Large Buoyant LDL-like Particles in Hepatic Lipase Deficiency

Johan H. Auwerx, Carol A. Marzetta, John E. Hokanson, and John D. Brunzell

Hepatic lipase (HL) is thought to play a role in processing very low density lipoprotein to low density lipoprotein (LDL). To analyze the relationship between HL and LDL, the density, size, and chemical composition of LDL isolated from 18 normal subjects and from three subjects with reduced or absent levels of HL activity were compared. In an HL-deficient subject, the major peak of apoprotein (apo) B-containing lipoproteins ('LDL') had a density of 1.023 g/ml and a diameter of 26.4 nm compared to male control subjects (1.044±0.006 g/ml and 25.3±0.3 nm). Two half-sisters of the HL-deficient subject with half the normal levels of HL activity had LDL that also were more buoyant and slightly larger than the LDL isolated from female control subjects. The peak density and average diameter of LDL were correlated with HL activity, consistent with the hypothesis that HL influenced formation and physical characteristics of typical LDL. Apo B-100 was the major apoprotein in the 'LDL' isolated from the HL-deficient subject and contained a greater proportion of triglyceride compared to the control subjects' LDL. The absence of HL appears to prevent the production of classical LDL. Our data support the hypothesis that HL helps determine normal LDL characteristics.


The formation of low density lipoproteins (LDL) involves both triglyceride (TG) hydrolysis with remodeling of very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL), as well as potential direct hepatic production of LDL particles (see reviews). A complicated picture of a 'delipidation cascade' is emerging in which the factors that determine whether VLDL will be removed from the circulation or undergo conversion into LDL is just being delineated. Several intravascular enzymes are thought to be involved in the formation of LDL, including lipoprotein lipase (LPL), lipid transfer protein, and hepatic triglyceride lipase (HL). Rubinstein et al. have hypothesized that HL is involved in the conversion of IDL to LDL; however, direct experimental data describing the mechanisms involved in this conversion are, as yet, undefined.

The conversion of VLDL remnants or IDL to LDL is thought to occur across the splanchnic bed where HL is located. Support for a role of HL among the factors involved in the processing of IDL to LDL has been derived from observations made on humans and animals with HL deficiency; humans with diseases associated with reduced levels of HL activity (hypothyroidism, uremia, chronic liver disease); and in experimentally induced HL deficiency in rats and monkeys. In these studies, reduced levels of HL were associated with an increase in IDL concentration and TG accumulation in LDL.

The present study was designed to examine the possible relationships between HL activity and LDL physical characteristics in normolipidemic subjects and in subjects with reduced or absent HL activity levels.

Methods

Study Subjects

Eighteen healthy Caucasian subjects (nine women and nine men), with a mean age of 35.4±11.3 years for women and 26.9±5.6 years for men (range, 18 to 48 years), were recruited for this study, and they participated on a voluntary basis. None of these subjects took any medication or had evidence of a lipoprotein disorder. All patients belonged to a single family with HL deficiency (Auwerx et al., unpublished data). The proband (PG) is a 50-year-old man with complete absence of HL activity in postheparin plasma. He was detected as having hypertriglyceridemia and was subsequently characterized as having b-VLDL and an E3/E3 phenotype. On all examinations, this man had no detectable HL activity in postheparin plasma. Two half-sisters, IG and JG, were also studied and had approximately half the normal levels of HL activity. The HL-deficient subject (PG) had been taking clofibrate since 1966 (which resulted in a drop of his TG from the 2000 to 4000 mg/dl range to less than 1000 mg/dl).
mg/dl, while IG and JG were not on lipid-lowering drugs or other medications that affect lipid metabolism.

Blood Collection

After an overnight fast, a blood sample was drawn into 0.1% disodium ethylenediaminetetraacetic acid. An intravenous heparin bolus of 60 IU/kg was then administered, and blood was collected in lithium-heparin tubes. Plasma was separated immediately by centrifugation and stored at either 4°C until lipoproteins were analyzed (started on the same day) or at -70°C until lipase activities were determined.

Density Gradient Ultracentrifugation

Discontinuous salt gradients were prepared in SW-41 ultracentrifugation tubes (Beckman Instruments, Palo Alto, CA) by underlayering 5.2 ml of saline (1.006 g/ml), 4 ml of a 1.006 g/ml solution, and 2.5 ml of plasma adjusted to a density of 1.21 g/ml as described. After centrifugation (15°C for 24 hours at 41,000 rpm), the samples were eluted from the bottom of the tube by pumping 1.85 g/ml of a dense solution (Fluorinert, 3M Company, St. Paul, MN) into the bottom of the tube at a rate of 0.8 ml/min. The effluent was monitored continuously at 280 nm and monitored by flow cell spectrophotometer (LKB Productor, Bromma, Sweden); 38 fractions of 0.31 ml each were collected for each sample. The density of every other fraction was measured at room temperature by pycnometry. The fractions selected to contain the material defining the major peak of LDL were pooled after density gradient ultracentrifugation (DGUC). The density ranges defining these fractions varied among the subjects and were based on the elution profiles of each LDL sample to ensure that the pooled samples contained the entire lipoprotein peak.

Single Vertical Spin for Apo lipoprotein B Containing Particles

This technique is a modification of the single vertical spin density ultracentrifugation developed by Chung et al., that optimizes the resolution of apo B-containing lipoproteins (SVS-apo B). A discontinuous salt gradient was formed in Sorvall TV-865B (Du Pont Company, Wilmington, DE) tubes by underlayering 5 ml of plasma adjusted to a density of 1.080 g/ml underneath 12 ml of a 1.006 g/ml NaCl solution. Samples were centrifuged at 65,000 rpm for 90 minutes at 10°C (total ω² of 2.36×10⁴) and then were fractionated from the bottom of the tube (flow rate 1.7 ml/min). Thirty-eight fractions were collected (0.45 ml/tube), and the total cholesterol was measured in each fraction. The buoyancy of LDL was characterized by its relative flotation number (RI) obtained by dividing the fraction number containing the LDL peak by the total number of fractions collected.

Sequential Flotation Procedures

Lipoproteins of d <1.063, 1.006 to 1.019 g/ml, or 1.019 to 1.063 g/ml were prepared by sequential ultracentrifugation in a 40.3 rotor (Beckman Instruments, Palo Alto, CA).

Nondenaturing Gradient Gel Electrophoresis

Four micrograms of protein from each d <1.063 g/ml lipoprotein sample were applied to alternate wells on 2% to 16% polyacrylamide gradient gels (Pharmacia, Uppsala, Sweden) and electrophoresed as described. Latex beads (Duke Scientific, Palo Alto, CA), thyroglobulin, and ferritin (Pharmacia) with diameters of 38.0, 17.0, and 12.2 nm, respectively, were run as standards. Each lane was scanned at 555 nm after staining for protein with Coomassie blue. The diameter of each LDL was determined by using a calibration curve generated from the standards.

Sodium Dodecyl Sulfate Polyacrylamide Gradient Gel Electrophoresis

Apoproteins from LDL isolated by DGUC were electrophoresed on 4% to 30% sodium dodecyl sulfate polyacryl- amide gradient gel electrophoresis (SDS-PAGE) as described previously. Apoproteins isolated from d <0.066 g/ml lipoproteins from a patient with LPL deficiency were run as standards.

Lipoprotein and Hepatic Triglyceride Lipase Activity

LPL and HL activity were measured in postheparin plasma by a method described previously. Aliquots of postheparin plasma were incubated with the substrate containing trioleoylglycerol, glycerol-tri-[1-14C]oleate (Amersham, Arlington Heights, IL), and lecithin for 60 minutes at 37°C. Enzyme activity is expressed as nanomoles of free fatty acids (FFA) released per minute per milliliter of plasma at 37°C. LPL activity was calculated as the lipolytic activity removed from plasma by incubation with a specific monoclonal antibody against LPL, and HL activity was determined as the activity remaining after incubation with the LPL antibody. For each assay, a lipase standard was included to correct for interassay variation, which averaged 7.2%.

In Vitro Incubation of Low Density Lipoprotein with Hepatic Lipase

The major peak of apo B-containing particles was isolated from the subject with HL deficiency (PG) and from a normal control by DGUC. Postheparin plasma from an LPL-deficient patient was obtained as a source of HL. The LPL-deficient patient had an LDL cholesterol of 19 mg/dl with an LDL peak density of 1.055 g/ml as determined by DGUC. Chylomicrons from the postheparin plasma were first removed by ultracentrifugation at 50,000 rpm at 4°C in a 60 Ti rotor for 60 minutes. The remaining plasma was used immediately in the incubation studies to minimize loss of HL activity. Two milliliters of postheparin plasma without chylomicrons from the LPL-deficient patient were used immediately in the incubation studies to minimize loss of HL activity. Two milliliters of postheparin plasma without chylomicrons from the LPL-deficient patient were used immediately in the incubation studies to minimize loss of HL activity. Two milliliters of postheparin plasma without chylomicrons from the LPL-deficient patient were used immediately in the incubation studies to minimize loss of HL activity.
Table 1. Lipid and Lipoprotein Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (yrs)</th>
<th>Total Chol (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-Chol (mg/dl)</th>
<th>LDL-Chol (mg/dl)</th>
<th>Apo B (g/ml)</th>
<th>HL activity (nmol FFA/min/ml)</th>
<th>LPL activity (nmol FFA/min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men (n=9)</td>
<td>26.9±5.6</td>
<td>161±42</td>
<td>71±24</td>
<td>51.10</td>
<td>103±26</td>
<td>88±22</td>
<td>147±53</td>
<td>156±62</td>
</tr>
<tr>
<td>Women (n=9)</td>
<td>35.4±11.3</td>
<td>175±20</td>
<td>56±19</td>
<td>64±15</td>
<td>102±14</td>
<td>84±19</td>
<td>85±51*</td>
<td>210±91*</td>
</tr>
<tr>
<td>PG</td>
<td>50</td>
<td>196</td>
<td>389</td>
<td>42</td>
<td>107</td>
<td>177</td>
<td>0</td>
<td>102</td>
</tr>
<tr>
<td>IG</td>
<td>42</td>
<td>183</td>
<td>66</td>
<td>101</td>
<td>72</td>
<td>62</td>
<td>39</td>
<td>261</td>
</tr>
<tr>
<td>JG</td>
<td>43</td>
<td>166</td>
<td>111</td>
<td>55</td>
<td>90</td>
<td>97</td>
<td>31</td>
<td>126</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD.

Chol=cholesterol, TG=triglyceride, LDL-Chol=LDL cholesterol, HDL-Chol=HDL cholesterol, HL=hepatic lipase, LPL=lipoprotein lipase.

*Statistically different from the control men, p<0.001.

Table 2. Low Density Lipoprotein Composition and Characteristics

<table>
<thead>
<tr>
<th>Subjects</th>
<th>LDL diameter* (nm)</th>
<th>LDL density† (g/ml)</th>
<th>Rf-value‡</th>
<th>Cholesteryl ester§</th>
<th>Triglyceride</th>
<th>Free cholesterol</th>
<th>Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Men (n=9)</td>
<td>25.3±0.3</td>
<td>1.044±0.006</td>
<td>0.296±.002</td>
<td>57.7±13.0</td>
<td>6.9±3.2</td>
<td>11.2±2.3</td>
<td>24.2±6.2</td>
</tr>
<tr>
<td>Women (n=9)</td>
<td>25.4±0.2</td>
<td>1.042±0.002</td>
<td>0.297±.0001</td>
<td>58.3±10.6</td>
<td>6.0±2.6</td>
<td>11.8±1.8</td>
<td>23.9±5.1</td>
</tr>
<tr>
<td>PG</td>
<td>26.4</td>
<td>1.023</td>
<td>0.405</td>
<td>20.6</td>
<td>40.6</td>
<td>6.9</td>
<td>31.9</td>
</tr>
<tr>
<td>IG</td>
<td>26.8 to 25.8</td>
<td>1.031 to 1.035</td>
<td>0.324</td>
<td>50.9</td>
<td>11.9</td>
<td>14.6</td>
<td>22.6</td>
</tr>
<tr>
<td>JG</td>
<td>26.5</td>
<td>1.027</td>
<td>0.324</td>
<td>53.0</td>
<td>14.3</td>
<td>11.3</td>
<td>21.4</td>
</tr>
</tbody>
</table>

* Determined by nondenaturing gradient gel electrophoresis; † determined by density gradient ultracentrifugation; ‡ Rf value = relative flotation value determined by single vertical spin for apoprotein B containing particles; § composition data expressed as lipid composition in mass percent.

LDL = low density lipoprotein.

The lipid and lipoprotein characteristics of the subjects with abnormalities in HL subjects were compared to the control subjects (Table 1). The control subjects had TG, total and LDL cholesterol, and plasma apo B concentrations below the 75th percentile and HDL cholesterol above the 25th percentile for each parameter as defined by the LRC Population Studies. No statistical differences were seen between the male and female subjects in any of these measurements. The control male subjects, however, had significantly lower HL activity than the control male subjects (85±51 vs. 147±53 nmol FFA/min/ml, respectively; p<0.001). In addition, the female control subjects had slightly higher LPL activities compared to the male control subjects, but these differences were not statistically significant (210±91 vs. 156±62, respectively).

In contrast to the control subjects, the HL-deficient subject (PG) had elevated plasma TG and apo B concentrations. IG and JG had approximately 40% of the HL activity compared to the control females (39 and 31, respectively, vs. 85 nmol FFA/min/ml). LPL activity was low normal in PG and JG and high normal in IG, but all were within the 90% confidence limits of normal controls.

The physical characteristics of LDL isolated from control subjects and the patients with abnormal HL activities were analyzed using DGUC, SVS-apo B, and non-denaturing gradient gel electrophoresis (GGE) (Table 2). In general, the patients with abnormal HL activity had a larger and more buoyant major lipoprotein fraction compared to the LDL from control subjects. An SVS-apo B lipoprotein cholesterol profile (Figure 1A) and a densitometric scan of d<1.063 lipoproteins separated by GGE from a control male subject (Figure 1B) with the Rf of 0.297 and diameter of 25.2 nm are representative of the male control group. The HL-deficient subject, PG (Figures 1C and 1D), had a major peak of lipoproteins (LDL) which was unusually buoyant (Rf=0.405) and large (26.4 nm) compared to the control subjects' LDL.

The "LDL" (1.016<d<1.036) isolated from PG had a higher percentage of TG and PL compared to the control LDL (Table 2). Based on SDS-PAGGE, 'LDL' isolated...
Fraction Number Decreasing Size —

Figure 1. Whole plasma lipoprotein cholesterol profile by single vertical spin for normal subject (A) and hepatic triglyceride lipase (HL)-deficient subject (C). In normal persons, very low density lipoprotein is found in fractions 30 to 38, intermediate density lipoprotein in 17 to 29, low density lipoprotein in 7 to 16, and high density lipoprotein in 1 to 7. Gradient gel electrophoresis of d<1.063 lipoproteins in normal subject (B) and HL-deficient subject is shown in D. Calculated diameters are in nanometers.

from PG by DGUC contained primarily apo B-100 and very little apo E (Figure 2). The apo E/apo B mass ratio of PG's 'LDL' was similar to the apo E/apo B in the control LDL determined in a subset of nine normal persons with peak densities between 1.036 and 1.049 g/ml (0.01 vs. 0.03±0.01, respectively).

The density and size characteristics of PG's major peak of lipoproteins were intermediate to LDL and IDL; therefore, additional DGUC analyses were done to determine the characteristics of these lipoproteins with reference to classically defined IDL (1.006<d<1.019) and LDL (1.019<d<1.063). The d<1.006 and 1.006<d<1.019 lipoprotein fractions were first isolated by sequential ultracentrifugation and then subjected to DGUC under identical conditions (Figure 3). The peak density of the major population of lipoproteins was 1.023 g/ml compared to 1.044 g/ml for male controls (see Table 2). The peak density of particles isolated within the IDL density range (d<1.019 g/ml) was 1.020 g/ml and contained approximately half of the mass of the original peak of apo B containing particles. The peak of the d>1.019 fraction was 1.027 g/ml. Although the major population of apo B containing lipoproteins isolated at an intermediate position to classically defined IDL and LDL, these particles contained primarily apo B-100 and are referred to as 'LDL' to differentiate them from IDL and LDL of the control subjects.

To determine whether HL could convert PG's 'LDL' to particles more typical of control LDL, an in vitro incubation study was done with postheparin plasma isolated from an LPL-deficient subject as the source of HL. Incubation of PG's 'LDL' with HL resulted in an increase in the peak density of the 'LDL' from 1.024 to 1.033 g/ml (Table 3). The control LDL incubated under identical conditions had only a slight increase in peak density from 1.036 to 1.038 g/ml. 'LDL' from PG and the normal subject incubated with purified LPL did not change the densities of these particles (data not shown).
Hepatic Lipase and LDL

Figure 3. Lipoprotein elution profiles by density gradient ultracentrifugation (a) d<1.006 fraction (--), 1.006<d<1.019 fraction (--), and d>1.019 fraction (--) first isolated by sequential ultracentrifugation from the hepatic lipase-deficient patient. Peak density in g/ml is given for each profile.

Table 3. Low Density Lipoprotein Incubation Study

<table>
<thead>
<tr>
<th></th>
<th>Before incubation</th>
<th>After incubation</th>
<th>Difference</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(peak density in g/ml*)</td>
<td>(peak density in g/ml*)</td>
<td></td>
</tr>
<tr>
<td>PG 'LDL'</td>
<td>1.024</td>
<td>1.033</td>
<td>0.009</td>
</tr>
<tr>
<td>Normal LDL</td>
<td>1.036</td>
<td>1.038</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Determined by density gradient ultracentrifugation and pycnometry.

The diameter of LDL correlated with its density in the control and abnormal subjects (Figure 4). Absolute HL deficiency (PG) was associated with the most buoyant 'LDL' particles, while half-normal levels of HL (IG and JG) were associated with 'LDL' intermediate to the buoyant particle in HL deficiency and normal LDL.

Discussion

The physical and chemical characteristics of the major apo B containing particles in the subjects with varying degrees of reduced HL activity were distinctive. The patient with HL deficiency had an accumulation of a TG-enriched, large and buoyant apo B containing lipoproteins as determined by DGUC, SVS-apo B, SDS-PAGE, and nondenaturing GGE. These observations correspond with previous studies that have shown the accumulation of TG-rich lipoproteins and slightly more buoyant 'LDL' in both familial and acquired forms of HL deficiency as described by analytical ultracentrifugation. Triglyceride-enriched 'LDL' from HL-deficient subjects differs dramatically from dense LDL isolated from subjects with hypertriglyceridemia. Deckelbaum et al. have shown that lipid transfer protein can exchange VLDL-TG with LDL cholesteryl ester, resulting in net transfer and an enrichment of LDL with TG in vitro. The subsequent action of lipoprotein lipase can then hydrolyze the TG in the in vitro modified LDL and result in smaller more dense LDL. Thus, the buoyant, TG-enriched, large 'LDL' seen in the HL-deficient subject (PG) are especially striking when compared to LDL found in hypertriglyceridemic subjects. The patients with intermediate levels of HL (IG and JG) also accumulated moderately TG-enriched large and buoyant LDL when compared to LDL isolated from the control subjects. It is unclear why buoyant 'LDL' persist in subjects with reduced or absent levels of HL activity when LPL is available. Perhaps these lipoproteins are poor substrates for LPL, similar to normal LDL.

The major apo B containing lipoproteins isolated from the HL-deficient subject (PG) have physical characteristics between classically defined IDL and LDL and contain primarily apo B-100. These lipoproteins probably represent the final product of VLDL and IDL metabolism in this subject. As illustrated in the subject with HL deficiency, the assumed final or terminal product of VLDL and IDL metabolism may not be completely isolated within the traditionally defined density or flotation intervals of LDL.

The abnormalities in the composition of 'LDL' from the HL-deficient subject may be due to a complex interplay between lipoprotein production and modification of the varying apo B containing particles and HL. HL, an enzyme synthesized by the liver, has been implicated in helping to determine the physical characteristics of LDL. Although there was no correlation between LDL size or density and HL activity among the normal subjects, this may be due to the small variation in LDL size and density, the large variability in HL activity (see Table 2), or the inability to 'normalize' these parameters to account for differences in age, body weight, and gender. However, an association between LDL size or density and HL activity existed when control subjects and those with HL deficiency were analyzed together.

Complete HL deficiency as in PG or partial deficiencies in HL activity seen in his half-sisters (IG and JG) are...
associated with a more buoyant and larger ‘LDL’. The effect of HL deficiency can help explain the alterations in lipid composition of the LDL particle. That is, HL catalyzes the hydrolysis of mono-, di-, triacylglycerol, and phospholipid, resulting in a decrease in TG/protein ratio, consistent with the TG and PL enrichment seen in the HL-deficient subject. Apoprotein composition was not unusual in the subjects with HL abnormalities, with apo B-100 being the major apoprotein in LDL from the normal subjects as well as in the ‘LDL’ from PG, IG, and JG. Although the LDL isolated for these studies were prepared by ultracentrifugation, which can lead to loss of apo E from the lipoproteins, all samples were subjected to only 24 hours of centrifugation (compared to >70 hours when using conventional sequential ultracentrifugation methods), and the samples were processed by the same techniques. Small amounts of apo E were detected by SDS-PAGE and radioimmunoassay in both LDL from the control subjects and the ‘LDL’ from the HL patient. However, no apo E enrichment of the ‘LDL’ fraction was detected.

The compositional differences between normal LDL and HL-deficient ‘LDL’ suggest that HL is responsible, in part, for VLDL to LDL processing. We therefore tested the potential of HL to convert the abnormally buoyant and large ‘LDL’ seen in the HL-deficient subject to particles more typical of normal LDL. PG’s ‘LDL’ were incubated with a source of HL from postheparin plasma isolated from an LPL-deficient subject. After incubation with HL, the mean density of the ‘LDL’ increased from 1.024 g/ml to 1.033 g/ml, consistent with this suggested role for HL.

In summary, we have described studies of the unique characteristics of the major population of apo B-containing lipoproteins isolated from a subject with HL deficiency and his two half-sisters with approximately half the normal levels of HL activity. These lipoproteins were in a density and size range between classically defined LDL and IDL; this emphasizes the problem with using conventional density and size ranges to define lipoprotein classes. The possible relationship between the physical characteristics of LDL and HL activity, as seen in the subjects with abnormal HL activity, is suggestive of a role for HL in partly determining LDL characteristics. The absence of HL appears to prevent the appearance of classical LDL and results in an accumulation of larger, more buoyant particles.

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