particles and atherosclerosis in cross-sectional and prospective investigations.

We thank Leila Kotite for advice and help with immunological procedures, Delia Ajoste and Kyee Yeo for expert technical assistance, and Barbara Paschke for help in manuscript preparation. This research was supported in part by a grant from the National Institutes of Health (HL-14237, Arteriosclerosis Specialized Center of Research).

Manuscript received 6 September 1991 and in revised form 5 December 1991.

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