Hyperlipoproteinemia with Albumin–Lipid Complex: A Novel Type of Hyperlipoproteinemia Associated with Insulin-Resistant Diabetes Mellitus

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We describe a new phenotype of hyperlipoproteinemia in two members of a family with a high degree of consanguinity. Both have a history of uncontrolled diabetes mellitus without ketoacidosis, and a family history of coronary artery disease at a relatively early age. A high degree of insulin resistance was found. The abnormal lipoprotein(s) has alpha-lipoprotein mobility on cellulose acetate electrophoresis and has a relative density of <1.006 as determined by ultracentrifugation of serum collected after a short fast. The fraction isolated by ultracentrifugation contains about half of the serum cholesterol and triglycerides and most of the phospholipids; the major protein component is albumin. Immunoelectrophoresis showed low concentrations of beta-lipoproteins in both sera, and two abnormal precipitin bands against monospecific antiserum to antilipoprotein A; a third member of the family showed only one abnormal precipitin band against the same antibody. We tentatively propose an abnormal gene(s) as the underlying mechanism. The insulin-resistant diabetes mellitus, probably inherited separately, may aggravate the hyperlipidemia.

Additional Keyphrases: heritable disorders · lipoproteins · immunoelectrophoresis · electrophoresis, cellulose acetate

Classically, hyperlipoproteinemias are divided into five major types with characteristic clinical and laboratory features (1). Rare disorders such as cerebrotendinous xanthomatosis (2), β-sitosterolemia, and xanthomatosis (3) are characterized by the accumulation of abnormal lipids. Here we describe three cases of what appears to be a new hyperlipoproteinemia syndrome involving the very-low-density lipoprotein (VLDL) fraction of the plasma. Two of these patients are female sibs, and the third is a female cousin in a family with parental consanguinity.

Case Histories

The family pedigree is shown in Figure 1.

Case 1: I.L. (IV-1). A 42-year-old married woman was referred to the clinic for management of hyperglycemia and hyperlipidemia of 13 years duration. Despite treatment with sulfonylureas and increasing doses of insulin, her hyperglycemia persisted.

Nothing in her medical history suggested ketoacidosis, coronary insufficiency, intermittent claudication, or cerebrovascular insufficiency, but she did have mild paresthesias. There were no symptoms related to thyroid dysfunction, liver disease, or renal failure. She denied taking alcohol or oral contraceptives. Her menopause was regular; and her medical history was otherwise negative.

Her parents (III-2,5 in Figure 1) were first cousins, her mother (III-5) died of coronary artery disease, with uremia and diabetes mellitus. Her brother (IV-2) has hyperlipidemia. Two of her maternal uncles (III-8,10) have diabetes mellitus and coronary artery disease. One maternal aunt (III-7; mother of Case 2) has hyperlipidemia and diabetes mellitus. A paternal aunt (III-3) is diabetic and the maternal grandmother (II-4) was also diabetic.

This middle-aged woman's height was 153 cm, weight 58 kg, blood pressure 165/85 mmHg, and pulse rate 72/min, with no posture-related changes. Her skin was normal except for small eruptive xanthomas over the elbows and the knees. There were no palmar tendinous xanthomas, no xanthelasmas, and no arcus senilis. The fundi showed lipemia retinalis, but were otherwise normal.

Case 2: G.M. (IV-9). This 18-year-old woman was referred to the clinic for uncontrolled hyperglycemia and hyperlipidemia. Two years before, eruptive xanthomas had appeared on her elbows, knees, and buttocks. Again, there was no history of ketoacidosis, and she was treated with sulfonylurea and insulin, but her hyperglycemia persisted. She had no history suggestive of coronary, peripheral arterial, or cerebrovascular insufficiency, and she had no paresthesias. She had noted a gradual loss of subcutaneous fat in her

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² Nonstandard abbreviations: LDL, VLDL, HDL, low-, very-low-, and high-density lipoproteins, respectively.

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cheeks over the past few years. Menarche occurred at the age of 12 years and since then menses was regular. Her past medical and developmental histories provided no pertinent information.

Physical examination showed absence of subcutaneous fat in the cheeks (Figure 2). There were eruptive xanthomas over the elbows (Figure 3), knees, and buttocks. Acanthosis nigricans was present in both axillary folds. This patient's height was 163 cm, weight 58 kg, blood pressure 135/85 mmHg, and pulse rate 80/min, with no posture-related changes. The fundi showed lipemia retinalis. She had prominent parotid glands and mildly enlarged tonsils of normal color. There was no hepatosplenomegaly, no palmar tendinous xanthomas, xanthelasmas, or arcus senilis. Results of the rest of the physical examination were unhelpful.

Case 3: H.M. (IV-6). This 34-year-old woman, sister of Case 2, was in good health, and results of previous laboratory screening studies, including blood glucose after fasting and serum lipids, were within normal limits. Physical examination revealed nothing abnormal.

Materials and Methods

Preparative ultracentrifugation of serum lipoproteins (d <1.006). Blood specimens were collected after 14 h of fasting, and blood cells and platelets were separated by low-speed centrifugation. The sera were mixed with salt solutions to give a final density of 1.006 kg/L. The serum lipoproteins were then isolated by centrifugation (Sorvall Model OTR:75B Ultracentrifuge, swinging-bucket rotor AH 650, 108 000 × g, 24 h, 4 °C) (4, 5). The d >1.006 supernates were collected quantitatively and analyzed.

Electrophoresis. Serum proteins and lipoproteins were separated and quantified by electrophoresis on cellulose acetate membranes (Helena Laboratories, Beaumont, TX 77704), which we scanned with a clinical densitometer (Helena Laboratories).

Immunoelectrophoresis. For immunoelectrophoresis we used 2-mm-thick Special Agar-Nobel (Difco Laboratories, Detroit, MI), 10 g/L in diethyl barbiturate buffer (18 mmol/L, pH 8.6). We filled the 1-mm-diameter wells with 10 μL of serum, and electrophoresed at 12 V/cm for 85 min. We filled the 60 × 2 mm trough, which was 3 mm from the sample well, with 100 μL of antiserum. After 24 h of diffusion in a moist chamber, the agar plate was washed and blotted several times, then dried and stained with Amido Black, 10 g/L in destaining solution (ethanol/conc. acetic acid/water, 30/6/64 by vol).

We used polyclonal antiserum (antiserum to human serum proteins) from Meloy Labs., Springfield, VA 22151, and monospecific antisera to human apo A and human apo B from Behringwerke, Marburg, F.R.G.

Insulin-tolerance test. To test the subjects' tolerance to insulin, we first administered 0.1, then 0.4 USP unit of monocomponent porcine regular insulin (Actrapid M.C.; Novo Laboratories, Copenhagen, Danemark) per kilogram body weight, intravenously, as a single dose. Blood glucose was measured before and 30, 60, 90, 120, and 180 min after these injections.

Blood chemistry determinations. Serum glucose, creatinine, total proteins, and albumin were determined by continuous-flow with the Technicon AutoAnalyzer II (6–9). Total and free cholesterol were colorimetrically determined (10). HDL-cholesterol was determined enzymically with a centrifugal analyzer (Centrifichem; Union Carbide Corp., Pleasantville, NY 10570) after precipitation of LDL and VLDL with dextran sulfate and magnesium chloride (11). Triglycerides were determined colorimetrically (12), serum phospholipids by the method of Sandhu (13). Alkaline phosphatase (EC 3.1.3.1) and aspartate and alanine aminotransferases (EC 2.6.1.1, 2.6.1.2) were measured in serum with the Centrifichem, with reagents supplied by Worthington Diagnostic Systems Inc., Freehold, NJ 07728. Serum thyroxin was radioimmunoassayed with kits (Amer-
sham International, Ameraham, Bucks, U.K.). Other laboratory determinations were done by the usual techniques.

Analysis of the VLDL fractions. Lyophilized preparations of the VLDL fractions of Cases 1 and 2 were sent to two independent laboratories for detailed analysis for lipids and proteins. Proteins were identified by the double-diffusion technique of Ouchterlony and sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to Weber and Osborn (14) after delipidation with ethanol/diethyl ether (3/1 by vol) according to Scanu and Edelstein (15). Detailed lipid analysis of the two cases was carried out according to Nestruck et al. (16). The nature of the protein components was further confirmed by immunoelectrophoresis, nephelometry, and electroimmunoassay.

Results

Results of the laboratory studies on the three women are shown in Table 1.

Electrophoresis. Lipoprotein electrophoresis showed a thick band in the alpha region in sera from both Cases 1 and 2 (Figure 4). The pre-beta band was normal, but the beta band was barely detectable. The respective proportions of the electrophoretic fractions for Cases 1 and 2 were as follows (normal values in parentheses): alpha-lipoproteins 82% and 74% (27-40%), pre-beta lipoproteins 15% and 14% (12-22%), beta lipoproteins 3% and 12% (45-56%). Dilution of the sera with isotonic saline did not change the electrophoretic pattern. Electrophoresis of serum proteins showed the following proportions: albumin 43% and 47% (57 ± 5%), alpha-globulins 8% and 7% (3 ± 1.5%), alpha-globulins 12% and 11.9% (9 ± 3%), beta-globulins 13% and 14% (14 ± 3%), and gamma-globulins 24% and 21% (15 ± 4%) for Cases 1 and 2, respectively.

Lipoprotein fractions obtained by preparative ultracentrifugation. After ultracentrifugation, a gelatinous, oily, deep amber-colored layer formed at the top of the serum samples from both cases, in contrast to sera from fasting normal controls. Chemical analysis of the d <1.006 fractions revealed that about 50% of the serum cholesterol and triglycerides and more than 90% of the serum phospholipids were concentrated in this layer (Table 2). Moreover, the VLDL fraction in each case contained at least fivefold more proteins than did the corresponding fraction in normals. When this lipid fraction was electrophoresed and stained for lipoproteins, it was shown to migrate exclusively in the alpha-lipoprotein region.

Insulin-tolerance test. Intravenous administration of insulin (0.1 USP unit/kg body weight) to patient 2 decreased blood glucose from a fasting value of 2.80 g/L to a minimum of 2.48 g/L within 45 min, a 11% decrease; 0.4 USP unit/kg body weight, given intravenously, decreased the blood sugar fasting value of 3.20 g/L to 2.15 g/L by 160 min, a 32% decrease. Results were similar for patient 1.

Immunoelectrophoresis. Immunoelectrophoresis of the sera from the three patients against polyvalent antisera to human serum proteins revealed no abnormality. As expected, patients 1 and 2 had less beta-lipoproteins than normal.

Table 1. Routine Blood Chemistry Data for the Three Cases

<table>
<thead>
<tr>
<th>Variable measured</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (fasting), g/L</td>
<td>3.65</td>
<td>3.85</td>
<td>1.15</td>
</tr>
<tr>
<td>Cholesterol, total, g/L</td>
<td>7.55</td>
<td>5.55</td>
<td>1.89</td>
</tr>
<tr>
<td>Cholesterol esters, g/L</td>
<td>5.03</td>
<td>2.78</td>
<td>2.69</td>
</tr>
<tr>
<td>HDL-cholesterol, g/L</td>
<td>0.13</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Triglycerides, g/L</td>
<td>38.20</td>
<td>36.20</td>
<td>2.38</td>
</tr>
<tr>
<td>Creatinine, mg/L</td>
<td>8.00</td>
<td>7.00</td>
<td>1.20</td>
</tr>
<tr>
<td>Phospholipids, g/L</td>
<td>7.50</td>
<td>5.25</td>
<td>1.20</td>
</tr>
<tr>
<td>Proteins, g/L&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60/40</td>
<td>58/38</td>
<td>73/41</td>
</tr>
<tr>
<td>Bilirubin, mg/L (total/direct)</td>
<td>4/2</td>
<td>16/8</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase, U/L</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Alanine aminotransferase, U/L</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>105</td>
<td>165</td>
<td>150</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>139</td>
<td>115&lt;sup&gt;b&lt;/sup&gt;</td>
<td>160</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>38</td>
<td>32</td>
<td>24</td>
</tr>
<tr>
<td>Leukocytes, no/mm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>9700</td>
<td>4700</td>
<td>1200</td>
</tr>
<tr>
<td>Urinary proteins, g/24 h</td>
<td>0.20</td>
<td>0.40</td>
<td>0.30</td>
</tr>
<tr>
<td>Serum thyroxin, µg/L</td>
<td>110</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>Alpha-antitrypsin, g/L</td>
<td>2.25</td>
<td>1.30</td>
<td>5.00</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values for albumin/globulin ratio in parentheses.

<sup>b</sup>The anemia of Case 2 was further studied by hemoglobin electrophoresis and bone-marrow iron-store examination, and iron-deficiency anemia was documented.

Fig. 4. Cellulose acetate electrophoresograms of serum lipoproteins of Cases 1 and 2 before (left) and after 10-fold dilution of the sera with saline (right), and control (C)

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especially patient 1. In comparison, patient 3 had a normal concentration of beta-lipoproteins (Figure 5, left).

However, when sera from the three patients were immunoelectrophoresed against monospecific anti-human apolipoprotein A antiserum, the main precipitin arcs in Cases 1 and 2 were shorter and less deep than normal. Furthermore, two precipitin arcs, partly fused with the main band, were observed on the cathodic side of the sample well. Patient 3 has a normal major precipitin arc in addition to only one small extra arc on the cathodic side (Figure 5, right).

**Lipid and protein compositions of the VLDL fractions:** Table 3 shows the percentage-by-weight composition of the isolated VLDL fractions. Triglycerides comprise approximately half of the total lipid content of samples from Cases 1 and 2, whereas the cholesterol in these samples is mainly in the free form (about 85%). The protein content is about fourfold that of normal VLDL.

**Identity of the protein of the VLDL fractions.** No evidence for any of the usual apolipoproteins in the VLDL fraction from either sample could be found by double-diffusion. The major protein component detected by sodium dodecyl sulfate–polyacrylamide gel electrophoresis appears to be albumin, along with a minor component with an $M_r$ of about 54,000 (Figure 6). The identity of albumin was confirmed and the $M_r$ 54,000 minor component was identified as alpha-antitrypsin by immunoelectrophoresis and nephelometry.

**Discussion**

The hyperlipoproteinemia syndromes have been divided into primary disorders, in which hyperlipoproteinemia is the principal manifestation of the disease, or secondary disorders related to an underlying disease process.

Among the primary hyperlipoproteinemias, Fredrickson et al. (17) differentiate five phenotypes, none of which has been associated with an abnormal increase in the lipoprotein fraction such as appears in our cases. In our patients, dilution of the serum fraction with isotonic saline did not affect its electrophoretic pattern; hence the lipid–apolipoprotein complex in this fraction appears to be firmly bound. Although on electrophoresis the abnormal component showed alpha mobility, freezing and thawing the pathological serum changed its electrophoretic behavior to more resemble the broad-beta type. Because freezing irreversibly alters the pattern of lipoproteins shown on paper electrophoresis (17), we suspected that we were dealing with an unusual apolipoprotein, as indicated by the apparently high concentration of $\alpha_1$-globulins in serum by protein electrophoresis. Because our patients gave no history of any recent acute inflammatory diseases, which would increase the concentration of the $\alpha_1$-globulins, we concluded that the increased $\alpha_1$-globulin concentrations in these patients most probably represented a primary event. In addition, the other biochemical findings in our cases differ from those of the classical phenotypes. For example, the cholesterol/triglyceride ratios in the isolated VLDLs in Cases 1 and 2 were 0.18 and 0.12, respectively, significantly lower than the ratio reported by Hazzard et al. (19) for normal persons (0.35 ± 0.08 (mean ± SD)) or patients with familial hypercholesterolemia (type IIa) (0.39 ± 0.05), endogenous lipemia (type IV) (0.27 ± 0.06), endogenous lipemia with chylomicrons (type V) (0.25 ± 0.08), or broad-beta disease (type III) (0.60 ± 0.11). Our normal reference value of 0.32 ± 0.09 (n = 5) is very similar to that reported by the above authors.

The unique identity of the VLDL fraction in our cases was demonstrated by the immunological and gel electrophoretic techniques. When we compared the composition of these fractions with that of normal VLDL, only albumin was detected as the major protein component. Furthermore, the lipid composition is also peculiar; the cholesterol in the two fractions, in contrast to normal VLDL, is mainly present in the unesterified form (about 88% vs about 45% for normal VLDL).

The protein and lipid composition of this VLDL is therefore quite different from that of any of the classical lipoproteins. The fact that albumin is the major protein component brings to mind the lipoprotein associated with cholestasis, namely lipoprotein X, but the properties of this unique lipoprotein also differ completely from those of this investi-

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**Fig. 5.** Immunoelectrophoregrams of serum proteins from Cases 1, 2, and 3 vs monospecific antihuman apolipoprotein B antibody (left) and monospecific antihuman apolipoprotein A antibody (right).

In each panel, the normal serum is in the upper well, patient's serum in the lower well.
Table 3. Lipid and Protein Composition of Samples 1 and 2, Normal VLDL, and Lipoprotein X

<table>
<thead>
<tr>
<th>g/kg of VLDL fraction</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Normal (ref. 1)</th>
<th>Lipoprotein X (ref. 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free cholesterol</td>
<td>160</td>
<td>140</td>
<td>40–80</td>
<td>230</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>40</td>
<td>30</td>
<td>160–220</td>
<td>20</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>420</td>
<td>550</td>
<td>450–650</td>
<td>30</td>
</tr>
<tr>
<td>Phospholipids*</td>
<td>140</td>
<td>60</td>
<td>150–200</td>
<td>660</td>
</tr>
<tr>
<td>Protein</td>
<td>240</td>
<td>220</td>
<td>60–100</td>
<td>60</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tr>
</tbody>
</table>

*Phosphatidylcholine + sphingomyelin were assumed to represent 90% of the phospholipids.

In conclusion, we believe that our three patients reported above suffer from a novel familial lipid metabolic disturbance. However, more such cases, with more detailed family studies, are required before the pattern of inheritance of this disorder can be established.

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References


