A protein band having the same migration as apolipoprotein (apo) B-48 was observed by SDS electrophoresis in the plasma very low density lipoprotein (VLDL) from 14 Type IV and three Type III hyperlipoproteinemic subjects and from six normal fasting subjects. The VLDL from five Type IV, three Type III, and one normal subject were separated into two subfractions, retained and nonretained, by immunofinity chromatography on monoclonal anti-apo B-100 Sepharose. Based on results of electrophoresis and radioimmunoassay, we have concluded that these two fractions represent apo B-48 and apo B-100 lipoproteins that we have named apo B-48 and apo B-100 VLDL. When compared to their respective apo B-100 VLDL, the apo B-48 VLDL from either Type III or Type IV was principally enriched in total lipids, in apo E, and had an electrophoretic migration similar to chylomicrons. This suggests that apo B-48 VLDL has the same origin (i.e., intestinal) in the two disorders. Both apo B-48 and apo B-100 VLDL were enriched in cholesteryl ester (CE) and depleted in triglyceride (TG) in Type III; however, both fractions were rich in TG and poor in CE in Type IV and in normal subjects. In addition, compared to their respective apo B-100 VLDL, the apo B-48 fraction was enriched in CE in Type III and in TG in Type IV. We conclude that, despite a possible similar origin for apo B-48 VLDL in Type III and in Type IV subjects, the composition of apo B-48 VLDL is variable and the CE/TG ratio is more characteristic of the type of hyperlipidemia than of the particular VLDL subfractions.

(Arteriosclerosis 5:201-211, March/April 1985)

Molecular weight,1 immunological,2-4 and metabolic heterogeneity5 of apolipoprotein (apo) B has been reported, and the literature has been recently reviewed by Kane.6 Using a series of specific anti-apo B-100 and anti-apo B-100/apo B-48 crossreacting monoclonal antibodies produced in our laboratory,2,3 we have demonstrated that an immunological heterogeneity was present in the very low density lipoproteins (VLDL) from familial dysbetalipoproteinemia (Type III hyperlipoproteinemia).7 Thus, we could separate total VLDL into apo B-48 VLDL and apo B-100 VLDL having different apolipoprotein and lipid composition. In addition, we observed immunological heterogeneity in apo B-100 particles.

The principal characteristic of lipoproteins in Type III hyperlipoproteinemia, which has been recently reviewed,7 it is the elevated triglyceride and cholesterol content in the plasma associated with the presence of an abnormal VLDL. In part, this is the result of an accumulation of cholesterol-rich remnant particles due to a defect in catabolism of both chylomicrons and VLDL.6-12 In contrast to Type III hyperlipoproteinemia, Type IV hyperlipoproteinemia is characterized by elevated plasma triglyceride without elevated low density lipoprotein (LDL) cholesterol, a condition that has been attributed to an overproduction of VLDL.13,14 Increased triglyceride synthesis with normal apo B production would result in the hypersecretion of large triglyceride-rich VLDL.15-18 In addition, a saturated or defective catabolism of the VLDL would be related to the increased triglyceride and VLDL content in the plasma.15, 16, 19
Based on the differences described in Type III and Type IV lipoprotein metabolism and on our recent results on VLDL heterogeneity in Type III, we asked three questions: 1) Is there apo B heterogeneity in Type IV VLDL and can apo B-48 VLDL be separated? 2) Is the composition of this fraction similar to that of apo B-48 VLDL from Type III subjects? 3) Are the apo B-100 VLDL similar or different in these two disorders? In this paper, we have used immunoaffinity chromatography to isolate two separate subfractions of VLDL in Type III and Type IV hyperlipoproteinemia and in one normal subject; we refer to these two fractions as apo B-48 VLDL and apo B-100 VLDL. In each case, the two fractions were characterized with respect to their lipid and apolipoprotein composition.

**Methods**

**Selection of Patients**

Patients were selected for their lipoprotein phenotype at the lipid clinic of the Clinical Research Institute of Montreal. The diagnosis of the lipid transport disorder had been established at a prior visit. The clinical data are summarized in Table 1.

**Diagnosis of Familial Dysbetalipoproteinemia, Type III**

We based our diagnosis on the presence of elevated plasma cholesterol (>250 mg/dl) with or without elevated plasma triglycerides (TG) (>200 mg/dl), the presence of a β-VLDL (comparison of electrophoreses of the <1.006 and >1.006 g/ml fractions from ultracentrifugation), and the E 2/2 apo E phenotype as determined by the technique of Bouthillier et al. These characteristics were associated with one or more of the following features: typical skin xanthomas or palmar creases pigmentation, equally high levels of plasma cholesterol and TG, and a VLDL cholesterol/triglyceride ratio of 0.30 or more. Three patients (two men, one woman) were selected for the Type III phenotype; one had atherosclerotic vascular disease and two had a VLDL cholesterol/TG ratio >0.3.

**Diagnosis of Hypertriglyceridemia, Type IV**

We based this diagnosis on elevated plasma triglycerides (>200 mg/dl) and VLDL cholesterol (>35 mg/dl) in the presence of normal LDL cholesterol (<190 mg/dl). The 14 patients (nine men and five women) had arcus cornaea and/or xanthelasma. The apo B-LDL level was <119 mg/dl indirectly excluding the diagnosis of a combined hyperlipidemia. Eight patients had previous atherosclerotic vascular disease. Three had secondary causes of hyperlipidemia (one with diabetes, one with hypothyroidism, and one with alcoholism). Their apo E phenotypes were distributed as follows: six had apo E 3/3, two had apo E 3/4, five had apo E 3/2, and one had apo E 4/2. Although a familial history was obtained for each case, no systematic sampling of first-degree relatives was carried out. Thus, the primary nature of the disease is only putative. The diagnosis of combined hyperlipidemia was excluded in all instances, and 11 patients were free of secondary causes of hyperlipidemia. Six of the 11 showed a positive familial context (cardiovascular disease or hypertriglyceridemia in one of the immediate family). The plasma samples were obtained while the patients were maintained on a normal North American diet with no drugs. Six normolipidemic volunteers were used to evaluate for the presence of apo B-48 in the VLDL fraction. Their characteristics are reported in Table 1.

Complete analysis of VLDL (separation on immunomanoaffinity columns, lipid and apolipoprotein analysis) were done for five males of the 14 Type IV subjects (without secondary causes, with a positive familial context, and with an apo B-LDL <105 mg/dl), the three Type III subjects and one normal subject. As indicated in Table 1, the five Type IV subjects studied in detail were representative of the Type IV selected. The study was approved by the Ethics Committee of the Institute and the informed consent of the patients was obtained.

**Table 1. Physical Characteristics and Plasma Lipid Levels of the Subjects**

<table>
<thead>
<tr>
<th>Pheno-type</th>
<th>No. and gender</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV 9M, 5F</td>
<td>49 ± 9 (37-67)</td>
<td>77.9 ± 15</td>
<td>721 ± 372 (202-1231)</td>
<td>277 ± 75 (162-368)</td>
<td></td>
</tr>
<tr>
<td>IV* 5M</td>
<td>49 ± 4 (41-57)</td>
<td>79.0 ± 15</td>
<td>692 ± 308 (493-1202)</td>
<td>263 ± 59 (218-362)</td>
<td></td>
</tr>
<tr>
<td>III* 2M, 1F</td>
<td>49 ± 8 (41-57)</td>
<td>74.9 ± 15</td>
<td>510 ± 238 (363-785)</td>
<td>466 ± 206 (284-690)</td>
<td></td>
</tr>
<tr>
<td>Normal 5M, 1F</td>
<td>42 ± 12 (25-62)</td>
<td>72.3 ± 13</td>
<td>118.4 ± 21 (86-143)</td>
<td>217 ± 23 (181-236)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; the range is given in parentheses.

*These patients were submitted to complete analysis.
Preparation of Lipoproteins

Blood was collected after a 14-hour fast into EDTA (1 mg/ml) and the red blood cells were removed by centrifugation (3000 rpm for 15 minutes). NaN₃ (0.02%) and phenylmethylsulfonyl fluoride (PMSF) (0.02%) were added to the plasma. Plasma lipoproteins were separated by floatation in a Beckman L5-65 ultracentrifuge (Beckman Instrument Incorporated, Spinco Division, Palo Alto, California) with a 50.2 Ti rotor at 5°C, 45,000 rpm for 20 hours. The VLDL chylomicron fraction was isolated at a density of <1.006 g/ml. LDL from a normal subject was isolated by sequential ultracentrifugation²¹ at 5°C between densities of 1.030 and 1.050 g/ml. All of the lipoprotein subfractions were dialyzed against phosphate-buffered saline (PBS) (0.015 M phosphate, 0.02% NaN₃ and 1 mM EDTA) containing 0.15 M NaCl, pH 7.2) containing 1 mM EDTA and 0.02% NaN₃ and stored at 4°C.

Monoclonal Antibodies and Antisera

We have previously described the production and characterization of monoclonal antibodies against human apo B²⁻³ and apo E.²⁻²² Affinity-purified rabbit antimouse IgG was purchased from Kirkegaard and Perry Laboratories, Incorporated (Gaithersburg, Maryland).

Immunoadsorbants and Affinity Chromatography

The preparation of the anti-apo B-100 monoclonal antibodies immunoadsorbants and the affinity chromatography of the VLDL have been reported.⁷ The anti-apo B-100 monoclonal antibodies 4G3 and SE11 were partially purified from ascitic fluid of hybridoma-bearing mice, by precipitation with 40% saturated ammonium sulfate. The precipitated proteins were redissolved and dialyzed against 0.1 M sodium bicarbonate (pH 8) and were then covalently coupled to Sepharose 4B.⁷ VLDL (5 mg protein) of each of three Type III, five Type IV, and one normal subject were applied on two columns connected in series containing respectively 15 ml of 5E11 Sepharose 4B and 10 ml of 4G3 Sepharose 4B. The nonretained fractions were collected after a first washing with 0.015 M PBS (pH 7), EDTA 1 mM. The bound lipoproteins were eluted with 0.05 M citric acid containing 1 M NaCl (pH 2.5). The eluted fractions were immediately dialyzed against 0.015 M PBS containing 0.02% NaN₃ and 1 mM EDTA.⁷

Radioimmunoassay of Apo B

This technique has been described previously.⁷ The radioimmunoassay (RIA) is based on competition between normal LDL immobilized on removablewells (Dynatech Laboratories, Incorporated, Alexandria, Virginia) and different dilutions of the sample to be tested for each antibody at its predetermined optimal dilution. Nonspecific binding was determined in wells in which the sample was replaced by LDL diluted to 80 μg/ml. All sample dilutions were made in 0.015 M PBS 1% bovine serum albumin. Each point is the mean of two values with a coefficient of variation less than 10%. Dilutions of LDL between 8 μg/ml and 0.016 μg/ml were included in each assay to prepare a standard curve.

Radioimmunoassay of Apo E

The quantitative determination of apo E in the different VLDL fractions was carried out by previously described procedures.²⁻²² Apo E (10 μg) purified by the technique of Weisgraber et al.²³ was kindly provided by Daniel Bouthillier and was labeled with 2 mCl of ¹²⁵I as described by Bolon and Hunter.²⁴ An apo E-pretitrated plasma stored at -20°C was used as the standard curve. All assays were performed using the monoclonal antibody 6C5.²²

Lipid Analysis

Lipids were analyzed in VLDL, apo B-100 VLDL, and apo B-48 VLDL fractions (100 μg protein) from each of the three Type III, five Type IV, and one normal subject, following the procedure of Kuksis et al.²⁵ The lyophilized samples were digested with 0.3 mg phospholipase C (Sigma Chemical Company, St Louis, Missouri); then 200 μg tridecanoyl glycerol (Serdary Research Laboratories, London, Ontario, Canada) was added as internal standard, and the lipids were extracted. The dried extract was redissolved in 80 μl pyridine and derivatized with 20 μl N, O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA, Pierce Chemical Company, Rockford, Illinois). The lipid-silyl ethers were analyzed by gas liquid chromatography (GLC) on 3% OV-1 in a single glass column (600 x 2 mm) using a Hewlett-Packard model 5880 gas chromatograph (Hewlett-Packard Company, Palo Alto, California), with a level-4 terminal, in the single-column compensation mode.

Phospholipid Analysis

Phospholipids were analyzed by thin layer chromatography (TLC) on silica gel G plates (Analtech Incorporated, Newark, DE) on the same fractions analyzed by GLC. Fractions (50 μg protein) were extracted following the procedure of Bligh and Dyer²⁶ by three volumes of chloroform/methanol (1:2, vol/vol). After removal of the protein precipitate by centrifugation, the chloroform phase was isolated by the addition of 1 volume of H₂O and 1 volume of chloroform, and the methanol/water phase was washed by 2 volumes of chloroform. After concentration of the chloroformic phases, the phospholipids were separated on TLC in the usual solvent system (CHCl₃/MeOH/acetic acid/water, 50:25:7:3 by volume), and revealed by iodine vapor. The TLC spots were scraped, and phosphorus analysis was made by the perchloric acid method as described.²⁷
Apolipoprotein Analysis

The apolipoprotein composition of the VLDL subfractions was assessed by sodium dodecyl sulfate (SDS) and alkaline urea polyacrylamide gel electrophoresis. To identify apo B-100 and apo B-48 in the VLDL and its subfractions, SDS polyacrylamide gel electrophoresis was carried out according to the method of Kane et al. To check for the presence of apo B-48 in the different Type IV VLDL, the samples were analyzed by SDS polyacrylamide electrophoresis followed by electrophoretic transfer to nitrocellulose paper according to the method of Towbin et al. Electrophoresis was performed on 1.5 mm thick slab gels containing 3% acrylamide. After migration, the separated proteins were electrophoretically transferred to nitrocellulose paper (0.45 μm pore size, Millipore). The nitrocellulose paper was then treated for immunological detection with the anti-apo B-48/apo B-100 cross-reacting antibody 2D8, following the technique of Marcel et al. and the identification of apo B was performed after autoradiography on XAR-5 Kodak films with intensifier screen (Cronex, Dupont). Paper electrophoresis in an albumin containing buffer was performed according to the method of Lees and Hatch. Protein concentration was measured by the Lowry technique.

Results

VLDL proteins from 14 patients with Type IV hyperlipoproteinemia, from three patients with Type III hyperlipoproteinemia, and from six normolipemic subjects were separated by SDS electrophoresis on 3% polyacrylamide gels. Typical patterns for the three groups are shown in Figure 1. As expected, apo B-48 was observed in the VLDL of all Type III subjects examined (Figure 1 A, lane 5, 50 μg protein; Figure 1 B, Lane 1, 100 μg protein). In addition, a protein having an apparent molecular weight identical to Type III apo B-48 was also observed in all Type IV VLDL samples (Figure 1A, Lanes 1-4) and in normal VLDL samples (Figure 1 B, Lanes 2-4). The staining intensity of the apo B-48 band appeared greatest with Type III VLDL, intermediate with Type IV VLDL, and least with normal VLDL.

The typical Type III profile, including the accumulation of apo B-48 in the plasma, is associated with the presence of the e2 allele in a homozygous form (apo E 2/2 phenotype). To see if the presence of apo B-48 in Type IV VLDL was associated with the e2 allele, we examined the apo E isoforms of all subjects studied. Of the 14 subjects, six were heterozygotes for the e2 allele. In Type IV hyperlipidemias, there was no correlation between the apparent apo B-48/apo B-100 ratio based on staining intensity and the presence of e2 allele in the heterozygous form. In Figure 1 A, the VLDL in Lanes 1 and 2 were prepared from subjects heterozygous for the e2 allele, whereas the VLDL in Lanes 3 and 4 were from subjects who did not have the apo E2 isoform. This observation was confirmed on the VLDL of the 14 Type IV subjects that had been separated by SDS electrophoresis and transferred electrophoretically to nitrocellulose paper, and for which the apo B had been detected by sequential incubations with a monoclonal antibody that cross-reacts with apo B-48 and apo B-100 (2D8), and 125I-antimouse IgG (results not shown). Again, the differences in the apparent apo B-48/apo B-100 ratio could not be associated with the presence of e2 allele. We did not find a significant difference in the level of triglyceride between the patients having the e2 allele (740 ± 422 mg/dl) and those without e2 allele (585 ± 380 mg/dl).

Separation of Apo B-48 VLDL and Apo B-100 VLDL

VLDL was separated into nonretained and retained fractions by immunoaffinity chromatography on anti-apo B-100 sepharose, a technique that has been previously described and validated. The separation of the VLDL and the analysis of the resulting fractions were performed on five Type IV subjects, three Type III subjects, and one normolipemic sub-

Figure 1. SDS, 3% polyacrylamide gel electrophoresis of VLDL from Type IV (A, Lanes 1 to 4), Type III (A, Lane 5; B, Lane 1), and normal subjects, (B, Lanes 2 to 4). 100 μg protein VLDL was applied on each gel except in A, Lane 5 where 50 μg protein was applied. Apo E phenotype was determined for Type IV VLDL as following: A, Lane 1: E3/E3; A, Lane 2: E4/E3; A, Lanes 3 and 4 E2/E3. Gels are stained with Coomassie blue.
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ject. The starting VLDL and the nonretained and retained fractions were systematically tested in RIA using four apo B-100-specific monoclonal antibodies (4G3, 5E11, 3A10, and 3F5) and with 1D1 and 2D8; these are two anti-apo B-100 monoclonal antibodies that cross-react with the apo B-48 of Type III VLDL and normal thoracic duct lymph chylomicrons.2,3 As shown in Figure 2, both the retained and nonretained fractions, as well as the starting VLDL, competed with LDL for antibodies 2D8 and 1D1, whereas only the retained fraction and starting VLDL showed reactivity with the four anti-apo B-100-specific antibodies. The same pattern of reactivity with the monoclonal antibodies was seen with the VLDL fractions from Type IV, Type III, and normal subjects. Therefore the nonretained fraction of Type IV and normal VLDL appeared immunochemically identical to apo B-48 VLDL of Type III. Based on these results, we have defined apo B-48 VLDL as the subfraction of particles isolated from fasting subjects at d < 1.006 g/ml that are not retained on the anti-apo B-100 immunoadsorbants, and apo B-100 VLDL as the subfraction of particles of d < 1.006 g/ml that are retained on the immunoadsorbants.

The VLDL fractions were examined on 3% polyacrylamide gel electrophoresis (Figure 3). As previously shown with Type III VLDL,7 apo B-48 from Type IV and normal subjects was present only in the nonretained fraction. Occasionally, as in Figure 3, the nonretained fraction obtained after a single passage was contaminated with a trace amount of apo B-100 that could be removed by rechromatography. Like the VLDL of Type III subjects, the apo B-100 and apo B-48 were associated with distinct VLDL particles in Type IV and normal subjects. The proportion of protein associated with apo B-48 VLDL was highest in the three Type III subjects (10.0% ± 1.1), intermediate in the five Type IV subjects (5.0% ± 0.8), and lowest in the one normal subject (2%).

**Paper Electrophoresis of Apo B-48 and Apo B-100 VLDL**

Type III and Type IV VLDL and their nonretained and retained fractions obtained by immunoaffinity chromatography were analyzed by paper electrophoresis. For Type IV, the apo B-100 VLDL had the same mobility as the bulk of the unfraccionated VLDL (prebeta), whereas the apo B-48 VLDL remained at the point of origin (Figure 4). The migrations of the Type III VLDL were identical to that previously described7 (data not shown), i.e., a broad β-band for apo B-100 VLDL and a band that remained at the origin for apo B-48 VLDL.

**Apolipoprotein Composition**

For Type III and Type IV, the proteins of starting VLDL and the two subfractions were separated by SDS polyacrylamide gel electrophoresis using 10% acrylamide in the presence of β-mercaptoethanol,
ject. The starting VLDL and the nonretained and retained fractions were systematically tested in RIA using four apo B-100-specific monoclonal antibodies (4G3, 5E11, 3A10, and 3F5) and with 1D1 and 2D8; these are two anti-apo B-100 monoclonal antibodies that cross-react with the apo B-48 of Type III VLDL and normal thoracic duct lymph chylomicrons. As shown in Figure 2, both the retained and nonretained fractions, as well as the starting VLDL, competed with LDL for antibodies 2D8 and 1D1, whereas only the retained fraction and starting VLDL showed reactivity with the four anti-apo B-100-specific antibodies. The same pattern of reactivity with the monoclonal antibodies was seen with the VLDL fractions from Type IV, Type III, and normal subjects. Therefore the nonretained fraction of Type IV and normal VLDL appeared immunologically identical to apo B-48 VLDL of Type III. Based on these results, we have defined apo B-48 VLDL as the subfraction of particles isolated from fasting subjects at d < 1.006 g/ml that are not retained on the anti-apo B-100 immunoadsorbents, and apo B-100 VLDL as the subfraction of particles of d < 1.006 g/ml that are retained on the immunoadsorbents.

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Apolipoprotein Composition

For Type III and Type IV, the proteins of starting VLDL and the two subfractions were separated by SDS polyacrylamide gel electrophoresis using 10% acrylamide in the presence of 8-mercaptoethanol,
and on alkaline urea, polyacrylamide gel. The results for Type III VLDL (not shown) were the same as previously reported and no qualitative difference could be seen between apo B-100 and apo B-48 VLDL in their apoprotein composition. The results for Type IV VLDL are shown in Figure 5. In Figure 5A, the starting VLDL and the two fractions seemed to be similar on 10% polyacrylamide gel. Because of its high molecular weight, apo B was found on the top of the gels. An intense band was seen with a molecular weight between the standards 43,000 and 30,000 corresponding to that of apo E (35,000). Near the bottom of the gels at approximately 10,000 to 20,000, a broad band was present in the region where apo A-II and the apo Cs would migrate. The fractions from Type IV VLDL were qualitatively similar to those from Type III.

On alkaline urea polyacrylamide gels (Figure 5 B), apo C-II, C-IIIa, C-IIIb, and C-IIIc were tentatively identified as major bands in the middle of the gels as well as apo C-I and apo E near the top of the gels. A weakly stained band just above the apo C-II may be apo D-2 as described by Chang et al. Starting VLDL and the two subfractions appeared qualitatively similar.

Apo E was measured by RIA in all Type IV and Type III VLDL and subfractions. The results are shown in Table 2. In Type IV, as in Type III, the apo E/protein ratio was significantly increased in the apo B-48 VLDL fraction, compared to starting VLDL and apo B-100 VLDL. This ratio was similar in starting VLDL and apo B-100 VLDL for Type IV, but higher in starting VLDL than in apo B-100 VLDL in Type III.

**Lipid Analysis**

Starting VLDL, as well as the nonretained and retained fractions, from the five Type IV subjects, three Type III subjects, and one normal subject were analyzed for their total lipid composition by gas-liquid chromatography following the method of Kuksis et al. and for their phospholipid composition by phosphorus assay. These results are summarized in Tables 3 to 6. Each value represents the mean for the number of subjects indicated, each analyzed in triplicate. The range was less than 3% between the three analyses, (intraassay variability) and all the samples were analyzed in the same experiment with the same standards.

Although not all comparisons were statistically significant in Type III (due to having only three subjects), the differences between B-48 VLDL and B-100 VLDL were similar to those previously reported for this type of patient. In Type III and Type IV pa-

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**Table 2. Relative Apo E Concentrations in Type III and Type IV VLDL Subfractions**

<table>
<thead>
<tr>
<th></th>
<th>Type III (n = 3)</th>
<th>Type IV (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apo B-100 VLDL</td>
<td>Apo B-48 VLDL</td>
</tr>
<tr>
<td>apo E/protein</td>
<td>0.43 ± 0.09</td>
<td>0.21 ± 0.02</td>
</tr>
</tbody>
</table>

The apo E concentration was determined by radioimmunoassay. The protein concentration was determined by Lowry assay.

*The difference between apo B-48 and apo B-100 VLDL by Student's paired t-test was significant at 2α < 0.05 for Type III and at 2α < 0.01 for Type IV (see underlined values).
Table 3. Chemical Composition of Type III, Type IV, and Normal VLDL, Apo B-100, and Apo B-48 VLDL

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Type III (n = 3)</th>
<th>Type IV (n = 5)</th>
<th>Normal (n = 1)</th>
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<tr>
<td></td>
<td>VLDL</td>
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<tr>
<td></td>
<td>B-100 VLDL</td>
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<tr>
<td>Total lipids</td>
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</tr>
<tr>
<td>Protein</td>
<td>92.5 ± 1.1</td>
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<tr>
<td>Lipids</td>
<td>4.1 ± 0.4*</td>
<td>3.9 ± 0.5*</td>
<td>3.5 ± 1.0*</td>
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</tbody>
</table>

The percentage composition by weight is expressed as the mean value ± SD of the number of indicated subjects. The protein content was determined by Lowry assay, and the total lipids by gas liquid chromatography.

Table 4. Lipid Composition of Type III, Type IV, and Normal VLDL, Apo B-100, and Apo B-48 VLDL

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Type III (n = 3)</th>
<th>Type IV (n = 5)</th>
<th>Normal (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VLDL</td>
<td>B-100 VLDL</td>
<td>B-48 VLDL</td>
</tr>
<tr>
<td></td>
<td>B-100 VLDL</td>
<td>B-48 VLDL</td>
<td></td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>7.3 ± 1.3*</td>
<td>7.3 ± 1.5</td>
<td>7.4 ± 1</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>32.8 ± 8</td>
<td>31.3 ± 0.5</td>
<td>38.1 ± 0.9</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>47.3 ± 8</td>
<td>46.3 ± 4</td>
<td>39.9 ± 3.7</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>12.2 ± 1.8</td>
<td>14.7 ± 2.6</td>
<td>12.9 ± 2.6</td>
</tr>
<tr>
<td>CE/FC†</td>
<td>2.66 ± 0.26</td>
<td>2.59 ± 0.4</td>
<td>2.94 ± 0.18</td>
</tr>
<tr>
<td>CE/TC‡</td>
<td>0.72 ± 0.20</td>
<td>0.68 ± 0.17</td>
<td>0.96 ± 0.15‡</td>
</tr>
</tbody>
</table>

*Values are expressed as the mean percentage ± SD by weight of total lipids as analyzed by gas liquid chromatography.
†Molar ratio of esterified cholesterol to unesterified cholesterol (EC/FC).
‡Weight ratio of cholesteryl ester to triglyceride.
§Difference between apo B-48 and apo B-100 VLDL by Student’s paired t test was significant at 2 < 0.1 for Type III and 2 < 0.05 for Type IV (see underlined values).

Table 5. Carbon Number Distribution of Cholesteryl Esters and Triglyceride In Type III, Type IV, and Normal VLDL and Subfractions

<table>
<thead>
<tr>
<th>Type III (n = 3)</th>
<th>Type IV (n = 5)</th>
<th>Normal (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VLDL</td>
<td>B-100 VLDL</td>
</tr>
<tr>
<td></td>
<td>B-100 VLDL</td>
<td>B-48 VLDL</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>17.9 ± 0.4*</td>
<td>19.5 ± 1.4</td>
</tr>
<tr>
<td>C18</td>
<td>70.8 ± 1.2</td>
<td>68.4 ± 1.2</td>
</tr>
<tr>
<td>C20</td>
<td>11.2 ± 1.1</td>
<td>12.1 ± 0.8</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C48</td>
<td>9.3 ± 0.4†</td>
<td>11.7 ± 2.5</td>
</tr>
<tr>
<td>C50</td>
<td>17.6 ± 2.9</td>
<td>18.3 ± 2.9</td>
</tr>
<tr>
<td>C52</td>
<td>43.3 ± 2.4</td>
<td>40.8 ± 2.3</td>
</tr>
<tr>
<td>C54</td>
<td>29.9 ± 5</td>
<td>27.2 ± 3.6</td>
</tr>
</tbody>
</table>

*Values are expressed as the mean percentage ± SD of total cholesteryl esters.
†Values are expressed as the mean percentage ± SD of total triglycerides.
‡The difference between apo B-48 and apo B-100 VLDL by Student’s t test was significant at 2α < 0.1 for Type III and 2α < 0.05 for Type IV (see underlined values).
patients, as well as in a normal subject, apo B-48 VLDL was enriched twofold in total lipids with respect to proteins, compared to apo B-100 VLDL or starting VLDL (Table 3). Lipid composition (Table 4) indicated that the usual lipid species are found in all fractions, particularly free cholesterol, cholesteryl esters, triglycerides, diglycerides, and some monoglycerides (not reported). This latter class is derived from lysophosphatidylcholine and perhaps by some degradation of diglycerides. This represents only about 0.3 to 0.4% of total lipids. Diglycerides were derived from phospholipids and sphingomyelins. Lysophosphatidylcholine, sphingomyelin and other phospholipids were analyzed more precisely based on their phosphorus content (see below). Triglycerides were the major component in all the samples; cholesteryl esters were the second most important class, followed by phospholipids and finally free cholesterol.

All Type III VLDL and subfractions had a triglyceride percentage lower, and cholesteryl esters and free cholesterol percentage higher, than in normal VLDL; as expected, Type III apo B-48 VLDL were richer in cholesteryl esters and poorer in triglycerides and phospholipids compared to apo B-100 VLDL; free cholesterol content was the same in Type III VLDL subfractions.

The Type IV VLDL composition was closer to that of the normal VLDL than to that of the Type III VLDL with, however, more cholesteryl esters and less phospholipid and triglyceride in Type IV VLDL than in normal VLDL. In contrast to Type III, Type IV apo B-48 VLDL were enriched in triglycerides and depleted in cholesteryl esters compared to apo B-100 VLDL. Free cholesterol content was the same in the two subfractions, whereas phospholipids were slightly more abundant in apo B-100 VLDL. Apo B-48 VLDL and apo B-100 VLDL compositions from one normal subject were comparable to that of Type IV, with more triglycerides and less cholesteryl esters in the apo B-48 fraction.

Lipid analysis permitted the distinction between the different carbon number classes for cholesteryl esters and triglycerides (Table 5). Cholesteryl esters with chains of 16, 18, and 20 carbons (as well as triglycerides with total carbon numbers of 48, 50, 52, and 54) were identified as independent and well-defined peaks on the chromatograms. As reported for total VLDL, 36 cholesterol ester with an 18-carbon fatty acid was the most important cholesteryl ester in all fractions of Type III, Type IV, and normal VLDL, and was increased in all apo B-48 VLDL compared to apo B-100 VLDL. For the triglycerides, 52 and 54 carbon number compounds were the most important, with a higher percentage for the 52. This latter class was in the same amount in apo B-48 VLDL and apo B-100 VLDL of Type IV and Type III. On the other hand, 54 acyl carbon triglycerides were increased in Type IV and Type III apo B-48 VLDL compared to apo B-100 VLDL at the expense of 48 acyl carbon triglycerides.

The results for phospholipids analysis are summarized in Table 6. They have not been previously reported for Type III and Type IV; however, in general, our results are similar to the composition reported for normal VLDL. 37 Phosphatidylcholine was the major phospholipid constituent in all fractions and seemed to be present in similar proportions in Type IV subfractions, but decreased in Type III apo B-48 VLDL compared to apo B-100 VLDL. Sphingomyelin was the second major component and was increased in apo B-48 VLDL compared to apo B-100 VLDL, particularly in Type III. No difference could be seen in lysophosphatidylcholine levels. The minor subclasses, phosphatidyethanolamine, and phosphatidylserine-phosphatidylinositol were present in all the subfractions.

### Discussion

We have previously described a method to separate apo B-100 VLDL and apo B-48 VLDL particles from a pool of plasma of Type III hyperlipemic patients, based on their immunological heterogeneity defined by monoclonal antibodies. In this paper, we used the same method to separate VLDL from plasma of five Type IV and of a new series of three Type III subjects into subfractions that contained particles...
with either apo B-100 or apo B-48 as the sole apo B species. We studied some physical and chemical properties of the starting VLDL and of these two subfractions to make two comparisons: 1) a comparison between the two groups of patients, and 2) a comparison between the two subfractions of VLDL, which were present in all individuals of both groups of patients. In addition, VLDL from one normal subject was submitted to the same analysis.

While the presence of apo B-48 VLDL has been documented in the VLDL from the fasting plasma of Type I, Type V, and Type III hyperlipidemic subjects, it has not been reported in fasting normal or Type IV subjects. Recently, Meng et al. using 3.25% SDS polyacrylamide gel electrophoresis showed the presence of apo B-48 in VLDL from Type III, but not from Type IV, subjects. On the other hand, Kane reported the presence of appreciable amounts of apo B-48 in VLDL from hypertriglyceridemic subjects without identifying the phenotype of the hypertriglyceridemia. In our studies, when we have applied 100 μg of VLDL protein from Type IV and normal subjects to 3.5% SDS polyacrylamide gels, we have consistently seen a band migrating at the same position as apo B-48. Although the reason for these discrepancies is unknown, it is apparently not related to the quantity of protein applied on the gels. In addition to its mobility in SDS electrophoresis, immunochemical characterization indicated that this band is indeed identical to apo B-48 from Type III VLDL or chylomicrons. By passing Type IV VLDL through anti-apo B-100 immunoaffinity columns, we separated starting VLDL into a nonretained fraction that contained only the protein with a mobility of apo B-48 and a retained fraction that contained only apo B-100. In RIA, the retained fraction reacted with all of the monoclonal antibodies, whereas the nonretained fraction reacted with only those monoclonal antibodies (1D1, 2D8) previously shown to cross-react with apo B-48.

These results exclude the possibility that the low molecular weight species seen in Type IV VLDL corresponds to the previously described apo B-50, an apo B species or fragment that has a migration similar to apo B-48 in SDS polyacrylamide gel electrophoresis and that reacts with some of the anti-B-100 monoclonal antibodies. Therefore, in the absence of contradicting evidence, we have considered the apo B-48 of Type IV and normal VLDL to be identical to that of Type III VLDL or chylomicrons. As in Type III VLDL, apo B-48 and apo B-100 are present on different particles in VLDL from Type IV and in normal subjects. While apo B-48 VLDL represents a relatively minor subpopulation of the total VLDL (5% for Type IV and 2% for normal subjects), it was present in all the subjects studied. In Type IV subjects, there was no obvious correlation in the apparent apo B-48/apo B-100 ratio and the e2 allele.

The comparison between the total VLDL from Type III and Type IV subjects indicated that the composition of apolipoproteins was qualitatively similar in the two syndromes and similar to the normal VLDL composition. The characteristic enrichment in apo E in the Type III plasma could be observed from the data of the RIA (Table 2). The lipid composition was characteristic of the syndrome with a CE/TG ratio elevated in Type III and low in Type IV, consistent with previous findings. Similar to Type IV VLDL, normal VLDL were also triglyceride-rich particles, according to reported data.

Apo B-100 VLDL is the major fraction of total VLDL in Type III, as it is in Type IV or in normal subjects. Thus, the differences described above between total VLDL from these two groups of patients were also present in the apo B-100 subfractions, and we noted particularly the difference in the electrophoretic migration (prebeta in the Type IV and a broad β band in the Type III), and the CE/TG ratio, which was much higher in Type III than in Type IV or normal VLDL.

Characterization of Type III VLDL subfractions from the three new patients confirms the differences between apo B-48 and apo B-100 VLDL previously reported for a pool of patients. Differences between the apo B-48 and apo B-100 VLDL from Type IV subjects became apparent when they were similarly analyzed. In addition, certain similarities between apo B-48 from Type III and Type IV subjects were seen when the two apo B-48 subfractions were compared with their respective apo B-100 VLDL; and these similarities include: 1) an increase in the ratio of total lipid-to-protein; 2) an enrichment in apo E; 3) an enrichment in cholesteryl ester with 14-carbon acyl chains and in triglycerides with a 54-carbons number; and 4) a similar electrophoretic mobility characteristic of chylomicrons. Thus, the apo B-48 VLDL may have a common origin, probably the intestine in Type III and Type IV hyperlipoproteinemia. On the other hand, we cannot exclude the possibility that an increased particle size of apo B-48 VLDL, as indicated by the increased lipid/protein ratio, rather than a common site of synthesis, is responsible for the similarities between the apo B-48 VLDL subfractions of Type III and Type IV hyperlipidemia.

In the case of Type III hyperlipoproteinemia, the presence of apo B-48 VLDL in fasting plasma can be rationalized by a defect in the chylomicron clearance due to the presence of a defective apo E isofrom. Because the disappearance of chylomicrons is extremely rapid in individuals with normal lipoprotein lipase activity, it is more difficult to explain the persistence of apo B-48 VLDL in fasting plasma of normal and Type IV subjects, but several possibilities may be considered: 1) The synthesis, assembly, and secretion of chylomicrons or intestinal VLDL may occur in the fasting stage; 2) The apo B-48 VLDL may represent chylomicron remnants that have escaped hepatic capture; and 3) Hepatic apo B-48 synthesis may occur in the human as has been described in the rat. These three possibilities will be considered in order.

Endogenous intestinal lipoprotein synthesis has been previously reported. Thus, lipoproteins of
the size of VLDL have been found in the fasting human jejunal mucosa, and intestinal VLDL can be isolated from fasting chylous urine, thoracic duct, and mesenteric lymph. The apo B species (apo B-48 or apo B-100) that is present in such endogenous intestinal VLDL has not been reported. After incubation with plasma, intestinal VLDL acquires the characteristics of plasma VLDL. However, unlike the apo B-48 VLDL described here that has an electrophoretic mobility of chylomicrons, the intestinal VLDL has a pre-beta migration. Therefore, in at least one physical property, the Type IV apo B-48 VLDL differs from previously described endogenous intestinal VLDL.

Type IV apo B-48 VLDL could also represent chylomicron remnants that have escaped hepatic capture, in spite of this fraction’s enrichment in apo E, which presumably can be recognized by the hepatic receptor. Such chylomicron remnants accumulate in Type III hyperlipidemia because of the defective apo E. However, the difference in the CE/TG ratio between Type III and Type IV VLDL (noted earlier for apo B-100 VLDL) was even more pronounced on comparing Type III with Type IV apo B-48 VLDL. Type III apo B-48 VLDL had not only a higher CE/TG ratio than Type IV apo B-48 VLDL, but also the highest CE/TG ratio of any VLDL fraction, whereas Type IV apo B-48 VLDL had the lowest CE/TG ratio of any VLDL fraction. The difference in the CE content may reflect the time the lipoproteins have been circulating because they can be enriched by the combined actions of lecithin-cholesterol acyltransferase and cholesteryl ester transfer protein. In addition, the higher TG content of Type IV apo B-48 VLDL also reflects the saturation of the lipoprotein lipase as well as the composition of a newly secreted lipoprotein. The Type IV apo B-48 VLDL thus presents a CE/TG ratio characteristic of a recently secreted particle. This could suggest that despite a possible common origin (intestinal), the apo B-48 VLDL may be metabolized quite differently in Type III and in Type IV subjects. Therefore, the CE/TG ratio appears more characteristic of the type of hyperlipidemia than of the apo B-48 or apo B-100 VLDL subtraction. Although only one normal subject was studied, normal apo B-48 VLDL resembled the apo B-48 VLDL from Type IV subjects much more than that of Type III subjects, in CE/TG ratio.

In rats, hepatic apo B-48 synthesis has been shown but this has not been seen in humans. However, until it can be demonstrated directly that hepatic apo B-48 VLDL secretion does not occur in humans, there remains the possibility that the apo B-48 VLDL in fasting plasma is of hepatic origin.

In summary, we have isolated and characterized a subpopulation of VLDL from Type IV and Type III subjects that had as its sole apo B species a protein with a molecular weight and immunological properties indistinguishable from apo B-48. Some physical and chemical properties of the apo B-48 VLDL were similar in Type III or in Type IV, suggesting a common origin. The most striking difference between the apo B-48 VLDL fractions isolated from Type III and Type IV subjects was that the enrichment in cholesterol ester seen in Type III apo B-48 VLDL was not found in Type IV apo B-48 VLDL. The origin of apo B-48 VLDL in fasting Type IV subjects and the metabolic processes responsible for its characteristic properties remain speculative.

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