Lipoproteins in Familial Hyperalphalipoproteinemia

Wolfgang Patsch, Ilmar Kuisk, Charles Glueck, and Gustav Schonfeld

Familial hyperalphalipoproteinemia is determined by a major gene and is characterized by high levels of high density lipoprotein cholesterol and longevity. To describe the plasma lipoproteins in this condition more completely, a kindred consisting of the proband, her affected father, and her two affected brothers was studied. Fasting plasmas were analyzed for lipoprotein lipids by combined preparative ultracentrifugal and precipitation methods. Levels of apolipoprotein A-I and apolipoprotein A-II, the major apoproteins of high density lipoproteins, were assayed by radiolmmunoassay. The flotation properties of very low density, low density and high density lipoprotein were determined by zonal ultracentrifugation, and the isolated high density lipoprotein subfractions were characterized according to their lipid and apoprotein compositions. Total cholesterol of all subjects was normal, but triglycerides were elevated (above the 90th percentile) in the two brothers. High density lipoprotein cholesterol ranged from 150 to 165 mg/dl in the proband and from 72 to 89 mg/dl in her relatives. Apolipoprotein A-I was 318 mg/dl in the proband and 156 to 169 mg/dl in the siblings; respective apolipoprotein A-II values were 67 and 82 to 83 mg/dl. The high density lipoprotein cholesterol and apolipoprotein A-I and A-II values for the relatives are above the 95th percentile for sex and age, while the levels of the proband are the highest recorded in our laboratories. On zonal ultracentrifugation, very low density lipoprotein and low density lipoprotein had normal flotation properties. High density lipoprotein was divisible into three populations, HDL₂, HDL₃, and HDL₄ (S₁₁₀,0,0C₀, S₂, S₉, and S₁, respectively), each of which floated in its usual position. But HDL₃ was greatly elevated, accounting for most of the rise in the high density lipoprotein fraction. The compositions of the high density lipoprotein fractions with respect to the major lipid and apoprotein classes did not differ from normal. Thus, in this kindred, high density lipoprotein was quantitatively rather than qualitatively unusual. It is of interest that hypertriglyceridemia and hyperalphalipoproteinemia coexisted in the siblings. These concurrent elevations differ from the expected reciprocal relationship between high density lipoprotein and very low density lipoprotein levels in plasma, and suggests that in some subjects the two abnormalities may be independently transmitted. (Arteriosclerosis 1981; 1:156–161)
of two major HDL fractions (HDL2, HDL3) isolated from the plasma of five American subjects by preparative ultracentrifugation at fixed density limits may obscure or distort HDL subclasses in cases where flotation properties may be altered.

In contrast to preparative ultracentrifugation, zonal ultracentrifugation11–13 is both an analytic and preparative procedure, which isolates HDL into two major subclasses, HDL1 and HDL2, for further analysis; at the same time, it provides information on their flotation properties. Patsch et al.12 have recently demonstrated that HDL3 is heterogenous in its physicochemical properties and has at least two populations that can be distinguished, designated as HDL3L (S1 21.26°C, 2.9) and HDL3H (S1 21.26°C, 1.7). Although HDL3H is not readily not apparent in normal adult plasma, we believe that HDL3H is of metabolic significance since it may be the only HDL3 species present in some subjects.14,15

Because of the limitations of preparative ultracentrifugation, an adequate description of HDL populations in the plasma of subjects with dyslipoproteinemia should include characterization of HDL subclasses by zonal and/or analytic16 ultracentrifugation. Recently, we had the opportunity to use zonal ultracentrifugation to study the plasma lipoproteins of an individual with newly diagnosed (homozygous) familial hyperalphalipoproteinemia and her two brothers. The lipoprotein lipid and apoprotein levels of the proband, her father, and two brothers also were examined.

Methods

Study Subjects

Table 1 summarizes lipid, lipoprotein, and apolipoprotein levels in the hyperalphalipoproteinemic kindred members including the subject (II-3), her two brothers (II-1 and II-2), and her father (I-2). The mother died from carcinoma of the lung several years before our study. None of the subjects was taking medications. The subject as well as her brothers and father had normal thyroid, hepatic, renal, and endocrine function by laboratory testing and were healthy without a history of chronic disease. All family members abstained from alcohol for religious reasons. Their blood was sampled fasting on their usual ad libitum diets. None of the subjects ate unusual diets (i.e., with altered proportions of fat, carbohydrate, cholesterol, polyunsaturated or saturated fats). Body weights were stable.

Family history revealed that neither the maternal nor paternal grandparents had sustained morbid or lethal myocardial infarction or stroke. In addition, there was no history in the maternal or paternal aunts and/or uncles of morbid or lethal infarction or stroke.

Analytical Methods

After the subjects had fasted for 12 to 14 hours, venous blood samples were drawn into ethylenediaminetetraacetic acid (EDTA) tubes (1 mg/ml). Lipoprotein lipids were determined by the Autoanalyzer II technology on lipoproteins isolated by the combined ultracentrifugal and precipitation techniques described by the Lipid Research Clinics Program.17 Apolipoproteins A-I and A-II were determined by radioimmunoassay.18,19 Zonal ultracentrifugation was performed at 41,000 rpm at 12°C for 24 hours in a Beckman Ti-14 zonal rotor, using an L2-65B ultracentrifuge.11,12 A three-step NaBr gradient spanning the density range 1.00 to 1.40 g/ml was generated by the Beckman model 141 gradient pump, as described previously. The rotor effluent was monitored continuously at 280 nm. Ten ml fractions were collected. Across the HDL area, apo A-I and apo A-II were determined by radioimmunoassay (RIA) in zonal fractions after dialysis against 0.1 M saline, 10 mM NaN3, and 1 mM EDTA, pH 7.6. In addition, appropriate volume fractions were pooled, dialyzed, and concentrated by pressure filtration using a Millipore cell (Millipore Corporation, Bedford, Massachusetts) equipped with a Pellicon membrane.

Table 1. Lipoprotein-Lipid and Apolipoprotein Levels in Hyperalphalipoproteinemia

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>TG</th>
<th>TC</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>Apo A-I</th>
<th>Apo A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-2</td>
<td>72</td>
<td>M</td>
<td>95</td>
<td>192</td>
<td>85</td>
<td>89</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>II-1</td>
<td>53</td>
<td>M</td>
<td>274</td>
<td>239</td>
<td>107</td>
<td>77</td>
<td>0.72</td>
<td>156</td>
</tr>
<tr>
<td>II-2</td>
<td>50</td>
<td>M</td>
<td>354</td>
<td>195</td>
<td>35</td>
<td>89</td>
<td>2.54</td>
<td>169</td>
</tr>
<tr>
<td>Proband II-3</td>
<td>48</td>
<td>F</td>
<td>123</td>
<td>240</td>
<td>65</td>
<td>150</td>
<td>2.31</td>
<td>318</td>
</tr>
</tbody>
</table>

Lipoprotein-lipid and apolipoprotein levels are expressed as mg/dl plasma.

*Values are 5th and 90th percentile levels for indicated age and sex (see ref. 26).

TG = triglycerides; TC = total cholesterol; LDL-C and HDL-C = cholesterol in low density lipoprotein and high density lipoprotein. Apo = apolipoprotein.
(PTGL 04710) and operated at 5 to 12 psi. In the concentrated fractions, free cholesterol, esterified cholesterol, triglyceride, phospholipids, and proteins were determined. The composition of each HDL fraction was calculated from these measurements. The VLDL plus LDL flotation characteristics were also determined by zonal ultracentrifugation at 42,000 rpm, at 15°C, for 140 minutes, using a linear density gradient from 1.00 to 1.3 gm/ml.

Apolipoprotein components of concentrated HDL fractions were separated electrophoretically by polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate (SDS). Gels were stained, destained, and scanned, as described.

**Results**

The proband's total triglyceride and total cholesterol levels were within normal limits (table 1). However, her HDL cholesterol at 150 mg/dl was above the 99th percentile for her age and sex, and was the highest value ever recorded in our laboratory. The HDL-cholesterol levels tended to be low in all, and total triglycerides were high in the two brothers. The apo A-I and apo A-II concentrations in the proband and in her two siblings were high. The value of 318 mg/dl for apo A-I is the highest recorded in our laboratory.

On zonal ultracentrifugation, the VLDL and LDL flotation properties did not differ significantly from those of normolipemic controls (figure 1), but the zonal profile for HDL was striking (figure 2). It showed three distinct populations: HDL2 was present as a sharp symmetrical peak in the usual position for HDL2, but it was grossly increased in quantity; HDL3L, which also floated in the usual position, was present in approximately normal amounts; and HDL3D, not visible in normal plasma, appeared as a shoulder on the trailing edge of HDL3, probably because the amount of HDL3D was somewhat increased.

The apo A-I contents of the zonal fractions (quantified by radioimmunoassay) closely paralleled the absorbance at 280. Relatively, little apo A-II was present in HDL2; instead, the major peak of apo A-II was present in the HDL3L fraction. Therefore, the apo A-I/apo A-II molar ratio was highest in HDL2 (~14), declined to approximately 1.7 in the HDL3L area, and rose to 3 in the HDL3L area. However, there was a distinct peak of apo A-II in the HDL2 region (figure 2), indicating that apo A-II is a genuine component of HDL2 and not merely a cross-contaminating component from the adjacent HDL3. The major HDL peaks were pooled, as indicated in table 2, and subjected to SDS polyacrylamide gel electrophoresis (figure 3). Dye uptake in the apo A-I region represented more than 90% of the total HDL2 dye uptake, about 60% of the HDL3L dye uptake, and 70% of the HDL3D dye uptake, and the molar apo A-I/apo A-II dye uptake area ratios ranged from 18.3 to 1.5 to 4.1 (table 2). These molar ratios are similar to the ones obtained by radioimmunoassay (figure 2 insert).

### Table 2. Apoprotein Compositions of the Major High Density Lipoprotein (HDL) Fractions of the Proband with Hyperalphalipoproteinemia

<table>
<thead>
<tr>
<th>HDL fraction</th>
<th>% Area Apo A-I</th>
<th>% Area Apo D</th>
<th>Apo A-II</th>
<th>A-I/A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL2 (100-160)</td>
<td>93.8</td>
<td>3.2</td>
<td>3.0</td>
<td>18.3</td>
</tr>
<tr>
<td>HDL3 (200-250)</td>
<td>58.6</td>
<td>1.7</td>
<td>24.8</td>
<td>1.5</td>
</tr>
<tr>
<td>HDL3D (290-350)</td>
<td>70.4</td>
<td>19.3</td>
<td>10.4</td>
<td>4.1</td>
</tr>
</tbody>
</table>

HDL fractions were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis, and stained bands were scanned. Two gels were run on the same HDL fraction. Results are mean dye uptake areas as percent of apo A-I + apo A-II + apo D areas (see also figure 3). Numbers in parentheses refer to effluent volumes (see figure 2). The apo A-I/apo A-II molar ratios were obtained by dividing the dye uptake ratios of apo A-I to apo A-II by their molecular weight ratio.
Table 3. Lipid and Protein Compositions of the Major High Density Lipoprotein (HDL) Fractions of the Proband with Hyperalphalipoproteinemia

<table>
<thead>
<tr>
<th>HDL fraction</th>
<th>Protein % mass</th>
<th>PL % mass</th>
<th>TG (mg%)</th>
<th>UC (mg%)</th>
<th>CE (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL2</td>
<td>38</td>
<td>31.8</td>
<td>2.5</td>
<td>5.9</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>(38)</td>
<td>(28)</td>
<td>(8)</td>
<td>(5)</td>
<td>(19.6)</td>
</tr>
<tr>
<td>HDL3L</td>
<td>51.1</td>
<td>25.9</td>
<td>3.1</td>
<td>3.3</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>(47.6)</td>
<td>(28.6)</td>
<td>(5.8)</td>
<td>(3.1)</td>
<td>(18.6)</td>
</tr>
<tr>
<td>HDL3D</td>
<td>56.5</td>
<td>22.9</td>
<td>2.3</td>
<td>2.0</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>(59)</td>
<td>(22.4)</td>
<td>(3.4)</td>
<td>(1.7)</td>
<td>(13.5)</td>
</tr>
</tbody>
</table>

Each HDL fraction was analyzed in triplicate. Mean values for each component were used to calculate percentage compositions. PL = phospholipids; TG = triglycerides; UC = unesterified cholesterol; CE = esterified cholesterol; numbers in parentheses are means for HDL isolated from three normolipidemic individuals.

The proportions of the major lipid moieties and of protein in the HDL fractions are given in Table 3 and are not different from normal values. As expected, the protein contents of HDL2, HDL3L, and HDL3D were 38, 51, and 57% respectively, i.e., inversely related to S, rates. Concomitantly, the relative proportions of cholesterol esters, free cholesterol, and phospholipids decreased.

Zonal profiles of HDL in the two brothers showed an ultracentrifugal profile similar to that of normal subjects. However, the amounts of HDL2 were increased relative to HDL3 when compared with normal subjects (data not shown).

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** The major high density lipoprotein (HDL) subfractions of the proband (-----) and a normal control (----) isolated by zonal ultracentrifugation. In each case, 5 ml of plasma were analyzed. A. HDL2 peaks at ~130 ml, HDL3L at ~240 ml, HDL3D at ~310 ml. The S, (121,26,C) rates of these peaks are 5.9, 2.9, and 1.7 respectively. B. Apo A-I and apo A-II concentrations were determined by radioimmunoassay of the fractions collected at the end of the ultracentrifugation of the proband's plasma, and apo A-I/apo A-II molar ratios were calculated (inset).

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** High density lipoprotein (HDL) apoproteins of the proband separated by sodium dodecyl sulfate gel electrophoresis. Gels a, c, and d correspond to HDL2, HDL3L, and HDL3D respectively. Bands from top to bottom are: apo A-I (very dark band), apo D and apo A-II. The relative dye uptake areas produced by these bands are given in Table 2. Apo A-I and apo A-II have been identified by comparison with purified standards; the band beneath apo A-I had an apparent mw of 22,000 daltons, which we assigned to apo D (see refs. 12 and 33).
Discussion

Our aim was to characterize lipoproteins and apolipoproteins in the proband and in her first degree relatives. There was little doubt about the diagnosis of primary familial hyperalphalipoproteinemia in this subject and her first-degree relatives, all of whom had significant primary elevations of HDL cholesterol, without any causes for secondary hyperalphalipoproteinemia.1–4 The HDL-cholesterol levels were measured in the proband on five separate occasions, and ranged between 150 and 165 mg/dl. The father and one of the brothers were tested on two separate occasions, and each time had elevated HDL-cholesterol levels. The other brother was only tested once, and his HDL cholesterol also was unequivocally elevated. Although the proband’s mother was deceased, the proband’s hyperalphalipoproteinemia was thought possibly to be homozygous, since her father and both brothers also had primary hyperalphalipoproteinemia and the HDL cholesterol levels of the proband were approximately twice those previously observed for obligate heterozygotes.1–4

The striking elevations in HDL cholesterol26 were accompanied by equally striking elevations in apo A-I and apo A-II,27 the major apoproteins of HDL. Interestingly, the proband’s brothers, in addition to these high HDL cholesterol levels, also had high triglycerides and VLDL cholesterol. These concurrent elevations are in contrast to the reciprocal relationship between HDL cholesterol and triglycerides seen in population studies28 and the low HDL cholesterol levels observed in many patients with either endogenous or exogenous hypertriglyceridemia.29

In spite of the low levels of LDL lipids in plasma, on zonal ultracentrifugation, the LDL populations in the plasma of the proband had normal flotation characteristics.11 The results suggest that the proband’s LDL was not unusual in its physical-chemical properties. Similar conclusions may be drawn with respect to VLDL.

Although each HDL class appeared to be normal in its flotation properties and in its lipid and apoprotein composition,12 quantitative alterations were seen in HDL subclasses: HDL2 was grossly elevated and HDL3D was unusually well separated from HDL3L. This is probably due to the increased amounts of HDL2. Increased amount of normally floating HDL2 also were present in the brothers. These results confirm and extend those reported by Mendoza et al.8 as to the normalcy of HDL fractions in subjects with hyperalphalipoproteinemia.

We do not have experimental evidence to explain the mechanism of the increased HDL2 levels in the plasmas of the proband and her relatives. However, a hypothesis has been proposed that lipolysis is of major importance in determining HDL2 levels in hyperalphalipoproteinemia, based on findings that lipoprotein lipase activity is increased in this condition,4 and on in vitro experiments that provided experimental evidence that HDL2-like particles can be generated by lipolysis of VLDL in the presence of HDL3.30 Our findings support this hypothesis.

An alternative possibility involves the action of lecithin-cholesterol acyl transferase (LCAT). We demonstrated that HDL2-like particles could be produced from HDL3 by incubation of normal plasma in the absence of lipoprotein lipase activity.31 This interconversion was increased in the presence of LP-X,32 a source of free cholesterol and phosphatidyl choline, and could be inhibited by p-OH mercuribenzoate, suggesting that HDL3→HDL2 conversion was mediated by LCAT.33 However, LCAT activity was found to be normal in hyperalphalipoproteinemia,7,8 implying that LCAT-mediated conversion of HDL3 to HDL2 may be quantitatively of minor importance in hyperalphalipoproteinemia.

The gross elevation in HDL2 in the proband, qualitatively similar to earlier findings in heterozygous subjects,9 also is consistent with the sharply reduced morbidity and mortality from coronary heart disease in familial hyperalphalipoproteinemia2,5,6,8 HDL2 appears to be the major HDL subclass associated with “protection” from atherosclerosis. An additional factor also potentially related to “protection” from atherosclerosis in families with familial hyperalphalipoproteinemia and certainly manifested in this family are the concurrent low levels of the major “atherogenic” lipoprotein LDL.1,4,10 All four family members had low to exceptionally low LDL cholesterol levels; their HDL cholesterol/LDL cholesterol ratios also were strikingly high.

Although the proband and her father both had relatively low triglyceride levels similar to those previously observed in kindred with familial hyperalphalipoproteinemia,1–6,10 her two brothers had elevations of triglycerides and HDL cholesterol levels. This suggests that in some subjects hypertriglyceridemia and hyperalphalipoproteinemia may be inherited independently.

Acknowledgments

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