Isolation and characterization of two distinct species of human very low density lipoproteins lacking apolipoprotein E

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Abstract We have isolated two fractions of very low density lipoprotein particles in human plasma that lack apolipoprotein (apo) E by combined anti-apoE and heparin affinity chromatography of whole plasma followed by ultracentrifugation. The two fractions are distinguished by their ability to bind to heparin. Each of these fractions, designated "B" particles to distinguish them from very low density lipoproteins that contain apoE ("B,E" particles), comprises an appreciable fraction of total particles in very low density lipoproteins of normolipidemic and hypertriglyceridemic subjects. The heparin-unbound B particles, which have been reported previously by others, are larger and have negligible affinity for low density lipoprotein receptors. The heparin-bound B particles are smaller and do bind to low density lipoprotein receptors, albeit with much lower affinity than B,E particles. No differences in accessibility to limited protease digestion were found between apoB-100 in the two types of B particles. Our data indicate that a substantial fraction of human very low density lipoproteins lacks apoE, the principal ligand for lipoprotein receptors that mediate the terminal catabolism of these lipoproteins. Whereas the B particles that fail to bind to heparin are likely to represent a form of nascent lipoprotein, the origin of those B particles that bind to heparin remains to be determined.–**Campos, E., S. Jäckle, G. C. Chen, and R. J. Havel.** Isolation and characterization of two distinct species of human very low density lipoproteins lacking apolipoprotein E. *J. Lipid Res.* 1996. **37:** 1897–1906.

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Triglyceride-rich lipoproteins in blood plasma (ρ < 1.006 g/ml) exhibit marked heterogeneity in size and composition which is reflected in their metabolism (1–3). In the postabsorptive state, most of these lipoproteins, designated very low density lipoproteins (VLDL), contain apolipoprotein (apo) B-100 and are derived from the liver. VLDL also exhibit heterogeneity in apolipoprotein composition and this property can also be used to separate distinct subfractions. VLDL have been separated into apoE-poor and apoE-rich fractions by chromatography on heparin-Sepharose (4, 5) as well as by immunoaffinity chromatography (6, 7). Results obtained with these procedures are affected by technical factors. In particular, ultracentrifugation dissociates some apoE from lipoproteins (8, 9) and a number of investigators have applied heparin-affinity and immunoaffinity chromatography to ultracentrifugally isolated lipoprotein fractions (4, 10–17). Mindful of such alterations in lipoprotein structure, our group has applied these procedures to unfractionated blood plasma to obtain VLDL poor in apoE from rabbits (6) and humans (5). In rabbits, VLDL lacking apoE (obtained by immunoaffinity chromatography on columns of anti-apoE bound to Sepharose), designated B particles, are metabolized more slowly and are more likely to be converted to low density lipoproteins (LDL) than VLDL containing apoE (B,E particles) that bind to such columns (2, 6). In humans, VLDL B particles that fail to bind to heparin-Sepharose are not recognized by the LDL receptor on cultured fibroblasts, but after treatment with lipoprotein lipase, the particles acquire the capacity to bind to heparin and also to acquire apoE (18).

The current study arose from our observation that, as applied to fresh human blood plasma, the mass of B particles in VLDL that fails to bind to anti-apoE substantially exceeds the mass that fails to bind heparin (19). We have shown that two populations of B particles exist, distinguished by their affinity for heparin, which also differ in size, composition and LDL receptor-affinity. Each population accounts for an appreciable fraction of

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein.

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total particles in VLDL from normal and hypertriglyceridemic subjects.

METHODS

Subjects and blood sampling

Blood samples were taken by venipuncture from postabsorptive healthy normolipidemic volunteers and patients with primary hyperlipidemia from the UCSF Lipid Clinic who had fasted for 9–12 h. Blood was collected into disodium EDTA (final concentration 2 mM) containing protease inhibitors: polybrene (25 μg/ml), benzamidine (0.2 mg/ml), aprotinin (2 mg/ml), D-phenyl-L-prolyl-L-arginine chloromethyl ketone (1 μM), and lima bean and soy trypsin inhibitors (20 μg/ml each) (20) and immediately cooled on ice. Plasma was obtained by centrifugation at 2000 *g* for 30 min at 4°C.

Affinity chromatography and ultracentrifugation

The immunoglobulin G fraction of apoE antiserum raised in rabbits (21) was coupled to CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's recommendations. Immunoaffinity columns (1.5 × 9 cm containing 16 ml of the affinity matrix and 100 mg immunoglobulin) were constructed and maintained at 10°C. Before use, the columns were pretreated with normal human plasma to reduce nonspecific adsorption of proteins (22) and eluted with 3 M NaSCN, pH 7.4. Finally, the columns were washed extensively with 0.15 M NaCl, pH 7.4, containing 2 mM disodium EDTA and 0.3 mg benzamidine per ml (buffer A). The capacity of the columns, determined by applying increasing volumes of normolipidemic serum and measuring the amount of unbound apoE, was approximately 50 μg apoE, as estimated by radioimmunoassay (21).

Plasma samples of 0.25 to 1.0 ml were applied to these columns and the unbound fraction was eluted at 15 ml/h for 1 h, then at 30 ml/h for an additional h. Bound lipoproteins were then eluted at 30 ml/h with 30 ml of 3 M NaSCN containing bovine serum albumin (1 mg/ml) and benzamidine (0.3 mg/ml), pH 7.4. Eluted lipoproteins were immediately dialyzed against buffer A and the column was washed with 75 ml of buffer A.

Columns of heparin-Sepharose (Pharmacia) of 1.5 × 11 cm (20 ml) or 1.5 × 5.5 cm (10 ml) were constructed and maintained at 10°C. The columns were first pretreated with human LDL ($1.025 < \rho < 1.055$ g/ml) to reduce nonspecific binding and then eluted with 3 M NaCl containing bovine serum albumin (1 mg/ml). The columns were finally washed extensively with buffer A. The capacity of these columns, determined as described above, was approximately 500 μg and 250 apoE, respectively. Six ml of plasma or the unbound fraction from anti-apoE columns was applied and eluted at 30 ml/h for 1 h with buffer A. After an additional 4 h of washing with buffer A, the bound lipoproteins were eluted at 30 ml/h with 3 M NaCl containing 1.3 mM disodium EDTA and bovine serum albumin (1 mg/ml), pH 7.4.

Before ultracentrifugation, all fractions collected from the affinity columns were concentrated with membrane PA-10 in a microprotein dialyzer/concentrator (Biomolecular Dynamics, Beaverton, OR). VLDL were then isolated from these fractions by discontinuous density gradient ultracentrifugation (23) in a Beckman SW 41 rotor as previously described (7).

Analyses

Total and free cholesterol and triglycerides were estimated by enzymatic assays (24, 25) on the Cobas (Mira) Chemistry System (Roche). Total phospholipids were determined as lipid phosphorous × 25 (26). Protein concentration was estimated by the method of Lowry et al. (27) with bovine serum albumin as standard. ApoE was quantified by a radioimmunoassay (21) and apoB by rate immunonephelometry after hydrolysis of lipoprotein-triglycerides with lipoprotein lipase from bovine milk (28). C apoproteins were calculated by difference, assuming that the total protein of VLDL is composed of apoB, apoE, and apoCs. The diameter of lipoprotein particles was determined in electron photomicrographs of negatively stained samples (29) by a semiautomatic method with a magnetic digitizer (30).

Limited proteolysis and peptide mapping of apoB-100

VLDL fractions and LDL were treated with two enzymes of distinct specificities: *Staphylococcus aureus* V8 protease (500–700 units/mg protein) and endoprotease Lys-C (30 units/mg protein) as described (31), with the following modifications. Limited proteolysis was carried out at 37°C for various times by incubating LDL or VLDL fractions (0.03 mg of apoB/ml) in buffer A at a mass ratio of enzyme to substrate of 1:9. The enzyme reaction was stopped by boiling for 1 min in the presence of 2% SDS. Peptide maps were subsequently displayed after SDS gel electrophoresis in a 4–7% linear gradient of polyacrylamide with a discontinuous buffer system (32). Before electrophoresis, the digests were combined with SDS, glycerol, bromophenol blue, 2-mercaptoethanol, and thioglycolic acid to final concentrations of 2.5, 10, 0.003, 2, and 0.4%, respectively, after which the mixtures were boiled for 1 min. After electrophoresis, the gels were stained with silver.

LDL receptor-binding assay

Binding of lipoprotein fractions to the LDL receptor in endosomal membranes from livers of estradiol-treated rats was carried out as described (33). In brief, endosomal membranes containing 8–12 mg protein were mixed with 100 μl buffer (100 mM NaCl, 5 mM $CaCl_2$, 50 mM Tris HCl, containing 20 mg bovine serum albumin per ml) in the absence and presence of 30 mM EDTA, pH 7.5. Fifty μl of ^{125}I-labeled lipoprotein fraction (34) was added at the concentrations indicated in Fig. 1, and the mixture was incubated for 60 min at 4°C. Bound and unbound lipoproteins were separated by centrifugation in a Beckman 42.2 rotor (35). Although the LDL receptor-related protein is also present in these endosome fractions, it has been established that virtually all EDTA-sensitive binding of triglyceride-rich lipoproteins to these membranes reflects association with the LDL receptor (33). The affinity of human LDL for the LDL receptor in these membranes, estimated as the equilibrium dissociation constant (K_d), was 9.2 ± 2.2 μg protein/ml (7). As estimated from the ratio of apoE to C apoproteins, the labeling procedure caused a loss of 20–30% of apoE in human VLDL samples.

Statistical analysis

Differences between mean values were evaluated with a two-tailed Student's *t* test. Correlations between variables were evaluated by linear regression analysis.

RESULTS

Identification of two distinct particles poor in apoE (B particles) in human VLDL

The two populations of VLDL that failed to bind to heparin and anti-apoE, respectively, were very poor in apoE, as expected and shown below. In analogy with previous work in rabbits (6), both populations were designated as "B" particles, whereas those VLDL that bound to anti-apoE were designated as "B,E" particles. In six subjects with varying plasma triglyceride concentrations, the fraction of VLDL-cholesterol that failed to bind to anti-apoE exceeded the fraction that failed to bind to heparin (**Table 1**). On average, 40.3 ± 7.4% of total VLDL-cholesterol failed to bind to anti-apoE, whereas 21.8 ± 8.7% failed to bind to heparin. In three subjects the estimated amount of cholesterol in VLDL particles that bound to heparin was less than one-half of the total particles that were very poor in apoE. The data in Table 1 thus indicate that an appreciable fraction of B particles binds to heparin. Within-subject variation in distribution of VLDL-cholesterol in the two types of B particles was small (**Table 2**).

In order to isolate the heparin-bound B particles, the unbound fraction of plasma from anti-apoE immunoaffinity columns was concentrated and then applied to the heparin-affinity columns. VLDL in the recovered unbound and bound fractions, together with those in the bound fraction from the immunoaffinity column (B,E particles) were obtained by ultracentrifugation. **Table 3** shows various mass ratios of the three subfractions in six subjects, five of whom were mildly hypertriglyceridemic (plasma triglycerides less than 300 mg/dl). From the mass ratios of apoE:apoB, it is evident that both B particle fractions contain less than 1 molecule of apoE per particle. The ratio of cholesteryl esters to triglycerides, the major core components of the particles, was lower in the heparin-unbound B particles than was found in the heparin-bound B particles and B,E particles, but the ratio of free cholesterol to total cholesterol was the same. The ratio of free cholesterol to phospholipids was also lower in the unbound B particles than in the other two fractions. These observations suggested that the unbound B particles were larger than

TABLE 1. Distribution of cholesterol among three VLDL subfractions

Subject (Age/Sex)	Total Cholesterol	Triglycerides	ApoE	ApoE Phenotype	B Particles			B,E Particles[b]
					Anti-ApoE-Unbound	Heparin-Unbound	Heparin-Bound[a]	
	mg/dl plasma				*% of total VLDL cholesterol*			
28/M	142	95	3.2	3/3	32.7	28.6	4.1	67.3
48/F	200	53	1.4	3/3	32.5	21.5	11.0	67.5
64/M	208	106	3.8	3/3	45.0	16.6	28.4	55.0
73/F	213	168	5.2	3/3	45.9	14.1	31.8	54.1
60/F	278	170	6.2	3/3	36.8	35.7	1.1	64.2
37/M	243	230	3.6	3/3	49.6	14.4	35.2	50.4

[a]Cholesterol in unbound apoE immunoaffinity column fraction less that in unbound heparin-affinity column fraction.
[b]Cholesterol in total VLDL (taken as 100%) less that in unbound apoE-immunoaffinity column fraction.

TABLE 2. Distribution over time of VLDL cholesterol and apoE from two individuals in unbound fractions from heparin-affinity and apoE-immunoaffinity columns.

Subject Sample	Plasma Triglycerides	Heparin-Unbound	Anti-ApoE-Unbound	Plasma ApoE	Heparin-Unbound[c]	Anti-ApoE-Unbound[c]
	mg/dl	*% of total VLDL cholesterol*		*μg/ml*	*% of plasma apoE*	
1[a]						
a	265	22.5	46.1	44.0	1.2	1.4
b	184	20.5	42.9	27.1	3.6	3.7
c	230	14.4	49.6	35.8	3.4	6.7
Mean (SD)		19.1(4.2)	46.2(3.4)		2.7(1.3)	3.9(2.7)
2[b]						
a	92	13.9	44.6	32.5	3.4	0.8
b	106	13.1	37.6	35.6	6.7	1.1
c	102	13.2	49.4	37.8	0.7	1.0
Mean (SD)		13.4(0.4)	43.9(5.9)		3.6(3.0)	0.93(0.17)

[a]From blood samples taken over 3-month period.
[b]From blood samples taken over 9-month period.
[c]Measured in each unbound fraction before ultracentrifugation.

either the bound B particles or B,E particles and this was confirmed by electron microscopy in three of the six subjects (Table 3). The diameters of 95% of the particles in the heparin-unbound fractions were between 250 and 800 Å, whereas those of the heparin-bound B particles were between 250 and 650 Å and those of the B,E particles were between 150 and 500 Å. Based upon these data, we also refer to the heparin-unbound and bound B particles as "large" and "small" B particles, respectively.

As also shown in Table 3, the mass ratio of cholesteryl esters to apoB did not differ significantly among the three subfractions. ApoB-48 was found in each of the subfractions (see below).

The composition of each fraction from three hypertriglyceridemic subjects is shown in **Table 4.** As expected from the differences in size, the summed mass of surface components (phospholipids, free cholesterol, and protein) was higher in the bound fractions: 39.9% and 40.0% for the heparin-bound B particles and B,E particles, respectively, as compared with 32.8% for the heparin-unbound B particles. This difference was observed in each subject. The lipid composition of the heparin-bound B particles was very similar to that of the B,E particles, despite the paucity of apoE in the former. This low concentration of apoE cannot be ascribed to ultracentrifugal dissociation because the total unbound fraction from the anti-apoE column, assayed before ultracentrifugation, had a comparable mass of apoE (Table 3).

TABLE 3. Properties of three VLDL subfractions

Mass Ratios	A Heparin-Unbound B Particles	B Heparin-Bound B Particles	C B,E Particles
Free cholesterol / Total cholesterol	0.55 (0.076)	0.55 (0.065)	0.55 (0.072)
Cholesteryl esters / Triglycerides	0.11 (0.026)	0.17 (0.035)[a]	0.19 (0.072)
Free cholesterol / Phospholipids	0.32 (0.039)	0.36 (0.088)	0.39 (0.058)[b]
ApoE:ApoB[c]	0.012 (0.010)	0.034 (0.019)	0.32 (0.13)
Cholesteryl esters:ApoB	3.4 (1.4)	2.5 (1.2)	3.3 (0.9)
Particle diameter (Å)[d]	491 (6)	353 (22)	370 (32)

Values are mean and SD (n = 6).
[a]$P < 0.01$ (compared to A).
[b]$P < 0.05$ (compared to A).
[c]ApoE was measured in fractions before ultracentrifugation.
[d]n = 3 (200 particles measured in each sample).

TABLE 4. Composition of VLDL subfractions (% mass)

	Total	B Particles		B,E Particles
		A. Heparin-Unbound	B. Heparin-Bound	
Cholesteryl esters	9.0 (1.7)	5.8 (0.95)	10.4 (1.9)[a]	11.6 (2.9)
Triglycerides	52.7 (1.4)	61.3 (1.7)	49.3 (1.2)[b]	48.9 (1.3)
Free cholesterol	7.1 (0.62)	6.2 (0.47)	8.1 (1.3)	7.7 (0.51)
Phospholipids	19.2 (1.6)	17.8 (0.95)	19.1 (0.9)	18.8 (1.5)
Protein	12.2 (1.9)	8.8 (1.0)	12.7 (1.6)[a]	13.5 (1.8)
ApoB	3.6 (0.26)	2.2 (0.51)	3.1 (1.1)	3.7 (0.61)
ApoC	8.2 (2.2)	6.6 (1.0)	9.4 (0.52)[c]	9.0 (2.1)
ApoE	0.46 (0.14)	0.038 (0.011)	0.13 (0.052)	0.76 (0.40)
Mass ratios				
ApoE:ApoB	0.13 (0.031)	0.017 (0.0051)	0.044 (0.0085)	0.20 (0.080)
ApoE:ApoC	0.060 (0.025)	0.0057(0.0008)	0.014 (0.0049)	0.095 (0.062)

Mean and SD, (n = 3); all comparisons are with A.
[a]P < 0.05.
[b]P < 0.002.
[c]P < 0.02.

Binding properties of VLDL subfractions to the LDL receptor

The affinity of the heparin-unbound B particles for the LDL receptor was too low to measure (>500 μg protein per ml). By contrast, the heparin-bound B particles invariably bound with low, but measurable affinity to the receptor, and that of the B,E particles was several fold higher (**Table 5**). As compared with the affinity of human LDL for the rat LDL receptor (ca. 9 μg protein/ml) that of the B,E particles varied substantially from 1.6 to 74 μg/ml. This variation was not clearly related to the plasma triglyceride concentration of the subjects studied. Estimated maximal binding of the heparin-bound B particles and B,E particles was similar. The binding isotherms from one subject are shown in **Fig. 1.** As the binding affinity of the B,E particles resembled that of total VLDL particles, we measured the binding affinity of total VLDL from 11 additional subjects (**Table 6**). This confirmed the wide variation in binding affinity among subjects with high normal to elevated plasma triglyceride concentrations and showed that the affinity was not significantly related to the apoE/apoB mass ratio ($r = 0.49$, n = 13), to plasma apoE concentration ($r = 0.51$, n = 13), or apoE phenotype.

Conformation of apoB in VLDL subfractions

To determine whether the differing affinities of the two B particles for the LDL receptor were accompanied by altered conformation of apoB-100, accessibility to digestion with *Staphylococcus aureus* V8 protease was assessed. Two apoB-100 fragments similar in size to the T1 and T2 fragments generated by thrombin cleavage of total VLDL (31) were readily generated in both types of B particle as well as in B,E particles (**Fig. 2,** lanes 7–9 and 12–14) and, in all three VLDL subfractions, the complementary fragments similar in size to apoB-74 and apoB-26 that were produced from apoB-100 of LDL (Fig. 2, lanes 10 and 15) were not observed. This simi-

TABLE 5. Receptor-binding properties of three VLDL subfractions

Plasma Triglycerides	Total VLDL		Heparin-Unbound B Particles		Heparin-Bound B Particles		B,E Particles	
	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}
mg/dl								
162	66	40			382	50	65	40
168	44	40			108	55	29	35
193	4	5	>500	indeterminate	83	5	1.6	2
260	23	39			216	35	10	24
500	44	5			18	8	74	13

Equilibrium dissociation constant (K_d): μg protein/ml; the K_d for human LDL with this method is approximately 9 μg protein/ml (7). Maximal binding (B_{max}): μg protein/mg membrane protein.

larity among the three VLDL subfractions was also observed when digestion was performed with endoprotease Lys-C (result not shown).

DISCUSSION

We have described two novel fractions of human VLDL that contain little apoE. One of these, as found by others (5), lacks affinity for heparin, but the other fraction does bind to heparin. Inasmuch as most of the particles in the latter, novel fraction contain apoB-100 but lack apoE, their heparin-binding property can be attributed mainly or exclusively to the former protein. Both the total population of VLDL particles lacking apoE and that portion that lacked affinity for heparin were isolated from the unretained fractions. As pointed out by McConathy et al. (36), such particles are equivalent to those obtained by gel filtration, which is considered to be a particularly useful procedure for isolating native lipoprotein species. With gel filtration, all apoE in rat (8) or human (21) serum remains associated with plasma lipoproteins. Dilution of lipoproteins during gel filtration could theoretically lead to accumulation of some free apoE, but if such did occur, this apoE would have to reassociate with other particles during chromatography.

To isolate the B,E particles, it was necessary to elute them from anti-apoE affinity columns with sodium thiocyanate. Others have shown that any apoE on triglyceride-rich lipoproteins remains associated during a second chromatography of bound fractions under conditions similar to ours (22, 36). Studies in rabbits have shown major differences in the metabolism of B and B,E particles separated by anti-apoE immunoaffinity chromatography (2, 6), as have studies of the metabolism of heparin-unbound and heparin-bound VLDL in

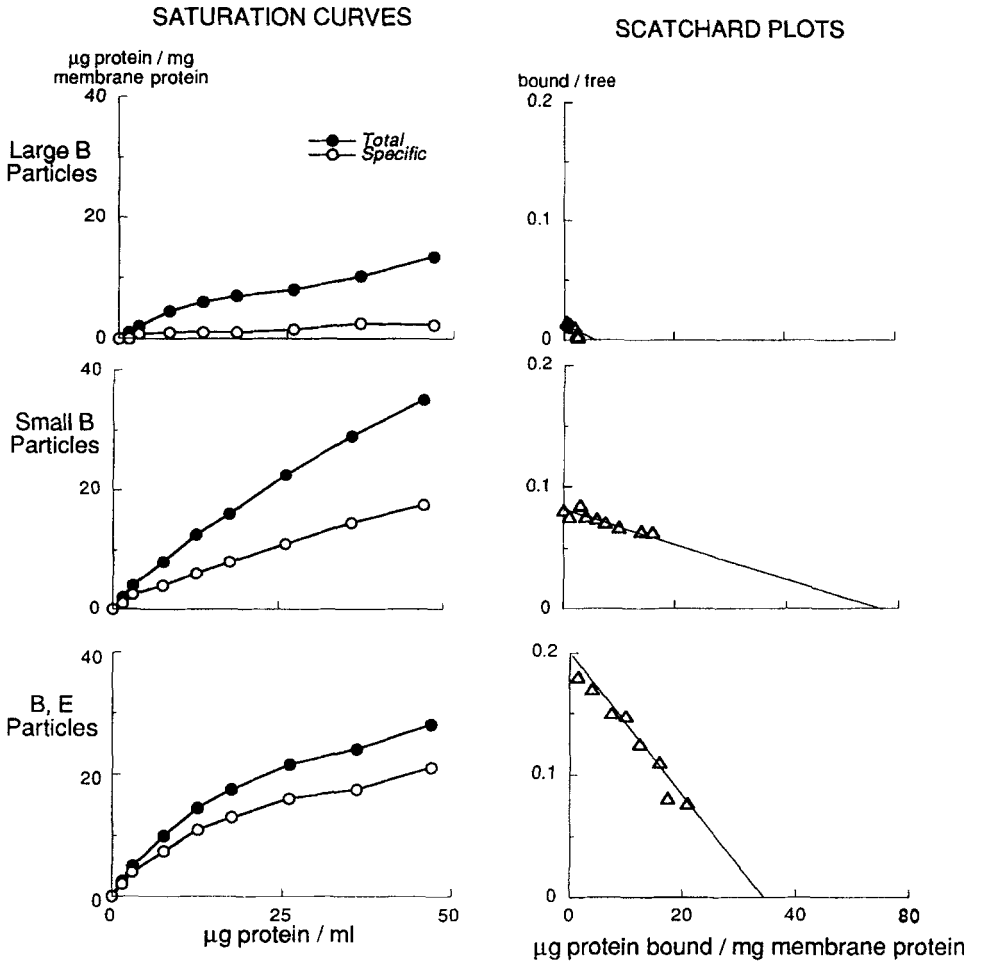

Fig. 1. Comparison of total and specific binding of large B, small B, and B,E particles to the LDL-receptor. Direct binding isotherms and Scatchard plots are shown. Specific (EDTA-sensitive) binding was calculated by subtracting EDTA-resistant from total binding. Values are the means of duplicate samples. Apparent maximal binding and K_d were estimated by the method of least squares.

TABLE 6. Binding affinities of VLDL in hypertriglyceridemic subjects

Subject	Plasma TG	Plasma ApoE	ApoE Phenotype	VLDL	
				ApoE/ApoB Mass Ratio	K_d
	mg/dl	*mg/dl*			*μg prot./ml*
1	162	3.6	3/3	0.10	66.2
2	168	6.9	4/3	0.15	43.9
3	175	4.9	3/3	0.13	15.5
4	193	5.3	4/3	0.12	4.0
5	215	4.0	3/2	-	30.0
6	225	5.0	4/4	0.13	63.2
7	252	6.1	4/3	-	5.0
8	260	5.0	4/3	0.16	22.8
9	263	12.6	3/2	0.26	7.4
10	278	10.3	3/3	0.15	16.4
11	346	20.6	3/3	0.17	11.9
12	429	6.5	3/3	-	22.0
13	484	17.0	3/2	0.14	19.8
14	500	9.7	3/3	0.15	44.3
15	561	14.1	3/2	0.17	8.0
16	992	24.0	4/2	0.27	10.5

humans (11). The heparin-unbound fraction of human VLDL has been shown to be enriched in phosphatidylethanolamine (5), a characteristic of nascent VLDL in rats (37).

In some earlier studies VLDL particles that contained appreciable apoE failed to bind heparin (12, 16). In these studies, however, VLDL were first isolated by ultracentrifugation, a procedure that may, in addition to dissociating some apoE, also alter the conformation of the remaining apoE or apoB-100. Leowsky et al. (14) observed a stronger correlation of the property of heparin affinity with the apoB rather than the apoE component of VLDL from diabetic subjects and suggested that apoB of smaller VLDL particles may interact with heparin in analogy with the studies of Gianturco et al. (38) and Krul et al. (39) on binding of VLDL subfractions to the LDL

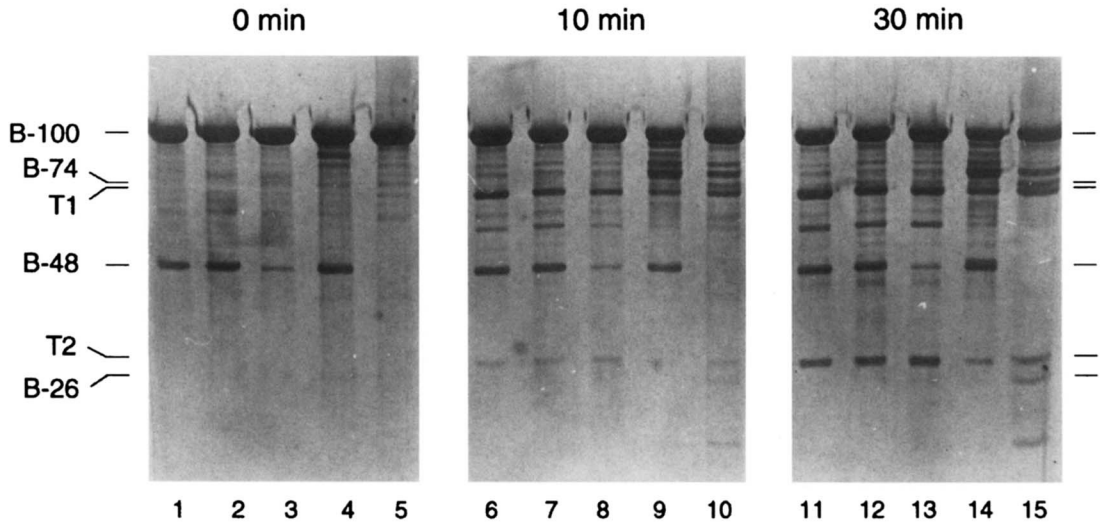

Fig. 2. Digestion by *Staphylococcus aureus* V8 protease of apoB-100 in total VLDL, large B particles, small B particles, B,E particles, and LDL, represented (from left to right, respectively) in lanes 1–5 (zero minutes), 6–10 (10 min incubation) and 11–15 (30 min incubation), as analyzed by SDS-PAGE with silver staining. Protein (0.2 μg) was applied to each lane. The mobilities of known apoB-100 fragments (B-74, B-26, T1, and T2) and apoB-48 are also indicated.

TABLE 7. Distribution of triglyceride-rich lipoprotein particles in fasting human blood plasma

Subfraction	% of Particles[a]
Chylomicron-derived (containing apoB-48)	10
Total VLDL (containing apoB-100)	90
Large B particles (lacking heparin affinity)	19
Small B particles (with heparin affinity)	15
B,E particles (apoB-50 epitope recognized by Mab JI-H exposed)	44
B,E particles (apoB-50 epitope recognized by Mab JI-H hidden)	12

[a]Estimates are approximations based upon molar concentration of apoB (one molecule per particle) in normotriglyceridemic subjects of current study and those of references 7 and 40.

receptor. Leowsky et al. (14) also used ultracentrifugally isolated VLDL in their studies, so that their observations cannot be compared directly with ours.

As observed previously (18), our large B particles lacking affinity for heparin were not recognized by the LDL receptor, but the small B particles that bind heparin exhibited low affinity receptor-binding. Such low affinity could reflect receptor-binding of only a fraction of the total population of small B particles and we cannot exclude the possibility that particles containing some apoE are responsible for the observed binding. It is unlikely that particles containing more than a single molecule of apoE are present, however, and, in our studies in rabbits, the metabolism of intermediate density lipoproteins (IDL) containing one apoE molecule did not appear to differ from that of IDL particles lacking apoE (6).

Our results also indicate that the affinity of ultracentrifugally isolated VLDL to the LDL receptor mainly reflects those VLDL particles that, on average, contain several molecules of apoE. As observed by others (38), this property of human VLDL varies widely among individuals and reflects the binding of apoE rather than apoB-100 to the receptor.

Although our results indicate that the two populations of B particles have different conformations that affect binding to heparin, we were unable to demonstrate different accessibility of component apoB-100 to limited protease digestion. In particular, unlike LDL, the small B particles failed to generate fragments similar in size to complementary apoB-74 and apoB-26 upon treatment with *Staphylococcus aureus* V8 protease and endoprotease Lys-C, although all VLDL particles as well as LDL were susceptible to cleavage by these two enzymes at residues 3198 and 3205, respectively (near the thrombin site at residue 3249) (31).

VLDL B particles that fail to bind heparin have been postulated to represent nascent VLDL (11, 18). The smaller B particles that bind to heparin may be a form of VLDL remnant that has lost its apoE, before conversion to IDL and LDL. In rabbits, however, evidence has been obtained that some nascent B particles never acquire apoE, whereas other nascent VLDL are B,E particles that lose apoE during the course of conversion to IDL and LDL (2, 6).

In the current study, some apoB-48 was found in B particles as well as in B,E particles, particularly in the large B particles, as shown in Fig. 2. Relatively little apoB-48 was found in small B particles, and it is possible that a small number of B-48, E particles is present in this fraction. Lipoproteins containing apoB-48, which comprise about 10% of total lipoprotein particles in VLDL obtained from fasting normolipidemic subjects (40), have been found to be rich in apoE overall (7). Evidence has also been obtained for a population of particles containing apoB-100, larger on average than most VLDL, that fails to bind to a monoclonal antibody (JI-H) whose epitope resides in the region of B-50 (7). These particles are rich in apoE relative to C apoproteins and thus distinct from the B particles found here. Such observations indicate heterogeneity of B,E particles with respect to apoB-100 conformation. As this and the current study were carried out by applying affinity chromatography to fresh human plasma, it appears that, in addition to lipoproteins containing apoB-48, at least four native species of VLDL containing apoB-100 can now be identified and quantified (**Table 7**). The two species of apoB-100, E particles together comprise about 56% of total VLDL, of which the fraction distinguished by lack of exposure of the epitope near B-50 accounts for about one-eighth (7). Finally, each of the two apoB fractions that have been isolated in the current work accounts for 15 to 20% of VLDL particles containing apoB-100. It is thus apparent that these latter fractions contribute substantially to VLDL heterogeneity and should be considered in future studies of the metabolism and pathophysiological properties of triglyceride-rich lipoproteins.

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