Remnants of Lipoproteins of Intestinal and Hepatic Origin in Familial Dysbetalipoproteinemia

John P. Kane, G. Chi Chen, Robert L. Hamilton, David A. Hardman, Mary J. Malloy, and Richard J. Havel

We used the low molecular weight form of apolipoprotein B (B-48) as a marker for the identification of remnant particles formed from chylomicrons in the plasma of patients with familial dysbetalipoproteinemia. In the serum of patients fasted 14 hours, the d < 1.006 g/cm$^3$ lipoproteins of prebeta mobility, separated by starch block electrophoresis, contained only the primary hepatogenous species of apolipoprotein B (B-100), and their lipid composition resembled that of normal prebeta very low density lipoproteins. In contrast, the fraction with beta mobility contained both the B-48 and B-100 proteins; the B-48 protein was found primarily among the largest particles. All fractions of beta mobility were greatly enriched with cholesteryl esters. The beta fraction thus contains remnant particles which appear to originate both from chylomicrons and hepatogenous very low density lipoproteins. It appears that these remnant particles share a common removal mechanism which is impaired in familial dysbetalipoproteinemia. (Arteriosclerosis 3:47-56, January/February 1983)

Familial dysbetalipoproteinemia (F. dys.) (type III hyperlipoproteinemia) is now recognized as a disorder in which remnant-like particles, formed from triglyceride-rich lipoproteins, accumulate in plasma. Clinically, it is associated with atherosclerosis of both the coronary and peripheral circulations. These remnant-like lipoprotein particles are enriched with cholesteryl esters and have beta mobility upon electrophoresis in agarose gel, in contrast with normal very low density lipoproteins (VLDL), which have prebeta mobility. The electrophoretic mobility of remnant particles is accounted for principally by deficiency of the C-apoproteins, which are normally the preponderant species in VLDL, with retention of appreciable quantities of less anionic apolipoprotein E (apo E). The initial stages of intravascular lipolysis appear to proceed relatively normally. Removal of remnant particles from the plasma normally occurs rapidly by means of receptor-mediated endocytosis in the liver. In F. dys., however, there is an impairment in the removal of these particles. This impairment is due in most cases to homozygosity for certain isoforms of apolipoprotein E, at least some of which do not interact normally with high affinity receptors on cells. The demonstrated abnormalities of apo E associated with F. dys. are alterations in the primary structure of the protein. It appears that the genes for the isoforms of apo E are allelic at a single locus. Because homozygosity for the abnormal isoforms is often observed in the absence of hyperlipoproteinemia, it has been postulated by Utermann and others that an additional genetic determinant for hyperlipoproteinemia is required for the appearance of F. dys.

Retention of orally administered retinyl esters in the "beta VLDL" fraction of the d < 1.006 g/cm$^3$ lipoproteins in F. dys. provides evidence that a portion of the remnant particles that comprise this electrophoretic fraction originates as chylomicrons. Recently, we have demonstrated that the B apoprotein of intestinal origin differs in apparent molecular weight and amino acid composition from that present in VLDL of hepatic origin. Further, this protein (B-48) appears to be under separate genetic control from that synthesized in the liver. A large body of evidence indicates that, unlike all the other known apolipoprotein species, the B apolipoproteins do not transfer among lipoprotein particles. Therefore, the
intestinal B-48 apolipoprotein should serve as a marker for particles of intestinal origin. Using zonal electrophoresis in starch block, we have isolated the beta migrating fraction of the d < 1.006 g/cm³ lipoproteins of serum from fasted patients with F. dys. In this fraction, we have demonstrated a subpopulation containing the intestinal species of apo B, indicating that remnants formed from chylomicrons are retained in the serum in F. dys., in addition to those derived from hepatogenous VLDL. This finding suggests a commonality in the hepatic receptor-mediated uptake of these remnant particles.

Methods

Characterization of Patients

Blood was drawn from five adults (three men, two women) with F. dys. who had fasted for 14 hours. They were not receiving drug treatment for their lipoprotein disorder, but one man was receiving allopurinol. Serum triglyceride levels varied among the patients between 250 and 700 mg/dl. The patients were free of disorders known to cause secondary hyperlipoproteinemia. They had no systemic disease other than atherosclerotic vascular disease, except for one man who had clinical gout and psoriasis. Criteria of primary F. dys. present in all patients were: 1) E2/2 phenotype determined by isoelectric focusing of the proteins of the VLDL; 2) a ratio of cholesterol to triglyceride greater than 0.42 in the VLDL; and 3) the presence of lipoproteins of beta electrophoretic mobility in the d < 1.006 g/cm³ lipoprotein fraction of plasma.

Blood was also drawn from a 42-year-old man with endogenous lipemia, for comparison of his triglyceride-rich lipoproteins with those of the patients with F. dys. He had no systemic disease other than coronary atherosclerosis. The d < 1.006 g/cm³ lipoproteins from this patient showed a cholesterol-to-triglyceride ratio of 0.33 and prebeta mobility on electrophoresis in agarose gel. The patient had an E3/2 phenotype. His total serum cholesterol level was 361 mg/dl and his triglyceride level was 850 mg/dl.

Preparation of Lipoproteins

As soon as clotting was complete (at 37°C), serum was removed. Sodium EDTA (1 mM), NaCl (0.04%), and gentamycin sulfate (0.5 mg/ml) were added to the serum and to all ultracentrifugal, chromatographic, and electrophoretic media to retard degradation of lipoproteins by hydroperoxidation and by bacteria. Four milliliter samples of serum were overlayered with 0.15 M saline in nitrocellulose tubes and centrifuged for 20 hours at 12°C in a Beckman model L ultracentrifuge using the 40.3 rotor. The supernatant fraction was recentrifuged under similar conditions to purify the d < 1.006 g/cm³ lipoproteins. The d > 1.006 g/cm³ fraction was adjusted to a nonprotein solvent density of 1.019 g/cm³ and recentrifuged for 24 hours. The supernatant fraction was recentrifuged at d = 1.019 g/cm³ (intermediate density lipoproteins, IDL). Similarly, low density lipoproteins (LDL) were isolated from the 1.019–1.063 g/cm³ density interval.

Starch Block Electrophoresis

Lipoproteins of the d < 1.006 g/cm³ fraction of serum containing 50 to 100 mg of protein were subjected to electrophoresis in starch block by a modification of the method of Kunkel and Trautman. The lipoproteins, dialyzed overnight against 0.5 M Na barbital buffer (pH 8.6), were mixed with insoluble potato starch which had been extensively washed with the buffer. The mixture was placed in the starting zone of a 0.5 x 12 x 40 cm block of similarly prepared starch. Electrophoresis was carried out for 16 hours at 100 mA (7°C). Serial sections of the block were extracted repeatedly with 0.15 M saline at 23°C. Mobilities of lipoproteins were confirmed by electrophoresis in agarose gel. For gel permeation chromatography, samples with beta and prebeta mobility were pooled separately and concentrated in an ultrafiltration cell with the UM-2 membrane (Amicon Corporation, Lexington, Massachusetts).

Gel Permeation Chromatography

The prebeta and beta fractions of the d < 1.006 g/cm³ lipoproteins were chromatographed on 1 x 85 cm columns of 4% agarose gel (Bio-Gel, A-15 m, 100–200 mesh, BioRad, Richmond, California), equilibrated in 0.2 N NaCl, with EDTA and antimicrobials as above, pH 7.5, at 23°C. Fractions of 1.6 ml were collected for analysis.

Lipoprotein Analyses

Lipids were extracted from lipoprotein fractions into 30 volumes of chloroform-methanol (2:1 vol/vol). Cholesterol and cholesteryl esters were measured enzymatically; triglycerides, by a fluorimetric technique; and phospholipids, as lipid phosphorus. Protein was measured by the Lowry technique with correction for hydration of the albumin standard. Electron microscopy of lipoprotein fractions was performed on samples negatively stained with potassium phosphotungstate. Particle diameters were measured on the photographic negative plates with a Nikon optical microcomparator (Model 6 C. Nippon Kogaku, KK, Tokyo, Japan). Apo B-48 antigen was identified by double immunodiffusion against a rabbit antiserum specific for the apo B-48 lipoprotein.

The species of apo B were separated by electrophoresis in 3.5% acrylamide gels with SDS. In selected lipoprotein subfractions, unstained B-100 and B-48 bands in the SDS gels were visualized with the aid of a Tyndall beam and excised. The total aminoacyl mass in each band was determined by amino acid analysis after hydrolysis at 105°C for 22 hours.
For characterization, the B-48 and B-100 proteins were isolated from the tetramethylurea-insoluble protein of the particles of the d < 1.006 g/cm³ fraction which had beta mobility, using preparative gel electrophoresis in SDS. The amino acid compositions of the isolated proteins were determined, after hydrolysis in 6 N HCl for 24 hours, with a two-column program on a Beckman model 121-M amino acid analyzer.

The circular dichroic (CD) spectra of isolated subfractions of the d < 1.006 g/cm³ lipoproteins and IDL fractions of the d < 1.006 g/cm³ lipoprotein fraction gave a single precipitin line in double immunodiffusion against rabbit antiserum specific for the B-48 protein.

Gel Chromatography of d < 1.006 g/cm³ Lipoproteins and of Their Prebeta and Beta Fractions

Elution patterns of the d < 1.006 g/cm³ lipoproteins of a patient with F. dys. and a patient with endogenous lipemia are shown in figure 1. In each case, an initial peak emerged at the void volume followed by the gradual appearance of smaller particles over a wide range of apparent Stokes radii. There was an overlap of the two preparations throughout the range of elution. The elution pattern of the prebeta starch block fraction of the d < 1.006 g/cm³ lipoproteins from the patient with F. dys. resembled that of VLDL from the patient with endogenous lipemia (figure 2 A). As in the VLDL of endogenous lipemia, the B-100 species was the sole form of apolipoprotein B detected in the prebeta VLDL of the patient with F. dys. The ratio of cholesterol to triglycerides was somewhat lower in the prebeta than in the VLDL of endogenous lipemia (0.14–0.25 vs 0.33, respectively). In its con-

Results

Identification of the B-48 and B-100 Proteins

Although patients had fasted at least 14 hours, the protein of whole d < 1.006 g/cm³ lipoprotein fraction from each patient with F. dys., which was separated by electrophoresis in SDS gel, contained bands of mobility identical to authentic B-100 and B-48 apolipoproteins. The amino acid analysis of putative B-48 protein isolated by preparative SDS gel electrophoresis is shown in table 1. The composition of the putative B-48 protein from the lipoproteins of a patient with F. dys. resembled that of B-48 protein isolated from human thoracic duct chylomicrons. Where differences between authentic B-48 and B-100 are the greatest, the correlation with chylomicron B-48 was most obvious, namely, in the content of glycine, methionine, isoleucine, and phenylalanine. The whole d < 1.006 g/cm³ lipoprotein fraction gave a mean residue mass of 112 for the protein moiety.

Table 1. Comparison of Amino Acid Compositions of Apo B Species from Dysbetalipoproteinemic Plasma with Those of the B-48 and B-100 Proteins

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residues/10⁴ residues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>From dysbeta-</td>
</tr>
<tr>
<td></td>
<td>lipoprotein-</td>
</tr>
<tr>
<td>protein plasma†</td>
<td>lipoprotein-</td>
</tr>
<tr>
<td></td>
<td>plasma†</td>
</tr>
<tr>
<td>Lysine</td>
<td>69.93</td>
</tr>
<tr>
<td>Histidine</td>
<td>25.29</td>
</tr>
<tr>
<td>Arginine</td>
<td>39.97</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>107.46</td>
</tr>
<tr>
<td>Threonine</td>
<td>66.92</td>
</tr>
<tr>
<td>Serine</td>
<td>85.80</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>114.90</td>
</tr>
<tr>
<td>Proline</td>
<td>36.98</td>
</tr>
<tr>
<td>Glycine</td>
<td>56.91</td>
</tr>
<tr>
<td>Alanine</td>
<td>67.13</td>
</tr>
<tr>
<td>Valine</td>
<td>51.80</td>
</tr>
<tr>
<td>Methionine</td>
<td>20.12</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>46.40</td>
</tr>
<tr>
<td>Leucine</td>
<td>128.30</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>35.42</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>42.67</td>
</tr>
</tbody>
</table>

*Three measurements were made, each at 22, 48, and 72 hours, and values extrapolated to zero time for threonine and serine; 72-hour values were taken for valine, isoleucine, and leucine.
†Mean of duplicate analyses after 22 hours of hydrolysis.
Figure 1. Separation of the d < 1.006 g/cm³ lipoproteins of serum of a patient with F. dys. (●—●) and a patient with endogenous lipemia (○—○) on a column of 4% agarose gel, 1 × 85 cm. Fractions of 1.6 ml were collected.

The compositions of gel-filtered fractions of the d < 1.006 g/cm³ lipoproteins of beta mobility from a patient with F. dys. are shown in table 2. All fractions were enriched in cholesteryl esters, about twofold that of normal prebeta lipoproteins. This occurred at the expense of triglycerides. The content of unesterified cholesterol was also increased slightly over that of normal prebeta VLDL. However, because the content of cholesteryl esters was so high, the ratio of free to esterified cholesterol was lower than that of normal prebeta VLDL (0.41 to 0.52 for the various fractions vs 0.97 for normal VLDL). Similar results were obtained on corresponding fractions from another patient with F. dys.

SDS gel electrophoretograms of the B apoproteins from the chromatographic fractions of the beta migrating portion of the d < 1.006 g/cm³ lipoproteins are shown in figure 5. All gels contained the B-100 protein. Appreciable amounts of B-48 protein were evident in the void volume fraction, with decreasing amounts in succeeding fractions; B-48 was only a trace constituent in the lipoproteins of the second peak. This pattern was typical of the beta starch block fractions of all the preparations of d < 1.006 g/cm³ lipoproteins in F. dys. The B-74 and B-26 pro-
Figure 3. Electron microscopic images of gel-filtered fractions of d < 1.006 g/cm³ lipoproteins with beta mobility. Fractions correspond to the points denoted by arrows in figure 2 B. A. Fraction I. B. Fraction II. C. Fraction III. D. Fraction IV. E. Fraction V. (60,000 ×; 1 mm = 166 Å).
Table 2. Composition of the Beta-Migrating Fraction of \( d < 1.006 \, \text{g/cm}^3 \) Lipoproteins

<table>
<thead>
<tr>
<th>Pooled column fraction</th>
<th>CE*</th>
<th>TG</th>
<th>FC</th>
<th>PL</th>
<th>Protein</th>
<th>Apo B</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>29.5</td>
<td>47.8</td>
<td>7.3</td>
<td>11.9</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>30.4</td>
<td>38.4</td>
<td>8.5</td>
<td>16.4</td>
<td>6.5</td>
<td>16</td>
</tr>
<tr>
<td>III</td>
<td>24.7</td>
<td>38.0</td>
<td>7.6</td>
<td>19.2</td>
<td>10.5</td>
<td>34</td>
</tr>
<tr>
<td>IV</td>
<td>25.3</td>
<td>33.8</td>
<td>7.6</td>
<td>20.6</td>
<td>12.7</td>
<td>50</td>
</tr>
<tr>
<td>V</td>
<td>23.4</td>
<td>33.6</td>
<td>7.3</td>
<td>20.3</td>
<td>15.4</td>
<td>51</td>
</tr>
</tbody>
</table>

The composition is given of the lipoproteins in the fractions designated in figure 2 B.

*CE = cholesteryl esters; FC = unesterified cholesterol; TG = triglycerides; PL = phospholipids.
†Too little for accurate measurement.

Figure 4. Particle size distributions of fractions of \( d < 1.006 \, \text{g/cm}^3 \) lipoproteins of beta mobility from a patient with F. dys., separated by gel chromatography in 4% agarose. Fraction numbers correspond to arrows in figure 2 B.

Figure 5. Electrophoresis of apolipoproteins from the beta starch block fraction in SDS. After separation by starch block electrophoresis, the lipoproteins of the beta fraction were subjected to gel permeation chromatography. From left to right are pooled Fractions I to V, corresponding to the bars in figure 2 B. The uppermost band is the B-100 protein; the second band from the top is the B-48 protein; the two prominent diffuse bands below are apolipoprotein E and the C apoproteins, respectively.
Table 3. Composition of IDL from Patients with Dysbetalipoproteinemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>CE</th>
<th>TG</th>
<th>FC</th>
<th>PL</th>
<th>Protein</th>
<th>Apo B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31.4</td>
<td>21.2</td>
<td>9.1</td>
<td>21.9</td>
<td>16.4</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>31.2</td>
<td>24.7</td>
<td>9.5</td>
<td>15.6</td>
<td>19.0</td>
<td>84</td>
</tr>
<tr>
<td>3</td>
<td>34.1</td>
<td>17.8</td>
<td>10.5</td>
<td>18.0</td>
<td>19.6</td>
<td>90</td>
</tr>
</tbody>
</table>

CE = cholesteryl esters; FC = unesterified cholesterol; TG = triglycerides; PL = phospholipids; IDL = intermediate density lipoproteins.

Proteins which are present in normal LDL were not detected. A determination of total aminoacyl mass of B-100 and B-48 for Fractions I through IV gave ratios of B-100 to B-48 of 1.7, 2.2, 3.9, and 19.3, respectively. The B-48 content of Fraction V was too low to measure. The ratio obtained from the void volume peak of a similar preparation from another dysbetalipoproteinemic patient was 1.2.

Intermediate Density Lipoproteins and Low Density Lipoproteins

The particle size distribution of IDL (1.006 < d < 1.019 g/cm³) from a patient with F. dys. is shown in figure 6. The particle diameters ranged downward from 415 Å, with a preponderance in the 260 Å to 360 Å range. The composition of IDL from three patients with F. dys. is shown in table 3. The content of cholesteryl ester varied from 31% to 34% and that of unesterified cholesterol from 9% to 10.5%. Apo B comprised 82% to 90% of the protein. B-100 was the only apo B species detected in this fraction on SDS gel electrophoresis. Apo B comprised 97% of the protein of LDL. The predominant species was B-100 with small amounts of the associated B-74 and B-26 proteins. There was no detectable B-48 protein in LDL.

Circular Dichroism of d < 1.006 Lipoproteins with Beta Mobility

Figure 7 shows the CD spectra of the d < 1.006 g/cm³ fraction of beta mobility, IDL, and LDL, all from a patient with F. dys. The former was taken from the region of Fraction V of a gel-filtered sample (figure 2 B) so as to avoid light scattering by the larger particles. The spectra of IDL and LDL resembled those observed for the corresponding fractions from normal individuals. However, the spectrum of the beta VLDL fraction had a deep trough about 208 nm. This resembled the spectrum observed with VLDL from cholesterol-fed rabbits. The magnitudes for the ellipticities for the three lipoproteins from F. dys. about 220 nm differed little, indicating that their contents of helix are similar. The differences in the spectra at the 208 nm trough and around the 195 nm peak were considerable and are probably due to the optical contributions of lipids. Similar results were obtained on the beta fraction from another patient with F. dys.
Discussion

The earliest observations on the metabolism of the core lipid constituents of chylomicrons indicated that the bulk of the triglycerides were removed by the peripheral tissues, chiefly by the lipoprotein lipase mechanism, whereas the cholesteryl esters were removed by the liver.\textsuperscript{35-37} Nestel et al.\textsuperscript{38} showed in dogs that functional hepatectomy caused a retention in the serum of cholesteryl esters, but not of triglycerides, after the injection of chylomicrons. This observation was confirmed in rats by Redgrave.\textsuperscript{39} Mjas et al.\textsuperscript{40} isolated remnant particles of intestinal origin after an injection of chylomicrons in functionally hepatectomized rats. They used the device of injecting before-hand an inhibitor of VLDL secretion, 4-aminopyrazoloquinoline, to clear the recipient animals' plasma of hepatogenous lipoproteins. They found the remnant particles to be largely spheres ranging from 250 Å to about 1500 Å in diameter, with a preponderance of particles in the 400 to 600 Å range. The largest remnants were found after the injection of large chylomicrons which emerged at the void volume of a column of 2% agarose gel. Remnants of plasma VLDL were also characterized. They ranged from 200 to 800 Å in diameter. All remnant particles were relatively enriched in free and esterified cholesterol, had reduced mobility upon electrophoresis, and their complements of C apolipoproteins were depleted so that the major proteins were apo B species and apo E. Two populations of cholesteryl ester-enriched lipoproteins, which were isolated by Sata et al.\textsuperscript{41} and by Havel and Kane\textsuperscript{42} from the serum of patients with F. dys., resemble the chylomicron and VLDL remnants of functionally hepatectomized rats, but heretofore there has been no unambiguous means of establishing their tissues of origin.

The identification of the B-48 apolipoprotein as the intestinal species of apo B appears to provide a marker by which daughter species of lipoproteins formed from chylomicrons may be detected. Although the d < 1.006 g/cm\textsuperscript{3} lipoproteins of human thoracic duct lymph contain some B-100 protein, it is likely that this is derived from VLDL entering the thoracic duct via hepatic lymphatics, because observations in the rat\textsuperscript{43-44} and the rabbit (R.J. Havel and J.P. Kane, unpublished observations) indicate that the B-48 protein is the sole B protein secreted by the intestine in those species.

It is possible that the human liver may secrete a protein identical to the intestinal B-48 protein. A protein with an apparent molecular weight identical to that of intestinal B-48 is secreted by rat liver.\textsuperscript{45-47} It is found in VLDL, LDL and in lower density fractions of LDL. It is not yet known whether it is structurally identical to the intestinal protein. In contrast, the absence of a protein identical to intestinal B-48 in human prebeta VLDL, LDL and LDL proteins was reported to be against the possibility that this protein is secreted by human liver. Again, the rat may be unusual among mammals in this respect because the VLDL of guinea pigs,\textsuperscript{48} rabbits,\textsuperscript{49} and monkeys (L.L. Rudel, personal communication) contain no B-48 proteins. Because we have isolated small amounts of a protein with the same apparent molecular weight as the intestinal B-48 protein from normal human LDL, but have demonstrated that it has a different amino acid composition,\textsuperscript{17} it was possible that the B-48 component in the beta migrating d < 1.006 g/cm\textsuperscript{3} lipoproteins of dysbetalipoproteinemia could have been this additional protein rather than the B-48 protein found in chylomicrons. Our amino acid analysis of the isolated protein, however, rules out the former possibility.

Observations on the newly recognized disorder, normotriglyceridemic abetalipoproteinemia, indicate that remnants of chylomicrons in humans essentially do not appear at densities greater than 1.006 g/cm\textsuperscript{3}; that is, the IDL and LDL of normal human serum originate in hepatic VLDL.\textsuperscript{18,45} This is consistent with recent observations on the metabolism of labeled B-48 protein in chylomicrons from rat mesenteric lymph.\textsuperscript{44} When injected into intact rats, the half-life was very short and only miniscule amounts of label were subsequently found transiently in lipoproteins of density > 1.006 g/cm\textsuperscript{3}. The abundance of B-48 protein among the d > 1.006 g/cm\textsuperscript{3} lipoproteins in the serum of our patients with F. dys. indicates that in this condition, too, lipoprotein remnants do not degrade into IDL- or LDL-like particles, despite impaired removal. The presence of B-100 as the sole species of apo B in prebeta VLDL from patients with F. dys., after separation of the triglyceride-rich lipoproteins on starch block electrophoresis, suggests that this population is exclusively hepatogenous VLDL.

The appearance of B-48 protein in the “beta VLDL” fraction on starch block electrophoresis after a 14-hour fast indicates that intestinal particles, presumably chylomicron remnants, do accumulate in F. dys.; hence, they evidently share the apo E-dependent endocytotic mechanism by which VLDL remnants are removed from plasma. Their appearance, predominantly with the circa 900 Å particles, among the “beta VLDL” indicates that most of them are larger than the bulk of VLDL remnants identified by the presence of B-100 protein. However, some hepatogenous remnants could be at least 600 Å in diameter, because some B-100 protein is found even among the largest particle fraction separated on agarose gel. Allowing for a greater apparent molecular weight of the B-100 protein,\textsuperscript{47} we found the B-48 protein to be in molar predominance in the fraction of beta VLDL with the largest particle diameters. Since the submission of this paper, a report has appeared describing a protein component with an apparent molecular weight identical to that of the B-48 protein in the beta migrating d < 1.006 g/cm\textsuperscript{3} lipoproteins of cholesterol-fed dogs and of humans with F. dys.\textsuperscript{48} Thus, the “beta VLDL” resulting from cholesterol feeding appears to contain remnant lipoproteins of intestinal, as well as hepatic, origin.
The observation of B-48 protein in the d < 1.006 g/cm³ lipoproteins of dysbetalipoproteinemia is in sharp contrast to our experience in endogenous lipemia. In most cases, no B-48 protein is detectable if triglyceride levels are below 600 to 700 mg/dl. In rare instances, trace quantities of a protein with the appropriate mobility in SDS gel for B-48 have been observed, however, possibly reflecting heterogeneity of mechanisms causing hypertriglyceridemia.

We found that the content of cholesteryl esters, both in the fraction rich in chylomicron-derived remnants and in the smaller, hepatic cholesteryl "beta VLDL," is much higher than in normal prebeta VLDL, extending the earlier observations of Hazzard et al. and others. The fact that remnant lipoproteins formed from chylomicrons and hepatic VLDL in F. dys. are heavily enriched in cholesteryl esters suggests that the long residence time in blood attendant to impaired receptor-mediated endocytosis allows the transfer of cholesteryl esters generated by lecithin-cholesterol acyltransferase (LCAT) to proceed further. In fact, the lipid compositions of both the B-48- and B-100-containing remnant populations resemble those of VLDL subjected to maximal transfer of cholesteryl esters by the LCAT-cholesteryl ester transfer mechanism in vitro.

The increased content of unesterified cholesterol in the beta migrating d < 1.006 g/cm³ lipoproteins is most impressive in the particles of largest diameters, where the content is two to three times that expected for normal chylomicrons. This reflects, at least in part, a substantial loss of the other amphipathic components, i.e., phospholipids and proteins from the particle surface. This finding has also been made in the d < 1.006 g/cm³ lipoproteins of cholesterol-fed rabbits. An abnormally large fraction of free cholesterol may be distributed into the cores of these lipoproteins in both F. dys. and in cholesterol-fed animals. It is likely that the striking contour of the CD spectrum in the region of 208 nm we have described here in beta VLDL from F. dys. reflects the abnormal lipid content of these particles, either cholesteryl ester or unesterified cholesterol, because it resembles closely the spectrum we have reported for the d < 1.006 g/cm³ lipoproteins and IDL of cholesterol-fed rabbits.

It is a reasonable assumption that the prebeta VLDL separated on starch block electrophoresis from the plasma of patients with F. dys. are newly secreted hepatic lipoproteins. They resemble normal VLDL in their lipid composition, particle diameters, and content of apolipoprotein B-100. The presence of both the intestinal and hepatic species of apolipoprotein B in the beta migrating fraction of the d < 1.006 g/cm³ lipoproteins in F. dys., however, indicates that this fraction is composed of remnant lipoproteins formed from both chylomicrons and hepatic VLDL, even after a standard 14-hour fast. Thus, these two species of remnants must share a common removal mechanism, which is impaired in F. dys. The changes in lipid composition that occur during the prolonged circulation of these particles in this disorder appear to affect the remnants of chylomicrons and VLDL alike.

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