Accumulation of an apoE-poor subfraction of very low density lipoprotein in hypertriglyceridemic men

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Abstract Studies were undertaken to investigate the mechanism of the marked accumulation of an apoE-poor very low density lipoprotein (VLDL) subfraction in untreated Type IV and IIb hypertriglyceridemic subjects. Heparin-Sepharose chromatography was used to separate large VLDL (S_f 60–400) from fasted subjects, into an apoE-poor, unbound fraction and an apoE-rich, bound fraction. As a percent of total VLDL protein, the apoE-poor fraction comprised 40 ± 4% of total VLDL in hypertriglyceridemic subjects versus 25% in normal subjects. Compared to the apoE-rich, bound fraction, this apoE-poor material was found to have a 5-fold lower ratio of apoE to apoC (0.20 ± 0.06 vs 0.91 ± 0.18, $P < 0.005$), but a 1.5-fold higher ratio of triglyceride to protein (11.41 ± 0.85 vs 7.97 ± 0.77, $P < 0.01$). In addition, the apoE-poor fraction was found to be enriched 2-fold in apoB-48 (10.30 ± 2.41% vs 5.73 ± 1.59% of total apoB, $P < 0.005$) compared to the apoE-rich fraction, suggesting that the apoE-poor fraction contains more chylomicron remnants. The amount of this apoE-poor VLDL was markedly reduced following a reduction in VLDL triglyceride levels (a decrease from 40 ± 4% to 21 ± 2% of VLDL protein following a 50% reduction in VLDL triglyceride levels). The large VLDL from Type I, III, and V hyperlipoproteinemic subjects subfractionated using heparin-Sepharose showed an equal distribution of apoE between the two fractions in contrast with the Type IV and IIb subjects. The separation of VLDL from Type I, III, and V subjects using heparin-Sepharose involves a mechanism other than apoE binding. Separation in the latter likely results from apoB-100 binding to heparin, as opposed to apoE binding of VLDL from Type IV and IIb subjects. — **Evans, A. J., M. W. Huff, and B. M. Wolfe.** Accumulation of an apoE-poor subfraction of very low density lipoprotein in hypertriglyceridemic men. *J. Lipid Res.* 1989. **30:** 1691–1701.

Supplementary key words hypertriglyceridemia • heparin-Sepharose chromatography

Triglyceride-rich very low density lipoproteins (VLDL) enter the circulation following synthesis and secretion mainly from the liver. VLDL are elevated in Type IV hypertriglyceridemia, due to both their overproduction and a decreased catabolism (1,2). In normal humans, VLDL are primarily converted to intermediate density lipoproteins (IDL) and low density lipoproteins (LDL) as a result of lipolysis, whereas in hypertriglyceridemic subjects, a large proportion of VLDL is removed without conversion to LDL (3, 4). Abnormalities of VLDL metabolism in hypertriglyceridemic subjects are predominantly in the large particles (S_f 60–400), indicating that this fraction may account for the metabolic heterogeneity of VLDL in these subjects compared to normal subjects (3–5). Plasma VLDL are a multidisperse population and vary in size from 300 to 800 Å (6). They contain apolipoproteins B-100, C, and E. ApoB-100 is required for particle formation, whereas apoE has an important role in modulating catabolism of these lipoproteins (7, 8). In studies using VLDL from hypertriglyceridemic subjects separated into subfractions by ultracentrifugation, it has been demonstrated that the size of VLDL particles determines whether apoE or apoB is the ligand for binding to cellular lipoprotein receptors (9, 10). Large VLDL utilize apoE and not apoB-100 as the ligand for binding to cell surface receptors whereas smaller VLDL and IDL use apoE and apoB-100 as binding ligands. The C apolipoproteins, in addition to the role of apoC-II in the activation of lipoprotein lipase, modulate the apoE-mediated hepatic catabolism of VLDL by inhibiting apoE-dependent binding and degradation (11, 12). VLDL from hypertriglyceridemic subjects contain variable amounts of apoB-48 (13), derived from intestinally synthesized chylomicrons (7, 14). Binding of chylomicron remnant particles to the lipoprotein remnant receptor is also apoE-mediated and does not involve binding of apoB-48 to a cell surface receptor (15).

Several studies have been conducted to assess metabolic and compositional heterogeneity of VLDL (16–19), including the use of heparin-Sepharose chromatography to separate human VLDL into an apoE-poor, unbound fraction and an apoE-rich, bound fraction (20). Weisgraber et al. (21) have demonstrated that the heparin-binding domain and the LDL receptor-binding domain of apoE are in the

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; LPL, lipoprotein lipase; HTGL, hepatic triglyceride lipase; IEF, isoelectric focusing.

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same region of the apoE molecule. This suggests that heparin-Sepharose may isolate a VLDL subfraction containing metabolically active apoE.

We have previously demonstrated that in VLDL from Type IV hypertriglyceridemic subjects, the unbound, apoE-poor particles tended to accumulate in plasma when compared to normal subjects (20). Metabolic studies with these subfractions in miniature pigs demonstrated that the apoE-poor fraction was metabolized at one-half the rate of its apoE-rich counterpart. The present studies were undertaken to confirm the accumulation of an apoE-poor subfraction in large VLDL in plasma from hypertriglyceridemic subjects. We wanted to determine whether the apoE-poor VLDL contains particles of intestinal origin by determining the apoB-48 content and assessing the effect of treatment for hypertriglyceridemia on this subfraction. We also wanted to determine whether an apoE-poor VLDL subfraction accumulates in patients with Types I, III, and V hyperlipoproteinemia.

MATERIALS AND METHODS

Subjects

Male subjects with primary hyperlipidemia were identified at the Lipid Clinic at University Hospital. They had no evidence of hepatic, renal, or other detectable metabolic disorders. The lipoprotein phenotypes were assessed according to the classification of Fredrickson, Goldstein, and Brown (22). The plasma was obtained from subjects expressing Type IV, IIb, III, V, and I phenotypes. All subjects had plasma triglyceride levels in excess of 300 mg/dl at the time of study and were fasted for 16 h prior to blood sampling. These studies were approved by the University of Western Ontario Health Sciences Standing Committee on Human Research and all subjects gave informed consent. The baseline data for the subjects used in this study are listed in **Table 1**. The Type I subject had profound fasting chylomicronemia (plasma TG, 3497 mg/dl) and was defined based on a total absence of any heparin-releasable lipoprotein lipase (LPL) activity. The LPL and hepatic triglyceride lipase (HTGL) activities were determined using the method of Blache, Bouthillier, and Davignon (23) and compared to post-heparin plasma samples from two normolipidemic, healthy volunteers given the same dose of heparin (Type I: LPL = 0.0, HTGL = 9.5 vs normals: LPL = 3.0, HTGL = 12.5; μmol of free fatty acid released per h per ml of post-heparin plasma, following a bolus injection of 10 I. U. heparin/kg of body weight). This subject was not apoC-II-deficient as determined by analytical isoelectric focusing gel electrophoresis. Three Type IV subjects were studied following treatment. One treated with diet alone (1500 calories per day with 30% of calories as fat) lost 6.0 kg of weight; another was treated with the same diet, losing 13.0 kg of weight, and gemfibrozil (300 mg, three times per day); and the third subject, with normal body weight, was treated with genfibrozil only (300 mg, twice per day).

Lipoprotein isolation

Approximately 50 ml of blood was collected from each fasting donor and placed in sterile tubes containing 0.15% EDTA. Plasma was separated at 4°C at 1000 *g* and chloramphenicol (Pentagone Pharma Inc., Montreal, Quebec) and gentamycin sulfate (Schering Canada Inc., Pointe Claire, Quebec) were added at concentrations of 0.050 mg/ml and 0.100 mg/ml, respectively. Plasma (20–25 ml) was placed under d 1.006 g/ml buffered saline (12–15 ml) (0.15 M NaCl–1 mM EDTA–1 mM Tris–10 μM phenylmethane sulfonyl fluoride–3 mM NaN_3–0.10 mM merthiolate) in a Beckman Quickseal Tube. With the exception of the Type I and V patients there were no detectable chylomicrons seen after centrifugation at 17,000 rpm for 30 min at 12°C in a Beckman 60 Ti rotor. VLDL (S_f 60–400) was isolated after 2 h at 40,000 rpm at 12°C in the Beckman 60 Ti rotor. The VLDL was then washed twice through an equal volume of buffered saline in a Beckman 50 Ti rotor spun at 40,000 rpm at 12°C for 16 h. The second wash served to eliminate contamination by albumin as determined by analytical isoelectric focusing and sodium dodecyl sulfate gel electrophoresis.

TABLE 1. Plasma and lipoprotein lipid concentrations of VLDL donors[a]

Lipoprotein Phenotype	Plasma		VLDL		LDL	HDL
	C	TG	C	TG	C	C
Type IV (n = 4)	278 ± 21	545 ± 76	109 ± 23	396 ± 47	139 ± 30	29 ± 4
Type IIb (n = 2)	419 ± 49	596 ± 11	114 ± 20	411 ± 60	269 ± 32	36 ± 2
Type III (n = 4)[b,c]	292 ± 31	643 ± 239	152 ± 38	472 ± 243	104 ± 27	35 ± 4
Type V (n = 2)	249 ± 110	1495 ± 466	ND	ND	ND	ND
Type I (n = 1)	262	3497	188	937	46	28

[a]Values are in mg/dl ± SEM; C, cholesterol; TG, triglyceride; ND, not determined.
[b]Three subjects were homozygous for apoE2 and the fourth was apoE3E1.
[c]One subject had a plasma triglyceride value of 775 mg/dl (without any detectable fasting chylomicronemia) accounting for the degree of variability for the plasma and VLDL triglycerides for the subjects studied. The VLDL cholesterol to plasma triglyceride ratio without this subject was 0.357 ± 0.043.

Heparin-Sepharose chromatography

Cyanogen bromide-activated Sepharose 4B was obtained from Pharmacia, Montreal, Quebec. Heparin (sodium salt, porcine mucosa, obtained from Calbiochem, La Jolla, CA) was bound to the Sepharose by the method of Iverius (24). The chromatography was carried out as described previously (20). Briefly, approximately 8 ml of swollen gel was packed in 1.0 × 15.0 cm columns and equilibrated against a buffer containing 2 mM phosphate (pH 7.4) 0.05 M NaCl, and 3 mM NaN_3. VLDL, dialyzed against the equilibrating buffer, were loaded on columns run at a flow rate of 6.0 ml/h at 4°C. The bound material was eluted in a stepwise manner with the same buffer containing 0.8 M NaCl. The same amount of VLDL protein (2–2.5 mg) from each subject was loaded on the columns. VLDL from most subjects was separated in duplicate. The VLDL fractions were collected and elution profiles were obtained by reading the absorbance of each fraction at 280 nm. Aliquots were taken from the most concentrated fractions of each subfraction for analysis by sodium dodecyl sulfate-polyacrylamide gradient gel electrophoresis. The remaining fractions were pooled for lipid and protein analysis and analytical isoelectric focusing gel electrophoresis. Prior to gel electrophoresis, the samples were delipidated with methanol–chloroform–diethyl ether 1:2:5 (v/v/v) followed by a wash with diethyl ether only (20).

Analytical isolectric focusing gel electrophoresis

Isoelectric focusing gel electrophoresis was carried out according to a modification (20) of methods published previously (25, 26). The gels were scanned by densitometry to determine the relative amounts of the various isoforms of apoE and apoC. Staining intensity was linear over the concentration range of VLDL protein loaded, as found previously (25, 26). ApoE phenotypes were determined by the method of Bouthillier, Sing, and Davignon (26).

Sodium dodecyl sulfate-polyacrylamide gradient gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gradient gel electrophoresis was used to assess the distribution of apoB-100 and apoB-48 between the two VLDL subfractions. The samples from each peak in the elution profile were solubilized in buffer containing 2% sodium dodecyl sulfate and incubated for 15 min at 60°C. The samples were loaded on 15 × 14 cm slab gels, 1.5 mm thick, consisting of a 4% acrylamide stacking gel (pH 6.8) and a 4–18% acrylamide gradient separating gel (pH 8.8). Samples were loaded at 36 μg/lane immediately after incubation. The sharpest bands corresponding to the two apoB species were obtained by electrophoresis at 90 v (constant voltage) in 0.1% sodium dodecyl sulfate until the tracking dye was within 1 cm of the bottom of the gel. The gels were fixed for 3 h in 10% acetic acid–15% isopropanol, stained in Coomassie Brilliant Blue R250 (dissolved in the fixing solution) for 2 h, and then destained in the fixing solution. The gels were then placed in 3% glycerol overnight and the relative amounts of apoB-100 and apoB-48 were determined by densitometry.

Analyses

Protein concentrations were determined by a modified method of Lowry et al. (27, 28). Cholesterol and triglycerides were measured using diagnostic kits from Boehringer Mannheim GmbH Diagnostica, Montreal, Quebec (Test Combination kit for triglycerides and C-system kit for cholesterol). HDL cholesterol was determined following precipitation with heparin-manganese chloride (29).

RESULTS

The subfractionation of VLDL (S_f 60–400) from a normal subject and from a Type IV hypertriglyceridemic subject on heparin-Sepharose columns is shown in **Fig. 1**.

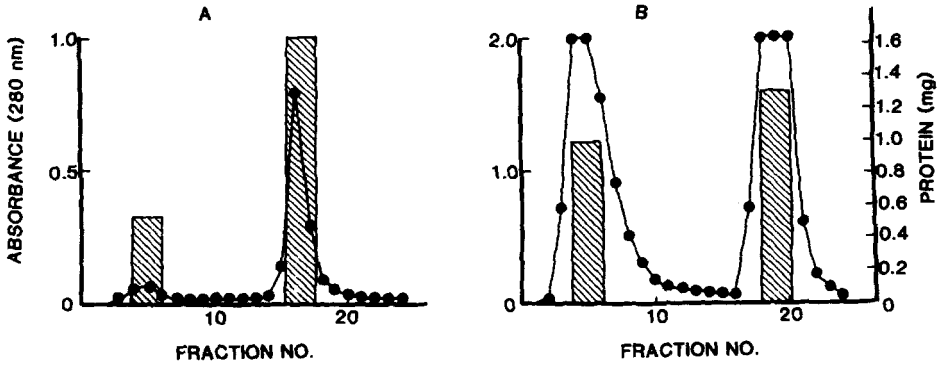

Fig. 1. Elution profiles of VLDL protein from a normal subject (A) and a Type IV hypertriglyceridemic subject (B) from heparin-Sepharose columns. VLDL protein was loaded and the subfractions were eluted as described in Materials and Methods. The elution profiles were obtained by measurement of the absorbance at 280 nm (●●). Due to the opalescence as a result of the high triglyceride content of the Type IV VLDL, the amounts and distribution of protein in the VLDL subfractions were measured by a modification (28) of the method of Lowry et al. (27) and are represented by the hatched bars.

These elution profiles demonstrate the substantial accumulation of the unbound, apoE-poor material in a Type IV subject. Although absorbance at 280 mm is influenced by opalescence associated with the elevated triglyceride content, the distribution of protein between the two subfractions also reveals the increased contribution of apoE-poor VLDL to the total VLDL in this subject. **Fig. 2** shows the results of analytical isoelectric focusing of whole VLDL and the two heparin affinity column fractions from a Type IV subject. It is apparent that the unbound fraction contains much less apoE relative to apoC.

Results for the Type IV and IIb subjects were pooled as no apparent differences were observed. For the Type IV and IIb subjects, a mean of 40 ± 4% of total VLDL protein was found in the unbound fraction. This contrasts markedly with the normal subjects studied previously, where less than 25% was unbound (20). The difference is shown in **Fig. 3**. The same amount of VLDL protein (2–2.5 mg) was loaded on the same amount of heparin-Sepharose in each study. Using samples from two Type IV subjects and one Type V subject, overloading the columns (3–3.5 mg) resulted in a 20–30% rise in the apoE/C ratio of the unbound fractions indicating that the binding capacity of the column had been exceeded. Underloading the columns (1–1.25 mg of VLDL protein) resulted in all of the VLDL binding to the column, indicating that other VLDL apoproteins such as apoB are binding to heparin (data not shown). **Table 2** shows the lipid and apolipoprotein compositions of the unbound and bound VLDL subfractions from Type IV, IIb, III, V and I subjects. The apoE and apoC isoforms in the subfractions from the Type IV and IIb subjects were quantitated by densitometry and appear as ratios of apoE to apoC for each subfraction in Table 2. The bound fraction had a significantly higher ratio of apoE to apoC (0.91 ± 0.18 vs 0.20 ± 0.06, $P < 0.005$). Since the amount of apoC was similar in each fraction, the unbound fraction in the Type IV and IIb subjects was designated as apoE-poor, whereas the bound fraction was apoE-rich. Although apoE can be lost from VLDL during ultracentrifugation, the second ultracentrifugation resulted in a 10-18% decrease in the apoE/apoC ratio in both subfractions as assessed by IEF. This suggests that the second ultracentrifugation step is not creating an apoE-poor fraction.

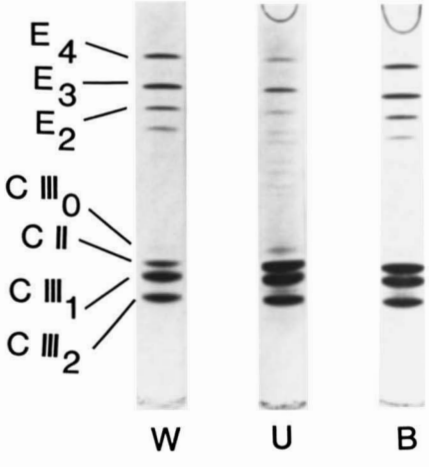

Fig. 2. Analytical isoelectric focusing gel electrophoresis of apolipoproteins from whole VLDL (W), unbound (U), and bound (B) VLDL subfractions, separated by heparin-Sepharose chromatography, from a Type IV hypertriglyceridemic subject. Gels were stained with Coomassie Blue G250 in perchloric acid.

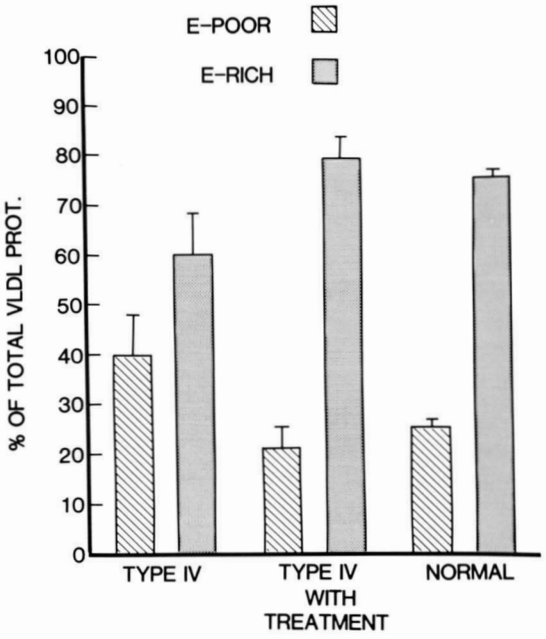

Fig. 3. The distribution of VLDL protein between apoE-poor and apoE-rich VLDL subfractions separated by heparin-Sepharose chromatography from Type IV hypertriglyceridemic subjects (before and after treatment to lower VLDL triglyceride concentrations) and normal subjects. Percent of total VLDL protein refers to the amount of VLDL protein in each subfraction as a percent of the total VLDL protein recovered from the column.

Table 3 demonstrates the reproducibility of the subfractionation of VLDL from Type IV subjects by heparin-Sepharose chromatography; it shows data for characteristics of the two fractions from duplicate columns run on the same VLDL sample. Little or no difference was detected for any of the parameters in the duplicate analyses. Reproducibility of the technique over time with the same subject in a steady state (in terms of plasma triglyceride) over a 24-h period is also demonstrated in Table 3. Again, little difference was observed in either subfraction.

Table 4 shows the characteristics of VLDL subfractions from VLDL samples from three Type IV subjects, obtained 1 to 10 months apart. Although changes in VLDL triglyceride concentrations were observed between sampling times, each subject remained hypertriglyceridemic. The basic characteristics of the VLDL separations were similar between each set of samples in that the triglyceride/protein ra-

TABLE 2. Characteristics of heparin-Sepharose VLDL fractions[a]

	Fraction	Type IV/IIb	Type III	Type I	Type V
C/TG	U	0.14 ± 0.02	0.34 ± 0.07	0.10	0.12 ± 0.03
	B	0.19 ± 0.02	0.38 ± 0.08	0.11	0.13 ± 0.07
C/Protein	U	1.66 ± 0.19	3.22 ± 0.95	2.18	1.82 ± 0.55
	B	1.42 ± 0.08	2.64 ± 0.83	1.67	1.24 ± 0.33
TG/Protein	U	11.41 ± 0.85	8.33 ± 1.35	21.23	16.95 ± 6.99
	B	7.97 ± 0.77[b]	5.40 ± 0.66	14.79	13.94 ± 6.65
ApoE/apoC	U	0.20 ± 0.06	0.33 ± 0.05	0.53	0.60 ± 0.02
	B	0.91 ± 0.18[c]	0.42 ± 0.13	0.58	0.72 ± 0.01
% ApoB-48	U	10.30 ± 2.41	15.50	58.00	ND
	B	5.73 ± 1.59[c]	7.30	21.00	ND

[a]Values are listed as mean ± SEM; C, cholesterol; TG, triglyceride; ND, not determined; U, unbound; B, bound.
[b]$P < 0.01$.
[c]$P < 0.005$.

tios remained elevated and the apoE-poor fraction comprised a significant proportion of the total VLDL pool. This indicates that the heparin-Sepharose separation of VLDL is reproducible with time in the same subjects, provided they remain hypertriglyceridemic.

Fig. 4 shows a typical sodium dodecyl sulfate-polyacrylamide gradient gel run on samples of whole VLDL and the two subfractions from a Type IV patient. The relative amounts of apoB-100 and apoB-48 were measured by densitometry, corrected for chromogenicity (30), and appear in

TABLE 3. Reproducibility of subfractionation by heparin-Sepharose chromatography of VLDL from Type IV subjects

Subject	VLDL TG	Column	Sampling Time	Fraction	C/TG[a]	C/Protein	TG/Protein	% VLDL Protein[b]
	mg/dl		*h*					
1[c]	346	1	0	E-poor	0.15	1.63	10.94	43
				E-rich	0.17	1.42	8.55	57
		2	0	E-poor	0.17	2.16	12.41	41
				E-rich	0.18	1.26	6.92	59
2[c]	394	1	0	E-poor	0.11	1.47	13.58	44
				E-rich	0.19	1.74	8.97	56
		2	0	E-poor	0.11	1.38	12.81	41
				E-rich	0.20	1.48	7.37	59
3[c]	231	1	0	E-poor	0.19	2.09	10.62	38
				E-rich	0.26	1.55	5.76	62
		2	0	E-poor	0.18	1.66	9.35	37
				E-rich	0.26	1.88	6.88	63
4[c]	337	1	0	E-poor	0.10	0.64	6.39	36
				E-rich	0.17	1.10	6.67	64
		2	0	E-poor	0.07	0.67	6.83	36
				E-rich	0.13	1.12	6.45	64
5[d]	563	1	0	E-poor	0.20	2.03	9.89	47
				E-rich	0.26	1.53	5.86	53
	471	2	0.5	E-poor	0.26	2.01	7.84	45
				E-rich	0.24	1.61	6.84	55
	469	3	1.0	E-poor	0.25	1.89	7.57	49
				E-rich	0.26	1.45	5.55	51
	519	4	1.5	E-poor	0.24	2.12	8.74	43
				E-rich	0.27	1.64	6.08	57
	424	5	24	E-poor	0.24	2.25	9.36	40
				E-rich	0.28	1.65	5.85	60

[a]C, cholesterol; TG, triglyceride.
[b]Percent of total protein refers to the amount of VLDL protein in each subfraction as a percent of the total VLDL protein recovered from the column.
[c]Equal amounts of VLDL protein were applied to two heparin-Sepharose columns and run simultaneously under identical conditions as described in Materials and Methods. Following the chromatography procedure, the absorbance (280 nm) in each fraction was measured. Elution profiles were identical in appearance for each set of duplicates. Aliquots from each VLDL subfraction were analyzed for lipids and protein.
[d]Equal amounts of VLDL protein, obtained at the indicated sampling times, were applied to five identical heparin-Sepharose columns and the VLDL fractions were eluted under identical conditions. Elution profiles (280 nm) were almost identical. Aliquots from each VLDL subfraction were analyzed for lipids and protein.

TABLE 4. Reproducibility of subfractionation by heparin-Sepharose chromatography of VLDL samples, from Type IV subjects obtained several months apart

Subject	VLDL Triglyceride	Sampling Time	Column Fraction	VLDL C/TG[a]	VLDL C/Protein	VLDL TG/Protein	VLDL ApoE/ApoC	VLDL % VLDL Protein[b]
	mg/dl	*mo*						
6[c]	875	0	E-poor	0.15	1.33	9.10	0.25	40
			E-rich	0.16	1.74	7.15	0.70	60
	513	1	E-poor	0.13	1.30	10.70	0.09	43
			E-rich	0.17	1.72	8.25	0.60	57
7[c]	965	0	E-poor	0.15	1.63	10.90	0.39	ND
			E-rich	0.18	1.42	8.55	1.14	ND
	654	4	E-poor	0.18	2.17	8.79	0.18	36
			E-rich	0.20	1.09	4.00	0.72	64
1[c]	443	1	E-poor	0.16	1.20	7.56	0.08	41
			E-rich	0.18	1.26	6.84	0.23	59
	702	10	E-poor	0.19	1.59	8.62	0.05	45
			E-rich	0.22	1.45	6.45	0.16	55

[a]C, cholesterol; TG, triglyceride; ND, not determined.
[b]Percent of total protein refers to the amount of VLDL protein in each subfraction as a percent of the total VLDL protein recovered from the column.
[c]Equal amounts of VLDL protein obtained at the indicated sampling times were applied to heparin-Sepharose columns and the fractions were eluted under identical conditions. Elution profiles (280 nm) were similar for each of the samples obtained from each patient. Aliquots of the VLDL fractions were assayed for lipids and protein.

Table 2 as the percentage of apoB-48 in total apoB. The unbound fraction had a significantly greater content of apoB-48 than did the bound fraction (10.30 ± 2.41% vs. 5.73 ± 1.59%, $P < 0.005$).

Lipid ratios for the Type IV and IIb VLDL subfractions, as shown in Table 2, demonstrate that the unbound, apoE-poor fraction has higher ratios of triglyceride to protein (11.41 ± 0.85 vs 7.97 ± 0.77 $P < 0.005$) and cholesterol to protein (1.66 ± 0.19 vs 1.42 ± 0.08, NS) compared to the apoE-rich fraction. Although not specifically measured in this study, these results indicate that the unbound, apoE-poor particles are somewhat larger in size than the apoE-rich particles.

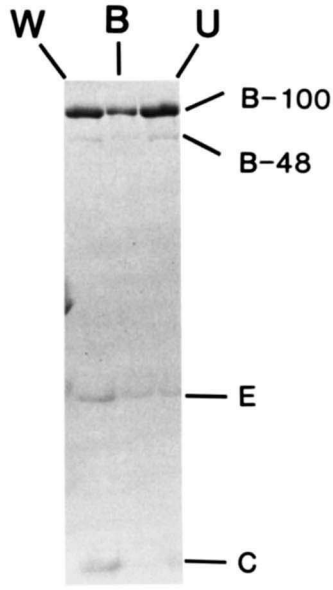

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gradient (4–18%) gel electrophoresis of apolipoproteins from whole VLDL (W), bound (B), and unbound (U) VLDL subfractions, separated by heparin-Sepharose chromatography, from a Type IV hypertriglyceridemic subject. Gels were stained with Coomassie Brilliant Blue R250.

Table 2 also shows that the two subfractions from subjects with Type III, V, and I hyperlipoproteinemia differ from those of Type IV and IIb subjects. Although the elution profiles and protein distribution between the subfractions (results not shown) were similar to the Type IV and IIb subjects (Fig. 1B and Fig. 3) the apoE distribution differed markedly. For the Type III subjects the enrichment of apoE relative to apoC in the bound fraction was only 1.3-fold over the unbound fraction compared to a 5.0-fold enrichment in the bound fraction from Type IV and IIb subjects (**Fig. 5**). The triglyceride to protein ratio was lower and cholesterol to protein ratio was higher in subjects with Type III hyperlipoproteinemia compared to the Type IV and IIb subjects, but these ratios did not differ significantly between the subfractions. Enrichment of apoB-48 in the unbound fraction was observed in the Type III subjects studied, as was the case for Type IV and IIb subjects. In contrast with the Type IV and IIb subjects, the unbound fractions from the Type V and I subjects had apoE to apoC ratios approximately 3-fold higher than the unbound fraction from the Type IV and IIb subjects, while the bound fraction in Type V and I subjects showed no enrichment in apoE over the unbound fraction. Table 2 and Fig. 5 show the similar apoE to apoC ratios between the unbound and bound fractions from the

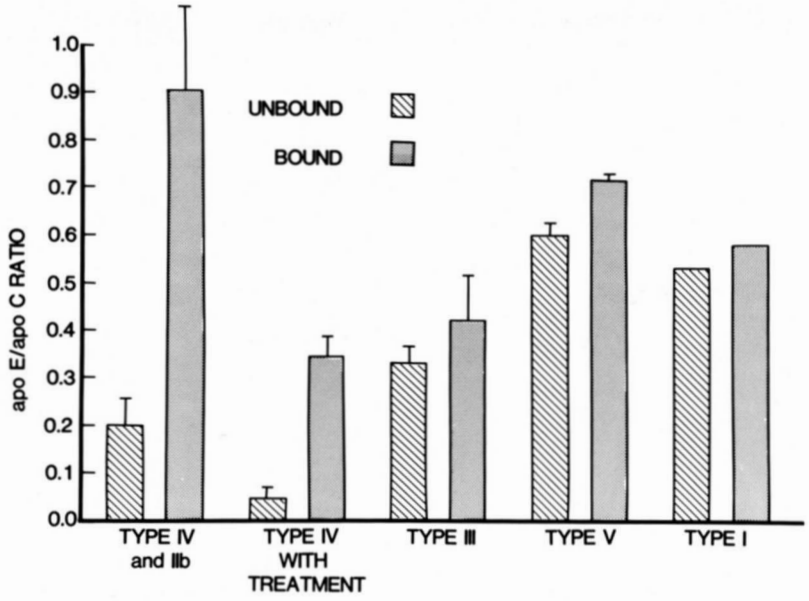

Fig. 5. Distribution of apoE (expressed as the ratio of apoE to apoC) between unbound and bound VLDL subfractions, separated by heparin-Sepharose chromatography, from Type IV and IIb hypertriglyceridemic subjects (n = 6), Type IV subjects who were treated (n = 3) to reduce VLDL triglyceride concentrations (see Materials and Methods: Subjects) and Type III (n = 4), V (n = 2), and I (n = 1) hyperlipoproteinemic subjects.

Type V and I subjects. In the subjects with Type V and Type I hyperlipoproteinemia, the triglyceride to protein and cholesterol to protein ratios are higher in the unbound versus the bound fraction and also higher than those seen in the Type IV and IIb subjects. As expected, the percentage of apoB-48 in the unbound fraction in the Type I subject was much higher (58% vs 10–15% of total apoB) compared to the other subjects, but showed a similar, 2-fold, enrichment in apoB-48 relative to the bound fraction. **Table 5** shows the changes in VLDL subfractions from three Type IV subjects receiving diet and/or gemfibrozil to lower plasma triglyceride concentrations (mean decrease was

TABLE 5. Lipid and apolipoprotein distribution of VLDL subfractions in Type IV subjects before and after treatment[a]

Subject		Plasma Triglyceride	VLDL Triglyceride	Column Fraction	C/TG	C/Protein	TG/Protein	ApoE/ApoC	% of Total VLDL Protein[b]
4[c]	C	664	372	U	0.178	1.77	9.96	0.118	34
				B	0.180	1.55	8.59	0.578	66
	T	425	ND	U	0.100	0.64	6.39	0.055	25
				B	0.166	1.10	6.67	0.274	75
8[d]	C	485	221	U	0.251	2.09	8.30	0.114	42
				B	0.318	1.99	7.50	0.576	58
	T	221	106	U	0.167	0.57	3.41	0.068	20
				B	0.146	0.46	3.11	0.352	80
9[e]	C	549	345	U	0.128	1.60	12.50	0.060	46
				B	0.152	1.55	10.22	0.496	54
	T	415	274	U	0.093	0.70	7.49	0.010	20
				B	0.152	0.98	6.64	0.395	80
Means	C	562 ± 52	312 ± 46	U	0.187 ± 0.036	1.82 ± 0.14	10.25 ± 1.22	0.097 ± 0.019	40 ± 4
				B	0.216 ± 0.051	1.70 ± 0.15	8.77 ± 0.79	0.550 ± 0.027	60 ± 4
	T	353 ± 66	190 ± 84	U	0.120 ± 0.024	0.64 ± 0.04	5.76 ± 1.22	0.044 ± 0.018	21 ± 2
				B	0.155 ± 0.006	0.85 ± 0.20	5.47 ± 1.18	0.340 ± 0.036	79 ± 2

[a]C, control (prior to treatment); T, following treatment; means ± SEM; C, cholesterol; TG, triglyceride; U, unbound; B, bound; ND, not determined.

[b]Percent of total protein refers to the amount of VLDL protein in each subfraction as a percent of the total VLDL protein recovered from the heparin-Sepharose column.

[c]Lipid-lowering diet (1500 calories per day, 30% as fat).

[d]Lipid-lowering diet + 900 mg gemfibrozil per day.

[e]Six hundred mg gemfibrozil per day.

209 ± 61 mg/dl). An elution profile of subject 8 shows a reduction in the amount of unbound, apoE-poor material (**Fig. 6**). As is shown in Table 5 and Fig. 3, there was marked reduction in the amount of VLDL protein in the unbound fraction (21 ± 2% vs 40 ± 4%) and a concomitant rise in the percentage in the bound fraction (79 ± 2% vs 60 ± 4%). Although the apoE to apoC ratios, and the ratios of triglyceride to protein and cholesterol to protein decreased in each subfraction with treatment, the 5-fold enrichment in apoE to apoC in the bound fraction (over the unbound fraction) was maintained (Table 5 and Fig. 5). The cholesterol to triglyceride ratio was lower in the unbound versus bound fraction both before and after treatment.

DISCUSSION

The results presented in this study show that there are two subpopulations of large VLDL (S_f 60–400) in Type IV and IIb hypertriglyceridemic subjects. The heparin-Sepharose chromatography technique used to separate large VLDL into apoE-poor and apoE-rich subfractions is based on the ability of apoE to bind to heparin (31). The striking feature of the VLDL from Type IV and IIb subjects was the substantial contribution made by the unbound, apoE-poor fraction to the expanded VLDL pool size in these individuals. Whether this is due to an enhanced production or decreased catabolism was not addressed in this study. In normal individuals, the unbound, apoE-poor fraction comprises a significantly smaller proportion of VLDL particles (Fig. 3).

Weisgraber et al. (21) have shown that apoE possesses two heparin binding sites, one of which is exposed when apoE is part of the lipoprotein complex and is located in the region of residues 142–147. This site is located in the region of apoE that has been demonstrated to contain the receptor-binding domain of the protein (32). This suggests that heparin-Sepharose binds a VLDL subfraction containing metabolically active apoE.

The elevated concentration of the apoE-poor subfraction in Type IV and IIb subjects suggests that this subfraction may make a substantial contribution to the overproduction and decreased catabolism of VLDL observed in these individuals (1, 2, 4). We demonstrated previously (20) that the apoE-poor subfraction from Type IV subjects was cleared at one-half the rate of the apoE-rich subfraction following injection of the radiolabeled subfractions into miniature pigs. We do not know if this is related to reduced lipolytic efficiency or if the apoE-poor fraction must first be converted to an apoE-rich particle prior to being removed from the VLDL density range. No such metabolic studies in man have yet been reported. However, Nestel et al. (33) suggest that the unbound, apoE-poor particles may be precursors of the apoE-rich subfraction.

In the Type IV and IIb subjects, the composition of the unbound fraction differs from that of the bound fraction. In addition to the much lower content of apoE in the unbound fraction, the triglyceride to protein ratio was significantly higher (Table 2). The twofold higher apoB-48 content in the apoE-poor fraction suggests that it contains more chylomicron remnants (14). The accumulation of apoB-48-containing lipoproteins in the apoE-poor subfraction may be due in part to its lack of apoE. It is unlikely that the apoB-48 associated with the bound fraction was involved in the binding to heparin. Weisgraber and Rall (34) showed that apoB-100 has seven distinct heparin-binding sites. While two of these sites are located in the N-terminal region of apoB-100 that coincides with apoB-48, these sites are of lower affinity for heparin than are the two heparin-binding sites, enriched in basic amino acids, that are unique to apoB-100 alone. These two sites are thought to comprise the receptor-binding domain of apoB-100. Furthermore, apoB-48 does not bind to lipoprotein receptors (15). Nestel and Billington (35) showed that large apoB-48 VLDL were removed more slowly from the circulation of Type V hyperlipoproteinemic subjects compared to large apoB-100 VLDL and that apoB-48 and B-100 were present in the distinct lipoprotein particles.

Examination of the distribution of apoE between the large VLDL subfractions from Type I, III, and V subjects indicated that the separation by heparin-Sepharose chromatography in these subjects involved a different binding mechanism than that observed in Type IV and IIb patients (Table 2 and Fig. 5). VLDL protein from Type I and V subjects was separated into unbound and bound fractions, similar to the Type IV and IIb subjects. In contrast to the Type IV and IIb separations, the apoE distributed more evenly between the unbound and bound fractions in the Type I and V subjects. The much larger triglyceride to protein ratios observed in both the unbound and bound fractions from the hyperchylomicronemic subjects indicate larger particle diameters for the S_f 60–400 fraction compared to the Type IV and IIb subjects. Studies by Bradley et al. (9), Bradley and Gianturco (36), and Krul et al. (10) have indicated that the diameter of the VLDL particle directly affects the conformation of apoB and apoE associated with these lipoproteins. In large VLDL (S_f>60) from Type IV and IIb subjects, the apoE receptor-binding domains were expressed, whereas the apoB-100 binding domains were poorly expressed. In smaller VLDL, IDL, and LDL, the apoE binding domains decrease in expression while those of apoB-100 increase in expression. Since the apoE from Type I and V subjects examined here distributed evenly between the unbound and bound fractions, this suggests that the binding of large VLDL from these subjects is not mediated by apoE, unlike Type IV and IIb VLDL. It could be that the very large particles from these subjects have some or most of the apoE masked by lipid, in a manner similar to that suggested by Weisgraber et al. (21), and only the apoE with exposed binding domains binds to heparin. ApoE in the unbound fraction from these individuals may also be masked

by the excess apoB-48 found in this fraction. Conversely, the apoB-100 binding regions may be exposed in some particles to a greater extent than those of apoE, leading to a separation based on apoB-100.

The results with the Type III S_f 60–400 VLDL were similar to those observed with Type I and V subjects in that there was a more even distribution of apoE between the unbound and bound fractions than was observed with the Type IV and IIb subjects (Table 2 and Fig. 5). The 1.3-fold enrichment of apoE relative to apoC in the bound fraction of VLDL from Type III subjects is similar to that reported previously by Brenninkmeijer et al. (37), who showed a 2-fold enrichment of apoE to apoC in heparin-Sepharose-bound VLDL from Type III subjects in contrast to a 10-fold enrichment in the bound fraction of Type IV VLDL. Weisweiller (38) has also reported the use of heparin-Sepharose columns to separate VLDL from Type III subjects into apoE-poor and apoE-rich fractions. However, this study did not include a quantitative assessment of apoE-enrichment in the bound fraction (38). The slight enrichment of apoE seen in the bound fraction of VLDL from Type III subjects in the present and previous studies (37, 38) suggests that apoE may not be the primary determinant of the VLDL fraction that binds to heparin. $ApoE_2$ binds poorly to the LDL receptor, due to one of several amino acid substitutions in the receptor binding (and heparin binding) domain (39). It is possible that the bound fraction of Type III VLDL includes particles in which the heparin binding domain of apoB-100 is exposed. The small enrichment in apoE in the bound fraction may be due to the residual binding to heparin by $apoE_2$. In vitro studies by Schneider et al. (40) have demonstrated that $apoE_2$ possesses a small and variable amount of LDL receptor binding (and thus, likely heparin binding) ability compared to $apoE_3$. Therefore, since apoE is not the major determinant for the separation of Type III VLDL, the heparin-Sepharose subfractions of Type III subjects are not the same as those of Type IV and IIb subjects.

Finally, we examined the effect of a reduction in plasma and VLDL triglyceride levels in three Type IV patients on the relative amounts of each subfraction in terms of VLDL protein and on the lipid composition of each subfraction. The results clearly demonstrated that a 40% reduction in total plasma triglyceride was associated with a decrease in the amount of unbound, apoE-poor VLDL in these subjects. The decrease in plasma triglyceride was accompanied by a 50% reduction in VLDL triglyceride levels. The elution profiles of the treated subjects approached that observed in a normal subject (Fig. 6). This was due to a substantial reduction in the contribution of apoE-poor VLDL to total VLDL protein (Table 5, Fig. 6). Our findings suggest that the accumulation of the apoE-poor material in Type IV subjects is in part due to an increased VLDL triglyceride concentration, and that treatment to lower VLDL triglyceride levels will lower apoE-poor VLDL production and/or enhance its catabolism. A reduced production rate is most likely, since Ginsburg, Le, and Gibson (41) have shown that the VLDL triglyceride reduction associated with weight loss in Type IV subjects was due entirely to decreased production of VLDL apoB.

Leowsky et al. (42) recently reported that treatment of poorly controlled, hypertriglyceridemic diabetic subjects to re-establish metabolic equilibrium resulted in an increase in the amount of VLDL protein that bound to heparin-Sepharose. This appears to support the findings of the present study; however, these authors did not indicate whether the bound fraction was enriched in apoE relative to the unbound fraction.

In summary, this study has shown that large VLDL in Type IV and IIb subjects contain a unique population of

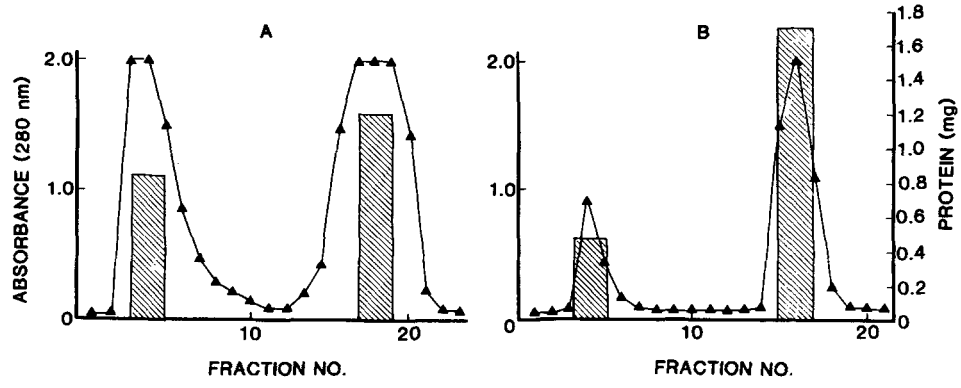

Fig. 6. Elution profiles of equal amounts of VLDL protein loaded on heparin-Sepharose columns from Type IV hypertriglyceridemic subject 8 (Table 5) before (A) and after (B) treatment to lower VLDL triglyceride levels. This subject was treated with a lipid-lowering diet (1500 calories per day with 30% of calories as fat) and gemfibrozil (300 mg, three times per day). Weight loss of 13.0 kg and a 52% reduction in VLDL triglyceride concentration was observed. Elution profiles were obtained by measurement of absorbance at 280 nm (▲–▲). The hatched bars represent the amount of protein in each fraction obtained by a modified Lowry method (27, 28).

particles, relatively poor in apoE and relatively enriched in apoB-48. The amount of this apoE-poor material can be reduced by caloric restriction and/or gemfibrozil treatment. Simons et al. (43) reported that apoB-48-rich chylomicron remnants are an independent predictor of risk of coronary artery disease. Packard et al. (44) and Reardon, Fidge, and Nestel (4) have demonstrated that the majority of large VLDL in Type IV subjects leaves plasma without conversion to LDL. It is possible that the unbound, apoE-poor material that accumulates in Type IV and IIb subjects could play a role in the increased incidence of premature atherosclerosis seen in some of these individuals. Further studies are required to elucidate the role of the apoE-poor material in the atherogenic process in hypertriglyceridemic subjects, as well as to explain the apparent inability of apoE in VLDL from hyperchylomicronemic individuals to bind to heparin.

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