Presence of Multiple Subpopulations of Lipoproteins of Intermediate Density in Normal Subjects

Thomas A. Musliner, Christine Giotas, and Ronald M. Krauss

Analysis of human plasma by gradient gel electrophoresis (GGE) revealed multiple distinct bands in the size range between very low density (VLDL) and low density lipoproteins (LDL). GGE of intermediate density lipoproteins (IDL) from normal subjects consistently showed two major bands with particle diameters in the range of 275 to 300 Å. The larger, usually predominant subspecies was designated IDL-1, and the smaller, IDL-2. GGE also demonstrated two sizes of subpopulations in the VLDL density range in all subjects. A discontinuous nonequilibrium density gradient ultracentrifugation technique was devised to isolate a series of fractions containing progressively smaller lipoproteins. Successive fractions showed progressive enrichment in cholesteryl-esters, depletion of triglyceride, slower migration on agarose, and depletion of apo E and the C apolipoproteins relative to apo B. The IDL-1 and IDL-2 subspecies appeared to represent two distributions of lipoproteins overlapping in size (particle diameter 280–300 Å and 268–284 Å, respectively) and buoyant density (d = 1.008–1.022 g/ml and d = 1.013–1.028 g/ml, respectively). Plasma from two patients with dysbetalipoproteinemia showed predominant increases in fractions normally containing small VLDL and IDL-1, while patients with severe hypertriglyceridemia had increases primarily in levels of larger VLDL. Heterogeneity of lipoprotein precursors could account for the discrete subspecies of LDL observed in human plasma. (Arteriosclerosis 6:79-87, January/February 1986)
We have applied the techniques of gradient gel electrophoresis (GGE) and a new method for nonequilibrium DGU to explore the possible heterogeneity of IDL in normal and hypertriglyceridemic human plasma. Gradient gel electrophoresis revealed the presence of subpopulations differing in size within the VLDL and IDL density ranges in normal subjects. Varying amounts of these species were also found in the plasma of patients with dysbetalipoproteinemia and severe hypertriglyceridemia.

**Methods**

**Plasma Samples**

After normolipidemic and hyperlipidemic adult volunteers had fasted for 12 to 14 hours, blood was collected into tubes containing disodium ethylene diaminetetraacetic acid (EDTA) at a final concentration of 1.5 mg/ml. The tubes were immediately placed on ice, and plasma was separated at 4°C by low-speed centrifugation. The two patients with dysbetalipoproteinemia had ratios of VLDL cholesterol to plasma triglycerides above 0.30, B-VLDL present shown to have a cysteine at residue 158 of the apo E amino acid sequence.

All subjects participating in this research gave their voluntary, fully informed consent, as required by SECNAVINST 3900.39. This study was approved by the Committee for the Protection of Human Subjects at our institution.

**Density Gradient Ultracentrifugation**

Major lipoprotein classes were isolated by sequential preparative ultracentrifugation under standard conditions. Sodium bromide solutions used to adjust densities were prepared by adding appropriate amounts of solid NaBr to a base d = 1.0063 g/ml solution, consisting of 11.422 g/liter NaCl and 0.01% EDTA. The final pH was adjusted to 7.4 with 10 M NaOH, and densities were checked with a DMA 46 Mettler/Paar density meter.

Nonequilibrium DGU was performed as follows: the plasma or isolated lipoprotein fraction to be analyzed was adjusted to d = 1.21 g/ml by the addition of solid NaBr or dialysis in NaBr solution at 4°C with four changes over a 24- to 48-hour period. A 4.5 ml sample was placed in a 9/16 in. × 3½ in. cellulose nitrate tube (12.5 ml) and sequentially overlayered with 3.0, 3.0, and 1.5 ml, respectively, of solutions of densities 1.020, 1.010, and 1.000 g/ml. The latter consisted of d = 1.0063 g/ml solution diluted 1:5 (vol/vol) with water. The tubes were centrifuged at 17°C in a Beckman SW 41 rotor with use of a Beckman L5-75 ultracentrifuge. The slowest setting for acceleration was used at the start of the run and increased to maximum after the rotor speed reached 5000 rpm. After 6 hours at 40,000 rpm, the rotor was allowed to coast to a stop without braking.

Following centrifugation, the tubes were scanned at 455 nm with a Transidyne RFT Scanning Densitometer mounted vertically. The contents of the tube were then withdrawn by pipetting four successive 1.0 ml fractions followed by six 0.5 ml fractions, designated 1–10 in order from the top of the tube. A background salt tube was generally included with each centrifugation, and the densities of the fractions collected from this tube were measured with a DMA 46 density meter.

For equilibrium DGU, the lipoprotein fraction to be analyzed was dialyzed to d = 1.020 g/ml in NaBr solution at 4°C with four changes over a 24-hour period. The following solutions were carefully layered in a 12.5 ml Beckman SW 41 rotor centrifuge tube: d = 1.040 g/ml (1 ml); d = 1.030 g/ml (3.5 ml); d = 1.020 g/ml (containing the lipoprotein sample, 3.5 ml); d = 1.010 g/ml (3.0 ml); and d = 1.000 g/ml (1 ml). The tubes were centrifuged at 40,000 rpm for 60 hours at 17°C in a Beckman SW 41 rotor in a Beckman L5–75 ultracentrifuge. After the rotor was allowed to coast to a stop without braking, the tubes were scanned at 455 nm as above. The contents were then withdrawn by pipetting in 1.0 ml fractions. Densities of the fractions were determined from background salt tubes included in the centrifugation.

**Polyacrylamide Gradient Gel Electrophoresis**

Electrophoresis of whole plasma or lipoprotein density subfractions was performed at 10°C by using 2–16% polyacrylamide gradient gels (Pharmacia PAA 2/16) for 24 hours at 125 V in Tris (0.09 M)-boric acid (0.08 M)-Na<sub>2</sub>EDTA (0.003 M) buffer (pH 8.3) as described elsewhere. Gels were fixed and stained for protein in a solution containing 0.1% Coomassie brilliant blue R-250, 50% ethanol, and 9% acetic acid (vol/vol) or for lipid with oil red O in 60% ethanol at 55°C. Gels were scanned at 555 nm (for Coomassie blue staining) or 530 nm (for oil red O staining) with a Transidyne RFT densitometer. Migration distances for each absorbance peak were determined and the molecular diameter corresponding to each peak was calculated from a calibration curve generated from the migration distance of standards of known diameter. These included carboxylated latex beads (Dow Chemical), thyroglobulin dimer, thyroglobulin, and apoB in Percoll (Pharmacia) with molecular diameters of 380 Å, 236 Å, 170 Å, and 122 Å, respectively. As previously reported, the standard deviation for measurement of particle diameter (eight determinations on four separate gel runs of an individual LDL sample) ranged from 2.0 to 2.8 Å (coefficient of variation, 0.8% to 1.0%).

GGE in the presence of sodium dodecyl sulfate (SDS) was performed by using 4% to 30% polyacrylamide gradient gels (Pharmacia PAA 4/30) in a buffer system containing 0.04 M Tris, 0.02 M sodium...
SUBPOPULATIONS OF PLASMA INTERMEDIATE DENSITY LIPOPROTEINS

Musliner et al. 81

acetate, 2 mM EDTA (pH 7.4) with 0.2% SDS. The sample buffer consisted of 10 mM Tris, 1 mM EDTA (pH 8.0) containing 2.5% SDS and 5% β-mercaptoethanol. Lipoproteins were delipidated prior to electrophoresis by using methanol and diethylether as described by Herbert et al.,18 dissolved in the sample buffer, and heated for 5 minutes at 100°C prior to application to the gel. Electrophoresis was carried out for approximately 4 hours at 150 V. 12°C. Gels were stained for protein as described above.

**Lipoprotein Electrophoresis on Agarose**

Electrophoresis on agarose was performed with Paragon (Beckman Instruments, Fullerton, California) Lipo Electrophoresis Kits. The conditions recommended by the manufacturer were followed, with the exception that the buffer provided was reconstituted in 1.0 liter rather than 1.5 liters of distilled water, and the electrophoresis time was extended to 45 minutes.

**Analytic Ultracentrifugation**

IDL subfractions, as well as lipoproteins of d < 1.063 g/ml, were analyzed by analytic ultracentrifugation in a Spinco Model E instrument with Schlieren optics, as previously described.7 The runs were performed at 26°C and 52,640 rpm with a salt density of 1.061 g/ml.

**Chemical Composition Determinations**

Phospholipid was determined by the method of Bartlett.19 Protein concentrations were determined by the Lowry procedure modified to include SDS.20 Total cholesterol and triglyceride concentrations were measured by using enzymatic methods on a System 3500 Gilford Computer Directed Analyzer (Gilford Instruments, Oberlin, Ohio). Assay reagents were purchased from Worthington Biochemical Corporation (Freehold, New Jersey). Free cholesterol and cholesteryl esters were determined by gas-liquid chromatography with a Hewlett-Packard 5830A gas chromatograph.21

**Results**

**Identification of Intermediate Size Lipoproteins in Whole Plasma by Gradient Gel Electrophoresis**

Analysis of whole human plasma by 2% to 16% GGE with lipid staining revealed the presence of multiple bands in the size range between VLDL and LDL. Densitometric scans of representative gels from a normolipidemic male and a mildly hypertriglyceridemic female are shown in Figure 1. As reported previously,12 LDL is present in multiple bands within the range of particle diameters 220 to 270 Å. Multiple peaks of larger diameter were also present, with a maximum size of 450 Å, the exclusion limit of the gel. The major included VLDL peak, with a particle diameter ranging between 340 to 420 Å, was followed by as many as three discrete, smaller peaks in the size range between 270 to 320 Å. As discussed below, peaks of 300 to 320 Å were usually due to the presence of Lp(a).

Plasma samples from six subjects were fractionated by ultracentrifugation to determine whether the multiple GGE peaks of diameter > 270 Å had characteristic density distributions. Examples of such separations are shown in Figure 1. The d < 1.006 g/ml fractions contained two major peaks of > 300 Å. The 1.006 < d < 1.019 g/ml fractions also showed two bands with particle diameters in the range of 276 to 296 Å. In both cases these two species corresponded to peaks of identical size on electrophoretograms of whole plasma, as did the major peak in the LDL fraction (1.019 < d < 1.063 g/ml). The electrophoretograms of the LDL fraction also revealed...
shoulders with particle diameters overlapping the smaller of the two IDL species. Gradient gel electrophoretograms of the d < 1.006 g/ml fraction from the plasma of an additional 24 normal subjects showed two or more major peaks in all cases. The 1.006 < d < 1.019 g/ml fraction from the plasma of eight additional normolipidemic subjects showed two IDL peaks in variable proportions in all but one of the subjects, regardless of whether discrete peaks were identified on GGE of whole plasma.

To further define possible modes in the distribution of lipoprotein bands in the 270 to 300 Å size range, 2–16% GGE was performed on the d < 1.063 g/ml lipoproteins in a group of 43 normolipidemic male and female subjects. Particle diameters of each distinct electrophoretic peak within 3 Å size intervals between 270 and 300 Å were tabulated and plotted on a histogram (Figure 2). Modes were observed in the 284 to 300 Å size interval and in the 274 to 282 Å range, as well as in the size range of LDL. The above results indicated that both VLDL and IDL contain more than one subspecies and prompted the use of other techniques to facilitate their separation.

Discontinuous Nonequilibrium Density Gradient Ultracentrifugation

Nonequilibrium DGU fractionation of the d < 1.019 g/ml lipoproteins isolated from normal plasma was performed as described in Methods, and the fractions were analyzed by 2% to 16% GGE. A representative gel is shown for these fractions in Figure 3; densitometric scans of a similar gel from a different normolipidemic subject are shown in Figure 4 A. The top 1 ml (fraction 1) contained the bulk of the VLDL. GGE showed this material to consist of a larger peak of particle diameter 365 to 400 Å with a smaller shoulder at 335 to 350 Å. Fractions 2 through 4 each contained a relatively homogeneous lipoprotein band of progressively smaller particle diameter, ranging from 330 to 295 Å. These lipoproteins appeared to form a continuous distribution, beginning with the smaller shoulder observed in fraction 1. This distribution of particles was designated “small VLDL,” because separate nonequilibrium DGU runs (not shown) of d < 1.006 and d > 1.006 g/ml lipoproteins revealed that the d < 1.006 g/ml fraction contained nearly all of the mass of particles of the size found in fractions 1 through 4. The size range of the major subspecies in fractions 5 and 6 (285 to 296 Å) corresponded to that of the predominant, larger subspecies demonstrable by GGE in the 1.006 < d < 1.019 g/ml ultracentrifugation fraction (Figure 1). It was designated IDL-1. Fraction 5 included an adjacent band of smaller diameter which increased in mass in successive fractions and generally reached a relative maximum in fraction 7 (Figures 3 and 4 A). This species was designated IDL-2. Fraction 8 contained only small amounts of lipoprotein, of similar size to the components identified in fraction 7. A similar pattern was seen when whole plasma was subjected to nonequilibrium (Figure 4 B), minimizing potential artifacts produced by prior ultracentrifugal fractionation.

Physical Properties of VLDL and IDL Subspecies

Fractions 5, 6, 7, and 8 from a nonequilibrium DGU run of the d < 1.063 g/ml lipoproteins from a normolipidemic subject were analyzed by equilibrium DGU.
Figure 4. A. Densitometric scans of oil red O-stained 2% to 16% gradient gels of the nonequilibrium DGU fractions isolated from the d < 1.019 g/ml lipoproteins from a normolipidemic subject. Particle diameters of the major peaks (in Å) were determined from calibration curves as described in Methods. The volumes of sample applied were: fraction 1: 5 μl; fractions 2 to 4: 20 μl; fractions 5 to 7: 15 μl. B. Oil red O-stained 2% to 16% gradient gel electrophoretograms of the nonequilibrium DGU fractions isolated from the whole plasma of a normolipidemic subject (1) and a subject with mild hypercholesterolemia (2) who had cholesterol of 294 mg/dl and triglycerides of 107 mg/dl. The volumes of sample applied to the gels were: whole plasma; 4 μl; fraction 1: 5 μl; fractions 2 to 6: 15 μl; fraction 7: 10 μl; fraction 8: 5 μl.

to determine the buoyant densities of the contained lipoprotein subspecies. Equilibrium DGU was performed as described in Methods and yielded a gradient ranging from 1.0075 to 1.044 g/ml. Densitometric scans at 455 nm of the equilibrium density gradient tubes are shown in Figure 5. Twelve 1 ml fractions were sequentially pipetted from each tube for analysis by 2% to 16% GGE. Similar fractions were taken from a background salt tube included in the same centrifugation run and densities measured as described in Methods.

Distribution of lipoprotein mass in fractions 5 through 8 displayed peaks at respective buoyant densities of 1.010, 1.013, 1.019, and 1.028 g/ml (Figure 5 A). GGE analysis of fractions obtained from the peaks of each of the four equilibrium DGU profiles (Figure 5 B) identified bands corresponding to the major species in the parent nonequilibrium DGU fractions. IDL-1 of particle diameter 280 to 300 Å, present as the major species in nonequilibrium DGU fractions 5-7, was identified in equilibrium DGU fractions of d = 1.008 to 1.022 g/ml. Nonequilibrium DGU fraction 5 contained another component with particle diameter larger than IDL-1, corresponding to the size range of small VLDL (300 to 330 Å, Figure 1), which was identified in equilibrium DGU fractions of d < 1.013 g/ml. IDL-2, the minor component in nonequilibrium DGU fractions 6 and 7 (Figure 1) with particle diameter 270 to 284 Å, was identified in equilibrium DGU fractions of d = 1.013 to 1.028 g/ml.
Particles in the LDL size and density range (d ≥ 1.028 g/ml) were predominant in nonequilibrium DGU fraction 8, which also contained small amounts of IDL-1 and IDL-2 in the more buoyant leading shoulder.

Analytic ultracentrifugation schlieren patterns of lipoproteins contained in fractions 4, 5, 6, and 7 from a normolipidemic subject showed peak S0 values for these fractions of 19.9, 16.6, 14.0, and 13.1, respectively.

**Compositional Analysis**

Triglycerides, free and esterified cholesterol, total protein, and phospholipid analysis of the non-equilibrium DGU fractions separated from the plasma of four normal subjects are tabulated in Table 1. Fraction 2 contained more than twice as much cholesterol, over 50% more protein, and nearly one-half of triglycerides compared with fractions 4, 5, 6, and 7 from a normolipidemic subject. A reference plasma sample is shown in the lane at the extreme right.

**VLDL and IDL Subspecies in Hyperlipidemic Patients**

Plasma samples from two patients with dysbeta-

lipoproteinemia were fractionated by nonequilibrium DGU and analyzed by GGE. The particle diameters of the major lipoprotein peaks in fractions 1 to 7 (not shown) were similar to those shown for the normolipidemic subjects (Figure 4). A disproportionate amount of the VLDL in fraction 1, however, appeared in the second peak of smaller particle diameter on GGE. Relative to the amount of LDL in fractions 8 + 9, the protein mass in small VLDL (fractions 2–4) and IDL (fractions 5–7) were increased an average of 14-fold and five-fold, respectively, in the two Type 3 patients compared with seven normal controls. This abnormal distribution was seen even at a time of excellent dietary control for one of the Type 3 patients (total plasma cholesterol 157 mg/dl, triglycerides 231 mg/dl).

Chemical composition of the nonequilibrium DGU fractions is shown for one of the patients with dysbeta-

lipoproteinemia (DR, Table 2) at a time when his

**Electrophoretic Mobility in Agarose**

Agarose electrophoresis of nonequilibrium DGU fractions 1 through 8 prepared from the d < 1.019 g/ml lipoproteins from a normolipidemic subject is shown in Figure 6. The VLDL in fraction 1 migrated more rapidly than the pre-β lipoproteins contained in whole plasma. Successive nonequilibrium DGU fractions migrated progressively more slowly, spanning the region from the pre-β to the β positions.

**Apolipoprotein Composition**

Delipidated samples of nonequilibrium DGU fractions 1 through 8 isolated from the d < 1.019 g/ml lipoproteins of a normolipidemic subject were subjected to GGE in the presence of SDS as described under Methods. As shown in Figure 7, the content of apo E and the C apolipoproteins relative to apo B decreased in successive fractions containing small VLDL, IDL-1, and IDL-2. Otherwise there were no distinct differences in apolipoprotein composition between the fractions.

**Table 1. Chemical Composition of Nonequilibrium Density Gradient Ultracentrifugation Fractions from Four Normolipidemic Male Subjects**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Triglyceride</th>
<th>Cholesterol ester</th>
<th>Phospholipid</th>
<th>Protein</th>
<th>Total cholesterol: protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58.0 ± 5.3</td>
<td>5.8 ± 0.7</td>
<td>8.4 ± 5.1</td>
<td>17.3 ± 0.8</td>
<td>10.5 ± 1.4</td>
</tr>
<tr>
<td>2</td>
<td>32.8 ± 3.8</td>
<td>6.5 ± 4.3</td>
<td>22.6 ± 7.9</td>
<td>21.8 ± 1.6</td>
<td>16.5 ± 2.2</td>
</tr>
<tr>
<td>3</td>
<td>29.1 ± 3.7</td>
<td>9.4 ± 1.0</td>
<td>22.1 ± 6.0</td>
<td>21.6 ± 1.0</td>
<td>18.0 ± 2.6</td>
</tr>
<tr>
<td>4</td>
<td>24.7 ± 3.5</td>
<td>9.6 ± 0.9</td>
<td>26.2 ± 5.4</td>
<td>21.7 ± 1.0</td>
<td>17.9 ± 2.5</td>
</tr>
<tr>
<td>5</td>
<td>20.8 ± 4.0</td>
<td>9.3 ± 0.6</td>
<td>31.3 ± 6.0</td>
<td>21.2 ± 0.7</td>
<td>17.4 ± 1.9</td>
</tr>
<tr>
<td>6</td>
<td>15.5 ± 2.7</td>
<td>11.2 ± 1.7</td>
<td>34.3 ± 8.3</td>
<td>21.4 ± 2.2</td>
<td>17.7 ± 4.9</td>
</tr>
<tr>
<td>7</td>
<td>11.3 ± 2.3</td>
<td>10.8 ± 2.5</td>
<td>40.1 ± 3.7</td>
<td>20.8 ± 1.2</td>
<td>17.0 ± 1.7</td>
</tr>
<tr>
<td>8</td>
<td>6.8 ± 1.1</td>
<td>10.2 ± 2.0</td>
<td>44.1 ± 2.3</td>
<td>19.5 ± 2.0</td>
<td>19.4 ± 2.2</td>
</tr>
<tr>
<td>9</td>
<td>4.4 ± 0.9</td>
<td>9.2 ± 1.9</td>
<td>44.5 ± 1.8</td>
<td>19.7 ± 1.5</td>
<td>22.2 ± 1.8</td>
</tr>
<tr>
<td>10</td>
<td>5.3 ± 0.5</td>
<td>8.1 ± 1.6</td>
<td>42.7 ± 1.0</td>
<td>18.9 ± 0.5</td>
<td>25.1 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SD, expressed as percent composition, with the sum of the mass of all components for each subject taken as 100%.

**Figure 6.** Agarose electrophoresis of the nonequilibrium DGU fractions from the d < 1.019 g/ml lipoproteins of a normolipidemic subject. A reference plasma sample is shown in the lane at the extreme right.
Table 2. Chemical Composition of Nonequilibrium Density Gradient Ultracentrifugation Fractions from a Subject with Dysbetalipoproteinemia

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Triglyceride</th>
<th>Free cholesterol</th>
<th>Cholesteryl ester</th>
<th>Phospholipid</th>
<th>Protein</th>
<th>Total cholesterol: protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52.5</td>
<td>7.6</td>
<td>25.9</td>
<td>8.3</td>
<td>5.8</td>
<td>5.77</td>
</tr>
<tr>
<td>2</td>
<td>25.6</td>
<td>9.3</td>
<td>34.3</td>
<td>21.5</td>
<td>9.3</td>
<td>4.71</td>
</tr>
<tr>
<td>3</td>
<td>20.8</td>
<td>9.2</td>
<td>36.2</td>
<td>22.7</td>
<td>11.1</td>
<td>4.09</td>
</tr>
<tr>
<td>4</td>
<td>19.1</td>
<td>9.7</td>
<td>36.7</td>
<td>22.6</td>
<td>11.9</td>
<td>3.89</td>
</tr>
<tr>
<td>5</td>
<td>18.7</td>
<td>10.6</td>
<td>35.1</td>
<td>22.1</td>
<td>13.5</td>
<td>3.39</td>
</tr>
<tr>
<td>6</td>
<td>18.9</td>
<td>10.1</td>
<td>33.9</td>
<td>21.9</td>
<td>15.2</td>
<td>2.89</td>
</tr>
<tr>
<td>7</td>
<td>18.6</td>
<td>9.4</td>
<td>31.4</td>
<td>23.7</td>
<td>16.9</td>
<td>2.41</td>
</tr>
<tr>
<td>8</td>
<td>17.2</td>
<td>7.7</td>
<td>30.0</td>
<td>22.9</td>
<td>22.2</td>
<td>1.70</td>
</tr>
<tr>
<td>9</td>
<td>13.5</td>
<td>8.4</td>
<td>31.9</td>
<td>21.9</td>
<td>24.3</td>
<td>1.66</td>
</tr>
<tr>
<td>10</td>
<td>9.2</td>
<td>5.4</td>
<td>19.3</td>
<td>27.9</td>
<td>38.3</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Values are expressed as percent composition, with the sum of the mass of all components taken as 100%.

In contrast to the findings in Type 3 subjects, analyses of nonequilibrium DGU fractions from four patients with severe hyperglyceridermia (855 to 1660 mg/dl) revealed disproportionate increases in large VLDL (>350 Å diameter). Fraction 1 contained 70% to 80% of the total VLDL + IDL protein mass in these patients, compared to 26% for a control group of 10 normolipidemic subjects and 30% to 35% for the patients with dysbetalipoproteinemia. GGE demonstrated bands in fractions 4 through 7 from hypertriglyceridermic subjects with particle diameters in the smaller end of the range observed for IDL-1 and IDL-2 in corresponding fractions from normolipidemic subjects.

Discussion

Human IDL have generally been regarded as a single class of lipoproteins generated from VLDL by lipolysis. In the studies reported here, two subpopulations of IDL were consistently identified as peaks or shoulders on gradient gel electrophoretograms of ultracentrifugal fractions from normal subjects. IDL-1 and IDL-2 in d = 1.006 to 1.019 g/ml fractions or in nonequilibrium DGU subfractions corresponded closely in size to peaks and shoulders present on 2% to 16% gradient gel electrophoretograms of uncentrifuged plasma from some of these subjects. Commonly, however, these peaks appeared to be obscured within larger bordering peaks on the whole plasma GGE profile. Although nonequilibrium DGU achieved a degree of separation of the IDL subspecies, they were generally not cleanly isolated by this technique. This is not unexpected in view of their overlapping size and buoyant densities, as established by GGE and equilibrium DGU.

Two subpopulations of VLDL were also consistently observed by 2% to 16% GGE of d < 1.006 g/ml fractions. The larger subpopulation (peak particle diameter >360 Å) was markedly increased in patients with severe hypertriglyceridermia. Lipoproteins similar in size to the smaller (300 to 350 Å) subpopulation were disproportionately increased in two patients with dysbetalipoproteinemia. Often no distinct transi-
tion was seen between small VLDL and IDL-1 (e.g., Figure 4), and it is possible that in such cases these bands represent a continuum of particles overlapping the conventional density used to separate VLDL and IDL. Composition analysis of small VLDL in normal subjects revealed enrichment in protein and cholesterol with depletion of triglyceride compared to large VLDL, as well as depletion of apo E and the C-apolipoproteins relative to apo B. With respect to size and a trend toward cholesterol enrichment, “small VLDL” resemble the β-VLDL that accumulate in patients with dysbetalipoproteinemia. In contrast to β-VLDL, “small VLDL” from normal subjects do not have increased apo E content relative to apo B and are less cholesterol-enriched. Similarities in composition suggest that “small VLDL” may correspond to the second pre-β species identified in some subjects by agarose electrophoresis.

Fractions relatively enriched in IDL-2 displayed further triglyceride depletion and cholesterol enrichment compared with those containing predominantly IDL-1. Apo E was still present, and overall no distinct apolipoprotein differences were detected between fractions containing predominantly IDL-1 vs IDL-2. In the absence of better means of separating these species, however, one can say little as to possible differences in their chemical or protein composition. Denser nonequilibrium DGU fractions containing mostly LDL had only trace amounts of apo E detectable by SDS GGE and were further enriched in cholesterol and depleted in triglyceride. IDL-2 and the largest subspecies isolated from the LDL density range (d = 1.025 to 1.063 g/ml) overlap in size distribution, yet can appear as distinct bands on GGE of whole plasma or nonequilibrium DGU fractions.

As shown in Figure 5, the hydrated densities of small VLDL (d < 1.010 g/ml), IDL-1 (d = 1.010 to 1.022 g/ml) and IDL-2 (d = 1.013 to 1.028 g/ml) extend outside of the conventional density ranges for VLDL and IDL. In particular, the two major IDL subpopulations comprise a distribution overlapping with small VLDL and large LDL. Conventionally prepared IDL fractions (1.006 < d < 1.019 g/ml) may contain lipoproteins included in the “small VLDL” distribution, while conventional LDL fractions (1.019 < d < 1.063 g/ml) are commonly contaminated with lipoproteins belonging to both the IDL-1 and IDL-2 particle distributions. Our findings are in agreement with those of Gibson et al., who found that an apo E containing lipoprotein subfraction isolated by 4% agarose chromatography displayed a distribution overlapping the traditional VLDL and LDL density intervals.

The question arises as to whether one or more of these subspecies could represent artifacts of the preparative or analytic tools employed for their study. Puppione et al. reported the presence of an unusual species in bovine IDL fractions (d = 1.006 to 1.020 g/ml) when prepared by ultracentrifugation at 16°C, but not when isolated at 37°C. These lipoproteins, however, ranged between 500 and 2000 Å in size, and would not be confused with the IDL subspecies identified here. Ultracentrifugation may result in the selective dissociation of apolipoproteins from the lipoprotein particle, and this might apply to apo E in the case of IDL. The fact that the IDL subspecies identified in our density gradient ultracentrifugation fractions coincide with bands observed by GGE of uncentrifuged plasma is evidence that the two species are not formed by ultracentrifugation, although conceivably some properties of the subspecies may be altered by this procedure. The relatively brief ultracentrifugation used in our nonequilibrium DGU technique would be expected to minimize such changes.

Clinical and experimental data suggest a role for cholesterol-enriched VLDL and IDL in atherosclerosis, since the density distributions of subpopulations within IDL overlap with each other and with those of VLDL and LDL, more refined analytic methods will be needed to study their potential metabolic or pathologic significance.

Acknowledgments

The authors appreciate the expert technical assistance of Joseph Josefa, Kathy McVicker, and Janet Selmek-Halsey. We are grateful to Frank Lindgren and his staff for performing the analytic ultracentrifugation procedures. Plasma samples from normal men and women were kindly provided by Peter Wood of Stanford University and Subr Roy of the University of Southern California. We also thank Scott Hambly for his help in manuscript preparation.

References

SUBPOPULATIONS OF PLASMA INTERMEDIATE DENSITY LIPOPROTEINS

Presence of multiple subpopulations of lipoproteins of intermediate density in normal subjects.

T A Musliner, C Giotas and R M Krauss

Arterioscler Thromb Vasc Biol. 1986;6:79-87
doi: 10.1161/01.ATV.6.1.79

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

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/6/1/79

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/