Heterozygous Hypobetalipoproteinemia with Fasting Chylomicronemia

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We describe a disorder in which low-density lipoprotein (LDL)-cholesterol and apolipoprotein B are in low concentration (0.47 mmol/L and 0.28 g/L, respectively) and chylomicrons are still present in plasma after an 18-h fast. The d < 1.006 fraction was isolated by flotation ultracentrifugation and the apolipoproteins were analyzed by electrophoresis, immunoblotting with anti-apolipoprotein B-100 antiserum, and isoelectric focusing. In the d < 1.006 fraction of the fasting serum, we found an apolipoprotein B form with the same apparent molecular mass as apolipoprotein B-48 and similar in amount to apolipoprotein B-100 (respective percentages, 46% and 54%). The monosialylated form of the apolipoprotein C-III was severely decreased. After an oral fat load, the repartition of the two species of apolipoprotein B did not change greatly (respective percentages, 60% and 40%), and the concentration of serum triglyceride increased only from 1.20 to 1.85 mmol/L.

Additional Keyphrases: apolipoproteins · chylomicrons · ultracentrifugation · isoelectric focusing · lipoproteins · heritable disorders

Two inherited diseases are characterized by the absence of chylomicrons, very-low-density lipoproteins (VLDL), and low-density lipoproteins (LDL) from plasma (1, 2).6 One of these is abetalipoproteinemia, an autosomal recessive disorder associated with a severe clinical picture that includes fat malabsorption, neurological disease, retinitis pigmentosa, and acanthocytosis. A distinctly different syndrome, hypobetalipoproteinemia, is inherited in an autosomal dominant pattern. Heterozygous hypobetalipoproteinemia has clinical and biochemical features similar to those of recessive abetalipoproteinemia. Heterozygous hypobetalipoproteinemia is characterized by a partial deficiency of LDL in plasma, and the patients are generally asymptomatic or display moderate clinical manifestations. In abetalipoproteinemia and homozygous hypobetalipoproteinemia, apolipoproteins B-100 and B-48 are both absent. Some disorders have been described in which apolipoprotein B-100 deficiency was associated with a possible synthesis of chylomicrons. Steinberg et al. (3) reported a case of asymptomatic hypobetalipoproteinemia with a fasting chylomicronemia. Later, Young et al. (4) re-examined the case and found that the lipoproteins of the patient contained an abnormal apolipoprotein B species (apolipoprotein B-37). Malloy et al. (5) described a "normotriglyceridemic abetalipoproteinemia" with secretion of chylomicrons in plasma despite the absence of normal LDL and VLDL. No apolipoprotein B-100 was detected in the patient's plasma but apolipoprotein B-48 remained. The syndrome was associated with obesity and neurological symptoms. In the apolipoprotein B-100 deficiency reported by Herbert et al. (6), the lipoprotein fraction d < 1.006 contained apolipoprotein B-48 but no apolipoprotein B-100, and the normal LDL were absent. Nevertheless, the patient had a syndrome of fat malabsorption that suggested limited capacity for chylomicron production (6). Takashima et al. (7) also reported a case of isolated apolipoprotein B-100 deficiency associated with fat malabsorption. We describe here a case of hypobetalipoproteinemia characterized by a relatively high proportion of an apolipoprotein B form of apparent molecular mass similar to that of apolipoprotein B-48 in fasting serum.

Materials and Methods

Case Report

The patient, a 47-year-old woman (height, 160 cm; weight, 68 kg), consulted for diarrhea and asthenia. The diarrhea had been evolving for many years. The asthenia, which had been evolving for six months, was accompanied by a 2-kg loss of weight, anorexia, slow digestion, and nausea.

Procedures

Lipid analyses. Cholesterol, triglycerides, and phospholipids were measured enzymatically with kits from Biomerieux (Marcy-l'Etoile, France). High-density lipoprotein (HDL) cholesterol was evaluated after heparin-Mn2+ precipitation (8) and later confirmed by (a) precipitation with phosphotungstate-Mg2+, with cholesterol measurement enzymatically (Biomerieux kit), and (b) precipitation with concanavalin A, with enzymatic measurement of cholesterol (Fournier Labs., Dijon, France). LDL cholesterol was determined with the Biomerieux kit. Apolipoproteins A-I and B were quantified.
by immunoprecipitation (Orion Diagnostica kits, Espoo, Finland). Apolipoprotein A-II was measured by electroimmunodiffusion on Sebia plates (Issy les Moulineaux, France). The results for the patient, for her children, and for normal subjects are given in Table 1. The five children of the patient all showed normal values for these lipid analyses except one, who had a slightly hypertriglyceridemia. Additional lipid and apolipoprotein analyses are given for the patient: the serum concentrations of apolipoprotein C-II (0.044 g/L), apolipoprotein C-III (0.054 g/L), and apolipoprotein E (0.020 g/L) were determined by single radial immunodiffusion (Daichii, Tokyo, Japan). Fecal fat excretion was 5.58 g/day.

Ultrasoundfugal fractionation of serum lipoproteins. Lipoproteins were separated by sequential density ultracentrifugation (24 h, 106 000 x g) with a 50 Ti rotor at 4 °C in a Model L-5 ultracentrifuge (Beckman Instruments, Palo Alto, CA). Three lipoprotein fractions were isolated after flotation at densities of 1.006, 1.063, and 1.21 kg/L (9). Each lipoprotein fraction was purified by flotation ultracentrifugation through a saline solution at the same density. The collected fractions were dialyzed and lyophilized.

Preparation of apolipoproteins. The lyophilized lipoproteins were delipidated with ethanol/diethyl ether (3/1 by vol) as described elsewhere (10).

Electrophoresis. For electrophoresis of lipoproteins we used a discontinuous polyacrylamide gel (Lipofilm; Sebia). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of apolipoproteins was carried out on 2–10% or 5–30% polyacrylamide gradient gel, by Laemmli’s method (11). The electrophoretograms were stained with Coomassie Brilliant Blue R-250 and quantified with a Cliniscan II densitometer (Helena Labs., Beaumont, TX). The relative molecular masses (M<sub>r</sub>) of the apolipoproteins B-74, B-48, and B-26 were determined by assuming an M<sub>r</sub> of 550 000 for apolipoprotein B-100 and using an electrophoresis calibration kit (no. 17-0446-01; Pharmacia, Uppsala, Sweden).

Fat feeding study. Serum lipids and the apolipoproteins of the d <1.006 fraction were studied after the patient had fasted for 18 h and after she ingested the following liquid formula: 30% cream, 377 g; sunflower oil, 24 g; granulated skimmed milk powder, 20 g; granulated sugar, 20 g; sweetening syrup, 20 mL; and water to make 500 mL. This meal contained 137 g of fat.

Isoelectric focusing. Isoelectric focusing was performed with a thin-layer (0.2 mm thick) 5% polyacrylamide gel containing urea, 8 mol/L, and 6% Pharmalyte (Pharmacia) between pH 4 and pH 6.5 (12). The electrode solutions were histidine, 0.2 mol/L, at the cathode and glutamic acid, 40 mmol/L, at the anode. The gel was prefocused at 500 V for 30 min. The apolipoproteins were dissolved in 10 mmol/L Tris·HCl (pH 8.6) buffer containing, per liter, 10 mmol of dithiothreitol and 8 mol of urea. Electrophoresing was performed with a constant voltage of 1500 V for 3 h. The gels were stained with Coomassie Brilliant Blue R-250.

Immunoblotting with anti-apolipoprotein B-100 antisera. For immunoblotting we used the method described by Burnette (13). Apolipoproteins were separated on a 2–10% gradient polyacrylamide gel and transferred to a nitrocellulose membrane according to Vaessen et al. (14). The membrane was then treated with goat anti-human apolipoprotein B-100 antiserum (Tago, Burlingame, CA) diluted 250-fold. Antigen–antibody complexes were detected with a second antibody of peroxidase-conjugated anti-goat Ig antibody (Miles Scientific, Naperville, IL) treated with 4-chloro-1-naphthol (Sigma, St. Louis, MO).

Analysis of phospholipids by HPLC. The lipids were extracted from serum with chloroform/methanol (2/1 by vol) (15). The samples were injected into a 25 cm × 4 mm column of Lichrosorb Si60 (5-μm particles; Merck, Darmstadt, F.R.G.) equipped with a 4 cm × 4 mm precolumn (40-μm particles) in a Model 1084A chromatograph (Hewlett-Packard, Waldbronn, F.R.G.). The flow rate was 2 mL/min, with gradient elution. Solvent A was acetonitrile/water/H<sub>3</sub>P0<sub>4</sub> 333 mmol/L (750/250/2 by vol); solvent B was acetonitrile/water/H<sub>3</sub>P0<sub>4</sub>, 333

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Table 1. Serum Lipid and Apolipoprotein Concentrations in the Patient and Her Children

<table>
<thead>
<tr>
<th>Sex, age (years)</th>
<th>Total cholesterol</th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
<th>Total triglycerides</th>
<th>Total phospholipids</th>
<th>Apolipoprotein concn, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient F 47</td>
<td>1.30</td>
<td>0.47</td>
<td>1.11</td>
<td>1.20</td>
<td>0.99</td>
<td>0.94</td>
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<tr>
<td>Patients' children</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 27</td>
<td>4.84</td>
<td>2.46</td>
<td>1.45</td>
<td>1.65</td>
<td>2.67</td>
<td>1.31</td>
</tr>
<tr>
<td>F 25</td>
<td>5.08</td>
<td>3.44</td>
<td>1.17</td>
<td>1.00</td>
<td>1.88</td>
<td>0.93</td>
</tr>
<tr>
<td>M 24</td>
<td>5.67</td>
<td>2.46</td>
<td>1.35</td>
<td>1.47</td>
<td>2.67</td>
<td>1.23</td>
</tr>
<tr>
<td>M 21</td>
<td>5.05</td>
<td>2.51</td>
<td>1.24</td>
<td>3.00</td>
<td>2.57</td>
<td>1.33</td>
</tr>
<tr>
<td>F 10</td>
<td>5.10</td>
<td>2.67</td>
<td>1.19</td>
<td>1.32</td>
<td>2.36</td>
<td>1.11</td>
</tr>
<tr>
<td>Normal subjects*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.98–5.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.59–4.92</td>
<td>0.91–2.07&lt;sup&gt;M&lt;/sup&gt;</td>
<td>0.34–1.82</td>
<td>2.06–3.61</td>
<td>0.95–1.75</td>
<td>0.30–0.60</td>
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<tr>
<td>3.63–6.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>0.91–2.46&lt;sup&gt;F&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3.89–6.73&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
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</tbody>
</table>

* These values (5th–95th percentile) are derived from populations of 30 subjects per age group (90, 45 males and 45 females).

<sup>b</sup> Ages 5–19 years; <sup>c</sup> ages 20–40 years; <sup>d</sup> ages 41–60 years.
mmol/L (990/10/2 by vol). The gradient system was as follows: 90% B, 3 min; changing to 40% B over 9 min; changing to 20% B over 4 min; then to 5% B over 2.5 min. Eluates were detected with an absorbance detector (LKB 2238 Uvicord S II; LKB, Bromma, Sweden) at 206 nm and a sensitivity of 0.1 A full-scale.

Fatty acid analyses. The fatty acids were extracted by the procedure of Folch et al. (15) and converted into methyl esters (16). The methyl esters were extracted with heptane, purified by thin-layer chromatography, and identified by gas–liquid chromatography with a Model 8500 chromatograph (Perkin-Elmer, Oak Brook, IL) with a 30-m fused silica capillary column with chemically bonded phase DB-1 (film thickness: 0.25 μm; J.W. Scientific, Touzart et Matignon, France). Fatty acids were quantified by using C19:0 as internal standard (Interchim, Montluçon, France).

Results

Electrophoresis of lipoproteins. We electrophoresed serum lipoproteins from fasting and nonfasting blood samples of the patient (Figure 1, lanes 1 and 2–4, respectively). Fasting sera from four of her children were also analyzed, for comparison (Figure 1, lanes 5–8). The patient’s fasting serum was characterized by (a) the presence of chylomicrons at the origin of the polyacrylamide gel, (b) a strong band migrating the same as VLDL, (c) a trace amount of normal LDL, and (d) a relatively strong HDL band. A densitometric scan of the electrophoretogram (excluding the chylomicrons) gave the relative percentages of 40% for VLDL, 10% for LDL, and 50% for HDL. The respective normal ranges are VLDL, 5–26%; LDL, 37–61%; and HDL, 21–48%. Also, the patient’s fasting serum was slightly turbid.

The patient’s child who displayed hypertriglyceridemia was characterized by an increased VLDL band on the electrophoresis pattern (Figure 1, lane 8; relative percentages: VLDL, 31%; LDL, 47%; HDL, 22%).

Analysis of apolipoproteins by SDS-PAGE. Three lipoprotein fractions were isolated from the patient’s fasting serum by flotation ultracentrifugation: chylomicrons/ VLDL at d <1.006, LDL at d <1.063, and HDL at d <1.21. Each lipoprotein fraction was delipidated and the apolipoprotein content was analyzed by Coomassie Blue-stained SDS-PAGE. The apolipoprotein pattern of the chylomicron/VLDL fraction (Figure 2A) showed, in comparison with VLDL isolated from a pool of normal sera: (a) a low amount of the high- Mr, band corresponding to apolipoprotein B-100 (20% of the total apolipoproteins, whereas an integral scan of the gel showed 60% apolipoprotein B-100 in control VLDL), and (b) two bands at Mr, 44 000 and 280 000. The 280 000 band was also detected in the LDL (Figure 2B).

Immunoblotting with anti-apolipoprotein B-100 antiserum. The d <1.006 fraction isolated from the patient was analyzed by immunoblotting with an anti-apolipoprotein B-100 antiserum. Figure 3 shows the results, and compares this fraction with LDL and VLDL isolated from a pool of normal sera. One essential band, corresponding to the apolipoprotein B-100, was revealed in the control VLDL (Figure 3, lane 3). In the control LDL (Figure 3, lane 2), two immunoreactive bands, at Mr, 410 000 and 150 000, were observed in addition to apolipoprotein B-100. We consequently assumed these to be the cleavage products B-74 and B-26 of the apolipoprotein B-100 (17). In the chylomicron/VLDL fraction of the patient (Figure 3, lane 1), a band was present at Mr, 280 000, which might correspond to the B-48 form secreted with the chylomicrons.

Fat feeding study. After the patient had fasted for 18 h, she was given an oral meal containing 137 g of fat. Blood samples were taken 2, 4, and 6 h afterwards. Serum concentrations of cholesterol, triglycerides, and phospholipids were determined before and after fasting (Table 2). The concentration of serum triglyceride increased from 1.20 to 1.65 mmol/L 4 h after the oral fat load.

Samples of fasting serum and of serum drawn 4 h after the oral fat load were subjected to ultracentrifugation, and the d <1.006 fraction was analyzed by SDS-PAGE. The relative proportions of the B-100 and B-48 forms, estimated by densitometric scanning of the Coomassie Blue-stained gel, were 54% and 46% in the

Fig. 1. Polyacrylamide gel electrophoresis of lipoproteins
The patient’s serum was analyzed after an 18-h fast (lane 1), and after the ingestion of an oral fat load: after 2 h, lane 2; after 4 h, lane 3; after 6 h, lane 4. Fasting sera of four of the patient’s children were analyzed in lanes 5–8

Fig. 2. SDS-PAGE of delipidated lipoprotein fractions: (A) 1, molecular mass standard for calibration; 1, control VLDL; 2, chylomicrons/ VLDL fraction of the patient; (B) 1, control VLDL; 2, chylomicrons/ VLDL fraction of the patient; 3, control LDL; 4, LDL fraction of the patient
fasting serum, and 40% and 60% in the serum drawn 4 h after the oral fat load.

Isoelectric focusing. The apolipoproteins C of the chylomicron/VLDL fraction of the patient were analyzed by isoelectric focusing in a gradient from pH 4 to pH 6.5 (Figure 4). The band corresponding to C-III was much less (densitometric scanning: C-IIIa, 10.3%; C-II, 34.8%; C-IIIb, 6.2%; C-IIIb, 48.7%) than in the pattern of control VLDL apolipoproteins C (C-IIIa, 5.5%; C-II, 23.0%; C-IIIb, 48.5%; C-IIIb, 23.0%).

HPLC chromatography of phospholipids. We used HPLC chromatography to estimate the phosphatidylcholine/sphingomyelin ratio. These two phospholipids were quantified by reference to standard phosphatidylcholine from egg yolk and standard sphingomyelin from bovine brain, respectively. In those conditions, the phosphatidylcholine/sphingomyelin ratio (by weight) of the patient’s total serum was 8.8 (normal range: 5.1–8.4, defined from 17 normal sera).

Fatty acid analysis. The relative percentages of arachidonic acid and linoleic acid were respectively lower and higher than the normal range: C 20:4n – 6, 4.5% (normal range: 6–9%); C 18:2n – 6, 28.3% (normal range: 20–25%).

Discussion

We describe here a syndrome associating (a) low serum concentrations of LDL-cholesterol (0.47 mmol/L) and apolipoprotein B (0.28 g/L) and (b) the presence of chylomicrons in serum after an 18-h fast. Chylomicrons were observed at the origin of the electrophoretogram, which also revealed a band in the VLDL region despite a very faint LDL band. Also, the patient responded to an oral fat load by an increase in triglyceride concentration, from 1.20 to 1.65 mmol/L. In addition to LDL, both chylomicrons and VLDL are usually absent from plasma in classical abetalipoproteinaemia and homozygous hypobetalipoproteinaemia (1). On the other hand, some cases of possible synthesis of chylomicrons despite apolipoprotein B-100 deficiency have been observed (3, 5–7). Heterozygous subjects for familial hypobetalipoproteinaemia contain VLDL and LDL in low concentration, and fasting chylomicronemia has also been observed in some patients after an overnight fast when high-fat meals had been eaten the day before (1). The patient’s HDL fraction appeared strongly stained with Sudan black on the electrophoretogram. The concentration of apolipoprotein A-I was found to be normal. The HDL cholesterol evaluated after heparin–Mn²⁺ precipitation (8) was within the normal range. Low HDL concentrations have been found in homozygous hypobetalipoproteinaemia (1) as well as in the syndromes studied by Steinberg et al. (3), Malloy et al. (5), and Herbert et al. (6), whereas variable HDL concentrations have been described in heterozygous hypobetalipoproteinaemia and recessive abetalipoproteinaemia (1).

A deficiency in the monosialylated apolipoprotein C-III was reported in abetalipoproteinaemia and in homozygous hypobetalipoproteinaemia (1) as well as in the cases of Malloy et al. (5), Herbert et al. (6), and Steinberg et al. (3). In the case described here, the C-III form was present in very low amounts, as shown by isoelectric focusing of the apolipoproteins of d <1.006. Although the total plasma phospholipid concentration was low, the phosphatidylcholine/sphingomyelin ratio was near the upper limit of the normal range. A reduction in the phosphatidylcholine/sphingomyelin ratio was described in abetalipoproteinaemia (1) and in the syndromes described by Malloy et al. (5) and Herbert et al. (6). The fatty acid repartition did not show considerable abnormalities; arachidonic acid was only slightly decreased.

Using ultracentrifugation, we isolated the d <1.006 fraction from the patient’s fasting serum. The apolipoproteins of the fraction were analyzed by Coomassie Blue-stained SDS-PAGE, and a Western-blot was carried out.
out with anti-apolipoprotein B-100 antiserum. The results showed (a) a low amount of the apolipoprotein B-100: 20% of the total apoproteins according to the densitometric scan of the Coomassie Blue staining, whereas apolipoprotein B-100 was estimated to be 60% in control VLDL, and (b) an additional band, revealed by the anti-apolipoprotein B-100 antiserum and appearing to be present in an amount similar to that of apolipoprotein B-100 according to the densitometric scan. The Mf of the additional band was suggestive of apolipoprotein B-48. Synthesis of apolipoprotein B-48 despite apolipoprotein B-100 deficiency was found in the cases reported by Malloy et al. (5), Herbert et al. (6), and Takashima et al. (7). Also, the d <1.006 fraction of our patient's fasting serum contained a protein with an Mf (44 000) consistent with that of apolipoprotein A-IV. In fasting plasma, 98% of apolipoprotein A-IV is located in the d >1.21 fraction (18). In lipemic plasma obtained from subjects after ingestion of a pint of cream, 10% of the plasma apolipoprotein A-IV was in the d <1.006 fraction and 90% in the d >1.21 fraction (18).

After an 18-h fast, the serum of our patient contained chylomicrons and an apolipoprotein B form showing the same Mf as apolipoprotein B-48. The amount of this apolipoprotein B was similar to that of apolipoprotein B-100 (46% and 54%, respectively). Four hours after an oral fat load, this concentration was slightly more increased than that of apolipoprotein B-100 (60% and 40%, respectively) and correlated with the slight increase in triglyceride concentration. However, the fat fecal excretion of our patient was within the normal range. In normotriglyceridemic abetalipoproteinemia (5), the absorption of triglycerides from the intestine and the secretion of chylomicrons were normal, although fat malabsorption was associated with the syndromes described by Herbert et al. (6) and Takashima et al. (7), and also with the unusual case of hypobetalipoproteinemia described by Steinberg et al. (3). In heterozygous hypobetalipoproteinemia, the fat absorption was reported to be usually normal (1, 2). A fasting chylomicronemia was observed in the cases described by Herbert et al. (6), Steinberg et al. (3), and also in heterozygotes for hypobetalipoproteinemia (1), which suggested prolonged fat absorption (1, 3, 6) and impaired chylomicron clearance (3). The patient of Steinberg et al. was later found to have inherited two abnormal apolipoprotein B alleles (18): one of these leads to the formation of an abnormal apolipoprotein B species (apolipoprotein B-37); the other one results in the production of apolipoprotein B-48 and apolipoprotein B-100, but the allele is associated with a low plasma concentration of apolipoprotein B-100. The majority of apolipoprotein B-37 was contained in HDL (4). An apolipoprotein B-39, found in VLDL/chylomicron and LDL fractions, was also described in hypobetalipoproteinemia by Collins et al. (20).

We have examined the five children of the patient, and all of them had total cholesterol and apolipoprotein B concentrations within the normal ranges. The important band at the electrophoretic migration distance of apolipoprotein B-48, observed by SDS-PAGE in the d <1.006 fraction of fasting serum for our patient, was not found in any of the children.

The case we have described is a heterozygous form of hypobetalipoproteinemia, characterized by a fasting chylomicronemia and by the relatively high proportion of an apolipoprotein B form, of Mf, similar to that of apolipoprotein B-48 in fasting serum.

References