Enhanced Metabolism of Normolipidemic Human Plasma Very Low Density Lipoprotein in Cultured Cells by Exogenous Apolipoprotein E-3

Shlomo Eisenberg, Gideon Friedman, and Tikva Vogel

In this investigation in cultured human fibroblasts, an attempt was made to determine the optimal metabolism of apolipoprotein (apo) B-100 lipoproteins from normolipidemic human subjects. We supplemented culture systems containing 125I-lipoproteins with exogenous recombinant or plasmatric apo E-3. Very low density lipoprotein (VLDL) fractions I, II, and III, and low density lipoproteins (LDL) were prepared from one E 4/3 and four E 3/3 subjects. Without added apo E-3, cellular metabolism (binding, cell association, and degradation) of the three VLDL fractions, but LDL was not affected. The effects of apo E-3 were specific, not observed with apo E-2, and not observed on receptor-negative cells. Exogenous apo E-3 also enhanced down-regulation of cellular sterol synthesis by the VLDLs, but not LDL, indicating increased particle catabolism by the cells. The optimal concentrations of exogenous apo E-3 were 4 to 8 μg protein/15 μg VLDL-protein, when most of the added apo E-3 became associated with the VLDL particles. Apo E-3 failed to associate with LDL. These results demonstrate that availability and association of adequate amounts of apo E-3 are crucial for optimal cellular metabolism of apo B-100 lipoproteins along the VLDL → LDL cascade.

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Cellular catabolism of very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) particles along the VLDL → IDL → low density lipoprotein (LDL) cascade may regulate LDL synthesis in both laboratory animals and in humans.1,2,3 For example, accelerated direct catabolism of VLDL remnants in the liver appears to account for the paucity of LDL in rats4,5 and other animal species.6 Even more important, it has been postulated that lack of direct catabolism of VLDL and LDL is responsible for increased LDL formation in the Watanabe heritable hyperlipidemic (WHHL) rabbit and in human subjects with familial hypercholesterolemia when functional LDL receptors are absent.7,8 In view of the potential importance of these "shunt" pathways as regulators of the apolipoprotein (apo) B cascade in general and of the LDL system in particular, it is essential to elucidate the mechanisms responsible for the cellular metabolism of lipoproteins other than LDL that contain apo B-100.

Previous studies of the metabolism of VLDL from normo- or hypertriglyceridemic subjects in cultured cells have demonstrated that functional apo E molecules (E-3 or E-4) are essential for the interactions of the lipoprotein with the LDL (B,E) receptor.9–14 Yet, normolipidemic VLDL density fractions I and II appear to lack the ability to regulate cellular sterol synthesis,9,10 even though apo E is present in these lipoproteins. Therefore, in the present investigation, we attempted to determine the maximal capacity for direct catabolism of normolipidemic human VLDL particles in cultured human skin fibroblasts. We provided the lipoproteins with exogenous recombinant or plasmatric apo E-3 and tested the hypothesis that adding the protein to lipoprotein cell systems would affect the cellular metabolism. Indeed, we found a many-fold enhancement of the metabolism of the VLDL.

Methods

Subjects

Five healthy normolipidemic subjects gave their consent to participate in the study. Each subject gave one unit of plasma during morning hours, after a 12- to 14-hour fast. The apo E profile of four subjects was E-3/3 and of the fifth, E-4/3.

Preparation of Lipoproteins

VLDL was separated at plasma density (1.006 g/ml) after 16 hours centrifugation in a 60 Ti rotor at 45,000 rpm, 4°C, in a Beckman L5-50 ultracentrifuge. The VLDL was spun once more at the same density. VLDL density subfractions I, II, and III were prepared on a NaCl gradient in an SW-41 rotor with the procedure of Lindgren et al.15 The flotation (Sf) rates of the three VLDLs were 100 to
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Millipore filter and were kept at 4°C. Lipoproteins were used within 10 days of preparation.

**Apolipoprotein E**

Two preparations of recombinant apo E-3 were used. The two preparations were identical to plasmatic apo E-3 in receptor binding assays and exhibited the same biological reactivity when tested in fibroblasts with VLDL and LDL. Plasmatic apo E-2 and apo E-3 were prepared from the VLDL of hyperlipidemic E-2/2 and E-3/3 subjects, respectively, following the procedure described by Stanley et al. The purity of the apo E-3 thus obtained is shown in Figure 1.

400, 60 to 100, and 20 to 60, respectively. LDL was separated at a density interval of 1.019 to 1.063 g/ml and was washed once in the high density solution. The purity of the lipoprotein fractions was ascertained by centrifugation in a zonal system. Lipoproteins were dialyzed against 0.15% NaCl, 20 mM Tris (pH = 7.4), and 0.01% EDTA solution and were radioiodinated to a specific activity of 300 to 600 cpm/ng protein. After iodination, the distribution of radioactivity between apo B, apo C, and apo E (means ± SEM) in VLDL-I was 24.7 ± 2.2%, 60.6 ± 2.7%, and 5.01 ± 1.1%, respectively; in VLDL-II, it was 28.7 ± 1.4%, 57.9 ± 1.2%, and 3.6%; and in VLDL-III, 46.9 ± 3.4%, 42.9 ± 2.8%, and 3.2 ± 0.9%. In LDL, 90.7 ± 0.7% of the radioactivity was associated with apo B. All lipoprotein preparations were sterilized by passage through a 0.45 μm filter.

**Re-association of Apo E-3 with Lipoproteins**

To determine the association of apo E-3 with lipoproteins, the following procedure was used. Lipoprotein protein (200 μg) was incubated with different amounts of plasmatic or recombinant apo E-3 (0 to 80 μg protein) at 37°C for 60 minutes. The incubation was carried out in 0.9% NaCl, 20 mM Tris, and 0.01% EDTA buffer (pH = 7.4) at a total volume of 0.5 ml. After the incubation, the sample was applied to the top of a 0.9x25 cm 8% agarose column, Bio-Gel A-1.5m, 100 to 200 mesh, (Bio-Rad, CA) and was eluted with the same buffer. Lipoproteins alone were recovered at the void volume, or the first 1 to 2 ml (tubes 1 and 2) of the column volume; apo E-3 alone eluted at the column volume of 9 to 12 ml (tubes 8 to 12). The column elution profile was monitored by absorbance at 280 nm. The lipoprotein peaks were identified, pooled, and dialyzed against water and 0.01% EDTA solution; aliquots of 15 μg protein were subjected to SDS-polyacrylamide gel electrophoresis.

**Cell Cultures**

Cultured human skin fibroblasts (HSF) were prepared as described previously and were used from the fifth to the 15th subculture. The cells (3.5 x 10⁴) were plated in 35 mm dishes (Falcon Labware, MD) and were allowed to grow in 2 ml Dulbecco-Vogt medium with 10% fetal calf serum (FCS) for 5 days. On the fifth day, the cultures were washed with 1 to 2 ml of phosphate-buffered saline (PBS) and were incubated for an additional 48 hours in medium containing 5 mg/ml human lipoprotein-deficient serum (LPDS). Experiments were performed on day 7 of culture.

**Table 1. Chemical Composition of VLDL Density Subfractions I, II, and III and of LDL**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Protein</th>
<th>TG</th>
<th>CE</th>
<th>FC</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-I</td>
<td>10.5 ± 0.6</td>
<td>65.6 ± 2.1</td>
<td>5.8 ± 0.9</td>
<td>3.2 ± 0.4</td>
<td>14.9 ± 1.6</td>
</tr>
<tr>
<td>VLDL-II</td>
<td>12.6 ± 0.4</td>
<td>56.2 ± 1.8</td>
<td>8.4 ± 1.1</td>
<td>4.1 ± 0.4</td>
<td>18.7 ± 1.1</td>
</tr>
<tr>
<td>VLDL-III</td>
<td>14.0 ± 1.0</td>
<td>49.2 ± 2.3</td>
<td>12.5 ± 1.6</td>
<td>4.2 ± 0.4</td>
<td>20.0 ± 1.1</td>
</tr>
<tr>
<td>LDL</td>
<td>22.5 ± 1.3</td>
<td>8.6 ± 0.6</td>
<td>37.3 ± 1.3</td>
<td>7.9 ± 0.3</td>
<td>23.7 ± 1.5</td>
</tr>
</tbody>
</table>

Values are mg/100 mg lipoprotein mass. Data are means ± SEM of six determinations of VLDL density subfractions prepared from the plasma of four normolipidemic subjects with apo E-3/3 phenotype, and one with E-4/3 phenotype. LDL was prepared from the same subjects.

TG = triglycerides, CE = cholesterol ester, FC = free cholesterol, PL = phospholipids.
Binding, Cell Association, and Proteolytic Degradation of ¹²⁵I-Lipoproteins

Binding, cell association, and proteolytic degradation of ¹²⁵I-labeled lipoproteins were determined as previously described. On the day of the experiment, the LPDS-containing medium was removed, the cells were washed with PBS and were incubated with ¹²⁵I-VLDL-I, ¹²⁵I-VLDL-II, ¹²⁵I-VLDL-III (15 μg protein/ml), or ¹²⁵I-LDL (10 μg protein/ml) in LPDS medium at 37°C. Unlabeled apo E-3 in buffer was added to part of the medium containing the ¹²⁵I-labeled lipoproteins; buffer alone was added to the samples without apo E-3. The medium was then added to the cultures and incubations were carried out for 6 hours. At the end of the incubation period, the medium was removed and examined for noniodide ¹²⁵I-protein degradation products. Dishes without cells and cultures incubated in the presence of a 40-fold excess of unlabeled lipoproteins were processed in parallel to the experimental samples. After removal of the medium, the cells were chilled on ice and were washed extensively with ice-cold PBS buffer containing 0.2% bovine serum albumin (BSA). The amount of ¹²⁵I-lipoproteins bound to the cells was determined by the release of radioactivity after incubation of the cells with medium containing 10 mg/ml of sodium heparin for 1 hour, according to the method of Goldstein et al. and Brown and Goldstein. Labeled lipids in the cells were extracted with chloroform/methanol (1:1, vol/vol) and the cell residues were dissolved in 1 ml of 0.5 N NaOH. The cell-associated lipoprotein protein was determined as the radioactivity remaining in the delipidated cells. The results are expressed as nanograms of ¹²⁵I lipoprotein protein bound, associated, or degraded per milligram of cell protein after subtraction of blank (cell-free) and nonspecific values.

Figure 3. Effects of exogenous apo E-3 on cellular metabolism of VLDL-I (ΔΔ), VLDL-II (□□), VLDL-III (○○), and LDL (■■) in cultured human skin fibroblasts. Data are of lipoproteins isolated from the plasma of a normolipidemic human subject whose apo E profile is E-4/3. Similar results were observed with lipoproteins isolated from the plasma of two subjects with apo E-3/3. Lipoproteins were incubated for 6 hours with up-regulated fibroblasts with or without the indicated amounts of exogenous recombinant apo E-3, as described in Methods.
Figure 4. SDS-PAGE of VLDL-I (gels 1 to 4) and LDL (gels 5, 6) after a 1-hour incubation with or without plasmatlc apo E-3 and chromatography on an 8% agarose column, as described in Methods. VLDL-I was prepared from the plasma of a mild hyperlipidemic subject and LDL from a normal lipemic subject. After chromatography, a loss of apo E from VLDL-I incubated without apo E-3 was observed (compare with Figure 2), but not from VLDL-I incubated with apo E-3. Lane 1. VLDL-I (200 μg protein) incubated without exogenous apo E-3. Lanes 2 to 4. VLDL-I incubated with 20, 40, or 60 μg apo E-3, respectively. Lanes 5 and 6. LDL (200 μg protein) incubated without (Lane 5) and with 80 μg apo E-3 (Lane 6).

Incorporation of 14C-Acetate to Sterols

The effects of added apo E-3 on the ability of the lipoproteins to regulate sterol synthesis was determined in up-regulated fibroblasts after 6-hour incubations with unla
cabeled VLDL fractions (30 μg lipoprotein protein) or with LDL (15 μg lipoprotein protein) in 1 ml LPDS medium. Recombinant apo E-3 was added to the samples at protein concentrations of 6 μg/ml (VLDL) or 4 μg/ml (LDL). After 6 hours, the medium was removed, the cell layers were washed with PBS and 0.2% BSA, and these were reincubated for 2 hours at 37°C with medium containing 10 μCi of 2-14C-sodium acetate, 55 mCi/mmol. Incorporation of acetate into sterol was determined as previously described. Cultures incubated without lipoproteins served to determine the capacity of the up-regulated cells to synthesize 14C-sterol. The values obtained from these cultures were taken as 100% values for incorporation of 14C-acetate to sterols.
The degradation of lipoprotein proteins were determined after a 6-hour incubation at 37°C, as described in Methods. HQ as indicated, with 6 μg protein (VLDL-I and VLDL-III) or 4 μg protein (LDL) of recombinant apo E-3. Binding, cell association, and degradation of lipoprotein proteins was determined as described in Methods.

Table 2. Effect of Exogenous Recombinant Apo E-3 on Binding, Cell Association, and Degradation of Lipoproteins in Human Skin Fibroblast Cultures

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Binding - apo E</th>
<th>Binding + apo E</th>
<th>Degradation - apo E</th>
<th>Degradation + apo E</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-I</td>
<td>7 ± 2</td>
<td>52 ± 5</td>
<td>17 ± 2</td>
<td>155 ± 30</td>
</tr>
<tr>
<td>VLDL-II</td>
<td>9 ± 3</td>
<td>75 ± 15</td>
<td>13 ± 7</td>
<td>189 ± 19</td>
</tr>
<tr>
<td>VLDL-III</td>
<td>12 ± 5</td>
<td>95 ± 8</td>
<td>17 ± 5</td>
<td>236 ± 40</td>
</tr>
<tr>
<td>LDL</td>
<td>42 ± 5</td>
<td>54 ± 6</td>
<td>146 ± 18</td>
<td>158 ± 14</td>
</tr>
</tbody>
</table>

Table 3. Effects of Exogenous Plasmatlc Apo E-2 and Apo E-3 on VLDL Metabolism in Cultured Skin Fibroblasts

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Apo E</th>
<th>Binding</th>
<th>Cell association</th>
<th>Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-I</td>
<td>none</td>
<td>5 ± 1</td>
<td>26 ± 5</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>E-2</td>
<td>12 ± 4</td>
<td>33 ± 6</td>
<td>63 ± 6</td>
<td></td>
</tr>
<tr>
<td>E-3</td>
<td>27 ± 2</td>
<td>104 ± 2</td>
<td>414 ± 8</td>
<td></td>
</tr>
<tr>
<td>VLDL-III</td>
<td>12 ± 6</td>
<td>18 ± 3</td>
<td>48 ± 5</td>
<td></td>
</tr>
<tr>
<td>E-2</td>
<td>16 ± 2</td>
<td>32 ± 1</td>
<td>139 ± 10</td>
<td></td>
</tr>
<tr>
<td>E-3</td>
<td>35 ± 2</td>
<td>141 ± 5</td>
<td>575 ± 10</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Effect of Exogenous Recombinant Apo E-3 on VLDL and LDL Metabolism in Cultured Normal and Homozygote FH Skin Fibroblasts

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Metabolic parameter</th>
<th>Normal cells - Apo E-3</th>
<th>Normal cells + Apo E-3</th>
<th>FH cells - Apo E-3</th>
<th>FH cells + Apo E-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-I</td>
<td>Binding</td>
<td>9 ± 2</td>
<td>53 ± 5</td>
<td>6 ± 2</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>VLDL-III</td>
<td>Binding</td>
<td>25 ± 3</td>
<td>548 ± 11</td>
<td>16 ± 2</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>LDL</td>
<td>Binding</td>
<td>15 ± 2</td>
<td>81 ± 4</td>
<td>9 ± 2</td>
<td>12 ± 3</td>
</tr>
</tbody>
</table>

The effects of optimal concentrations of exogenous apo E-3 on the cellular metabolism of VLDL-I, VLDL-II, VLDL-III, and LDL obtained from the plasma of four normotriglyceridemic E-3/3 subjects and one E-4/3 subject are shown in Table 2. Apo E-3 caused a many-fold increase of cellular metabolism (binding, cell association, and degradation) of all three VLDL fractions. VLDL degradation values (all three subfractions) were barely measurable without added apo E; upon addition of apo E-3, VLDL degradation was 500 to 1000 ng protein/mg cell protein/6 hours incubation. The effects on LDL were minimal.

Three experiments were performed to determine the specificity of the effects of exogenous apo E-3 on VLDL metabolism. In the first experiment, the effects of plasmatic apo E-3 were compared to those of plasmatic apo E-2 (Table 3). The effects were investigated with VLDL-I and VLDL-III from a normolipidemic E-3/3 subject. With both VLDL preparations, apo E-3 caused a marked increase of binding, association, and proteolytic degradation of the lipoproteins, while apo E-2 exhibited limited activity, especially in the degradation study. Similar results were found for VLDL from an E-2/2, type III patient (data not shown). These observations are consistent with previous reported data for β-VLDL and a variety of cell types. In the second experiment, the effects of recombinant apo E-3 on VLDL metabolism in LDL receptor negative cells were determined (Table 4). Without the

Table 4. Effect of Exogenous Recombinant Apo E-3 on VLDL and LDL Metabolism in Cultured Normal and Homozygote FH Skin Fibroblasts

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Metabolic parameter</th>
<th>Normal cells - Apo E-3</th>
<th>Normal cells + Apo E-3</th>
<th>FH cells - Apo E-3</th>
<th>FH cells + Apo E-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-I</td>
<td>Binding</td>
<td>9 ± 2</td>
<td>53 ± 5</td>
<td>6 ± 2</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>VLDL-III</td>
<td>Binding</td>
<td>25 ± 3</td>
<td>548 ± 11</td>
<td>16 ± 2</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>LDL</td>
<td>Binding</td>
<td>15 ± 2</td>
<td>81 ± 4</td>
<td>9 ± 2</td>
<td>12 ± 3</td>
</tr>
</tbody>
</table>

Values are ng protein/mg cell protein/6 hrs. Results are of triplicate determinations of 125I-labeled VLDL-I, VLDL-III, and LDL metabolism in cultured, up-regulated skin fibroblasts in cells obtained from a normolipidemic human subject and receptor-negative homozygote FH patients. Lipoproteins were added at protein concentrations of either 15 (VLDL-I and -III) or 10 (LDL) μg protein/ml and supplemented, as indicated, with 6 μg protein (VLDL-I and VLDL-III) or 4 μg protein (LDL) of recombinant apo E-3. Binding, cell association, and degradation of lipoprotein proteins were determined after a 6-hour incubation at 37°C, as described in Methods. FH = familial hypercholesterolemia.
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Table 5. Suppression of 125I-VLDL Proteolytic Degradation on Cultured Fibroblasts by Unlabeled Lipoproteins

<table>
<thead>
<tr>
<th>Apo E-3</th>
<th>Lipoprotein added</th>
<th>VLDL-I</th>
<th>VLDL-II</th>
<th>VLDL-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>50†</td>
<td>23</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>779</td>
<td>931</td>
<td>1078</td>
</tr>
<tr>
<td>4 LDL, 400 μg</td>
<td>41</td>
<td>27</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>4 LDL, 400 μg + E-3, 160 μg</td>
<td>11</td>
<td>trace</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>4 LDL, 400 μg</td>
<td>53</td>
<td>54</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>4 LDL, 1000 μg</td>
<td>31</td>
<td>22</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>4 LDL, 2000 μg</td>
<td>22</td>
<td>14</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

*Values are μg protein. †Values are ng protein/μg cell protein/6 h.

Data are means of two determinations. The difference between duplicate values was less than 10% of the mean. Proteolytic degradation of 125I-labeled VLDL-I, -II, or -III (10 μg protein) was determined after a 6-hour incubation at 37°C, as described in Methods. Similar results were obtained for binding and cell-associated values.

Table 6. Effect of Exogenous Recombinant Apo E-3 on Regulation of Cholesterol Synthesis by Lipoproteins in Human Skin Fibroblast Cultures

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Cholesterol synthesis*</th>
<th>- apo E</th>
<th>+ apo E</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100.0</td>
<td>112.7 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>VLDL-I</td>
<td>97.9 ± 9.4</td>
<td>75.7 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>VLDL-II</td>
<td>101.0 ± 6.8</td>
<td>55.0 ± 11.8</td>
<td></td>
</tr>
<tr>
<td>VLDL-III</td>
<td>96.9 ± 15.1</td>
<td>45.8 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>42.2 ± 7.3</td>
<td>41.0 ± 1.9</td>
<td></td>
</tr>
</tbody>
</table>

*Cholesterol synthesis in cells incubated with lipoprotein-deficient serum without added lipoproteins was regarded as 100%.

Values are percent of control and are the means ± SEM of four experiments carried out with lipoproteins isolated from the plasma of three normolipidemic human subjects, two with apo E of E-3/3, and one with E-4/3. The amounts of exogenous apo E added were 6 μg/ml for VLDL (30 μg lipoprotein protein) or 4 μg/ml for LDL (15 μg lipoprotein protein). Incorporation of 14C-aceta to sterols was determined after a 6-hour incubation of up-regulated cells with unlabeled lipoproteins, as described in Methods.

addition of apo E-3, the binding, association, and degradation of all lipoproteins tested (VLDL-I, VLDL-III, and LDL) in the receptor negative cells were negligible. As expected, exogenous apo E-3 was ineffective in these cells as compared to normal fibroblasts. In the third experiment, VLDL alone, apo E-3-enriched VLDL, and several concentrations of LDL were used to block receptor-specific metabolism of 129I-labeled VLDL-I, -II, and III (Table 5). While all conditions caused pronounced inhibition of 125I-VLDL degradation, apo E-3-enriched VLDLs were more effective, followed by unlabeled LDL. These results again indicated that specific B,E receptors are involved with uptake and degradation by the cells of apo E-3-enriched VLDLs.

To ascertain that exogenous apo E-3 increases VLDL particle metabolism and not merely protein uptake, the effects on cellular sterol synthesis were determined (Table 6). Apo E-3 alone caused a slight increase of cellular sterol synthesis, presumably due to increased efflux of cholesterol from the cells. VLDL alone did not down-regulate sterol synthesis, while LDL had a pronounced effect. Addition of apo E-3 caused marked down-regulation of the incorporation of 14C-aceta to sterol in incubations with VLDL populations without any effect on the regulation of sterol synthesis by LDL.

Discussion

The present study is part of an investigation into the nature of metabolic pathways that are responsible for direct catabolism of lipoproteins along the VLDL → IDL → LDL cascade. Such shunt pathways are undoubtedly responsible for the paucity of LDL in rats and other laboratory animals. The situation in humans is less clear. Kinetic analysis of human VLDL-apo B turnover data indicates that in normolipidemic subjects, as much as 50% of the VLDL-apo B is degraded before the formation of LDL. This presumably occurs via interaction of functional apo E molecules in VLDL with LDL receptors. Yet measurements of the interactions of normal VLDL (especially density fractions I and II) with the receptor failed to demonstrate significant catabolism of the lipoprotein in cultured cells. The results of the present investigation definitely show pronounced metabolism of normal VLDL populations by LDL receptors in fibroblasts when functional recombinant or plasmatic apo E-3 was added to intact, albeit centrifugally prepared, lipoproteins. These findings indicate that the reported inability of normal VLDL-I and VLDL-II to interact with the LDL receptor is due, at least in part, to loss of apo E from the lipoproteins during centrifugation and not to inherent properties of the lipoprotein particles themselves.

Investigations on the metabolism of VLDL in cultured cells have demonstrated that functional apo E molecules (E-3 and E-4) play an essential role in the catabolism of the lipoprotein through receptor-mediated processes. Several investigators have determined the effects of adding apo E-3 on the binding, cellular metabolism, or both of a variety of VLDL and B-VLDL particles in tissue culture systems. In some of these studies, inactive apo E-2 or chemically modified apo E-3 were replaced by active apo E-3. In other studies, apo E and other apoproteins in VLDL were degraded by either thrombin or trypsin treatment, and new, intact apo E-3 was added. All these studies demonstrated that apo E-3 restores the biological activity of the particles.

The approach used in the present investigation, however, was different. We attempted to determine the amounts of apo E-3 necessary for optimal cellular reactivity of normal VLDL from humans with E-3/3 or E-4/3 phenotype. With this novel approach, we demonstrated that maximal cellular metabolism of normal VLDL was achieved when the apo E/apo B weight ratio was about 0.3 to 0.5 (with higher concentrations of apo E-3, the excess protein apparently failed to associate with the VLDL). At these optimal concentrations, apo E constitutes 15% to 25% of the total VLDL protein and each particle contains 5 to 9 molecules of apo E. The reported content...
of apo E in normolipidemic VLDL fractions prepared by centrifugation and separated by gel chromatography on agarose columns, however, is about half, 7.5% to 11.6% of the total protein. These concentrations are perhaps less than optimal.

The findings reported here demonstrate a re-association of apo E-3 with centrifugally prepared VLDL fractions that leads to a many-fold enhancement of their cellular interactions. This effect is specific for apo E-3, similar for recombinant and the plasmatic protein, and dependent on the presence in the cells of functional LDL (B,E) receptors. The lack of response of normal LDL is presumably due to the inability of the apo E to re-associate with LDL particles. These observations would appear to indicate that the ability of apo E-3 to associate with different lipoproteins is different and reflects as yet unknown chemical and/or physical properties of the particles. That is if indeed the case, then such properties may play an essential role in regulating lipoprotein catabolism along the VLDL → IDL → LDL cascade.

In addition, however, we believe that the magnitude of cell catabolism of the apo E-3-enriched VLDL particles is less than would be expected from their apo E content. With 5 to 9 molecules of apo E-3 per VLDL particle, we expected to find cell reactivity that is several-fold higher than that of LDL. Such high reactivities were described for the cholesterol-rich apo E-HDL cholesterol from cholesteryl-fed animals. With VLDL, however, this was not expected even when the particles were maximally enriched with apo E-3. Is it possible that the biological expression of the apo E-3 in VLDL is incomplete? The presence of apo C in chylomicrons and Intralipid reportedly interferes with the apo E-3 without losses of apo C. Therefore, in addition to the apo E-3 without losses of apo C. Therefore, in addition to the presence of apo C, the regulation of apo E expression in lipoproteins may depend upon properties such as lipid composition, size, radius of curvature, or specific interactions with other lipoprotein constituents.

The considerations discussed above lead us to suggest that the process of catabolism of apo B-100/apo E lipoproteins via specific interactions of functional apo E molecules with LDL receptor is more complex than heretofore realized and is dependent on several different processes. These are: the availability of optimal amounts of functional apo E molecules, the capacity of the lipoproteins to associate apo E, and the biological expression of apo E on different particles. The pathways that regulate these processes are poorly understood and should be evaluated in future studies.

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Index Terms: VLDL catabolism • apo E-3 • LDL • cultured human fibroblasts • cholesterol synthesis
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