Plasma Lipoproteins in Familial Hepatic Lipase Deficiency

Philip W. Connelly, Graham F. Maguire, Maureen Lee, and J. Alick Little

We have studied the lipoproteins, apolipoproteins, and postheparin lipase activities in an extended pedigree with familial hepatic lipase deficiency. A deficiency of hepatic lipase was found in three of five brothers and in one of their children. Triglyceride enrichment of low density and high density lipoproteins was identified as the constitutive phenotype. Very low density lipoprotein was observed in hepatic lipase-deficient subjects, but it was absent when the plasma triglyceride concentration was less than 1 μM/l. The hepatic lipase-deficient subjects had normal or elevated low density lipoprotein cholesterol and high density lipoprotein cholesterol concentrations. Hyperprebetalipoproteinemia, hyperbetalipoproteinemia, and hyperalphalipoproteinemia were observed in both affected and unaffected family members. Compared with the unaffected family members, the hepatic lipase-deficient subjects had no significant differences in very low density lipoprotein cholesterol, very low density lipoprotein triglyceride, or low density lipoprotein cholesterol. These observations are consistent with the presence of additional genes causing hyperlipidemia in this family, independent of the deficiency of hepatic lipase.

Lipoprotein lipase (LPL) and hepatic lipase (HL) are enzymes that are released into the plasma by the intravenous injection of heparin.1 LPL has been established as important in the catabolism of chylomicrons and very low density lipoproteins (VLDL).1 Postheparin plasma LPL activity is negatively correlated with the concentration of VLDL and positively correlated with high density lipoprotein (HDL) cholesterol concentration.2-5 The role of HL in lipoprotein metabolism is only partly understood.1-5 Postheparin plasma HL activity has been reported to be positively correlated with VLDL concentration and negatively correlated with HDL cholesterol concentration.2-5 It is known that in vitro HL will hydrolyze HDL triglyceride and phospholipid.4 Intermediate density lipoproteins (IDL) and low density lipoproteins (LDL) with a density between 1.019 and 1.045 have also been shown to be in vitro substrates of HL.7,8 The inhibition of HL in vivo by infusion of anti-HL antibody into rats9,10,11 and cynomolgous monkeys12 has been found to result in the increase of HDL and IDL. However, there is no agreement on the temporal relationships of these changes and no consistent changes in LDL between species. In the study on the primate model,12 VLDL triglyceride concentrations increased 60% to 300% after infusion of anti-HL antibody. These authors did not report whether the VLDL contained particles of β electrophoretic mobility.

The discovery of familial HL deficiency13 has provided an opportunity to investigate the role of HL in lipoprotein metabolism in humans. The proband and his brother were markedly hypertriglyceridemic, with hyperprebetalipoproteinemia, β-VLDL, normal or elevated concentrations of LDL cholesterol and HDL cholesterol, and elevated concentrations of LDL and HDL triglyceride and phospholipid. Recently another family with HL deficiency and virtually identical qualitative lipoprotein abnormalities has been described.14 The brothers studied in our laboratory have significant ischemic vascular disease and plasma apolipoprotein (apo) B levels approximately twice the normal level,13 in contrast to the Scandinavian subjects,14 who had no apparent ischemic vascular disease and normal levels of plasma apo B. The turnover of apo B has been studied in one of the Scandinavian subjects, and it was found that the conversion of VLDL to LDL was reduced by 50%, and the conversion of IDL to LDL was reduced to 5% of the control values.15 It was also reported that by rate zonal ultracentrifugation there was no LDL16. This is in contrast to the subjects reported by this laboratory in whom LDL was identified in significant quantities by preparative and analytical ultracentrifugation.13 β-VLDL is also a characteristic of dysbetalipoproteinemia.16 β-VLDL are cholesterol-rich remnants of VLDL and chylomicron metabolism that accumulate in dysbetalipoproteinemia because of the presence of the poorly functional form of apo E, apo E2. In contrast to HL deficiency, subjects with dysbetalipoproteinemia have relatively low LDL and HDL cholesterol concentrations16 and normal postheparin lipolytic activity.17 Recent studies on the β-VLDL from Subject B2 have shown that this cholesterol-rich lipoprotein was effective in binding to...
fibroblasts and in stimulation of fibroblast acyl-CoA: cholesterol acyltransferase. This demonstrated that the apoproteins of the β-VLDL in HL deficiency are functional as ligands for the apo B,E receptor.

It is possible that some of the lipoprotein abnormalities observed in the HL deficiency in the family we described are due to the co-inheritance of additional genes for hyperlipidemia. To study the phenotype expression of HL deficiency, we expanded our investigation to additional siblings of the proband, their spouses, and children of the proband and his siblings.

**Methods**

**Plasma Lipid and Lipoproteins**

The plasma lipid and lipoprotein profiles were determined by using the Lipid Research Clinics protocol. Blood samples were obtained from subjects after a 12- to 16-hour fast and were collected into tubes containing Na₂ ethylenediaminetetraacetic acid (EDTA). The total cholesterol and triglycerides of plasma and the lipoprotein fractions were measured by the Technicon AA-II method standardized with the Centers for Disease Control (Atlanta, GA). The plasma was centrifuged with a Beckman 50.3 rotor in an L8-80 ultracentrifuge at 45,000 rpm for 18 hours to isolate the VLDL. The VLDL cholesterol and triglyceride were calculated as the differences between the plasma and d>1.006 g/ml values. The HDL cholesterol and triglyceride were determined on the supernatant fraction (VLDL) and the d>1.006 g/ml fraction was performed by using the Lipid Research Clinics protocol. The plasma lipid and lipoprotein profiles were determined by using the Lipid Research Clinics protocol. The total cholesterol and triglycerides of plasma and the lipoprotein fractions were measured by the Technicon AA-II method standardized with the Centers for Disease Control (Atlanta, GA). The plasma was centrifuged with a Beckman 50.3 rotor in an L8-80 ultracentrifuge at 45,000 rpm for 18 hours to isolate the VLDL. The VLDL cholesterol and triglyceride were calculated as the differences between the plasma and d>1.006 g/ml values. The HDL cholesterol and triglyceride were determined on the supernatant fraction (VLDL) and the d>1.006 g/ml fraction was performed by using the Lipid Research Clinics protocol.

**Agarose Electrophoresis of Lipoproteins**

Agarose electrophoresis of the plasma, d<1.006 g/ml fraction (VLDL) and the d>1.006 g/ml fraction was performed as previously described. β-VLDL was identified as lipoproteins with a d<1.006 g/ml and a β electrophoretic mobility.

**Isoelectric Focusing of Very Low Density Apolipoprotein**

The VLDL were dialyzed against 0.01% Na₂ EDTA, pH 8.2. They were then aliquoted, lyophilized, and delipidated with ethanol/diethyl ether (3:1). The apolipoproteins were separated by isoelectric focusing by using LKB ampholines and a pH gradient from 4 to 8 as previously described.

**Assay of Lipoprotein Lipase and Hepatic Lipase**

LPL and HL were released into the blood by the administration of a bolus of heparin (100 units/kg body weight). The postheparin plasma was collected one-half hour after injection of heparin, and the activity of LPL and HL were determined by using 14C-triolein emulsified with Triton X-100 as a substrate. The total lipolytic activity was assayed in the presence of normal plasma, which served as a source of apo C-II. The activity of HL was determined after inhibition of LPL by pre-incubation with protamine sulfate. LPL activity was taken as the difference between the total lipolytic activity and the HL activity. The linearity of the assay was established for each subject. The activities are expressed as the micromoles of free fatty acid released per milliliter of plasma per hour.

**Heparin-Sepharose Column Chromatography for Separation of Lipoprotein Lipase and Hepatic Lipase**

LPL and HL were separated by heparin-Sepharose chromatography. Heparin-Sepharose CL6B (Pharmacia, Montreal, Canada) was suspended in a buffer consisting of 0.15 M NaCl, 5 mM Nabarbital, and 20% glycerol (pH 7.4) and was packed into a 3 ml disposable syringe to a height of 2.5 cm. A 2 ml aliquot of postheparin plasma was mixed with 2 ml of 0.5 M NaCl, 5 mM Nabarbital, and 40% glycerol (pH 7.4). This was applied to the column, and 1 ml fractions were collected. The plasma was followed by 8 ml of 0.3 M NaCl, 5 mM Nabarbital, and 20% glycerol (pH 7.4). HL was eluted with 8 ml of 0.75 M NaCl, 5 mM Nabarbital, and 20% glycerol (pH 7.4). This was followed by 8 ml of 1.3 M NaCl, 5 mM Nabarbital, and 20% glycerol (pH 7.4) to elute LPL.

Lipase activity was assayed by using Triton X-100 emulsified triolein as described above, with the exception that the free fatty acid released was assayed by using an enzymatic method of detection.

**Assay of Apolipoproteins A-I and B**

Plasma apo A-I and apo B were assayed simultaneously by rocket immunnoassay with an agarose gel containing antibodies to apo A-I and apo B (Gelman, Montreal, Canada).

**Statistical Analyses**

Statistical analyses of the data were performed by using the routines of the SAS statistical package. Before the analysis was performed, each variable was tested to determine whether it was normally distributed. It was found that plasma triglyceride (TG), VLDL cholesterol (VLDL C), VLDL TG, LDL TG, and HDL TG were not normally distributed. Therefore, we tried to normalize the data. Each of these lipids primarily represents the volume of lipoprotein particles, which is represented by:

\[(\frac{4\pi}{3})r^3\]

The test of normality (taking the cube root of these values) was successful. Further statistical analyses of these variables were performed with the transformed data. Comparisons between the HL-deficient subjects and the unaffected subjects were performed by using the analysis of variance for samples of unequal size, including age as a covarant.

**Lipoprotein Phenotype**

The lipoprotein phenotype was determined by using the 95th percentile values and established algorithms, with the exceptions that the α-lipoprotein phenotype was assigned independent of the other lipoprotein fractions, and the 90th percentile values were used to identify hyperalphalipoproteinemia.
The pedigree, including the 20 members of the family examined for this study, is shown in Figure 1. The age and gender of each subject is shown in Table 1.

The procedures followed were in accordance with the ethical standards of the University of Toronto Committee on Human Experimentation.

Subjects

Results

Twenty family members were screened for this study. The proband, B1 (indicated in Figure 1 by the arrow), and his brother, B2, were previously shown to have HL deficiency. Further characterization of the postheparin plasma lipase activity by heparin-Sepharose chromatography was carried out. The proband and B2 had unde-
The lipid and lipoprotein results for each subject are compared with normal ranges. These results for the children of B2 are consistent with inheritance of hyperlipidemia from their father independent of HL deficiency. One child of B3 and L3, C16, had hyperbetalipoproteinemia, hyperalphalipoproteinemia, and HL activity about one-sixth of the mean HL activity of the 17 family members, excluding B1, B2, and B3. One child of B6 and L4, C12, had hyperbetalipoproteinemia, while the second child, C14, was normolipemic.

**Hepatic Lipase Activity and Lipoprotein Composition**

The initial study of B1 and B2 indicated that the characteristic features of their lipemia were the presence of β-VLDL and triglyceride- and phospholipid-rich LDL and HDL. The results of analysis of LDL and HDL triglyceride concentrations are shown in Table 1. The LDL triglyceride and HDL triglyceride concentrations of B1, B2, B3, and C16 were all greater than two standard deviations above the mean values for the other 16 family members. The increased concentration of triglyceride in LDL and HDL did not correlate with the VLDL triglyceride concentration in these subjects (see below).

The presence of β-VLDL was determined qualitatively by agarose electrophoresis of the ultracentrifugally isolated VLDL fraction. Subjects B1, B2, B3, and C16 had a normal HL enzyme activity and hyperbetalipoproteinemia, while B6 had an HL activity of 2.59 and was normolipemic.

Investigation of the spouses showed that only L1, the spouse of B1, had hyperbetalipoproteinemia. Spouses L3, L4, and L6 were normolipemic, while L2 had hyperalphalipoproteinemia. The activities of postheparin HL and LPL and the concentration of apo A-I and apo B and the plasma lipids and lipoproteins in the family members are shown in Table 1. The lipid and lipoprotein results for each subject were classified according to the Lipid Research Clinics Population Study age- and gender-specific percentile ranges. Subjects B1, B2, B3, and C16 had complete inactivity of normal HL The only lipolytic activity to elute from the columns was characteristic for LPL.

### Table 2. Comparison of Plasma Lipid, Lipoprotein, Apolipoprotein, and Lipase Values in Four Hepatic Lipase-deficient and 16 Unaffected Family Members

<table>
<thead>
<tr>
<th></th>
<th>HL-deficient (n=4)</th>
<th>HL-normal (n=16)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL*</td>
<td>0.24±0.47</td>
<td>6.6±2.71</td>
<td>p=0.0004</td>
</tr>
<tr>
<td>LPL</td>
<td>14.20±3.58</td>
<td>9.3±3.12</td>
<td>p=0.022</td>
</tr>
<tr>
<td>Chol (mM/l)</td>
<td>7.03±0.91</td>
<td>6.02±0.87</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mM/l)</td>
<td>4.77±3.22</td>
<td>1.81±1.24</td>
<td>p=0.049</td>
</tr>
<tr>
<td>VLDL C (mM/l)</td>
<td>1.51±1.52</td>
<td>0.59±0.53</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL TG (mM/l)</td>
<td>2.46±2.57</td>
<td>1.20±1.15</td>
<td>NS</td>
</tr>
<tr>
<td>LDL C (mM/l)</td>
<td>3.27±0.98</td>
<td>3.76±0.74</td>
<td>NS</td>
</tr>
<tr>
<td>LDL TG (mM/l)</td>
<td>1.41±0.37</td>
<td>0.44±0.15</td>
<td>p=0.0007</td>
</tr>
<tr>
<td>HDL C (mM/l)</td>
<td>1.69±0.64</td>
<td>1.54±0.43</td>
<td>NS</td>
</tr>
<tr>
<td>HDL TG (mM/l)</td>
<td>0.60±0.08</td>
<td>0.17±0.07</td>
<td>p=0.0003</td>
</tr>
<tr>
<td>Apo A-I (g/l)</td>
<td>1.91±0.22</td>
<td>1.65±0.20</td>
<td>p=0.024</td>
</tr>
<tr>
<td>Apo B (g/l)</td>
<td>1.70±0.26</td>
<td>1.31±0.26</td>
<td>NS</td>
</tr>
</tbody>
</table>

*The lipase activities are expressed as micromoles of free fatty acid released/milliliter of plasma/hour. NS=not significant, C=cholesterol. See Table 1 legend for an explanation of the other abbreviations.

Hyperlipidemia was found in children from each of the family units studied. The children of the proband (C2, C3, C4) had various combinations of hyperbeta-, hyperbetalipoproteinemia. The children of B2 and L2 had either a normal lipoprotein phenotype (C10), hyperbetalipoproteinemia (C9), or hyperalphalipoproteinemia (C5 and C6). Thus, hyperlipoproteinemia in these children was present along with HL activity within the normal range. These results for the children of B2 are consistent with inheritance of hyperlipidemia from their father independent of HL deficiency. One child of B3 and L3, C16, had hyperbetalipoproteinemia, hyperalphalipoproteinemia, and HL activity about one-sixth of the mean HL activity of the 17 family members, excluding B1, B2, and B3. One child of B6 and L4, C12, had hyperbetalipoproteinemia, while the second child, C14, was normolipemic.

**Apolipoprotein E Phenotype of Family Members**

Since β-VLDL is characteristic of patients with Type III hyperlipoproteinemia who are homozygous for apo E2, the apo E phenotype of each subject was determined. Patients B1, B2, B3, and C16 had an E3/E3 phenotype.
Comparison of Family Members with or without Hepatic Lipase Deficiency

The family members were divided into those with HL deficiency and those without HL deficiency. The HL-deficient group consisted of four subjects, B1, B2, B3, and C16, with the group without HL deficiency consisting of the remaining 16 family members tested at this screening. The group without HL deficiency consisted of younger individuals and more female subjects relative to the HL-deficient group. It was necessary to use an analysis of variance, with the data grouped according to gender and with age as a covariant, to determine the significant differences independent of the variations due to age and gender. The mean and standard deviation of each measured parameter is shown for each group in Table 2. In the HL-deficient group, besides a significantly lower HL activity, the LDL triglyceride and HDL triglyceride concentrations were significantly higher. In addition, the HL-deficient group had higher mean LPL activity and apo A-I concentrations. However, VLDL cholesterol and triglyceride, LDL cholesterol, and HDL cholesterol were similar in the two groups.

Correlation Analysis of Data for Hepatic Lipase-deficient Subjects

The concentrations of lipoproteins and apoproteins in plasma reflects the balance between anabolic and catabolic processes. The analysis of correlations between lipoproteins, apoproteins, and lipases is one possible means of detecting potential mechanistic relationships among these factors.

The correlation matrix of the data for the four HL-deficient subjects is presented in Table 3. The correlation of HL activity was not tested, since three of the four values were 0, which did not allow statistical comparisons to be made. LPL activity was negatively correlated with VLDL triglyceride and VLDL cholesterol ($r = -0.96$, $p = 0.037$ and $r = -0.97$, $p = 0.028$, respectively). LPL activity also correlated positively with HDL cholesterol ($r = 0.99$, $p = 0.006$), apo A-I ($r = 0.968$, $p = 0.032$), and LDL cholesterol ($r = 0.986$, $p = 0.014$). HDL cholesterol, in addition to the correlation with LPL, was positively correlated with apo A-I ($r = 0.983$, $p = 0.017$) and LDL cholesterol ($r = 0.977$, $p = 0.028$). HDL cholesterol was negatively correlated with VLDL cholesterol ($r = -0.95$, $p = 0.05$) and VLDL triglyceride ($r = -0.935$, $p = 0.065$). LDL cholesterol, in addition to the correlation with LPL and HDL cholesterol, was positively correlated with apo A-I ($r = 0.968$, $p = 0.034$) and negatively correlated with VLDL cholesterol ($r = -0.939$, $p = 0.061$). The negative correlation of apo A-I with VLDL cholesterol and VLDL triglyceride did not reach significance.

The correlations among VLDL, HDL, apo A-I, and LPL were investigated further by a stepwise multiple regression analysis. HDL cholesterol was chosen as the dependent variable since it represents primarily the cholesteryl esters formed in plasma by the action of lecithin: cholesterol acyltransferase and reflects the steady state between this process and transfer of cholesteryl esters to tissues and other lipoproteins. Regression analysis of the data for the four HL-deficient subjects (Table 4) showed that LPL activity accounted for 99% of the variability in HDL cholesterol and that when LPL was in the model, apo A-I did not meet the 0.10 significance level. Apo A-I without LPL in the model accounted for 97% of the variability in HDL cholesterol.

Lipoproteins, Lipases, and Apoproteins of Unaffected Children

A total of nine children were found to be unaffected according to the criteria chosen to identify HL deficiency. These included four female and five male subjects. The female and male children were compared for differences in age, lipoprotein concentration, lipase activities, or apoprotein concentrations (Table 5). Significant differences were found in the concentration of LDL cholesterol

<p>| Table 3. Correlation Coefficients for Four Hepatic Lipase-deficient Family Members |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>VLDL TG</th>
<th>VLDL C</th>
<th>LDL TG</th>
<th>LDL C</th>
<th>HDL TG</th>
<th>HDL C</th>
<th>Apo A-I</th>
<th>Apo B</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL TG</td>
<td>0.999</td>
<td>0.589</td>
<td>-0.934</td>
<td>0.654</td>
<td>-0.935</td>
<td>-0.865</td>
<td>0.866</td>
<td>-0.963</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>NS</td>
<td>0.066</td>
<td>NS</td>
<td>0.065</td>
<td>NS</td>
<td>0.037</td>
<td>0.037</td>
</tr>
<tr>
<td>VLDL C</td>
<td>-0.578</td>
<td>-0.939</td>
<td>0.621</td>
<td>-0.950</td>
<td>-0.883</td>
<td>0.878</td>
<td>-0.972</td>
<td>-0.972</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>0.061</td>
<td>NS</td>
<td>0.050</td>
<td>NS</td>
<td>0.028</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LDL TG</td>
<td>-0.263</td>
<td>0.859</td>
<td>-0.342</td>
<td>-0.167</td>
<td>0.803</td>
<td>-0.380</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LDL C</td>
<td>-0.389</td>
<td>0.972</td>
<td>0.966</td>
<td>0.890</td>
<td>0.986</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>0.028</td>
<td>0.034</td>
<td>NS</td>
<td>0.014</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HDL TG</td>
<td>-0.344</td>
<td>-0.188</td>
<td>0.614</td>
<td>0.429</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>-0.034</td>
<td>NS</td>
<td>0.983</td>
<td>0.994</td>
<td>NS</td>
<td>0.066</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HDL C</td>
<td>0.017</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>-0.688</td>
<td>0.968</td>
<td>NS</td>
<td>0.032</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>-0.797</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LPL</td>
<td>-0.688</td>
<td>0.968</td>
<td>NS</td>
<td>0.032</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

See the Table 1 and Table 2 legends for explanations of the abbreviations.
Table 4. Multiple Regression Analysis of Dependent Variable, High Density Lipoprotein Cholesterol

<table>
<thead>
<tr>
<th>Group*</th>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>Partial r</th>
<th>Model r</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>LPL</td>
<td>0.177</td>
<td>0.014</td>
<td>0.987</td>
<td>0.987</td>
<td>153.9</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Constant</td>
<td>-0.825</td>
<td>0.207</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Apo A-I</td>
<td>2.87</td>
<td>0.375</td>
<td>0.967</td>
<td>0.967</td>
<td>58.9</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Constant</td>
<td>-3.80</td>
<td>0.719</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>VLDL TG</td>
<td>-0.602</td>
<td>0.029</td>
<td>0.953</td>
<td>1.000</td>
<td>3653.7</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>0.078</td>
<td>0.004</td>
<td>0.047</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Constant</td>
<td>1.553</td>
<td>0.057</td>
<td></td>
<td>1.000</td>
<td>3653.7</td>
<td>0.012</td>
</tr>
<tr>
<td>B</td>
<td>Apo A-I</td>
<td>1.746</td>
<td>0.156</td>
<td>0.057</td>
<td>0.999</td>
<td>1089.2</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Constant</td>
<td>-2.020</td>
<td>0.220</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Apo A-I</td>
<td>1.296</td>
<td>0.061</td>
<td>0.954</td>
<td>0.998</td>
<td>453.6</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>VLDL C</td>
<td>-0.255</td>
<td>0.040</td>
<td>0.044</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Constant</td>
<td>-0.444</td>
<td>0.115</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Group identification: A=the four hepatic lipase-deficient subjects, B=the four unaffected female children, C=the five unaffected male children.
SE=standard error. See the Table 1 legend for an explanation of the other abbreviations.

Table 5. Comparison of Plasma Lipid, Lipoprotein, Apolipoprotein, and Lipase Values between the Unaffected Female and Male Children

<table>
<thead>
<tr>
<th></th>
<th>Female (n=4)</th>
<th>Male (n=5)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL*</td>
<td>4.6±2.76</td>
<td>8.5±2.87</td>
<td>p=0.072</td>
</tr>
<tr>
<td>LPL</td>
<td>7.8±3.19</td>
<td>9.2±2.87</td>
<td>NS</td>
</tr>
<tr>
<td>Chol (mM/l)</td>
<td>5.22±0.84</td>
<td>6.36±0.76</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mM/l)</td>
<td>1.63±1.67</td>
<td>1.92±1.14</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL C (mM/l)</td>
<td>0.57±0.80</td>
<td>0.76±0.59</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL TG (mM/l)</td>
<td>1.15±1.89</td>
<td>1.19±0.78</td>
<td>NS</td>
</tr>
<tr>
<td>LDL C (mM/l)</td>
<td>3.02±0.51</td>
<td>4.29±0.65</td>
<td>p=0.016</td>
</tr>
<tr>
<td>LDL TG (mM/l)</td>
<td>0.42±0.13</td>
<td>0.44±0.15</td>
<td>NS</td>
</tr>
<tr>
<td>HDL C (mM/l)</td>
<td>1.63±0.51</td>
<td>1.31±0.27</td>
<td>NS</td>
</tr>
<tr>
<td>HDL TG (mM/l)</td>
<td>0.17±0.05</td>
<td>0.14±0.06</td>
<td>NS</td>
</tr>
<tr>
<td>Apo A-I (g/l)</td>
<td>1.71±0.15</td>
<td>1.52±0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Apo B (g/l)</td>
<td>1.10±0.34</td>
<td>1.42±0.15</td>
<td>p=0.047</td>
</tr>
<tr>
<td>Age</td>
<td>31.2±6.6</td>
<td>28.8±4.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

*The lipase activities are expressed as micromoles free fatty acid released/milliliter plasma/hour. NS=not significant. See the Table 1 legend for an explanation of other abbreviations.

(p=0.015) and HL activity (p=0.072). Lower LDL cholesterol levels and HL activity in women compared with men has been reported in other populations. Higher HDL triglyceride levels in women compared with men has been observed by others, but was not seen in this relatively small group.

Correlation Analysis of Data for Unaffected Children

The correlation matrix for the four unaffected female children is shown in Table 6. HL was positively correlated with VLDL triglyceride (r=0.992, p=0.008), VLDL cholesterol (r=0.995, p=0.005), LDL triglyceride (r=0.986, p=0.014), HDL triglyceride (r=0.95, p=0.05), and apo B (r=0.98, p=0.02). HL was negatively correlated with HDL cholesterol (r=-0.96, p=0.04), and apo A-I (r=-0.999, p=0.001). LDL triglyceride, in addition to the correlation with HL, was positively correlated with VLDL triglyceride (r=0.988, p=0.012), VLDL cholesterol (r=0.989, p=0.011), HDL triglyceride (r=0.985, p=0.015), and apo B (r=0.996, p=0.004). LDL triglyceride was negatively correlated with apo A-I (r=-0.980, p=0.019). HDL triglycer-
Table 6. Correlation Coefficients for Four Unaffected Female Children

<table>
<thead>
<tr>
<th></th>
<th>VLDL TG</th>
<th>VLDL C</th>
<th>LDL TG</th>
<th>LDL C</th>
<th>HDL TG</th>
<th>HDL C</th>
<th>Apo A-I</th>
<th>Apo B</th>
<th>LPL</th>
<th>HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL TG</td>
<td>1.000</td>
<td>0.988</td>
<td>0.717</td>
<td>0.951</td>
<td>-0.950</td>
<td>-0.991</td>
<td>0.972</td>
<td>-0.859</td>
<td>0.992</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.012</td>
<td>NS</td>
<td>0.049</td>
<td>0.050</td>
<td>0.009</td>
<td>0.028</td>
<td>NS</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>VLDL C</td>
<td>-0.989</td>
<td>0.721</td>
<td>0.955</td>
<td>-0.952</td>
<td>-0.994</td>
<td>0.975</td>
<td>-0.880</td>
<td>0.995</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.011</td>
<td>0.045</td>
<td>0.048</td>
<td>0.006</td>
<td>0.025</td>
<td>0.005</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL TG</td>
<td>-0.814</td>
<td>0.985</td>
<td>-0.903</td>
<td>-0.980</td>
<td>0.996</td>
<td>-0.779</td>
<td>0.987</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>0.015</td>
<td>0.097</td>
<td>0.019</td>
<td>0.004</td>
<td>0.013</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL C</td>
<td>-0.874</td>
<td>-0.497</td>
<td>-0.699</td>
<td>0.850</td>
<td>-0.278</td>
<td>0.729</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL TG</td>
<td>-0.854</td>
<td>-0.956</td>
<td>0.996</td>
<td>0.704</td>
<td>0.968</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>0.044</td>
<td>0.004</td>
<td>NS</td>
<td>0.032</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL C</td>
<td>0.968</td>
<td>-0.879</td>
<td>0.971</td>
<td>-0.956</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.032</td>
<td>NS</td>
<td>0.029</td>
<td>0.044</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo A-I</td>
<td>-0.971</td>
<td>0.880</td>
<td>-0.999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.029</td>
<td>NS</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo B</td>
<td>-0.741</td>
<td>0.980</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>0.020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>-0.859</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See the Table 1 legend for an explanation of the abbreviations.

The correlation coefficients were different for the five unaffected male children. The only correlations that reached statistical significance were between HDL cholesterol and plasma apo A-I (r = 0.978, p = 0.0042); plasma cholesterol and apo B (r = 0.933, p = 0.021); and VLDL cholesterol and VLDL triglyceride (r = 0.964, p = 0.008). The correlations of LDL cholesterol and plasma apo B (r = 0.818, p = 0.091); of age and HDL activity (r = -0.729, p = 0.162); of VLDL cholesterol and HDL cholesterol (r = -0.704, p = 0.185); of HDL and LDL TG (r = -0.809, p = 0.097); and of LPL and HDL TG (r = -0.864, p = 0.059) were the only other correlations with significance levels below 0.20.

Discussion

The current study extends the previous observation of two subjects with HL deficiency to other family members. The presence of HL deficiency in three of five brothers and in one of their children confirms the familial nature of this syndrome. Hypertriglyceridemia of both LDL and HDL is the constitutive lipoprotein phenotype of HL deficiency.

The parents of the proband and his siblings were not available at the time of this investigation. A previous investigation of the mother (A1) at 82 years of age showed HL activity of 2.6 μmol of free fatty acid released/milliliter of plasma/hour. We do not have a reference range for HL activity of 82-year-old women; however, the value is below the mean that was obtained in this study for the four female children. The heritability of HL activity has been investigated by Kuusi et al.28 In a study of monozygotic and dizygotic male twins with ages between 48 and 63 years, they reported that the influence of heredity was detectable for postheparin HL activity but not for postheparin LPL activity. The presence of the HL-deficient phenotype in C16 suggests a complex mechanism as the underlying cause of deficiency. However, it should be noted that because variation in age and gender has a significant effect upon postheparin HL activity, the comparison of the activities obtained for parents and their children may be complicated.

Apparently, HL deficiency does not result in a constitutive hyperbetalipoproteinemia. However, the present study suggests that HL deficiency associated with other genetic and acquired factors may make individuals more likely to develop hyperbetalipoproteinemia and hyperbetalipoproteinemia.

The presence of β-VLDL was found to be a variable feature of HL deficiency. It was absent at times (B3), and its presence was related to the concentration of VLDL. We speculate that this may be related to diet, obesity, and energy balance. The presence of β-VLDL in the absence of HL activity demonstrates that LPL activity alone is sufficient for the formation of β-VLDL. Rubinstein et al.29 noted differences in the distribution of apo E among the lipoproteins of B1 and B2 after heparin-induced lipolysis.
This may have been due to the presence of different amounts of β-VLDL in these two patients. Further studies by Gibson and Brown have added support to the hypothesis that hydrolysis of VLDL lipids by HL is important for the transfer of apo E to HDL.

Schonfeld et al. have shown that treatment of subclasses of VLDL with lipoprotein lipase increased the expression of apo B-dependent binding to fibroblast apo B,E receptors. Recently, Aviram et al. have shown that modification of triglyceride-rich LDL by HL enhanced degradation of LDL by fibroblast apo B,E receptors. Thus, HL may be important in vivo for the expression of apo B-dependent binding of LDL to cell surface lipoprotein receptors and in determining the ligand for the apo B,E receptor-dependent clearance of apo B-containing particles. A rate-limiting role of HL in the conversion of VLDL to IDL and LDL was not observed in subjects with detectable HL activity, and this role is probably specific to the rare cases of HL deficiency.

HL-deficient subjects have normal or elevated levels of LDL and HDL cholesterol. Because of the significant amount of triglyceride in these lipoproteins, a measurement of cholesterol underestimates their mass. LDL triglyceride and HDL triglyceride were not correlated with the cholesterol concentration of their respective lipoprotein fractions in either the HL-deficient subjects or the unaffected female children. This suggests that LDL and HDL triglyceride represents metabolically distinct subsets of these lipoprotein classes.

The HL-deficient subjects had normal or elevated LPL activity and normal or elevated LDL and HDL cholesterol. Correlation and multiple regression analyses demonstrated the significance of LPL in determining the concentration of HDL cholesterol and LDL cholesterol in the HL-deficient subjects. In the absence of HL, the positive relationship of LPL and HDL cholesterol was more obvious than previously noted in normal subjects. The positive relationship of LPL and LDL cholesterol is unique. The current analysis groups IDL (density between 1.006 g/ml and 1.019 g/ml) and LDL (1.019 g/ml to 1.063 g/ml) as LDL. We have analyzed the quantity of the VLDL, LDL, and HDL using either a fixed-angle rotor or a swinging-bucket rotor, and we find that a significant proportion of the lipid mass is in the LDL density range (data not shown).

Together, these observations indicate that there is a formation of LDL (i.e., lipoproteins with a density between 1.019 g/ml and 1.063 g/ml) in HL deficiency. This is consistent with the relative activity of LPL and HL toward VLDL in vitro and in vivo. There would appear to be differences between the family studied here and the subject investigated by Demant et al., not the least of which is the presence in this family of hyperlipidemia independent of HL deficiency.

Comparison of the lipoprotein, apolipoprotein, and lipase values between the HL-deficient subjects and the unaffected members of the family provided some interesting differences. The LPL activity was significantly higher in the HL-deficient subjects than in the remainder of the family. This could be a result of some primary defect in the regulation of the expression of LPL and HL activity, or it may be an adaptation secondary to the primary deficiency of HL.

HL activity in the four unaffected female children correlated significantly with each lipid and apolipoprotein parameter except LDL cholesterol. The positive correlation of HL with LDL triglyceride and HDL triglyceride was the opposite of the relationship expected between the enzyme and its substrate. This suggests that the relationship between HL and its substrate lipoproteins is only apparent when HL activity is very low. The negative correlation of HL with HDL cholesterol and apo A-I suggested a causal relationship. However, the results of the multiple regression analysis with HDL cholesterol as the dependent variable showed that VLDL triglyceride and LPL accounted for 100% of the variability in HDL cholesterol. Either VLDL cholesterol or HL activity could be substituted for VLDL triglyceride. The strong relationship between HDL cholesterol and LPL activity suggests that LPL is causally related to the hyperalphalipoproteinemia that is present in this family.

Our results indicate a highly significant association between VLDL cholesterol and VLDL triglyceride. As a result, HL activity was significantly correlated with both of these values in the female children studied. This contrasted with the results for the male children, for whom no significant correlation was found between HL activity and VLDL. Applebaum-Bowden et al. also observed a significant association of HL activity and VLDL triglyceride in women but not in men. However, they did not observe a significant association of VLDL cholesterol with HL activity. These investigators also observed a significant negative association between age and HL activity in men but not in women. A similar correlation was seen in the present data set, but it did not reach statistical significance. This was undoubtedly due in part to the small number of persons in the present study. However, the possible age-dependent decrease in HL activity in men may affect the phenotypic expression of HL deficiency. The proband and his HL-deficient brothers were first investigated at the ages of 36 and 39 years. It is possible that had investigation of these persons been carried out at an earlier age, HL activity may have been detectable.

Hyperprebetalipoproteinemia, hyperbetalipoproteinemia, and hyperalphalipoproteinemia were observed in both affected and unaffected family members. Compared with the unaffected family members, the HL-deficient subjects had no significant differences in VLDL cholesterol, VLDL triglyceride, or LDL cholesterol. These observations are consistent with the presence of additional genes causing hyperlipidemia in this family independent of the deficiency of HL.

Premature ischemic vascular disease was present in the proband and his affected brothers in the presence of hyperalphalipoproteinemia and significant disturbances in the metabolism of all the lipoproteins.

It was not possible to decide whether the characteristic lipoproteins of HL deficiency (the triglyceriderich LDL and HDL and the β-VLDL), or whether the hyperalphalipoproteinemia that was unrelated to HL deficiency, was atherogenic in this family. A follow-up of these subjects might provide an answer to this question.
REFERENCES


Index Terms: hepatic lipase deficiency • lipoprotein lipase • β-very low density lipoproteins • triglyceride-rich low density lipoproteins • triglyceride-rich high density lipoproteins
Plasma lipoproteins in familial hepatic lipase deficiency.
P W Connelly, G F Maguire, M Lee and J A Little

doi: 10.1161/01.ATV.10.1.40

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/10/1/40

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/